

SPECTRAL MANIPULATION IMPROVES GROWTH AND QUALITY ATTRIBUTES OF
LEAFY GREENS GROWN INDOORS

By

Qingwu Meng

A DISSERTATION

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

Horticulture—Doctor of Philosophy

2018

ABSTRACT

SPECTRAL MANIPULATION IMPROVES GROWTH AND QUALITY ATTRIBUTES OF LEAFY GREENS GROWN INDOORS

By

Qingwu Meng

Specialty food production in controlled environments is gaining momentum with an increasing interest in supplying fresh, local, and nutritious produce throughout the year. Plant phenotypic plasticity enables trait manipulation through adjusting environmental variables such as light (quantity, quality, and duration). Changing the spectral composition can alter photosynthetic energy and photomorphogenic signals, thereby influencing yield, morphology, and secondary metabolism. Blue and red light are typically used in sole-source lighting because of their high photosynthetic photon efficacy. In contrast, potential benefits of other wavebands such as green and far-red light have been less explored. To elucidate how various combinations of blue, green, red, and far-red light regulate growth and quality attributes of leafy greens, we conducted experiments in controlled-environment growth rooms with sole-source lighting from adjustable light-emitting diodes and/or in a greenhouse. Plants were grown in a deep-flow-technique hydroponic system and/or in a soilless substrate. Here, adding far-red light to blue and red light elicited the shade-avoidance response of lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) seedlings, increasing leaf expansion, light capture, and biomass. These responses were more pronounced under a high ratio of blue to red light or a low photosynthetic photon flux density. Spectral interactions were further investigated among blue, green, and far-red light in mature hydroponic lettuce and kale (*Brassica oleracea* var. *sabellica*). In a red-light background, substituting green or far-red light for blue light antagonized blue light-induced growth suppression and pigment accumulation. However, responses under increasing green light were

confounded by decreasing blue light, which can also trigger the shade-avoidance response. This was addressed by a following experiment, in which red light was substituted with green light at various blue photon flux densities. With or without green light, increasing blue light decreased biomass and leaf size of red-leaf lettuce but increased red foliage coloration and concentrations of several essential nutrients. Green light marginally influenced biomass under low blue light but decreased it under high blue light. Thus, green light effects depend on interactions among blue, green, and red light in specific spectral contexts. In addition, consumers preferred lettuce grown under sole-source lighting compared with those grown in a greenhouse. Finally, sequential alternations of spectra revealed lasting effects of initial lighting treatments and dynamic lettuce growth responses over time. Collectively, these studies reveal how crop traits can be improved by wavebands beyond static red and blue light and help uncover complex spectral interactions in whole-plant physiology of herbaceous plants.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Erik Runkle, my major professor, for his academic guidance and support as well as Drs. Cary Mitchell, Jennifer Boldt, and Roberto Lopez for serving on my advisory committee and providing valuable advice throughout my experiments and dissertation writing. I greatly appreciate experimental assistance from Steve Brooks, Nathan Kelly, Nate DuRussel, Zach Argo, Dr. Yujin Park, and Cathy Whitman (Michigan State University Department of Horticulture); help with elemental and phytonutrient analysis from Dr. Jennifer Boldt, Doug Sturtz, and Dr. Chris Ranger (USDA Agricultural Research Service); help with lettuce sensory tests from Dr. Sungeun Cho, Edward Szczygiel, and Shelby Cieslinski (Michigan State University Department of Food Science and Human Nutrition); and technical support with from Charles Brunault, Dr. David Hamby, Rodrigo Pereyra, Alan Sarkisian, and Dr. Dorian Spero (OSRAM Innovation). I also wish to thank Drs. Randy Beaudry, Roberto Lopez, Dan Brainard, Bert Cregg, Maria Jose, and Emily Merewitz for allowing me to use scientific instruments for plant measurements. Funding and/or donations from Michigan State University AgBioResearch (including Project GREEN GR15-025 and GR17-072), the USDA National Institute of Food and Agriculture, Hatch project 192266, and industry partners (OSRAM Opto Semiconductors, OSRAM Innovation, Grodan, and JR Peters, Inc.) are greatly appreciated. I am grateful for the excellent support and help from everyone in the Department of Horticulture. Last but not least, I could not have accomplished my goals without enduring love and support from my family and friends, especially Dr. Patrick Kelley, Jinfang Tian, Phyllis Kelley, Bill Kelley, Erin Kelley, Jing Xiao, Dr. Yujin Park, Dr. Diep Tran, Kellie Walters, Kristen Andersen, Emily Pawa, Wei-Kuang Lin, Songwen Zhang, and Dr. Danve Castroverde.

TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	ix
SECTION I	1
FAR-RED LIGHT INTERACTS WITH BLUE AND RED LIGHT TO REGULATE GROWTH, MORPHOLOGY, AND PIGMENTATION OF LETTUCE AND BASIL SEEDLINGS.....	1
<i>Abstract.</i>	3
Introduction	4
Materials and methods	7
<i>Plant material and propagation</i>	7
<i>Lighting treatments</i>	9
<i>Data collection and analysis</i>	10
Results	11
<i>Experiment I</i>	11
<i>Experiment II</i>	14
Discussion	15
Acknowledgments	22
APPENDIX	23
LITERATURE CITED	32
SECTION II	38
GREEN OR FAR-RED LIGHT ANTAGONIZES BLUE LIGHT IN REGULATION OF LETTUCE AND KALE GROWTH	38
<i>Abstract.</i>	40
Introduction	41
Materials and methods	44
<i>Plant material and propagation</i>	44
<i>Production culture and environment</i>	45
<i>Lighting treatments</i>	46
<i>Data collection and analysis</i>	47
Results	48
<i>Shoot weight</i>	48
<i>Plant morphology</i>	49
<i>Chlorophyll fluorescence, pigmentation, and leaf number</i>	50
Discussion	51
Acknowledgements	57
APPENDIX	58
LITERATURE CITED	67

SECTION III	71
BLUE LIGHT INTERACTS WITH GREEN LIGHT TO INFLUENCE GROWTH AND PREDOMINANTLY CONTROLS QUALITY ATTRIBUTES OF LETTUCE	71
<i>Abstract</i>	73
Introduction	74
Materials and methods	78
<i>Plant material and propagation</i>	78
<i>Lighting treatments</i>	79
<i>Production culture and environment</i>	80
<i>Data collection and analysis</i>	81
Results	84
<i>Biomass</i>	84
<i>Morphology</i>	85
<i>SPAD and Fv/Fm</i>	85
<i>Foliage coloration</i>	86
<i>Sensory attributes</i>	86
<i>Essential nutrients</i>	86
Discussion	87
Acknowledgements	96
APPENDIX	97
LITERATURE CITED	106
 SECTION IV	 112
GROWTH RESPONSES OF RED-LEAF LETTUCE TO TEMPORAL CHANGES IN LIGHT QUALITY	 112
<i>Abstract</i>	114
Introduction	115
Materials and methods	118
<i>The propagation phase</i>	118
<i>The production phase</i>	119
<i>Environmental conditions</i>	120
<i>Lighting treatments</i>	120
<i>Data collection and analysis</i>	121
Results	122
<i>Static lighting treatments for young and mature lettuce</i>	122
<i>Temporal lighting combinations for mature lettuce</i>	124
<i>The effects of initial and eventual lighting treatments on mature lettuce</i>	124
Discussion	126
Acknowledgements	133
APPENDIX	134
LITERATURE CITED	145

LIST OF TABLES

Table I-1. Spectral characteristics of the lighting treatments in experiments I and II comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The photon flux density of green (G; 500–600 nm) light is also provided. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency and spectral data from 350 to 800 nm (Sager et al., 1988)] were calculated. The estimated phytochrome photoequilibrium (PPE) was calculated according to Sager et al. (1988).
..... 24

Table II-1. The pH, electrical conductivity, and temperature (mean $\pm$ standard deviation from daily measurements) of nutrient solutions for ten lighting treatments in three experimental replications (rep.). Plants were grown hydroponically under a 20-h photoperiod and various mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 59

Table II-2. The air temperature, CO₂ concentration, and relative humidity (mean $\pm$ standard deviation from hourly averages) for ten lighting treatments in three experimental replications (rep.). Plants were grown hydroponically under a 20-h photoperiod and various mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 60

Table II-3. Spectral characteristics of ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. Integrated parameters include the photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency (McCree, 1972) and spectral data from 350 to 800 nm]. The estimated phytochrome photoequilibrium (PPE) was calculated as the proportion of active phytochromes in the total phytochrome pool according to Sager et al. (1988). The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 61

Table III-1. Spectral characteristics of nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes (LEDs). The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Photon flux densities over 1-nm increments were integrated as the photosynthetic photon flux density (PPFD; 400–700 nm) and the total photon flux density (TPFD; 400–800 nm), which includes far-red (FR; 700–800 nm) light. The yield photon flux density (YPFD; 300–800 nm) was the product of the spectral distribution and relative quantum efficiency (Sager et al., 1988). The phytochrome photoequilibrium (PPE) was estimated

according to Sager et al. (1988). The color-rendering index (CRI) was calculated based on the spectral distribution using the online LED ColorCalculator by OSRAM Sylvania. 98

Table III-2. The pH, electrical conductivity, and water temperature [mean $\pm$ standard deviation in each replication (rep.)] of nutrient solutions for nine lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 99

Table IV-1. The pH, electrical conductivity, and water temperature (mean $\pm$ standard deviation) of nutrient solutions for six lighting treatment plots in two blocks during the lettuce production phase. Plants were grown under warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 135

Table IV-2. Spectral characteristics of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Integrated parameters include the photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency (McCree, 1972) and spectral data from 300 to 800 nm]. The estimated phytochrome photoequilibrium (PPE) was calculated as described by Sager et al. (1988). 136

Table IV-3. Temporal lighting combinations during lettuce propagation and production. Plants were grown under static or alternate lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 137

LIST OF FIGURES

Figure I-1. Spectral distributions of six lighting treatments in experiment I comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.	25
Figure I-2. Spectral distributions of six lighting treatments in experiment II comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.	26
Figure I-3. Shoot and root fresh and dry weights and weight partitioning of lettuce ‘Rex’ and ‘Cherokee’ and basil ‘Genovese’ grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).	27
Figure I-4. Hypocotyl caliper and length, leaf length, and leaf number of lettuce ‘Rex’ and ‘Cherokee’ and basil ‘Genovese’ grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).	28
Figure I-5. Relative specific chlorophyll content (SPAD) and foliage color coordinates [a^* for greenness–redness (negative–positive) and b^* for blueness–yellowness (negative–positive)] of lettuce ‘Rex’ and ‘Cherokee’ and basil ‘Genovese’ grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).	29
Figure I-6. Shoot fresh and dry weights, leaf length and width, and hypocotyl length of lettuce ‘Rex’ and ‘Rouxai’ grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).	30
Figure I-7. Relative specific chlorophyll content (SPAD) and leaf number of lettuce ‘Rex’ and ‘Rouxai’, and the green-red color coordinate [a^* for greenness–redness (negative–positive)] of lettuce ‘Rouxai’ grown under six sole-source lighting treatments comprised of blue (B; 400–500	

nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). 31

Figure II-1. Spectral distributions of ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 62

Figure II-2. Lettuce 'Rex' and 'Rouxai' 27 and 30 d after sowing, respectively. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 63

Figure II-3. Shoot fresh and dry weight, length and width of the fifth most mature leaf, plant diameter, leaf area, and petiole length of lettuce 'Rex', lettuce 'Rouxai', and kale 'Siberian'. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). 64

Figure II-4. The maximum quantum efficiency of photosystem II (Fv/Fm), relative chlorophyll concentration (SPAD), and leaf number of lettuce 'Rex', lettuce 'Rouxai', and kale 'Siberian'. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). 65

Figure II-5. Lab color space analysis (L^* , lightness; a^* , green–red; b^* , blue–yellow) for foliage coloration of lettuce 'Rouxai'. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). 66

Figure III-1. Spectral distributions of nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes. The greenhouse treatment received sunlight with supplemental

high-pressure sodium lighting. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 100

Figure III-2. Lettuce ‘Rouxai’ 32 d after sowing from the first replication. Plants were grown under nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes or a greenhouse treatment that received sunlight supplemented with high-pressure sodium lighting. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 101

Figure III-3. Shoot fresh and dry weights, leaf length and width, plant diameter, leaf number, the SPAD index, and maximum quantum efficiency of photosystem II (F_v/F_m) of lettuce ‘Rouxai’ grown under nine sole-source lighting treatments, with or without green light, or in a greenhouse. Equations, p-values, coefficients of determination (R^2), and percentage changes are given for linear responses to the blue photon flux density ($\alpha = 0.05$) with green light (solid lines and black text) and without (dashed lines and gray text) green light. At any blue photon flux density, an asterisk indicates that means with and without green light are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors..... 102

Figure III-4. Lab color space analysis of lettuce ‘Rouxai’ grown under nine sole-source lighting treatments, with or without green light, or in a greenhouse. Means followed by different letters within each parameter are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Black and gray letters are associated with filled circles (without green light) and empty circles (with green light), respectively. Error bars show standard errors. 103

Figure III-5. Sensory ratings on lettuce ‘Rouxai’ by 164 panelists (86 and 78 in two replications). Plants were grown under five sole-source lighting treatments or in a greenhouse. The number for each waveband [blue (B; 400–500 nm), green (G; 500–600 nm), or red (R; 600–700 nm)] is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each category are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors. 104

Figure III-6. Concentrations of macronutrients [nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), and sulfur (S)] and micronutrients [iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo)] in leaf tissues of lettuce ‘Rouxai’. Plants were grown under five sole-source lighting treatments or in a greenhouse. The number for each waveband [blue (B; 400–500 nm), green (G; 500–600 nm), or red (R; 600–700 nm)] is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each element are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors. 105

Figure IV-1. Spectral distributions of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 138

Figure IV-2. Shoot fresh and dry weights and leaf length on days 11 and 25 of lettuce ‘Rouxai’ grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors..... 139

Figure IV-3. Lab color space parameters on days 11 and 25 of lettuce ‘Rouxai’ grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters in each graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors. 140

Figure IV-4. Shoot fresh and dry weights, leaf length, and the a^* color space coordinate of lettuce ‘Rouxai’ on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and treatment applied during day 11–25 are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). NS, non-significant. Error bars show standard errors. 141

Figure IV-5. The effects of initial (applied day 0–11) and eventual (applied day 11–25) lighting treatments on pooled final shoot fresh and dry weights and leaf length of lettuce ‘Rouxai’ on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors..... 142

Figure IV-6. The effects of initial (applied day 0–11) and eventual (applied day 11–25) lighting treatments on pooled final Lab color space parameters of lettuce ‘Rouxai’ on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors..... 143

Figure IV-7. Relative shoot dry weight of lettuce ‘Rouxai’ on day 25 plotted against the relative photosynthetic photon flux density (PPFD), relative yield photon flux density (YPPFD), relative leaf length, relative PPFD $\times$ relative leaf length, and relative YPPFD $\times$ relative leaf length. Plants

were grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Data were averaged for each lighting treatment from two blocks. Linear regression equations, coefficients of determination, and p-values for slopes are provided. The only significant linear relationship occurs between relative YPFD $\times$ relative leaf length and relative shoot dry weight ($\alpha = 0.05$)..... 144

SECTION I

FAR-RED LIGHT INTERACTS WITH BLUE AND RED LIGHT TO REGULATE GROWTH, MORPHOLOGY, AND PIGMENTATION OF LETTUCE AND BASIL SEEDLINGS

Far-red light interacts with blue and red light to regulate growth, morphology, and pigmentation of lettuce and basil seedlings

Qingwu Meng and Erik S. Runkle*

Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI 48824-1325, USA

*Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890. E-mail address:

runkleer@msu.edu (E.S. Runkle)

Abstract.

Although outside the photosynthetic photon flux density (PPFD) waveband (400–700 nm), far-red (FR; 700–800 nm) light regulates photomorphogenesis and photochemistry, thereby meriting consideration in sole-source plant-lighting applications. We investigated how FR light interacted with the ratio of blue (B; 400–500 nm) to red (R; 600–700 nm) light (B:R) and PPFD to regulate seedling growth under sole-source lighting. We postulated that adding FR light to B+R light would increase leaf expansion and thus light capture to promote whole-plant photosynthesis, but that FR effects would depend on B:R and PPFD. In experiment I, lettuce (*Lactuca sativa*) ‘Rex’ and ‘Cherokee’ and basil (*Ocimum basilicum*) ‘Genovese’ were continuously irradiated by 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B and/or R light [B₃₀R₁₅₀ (low B:R), B₉₀R₉₀ (high B:R), R₁₈₀, or B₁₈₀, where subscripts indicate respective photon flux densities in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$] with or without 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light. Twelve and 16 days after seed sow for lettuce and basil, respectively, the addition of FR light increased leaf length and shoot weight of all crops with more pronounced impacts under high B:R than low B:R. It also increased root dry weight of basil and lettuce ‘Cherokee’. Adding FR to B+R light reduced specific chlorophyll content in lettuce by 10–20%, but not in basil. Red pigmentation of lettuce ‘Cherokee’ increased with increasing B:R but decreased with the inclusion of FR light. In experiment II, we grew lettuce ‘Rex’ (for 8 days) and ‘Rouxai’ (for 10 days) under B₉₀R₉₀ (low PPFD) or B₁₈₀R₁₈₀ (high PPFD) at the same B:R with or without FR light at 30 or 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Additional FR light increased lettuce shoot weight and extension growth but reduced relative specific chlorophyll content under both PPFDs, although FR effects were attenuated under the high PPFD. Shoot dry weight, relative specific chlorophyll content, and red foliage pigmentation increased with PPFD. We conclude that FR enrichment improves photosynthetic light capture and thus promotes crop

growth under sole-source lighting, and that its effects are especially pronounced under high B:R and a low PPFD.

Keywords: controlled environment, leafy greens, light-emitting diode, light quality, phytochrome, sole-source lighting, vertical farming.

Abbreviations: AFB, AUXIN SIGNALING F-BOX; B, blue; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; DFR, dihydroflavonol 4-reductase; DLI, daily light integral; FR, far red; G, green; HAT4, HOMEODOMAIN-BOX FROM ARABIDOPSIS THALIANA; LDOX, leucoanthocyanidin dioxygenase; LEDs, light-emitting diodes; PIF, phytochrome-interacting factor; PIL1, PIF3-LIKE1; PPE, phytochrome photoequilibrium; PPFD, photosynthetic photon flux density; R, red; TIR1, TRANSPORT INHIBITOR RESPONSE; TPDF, total photon flux density; UF3GT; UDP-glucose:flavonoid 3-O-glucosyltransferase; YPFD, yield photon flux density.

Introduction

The increasing relevance of indoor vertical farming in urban areas demands precision in environmental control to optimize crop growth and elicit desired characteristics. Since plant photosynthesis, morphology, and secondary metabolism are regulated by light quality, quantity, and duration, the intensity and spectral composition of sole-source lighting is of paramount importance. Mixtures of blue (B; 400–500 nm) and red (R; 600–700 nm) light-emitting diodes (LEDs) have been prevalent primarily because of their high electrical efficiency and adequacy for normal plant growth (Yorio et al., 1998). As the ratio of B to R light (B:R) increases,

vegetable and ornamental crops typically have reduced weight and extension growth but enhanced pigmentation and nutritional value (Son and Oh, 2013; Kopsell et al., 2015; Wollaeger and Runkle, 2015), although growth responses to B:R can vary depending on species (Hernández and Kubota, 2016). Increasing the photosynthetic photon flux density (PPFD; 400–700 nm) or the photosynthetic daily light integral (DLI) can promote crop-growth rates as plants undergo photosynthetic acclimation in high light. For example, increasing the PPFD from 50 to 550 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased fresh and dry weight of tomato (*Solanum lycopersicum*) (Fan et al., 2013). Similarly, increasing the DLI from 8 to 22 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ increased fresh weight of butterhead lettuce (*Lactuca sativa*) ‘Ostinata’ (Both et al., 1997). Nonetheless, the target PPFD and DLI for sole-source lighting of leafy greens are usually moderated to avoid photodamage and tipburn and to maximize return on investment. Although outside the PPFD range, far-red (FR; 700–800 nm) light mediates a wide array of physiological processes including photomorphogenesis, photochemistry, flowering, and anthocyanin production (Franklin, 2008; Carvalho and Folta, 2014; Zhen and van Iersel, 2017; Park and Runkle, 2017, 2018). Therefore, its biological significance and potential commercial applications in controlled-environment agriculture merit further investigation.

The varying spectral distributions of sunlight above and below a canopy inform plants of locations and surroundings (Smith, 2000). The upper canopy receives a broad spectrum with similar B, green (G; 500–600 nm), R, and FR photon fluxes, whereas the spectrum in the lower canopy has significantly reduced B, G (reduced less than B or R light), and R light but abundant FR light (Vandenbussche et al., 2005; Franklin, 2008). The ratio of R to FR light (R:FR) is approximately 1.2 in broad daylight and as low as 0.05 in vegetative shade (Franklin and Whitelam, 2005). Both low B light (e.g., $<50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and low R:FR can elicit shade-

avoidance responses, such as accelerated shoot elongation, increased leaf expansion, upward leaf orientation (or hyponasty), reduced branching, and early flowering (Vandenbussche et al., 2005). Signaling of these morphological and developmental changes occurs through photoreceptors including R- and FR-absorbing phytochromes and B- and ultraviolet-A-absorbing cryptochromes. However, genes expressed under low B light and low R:FR are distinctly different in regulation of shade avoidance (Pedmale et al., 2016).

Independent of the auxin pathway, low B light promotes extension growth by inducing direct interactions of cryptochromes 1 and 2 with phytochrome-interacting factors (PIFs) 4 and 5, followed by the binding of cryptochrome 2 and PIFs 4 and 5 to the promoters of growth-related genes (Pedmale et al., 2016). On the other hand, low R:FR increases extension growth by enabling stable functions of PIFs 4, 5, and 7 to increase auxin and gibberellin accumulation through phytochrome B, albeit with antagonism from phytochrome A (Franklin, 2008; Hornitschek et al., 2012; Li et al., 2012). Phytochromes can be converted from an inactive R-absorbing form (P_R) to an active FR-absorbing form (P_{FR}) under R light, and vice versa under FR light (Smith, 2000). Phytochrome B is stabilized under R light to degrade PIFs 4 and 5, subsequently repressing genes related to shade avoidance such as *HOMEODOMAIN-LESS 4* (*HAT4*) and *PIF3-LIKE1* (*PIL1*) (Lorrain et al., 2008; Zhang et al., 2011). Low R:FR alleviates repression of PIFs and downstream shade-related transcripts from active phytochrome B by decreasing the proportion of P_{FR} in the total phytochrome pool [or the phytochrome photoequilibrium (PPE)] (Franklin, 2008). In addition, dephosphorylated PIF7 accumulates with reduced active phytochrome B under low R:FR and increases expression of auxin biosynthetic genes to directly promote growth (Li et al., 2012).

Besides low B light and low R:FR, a low PPFD can also trigger the shade-avoidance

response (e.g., increased hypocotyl elongation) through a different mechanism, which remains poorly understood except for its regulation of auxin signaling (Hersch et al., 2014; Pedmale et al., 2016). Low PPFD responses are likely mediated by multiple photoreceptors including phytochromes, cryptochromes, and phototropins (Pedmale et al., 2016). In *Arabidopsis thaliana*, a low PPFD decreased auxin content but enhanced auxin sensitivity (Hersch et al., 2014). Moreover, auxin signaling was augmented at a low PPFD compared to a high PPFD under low R:FR, but not under high R:FR (Hersch et al., 2014), indicating that R:FR interacted with the PPFD. However, ramifications of this interaction in regulation of crop yield and quality remain inconclusive, especially for specialty food crops grown in controlled environments. In addition, because the B photon flux, R:FR, and PPFD converge in target growth responses through different pathways (Pedmale et al., 2016), they interact with each other and add complexity to photocontrol of desired crop phenotypes.

The objective of this study was to investigate how FR light interacted with B:R (experiment I) and the PPFD (experiment II) to influence shoot and root weights, morphological traits, and foliage pigmentation of lettuce and basil seedlings grown indoors under sole-source LED lighting. We postulated that the addition of FR light to B+R light would promote biomass accumulation and extension growth of both lettuce cultivars and basil but decrease pigmentation of red oakleaf lettuce, and that the magnitude of FR effects would depend on B:R and the PPFD.

Materials and methods

Plant material and propagation

In experiment I, seeds of green butterhead lettuce ‘Rex’, red oakleaf lettuce ‘Cherokee’, and basil (*Ocimum basilicum*) ‘Genovese’ (Johnny’s Selected Seeds, Winslow, ME) were sown in a

peat-perlite medium (70% peat moss, 21% perlite, and 9% vermiculite, Suremix; Michigan Grower Products, Inc., Galesburg, MI) on 27 Sept. 2015 and 13 Oct. 2015 for two replications. In experiment II, seeds of lettuce ‘Rex’ and red oakleaf lettuce ‘Rouxai’ (Johnny’s Selected Seeds) were sown in the same medium on 5 Mar. 2016, 22 Mar. 2016, and 11 June 2017 for three replications. Throughout the experiment, the medium was subirrigated daily with reverse-osmosis water supplemented with a water-soluble fertilizer (13N–5P–13K MSU Plug Special; Greencare Fertilizers, Inc., Kankakee, IL) to supply the following nutrients (in $\text{mg}\cdot\text{L}^{-1}$): 50 N, 19 P, 50 K, 23 Ca, 3.9 Mg, 1.0 Fe, 0.52 Mn, 0.52 Zn, 0.52 Cu, 0.31 B, and 0.10 Mo. Seed trays (individual cells measuring 2.7×2.7 cm; 12.0-mL volume) were covered with transparent plastic humidity domes and placed under continuous cool-white fluorescent light (F96T12; Philips, Amsterdam, the Netherlands) in a walk-in refrigerated growth room at a temperature set point of 20 °C. The PPFD at plug height was maintained at $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from day 0 to 2 and $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from day 2 to 3. In experiment I, lettuce and basil seedlings were transferred to six LED chambers (OSRAM Opto Semiconductors, Northville, MI), as described by Wollaeger and Runkle (2013), in a separate growth room at a temperature set point of 23 °C on day 3 and 4, respectively, when humidity domes were removed. In experiment II, lettuce seedlings were transferred to the six LED chambers on day 3 and grown at a temperature set point of 23 °C, but humidity domes were removed on day 4. Air temperature and plant-canopy temperature were measured with thermocouples (0.13-mm type E; Omega Engineering, Inc., Stamford, CT) and infrared sensors (OS36-01-K-80F; Omega Engineering, Inc.), respectively, and were recorded by a datalogger (CR10; Campbell Scientific, Logan, UT) throughout the experiments. Quantum sensors (LI-190R; LI-COR, Inc., Lincoln, NE) were leveled and positioned on the base of each LED chamber to measure the PPFD. Temperature and light data were recorded every 10 s and

averaged every 10 min by the datalogger.

Lighting treatments

In experiment I, lettuce and basil seedlings were grown from day 3 to 12 and from day 4 to 16, respectively, under six LED lighting treatments delivering mixtures of B (peak = 447 nm), R (peak = 661 nm), and FR (peak = 732 nm) light (OSRAM Opto Semiconductors) at a constant PPFD of $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a 24-h photoperiod: B₃₀R₁₅₀, B₃₀R₁₅₀FR₃₀, B₉₀R₉₀, B₉₀R₉₀FR₃₀, R₁₈₀FR₃₀, and B₁₈₀FR₃₀, where the number following each waveband indicates its respective photon flux density at plant height in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The use of continuous lighting was to achieve the highest DLI of $15.6 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ at the desired PPFD. Light was measured at plant height at ten locations across the base of each LED chamber with a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT). The photon flux density of each waveband was adjusted using a channel-specific slider integrated adjacent to the circuit board in each LED fixture for each LED chamber. Aluminum wire mesh was placed beneath the middle two-thirds of each LED fixture to improve light uniformity. The spectral characteristics and distributions of all treatments are provided in Table I-1 and Figure I-1. The yield photon flux density (YPPFD) was calculated by multiplying relative quantum efficiency and spectral data from 350 to 800 nm (Sager et al., 1988). The phytochrome photoequilibrium (PPE) was estimated based on phytochrome absorption coefficients and spectral data (Sager et al., 1988).

In experiment II, lettuce ‘Rex’ and ‘Rouxai’ seedlings were grown in the same LED chambers from day 3 to 8 or 10, respectively, under six B, R, and FR LED lighting treatments at a PPFD of 180 or $360 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a 24-h photoperiod: B₉₀R₉₀, B₉₀R₉₀FR₃₀, B₉₀R₉₀FR₇₅, B₁₈₀R₁₈₀, B₁₈₀R₁₈₀FR₃₀, and B₁₈₀R₁₈₀FR₇₅, where the numbers indicate the same denotation as previously described. The methodology for light measurements and adjustments was the same as

for experiment I. The spectral characteristics and distributions of all treatments are provided in Table I-1 and Figure I-2.

Data collection and analysis

In experiment I, destructive measurements on 10 plants per cultivar, treatment, and replication were conducted on day 12 for lettuce and day 16 for basil. In experiment II, the same measurements except for roots were conducted on day 8 and 10 for lettuce ‘Rex’ and ‘Rouxai’, respectively. Shoot and root fresh weights were measured with an analytical balance (AG245; Mettler Toledo, Greifensee, Switzerland). Before root fresh weight measurements, roots were cleaned in clear water, tapped with paper towel, and stored on a ventilated table until external water evaporated. Subsequently, shoots and roots were dried in a heated oven (model 630; Napco Scientific Company, Tualatin, OR) at 80 °C for ≥ 5 d before dry weight measurements. Length of the most mature true leaf, hypocotyl length, and leaf number of each seedling were measured. Stem caliper was measured with a digital micrometer (Mitutoyo Corporation, Kawasaki, Japan). Relative specific chlorophyll content was measured with an instant chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan) and averaged from three measurements on the first true leaf of each seedling. Foliage color profiles of red-leaf lettuce cultivars and basil were quantified with a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc.) using $L^*a^*b^*$ coordinates defined by the International Commission on Illumination. Data for L^* are not reported here. The greater a^* was, the redder the leaf, whereas the smaller a^* was, the greener the leaf. The greater b^* was, the yellower the leaf, whereas the smaller b^* was, the bluer the leaf.

Both experiments were based on a randomized complete block design with temporal repetition as the block and the lighting treatment as the fixed factor. All data for each cultivar in

each experiment were pooled from all replications and analyzed with SAS (version 9.4; SAS Institute, Cary, NC) using the PROC MIXED (for continuous data) and PROC GLIMMIX (for discrete data) procedures and Tukey's honestly significant difference test ($\alpha = 0.05$).

Results

Experiment I

The addition of $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light to B+R light, which increased the total photon flux density (TPFD; 400–800 nm) by 17%, generally increased shoot fresh and dry weights of lettuce and basil (Figure I-3A–C). However, the magnitude of this increase was greater under B₉₀R₉₀ than under B₃₀R₁₅₀ for all crops. For example, FR light did not affect shoot weight of lettuce 'Rex' under B₃₀R₁₅₀ but increased its shoot fresh (and dry) weight by 42% (43%) under B₉₀R₉₀. In addition, FR light increased shoot fresh (and dry) weight of lettuce 'Cherokee' by 17% (17%) under B₃₀R₁₅₀ but by 48% (44%) under B₉₀R₉₀. Without FR light, shoot fresh (and dry) weight of lettuce 'Rex' and 'Cherokee' was 18% (17%) and 23% (22%) lower, respectively, under B₉₀R₉₀ than under B₃₀R₁₅₀. In contrast, with additional FR light, shoot fresh and dry weights of these two lettuce cultivars was similar under B₃₀R₁₅₀ and B₉₀R₉₀. With or without FR light, shoot weight of basil was generally not influenced by B:R, except that its shoot fresh weight was 13% greater under B₉₀R₉₀ than under B₃₀R₁₅₀. Plants grown under R₁₈₀FR₃₀ had similar shoot weight to those grown under B₃₀R₁₅₀FR₃₀, except for shoot dry weight of lettuce 'Rex', which was lower under R₁₈₀FR₃₀. Without R light, shoot weight under B₁₈₀FR₃₀ was similarly low as that under B₉₀R₉₀ for lettuce and as that under B₃₀R₁₅₀, B₉₀R₉₀, and R₁₈₀FR₃₀ for basil.

Although adding FR light to B+R light did not influence root weight of lettuce 'Rex', it

increased root fresh (and dry) weight of lettuce ‘Cherokee’ grown under B₃₀R₁₅₀ by 27% (25%) and that of basil grown under B₃₀R₁₅₀ and B₉₀R₉₀ by 25% (18%) and 55% (26%), respectively (Figure I-3D–F). Increasing B:R from 0.2 (B₃₀R₁₅₀) to 1.0 (B₉₀R₉₀) decreased root dry weight, but not root fresh weight, of lettuce ‘Rex’ by 19% with or without FR light, and decreased root fresh (and dry) weight of lettuce ‘Cherokee’ by 25% (29%) with FR light. In contrast, the high B:R increased root fresh weight, but not dry weight, of basil grown without and with FR light by 18% and 46%, respectively. For lettuce ‘Rex’, root fresh and dry weights under R₁₈₀FR₃₀ and B₁₈₀FR₃₀ were the lowest. For lettuce ‘Cherokee’, root fresh and dry weights were similar under R₁₈₀FR₃₀, B₉₀R₉₀, and B₉₀R₉₀FR₃₀ and were the lowest under B₁₈₀FR₃₀. Root fresh weight of basil under B₁₈₀FR₃₀ was comparable to all other treatments except B₉₀R₉₀FR₃₀. Root dry weight of basil was similar under B₃₀R₁₅₀, B₉₀R₉₀, R₁₈₀FR₃₀, and B₁₈₀FR₃₀. and comparable to those under B₃₀R₁₅₀ for basil. For lettuce, the root-to-shoot ratio was lower under B₉₀R₉₀FR₃₀ than under the other three B+R+FR treatments (i.e., B₃₀R₁₅₀, B₃₀R₁₅₀FR₃₀, and B₉₀R₉₀) and was generally the lowest under R₁₈₀FR₃₀ and B₁₈₀FR₃₀ (Figure I-3G–H). In contrast, the root-to-shoot ratio of basil was the highest under B₉₀R₉₀FR₃₀ based on fresh weight but was not affected by additional FR light (irrespective of B:R) based on dry weight (Figure I-3I).

The addition of FR light to B+R light did not affect hypocotyl caliper of lettuce but increased that of basil grown under B₉₀R₉₀ by 13% (Figure I-4A–C). Hypocotyl caliper of lettuce ‘Rex’ was the lowest under R₁₈₀FR₃₀ followed by B₁₈₀FR₃₀. Hypocotyl length of lettuce ‘Rex’ and ‘Cherokee’ grown under R₁₈₀FR₃₀ was 169–246% and 116–215% greater, respectively, than that under all other treatments. Adding FR light to B+R light did not affect hypocotyl length of lettuce ‘Rex’ but increased that of lettuce ‘Cherokee’ and basil grown under B₉₀R₉₀ by 33% and 37%, respectively. Without FR light, increasing B:R from 0.2 to 1.0 decreased hypocotyl length

of basil by 19%, whereas inclusion of FR light counteracted this effect. Hypocotyls of all crops were shorter under B₁₈₀FR₃₀ than under R₁₈₀FR₃₀.

The addition of FR light increased leaf length of all crops, especially under B₉₀R₉₀ (Figure I-4D–F). For example, FR light increased leaf length of lettuce ‘Rex’, lettuce ‘Cherokee’, and basil by 25%, 22%, and 20%, respectively, under B₃₀R₁₅₀ but by 63%, 66%, and 28%, respectively, under B₉₀R₉₀. Without FR light, leaf length of lettuce ‘Rex’ and ‘Cherokee’ grown under B₉₀R₉₀ was 12% and 20% shorter, respectively, than that under B₃₀R₁₅₀; however, B:R did not influence leaf length of basil. Lettuce leaves were the most elongated under R₁₈₀FR₃₀ followed by B₁₈₀FR₃₀ and B₉₀R₉₀FR₃₀. In contrast, basil leaves were the longest under FR-including treatments except R₁₈₀FR₃₀. The addition of FR light decreased leaf number of lettuce ‘Rex’ and basil grown under B₉₀R₉₀ by one but did not influence that of lettuce ‘Cherokee’ (Figure I-4G–I). Lettuce ‘Rex’ and ‘Cherokee’ developed the fewest leaves under B₁₈₀FR₃₀ among all treatments.

When added to B+R light, FR light decreased relative specific chlorophyll content of lettuce ‘Rex’ by 16–19% under B₃₀R₁₅₀ and B₉₀R₉₀ and that of lettuce ‘Cherokee’ by 10% under B₉₀R₉₀, but did not affect basil (Figure I-5A–C). Without FR light, increasing B:R from B₃₀R₁₅₀ to B₉₀R₉₀ increased relative specific chlorophyll content of both lettuce cultivars by 9–10%, but did not impact basil. Relative specific chlorophyll content was the lowest under R₁₈₀FR₃₀ for all crops. Light quality also influenced red-green foliage coloration of red-leaf lettuce ‘Cherokee’ but had minimal effects on that of green leaf lettuce ‘Rex’ and basil (Figure I-5D–F). Adding FR light to B+R light decreased red foliage coloration (or a^*) of lettuce ‘Cherokee’, indicating reduced anthocyanin concentration. Increasing B:R from 0.2 to 1.0 intensified leaf redness regardless of FR light. Leaf yellowness increases with increasing b^* , whereas leaf greenness increases with increasing relative specific chlorophyll content. The trends for b^* were generally

opposite of those for relative specific chlorophyll content (Figure I-5A–C, G–I), showing consistent spectral effects on leaf chlorophyll accumulation and coloration in all crops.

Experiment II

Increasing the FR photon flux density from 0 to 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased shoot fresh weight of lettuce ‘Rex’ and ‘Rouxai’ grown under the low PPFD (i.e., B₉₀R₉₀) by 42% and 51%, respectively, but not under the high PPFD (i.e., B₁₈₀R₁₈₀) (Figure I-6A–B). In contrast, it increased shoot dry weight of both lettuce cultivars regardless of the PPFD, but the magnitude of the increase was greater under the lower PPFD. For example, FR light increased shoot dry weight of lettuce ‘Rex’ and ‘Rouxai’ by up to 35% and 57%, respectively, under the low PPFD but only up to 20% and 24%, respectively, under the high PPFD. Without FR light, shoot fresh weight of lettuce ‘Rex’ and ‘Rouxai’ was 38% and 44% greater, respectively, under the high PPFD than under the low PPFD. At any FR photon flux density, the high PPFD increased shoot dry weight of lettuce ‘Rex’ and ‘Rouxai’ by 37–54% and 22–63%, respectively, compared to the low PPFD.

Adding FR light to B+R light increased leaf length of both lettuce cultivars similarly, with a greater effect under the low PPFD than under the high PPFD (Figure I-6C–D). For example, adding 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light increased leaf length of lettuce ‘Rex’ and ‘Rouxai’ by 71% and 47%, respectively, under the low PPFD but by only 25% and 10%, respectively, under the high PPFD. Although the high PPFD increased leaf length slightly (by 11–12%) compared to the low PPFD in the absence of FR light, leaf length of both lettuce cultivars was 12–18% lower under the high PPFD than under the low PPFD when 30 or 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ FR light was added. Increasing FR photon flux density from 0 to 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased lettuce leaf width by 21–24% under the low PPFD, but not under the high PPFD. Lettuce leaf width was 19–22% greater

under the high PPFD than under the low PPFD without FR light, but was similar under the low and high PPFDs when FR light was added. FR light increased hypocotyl length of lettuce ‘Rex’ by up to 64% and 30% under the low and high PPFDs, respectively, and increased that of lettuce ‘Rouxai’ by up to 27% under the low PPFD, but not under the high PPFD (Figure I-6E–F). At the same added FR photon flux density, increasing the PPFD from 180 to 360 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ did not influence hypocotyl length of lettuce ‘Rouxai’ but decreased that of lettuce ‘Rex’ by 20–34%. Without FR light, hypocotyl length of the two lettuce cultivars was similar under both PPFDs.

Relative specific chlorophyll content decreased by 19–24% for green leaf lettuce ‘Rex’ and by 10% for red-leaf lettuce ‘Rouxai’ as FR photon flux density increased from 0 to 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ irrespective of the PPFD (Figure I-7A–B). At the same FR photon flux density, the SPAD index was 11–19% and 21–23% greater under the high PPFD than under the low PPFD for lettuce ‘Rex’ and ‘Rouxai’, respectively. Lettuce ‘Rouxai’ grown under the high PPFD developed redder foliage (greater a^*) than that grown under the low PPFD (Figure I-7C). Under the high PPFD, red pigmentation increased as FR photon flux density increased from 0 to 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Leaf development of the two lettuce cultivars was generally similar in all PPFD and FR treatments (Figure I-7D–E).

Discussion

The addition of FR light to B+R light generally increased shoot weight and leaf expansion but decreased relative specific chlorophyll content and red foliage pigmentation. The magnitude of these FR-induced responses depended on B:R and the PPFD. In most cases, FR light effects were pronounced under the high B:R (experiment I) and the low PPFD (experiment II) but attenuated under the low B:R and the high PPFD, likely because the same photon flux density of

FR light decreased the estimated PPE more under the high B:R and the low PPFD. When added to B+R light, FR light decreased the PPE and allowed for the proper functions of PIFs in the shade-avoidance response (Franklin, 2008). Adding $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light decreased the estimated PPE by 5% and 8% under B₃₀R₁₅₀ and B₉₀R₉₀, respectively, in experiment I and by 4% and 7% under B₁₈₀R₁₈₀ and B₉₀R₉₀, respectively, in experiment II (Table I-1). In addition, adding $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light decreased the estimated PPE by 9% and 17% under B₁₈₀R₁₈₀ and B₉₀R₉₀, respectively, in experiment II (Table I-1). A decrease in the estimated PPE with the addition of FR light (peak = 731 nm) has been associated with linear increases in shoot dry weight, stem length, and leaf area as well as a linear reduction in specific chlorophyll content of ornamental seedlings such as geranium (*Pelargonium ×hortorum*) ‘Pinto Premium Orange Bicolor’ and snapdragon (*Antirrhinum majus*) ‘Trailing Candy Showers Yellow’ (Park and Runkle, 2017, 2018). Similar manipulation of the PPE with FR light also increased plant height of other ornamental crops grown under sole-source and photoperiodic lighting (Craig and Runkle, 2013, 2016; Mah et al., 2018).

In arabidopsis, F-box proteins TRANSPORT INHIBITOR RESPONSE (TIR1) and AUXIN SIGNALING F-BOX (AFB) are positive regulators in the auxin signaling pathway (Yu et al., 2013). The increase in *AFB1* expression mediated by low R:FR (or low PPE) was greater under a low PPFD than under a high PPFD (Hersch et al., 2014). Therefore, the interaction between FR light and PPFD in the present study could at least partly be explained by differential auxin responses under different PPE. In contrast, the effects of additional FR light on ornamental seedlings were reportedly independent of the PPFD at a fixed B photon flux density (Park and Runkle, 2018). However, the increments of FR light in that study varied under the low and high PPFDs to maintain a similar PPE at each increment; therefore, there was no interaction between

the PPFD and the PPE, not FR light at a certain photon flux density. The seemingly contradictory conclusions are reconciled by distinguishing between the PPE and FR light.

The interactions between FR light and B:R or the PPFD depend on the B photon flux density. B light modulates cryptochromes to generally suppress stem elongation, leaf expansion, and shoot weight but promotes chlorophyll and anthocyanin concentration (Stutte, 2009; Son and Oh, 2013; Wollaeger and Runkle, 2015). In *Arabidopsis*, cryptochromes 1 and 2 mediate extension growth, whereas cryptochrome 1 controls anthocyanin accumulation in B light (Bouly et al., 2007; Pedmale et al., 2016). In the present study, additional FR light antagonized these B light effects in an R-light background. Low R:FR signals the shade-avoidance response through phytochromes (Franklin and Whitelam, 2005), whereas a unique set of growth-promoting genes repressed in ample B light are expressed in low B light through a cryptochrome-dependent pathway (Zhang et al., 2011; Pedmale et al., 2016). Therefore, the antagonistic effects of FR and B light on growth responses can be attributed to their convergent control of functionally similar genes by both phytochromes and cryptochromes.

Additional FR light increased leaf expansion and radiation capture by the plant for photosynthesis, consequently contributing to weight gain. Similarly, increased leaf expansion by FR light (peak = 731 or 734 nm) was associated with increased shoot dry weight of baby leaf lettuce ‘Red Cross’ and ornamental seedlings (Li and Kubota, 2009; Park and Runkle, 2017). Besides controlling morphological traits as a signal, FR light can drive photosynthesis directly as an energy source. Far-red light preferentially excites photosystem I to channel electrons from over-excitation of photosystem II by shorter wavelengths (Myers, 1971; Zhen and van Iersel, 2017). Adding $110 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light (peak = 735 nm) to B+R and warm-white light at PPFDs ranging from 50 to $750 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ consistently increased the quantum efficiency of

photosystem II of lettuce ‘Green Towers’ by 7% and 4%, respectively (Zhen and van Iersel, 2017). The additional FR light also increased the net photosynthetic rate (Zhen and van Iersel, 2017). Considering that the relative quantum efficiency curve extends into the FR region (McCree, 1972), the defined PPFD range (400–700 nm) fails to include direct contributions of FR light to photochemistry and photosynthesis. When all photons between 350 and 800 nm were considered, the YPFDF in the present study increased slightly with additional FR light, showing that FR light increased photosynthetic energy (Table I-1). However, interpretations of FR light effects on whole-plant photosynthesis depend on the context in which FR light is provided. For example, adding FR light to constant B+R light increased shoot dry weight of geranium, petunia (*Petunia ×hybrida*), and snapdragon, whereas substituting FR light for R light to maintain a constant TPFDF did not influence it (Park and Runkle, 2017). Similarly, adding FR light to fixed B+R light combinations in the present study promoted shoot dry mass accumulation of lettuce and basil through its dual functions in enhancing extension growth and photosynthesis. In both experiments, adding 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light to B₉₀R₉₀ increased the TPFDF by 17% but increased shoot fresh and dry weights of lettuce and basil seedlings by 22–48%. This shows that although additional FR light can directly contribute to net photosynthesis, its enhancement of leaf expansion also plays a significant role in regulating whole-plant photosynthesis.

The net photosynthetic rate increases with the PPFD up to the light saturation point (typically $\geq 400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for lettuce) in the photosynthetic light-response curve (Tennessen et al., 1994; Wang et al., 2016; Joshi et al., 2017; Jishi et al., 2018). In the present study, doubling the PPFD from 180 to 360 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (or doubling the DLI from 15.6 to 31.1 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) increased shoot dry mass of lettuce ‘Rex’ and ‘Rouxai’ with and without additional FR light. Similarly, shoot dry mass of lettuce ‘Okayama-saradana’ and ‘Waldmann’s Green’ increased as the PPFD

increased from 85 to 170 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and from 200 to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, under a 16-h photoperiod irrespective of light quality (Yanagi et al., 1996; Cope et al., 2014; Snowden et al., 2016). In addition, shoot, root, and leaf dry weights of petunia, geranium, and coleus (*Solenostemon scutellarioides*) increased as the PPFD increased from 96 to 288 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with or without additional FR light (Park and Runkle, 2018).

During photosynthetic acclimation, a high PPFD increases production of photosystem II, ribulose-1,5-bisphosphate carboxylase/oxygenase (or RuBisCo), electron transport complexes, and ATP synthases, whereas a low PPFD decreases these (Bailey et al., 2001; Walters, 2005). Therefore, promotion of plant growth under a higher, but not saturating, PPFD can be attributed to increased light energy coupled with an enhanced photosynthetic apparatus. Because the quantum yield of photosystem II decreases as the PPFD increases (Zhen and van Iersel, 2017), an increase in shoot weight was not linear to an increase in the PPFD. In the present study, doubling the PPFD in the presence of FR light decreased extension growth but increased shoot dry mass of lettuce, indicating that the increased light energy overcame the restricted light interception. Similarly, increased dry mass of tomato plants was accompanied by reduced plant height and specific leaf area as well as increased leaf thickness as the PPFD increased from 50 to 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fan et al., 2013). A high PPFD also decreased specific leaf area but increased growth of other vegetable and ornamental crops (Snowden et al., 2016; Park and Runkle, 2018). In contrast, plants grown under a low PPFD increase light capture to maximize photosynthesis by developing larger leaves and increasing the abundance of light-harvesting complexes (Walters, 2005).

The effects of FR light and B:R on root growth and leaf development were inconsistent among crops tested. For example, the addition of 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light to B+R light

promoted root growth of basil under the low and high B:R, increased that of lettuce ‘Cherokee’ only under the low B:R, but did not affect that of lettuce ‘Rex’. In addition, it decreased the root-to-shoot ratio of both lettuce cultivars under the high B:R, but did not influence biomass partitioning under the low B:R or for basil. The addition of $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light decreased leaf number of lettuce ‘Rex’ and basil under the high B:R, but not under the low B:R or for lettuce ‘Cherokee’, in experiment I; however, it did not affect that of lettuce ‘Rex’ but increased that of lettuce ‘Rouxai’ in experiment II. Different leaf development responses of lettuce ‘Rex’ in the two experiments may be attributed to different harvest dates. Increasing B:R decreased root growth of lettuce ‘Rex’ irrespective of FR light, decreased that of lettuce ‘Cherokee’ only in the presence of FR light, but did not affect that of basil. These interactions indicate that FR light and B:R can regulate root growth and leaf development depending on the spectral condition, crop type, and crop age.

An increase in B:R or the PPFD promoted red foliage pigmentation (or anthocyanin concentration) of red-leaf lettuce in the present study. Similarly, an increase in the B fraction increased anthocyanin concentration of lettuce ‘Red Cross’ grown under white light (Li and Kubota, 2009). Through cryptochrome 1, B light can upregulate expression of the gene encoding chalcone synthase, which is a key precursor in the anthocyanin pathway (Ahmad et al., 1995; Jenkins et al., 2001; Meng et al., 2004; Chatterjee et al., 2006). Besides B, ultraviolet-A, and ultraviolet-B radiation, high light is another abiotic stress eliciting accumulation of anthocyanins, which protect plants from photodamage (Page et al., 2012). High light upregulates transcription factor MYB112, which controls expression of flavonoid biosynthetic genes including those dedicated to anthocyanin production (Lotkowska et al., 2015). Increasing the PPFD from 200 to $290 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased total anthocyanin content of lettuce ‘Hongyeom Jeockchukmyeon’

grown under B+R+white light with an 18-h photoperiod (Kang et al., 2013). Similarly, providing $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of end-of-production B and/or R supplemental lighting to greenhouse-grown red-leaf lettuce intensified red foliage pigmentation compared to the non-lighted control (Owen and Lopez, 2015).

In the present study, additional FR light at $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reduced red pigmentation of lettuce ‘Cherokee’ grown under low and high B:R. A higher dose of FR light (i.e., $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) did not influence leaf redness of lettuce ‘Rouxai’ under the low PPFD but increased it under the high PPFD. In a white-light background, low R:FR typically reduces anthocyanin concentration in a wide range of species such as mature lettuce, tomato, and prairie plants by regulating the PPE (Kerckhoffs et al., 1992; Alokam et al., 2002; Li and Kubota, 2009). In contrast, FR light can promote anthocyanin production in approximately five-day-old arabidopsis and kale (*Brassica napus*) seedlings when delivered alone or with R light (Neff and Chory, 1998; Carvalho and Folta, 2014; Li et al., 2014). Upon activation by FR light, phytochrome A represses CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and allows transcription factor MYB75 to promote expression of genes encoding anthocyanin biosynthetic enzymes including dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UGT) (Li et al., 2014). On the other hand, phytochrome B represses expression of the chalcone synthase gene and consequently anthocyanin accumulation under FR light in five-day-old arabidopsis seedlings (Zheng et al., 2013). Therefore, the inconsistent results on FR-mediated anthocyanin accumulation can at least partly be attributed to the opposing effects of phytochromes A and B on photomorphogenesis and pigmentation under FR light and complex interactions of phytochromes and cryptochromes depending on the spectral context, species, and developmental stage.

In conclusion, the addition of FR light to constant B+R light generally increased shoot weight and extension growth but reduced relative specific chlorophyll content and anthocyanin concentration of lettuce and basil seedlings. Far-red light contributed to whole-plant photosynthesis by optimizing light capture and improving photochemistry. It interacted with B:R and PPFD in regulation of plant growth, morphology, and pigmentation. In general, the effects of FR light were more pronounced under the higher B:R and the lower PPFD but were attenuated under the lower B:R and the higher PPFD. These complex interactions can be attributed to coaction of phytochromes mediated by R and FR light and cryptochromes mediated by B light in plant signaling. Although FR LEDs are currently less efficient than B or R LEDs, the use of moderate FR light can achieve comparable seedling growth as doubling B+R light and thus merits consideration in commercial farms.

Acknowledgments

We thank Nate DuRussel for technical assistance and Randy Beaudry and Bert Cregg for instruments. This work was supported by Michigan State University AgBioResearch Project GREEN GR15-025, OSRAM Opto Semiconductors, and the USDA National Institute of Food and Agriculture, Hatch project 192266.

APPENDIX

Table I-1. Spectral characteristics of the lighting treatments in experiments I and II comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The photon flux density of green (G; 500–600 nm) light is also provided. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency and spectral data from 350 to 800 nm (Sager et al., 1988)] were calculated. The estimated phytochrome photoequilibrium (PPE) was calculated according to Sager et al. (1988).

Experiment I						
	B ₃₀ R ₁₅₀	B ₃₀ R ₁₅₀ FR ₃₀	B ₉₀ R ₉₀	B ₉₀ R ₉₀ FR ₃₀	R ₁₈₀ FR ₃₀	B ₁₈₀ FR ₃₀
	Single-band photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
B	31.0	30.9	89.4	88.7	0.1	179.7
G	0.5	0.5	0.8	0.8	0.4	1.2
R	148.7	148.7	90.3	91.0	179.9	2.7
FR	0.6	29.8	0.6	29.9	31.0	29.1
	Integrated photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
PPFD	180.2	180.1	180.5	180.5	180.5	183.6
TPFD	180.9	209.9	181.1	210.4	211.5	212.7
YPFD	162.4	167.7	151.4	156.9	173.7	142.1
	Light ratio					
B:R	0.2	0.2	1.0	1.0	0.0	65.7
B:FR	49.1	1.0	138.6	3.0	0.0	6.2
R:FR	235.2	5.0	140.0	3.0	5.8	0.1
PPE	0.880	0.833	0.858	0.789	0.844	0.351
Experiment II						
	B ₉₀ R ₉₀	B ₉₀ R ₉₀ FR ₃₀	B ₉₀ R ₉₀ FR ₇₅	B ₁₈₀ R ₁₈₀	B ₁₈₀ R ₁₈₀ FR ₃₀	B ₁₈₀ R ₁₈₀ FR ₇₅
	Single-band photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
B	90.3	89.9	90.9	181.3	179.4	179.6
G	0.8	0.8	0.8	1.6	1.5	1.5
R	90.5	90.9	89.9	181.3	179.0	180.1
FR	0.8	29.9	74.7	1.7	29.7	75.1
	Integrated photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
PPFD	181.5	181.5	181.6	364.1	360.0	361.2
TPFD	182.3	211.4	256.2	365.8	389.7	436.3
YPFD	152.4	157.2	163.7	305.3	306.3	314.4
	Light ratio					
R:FR	114.7	3.0	1.2	107.0	6.0	2.4
B:R	1.0	1.0	1.0	1.0	1.0	1.0
B:FR	114.5	3.0	1.2	107.0	6.0	2.4
PPE	0.859	0.795	0.713	0.858	0.826	0.780

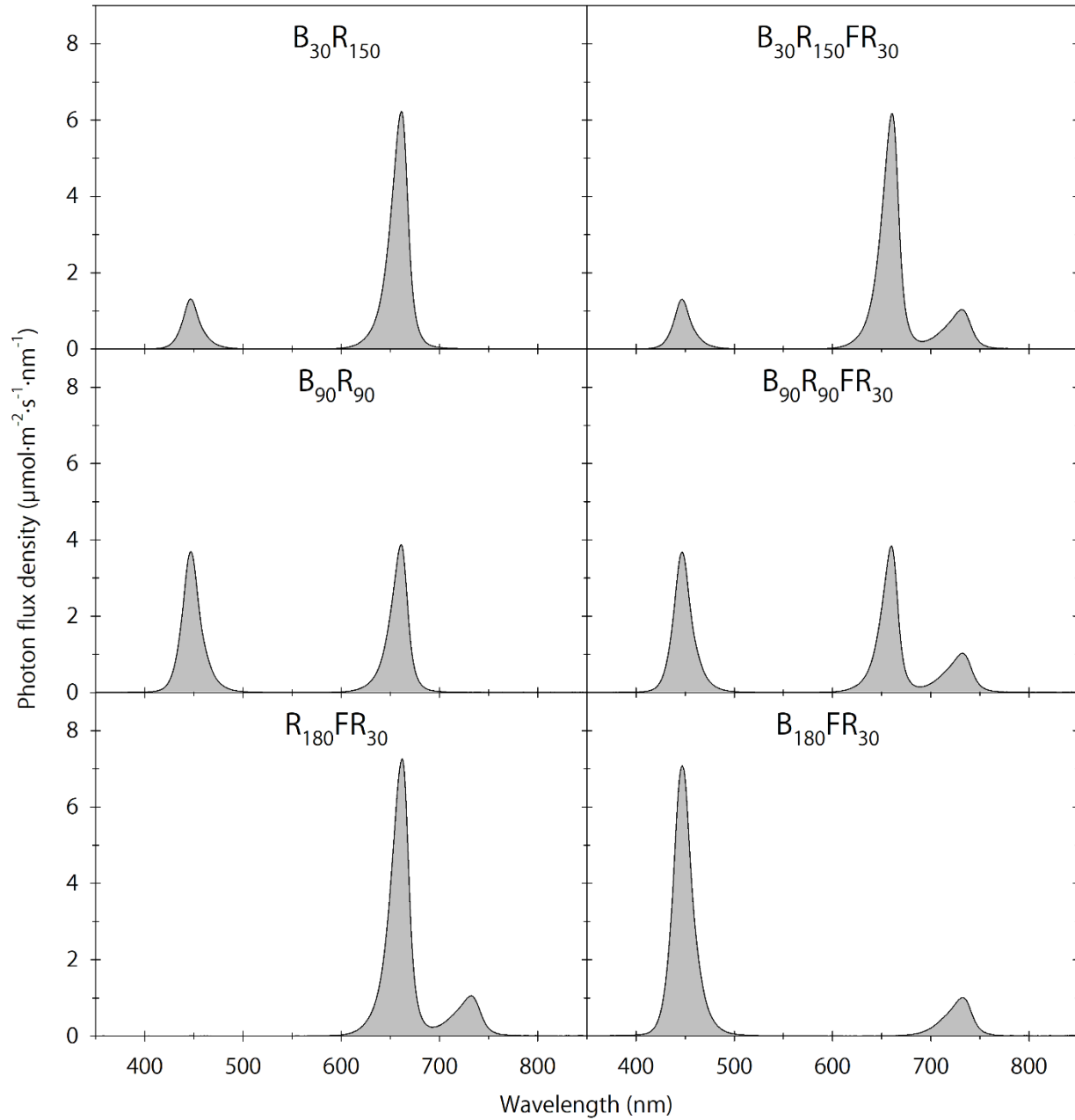


Figure I-1. Spectral distributions of six lighting treatments in experiment I comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

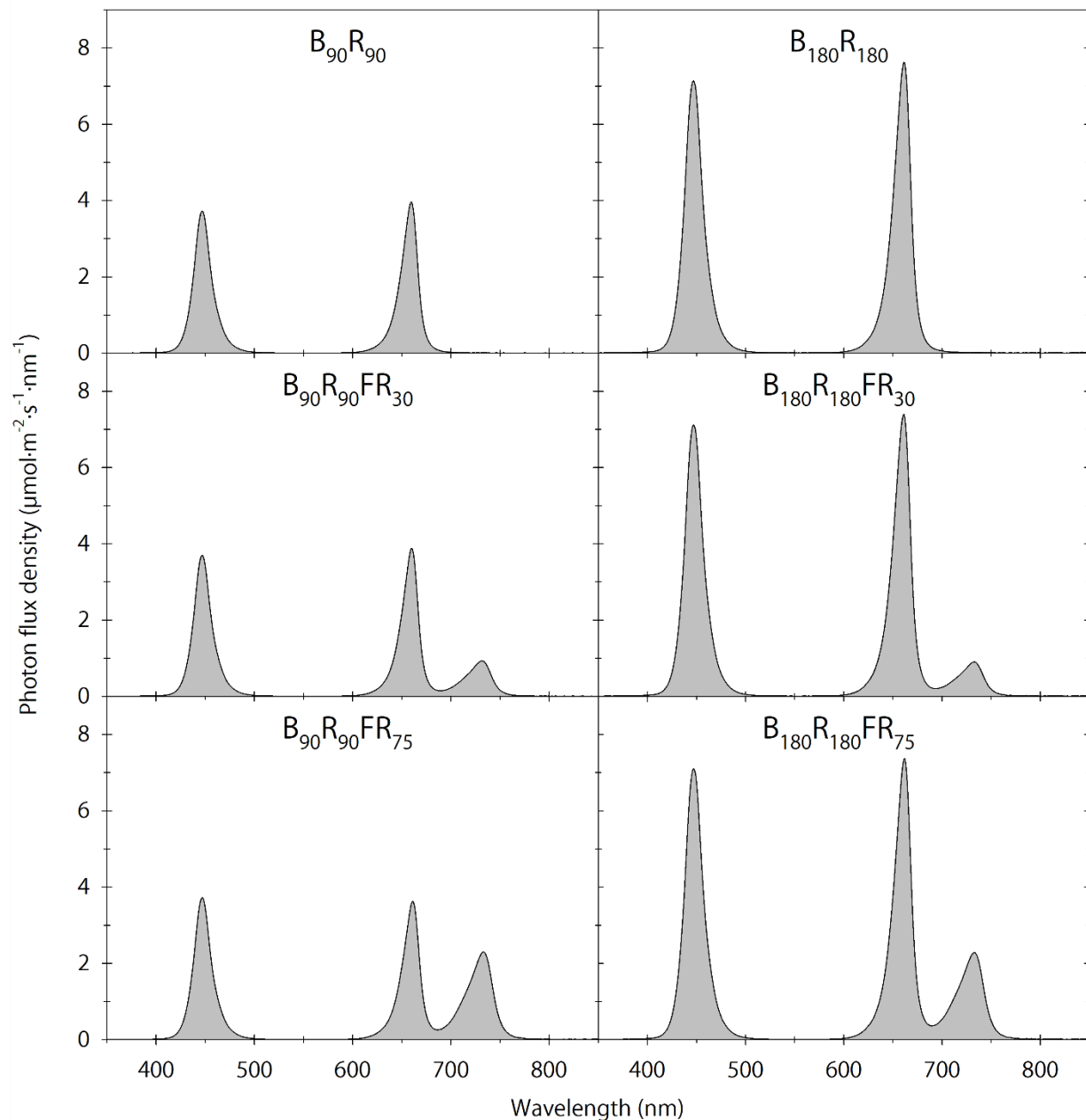


Figure I-2. Spectral distributions of six lighting treatments in experiment II comprised of mixed blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

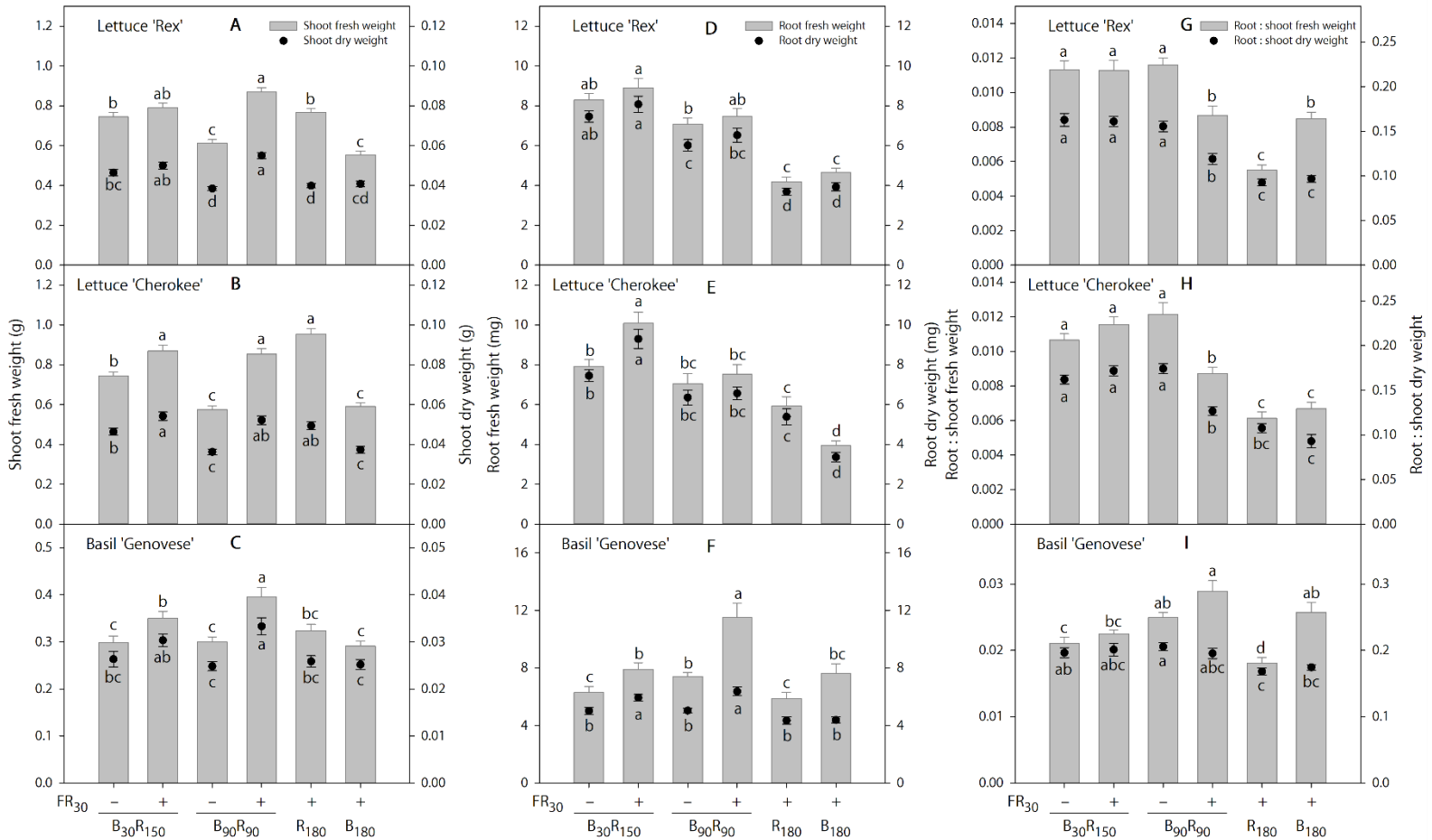


Figure I-3. Shoot and root fresh and dry weights and weight partitioning of lettuce 'Rex' and 'Cherokee' and basil 'Genovese' grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

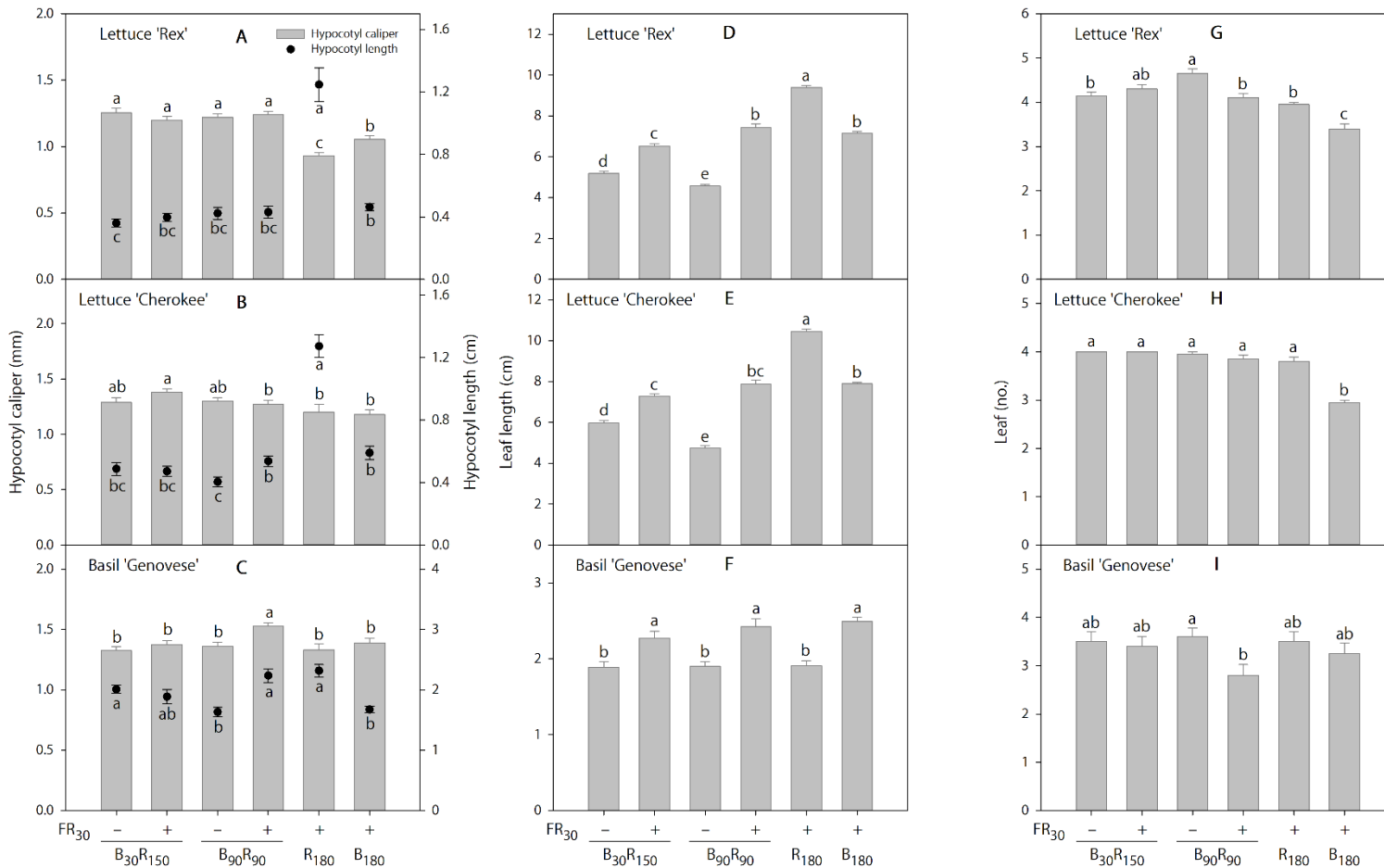


Figure I-4. Hypocotyl caliber and length, leaf length, and leaf number of lettuce 'Rex' and 'Cherokee' and basil 'Genovese' grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

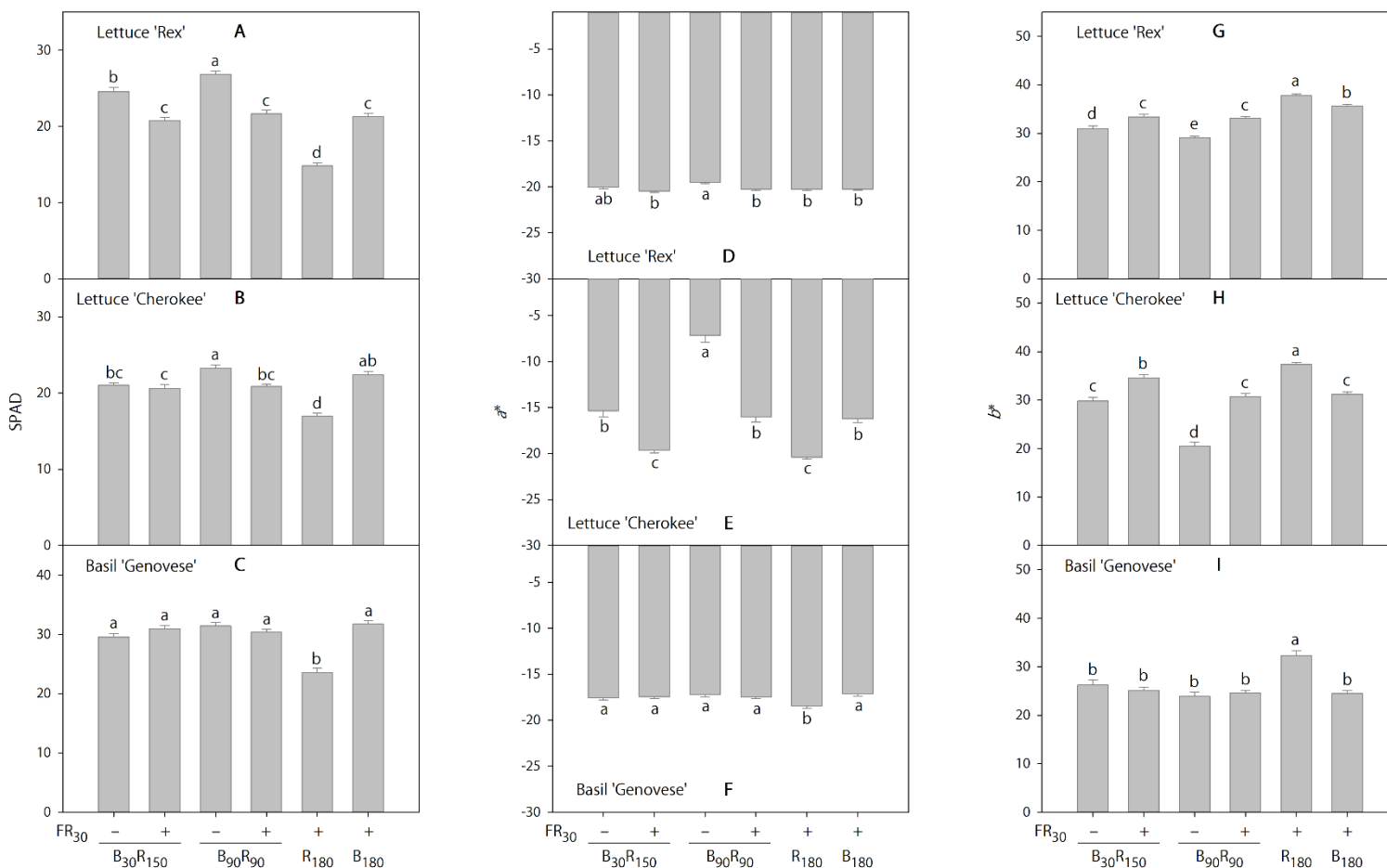


Figure I-5. Relative specific chlorophyll content (SPAD) and foliage color coordinates [a^* for greenness–redness (negative–positive) and b^* for blueness–yellowness (negative–positive)] of lettuce ‘Rex’ and ‘Cherokee’ and basil ‘Genovese’ grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).

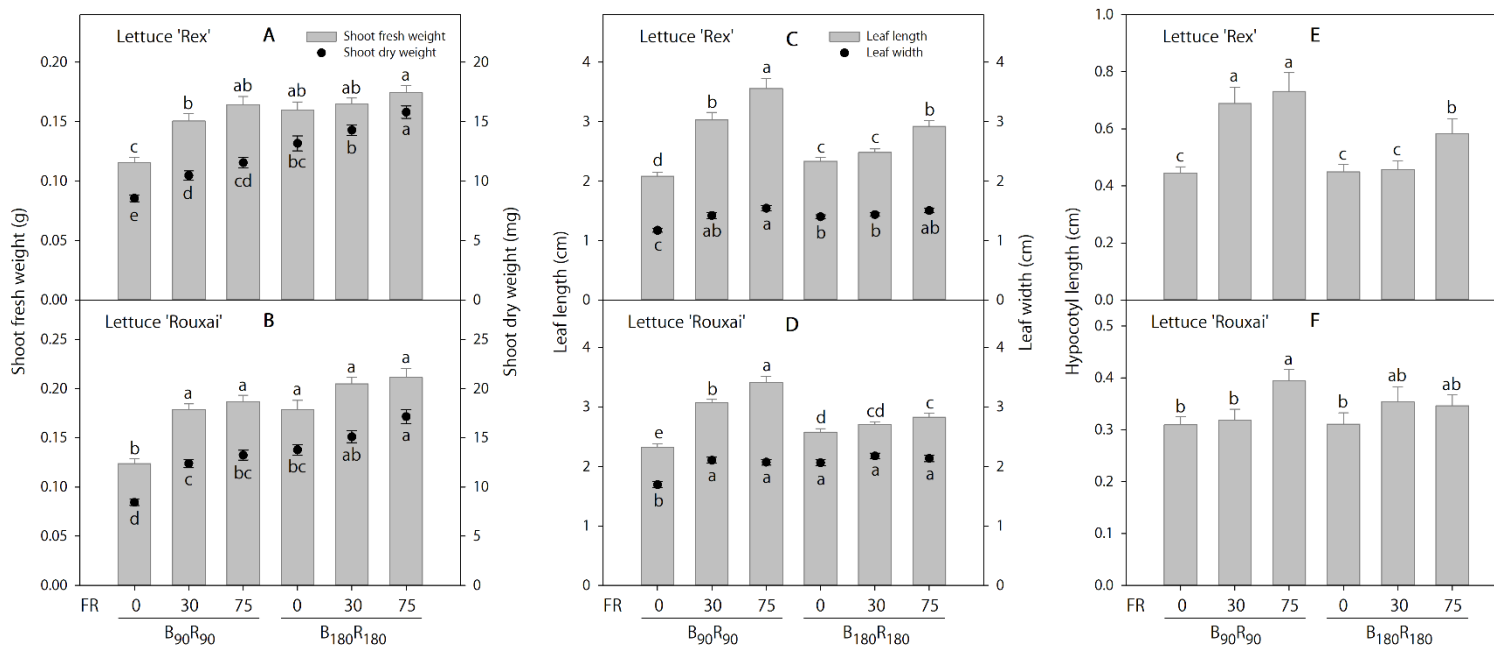


Figure I-6. Shoot fresh and dry weights, leaf length and width, and hypocotyl length of lettuce 'Rex' and 'Rouxai' grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

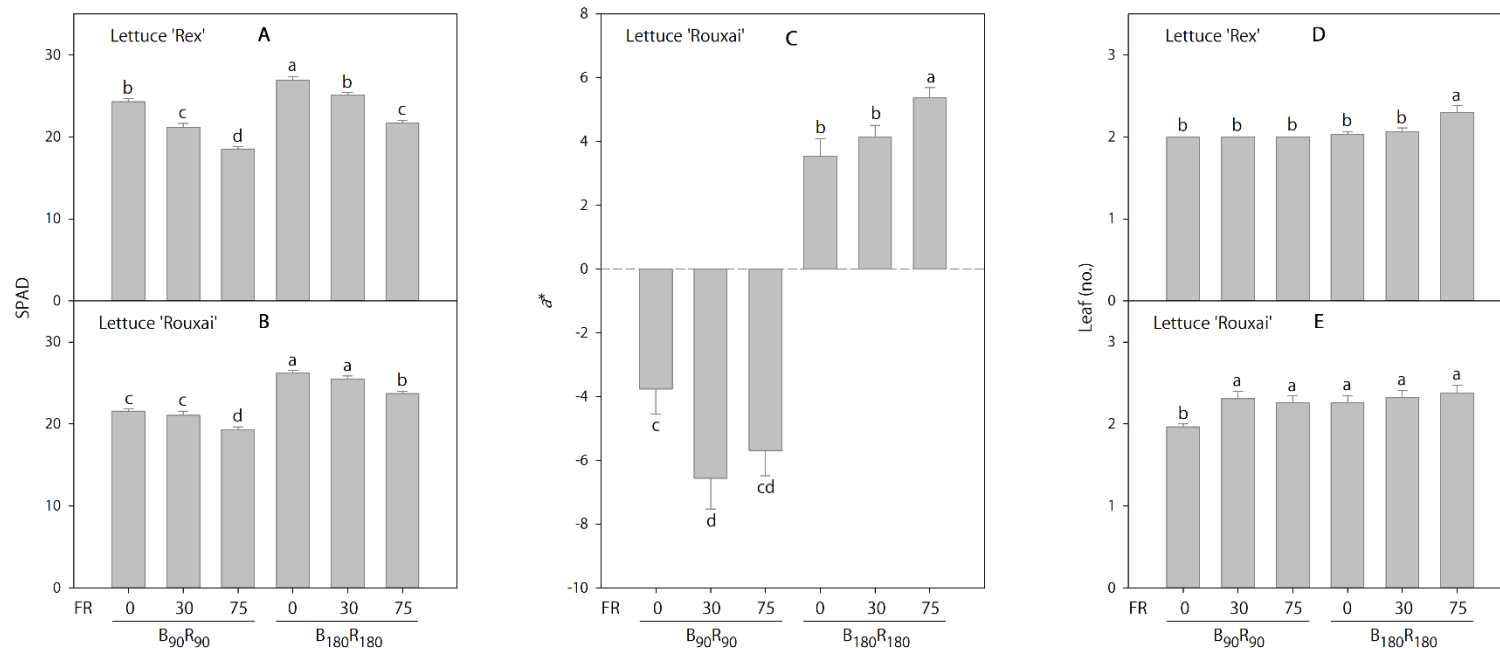


Figure I-7. Relative specific chlorophyll content (SPAD) and leaf number of lettuce 'Rex' and 'Rouxai', and the green-red color coordinate [a^* for greenness–redness (negative–positive)] of lettuce 'Rouxai' grown under six sole-source lighting treatments comprised of blue (B; 400–500 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes. The number following each waveband is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

LITERATURE CITED

LITERATURE CITED

- Ahmad, M., Lin, C. and Cashmore, A.R., 1995. Mutations throughout an *Arabidopsis* blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. *Plant J.* 8:653–658.
- Alokam, S., Chinnappa, C.C. and Reid, D.M., 2002. Red/far-red light mediated stem elongation and anthocyanin accumulation in *Stellaria longipes*: differential response of alpine and prairie ecotypes. *Can. J. Bot.* 80:72–81.
- Bailey, S., Walters, R.G., Jansson, S. and Horton, P., 2001. Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses. *Planta* 213:794–801.
- Both, A.J., Albright, L.D., Langhans, R.W., Reiser, R.A. and Vinzant, B.G., 1997. Hydroponic lettuce production influenced by integrated supplemental light levels in a controlled environment agriculture facility: experimental results. *Acta Hortic.* 418:45–52.
- Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R. and Ahmad, M., 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J. Biol. Chem.* 282:9383–9391.
- Carvalho, S.D. and Folta, K.M., 2014. Sequential light programs shape kale (*Brassica napus*) sprout appearance and alter metabolic and nutrient content. *Hortic. Res.* 1:8.
- Chatterjee, M., Sharma, P. and Khurana, J.P., 2006. Cryptochrome 1 from *Brassica napus* is up-regulated by blue light and controls hypocotyl/stem growth and anthocyanin accumulation. *Plant Physiol.* 141:61–74.
- Cope, K.R., Snowden, M.C. and Bugbee, B., 2014. Photobiological interactions of blue light and photosynthetic photon flux: effects of monochromatic and broad-spectrum light sources. *Photochem. Photobiol.* 90:574–584.
- Craig, D.S. and Runkle, E.S., 2013. A moderate to high red to far-red light ratio from light-emitting diodes controls flowering of short-day plants. *J. Am. Soc. Hortic. Sci.* 138:167–172.
- Craig, D.S. and Runkle, E.S., 2016. An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants. *Environ. Exp. Bot.* 121:132–138.
- Fan, X.X., Xu, Z.G., Liu, X.Y., Tang, C.M., Wang, L.W. and Han, X.L., 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Sci. Hortic.* 153:50–55.
- Franklin, K.A., 2008. Shade avoidance. *New Phytol.* 179:930–944.

- Franklin, K.A. and Whitelam, G.C., 2005. Phytochromes and shade-avoidance responses in plants. *Ann. Bot.* 96:169–175.
- Hernández, R. and Kubota, C., 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot.* 121:66–74.
- Hersch, M., Lorrain, S., de Wit, M., Trevisan, M., Ljung, K., Bergmann, S. and Fankhauser, C., 2014. Light intensity modulates the regulatory network of the shade avoidance response in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 201320355.
- Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S. and Xenarios, I., 2012. Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant J.* 71:699–711.
- Jenkins, G.I., Long, J.C., Wade, H.K., Shenton, M.R. and Bibikova, T.N., 2001. UV and blue light signalling: pathways regulating chalcone synthase gene expression in *Arabidopsis*. *New Phytol.* 151:121–131.
- Jishi, T., Matsuda, R. and Fujiwara, K., 2018. Effects of photosynthetic photon flux density, frequency, duty ratio, and their interactions on net photosynthetic rate of cos lettuce leaves under pulsed light: explanation based on photosynthetic-intermediate pool dynamics. *Photosynth. Res.* 136:371–378.
- Joshi, J., Zhang, G., Shen, S., Supaibulwatana, K., Watanabe, C.K. and Yamori, W., 2017. A combination of downward lighting and supplemental upward lighting improves plant growth in a closed plant factory with artificial lighting. *HortScience* 52:831–835.
- Kang, J.H., KrishnaKumar, S., Atulba, S.L.S., Jeong, B.R. and 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.
- Kerckhoffs, L.H.J., Kendrick, R.E., Whitelam, G.C. and Smith, H., 1992. Extension growth and anthocyanin responses of photomorphogenic tomato mutants to changes in the phytochrome photoequilibrium during the daily photoperiod. *Photochem. Photobiol.* 56:611–615.
- Kopsell, D.A., Sams, C.E. and Morrow, R.C., 2015. Blue wavelengths from LED lighting increase nutritionally important metabolites in specialty crops. *HortScience* 50:1285–1288.
- Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.S. and Ecker, J.R., 2012. Linking photoreceptor excitation to changes in plant architecture. *Genes Dev.* 26:785–790.
- Li, Q. and Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67:59–64.
- Li, T., Jia, K.P., Lian, H.L., Yang, X., Li, L. and 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.
- Lorrain, S., Allen, T., Duek, P.D., Whitelam, G.C. and Fankhauser, C., 2008. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J. 53:312–323.
- Lotkowska, M.E., Tohge, T., Fernie, A.R., Xue, G.P., Balazadeh, S. and Mueller-Roeber, B., 2015. The Arabidopsis transcription factor MYB112 promotes anthocyanin formation during salinity and under high light stress. Plant Physiol. 00605.2015.
- Mah, J.J., Llewellyn, D. and Zheng, Y., 2018. Morphology and flowering responses of four bedding plant species to a range of red to far red ratios. HortScience 53:472–478.
- McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9:191–216.
- Meng, X., Xing, T. and Wang, X., 2004. The role of light in the regulation of anthocyanin accumulation in *Gerbera hybrida*. Plant Growth Regul. 44:243–250.
- Myers, J., 1971. Enhancement studies in photosynthesis. Ann. Rev. Plant Physiol. 22:289–312.
- Neff, M.M. and Chory, J., 1998. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol. 118:27–35.
- Owen, W.G. and 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.
- Page, M., Sultana, N., Paszkiewicz, K., Florance, H. and Smirnoff, N., 2012. The influence of ascorbate on anthocyanin accumulation during high light acclimation in *Arabidopsis thaliana*: further evidence for redox control of anthocyanin synthesis. Plant Cell Environ. 35:388–404.
- Park, Y. and 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.
- Park, Y. and Runkle, E.S., 2018. Far-red radiation and photosynthetic photon flux density independently regulate seedling growth but interactively regulate flowering. Environ. Exp. Bot. <https://doi.org/10.1016/j.envexpbot.2018.06.033>.
- Pedmale, U.V., Huang, S.S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A., Sridevi, P., Nito, K., Nery, J.R. and Ecker, J.R., 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. Cell 164:233–245.
- Sager, J.C., Smith, W.O., Edwards, J.L. and Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. Am. Soc. Agric. Eng. 31:1882–1889.

- Smith, H., 2000. Phytochromes and light signal perception by plants—an emerging synthesis. *Nature* 407:585–591.
- Snowden, M.C., Cope, K.R. and Bugbee, B., 2016. Sensitivity of seven diverse species to blue and green light: interactions with photon flux. *PloS ONE* 11(10):e0163121.
- Son, K.H. and 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.
- Stutte, G.W., 2009. Light-emitting diodes for manipulating the phytochrome apparatus. *HortScience* 44:231–234.
- Tennessen, D.J., Singaas, E.L. and Sharkey, T.D., 1994. Light-emitting diodes as a light source for photosynthesis research. *Photosynth. Res.* 39:85–92.
- Vandenbussche, F., Pierik, R., Millenaar, F.F., Voesenek, L.A. and Van Der Straeten, D., 2005. Reaching out of the shade. *Curr. Opin. Plant Biol.* 8:462–468.
- Walters, R.G., 2005. Towards an understanding of photosynthetic acclimation. *J. Exp. Bot.* 56:435–447.
- Wang, J., Lu, W., Tong, Y. and 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.* 7:250.
- Wollaeger, H.M. and Runkle, E.S., 2013. Growth responses of ornamental annual seedlings under different wavelengths of red light provided by light-emitting diodes. *HortScience* 48:1478–1483.
- Wollaeger, H.M. and Runkle, E.S., 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. *HortScience* 50:522–529.
- Yanagi, T., Okamoto, K. and Takita, S., 1996. Effects of blue, red, and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. *Acta Hortic.* 440:117–122.
- Yorio, N.C., Wheeler, R.M., Goins, G.D., Sanwo-Lewandowski, M.M., Mackowiak, C.L., Brown, C.S., Sager, J.C. and Stutte, G.W., 1998. Blue light requirements for crop plants used in bioregenerative life support systems. *Life Support Biosph. Sci.* 5:119–128.
- Yu, H., Moss, B., Jang, S.S., Prigge, M.J., Klavins, E., Nemhauser, J. and Estelle, M., 2013. Mutations in the TIR1 auxin receptor that increase affinity for Aux/IAA proteins result in auxin hypersensitivity. *Plant Physiol.* 162:295–303.
- Zhang, T., Maruhnich, S.A. and Folta, K.M., 2011. Green light induces shade avoidance symptoms. *Plant Physiol.* 157:1528–1536.

- Zhen, S. and van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. *J. Plant Physiol.* 209:115–122.
- Zheng, X., Wu, S., Zhai, H., Zhou, P., Song, M., Su, L., Xi, Y., Li, Z., Cai, Y., Meng, F. and Yang, L., 2013. *Arabidopsis* phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. *Plant Cell* tpc.112.107086.

SECTION II

GREEN OR FAR-RED LIGHT ANTAGONIZES BLUE LIGHT IN REGULATION OF LETTUCE AND KALE GROWTH

Green or far-red light antagonizes blue light in regulation of lettuce and kale growth

Qingwu Meng, Nathan Kelly, and Erik S. Runkle*

Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI
48824-1325, USA

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

Abstract.

Although red (R; 600–700 nm) and blue (B; 400–500 nm) light can be sufficient for plants grown indoors, other wavebands such as green (G; 500–600 nm) and far red (FR; 700–800 nm) can also regulate photosynthesis, plant morphology, and secondary metabolism. The objective of this study was to determine how substitutions of B light with G and/or FR light influence growth of leafy greens grown indoors under light-emitting diodes (LEDs). We postulated that G and/or FR light would trigger the shade-avoidance response and thus promote biomass accumulation through increased light interception. We grew lettuce (*Lactuca sativa* ‘Rex’ and ‘Rouxai’) and kale (*Brassica oleracea* var. *sabellica* ‘Siberian’) under warm-white (WW) LEDs at $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (400–800 nm) for 9–11 days and then transplanted seedlings into a hydroponic system with ten different lighting treatments. The air temperature (20 °C), photoperiod (20 hours), total photon flux density ($180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 400–800 nm), and fertility were maintained the same across treatments. In addition to WW and equalized-white (EQW) controls, combinations of B (peak = 449 nm), G (peak = 526 nm), and FR (peak = 733 nm) LEDs, each at 0, 20, 40, or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, were delivered in a R background (peak = 664 nm) of $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. One month after seed sow, we collected data on shoot weight, leaf morphology, and pigmentation. Substituting G or FR light for B light promoted leaf expansion and increased shoot weight but decreased chlorophyll concentration in all crops. For example, lettuce ‘Rex’ grown under $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G + $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R light was 38% greater in plant diameter and 54% greater in shoot dry weight compared to those under $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B + $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R light. Substituting B light with G light at $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ also reduced red coloration of lettuce ‘Rouxai’. At the same photon flux density, FR light increased leaf expansion and decreased red foliage coloration more than G light. We conclude that G and/or FR light can

counter B-light-induced growth inhibition and trigger the shade-avoidance response, accelerating plant growth while decreasing pigment concentration.

Keywords: controlled-environment agriculture, cryptochrome, phytochrome, shade-avoidance response, sole-source lighting, whole-plant photosynthesis.

Abbreviations: B, blue; EQW, equalized white; FR, far red; G, green; LED, light-emitting diode; PIF, phytochrome-interacting factor; PPE, phytochrome photoequilibrium; PPFD, photosynthetic photon flux density; R, red; TPDF, total photon flux density; W, white; WW, warm white; YPDF, yield photon flux density.

Introduction

Light is both an energy source and a signal to higher plants. Biologically relevant wavelengths from ultraviolet to far-red radiation create an energy gradient, the variation of which enables plants to sense and survive in various environmental conditions. Photosynthetically active radiation, by definition, ranges from 400 to 700 nm including blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) light. Light-emitting diode (LED) fixtures developed for horticultural applications have been generally comprised of B+R light because of their high photosynthetic and electrical efficacy. In contrast, G light is rarely included in sole-source lighting mainly because G LEDs are highly inefficient due to physical challenges in optoelectronics. In addition, G light is perceived as less useful to plant growth than B and R light because of its lower quantum yield and weaker absorption by chlorophylls (McCree, 1972). However, the dip in the quantum yield curve by McCree (1972) occurs in the B waveband (475

nm) rather than the G waveband. In addition, the integrated quantum yield is 18% greater in G than in B based on 100-nm bands. Because the McCree curve was based on instantaneous single-leaf measurements in low light, it is not necessarily a predictor of long-term whole-plant photosynthesis, at least partly because of adaptive morphological changes. Moreover, a substantial amount (70–80%) of G light is absorbed by the leaf, especially the abaxial side (McCree, 1972; Brodersen and Vogelmann, 2010). G light penetrates further in the leaf profile than B or R light, scatters between cellular components within the leaf, and is transmitted to the lower canopy to constitute a shade signal (Klein, 1992; Vandenbussche et al., 2005; Terashima et al., 2009; Zhang et al., 2011). As a signal, G light can evoke shade-avoidance responses such as promotion of hypocotyl elongation, stem extension, leaf expansion, and upward leaf orientation (Zhang et al., 2011; Wang and Folta, 2013). It can also reverse B light-induced responses such as inhibition of extension growth, stimulation of stomata opening, and promotion of anthocyanin accumulation (Folta, 2004; Folta and Maruhnich, 2007; Wang and Folta, 2013). Taken together, G light has pivotal ramifications in both photosynthesis and signaling.

The role of G light in whole-plant photosynthesis has been investigated, albeit with inconsistent outcomes. G light can increase shoot weight and extension growth of some vegetable and ornamental seedlings. For example, partial substitution of R light with 24% G fluorescent light in a 16%B+84%R background at a photosynthetic photon flux density (PPFD; 400–700 nm) of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased shoot fresh and dry weight and leaf area of lettuce (*Lactuca sativa*) ‘Waldmann’s Green’ but did not influence net photosynthesis or chlorophyll concentration (Kim et al., 2004a). In addition, substituting G light (peak = 516 nm) for B light in a 50%B+50%R background at a PPFD of $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased shoot fresh weight of tomato (*Solanum lycopersicum*); shoot dry weight of petunia (*Petunia ×hybrida*); height of

impatiens (*Impatiens walleriana*), tomato, and salvia (*Salvia splendens*); and leaf area of tomato (Wollaeger and Runkle, 2014). In contrast, shoot dry mass, net assimilation, and specific leaf area of lettuce ‘Waldmann’s Green’ were similar under varying G light percentages from 2% to 41% at a PPFD of 200 or 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Snowden et al., 2016). The peak wavelengths of G light in some of these studies were not reported and could have contributed to the different outcomes observed because photosynthesis can depend on the peak wavelength of G light. For example, the net photosynthetic rate of lettuce ‘Banchu Red Fire’ was greater under G light with a peak of 510 nm than with a peak of 520 or 530 nm (Johkan et al., 2012). Supplemental G light is neutral to plant growth, pigmentation, and phytochemical accumulation if it is already abundant in a white (W) spectrum. For example, shoot weight and leaf dimensions of baby leaf lettuce ‘Red Cross’ were similar when 43% of cool-W fluorescent light was substituted with 130 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ G light (peak = 526 nm), which increased G light percentage of the PPFD from 52% to 70% (Li and Kubota, 2009). Therefore, the context in which G light is delivered can affect plant responses.

Far-red (FR; 700–800 nm) light extends beyond the PPFD range but can also drive photochemistry and photosynthesis, although the quantum yield of FR light is low (McCree, 1972; Pettai et al., 2005; Zhen and van Iersel, 2017). The light-dependent reaction of photosynthesis begins with excitation of photosystem II preferentially by slightly shorter wavelengths (≤ 680 nm) followed by excitation of photosystem I preferentially by slightly longer wavelengths (≥ 700 nm). Simultaneous delivery of FR light with B+R or W light helps prevent overexcitation of photosystem II and balance electron flow in the photosynthetic machinery, thereby increasing the quantum yield of photosystem II (Myers, 1971; Zhen and van Iersel, 2017). As a signal, FR light can also modulate phytochrome activity and thus control a wide

range of phenotypic responses. It converts phytochromes to their inactive form, whereas R light converts them to their active form (Sager et al., 1988). The addition of FR light can elicit the shade-avoidance response through phytochromes, modify stem and leaf morphology, and increase whole-plant net assimilation of baby leaf lettuce and ornamental seedlings (Li and Kubota, 2009; Park and Runkle, 2017).

Both G and FR light mediate photosynthesis and the shade-avoidance response; however, a knowledge gap exists with respect to their comparative and cumulative effects. Moreover, the increasing popularity of W LEDs in horticultural lighting necessitates testing of multi-waveband combinations for indoor production of specialty crops. Therefore, the objectives of our study were to: 1) investigate how substitutions of G and/or FR light for B light influence shoot weight, morphology, and pigmentation of leafy greens, and 2) evaluate different types of W LEDs. We postulated 1) G and/or FR light, when substituted for B light, would promote extension growth, increase shoot weight, and decrease pigment concentration, and 2) W LEDs, which include both G and FR light, would increase crop yield compared to B+R LEDs.

Materials and methods

Plant material and propagation

Seeds of green butterhead lettuce ‘Rex’, red oakleaf lettuce ‘Rouxai’, and kale (*Brassica oleracea* var. *sabellica*) ‘Siberian’ were obtained from a seed producer (Johnny’s Selected Seeds, Winslow, ME) and sown in a soilless rockwool substrate arranged as 200 2.5-cm plugs per sheet (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada), which was presoaked in deionized water with an adjusted pH of 4.4–4.5 using diluted (1:31) 95–98% sulfuric acid (J.Y. Baker, Inc., Phillipsburg, NJ). This experiment was performed three times with seeds of lettuce ‘Rouxai’

sown on 26 June, 4 Sept., and 19 Oct. 2017, and seeds of lettuce ‘Rex’ and kale ‘Siberian’ sown 2 d later in each replication. Seed plugs were placed in plastic trays and covered with transparent humidity domes to prevent seed desiccation during germination. The humidity domes were subsequently removed 4 d after seed sow. Seeds and seedlings were germinated and grown in a ventilated and refrigerated growth compartment in the Controlled-Environment Lighting Laboratory (Michigan State University, East Lansing, MI) at an air temperature setpoint of 20 °C and ambient CO₂ under a 24-h photoperiod and a total photon flux density (TPFD; 400–800 nm) of 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from warm-white (WW; peak = 639 nm, correlated color temperature = 2700 K) LEDs (PHYTOFY RL; OSRAM, Beverley, MA). Seedlings were irrigated with deionized water supplemented with a water-soluble fertilizer (13N–3P–15K Orchid RO Water Special; Greencare Fertilizers, Inc., Kankakee, IL) to supply the following nutrients (in $\text{mg}\cdot\text{L}^{-1}$): 100 N, 23 P, 115 K, 62 Ca, 15 Mg, 1.4 Fe, 0.68 Mn, 0.34 Zn, 0.14 B, 0.34 Cu, and 0.14 Mo. The electrical conductivity ranged from 1.0 to 1.2 $\text{mS}\cdot\text{cm}^{-1}$. pH was routinely adjusted to 5.5–5.8 using potassium bicarbonate.

Production culture and environment

All lettuce and kale seedlings in rockwool cubes were transplanted into a deep-flow hydroponic system with three vertically stacked layers (Indoor Harvest, Houston, TX) on 7 July, 15 Sept., and 30 Oct. 2017 for three replications to receive ten different lighting treatments. The plants were spaced on 20-cm horizontal and 15-cm diagonal centers on 36-cell floating rafts (60.9 × 121.9 × 2.5 cm; Beaver Plastics, Ltd; Acheson, AB, Canada). They were grown at an air temperature setpoint of 20 °C and ambient CO₂ under a 20-h photoperiod (0200–2200 HR) with roots fully submerged in constantly recirculating deionized water supplemented with the same water-soluble fertilizer as for seedlings to supply the following nutrients (in $\text{mg}\cdot\text{L}^{-1}$): 150 N, 35

P, 173 K, 92 Ca, 23 Mg, 2.0 Fe, 1.0 Mn, 0.51 Zn, 0.21 B, 0.51 Cu, and 0.21 Mo. The pH, electrical conductivity, and temperature of the nutrient solutions for all lighting treatments were measured daily throughout the experiment with a pH and electrical conductivity meter (HI9814; Hanna Instruments, Woonsocket, RI). The averages are provided in Table II-1. The nutrient solution was constantly oxygenated with an air stone (20.3×2.5 cm; Active Aqua AS8RD; Hydrofarm, Petaluma, CA) and a 60-W air pump (Active Aqua AAPA70L; Hydrofarm).

Ventilation and air-conditioning units (HBH030A3C20CRS; Heat Controller, LLC., Jackson, MI) ran on a wireless thermostat controller (Honeywell International, Inc., Morris Plains, NJ) to promote air flow and maintain the air temperature setpoint. Thermocouples (0.13-mm type E; Omega Engineering, Inc., Stamford, CT), infrared temperature sensors (OS36-01-K-80F; Omega Engineering, Inc.), light quantum sensors (LI-190R; LI-COR, Inc., Lincoln, NE), CO₂ sensors (GMD20; Vaisala, Inc., Louisville, CO), and relative humidity and temperature probes (HMP110; Vaisala, Inc.) were used to monitor corresponding environmental parameters. One or two sensors of each type were positioned in representative locations of the growth room. These environmental data were collected once every 10 s with hourly averages recorded using a datalogger (CR1000; Campbell Scientific, Inc., Logan, UT) coupled with a multiplexer (AM16/32B; Campbell Scientific, Inc.). Mean air temperature (20.0–21.2 °C), CO₂ concentration (379–402 ppm), and relative humidity (44–58%) data for all lighting treatments throughout the experiment were taken from their closest sensors and are provided in Table II-2.

Lighting treatments

From transplant to harvest, plants were grown under ten different lighting treatments consisting of B (peak = 449 nm), G (peak = 526 nm), R (peak = 664 nm), FR (peak = 733 nm), WW, and equalized-white (EQW; peak = 559 nm, 6500 K) LEDs, which were all housed in the

same adjustable LED fixture with seven independent color channels (PHYTOFY RL; OSRAM). The light output of each color channel is controlled at $1\text{-}\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increments using proprietary software (Spartan Control Software; OSRAM). Three LED fixtures ($67.3 \times 29.8 \times 4.3$ cm each) were positioned 43 cm above each treatment canopy and spaced on 41-cm centers to ensure light uniformity. All lighting treatments delivered the same TPDF of $180\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from WW, EQW, or constant R light at $120\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with eight combinations of B, G, and FR light supplying the remaining $60\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$: B₆₀R₁₂₀, B₄₀G₂₀R₁₂₀, B₂₀G₄₀R₁₂₀, G₆₀R₁₂₀, B₄₀R₁₂₀FR₂₀, B₂₀R₁₂₀FR₄₀, R₁₂₀FR₆₀, B₂₀G₂₀R₁₂₀FR₂₀, WW₁₈₀, and EQW₁₈₀. The number following each waveband indicates its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All LEDs were scheduled in the control software to run the designated spectral combinations from 0200 to 2200 HR daily. The spectral distributions of all lighting treatments were measured using a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT) and adjusted in the control software based on the TPDF averaged from seven representative locations, where individual plants were located on the floating raft (Table II-3, Figure II-1). The yield photon flux density (YPFD) was the product of the spectral data and relative quantum efficiency from 350 to 800 nm (McCree, 1972) (Table II-3). The estimated phytochrome photoequilibrium (PPE) was calculated as the proportion of FR-absorbing, active phytochromes in the total phytochrome pool based on the spectral data and absorption coefficients of phytochromes (Sager et al., 1988) (Table II-3).

Data collection and analysis

Photographs of a representative plant under each lighting treatment were taken under white fluorescent light to document visual appearance (Figure II-2). Immediately after, destructive measurements were conducted on 10 plants per cultivar from each treatment and replication approximately 30 d after seed sow. For each plant, shoot fresh and dry weight, plant diameter,

length and width of the fifth most mature leaf, leaf area (only collected for kale), petiole length (only collected for kale), and leaf number were measured. Average relative chlorophyll concentration was determined using a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan). Chlorophyll fluorescence was measured on dark-adapted leaves 30 min after dark adaption using a multi-mode chlorophyll fluorometer (OS5p; Opti-Sciences, Inc., Hudson, NH) to obtain the maximum quantum efficiency of photosystem II (Fv/Fm). A Lab color space analysis was conducted using a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc.) on leaves of lettuce ‘Rouxai’ to quantify foliage coloration. L^* ranges from 0 (the darkest black) to 100 (the brightest white) to indicate lightness. With the true neutral gray being 0, a^* is the scale of green (in the negative direction) to red (in the positive direction), whereas b^* is the scale of blue (in the negative direction) to yellow (in the positive direction). Shoots were dried in an oven (Blue M, Blue Island, IL) at 60 °C for 5 d before dry weight measurements.

The experiment was a randomized complete block design with time as the block. All data from three replications were combined and analyzed with the PROC MEANS, PROC MIXED, and PROC GLIMMIX procedures and Tukey’s honestly significant difference test ($\alpha = 0.05$) in SAS (version 9.4; SAS Institute, Inc., Cary, NC).

Results

Shoot weight

Shoot fresh and dry weight of the two lettuce cultivars and kale increased with the ratio of G to B light (G:B) (Figure II-3A–C). Shoot fresh (and dry) weight of lettuce ‘Rex’, lettuce ‘Rouxai’, and kale grown under G₆₀R₁₂₀ was 72% (54%), 79% (63%), and 50% (34%) greater, respectively, than under B₆₀R₁₂₀. Similarly, increasing the ratio of FR to B light (FR:B) from

0:60 to 60:0 increased shoot fresh weight of lettuce ‘Rex’, lettuce ‘Rouxai’, and kale by 37%, 91%, and 43%, respectively. Although shoot dry weight increased when B light was substituted with FR light at 0–40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, it was 14% lower, 38% greater, and similar for lettuce ‘Rex’, lettuce ‘Rouxai’, and kale, respectively, when FR light increased from 40 to 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. When B light was substituted with G or FR light at the same photon flux density, shoot fresh weight of most crops was similar, except it was 26% greater under G₆₀R₁₂₀ than under R₁₂₀FR₆₀ for lettuce ‘Rex’; however, shoot dry weight of the two lettuce cultivars was variable. Shoot dry weight of lettuce ‘Rex’ under FR light at 20 or 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was greater than under the same photon flux densities of G light. Shoot dry weight of lettuce ‘Rouxai’ was greater under 40 or 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light than under the same photon flux densities of G light. Plants grown under both G and FR light at 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (i.e., B₂₀G₂₀R₁₂₀FR₂₀) generally had greater shoot fresh and dry weight than those grown under either G or FR light at 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (i.e., B₄₀G₂₀R₁₂₀ or B₄₀R₁₂₀FR₂₀), showing additive responses in weight gain. Shoot fresh and dry weight of all crops were comparable under B₂₀G₂₀R₁₂₀FR₂₀, B₂₀R₁₂₀FR₄₀, and WW₁₈₀. Compared to shoot fresh and dry weight under WW₁₈₀, those under EQW₁₈₀ were 16% and 19–25% lower, respectively, for lettuce but were similar for kale.

Plant morphology

Leaf length and width of all crops, plant diameter of lettuce, and leaf area and petiole length of kale increased as B light was substituted with increasing G or FR light (Figure II-3D–I). Compared to plants grown under B₆₀R₁₂₀, lettuce ‘Rex’, lettuce ‘Rouxai’, and kale had 39%, 24%, and 23% greater leaf length and 34%, 27%, and 32% greater leaf width under G₆₀R₁₂₀, respectively, and 94%, 65%, and 31% greater leaf length and 42%, 50%, and 22% greater leaf width under R₁₂₀FR₆₀, respectively. All crops grown under B₂₀G₂₀R₁₂₀FR₂₀ had similar leaf

length and width to those grown under G₆₀R₁₂₀, B₂₀R₁₂₀FR₄₀, and WW₁₈₀. Leaf length and width were 10–11% lower under EQW₁₈₀ than under WW₁₈₀ for lettuce ‘Rex’ but similar under the two white treatments for the other crops. Leaf length was generally greater under FR light than G light at the same photon flux density for lettuce, but not kale. Plant diameter of lettuce followed a similar trend to leaf length; substituting B light with G or FR light increased plant diameter by up to 38% or 32%, respectively, for lettuce ‘Rex’ and by up to 86% or 63%, respectively, for lettuce ‘Rouxai’. Lettuce grown under WW₁₈₀ had a similar diameter to those grown under B₂₀R₁₂₀FR₄₀, B₂₀G₂₀R₁₂₀FR₂₀, and EQW₁₈₀. Leaf area and petiole length of kale increased with G:B and FR:B. For example, kale developed 59% and 41% larger leaves and 54% and 158% longer petioles under G₆₀R₁₂₀ and R₁₂₀FR₆₀, respectively, than under B₆₀R₁₂₀. Petiole length of kale was 26%, 61%, and 68% greater under FR light than under G light at 20, 40, and 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Kale grown under B₂₀G₂₀R₁₂₀FR₂₀, WW₁₈₀, and EQW₁₈₀ had similar leaf area and petiole length.

Chlorophyll fluorescence, pigmentation, and leaf number

Substituting B light with G or FR light reduced Fv/Fm of lettuce by up to 5% or 10–12%, respectively (Figure II-4A–B). At the same photon flux density, FR light decreased Fv/Fm of lettuce more than G light did. In contrast, Fv/Fm of kale was not influenced by G light but was reduced by 4–6% under R₁₂₀FR₆₀ and B₂₀G₂₀R₁₂₀FR₂₀ compared to B₆₀R₁₂₀ (Figure II-4C). Relative specific chlorophyll content (the SPAD value) in lettuce and kale leaves decreased by up to 17–22% or 35–48% as the substitution of B with G or FR light increased from 0 to 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively (Figure II-4D–F). As low as 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light reduced the SPAD value of lettuce ‘Rex’ and kale more than 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light did. Compared to B₆₀R₁₂₀, lettuce and kale grown under broad-spectrum light that included FR light (i.e.,

B₂₀G₂₀R₁₂₀FR₂₀, WW₁₈₀, and EQW₁₈₀) had lower relative specific chlorophyll content.

Leaf number of lettuce ‘Rex’ and ‘Rouxai’ increased slightly under G₆₀R₁₂₀, B₂₀G₂₀R₁₂₀FR₂₀, and WW₁₈₀ compared to B₆₀R₁₂₀ (Figure II-4G–H). Lettuce ‘Rex’ and kale developed one and two more leaves, respectively, under G light than under FR light at the same photon flux density of 40 or 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure II-4G, II-4I). The leaf color of lettuce ‘Rouxai’ was generally brighter, greener, and yellower under G₆₀R₁₂₀ and R₁₂₀FR₆₀ compared to B₆₀R₁₂₀, whereas most other light combinations did not influence color profiles (Figure II-5A–C). Foliage coloration of lettuce ‘Rouxai’ was similar under the two white light treatments (WW₁₈₀ and EQW₁₈₀), although leaves under EQW₁₈₀ were slightly brighter and yellower compared to B₆₀R₁₂₀.

Discussion

In the same R-light background, substituting G and/or FR light for B light increased shoot weight of lettuce and kale (Figure II-3). This increase can be attributed to both net photosynthesis and morphology because the relative growth rate is the product of the net assimilation rate and the leaf area ratio (Evans, 1972; Lambers et al., 2008). Both G and FR light directly contribute to net photosynthesis. G light is often regarded as less effective than R or B light at driving photosynthesis, at least partly because the quantum yield of G light is seemingly lower than that of R or B light, according to the relative quantum yield curve developed by McCree (1972), although this is a misconception. The quantum yield accounts for absorbed photons, rather than incident photons, because it is calculated from the leaf action spectrum and absorption data (McCree, 1972). Therefore, the efficacy of incident photons is reflected in the action spectrum, not the relative quantum yield curve. The action spectrum reveals a major peak in the R region and similar efficacy of B and G light in photosynthesis. For the peak wavelengths

of the LEDs used in our study, the net photosynthetic rate under B light (peak = 450 nm) and G light (peak = 525 nm) is 54–57% of that under R light (peak = 660 nm) (McCree, 1972). Based on these interpretations of the McCree curve, changing G:B generally should not affect net photosynthesis under a low PPFD. For example, the net assimilation rate of lettuce ‘Waldmann’s Green’ was not influenced by increasing G light from 2% to 41% or increasing B light from 10% to 30% at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Snowden et al., 2016). Likewise, the net photosynthetic rate of the same lettuce cultivar remained unchanged when substituting 29% of R light with G light in a 16%B+84%R mixture at $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Kim et al., 2004a). Moreover, G light penetrates deeper in the leaf profile compared to R or B light and drives photosynthesis through abundant lower chloroplasts (Sun et al., 1998; Terashima et al., 2009; Brodersen and Vogelmann, 2010).

Although outside the PPFD waveband, FR light is a major excitation source for photosynthetic machinery; photosystems II and I are preferentially excited by R and FR light, respectively (Zhen and van Iersel, 2017). The addition of FR light (peak = 735 nm) in a B+R or W spectrum increased the quantum yield of photosystem II of lettuce ‘Green Towers’ by restoring the energy balance between the two photosystems in the photosynthetic electron transport chain, thereby increasing net photosynthesis (Zhen and van Iersel, 2017). In contrast, in our study, increasing FR:B or G:B reduced the maximum quantum yield of photosystem II, especially under FR light. This discrepancy could be attributed to ways in which FR light was included: Zhen and van Iersel (2017) added FR light to a constant PPFD of B+R or W light, but in our study, we substituted FR light for B light to maintain a constant TPFD. Because chlorophyll fluorescence measures how absorbed photons are used besides photochemistry and heat dissipation, the relative quantum yield curve of McCree (1972) based on absorbed photons

is relevant. The quantum yield between 700 and 725 nm is substantially lower than between 400 and 700 nm. Consequently, adding FR light to a fixed spectrum can increase quantum yield, but substituting FR light for a portion of the PPFD can reduce it. This dichotomy has been previously observed: adding up to $64 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light (peak = 731 nm) to $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of 20%B+80%R light increased the net assimilation rate (or shoot dry weight per unit leaf area) of geranium (*Pelargonium × hortorum*) and snapdragon (*Antirrhinum majus*); however, substituting 32 or $64 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light for R light decreased the net assimilation rate of geranium and did not influence that of snapdragon (Park and Runkle, 2017). Furthermore, substituting FR or G light for B light in our study was coupled with a reduced ratio of B to R light (B:R). Decreasing B:R at a constant PPFD of $171 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reduced Fv/Fm in lettuce ‘Grand Rapids TBR’ (Son and Oh, 2013). In our study, B:R decreased as B light was substituted with FR or G light, which may also have caused the decreases in Fv/Fm besides the effects of FR light. Increasing FR:B or G:B while decreasing B:R decreased Fv/Fm and chlorophyll concentration but increased whole-plant photosynthesis because of greater light capture by enlarged leaves, which likely compensated for the reduced quantum yield.

Both G and FR light can promote extension growth by triggering the shade-avoidance response through photoreceptors (Franklin and Whitelam, 2005; Zhang et al., 2011). With increasing substitution of B light with G or FR light, increases in shoot fresh and dry weight were accompanied by increases in leaf length, width, and area for lettuce and kale (Figure II-3). A low ratio of R to FR light (R:FR) is a shade indicator that acts upon phytochromes to promote stem and petiole elongation, leaf expansion, hyponasty, and flowering while reducing branching (Franklin and Whitelam, 2005; Vandenbussche et al., 2005; Park and Runkle, 2017). When plants are exposed to a low R:FR, phytochrome B partially converts to its inactive form and

dissociates from phytochrome-interacting factors (PIF) 4 and 5, which then accumulate and promote expression of genes involved in elongation growth (Franklin, 2008). Prolonged exposure to a low R:FR can also increase gibberellin synthesis, which facilitates the functions of PIFs and induces sustained extension growth (Franklin, 2008). In our study, leaf length, width, and area, plant diameter, and petiole length increased with decreasing R:FR (Figure II-3). As a result, larger leaves enhanced light capture for photosynthesis.

Although G light is not absorbed as well as R or B light at the upper canopy, it is transmitted further into the canopy. Therefore, the spectral distribution in vegetative shade under sunlight is rich in FR light and to a lesser extent, G light (Vandenbussche et al., 2005). The addition of 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of 44%B+56%R light induced shade-avoidance symptoms including promotion of petiole elongation and hyponasty in wild-type arabidopsis (*Arabidopsis thaliana*) as well as its phytochrome and cryptochrome mutants (Zhang et al., 2011). Although cryptochromes mainly absorb B light and ultraviolet-A radiation, they also absorb and respond to G light (Liu et al., 2008). The shade-avoidance response induced by G light is likely mediated by cryptochromes, possibly together with an unknown G light receptor through a mechanism different from that for FR light (Folta, 2004; Zhang et al., 2011; Wang and Folta, 2013). In our study, increased leaf expansion under FR and G light contributed to increased whole-plant photosynthesis and shoot weight; however, FR light elicited more pronounced shade-avoidance symptoms than G light did when delivered at the same photon flux density. The attenuated shade-avoidance response under G light can at least partly be attributed to suppression of G-absorbing cryptochromes on expression of shade-induced genes, which is promoted under FR light (Zhang et al., 2011; Wang and Folta, 2013). In addition, lettuce and kale grown under combined G and FR light generally had larger leaves and greater shoot weight

than those grown under either G or FR light, showing additive effects of G and FR light on leaf morphology and shoot weight.

Besides a low R:FR and enriched green light, insufficient or low B light can also signal the shade-avoidance response through cryptochrome-mediated regulation of PIF4 and PIF5 (Keuskamp et al., 2010; Keller et al., 2011; Pedmale et al., 2016). When B light is sufficiently high, cryptochromes in arabidopsis actively suppress PIF4 while cryptochrome 2 and PIF5 are reduced, resulting in normal photomorphogenesis (Pedmale et al., 2016). In contrast, low B light allows cryptochromes 1 and 2 to physically interact with and stabilize PIF4 and PIF5, resulting in ample PIF proteins to promote expression of growth-related genes and increase hypocotyl growth (Pedmale et al., 2016). In our study, the absolute amount of B light decreased when B light was substituted with G or FR light. Therefore, reduced B light likely enhanced the shade-avoidance symptoms together with increased G or FR light. Here, B photon flux density was not kept constant because we intended to investigate the interaction between B and G light at the same TPFD. With increasing G:B, G light (peak = 525, 559, or 563 nm) can reverse B light effects on various physiological processes, such as hypocotyl elongation and stomatal opening, at least partly by antagonizing degradation of cryptochrome 2 induced by ample B light (Folta, 2004; Banerjee et al., 2007; Bouly et al., 2007; Folta and Maruhnich, 2007). A G:B of 2:1 and a peak wavelength of 540 nm were most effective at reversing B-light-controlled stomatal opening (Frechilla et al., 2000; Talbott et al., 2002). Although the addition of G light to B+R light lowered stomatal conductance of lettuce ‘Waldmann’s Green’, it increased shoot weight rather than limit carbon fixation (Kim et al., 2004b). Our results on lettuce and kale suggest that G light antagonizes B-light-induced inhibition of extension growth, in agreement with previous studies on arabidopsis (Folta, 2004; Bouly et al., 2007). The B and G reversibility is also evident in

anthocyanin accumulation of arabidopsis and lettuce ‘Red Sails’, which is upregulated under B light through cryptochrome 1 but reduced by additional G light in a fluence-rate-dependent manner (Bouly et al., 2007; Zhang and Folta, 2012; Wang and Folta, 2013). Similarly, in our study, a decrease in a^* with increasing G:B showed attenuated red foliage coloration in lettuce ‘Rouxai’ under G light.

The inclusion of G light in a B+R spectrum can create W light and thus improve visual quality for assessment of plant health. W light is generally created by covering B LEDs with a phosphor coating, the material of which can alter the W spectrum. The WW and EQW LEDs used in our study had broad spectra covering 400 to 750 nm with predominately R light (54%) and G light (61%), respectively. Lettuce and kale growth were similar under WW₁₈₀ and B₂₀G₂₀R₁₂₀FR₂₀ because they both included G and FR light in a B+R background. Shoot weight was lower under EQW LEDs than under WW LEDs for lettuce, but not kale, in the environmental conditions tested at 20 °C. However, unpublished results from Q. Meng and E.S. Runkle (Michigan State University) in a subsequent experiment have shown similar growth of lettuce ‘Rouxai’ under these two types of W LEDs when air temperature was 22 °C, indicating a possible interaction between light quality and temperature in regulation of crop growth.

In conclusion, substituting G and/or FR light for B light in a constant R background increased leaf expansion, light capture, and shoot weight of lettuce and kale despite reduced Fv/Fm and chlorophyll concentration. We demonstrated that G light was effective at driving whole-plant photosynthesis under sole-source lighting. G and FR light both induced shade-avoidance symptoms and antagonized B light in control of extension growth and pigmentation; however, FR light was a more potent shade signal than G light when delivered at the same photon flux density. The inclusion of G light is also useful for creating a visually pleasant W

light environment. Since G LEDs are inefficient at converting electrical energy to light, efficient W LEDs are a suitable alternative to provide G light when desired.

Acknowledgements

We thank David Hamby, Rodrigo Pereyra, Charles Brunault, Alan Sarkisian, and Dorian Spero from OSRAM Innovation for lighting support; Steve Brooks for technical assistance; Randy Beaudry, Dan Brainard, Roberto Lopez, and Emily Merewitz for instruments; and Nathan Kelly, Yujin Park, and Nate DuRussel for experimental assistance. This work was supported by Michigan State University AgBioResearch Project GREEN GR17-072 and the USDA National Institute of Food and Agriculture, Hatch project 192266.

APPENDIX

Table II-1. The pH, electrical conductivity, and temperature (mean $\pm$ standard deviation from daily measurements) of nutrient solutions for ten lighting treatments in three experimental replications (rep.). Plants were grown hydroponically under a 20-h photoperiod and various mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Lighting treatment	pH			Electrical conductivity ($\text{mS}\cdot\text{cm}^{-1}$)			Water temperature ($^{\circ}\text{C}$)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
B ₆₀ R ₁₂₀	5.9 $\pm$ 0.2	5.8 $\pm$ 0.1	6.1 $\pm$ 0.4	1.38 $\pm$ 0.07	1.82 $\pm$ 0.06	1.59 $\pm$ 0.08	20.6 $\pm$ 0.2	22.9 $\pm$ 0.5	20.9 $\pm$ 0.2
B ₄₀ G ₂₀ R ₁₂₀	5.9 $\pm$ 0.2	5.8 $\pm$ 0.1	6.1 $\pm$ 0.4	1.38 $\pm$ 0.07	1.82 $\pm$ 0.06	1.59 $\pm$ 0.08	20.6 $\pm$ 0.2	22.9 $\pm$ 0.5	20.9 $\pm$ 0.2
B ₂₀ G ₄₀ R ₁₂₀	5.9 $\pm$ 0.3	5.6 $\pm$ 0.1	6.1 $\pm$ 0.4	1.33 $\pm$ 0.10	1.67 $\pm$ 0.11	1.59 $\pm$ 0.08	21.4 $\pm$ 0.3	21.9 $\pm$ 0.3	20.9 $\pm$ 0.2
G ₆₀ R ₁₂₀	5.9 $\pm$ 0.3	5.6 $\pm$ 0.1	5.9 $\pm$ 0.3	1.33 $\pm$ 0.10	1.67 $\pm$ 0.11	1.57 $\pm$ 0.08	21.4 $\pm$ 0.3	21.9 $\pm$ 0.3	21.0 $\pm$ 0.1
B ₄₀ R ₁₂₀ FR ₂₀	5.8 $\pm$ 0.2	5.6 $\pm$ 0.3	5.9 $\pm$ 0.3	1.30 $\pm$ 0.07	1.70 $\pm$ 0.08	1.57 $\pm$ 0.08	22.0 $\pm$ 0.2	21.8 $\pm$ 0.2	21.0 $\pm$ 0.1
B ₂₀ R ₁₂₀ FR ₄₀	5.8 $\pm$ 0.2	5.6 $\pm$ 0.3	5.9 $\pm$ 0.3	1.30 $\pm$ 0.07	1.70 $\pm$ 0.08	1.57 $\pm$ 0.08	22.0 $\pm$ 0.2	21.8 $\pm$ 0.2	21.0 $\pm$ 0.1
R ₁₂₀ FR ₆₀	5.8 $\pm$ 0.3	5.6 $\pm$ 0.3	5.9 $\pm$ 0.3	1.34 $\pm$ 0.06	1.70 $\pm$ 0.06	1.54 $\pm$ 0.09	21.9 $\pm$ 0.3	23.6 $\pm$ 0.6	21.8 $\pm$ 0.2
B ₂₀ G ₂₀ R ₁₂₀ FR ₂₀	5.8 $\pm$ 0.3	5.6 $\pm$ 0.3	5.9 $\pm$ 0.3	1.34 $\pm$ 0.06	1.70 $\pm$ 0.06	1.54 $\pm$ 0.09	21.9 $\pm$ 0.3	23.6 $\pm$ 0.6	21.8 $\pm$ 0.2
WW ₁₈₀	5.8 $\pm$ 0.3	5.8 $\pm$ 0.3	5.9 $\pm$ 0.3	1.35 $\pm$ 0.07	1.76 $\pm$ 0.07	1.54 $\pm$ 0.09	21.8 $\pm$ 0.3	23.6 $\pm$ 0.5	21.8 $\pm$ 0.2
EQW ₁₈₀	5.8 $\pm$ 0.3	5.8 $\pm$ 0.3	5.9 $\pm$ 0.2	1.35 $\pm$ 0.07	1.76 $\pm$ 0.07	1.57 $\pm$ 0.08	21.8 $\pm$ 0.3	23.6 $\pm$ 0.5	21.6 $\pm$ 0.1

Table II-2. The air temperature, CO₂ concentration, and relative humidity (mean $\pm$ standard deviation from hourly averages) for ten lighting treatments in three experimental replications (rep.). Plants were grown hydroponically under a 20-h photoperiod and various mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Lighting treatment	Air temperature (°C)			CO ₂ concentration (ppm)			Relative humidity (%)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
B ₆₀ R ₁₂₀	20.0 $\pm$ 0.2	20.7 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
B ₄₀ G ₂₀ R ₁₂₀	20.0 $\pm$ 0.2	20.7 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
B ₂₀ G ₄₀ R ₁₂₀	20.0 $\pm$ 0.2	20.4 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
G ₆₀ R ₁₂₀	20.0 $\pm$ 0.2	20.4 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
B ₄₀ R ₁₂₀ FR ₂₀	20.3 $\pm$ 0.2	20.4 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
B ₂₀ R ₁₂₀ FR ₄₀	20.3 $\pm$ 0.2	20.4 $\pm$ 0.6	20.1 $\pm$ 0.5	379 $\pm$ 22	402 $\pm$ 41	402 $\pm$ 41	58 $\pm$ 12	44 $\pm$ 3	44 $\pm$ 8
R ₁₂₀ FR ₆₀	20.3 $\pm$ 0.2	21.2 $\pm$ 1.0	20.6 $\pm$ 0.5	379 $\pm$ 22	389 $\pm$ 33	402 $\pm$ 41	58 $\pm$ 12	45 $\pm$ 4	44 $\pm$ 8
B ₂₀ G ₂₀ R ₁₂₀ FR ₂₀	20.3 $\pm$ 0.2	21.2 $\pm$ 1.0	20.6 $\pm$ 0.5	379 $\pm$ 22	389 $\pm$ 33	402 $\pm$ 41	58 $\pm$ 12	45 $\pm$ 4	44 $\pm$ 8
WW ₁₈₀	20.5 $\pm$ 0.3	20.7 $\pm$ 0.6	20.6 $\pm$ 0.5	369 $\pm$ 20	402 $\pm$ 41	402 $\pm$ 41	57 $\pm$ 10	44 $\pm$ 3	44 $\pm$ 8
EQW ₁₈₀	20.5 $\pm$ 0.3	20.7 $\pm$ 0.6	20.6 $\pm$ 0.5	369 $\pm$ 20	402 $\pm$ 41	402 $\pm$ 41	57 $\pm$ 10	44 $\pm$ 3	44 $\pm$ 8

Table II-3. Spectral characteristics of ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. Integrated parameters include the photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency (McCree, 1972) and spectral data from 350 to 800 nm]. The estimated phytochrome photoequilibrium (PPE) was calculated as the proportion of active phytochromes in the total phytochrome pool according to Sager et al. (1988). The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

	B ₆₀ R ₁₂₀	B ₄₀ G ₂₀ R ₁₂₀	B ₂₀ G ₄₀ R ₁₂₀	G ₆₀ R ₁₂₀	B ₄₀ R ₁₂₀ FR ₂₀	B ₂₀ R ₁₂₀ FR ₄₀	R ₁₂₀ FR ₆₀	B ₂₀ G ₂₀ R ₁₂₀ FR ₂₀	WW ₁₈₀	EQW ₁₈₀
Single-band photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)										
B	58.3	41.1	19.8	3.5	40.3	20.2	0.2	20.2	12.1	15.7
G	0.6	20.9	38.7	59.4	0.9	0.8	0.4	19.9	53.6	107.2
R	115.1	119.3	118.1	118.5	115.4	116.3	116.0	116.6	101.3	50.4
FR	1.0	1.2	1.2	1.3	21.1	41.4	58.0	21.3	18.8	6.2
Integrated photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)										
PPFD	174.1	181.4	176.6	181.4	156.6	137.3	116.6	156.7	166.9	173.3
TPFD	175.1	182.6	177.9	182.7	177.7	178.7	174.6	178.0	185.7	179.4
YPFD	150.9	158.5	155.3	159.5	141.6	130.6	117.4	142.9	153.0	153.4
Light ratio										
R:FR	112.7	98.1	95.0	93.0	5.5	2.8	2.0	5.5	5.4	8.2
B:R	0.5	0.3	0.2	0.0	0.3	0.2	0.0	0.2	0.1	0.3
B:G	90.0	2.0	0.5	0.1	44.0	24.8	0.4	1.0	0.2	0.1
B:FR	57.2	33.8	15.9	2.8	1.9	0.5	0.0	0.9	0.6	2.5
PPE	0.872	0.876	0.879	0.883	0.837	0.807	0.784	0.843	0.829	0.850

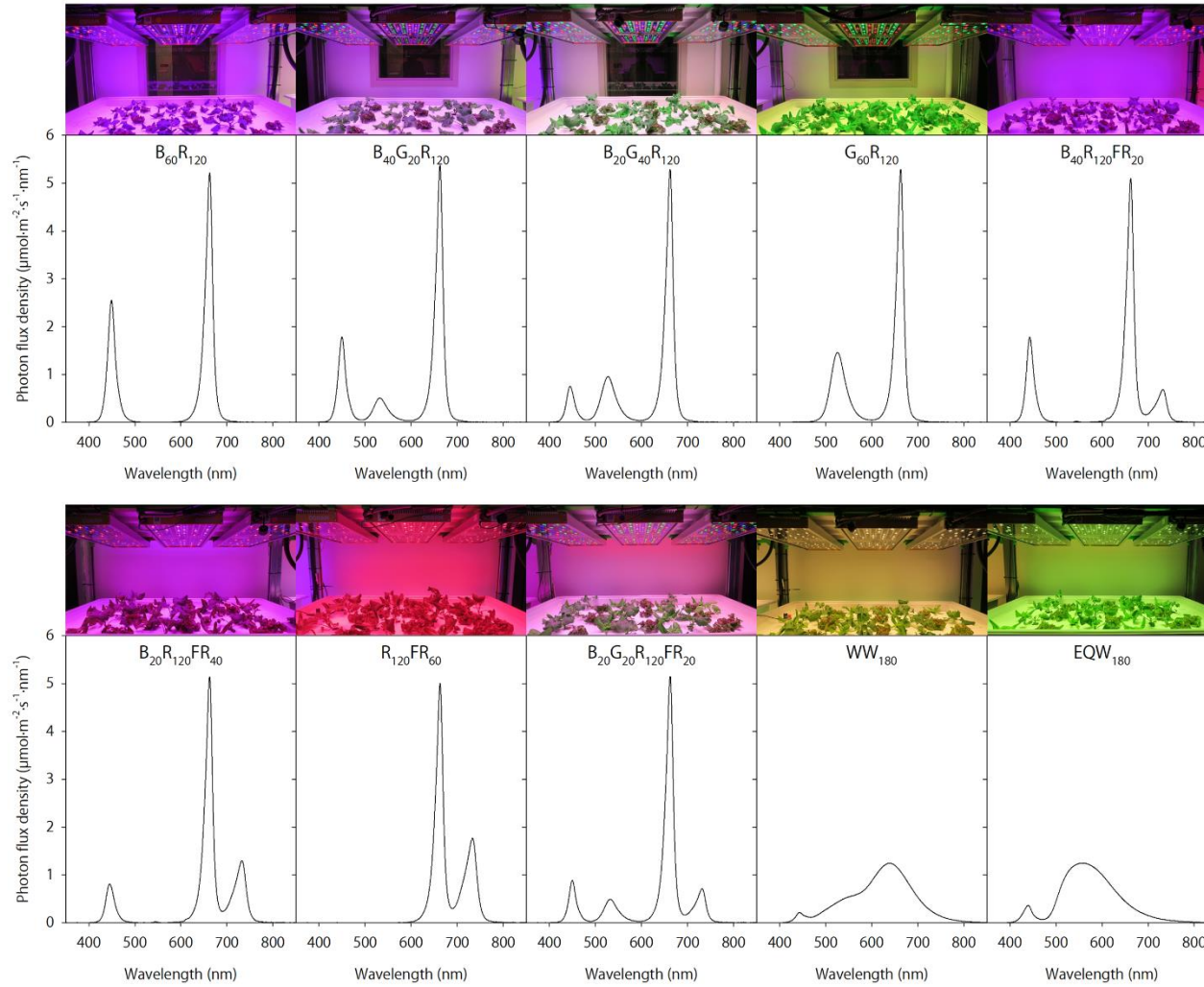


Figure II-1. Spectral distributions of ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

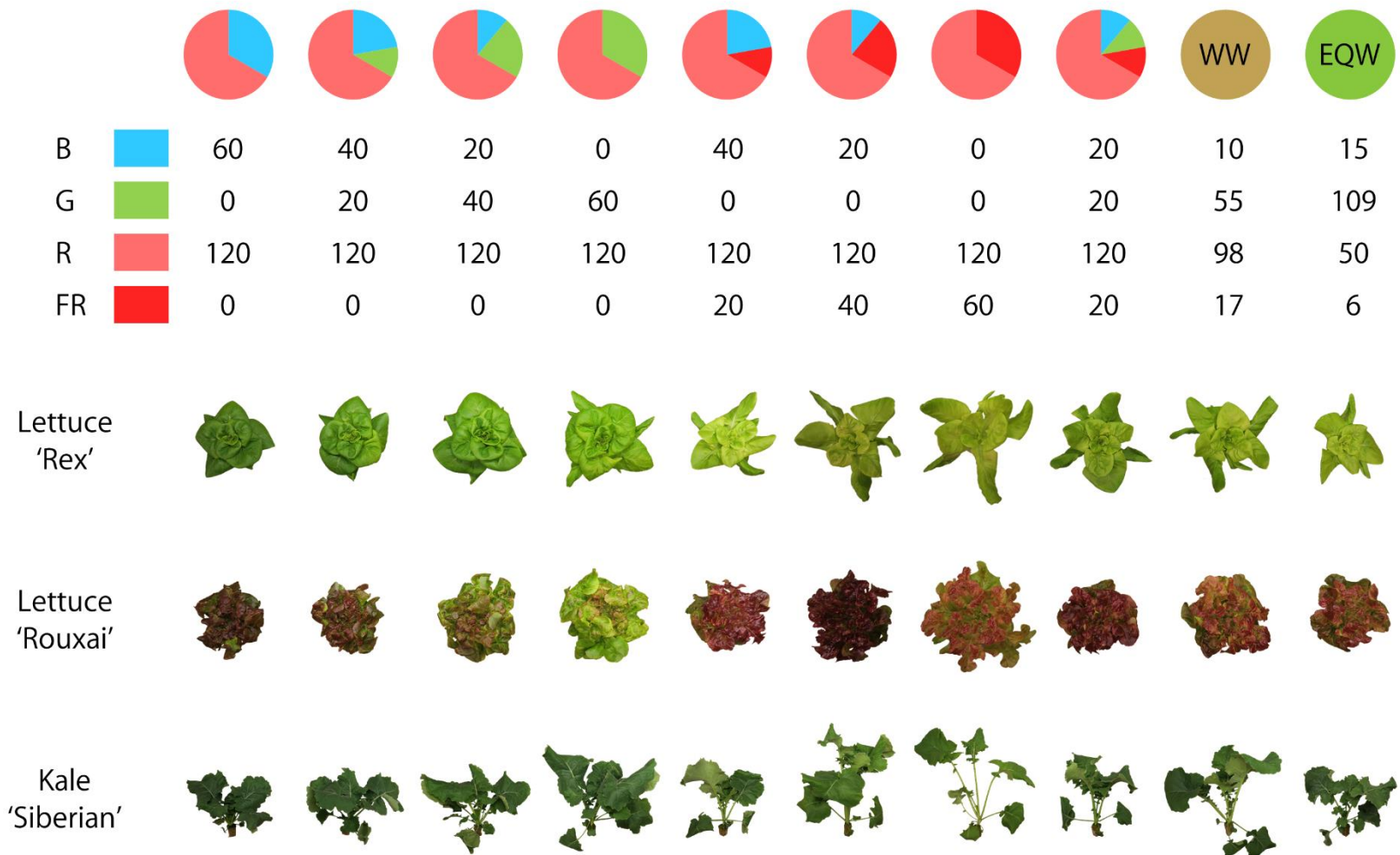


Figure II-2. Lettuce 'Rex' and 'Rouxai' 27 and 30 d after sowing, respectively. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

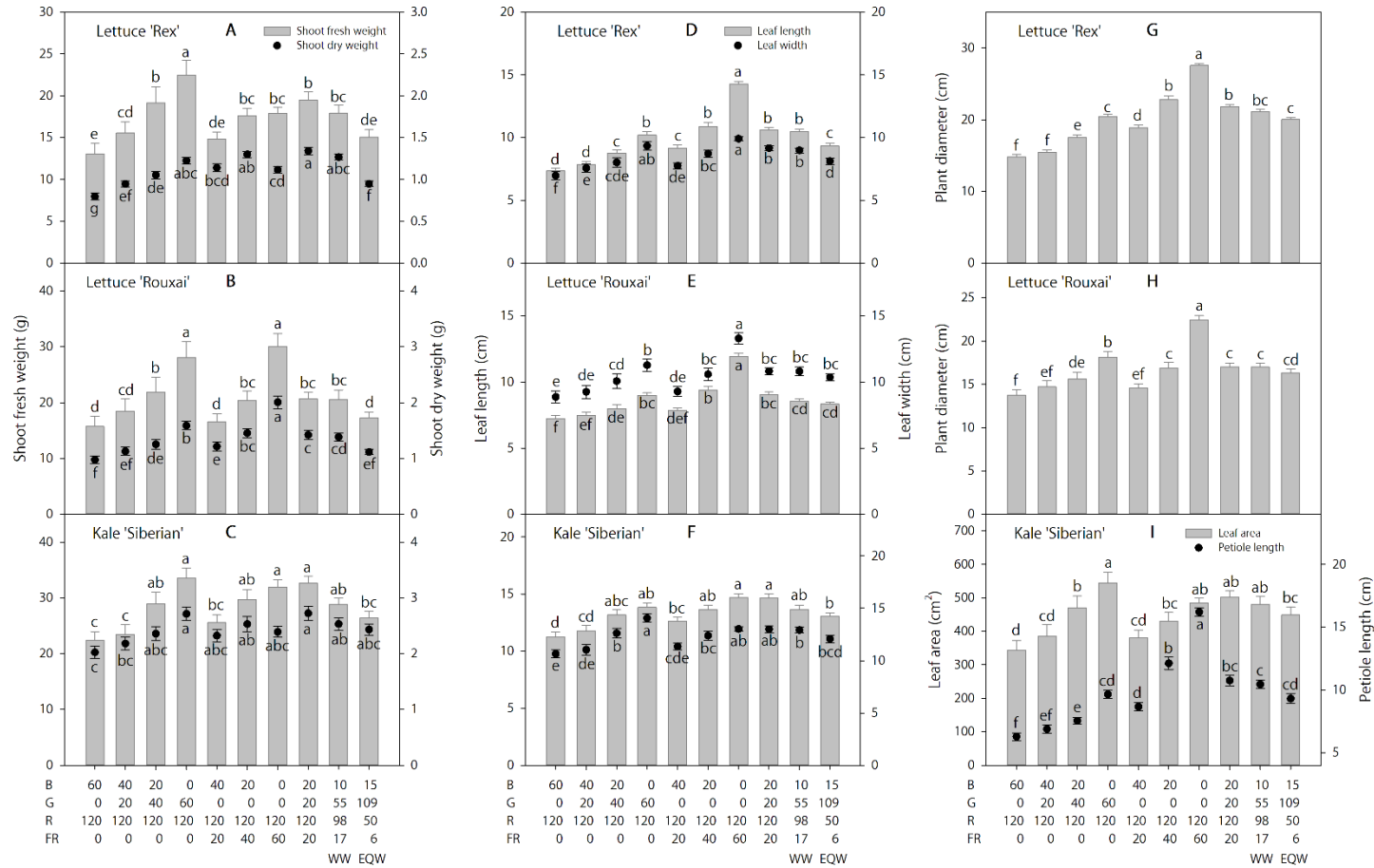


Figure II-3. Shoot fresh and dry weight, length and width of the fifth most mature leaf, plant diameter, leaf area, and petiole length of lettuce ‘Rex’, lettuce ‘Rouxai’, and kale ‘Siberian’. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).

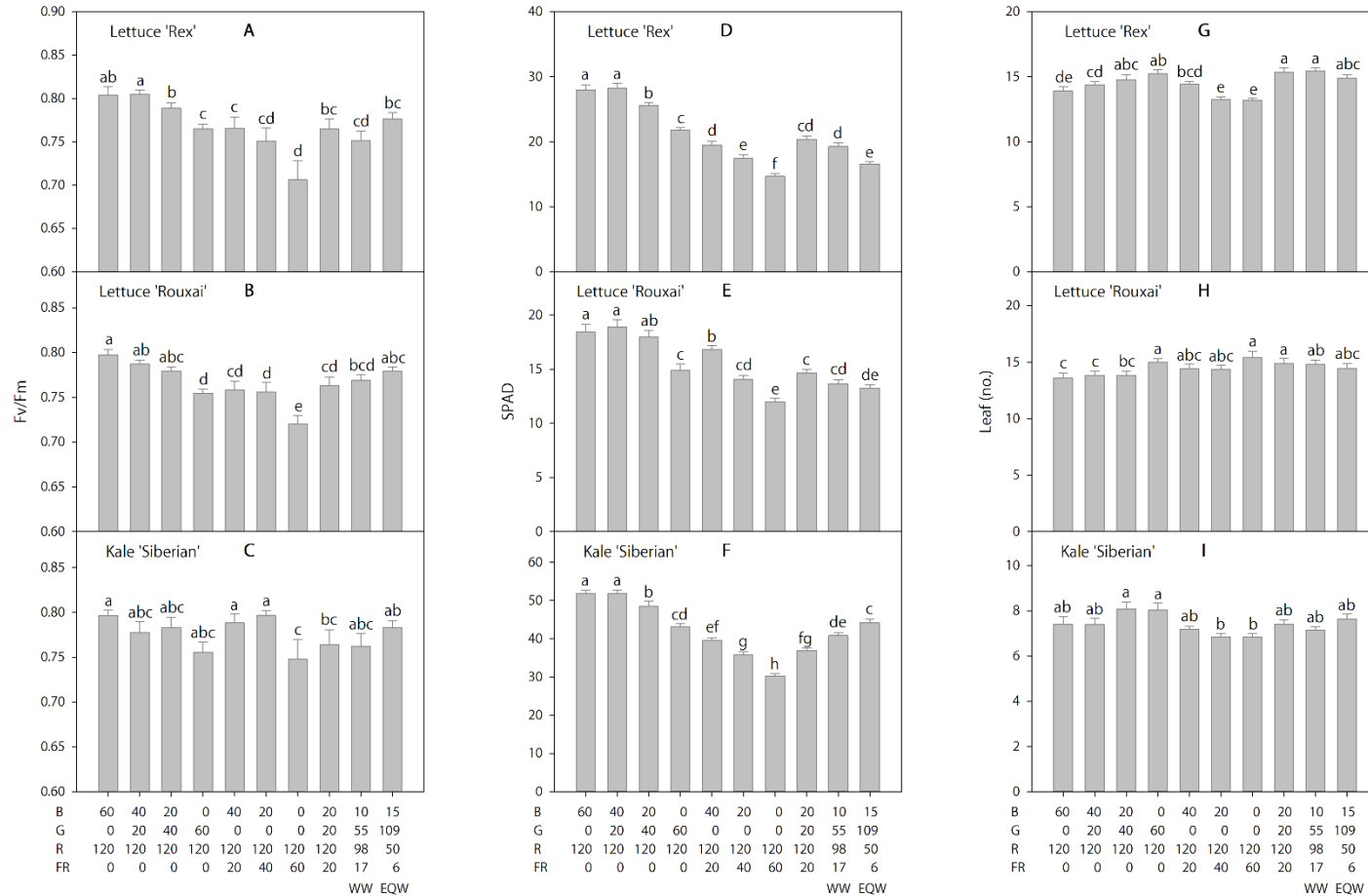


Figure II-4. The maximum quantum efficiency of photosystem II (Fv/Fm), relative chlorophyll concentration (SPAD), and leaf number of lettuce 'Rex', lettuce 'Rouxai', and kale 'Siberian'. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$).

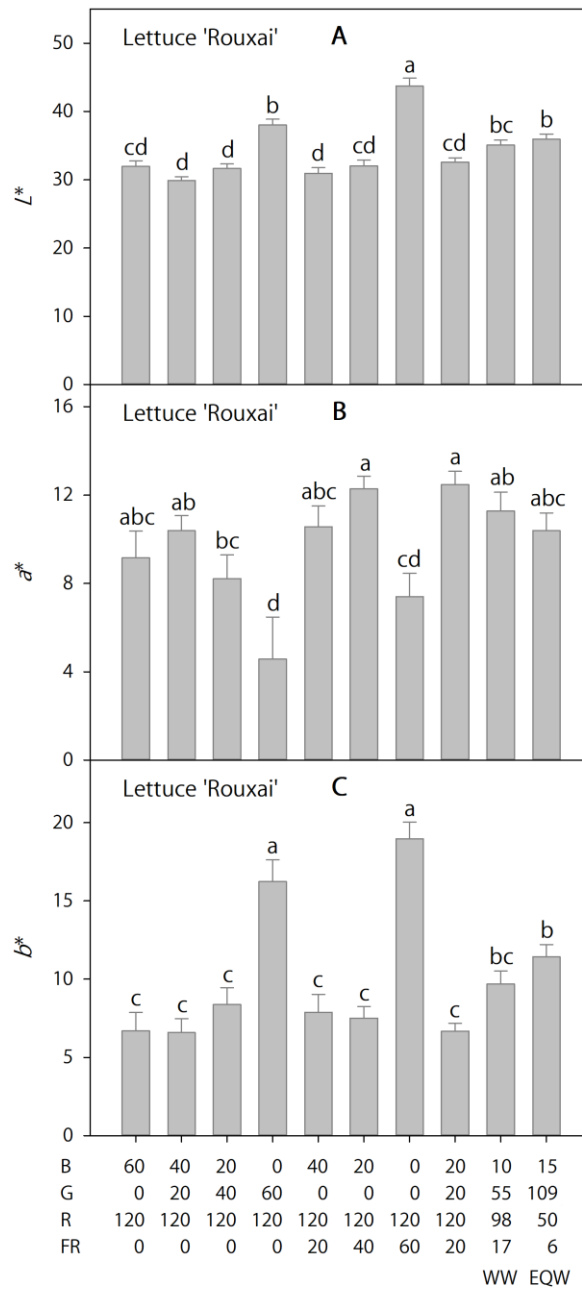


Figure II-5. Lab color space analysis (L^* , lightness; a^* , green–red; b^* , blue–yellow) for foliage coloration of lettuce ‘Rouxai’. Plants were grown under ten lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm); warm-white (WW); or equalized-white (EQW) light-emitting diodes. The number for each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and cultivar are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$).

LITERATURE CITED

LITERATURE CITED

- Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R. and Batschauer, A., 2007. The signaling state of *Arabidopsis* cryptochrome 2 contains flavin semiquinone. *J. Biol. Chem.* 282:14916–14922.
- Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R. and Ahmad, M., 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J. Biol. Chem.* 282:9383–9391.
- Brodersen, C.R. and Vogelmann, T.C., 2010. Do changes in light direction affect absorption profiles in leaves? *Funct. Plant Biol.* 37:403–412.
- Evans, G.C., 1972. The quantitative analysis of plant growth. Blackwell Scientific Publications, Oxford.
- Folta, K.M., 2004. Green light stimulates early stem elongation, antagonizing light-mediated growth inhibition. *Plant Physiol.* 135:1407–1416.
- Folta, K.M. and Maruhnich, S.A., 2007. Green light: a signal to slow down or stop. *J. Exp. Bot.* 58:3099–3111.
- Franklin, K.A. and Whitelam, G.C., 2005. Phytochromes and shade-avoidance responses in plants. *Ann. Bot.* 96:169–175.
- Franklin, K.A., 2008. Shade avoidance. *New Phytol.* 179:930–944.
- Frechilla, S., Talbott, L.D., Bogomolni, R.A. and Zeiger, E., 2000. Reversal of blue light-stimulated stomatal opening by green light. *Plant Cell Physiol.* 41:171–176.
- Johkan, M., Shoji, K., Goto, F., Hahida, S.N. and Yoshihara, T., 2012. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. *Environ. Exp. Bot.* 75:128–133.
- Keller, M.M., Jaillais, Y., Pedmale, U.V., Moreno, J.E., Chory, J. and Ballaré, C.L., 2011. Cryptochrome 1 and phytochrome B control shade-avoidance responses in *Arabidopsis* via partially independent hormonal cascades. *Plant J.* 67:195–207.
- Keuskamp, D.H., Sasidharan, R. and Pierik, R., 2010. Physiological regulation and functional significance of shade avoidance responses to neighbors. *Plant Signal. Behav.* 5:655–662.
- Kim, H.H., Goins, G.D., Wheeler, R.M. and Sager, J.C., 2004a. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. *HortScience* 39:1617–1622.

- Kim, H.H., Goins, G.D., Wheeler, R.M. and Sager, J.C., 2004b. Stomatal conductance of lettuce grown under or exposed to different light qualities. *Ann. Bot.* 94:691–697.
- Klein, R.M., 1992. Effects of green light on biological systems. *Biol. Rev.* 67:199–284.
- Lambers, H., Chapin, F.S. and Pons, T.L., 2008. Growth and allocation. In *Plant Physiological Ecology* (pp. 312-374). Springer, New York, NY.
- Li, Q. and Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67:59–64.
- Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D. and Lin, C., 2008. Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. *Science* 322:1535–1539.
- McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agric. Meteorol.* 9:191–216.
- Myers, J., 1971. Enhancement studies in photosynthesis. *Ann. Rev. Plant Physiol.* 22:289–312.
- Park, Y. and 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.
- Pedmale, U.V., Huang, S.S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A., Sridevi, P., Nito, K., Nery, J.R. and Ecker, J.R., 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* 164:233–245.
- Pettai, H., Oja, V., Freiberg, A. and Laisk, A., 2005. The long-wavelength limit of plant photosynthesis. *FEBS Letters* 579:4017–4019.
- Sager, J.C., Smith, W.O., Edwards, J.L. and Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. Am. Soc. Agric. Eng.* 31:1882–1889.
- Snowden, M.C., Cope, K.R. and Bugbee, B., 2016. Sensitivity of seven diverse species to blue and green light: interactions with photon flux. *PloS ONE* 11(10):e0163121.
- Son, K.H. and 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.
- Sun, J., Nishio, J.N. and Vogelmann, T.C., 1998. Green light drives CO₂ fixation deep within leaves. *Plant Cell Physiol.* 39:1020–1026.
- Talbott, L.D., Nikolova, G., Ortiz, A., Shmayevich, I. and Zeiger, E., 2002. Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. *Am. J. Bot.* 89:366–368.

- Terashima, I., Fujita, T., Inoue, T., Chow, W.S. and Oguchi, R., 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. *Plant Cell Physiol.* 50:684–697.
- Vandenbussche, F., Pierik, R., Millenaar, F.F., Voesenek, L.A. and Van Der Straeten, D., 2005. Reaching out of the shade. *Curr. Opin. Plant Biol.* 8:462–468.
- Wang, Y. and Folta, K.M., 2013. Contributions of green light to plant growth and development. *Am. J. Bot.* 100:70–78.
- Wollaeger, H.M. and Runkle, E.S., 2014. Growth of impatiens, petunia, salvia, and tomato seedlings under blue, green, and red light-emitting diodes. *HortScience* 49:734–740.
- Zhang, T., Maruhnich, S.A. and Folta, K.M., 2011. Green light induces shade avoidance symptoms. *Plant Physiol.* 157:1528–1536.
- Zhang, T. and Folta, K.M., 2012. Green light signaling and adaptive response. *Plant Signal. Behav.* 7:75–78.
- Zhen, S. and van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. *J. Plant Physiol.* 209:115–122.

SECTION III

**BLUE LIGHT INTERACTS WITH GREEN LIGHT TO INFLUENCE GROWTH AND
PREDOMINANTLY CONTROLS QUALITY ATTRIBUTES OF LETTUCE**

Blue light interacts with green light to influence growth and predominantly controls quality attributes of lettuce

Qingwu Meng¹, Jennifer Boldt², and Erik S. Runkle^{1*}

¹ Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI 48824-1325, USA

² USDA-ARS, Greenhouse Production Research Group, 2801 W. Bancroft Street, Toledo, OH 43606, USA

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

Abstract.

Adding green (G; 500–600 nm) light to blue (B; 400–500 nm) and red (R; 600–700 nm) light creates white light to improve crop inspection at indoor farms. Although G light can drive photosynthesis and elicit the shade-avoidance response, its effects on plant growth and morphology have been inconsistent. We postulated that G light would counter suppression of crop growth and promotion of secondary metabolism by B light depending on the B photon flux density. Lettuce (*Lactuca sativa*) ‘Rouxai’ was grown in a growth room under nine sole-source light-emitting diode (LED) treatments with a 20-hour photoperiod or in a greenhouse. At the same photosynthetic photon flux density of $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, plants were grown under warm-white LEDs or increasing B photon flux densities at 0, 20, 60, and $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with or without substituting the remaining R light with $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light. Biomass and leaf expansion negatively correlated with the B photon flux density with or without G light. For example, increasing the B photon flux density decreased fresh and dry weights by up to 63% and 54%, respectively. The inclusion of G light did not affect shoot dry weight at 0 or $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light, but decreased it at 60 or $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light. Results suggest that the shade-avoidance response is strongly elicited by low B light and repressed by high B light, rendering G light ineffective at controlling morphology. Moreover, substituting R light with G light likely reduced the quantum yield. Otherwise, G light barely influenced morphology, foliage coloration, essential nutrients, or sensory attributes regardless of the B photon flux density. Increasing the B photon flux density increased red foliage coloration and concentrations of several macronutrients (e.g., nitrogen and magnesium) and micronutrients (e.g., zinc and copper). Consumers preferred plants grown under sole-source lighting over those grown in the greenhouse, which were more bitter and less acceptable, flavorful, and sweet. We conclude that lettuce phenotypes are

primarily controlled by B light, and G light maintains or suppresses lettuce growth depending on the B photon flux density.

Keywords: essential nutrients, indoor farming, LEDs, morphology, red light, sensory quality

Abbreviations: B, blue; DLI, daily light integral; FR, far red; G, green; LED, light-emitting diode; PIF, phytochrome-interacting factor; PPFD, photosynthetic photon flux density; R, red; TPDF, total photon flux density; WW, warm white; YPFD, yield photon flux density.

Introduction

With an emergent interest in producing local, fresh, and nutritious food throughout the year, indoor farming has recently been expanding rapidly in urban and peri-urban areas. High-value, fast-growing, and short-stature crops, such as leafy greens and herbs, are common crop types suitable for commercial indoor vertical farming. Light-emitting diodes (LEDs) are the predominant light source in indoor vertical production systems because they can be placed close to the crop canopy, are energy efficient and long-lasting, and have customizable spectral distributions (Massa et al., 2008). Because light quality influences physiological processes including photosynthesis, photomorphogenesis, and secondary metabolism, characterizing and understanding crop spectral responses is crucial to achieving desired yield and quality attributes, such as shape, texture, nutritional value, and organoleptic properties. Biologically relevant wavebands delivered by LEDs include ultraviolet (280–400 nm), blue (B; 400–500 nm; typical peak wavelength = 450 nm), green (G; 500–600 nm; typical peak wavelength = 525 nm), red (R; 600–700 nm; typical peak wavelength = 660 nm), and far red (FR; 700–800 nm; typical peak

wavelength = 735 nm). On one hand, changing the spectral composition can shift the energy balance to affect quantum efficiency and photoprotective mechanisms involving secondary metabolic compounds (Hogewoning et al., 2012; Kopsell et al., 2015). On the other hand, the spectral composition, such as the ratio of R to FR light, can evoke the shade-avoidance response to modify morphological traits (Franklin, 2008).

A typical green leaf of plants grown in growth chambers absorbed about 92% of B light (450 nm), 92% of R light (660 nm), and 81% of G light (525 nm) (McCree, 1972). Light transmission and reflection are higher for G light than for B or R light. Although chlorophylls *a* and *b* on the adaxial leaf surface absorb most B and R light and little G light, up to 80% of G light is transmitted through the mesophyll and penetrates deeper in the leaf profile (Terashima et al., 2009; Brodersen and Vogelmann, 2010). For a wide range of grain, oilseed, vegetable, and other crops grown in growth chambers, the relative quantum yield based on absorbed photons in low light (around $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was about 0.75, 0.74, and 0.93 for B (450 nm), G (525 nm), and R light (660 nm), respectively (McCree, 1972; Sager et al., 1988). More recently, quantum yields at various wavelengths were quantified again for cucumber (*Cucumis sativus*) (Hogewoning et al., 2012). In low light, the quantum yield based on absorbed photons was the highest for R light, followed by G light and then B light, whereas the quantum yield based on incident photons was the highest for R light and similarly lower for B and G light (about 70% of the highest yield) (Hogewoning et al., 2012). The higher quantum yield under absorbed G light than B light can be attributed to deeper penetration of G light into the mesophyll and its prolonged light paths in the leaf through scattering (Smith et al., 2017). Therefore, G light drives photosynthesis effectively despite its relatively weak absorption by chlorophylls and widely misconceived low quantum efficiency. The spectral composition in the background and spectra compared need to be clearly

defined when evaluating the effects of additional G light on photosynthesis. For example, in theory, substituting incident B light with G light would not change the quantum yield, whereas substituting incident R light with G light would decrease it.

A low ratio of R to FR light, low B light, and inclusion of G light can all trigger the shade-avoidance response, such as promotion of extension growth, acceleration of flowering, and hyponasty (Smith and Whitelam, 1997; Keuskamp et al., 2011; Zhang et al., 2011). The shade-avoidance response is mediated by phytochromes, such as phytochrome B, at a low ratio of R to FR light; by cryptochromes 1 and 2 in low B light; and through a less understood mechanism in the presence of G light (Smith and Whitelam, 1997; Zhang et al., 2011; Pedmale et al., 2016). Increased leaf expansion under shade signals such as a low ratio of R to FR light can increase light capture, thereby promoting whole-plant photosynthesis (Park and Runkle, 2017). Many shade-induced genes upregulated by FR light can also be activated by G light, albeit suppression of gene expression by cryptochromes in G light without FR light (Zhang et al., 2011). At the whole-plant level, inclusion of 24% G light at a fixed B photon flux density promoted leaf expansion and biomass accumulation of lettuce (*Lactuca sativa*) ‘Waldmann’s Green’ (Kim et al., 2004). However, other studies suggested a relatively passive role of G light in plant growth and morphology. For example, the inclusion of 10% G light did not influence leaf shape or biomass of lettuce ‘Green Skirt’ (Kang et al., 2016). In addition, increasing the G light fraction from 0% to 41% did not influence shoot dry weight but decreased the leaf area index of lettuce ‘Waldmann’s Green’ by 11% at a photosynthetic photon flux density (PPFD; 400–700 nm) of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, but not $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Snowden et al., 2016). These inconsistent findings on G light necessitate a detailed investigation that eliminates possible confounding factors to elucidate spectral interactions.

Energetic B photons can elicit accumulation of essential nutrients and secondary metabolites that have nutritional value and impart flavor (Son and Oh, 2013; Kopsell et al., 2015). For example, increasing the B photon flux density (or fraction) increased concentrations of total phenolics and flavonoids and promoted antioxidant capacity of lettuce ‘Sunmang’ and ‘Grand Rapids TBR’ (Son and Oh, 2013). In addition, partial substitution of white light with B light increased concentrations of anthocyanins, xanthophylls, and β -carotenes (Li and Kubota, 2009). Increases in anthocyanins by high B light can be attributed to increased activity of phenylalanine ammonia-lyase, a key enzyme in the biosynthetic pathways of polyphenol compounds (Heo et al., 2012). Because secondary metabolites such as phenolic compounds are bitter (Tomás-Barberán and Espín, 2001), B light can potentially affect consumer preferences of organoleptic properties. However, few studies on LED lighting have investigated spectral regulation of crop flavor and texture. In one study, compared with B+R light, B+R+white light and white fluorescent light improved sensory attributes, such as shape, color, sweetness, and crisp texture, of lettuce ‘Capitata’, suggesting influence of G light (Lin et al., 2013).

In Section II, substituting $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light with G light in a background of $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R light increased biomass accumulation and extension growth of lettuce ‘Rex’ and ‘Rouxai’ and kale (*Brassica oleracea* var. *sabellica* ‘Siberian’). However, because the B photon flux density decreased with incremental additions of G light, promotion of plant growth under enriched G light could also be attributed to reduced B light. To decouple the effects of G light from those of B light, R light was partially substituted with G light at various B photon flux densities in the present study. For red-leaf lettuce ‘Rouxai’, we postulated that (1) increasing the B photon flux density, with or without G light, would decrease leaf expansion and biomass, increase accumulation of anthocyanins, macronutrients, and micronutrients, and intensify the

bitter taste; (2) partial substitution of R light with G light would counter the effects of B light in a B photon flux density-dependent manner and elicit the shade-avoidance response, thereby increasing light interception and biomass; and (3) plant growth under sole-source lighting with low B light would be greater than that in a greenhouse environment.

Materials and methods

Plant material and propagation

Seeds of red oakleaf lettuce ‘Rouxai’ were obtained from a seed producer (Johnny’s Selected Seeds, Winslow, ME) and sown in a rockwool substrate sheet composed of 200 2.5-cm-wide square plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) on 11 Jan. and 19 Feb. 2018 for two replications. The substrate was held in a plastic tray and presoaked in deionized water with an adjusted pH of 4.3 using diluted (1:31) 95–98% sulfuric acid (J.Y. Baker, Inc., Phillipsburg, NJ). During the first 24 h, seed trays were covered with transparent humidity domes and placed in a growth room at 20 °C under continuous lighting from warm-white LEDs (2700 K, PHYTOFY RL; OSRAM, Beverley, MA) at a total photon flux density (TPFD; 400–800 nm) of $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. On day 1, the air temperature, photoperiod, and TPFD were changed to 22 °C, 20 h, and $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. The substrate was subirrigated with a nutrient solution (pH = 5.7–5.9, electrical conductivity = $1.2\text{--}1.4 \text{ mS}\cdot\text{cm}^{-1}$) to supply the following nutrients (in $\text{mg}\cdot\text{L}^{-1}$): 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 nutrient solution used from day 1 to 13 was made by supplementing deionized water with a water-soluble fertilizer (12N–4P₂O₅–16K₂O RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA). The pH was adjusted to the desired range by additions of potassium

bicarbonate and/or diluted sulfuric acid.

Lighting treatments

On day 4, after humidity domes were removed, 35 lettuce seedlings were transferred from warm-white light to each of nine light-quality treatments at the same TPFD of $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under a 20-h photoperiod. Plants were exposed to four B photon flux densities in a R background without G light (R₁₈₀, B₂₀R₁₆₀, B₆₀R₁₂₀, and B₁₀₀R₈₀) or with a substitution of G light for R light (G₆₀R₁₂₀, B₂₀G₆₀R₁₀₀, B₆₀G₆₀R₆₀, and B₁₀₀G₆₀R₂₀). The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Additional plants were kept under warm-white light. The peak wavelengths of B, G, R, and warm-white LEDs (PHYTOFY RL; OSRAM) were 449, 526, 664, and 639 nm, respectively. The LED hardware was paired with software (PHYTOFY Control Software; OSRAM) to create the lighting treatments and schedules. The color channels in three identical LED fixtures ($67.3 \times 29.8 \times 4.3$ cm each, placed 41 cm apart) per treatment were independently controlled to deliver the desired spectral treatments, which were measured with a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT) at plant canopy (46 cm below the LED fixtures) and were averaged from seven locations for each treatment (Figure III-1). Single-band photon flux densities for B, G, R, and FR light, integrated photon flux densities (e.g., TPFD and PPFD), and light ratios are shown in Table III-1. The yield photon flux density (YPFD; 300–800 nm) was the product of the spectral distribution and relative quantum yield from Sager et al. (1988). The phytochrome photoequilibrium describes the fraction of FR-absorbing phytochromes in the total phytochrome pool and was estimated based on the spectral distribution and phytochrome absorption coefficients (Sager et al., 1988). The color rendering index, which measures how well a light source reveals object colors compared to a natural light source, was calculated with the online LED ColorCalculator by OSRAM Sylvania (Wilmington,

MA). The photosynthetic daily light integral (DLI, 400–700 nm) was $13.0 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

Production culture and environment

On day 13, lettuce seedlings were transplanted into a deep-flow-technique hydroponic system on three-layer growing racks (Indoor Harvest, Houston, TX) in the same light and temperature environment as before. Plants were positioned on 36-cell floating rafts ($60.9 \times 121.9 \times 2.5 \text{ cm}$; Beaver Plastics, Ltd; Acheson, AB, Canada) in flood tables ($1.22 \times 0.61 \times 0.18 \text{ m}$; Active Aqua AAHR24W; Hydrofarm, Petaluma, CA). A nutrient solution was constantly recirculated by a water pump in a reservoir and oxygenated by an air stone ($20.3 \times 2.5 \text{ cm}$; Active Aqua AS8RD; Hydrofarm) connected to an air pump (Active Aqua AAPA70L; Hydrofarm). It was made of deionized water supplemented with a water-soluble fertilizer (12N–4P–16K RO Hydro FeED; JR Peters, Inc.) and potassium bicarbonate to supply the following nutrients (in $\text{mg} \cdot \text{L}^{-1}$): 150 N, 50 P, 200 K, 88 Ca, 58 Mg, 47 S, 2.1 Fe, 0.63 Mn, 0.68 Zn, 0.15 B, 0.56 Cu, and 0.15 Mo. The pH, electrical conductivity, and water temperature for each rack housing three lighting treatments were measured daily with a pH and electrical conductivity meter (HI9814; Hanna Instruments, Woonsocket, RI) (Table III-2). When pH dropped below 5.1, it was increased to 5.6–5.9 using potassium bicarbonate. The nutrient solution tank was replenished with deionized water before the water pump surfaced. No additional fertilizers were added throughout the experiment. The PPFD, air temperature, CO_2 concentration, and relative humidity were monitored with sensors and recorded as described in Chapter 2. The mean air temperature, CO_2 concentration, and relative humidity (mean $\pm$ standard deviation) in the growth room were $22.4 \pm 0.6 \text{ }^\circ\text{C}$, $410 \pm 50 \text{ ppm}$, and $34\% \pm 10\%$, respectively, in replication 1 and $22.5 \pm 0.6 \text{ }^\circ\text{C}$, $398 \pm 35 \text{ ppm}$, and $35\% \pm 7\%$, respectively, in replication 2.

Additional lettuce seedlings grown under warm-white light were transferred on day 13 to a

glass-glazed greenhouse at 22 °C with an environmental control system (Integro 725; Priva, De Lier, the Netherlands). During a 16-h photoperiod (set different from 20 h indoors to achieve comparable DLIs), supplemental lighting from high-pressure sodium lamps (PL2000; P.L. Light Systems Inc., Beamsville, ON, Canada) automatically switched on to provide an additional PPFD of 60–90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at plant height when the ambient PPFD was $<185 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and switched off when it was $>370 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Supplemental lighting was manually turned off on day 23 in replication 2 because of an overabundance of sunlight. Supplemental lighting contributed approximately 22% and 13% of the DLIs during two replications. Plants were transplanted into 10-cm plastic pots filled with a peat-perlite medium (Suremix; Michigan Grower Products, Inc., Galesburg, MI) and irrigated with reverse-osmosis water supplemented with the same fertilizer (12N–4P–16K RO Hydro FeED; JR Peters, Inc.) at the same nutrient concentrations as for plants in the growth room. The initial pH and electrical conductivity of the nutrient solution were 5.6 and $1.6 \text{ mS}\cdot\text{cm}^{-1}$, respectively. The pH was maintained at around 5.6 using potassium bicarbonate throughout the experiment. An infrared thermocouple (OS36-01-K-80F; Omega Engineering, Inc., Stamford, CT) and a line quantum sensor (Apogee Instruments, Inc., Logan, UT) were used to measure leaf temperature and the PPFD, respectively, at plant height. Hourly average data were calculated from instantaneous measurements every 10 s with a data logger (CR10; Campbell Scientific, Logan, UT). The mean leaf temperature and DLI (mean $\pm$ standard deviation) were $21.8 \pm 2.3 \text{ }^{\circ}\text{C}$ and $16.1 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively, in replication 1 and $22.7 \pm 2.2 \text{ }^{\circ}\text{C}$ and $19.8 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively, in replication 2.

Data collection and analysis

On day 33 in replication 1 and day 30 in replication 2, growth data were collected on eight plants per treatment in destructive measurements. Shoot fresh and dry weights [following ≥ 5 d in

a drying oven (Blue M, Blue Island, IL) at 60 °C] were measured with an analytical balance (GX-1000; A&D Store, Inc., Wood Dale, IL). Plant diameter, leaf length and width of the sixth true leaf, and leaf number (when >3 cm) were recorded. Relative specific chlorophyll content (the SPAD index) was measured with a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan). The average SPAD index for each sample was taken from three measurements at different locations of each plant. Foliage coloration was quantified with a portable colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc.) as the International Commission on Illumination $L^*a^*b^*$ color space coordinates. L^* indicates leaf brightness, ranging from 0 (the darkest black) to 100 (the brightest white). The positive directions of a^* and b^* indicate redness and yellowness, respectively, whereas their negative directions indicate greenness and blueness, respectively. The maximum quantum efficiency of photosystem II (F_v/F_m) was measured on dark-adapted leaves (for 30 min) with a multi-mode chlorophyll fluorometer (OS5p; Opti-Sciences, Inc., Hudson, NH).

Consumer sensory tests were performed at the Sensory Evaluation Laboratory in the Department of Food Science and Human Nutrition at Michigan State University following protocols as described in Szczygiel et al. (2017). On day 36 in replication 1 and day 32 in replication 2, organoleptic properties of lettuce leaves from each of six treatments (R_{180} , $B_{20}R_{160}$, $B_{20}G_{60}R_{100}$, $B_{100}R_{80}$, $B_{100}G_{60}R_{20}$, and greenhouse) were evaluated by 86 and 78 sensory panelists of over 18 years old, respectively, who consumed lettuce at least once a month. The panelists were recruited using the Michigan State University Paid Research Pool by Sona Systems. Each panelist was presented with six coded samples in a random order and was asked to rate overall acceptability, appearance, color, texture, overall flavor, and aftertaste on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. The levels of bitterness and sweetness were

measured on a 5-point Likert scale, where 1 = not at all bitter (or sweet) and 5 = extremely bitter (or sweet). How the samples met expectations of red-leaf lettuce was measured on a 5-point Likert scale, where 1 = much worse than expected and 5 = much better than expected. Willingness to buy was measured on a 5-point Likert scale, where 1 = definitely would not purchase and 5 = definitely would purchase. Subsequently, panelists were asked their age, gender, and consumption frequencies of lettuce, cruciferous vegetables, and coffee.

Elemental analysis was conducted at the U.S. Department of Agriculture Agricultural Research Service (Toledo, OH) on lettuce leaf tissues from the same six treatments as for sensory analysis. Dry tissues were ground using a mortar and a pestle. Foliar nitrogen content was measured with a CHN analyzer (vario MICRO cube; Elementar, Hanau, Germany) using approximately 2.5-g dry lettuce tissue in tin capsules (EA Consumables, Pennsauken, NJ). Other macronutrients and micronutrients were quantified with inductively coupled plasma optical emission spectrometry (iCAP 6300 Duo ICP-OES Analyzer; Thermo Fisher Scientific Inc., Waltham, MA) based on the modified Environmental Protection Agency method 3051 with an extra hydrogen peroxide step. Spinach leaves (NIST standard reference material 1570a) were included for every 20 samples. Peach leaves (NIST standard reference material 1547) were included for every 40 samples. 5-mL nitric acid was combined with approximately 0.25-g dry lettuce tissue in a Teflon vessel. Samples were placed in a microwave for digestion (MARS 6; CEM Corporation, Matthews, NC), in which temperature was increased to 200 °C in 15 min, maintained at 200 °C for 15 min, and then decreased to room temperature. After the addition of 1.5-mL hydrogen peroxide in each sample, samples were reheated to 200 °C, remained at that temperature for 5 min, and then cooled to room temperature. Samples were filtered (Whatman qualitative filter paper, Grade 2; Whatman plc, Maidstone, UK) after the addition of 12-mL

deionized water in each sample. A 1.3-mL aliquot of the solution was diluted (1:10) with deionized water for elemental analysis in the ICP-OES analyzer.

The experiment was performed twice in time and followed a randomized complete block design. All data were pooled from two replications because the treatment-by-replication interaction was not significant ($P > 0.05$), or the response trends were similar between replications. Data were analyzed in SAS (version 9.4; SAS Institute, Inc., Cary, NC) with the PROC MEANS, PROC MIXED, and PROC GLIMMIX procedures and Tukey's honestly significant difference test ($\alpha = 0.05$). Photographs of a representative plant from each treatment were taken to show visual differences (Figure III-2).

Results

Biomass

Irrespective of the presence of G light, there were linear negative relationships between the B photon flux density and biomass accumulation. At a PPFD of $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, increasing the B photon flux density from 0 to $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased shoot fresh and dry weights by 58% and 46%, respectively, in a R-light background and by 63% and 54%, respectively, with $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light in substitution of R light (Figure III-3). The effects of G light varied depending on the B photon flux density. G light did not influence shoot fresh weight when B light was absent, increased it by 18% at B₂₀, but decreased it by 29% and 19% at B₆₀ and B₁₀₀, respectively. G light did not influence shoot dry weight at B₀ or B₂₀ but decreased it by 26% and 20% at B₆₀ and B₁₀₀, respectively. Shoot fresh and dry weights were similar under WW₁₈₀ and G₆₀R₁₂₀. Plants grown in the greenhouse had shoot fresh and dry weights comparable to those grown under B₁₀₀R₈₀, although they received higher DLIs.

Morphology

Increasing the B photon flux density in the presence of G light decreased leaf length linearly by up to 17% (Figure III-3). Without G light, leaf length decreased by 13% from B₀ to B₂₀ but did not change beyond B₂₀. Substituting R light with G light did not affect leaf length at any B photon flux density delivered. Plants grown under WW₁₈₀ and in the greenhouse had similar leaf length to those grown under R₁₈₀ and G₆₀R₁₂₀. Increasing the B photon flux density from 0 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased leaf width by 34% and 29% with and without G light, respectively. Leaf width was similar with or without G light at B₀, B₂₀, and B₆₀, but was 9% lower with G light at B₁₀₀. Leaves were the widest under R₁₈₀, G₆₀R₁₂₀, and WW₁₈₀ and the narrowest under B₁₀₀G₆₀R₂₀ and in the greenhouse. Plant diameter decreased linearly with an increasing B photon flux density, by up to 21% with G light and 18% without G light. G light decreased plant diameter by 9% at B₆₀ but did not affect it at the other B photon flux densities. Without G light, plants had two or three more leaves at B₀ than at B₂₀ and B₁₀₀. In the presence of G light, plants developed three more leaves at B₀ and B₂₀ (and under WW₁₈₀) than at B₆₀ and B₁₀₀ (and in the greenhouse). Substituting G light for R light increased leaf number by three at B₂₀, but not at the other B photon flux densities.

SPAD and Fv/Fm

With or without G light, increasing the B photon flux density from 0 to 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased the SPAD index by 12–13% and saturated this response (Figure III-3). The inclusion of G light decreased the SPAD index by 9% at B₆₀, but not at the other B photon flux densities. Increasing the B photon flux density from 0 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ did not affect Fv/Fm in the absence of G light but increased it by 3% in the presence of G light. Fv/Fm was similar under WW₁₈₀ and at B₀ and B₂₀ and similar in the greenhouse and at B₁₀₀.

Foliage coloration

With or without G light, increasing the B photon flux density from 0 to 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased brightness (L^*) and yellowness (b^*) and increased redness (a^*) of foliage directly exposed to light (Figure III-2, III-4). Colors generally saturated with 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light, except in the presence of G light, foliage redness was saturated at 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light. Substituting G light for R light did not influence foliage coloration at any B photon flux density. Foliage coloration of plants grown under WW₁₈₀, which included B₁₂, was in between the B₀ and B₂₀ treatments. Plants grown in the greenhouse had similar foliage coloration to those grown at B₁₀₀R₈₀.

Sensory attributes

Regardless of differences in leaf color, plant appearance and color were rated similarly by panelists across all tested treatments (Figure III-5). Substituting G light for R light did not influence any sensory attribute at B₂₀ or B₁₀₀. Ratings on overall acceptability, flavor, aftertaste, meeting expectations, and willingness to buy were 9–13%, 15–18%, 15–17%, 13–19%, and 15–20% lower, respectively, for greenhouse-grown plants than for plants grown under the five sole-source lighting treatments. These sensory attributes were similar under the five sole-source lighting treatments. Leaf texture was rated 6% lower under B₁₀₀G₆₀R₂₀ than under R₁₈₀. Bitterness of greenhouse-grown plants was rated 37–50% higher than that under the five sole-source lighting treatments. In the absence of G light, increasing the B photon flux density from 0 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased the sweetness rating by 14%. Sweetness was rated similarly low for plants grown in the greenhouse and under B₁₀₀G₆₀R₂₀.

Essential nutrients

There were no treatment effects on phosphorus, calcium, iron, and boron concentrations

(Figure III-6). Substituting G light for R light at B₂₀ or B₁₀₀ did not affect any macronutrient or micronutrient concentrations. In the absence of G light, increasing the B photon flux density from 0 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased nitrogen, magnesium, sulfur, zinc, and copper concentrations by 15%, 10%, 19%, 19%, and 45%, respectively, but did not affect the other nutrient concentrations. With G light, increasing the B photon flux density from 20 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased nitrogen and sulfur concentrations by 10% and 29%, respectively, but did not influence the other nutrient concentrations. Plants grown in the greenhouse were 22–26%, 44–54%, 61–70% lower in potassium, manganese, and molybdenum concentrations, respectively, and 36–71% higher in magnesium concentration compared with those grown under five sole-source lighting treatments. The nitrogen concentration in greenhouse-grown plants was similar to that under R₁₀₀ and B₂₀R₁₆₀. The sulfur, zinc, and copper concentrations in greenhouse-grown plants were similar to those under B₁₀₀G₆₀R₂₀.

Discussion

In Section II, incremental substitutions of G light for B light in B₆₀R₁₂₀ increased biomass and leaf expansion of lettuce ‘Rex’, lettuce ‘Rouxai’, and kale ‘Siberian’. Similarly, substituting 14 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for B₄₂ or B₆₀ in a R-light background increased fresh and dry weights and leaf area of red-leaf lettuce ‘Sunmang’ (Son and Oh, 2015). However, since B light was not kept constant, increased plant growth could be attributed to diminishing B light rather than increasing G light. In the present study, G light in substitution of R light had variable effects at multiple fixed B photon flux densities. In the absence of B light, G light did not influence any parameters measured. Under low B light (B₂₀), G light increased shoot fresh weight and leaf number. Under moderate B light (B₆₀), G light decreased shoot fresh and dry weights, plant

diameter, and the SPAD index. Under high B light (B_{100}), G light decreased shoot fresh and dry weights and leaf width. This interaction between B and G light is a novel discovery that adds complexity to spectral responses in plants although the changing R photon flux density could also be part of the interaction. A similar study confirmed that increasing the B photon flux density from 0 to $45 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased lettuce growth and leaf expansion, but there were no effects of $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light at the B photon flux densities tested (Kang et al., 2016). In comparison, the dependence of G light effects on the B photon flux density was found in our study using a wider range of B photon flux densities (between 0 to $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and a higher G photon flux density ($60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

The minimal effects of G light under low B light were consistent with some previous studies. For example, at a PPFD of $173 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, substituting $17 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R light with G light did not influence biomass and leaf area of lettuce ‘Grand Rapids TBR’ at B_{22} and B_{42} , although it increased those of lettuce ‘Sunmang’ at B_{42} , but not at B_{22} (Son and Oh, 2015). The low G photon flux density (G_{17}) was regarded as less effective than a higher one (G_{36}) at promoting growth rates in a quantitative manner; however, the high G photon flux density (G_{60}) in our study marginally influenced growth under low B light. In addition, G light in $B_{20}G_{28}R_{52}$ was interpreted as neither promotive nor inhibitory for growth and morphology of cucumber ‘Cumlaude’ because the data fit dose-response relationships with the B photon flux density, although $B_{20}R_{80}$ was not provided as a direct comparison (Hernández and Kubota, 2016). Similarly, under low B light ($21\text{--}28 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), increasing the G photon flux density from 3 to $82 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ did not affect dry mass or net assimilation of lettuce ‘Waldmann’s Green’ (Snowden et al., 2016). While these findings suggested G light neither promoted nor suppressed plant growth, other studies indicated positive or negative roles

of G light under low B light. For example, substituting $36 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R light in B₂₄R₁₂₆ (from LEDs) with G light (from filtered fluorescent lamps) increased leaf area, shoot fresh weight, and shoot dry weight of lettuce ‘Waldmann’s Green’ (Kim et al., 2004); however, these results could be confounded by increases in diffuse light or leaf temperature due to the use of green fluorescent lamps (Snowden et al., 2016). On the contrary, substituting G light for half the R light in R₁₆₀ decreased leaf area and shoot fresh and dry weights of tomato (*Solanum lycopersicum* ‘Early Girl’), salvia (*Salvia splendens* ‘Vista Red’), and petunia (*Petunia* $\times$ hybrida ‘Wave Pink’) seedlings, but not impatiens (*Impatiens walleriana* ‘SuperElfin XP Red’) seedlings (Wollaeger and Runkle, 2014). This indicates that different plant species can vary in their responses to G light.

In the present study, at the same PPFD, substituting G light for R light decreased biomass accumulation under moderate to high B light. This could at least partly be explained by photosynthetic differences. Substituting $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light at a PPFD of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased the photosynthetic rate of lettuce ‘Green Skirt’ at B₁₅, B₃₀, and B₄₅ (but increased it at B₀), although it did not affect leaf shape or plant growth (Kang et al., 2016). However, the photosynthetic rate of lettuce ‘Waldmann’s Green’ was similar with and without G light at B₂₄ (Kim et al., 2004). In our study, substituting $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light decreased the YPF_D at B₀, B₂₀, B₆₀, and B₁₀₀ by 3%, 4%, 8%, and 4%, respectively. Therefore, less biomass with G light at B₆₀ can be attributed to a lower YPF_D, as well as reduced plant diameter and chlorophyll content, which reduced both photosynthesis and light interception. Growth inhibition under G light at B₁₀₀ was mainly associated with reduced leaf width and thus light interception rather than the small decrease in the YPF_D, which did not affect shoot dry weight or leaf expansion at B₀ and B₂₀. These results substantiate a previous notion that G light,

when added to B+R light, can negatively influence plant growth (Went, 1957; Folta and Maruhnich, 2007). In contrast, butterhead lettuce grown under B₄₀R₁₆₀ and B₆₇G₆₇R₆₇ with a 12-h photoperiod had similar shoot fresh weight, leaf area, and leaf number (Bian et al., 2018). These plants were grown under white fluorescent lamps until day 14 and received lighting treatments at a DLI of 8.6 mol·m⁻²·d⁻¹ from day 14 to 34. However, in our study, lighting treatments were applied to plants earlier, longer (from day 4 to day 30 or 33), and at a 50% higher DLI, which could cause different responses.

The relative growth rate of a plant is a function of its leaf area ratio and the net assimilation rate (Lambers et al., 2008). The leaf area ratio determines the amount of light captured to drive photosynthesis. In this study, reduced plant size was associated with reduced biomass accumulation. Increasing the B photon flux density linearly decreased shoot fresh and dry weights as well as leaf width and plant diameter of lettuce ‘Rouxai’, showing suppression of B light on yield and extension growth. Similarly, incremental substitutions of B light for R light (from 0 to 100 μmol·m⁻²·s⁻¹ at a PPFD of 171 μmol·m⁻²·s⁻¹) decreased shoot fresh and dry weights of red-leaf lettuce ‘Sunmang’ by up to 71% and 61%, respectively, and decreased leaf area by up to 72% (Son and Oh, 2013). In our study, B light decreased leaf width more than leaf length, indicating that inhibitory effects of B light on leaf expansion were unequal on transverse directions. Plants use photoreceptors, such as cryptochromes, to gauge the incident B photon flux density (Casal, 2000; Lin, 2000). Extension growth in response to the B photon flux density are mediated by dynamic, direct interactions between cryptochromes and phytochrome-interacting factors (PIFs), which are basic helix-loop-helix transcription factors (Pedmale et al., 2016). At a low B photon flux density, cryptochromes 1 and 2 interact with PIFs 4 and 5 to promote expression of growth-related genes, whereas at a high B photon flux density, suppression of PIFs

4 and 5 by cryptochromes and proteasomal degradation of cryptochrome 2 and PIF5 together inhibit extension growth (Pedmale et al., 2016). Reduced B photon flux densities can elicit the shade-avoidance response, such as stem and hypocotyl elongation and hyponasty, involving regulation of DELLA proteins through gibberellin, control of auxin, and changes in cell wall extensibility through expansins and xyloglucan endotransglucosylase/hydrolases (Pierik et al., 2004; Djakovic-Petrovic et al., 2007; Sasidharan et al., 2008; Pierik et al., 2009).

In most cases, B light inhibits stem elongation and leaf expansion; however, the effects of B light on extension growth in some studies were inconsistent with this paradigm, possibly because of confounding wavebands, interacting factors, or species- or cultivar-specific sensitivity. For example, using high-pressure sodium and metal halide lamps, increasing the B light fraction from 6% to 26% decreased cell expansion and thus leaf expansion in soybean (*Glycine max* ‘Hoyt’); however, increasing the B light fraction from 0% to 6% increased cell expansion and division in lettuce ‘Grand Rapids’ (Dougher and Bugbee, 2004). Filter conversion of B light to yellow light (580–600 nm) to achieve 0% B light might be a confounding factor because yellow light appeared to suppress lettuce growth (Dougher and Bugbee, 2001). In a subsequent study, changing the B light fraction between 11% and 28% did not influence the leaf area index or dry mass of lettuce ‘Waldmann’s Green’ (Snowden et al., 2016), although other wavebands (e.g., G and R light) could have confounded the outcomes because B light was emitted from broad-spectrum LEDs. Cucumber ‘Cumlaude’ grown under 100% B LEDs at $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were taller than those grown under R or R+B LEDs, and had a greater leaf area than those grown under B₇₅R₂₅ (Hernández and Kubota, 2016). The lack of growth inhibition under 100% B light was partly attributed to a low phytochrome photoequilibrium of 0.5 (Hernández and Kubota, 2016); however, 100% B light suppressed leaf expansion and decreased shoot dry weight of

lettuce and salvia ‘Vista Red’ (Wollaeger and Runkle, 2015; Wang et al., 2016). Therefore, atypical sensitivity of specific species and cultivars to B light alone is possible.

Although G light can elicit the shade-avoidance response (Zhang et al., 2011; Zhang and Folta, 2012; Wang and Folta, 2013), there was no evidence that it did so in this study. First, growth and morphological responses to G light can change dynamically depending on plant age, which may contribute to some discrepancies in earlier studies on plants of different developmental stages. For example, partial substitution of white light (B₃₇G₈₆R₅₈) with G light (B₃₁G₁₀₄R₄₅) increased fresh and dry weights and shoot diameter of lettuce ‘Outredgeous’ 14 and 21 d after sowing, but did not affect fresh and dry weights or leaf area on day 28 (Mickens et al., 2018). Therefore, plants harvested on day 30 and 33 in our study may be less responsive to G light in the maturation phase than in the lag phase. Second, low B light is a strong shade signal that may saturate the shade-avoidance response (Pierik et al., 2004; Keuskamp et al., 2011), rendering additional G light futile in morphological control. Third, predominant suppression of extension growth by high B light may override weaker control of extension growth by G light. For example, leaf area and shoot fresh and dry weights of impatiens ‘SuperElfin XP Red’, salvia ‘Vista Red’, and petunia ‘Wave Pink’ seedlings were similar under B₁₆₀ and B₈₀G₈₀ (Wollaeger and Runkle, 2014).

Taken together with antagonism between B and G light with fixed R light from Chapter 2, the effects of G light depend on the specific spectral context. Without the shade-avoidance response as a confounding factor under moderate or high B light, G light was evidently less effective than R light at promoting lettuce growth. This is supported by observations that G light is less effective than R light at driving photosynthesis (McCree, 1972; Hogewoning et al., 2012; Kang et al., 2016). Thus, the comparable effectiveness of G and R light with little or no B light

can be attributed to a strong shade-avoidance signal, which G light either sustained or at least did not negate. Morphological adaptation to this signal overrode different photosynthetic efficacies between G and R light, resulting in similar whole-plant photosynthesis. Isolating the role of G light in the low B light-induced shade-avoidance response warrants further investigation.

Increasing the DLI typically increases shoot dry weight of lettuce (Both et al., 1997; Kitaya et al., 1998). However, shoot dry weight was similarly low for plants grown under B₁₀₀R₈₀ and in the greenhouse although the average DLI in the greenhouse was 24–53% higher than in the growth room. Confounding factors in the greenhouse environment may include air movement, light quality, light intensity (the DLI and fluctuating light throughout the day), photoperiod, photoinhibition under high light, vapor pressure deficit, and the growing method (hydroponics vs. soilless substrate). These uncontrolled variables should be considered when comparing results between the greenhouse and the growth room.

Anthocyanin accumulation was low in foliage without direct exposure to light due to shading from other leaves. The inclusion of B light at 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was generally sufficient to saturate top foliage coloration and thus anthocyanin accumulation of lettuce ‘Rouxai’, whereas G light did not influence foliage coloration at a fixed B photon flux density. Similarly, anthocyanin concentration of red-leaf lettuce ‘Red Cross’ was increased by substituting 130 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light, but not G light, for white light at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Li and Kubota, 2009). Compared with R light, B light or B+R light at 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased anthocyanin concentration of red-leaf lettuce ‘Banchu Red Fire’ (Johkan et al., 2010). In addition, 2-h predawn applications of B light at 45 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased anthocyanin concentration in greenhouse-grown red-leaf lettuce ‘Lollo Rossa’ (Ouzounis et al., 2015). In arabidopsis (*Arabidopsis thaliana*), cryptochrome 1 mediates anthocyanin accumulation under B light by upregulating flavonoid

biosynthetic enzymes such as chalcone synthase (Jenkins et al., 1995; Christie and Briggs, 2001; Bouly et al., 2007). Cryptochrome 1 is responsible for B light-induced anthocyanin accumulation in rapeseed (*Brassica napus*) besides arabidopsis (Chatterjee et al., 2016). Cryptochrome 2 can also regulate anthocyanin production under low B light, but not high B light, in which cryptochrome 2 undergoes rapid degradation (Christie and Briggs, 2001; Wang et al., 2001; Pedmale et al., 2016).

High light is an environmental stress that can elicit anthocyanin accumulation for protection against photodamage (Page et al., 2012). Acclimation to high light is accompanied by increases in flavonoid biosynthesis transcripts in arabidopsis (Page et al., 2012). Therefore, high anthocyanin concentration in greenhouse-grown lettuce could be attributed to the high DLI. However, phytonutrients such as anthocyanins, phenolic secondary metabolites, and glucosinolates, which can accumulate under B light or high light, impart bitter and astringent tastes to fruits and vegetables while increasing potential health benefits (Tomás-Barberán and Espín, 2001; Kopsell et al., 2015). Compared with lettuce grown under sole-source lighting, the lower ratings of greenhouse-grown lettuce on acceptability, flavor, aftertaste, and willingness to buy were associated with the higher ratings of bitterness. Consumers are generally averse to bitter plant foods regardless of their health-promoting properties, which presents a dilemma for food producers (Drewnowski and Gomez-Carneros, 2000). However, bitter-tasting lettuce may be tolerable if mixed with other types of salad greens and dressings. In our study, lettuce grown under sole-source lighting had similar foliage coloration but lower bitterness than the greenhouse counterpart, indicating similar anthocyanin accumulation but higher concentrations of other bitter compounds in the greenhouse. Therefore, sole-source lighting can enable desirable coloration without negatively affecting sensory factors and consumer preferences. Although high

B light slightly decreased sweetness perception and texture liking, it did not influence the other sensory attributes.

While substituting G light for R light did not affect macronutrient and micronutrient concentrations in lettuce ‘Rouxai’, increasing B light increased concentrations of nitrogen, magnesium, sulfur, zinc, and copper. Similarly, substituting 10% G light for R light did not affect macronutrient and micronutrient concentrations of broccoli (*Brassica oleracea* var. *italica*) microgreens at 5% or 20% B light, whereas increasing the B light fraction from 5% to 20% at a PPFD of $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased concentrations of calcium, potassium, and sulfur (Kopsell et al., 2014). In addition, concentrations of macronutrients (phosphorus, potassium, magnesium, calcium, and sulfur) and micronutrients (boron, copper, iron, manganese, molybdenum, sodium, and zinc) in broccoli microgreens were higher under B₄₁ than under B₄₂R₃₀₈ applied for 5 d before harvest (Kopsell and Sams, 2013). This suggests that accumulation of essential macronutrients and micronutrients is primarily mediated by relative B light (its fraction of the PPFD) rather than absolute B light (its photon flux density). Promotion of nutrient uptake by B light is associated with increased stomatal opening, membrane permeability, proton extrusion, and ion transporters (Spalding, 2000; Babourina et al., 2002; Kopsell et al., 2014). Greenhouse-grown lettuce had lower concentrations of potassium, manganese, and molybdenum and higher concentrations of magnesium and copper than lettuce grown under sole-source lighting. These differences could be attributed to different DLIs. For example, increasing the PPFD from 105 to $315 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ decreased concentrations of some macronutrients and micronutrients in some *Brassica* species and ornamental crops, but not others (Gerovac et al., 2016; Craver et al., 2018). Reduced nutrient concentrations under high light could be attributed to dilution of nutrients at high shoot dry weight (Craver et al., 2018). However, greenhouse-grown lettuce in our study had

both low concentrations of certain nutrients and low shoot dry weight, thereby low total content of those nutrients, which was possibly influenced by other environmental variables.

In conclusion, lettuce growth, morphology, and coloration were primarily influenced by B light with or without G light. Increasing B light increased concentrations of several macronutrients and micronutrients. Interestingly, the role of G light in growth regulation depended on the B photon flux density. While substituting G light for R light generally did not influence lettuce growth under little or low B light, it decreased the yield under moderate or high B light. Sensory attributes and consumer preferences were generally unaffected by the quality of sole-source lighting but unfavorable for greenhouse-grown plants. Temporally changing the spectrum can potentially optimize both yields and coloration. For example, a spectrum with a low ratio of B to R light maximizes yields during production, whereas a subsequent spectrum with a high ratio of B to R light enhances red foliage coloration one week before harvest.

Acknowledgements

We thank David Hamby, Rodrigo Pereyra, Charles Brunault, Alan Sarkisian, and Dorian Spero from OSRAM Innovation for lighting support; Nathan Kelly for experimental assistance; Steve Brooks for technical assistance; Sungeun Cho, Edward Szczygiel, and Shelby Cieslinski from Michigan State University Department of Food Science and Human Nutrition for help with consumer preference tests; Jennifer Boldt, Douglas Sturtz, and Chris Ranger from USDA-ARS for help with elemental analysis; Randy Beaudry, Dan Brainard, Roberto Lopez, and Emily Merewitz for instruments; and material donations from Grodan and JR Peters, Inc. This work was supported by Michigan State University AgBioResearch (including Project GREEN GR17-072) and the USDA National Institute of Food and Agriculture, Hatch project 192266.

APPENDIX

Table III-1. Spectral characteristics of nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes (LEDs). The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Photon flux densities over 1-nm increments were integrated as the photosynthetic photon flux density (PPFD; 400–700 nm) and the total photon flux density (TPFD; 400–800 nm), which includes far-red (FR; 700–800 nm) light. The yield photon flux density (YPFD; 300–800 nm) was the product of the spectral distribution and relative quantum efficiency (Sager et al., 1988). The phytochrome photoequilibrium (PPE) was estimated according to Sager et al. (1988). The color-rendering index (CRI) was calculated based on the spectral distribution using the online LED ColorCalculator by OSRAM Sylvania.

	LED lighting treatment								
	R ₁₈₀	G ₆₀ R ₁₂₀	B ₂₀ R ₁₆₀	B ₂₀ G ₆₀ R ₁₀₀	B ₆₀ R ₁₂₀	B ₆₀ G ₆₀ R ₆₀	B ₁₀₀ R ₈₀	B ₁₀₀ G ₆₀ R ₂₀	WW ₁₈₀
	Single-band photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)								
B	0.1	3.4	19.6	24.3	58.6	62.1	99.6	103.1	12.1
G	0.5	60.7	0.8	58.9	1.0	58.8	0.7	60.1	51.9
R	180.1	120.8	158.9	99.1	121.9	57.5	83.1	23.2	98.4
FR	1.6	1.5	1.8	1.2	1.3	0.7	0.8	0.4	18.6
	Integrated photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)								
PPFD	180.7	185.0	179.3	182.3	181.6	178.4	183.4	186.3	162.4
TPFD	182.3	186.5	181.1	183.5	182.9	179.1	184.2	186.7	181.0
YPFD	167.7	162.8	163.1	156.5	158.2	146.1	151.8	145.4	148.9
	Light ratio								
B:R	0.0	0.0	0.1	0.2	0.5	1.1	1.2	4.5	0.1
B:G	0.2	0.1	25.6	0.4	58.4	1.1	137.9	1.7	0.2
G:R	0.0	0.5	0.0	0.6	0.0	1.0	0.0	2.6	0.5
R:FR	111.2	80.6	86.8	85.5	91.9	84.6	103.7	57.5	5.3
PPE	0.883	0.882	0.880	0.876	0.869	0.855	0.855	0.796	0.828
	Visual quality								
CRI	42	38	-58	58	-250	61	-222	51	97

Table III-2. The pH, electrical conductivity, and water temperature [mean $\pm$ standard deviation in each replication (rep.)] of nutrient solutions for nine lighting treatments comprised of mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Lighting treatment	pH		Electrical conductivity ($\text{mS}\cdot\text{cm}^{-1}$)		Water temperature ($^{\circ}\text{C}$)	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
R ₁₈₀	5.9 $\pm$ 0.7	5.8 $\pm$ 0.7	1.80 $\pm$ 0.16	1.77 $\pm$ 0.12	23.1 $\pm$ 0.3	23.5 $\pm$ 0.2
G ₆₀ R ₁₂₀	5.9 $\pm$ 0.7	5.8 $\pm$ 0.7	1.80 $\pm$ 0.16	1.77 $\pm$ 0.12	23.1 $\pm$ 0.3	23.5 $\pm$ 0.2
B ₂₀ R ₁₆₀	5.9 $\pm$ 0.7	5.8 $\pm$ 0.7	1.80 $\pm$ 0.16	1.77 $\pm$ 0.12	23.1 $\pm$ 0.3	23.5 $\pm$ 0.2
B ₂₀ G ₆₀ R ₁₀₀	5.9 $\pm$ 0.6	5.7 $\pm$ 0.5	1.76 $\pm$ 0.12	1.77 $\pm$ 0.10	23.6 $\pm$ 0.5	23.9 $\pm$ 0.2
B ₆₀ R ₁₂₀	5.9 $\pm$ 0.6	5.7 $\pm$ 0.5	1.76 $\pm$ 0.12	1.77 $\pm$ 0.10	23.6 $\pm$ 0.5	23.9 $\pm$ 0.2
B ₆₀ G ₆₀ R ₆₀	5.9 $\pm$ 0.6	5.7 $\pm$ 0.5	1.76 $\pm$ 0.12	1.77 $\pm$ 0.10	23.6 $\pm$ 0.5	23.9 $\pm$ 0.2
B ₁₀₀ R ₈₀	5.9 $\pm$ 0.5	5.5 $\pm$ 0.5	1.80 $\pm$ 0.11	1.79 $\pm$ 0.08	24.0 $\pm$ 0.7	23.8 $\pm$ 0.1
B ₁₀₀ G ₆₀ R ₂₀	5.9 $\pm$ 0.5	5.5 $\pm$ 0.5	1.80 $\pm$ 0.11	1.79 $\pm$ 0.08	24.0 $\pm$ 0.7	23.8 $\pm$ 0.1
WW ₁₈₀	5.9 $\pm$ 0.6	5.5 $\pm$ 0.6	1.79 $\pm$ 0.09	1.83 $\pm$ 0.08	23.4 $\pm$ 0.5	23.1 $\pm$ 0.2

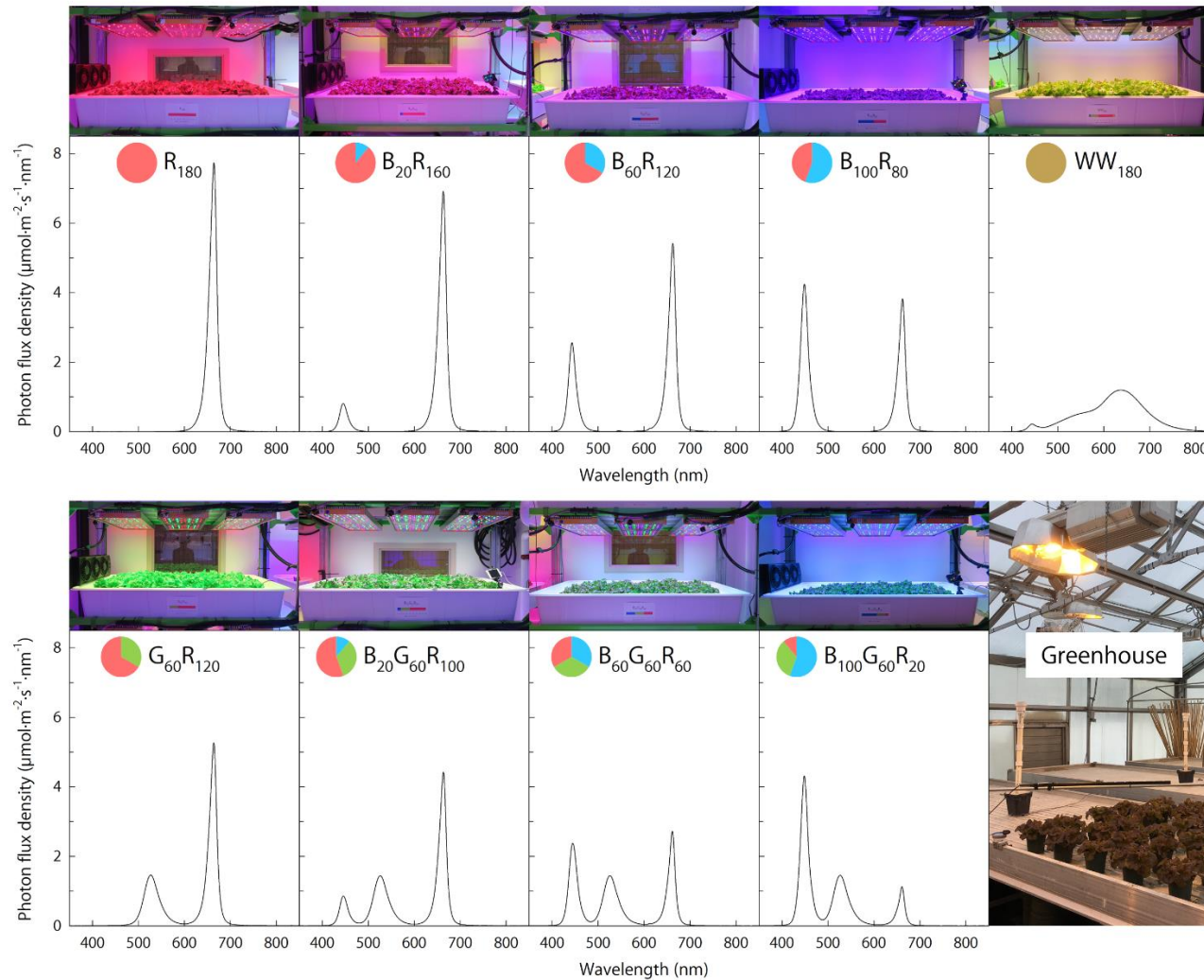


Figure III-1. Spectral distributions of nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes. The greenhouse treatment received sunlight with supplemental high-pressure sodium lighting. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

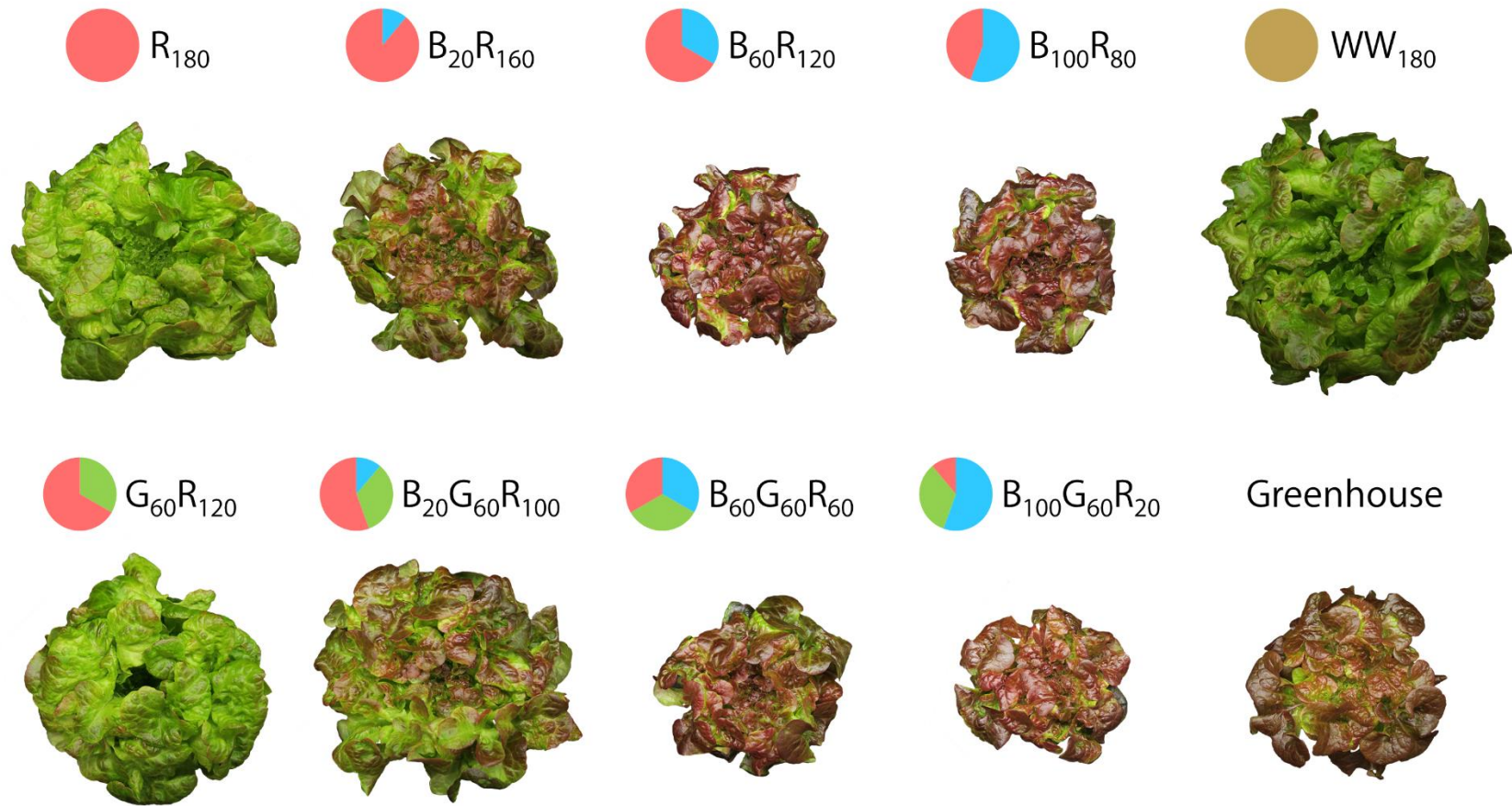


Figure III-2. Lettuce 'Rouxai' 32 d after sowing from the first replication. Plants were grown under nine sole-source lighting treatments delivered by mixtures of blue (B; 400–500 nm), green (G; 500–600 nm), and red (R; 600–700 nm) or warm-white (WW) light-emitting diodes or a greenhouse treatment that received sunlight supplemented with high-pressure sodium lighting. The number following each waveband is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

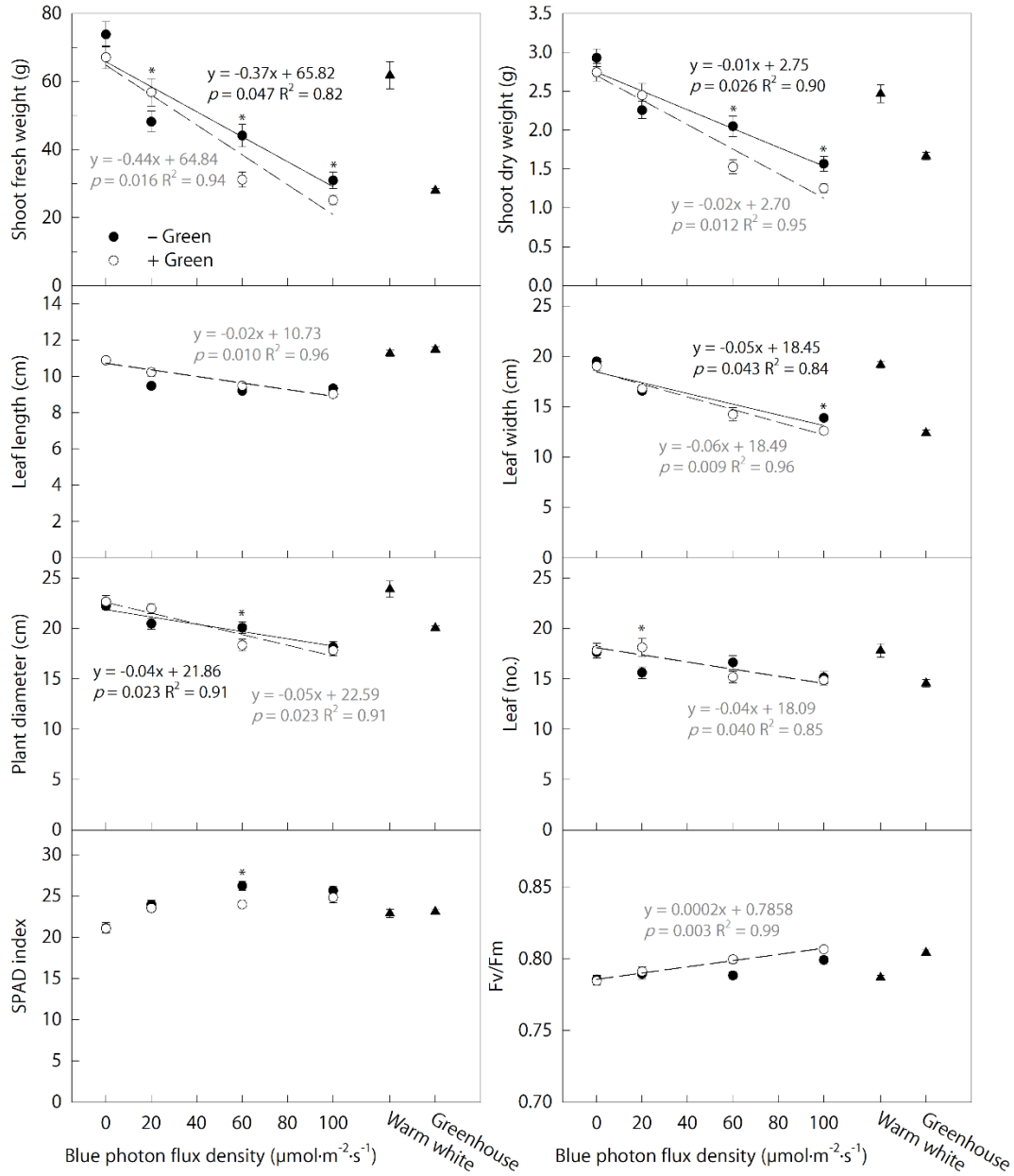


Figure III-3. Shoot fresh and dry weights, leaf length and width, plant diameter, leaf number, the SPAD index, and maximum quantum efficiency of photosystem II (Fv/Fm) of lettuce 'Rouxai' grown under nine sole-source lighting treatments, with or without green light, or in a greenhouse. Equations, p-values, coefficients of determination (R^2), and percentage changes are given for linear responses to the blue photon flux density ($\alpha = 0.05$) with green light (solid lines and black text) and without (dashed lines and gray text) green light. At any blue photon flux density, an asterisk indicates that means with and without green light are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

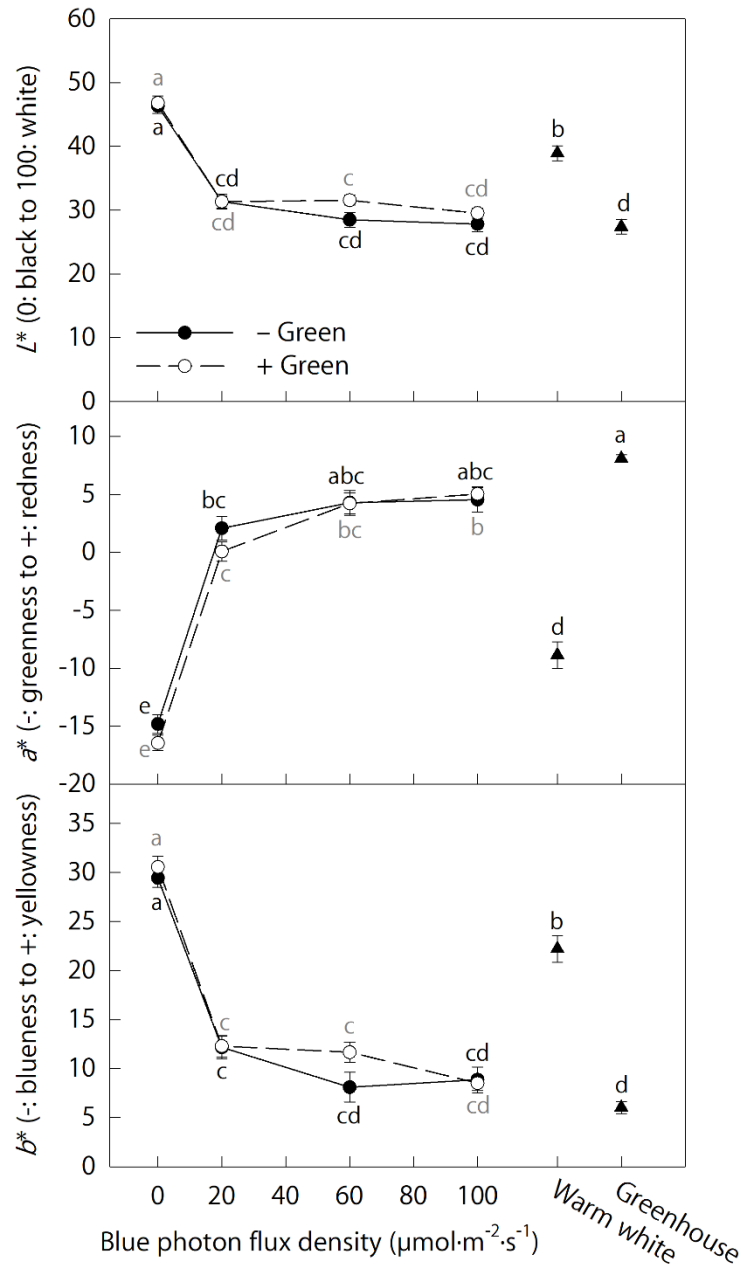


Figure III-4. Lab color space analysis of lettuce 'Rouxai' grown under nine sole-source lighting treatments, with or without green light, or in a greenhouse. Means followed by different letters within each parameter are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Black and gray letters are associated with filled circles (without green light) and empty circles (with green light), respectively. Error bars show standard errors.

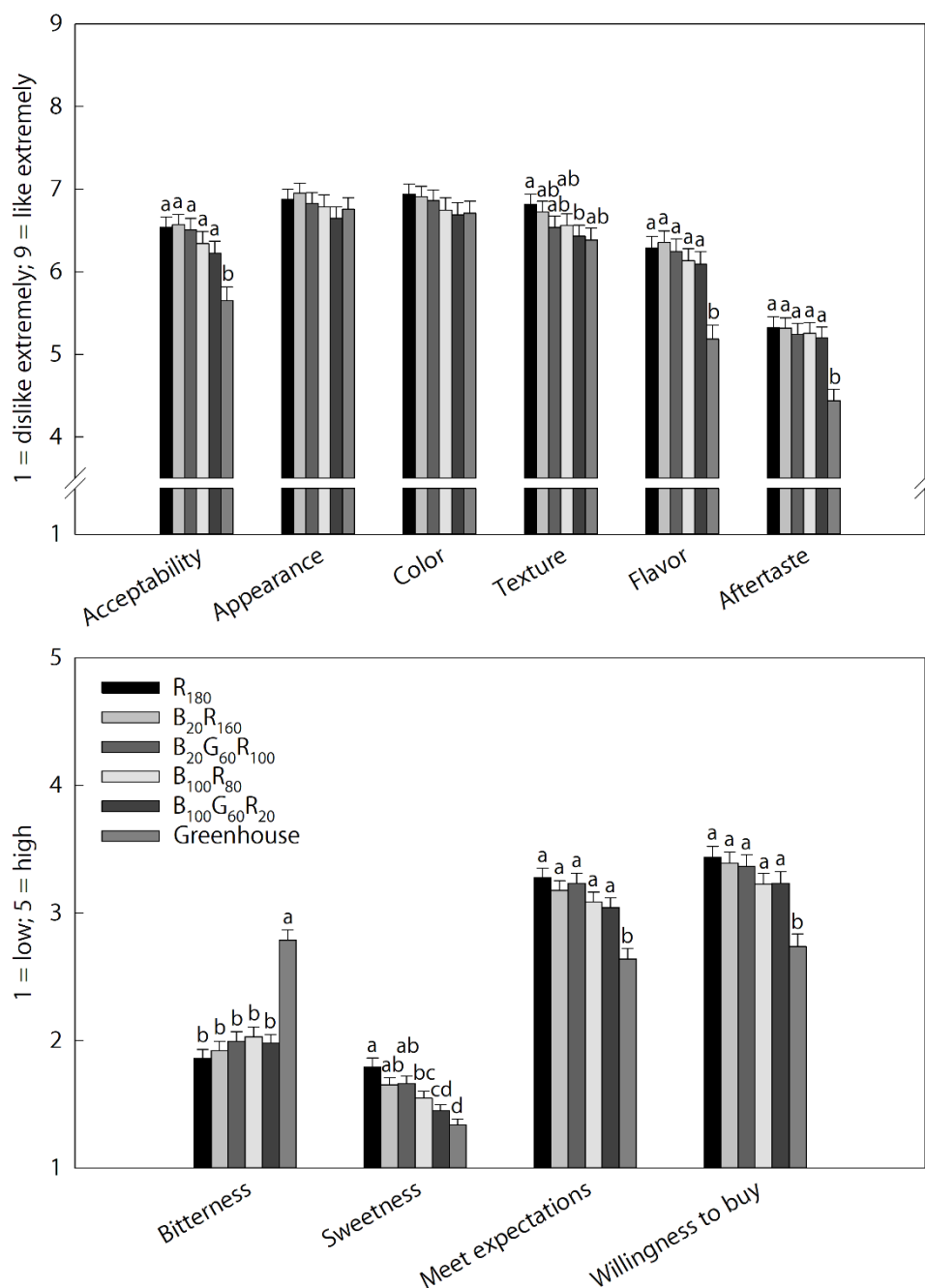


Figure III-5. Sensory ratings on lettuce ‘Rouxai’ by 164 panelists (86 and 78 in two replications). Plants were grown under five sole-source lighting treatments or in a greenhouse. The number for each waveband [blue (B; 400–500 nm), green (G; 500–600 nm), or red (R; 600–700 nm)] is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each category are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

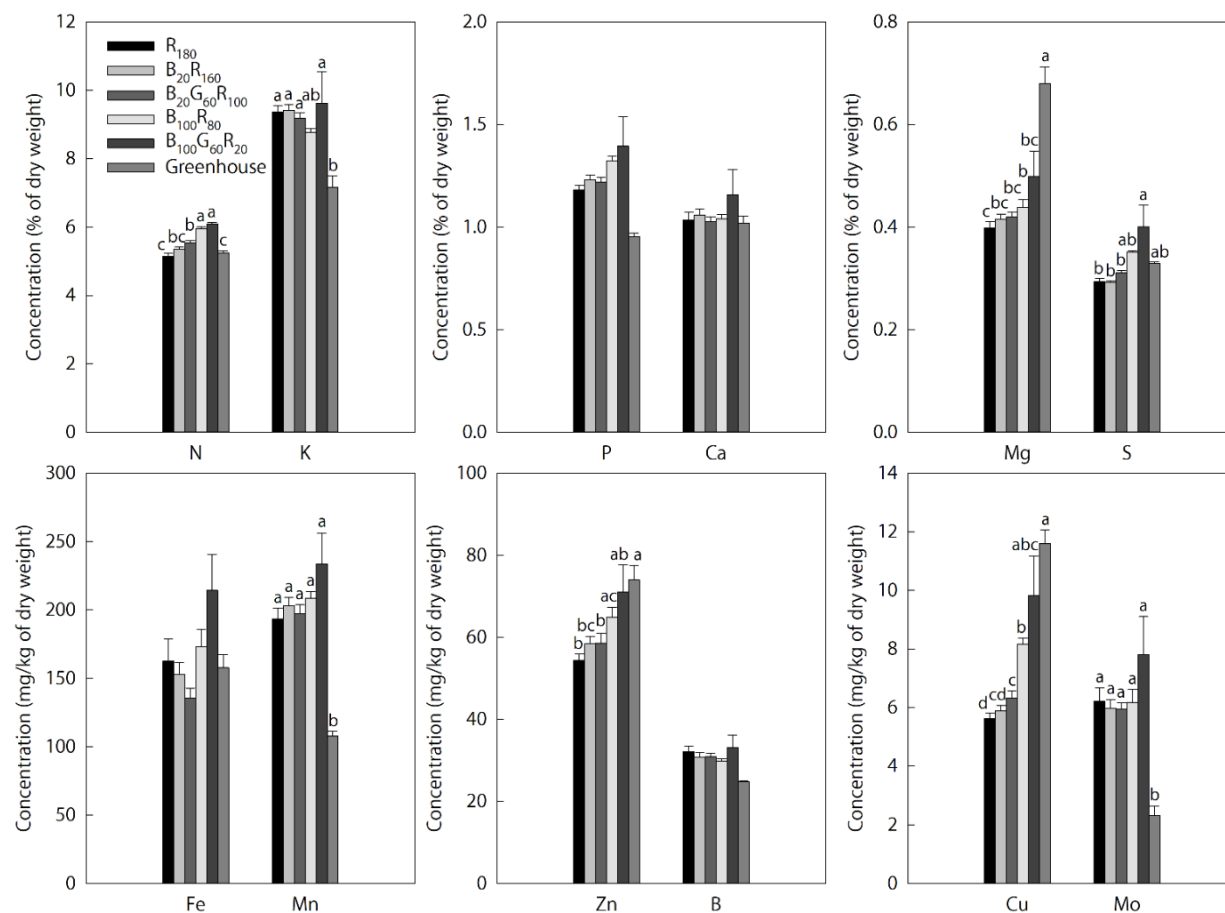


Figure III-6. Concentrations of macronutrients [nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), and sulfur (S)] and micronutrients [iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo)] in leaf tissues of lettuce 'Rouxai'. Plants were grown under five sole-source lighting treatments or in a greenhouse. The number for each waveband [blue (B; 400–500 nm), green (G; 500–600 nm), or red (R; 600–700 nm)] is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each element are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

LITERATURE CITED

LITERATURE CITED

- Babourina, O., Newman, I. and Shabala, S., 2002. Blue light-induced kinetics of H^+ and Ca^{2+} fluxes in etiolated wild-type and phototropin-mutant *Arabidopsis* seedlings. *Proc. Natl. Acad. Sci.* 99:2433–2438.
- Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R. and Batschauer, A., 2007. The signaling state of *Arabidopsis* cryptochrome 2 contains flavin semiquinone. *J. Biol. Chem.* 282:14916–14922.
- Bian, Z., Yang, Q., Li, T., Cheng, R., Barnett, Y. and Lu, C., 2018. Study of the beneficial effects of green light on lettuce grown under short - term continuous red and blue light-emitting diodes. *Physiol. Plant.* 164:226–240.
- Both, A.J., Albright, L.D., Langhans, R.W., Reiser, R.A. and Vinzant, B.G., 1994. Hydroponic lettuce production influenced by integrated supplemental light levels in a controlled environment agriculture facility: experimental results. *Acta Hortic.* 418:45–51.
- Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R. and Ahmad, M., 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J. Biol. Chem.* 282:9383–9391.
- Brodersen, C.R. and Vogelmann, T.C., 2010. Do changes in light direction affect absorption profiles in leaves? *Funct. Plant Biol.* 403–412.
- Casal, J.J., 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochem. Photobiol.* 71:1–11.
- Chatterjee, M., Sharma, P. and Khurana, J.P., 2006. Cryptochrome 1 from *Brassica napus* is up-regulated by blue light and controls hypocotyl/stem growth and anthocyanin accumulation. *Plant Physiol.* 141:61–74.
- Christie, J.M. and Briggs, W.R., 2001. Blue light sensing in higher plants. *J. Biol. Chem.* 276:11457–11460.
- Craver, J.K., Boldt, J.K. and Lopez, R.G., 2018. Radiation intensity and quality from sole-source light-emitting diodes affect seedling quality and subsequent flowering of long-day bedding plant species. *HortScience* 53:1407–1415.
- Djakovic-Petrovic, T., de Wit, M., Voesenek, L.A. and Pierik, R., 2007. DELLA protein function in growth responses to canopy signals. *Plant J.* 51:117–126.
- Dougher, T.A. and Bugbee, B., 2001. Evidence for yellow light suppression of lettuce growth. *Photochem. Photobiol.* 73:208–212.

- Dougher, T.A. and Bugbee, B., 2004. Long-term blue light effects on the histology of lettuce and soybean leaves and stems. *J. Am. Soc. Hortic. Sci.* 129:467–472.
- Drewnowski, A. and Gomez-Carneros, C., 2000. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.* 72:1424–1435.
- Folta, K.M. and Maruhnich, S.A., 2007. Green light: a signal to slow down or stop. *J. Exp. Bot.* 58:3099–3111.
- Franklin, K.A., 2008. Shade avoidance. *New Phytol.* 179:930–944.
- Gerovac, J.R., Craver, J.K., Boldt, J.K. and Lopez, R.G., 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of *Brassica* microgreens. *HortScience* 51:497–503.
- Jenkins, G.I., Christie, J.M., Fuglevand, G., Long, J.C. and Jackson, J.A., 1995. Plant responses to UV and blue light: biochemical and genetic approaches. *Plant Sci.* 112:117–138.
- Johkan, M., Shoji, K., Goto, F., Hashida, S.N. and 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.
- Heo, J.W., Kang, D.H., Bang, H.S., Hong, S.G., Chun, C.H. and Kang, K.K., 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. *Korean J. Hortic. Sci. Technol.* 30:6–12.
- Hernández, R. and Kubota, C., 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot.* 121:66–74.
- Hogewoning, S.W., Wientjes, E., Douwstra, P., Trouwborst, G., Van Ieperen, W., Croce, R. and Harbinson, J., 2012. Photosynthetic quantum yield dynamics: from photosystems to leaves. *Plant Cell* 24:1921–1935.
- Kang, W.H., Park, J.S., Park, K.S. and Son, J.E., 2016. Leaf photosynthetic rate, growth, and morphology of lettuce under different fractions of red, blue, and green light from light-emitting diodes (LEDs). *Hortic. Environ. Biotechnol.* 57:573–579.
- Keuskamp, D.H., Sasidharan, R., Vos, I., Peeters, A.J., Voesenek, L.A. and Pierik, R., 2011. Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in *Arabidopsis* seedlings. *Plant J.* 67:208–217.
- Kim, H.H., Goins, G.D., Wheeler, R.M. and Sager, J.C., 2004. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. *HortScience* 39:1617–1622.
- Kitaya, Y., Niu, G., Kozai, T. and Ohashi, M., 1998. Photosynthetic photon flux, photoperiod, and CO₂ concentration affect growth and morphology of lettuce plug transplants.

HortScience 33:988-991.

- Kopsell, D.A. and Sams, C.E., 2013. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Am. Soc. Hortic. Sci.* 138:31–37.
- Kopsell, D.A., Sams, C.E., Barickman, T.C. and Morrow, R.C., 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. *J. Am. Soc. Hortic. Sci.* 139:469–477.
- Kopsell, D.A., Sams, C.E. and Morrow, R.C., 2015. Blue wavelengths from LED lighting increase nutritionally important metabolites in specialty crops. *HortScience* 50:1285–1288.
- Lambers, H., Chapin, F.S. and Pons, T.L., 2008. Growth and allocation, in: *Plant Physiological Ecology*. Springer, New York, NY.
- Li, Q. and Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67:59–64.
- Lin, C., 2000. Plant blue-light receptors. *Trends Plant Sci.* 5:337–342.
- Lin, K.H., Huang, M.Y., Huang, W.D., Hsu, M.H., Yang, Z.W. and Yang, C.M., 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). *Sci. Hortic.* 150:86–91.
- Massa, G.D., Kim, H.H., Wheeler, R.M. and Mitchell, C.A., 2008. Plant productivity in response to LED lighting. *HortScience* 43:1951–1956.
- McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agric. Meteorol.* 9:191–216.
- Mickens, M.A., Skoog, E.J., Reese, L.E., Barnwell, P.L., Spencer, L.E., Massa, G.D. and Wheeler, R.M., 2018. A strategic approach for investigating light recipes for ‘Outredgeous’ red romaine lettuce using white and monochromatic LEDs. *Life Sci. Space Res.* <https://doi.org/10.1016/j.lssr.2018.09.003>
- Ouzounis, T., Razi Parjikolaei, B., Fretté, X., Rosenqvist, E. and Ottosen, C.O., 2015. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in *Lactuca sativa*. *Front. Plant Sci.* 6:19.
- Page, M., Sultana, N., Paszkiewicz, K., Florance, H. and Smirnoff, N., 2012. The influence of ascorbate on anthocyanin accumulation during high light acclimation in *Arabidopsis thaliana*: further evidence for redox control of anthocyanin synthesis. *Plant Cell Environ.* 35:388–404.
- Park, Y. and 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.
- Pedmale, U.V., Huang, S.S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A., Sridevi, P., Nito, K., Nery, J.R. and Ecker, J.R., 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* 164:233–245.
- Pierik, R., Whitelam, G.C., Voeselek, L.A., De Kroon, H. and Visser, E.J., 2004. Canopy studies on ethylene-insensitive tobacco identify ethylene as a novel element in blue light and plant-plant signalling. *Plant J.* 38:310–319.
- Pierik, R., Djakovic-Petrovic, T., Keuskamp, D.H., de Wit, M. and Voeselek, L.A., 2009. Auxin and ethylene regulate elongation responses to neighbor proximity signals independent of gibberellin and DELLA proteins in Arabidopsis. *Plant Physiol.* 149:1701–1712.
- Sager, J.C., Smith, W.O., Edwards, J.L. and Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. Am. Soc. Agric. Eng.* 31:1882–1889.
- Sasidharan, R., Chinnappa, C.C., Voeselek, L.A. and Pierik, R., 2008. The regulation of cell wall extensibility during shade avoidance: a study using two contrasting ecotypes of *Stellaria longipes*. *Plant Physiol.* 148:1557–1569.
- Smith, H.L., McAusland, L. and Murchie, E.H., 2017. Don't ignore the green light: exploring diverse roles in plant processes. *J. Exp. Bot.* 68:2099–2110.
- Smith, H. and Whitelam, G.C., 1997. The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant Cell Environ.* 20:840–844.
- Snowden, M.C., Cope, K.R. and Bugbee, B., 2016. Sensitivity of seven diverse species to blue and green light: interactions with photon flux. *PLoS ONE* 11(10):e0163121.
- Spalding, E.P., 2000. Ion channels and the transduction of light signals. *Plant Cell Environ.* 23:665–674.
- Son, K.H. and 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.
- Son, K.H. and 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.
- Szczygiel, E.J., Harte, J.B., Strasburg, G.M. and Cho, S., 2017. Consumer acceptance and aroma characterization of navy bean (*Phaseolus vulgaris*) powders prepared by extrusion and conventional processing methods. *J. Sci. Food Agric.* 97:4142–4150.
- Terashima, I., Fujita, T., Inoue, T., Chow, W.S. and Oguchi, R., 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic

- question of why leaves are green. *Plant Cell Physiol.* 50:684–697.
- Tomás-Barberán, F.A. and Espín, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric.* 81:853–876.
- Wang, Y. and Folta, K.M., 2013. Contributions of green light to plant growth and development. *Am. J. Bot.* 100:70–78.
- Wang, H., Ma, L.G., Li, J.M., Zhao, H.Y. and Deng, X.W., 2001. Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* 294:154–158.
- Wang, J., Lu, W., Tong, Y. and 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.* 7:250.
- Went, F.W., 1957. The experimental control of plant growth. *Chronica Botanica*, Waltham, MA.
- Wollaeger, H.M. and Runkle, E.S., 2014. Growth of impatiens, petunia, salvia, and tomato seedlings under blue, green, and red light-emitting diodes. *HortScience* 49:734–740.
- Wollaeger, H.M. and Runkle, E.S., 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. *HortScience* 50:522–529.
- Zhang, T. and Folta, K.M., 2012. Green light signaling and adaptive response. *Plant Signal. Behav.* 7:75–78.
- Zhang, T., Maruhnich, S.A. and Folta, K.M., 2011. Green light induces shade avoidance symptoms. *Plant Physiol.* 111.180661.

SECTION IV

GROWTH RESPONSES OF RED-LEAF LETTUCE TO TEMPORAL CHANGES IN LIGHT QUALITY

Growth responses of red-leaf lettuce to temporal changes in light quality

Qingwu Meng and Erik S. Runkle*

Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI
48824-1325, USA

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

Abstract.

The spectrum of horticultural lighting is typically static for indoor production of leafy greens. However, temporal spectrum differentiation for distinct developmental phases can potentially control age-specific desirable traits. Spectral effects can be persistent yet dynamic as plants mature, necessitating characterization of time-dependent responses. We grew red-leaf lettuce (*Lactuca sativa*) ‘Rouxai’ in a growth room at 23 °C and under a 20-h photoperiod created by warm-white (WW), blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and/or far-red (FR; 700–800 nm) light-emitting diodes. From day 0 to 11, plants received six static light-quality treatments with the same total photon flux density (400–800 nm): WW₁₈₀, R₁₈₀, B₂₀R₁₆₀, B₂₀G₆₀R₁₀₀, B₂₀R₁₀₀FR₆₀, or B₁₈₀ (subscripts denote photon flux densities in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). On day 11, plants grown under each of the six treatments were transferred to all treatments, which created 36 temporal spectrum alternations. Data on plant growth, morphology, and coloration were collected on days 11 and 25. Increasing B light from 0 to 100% in static treatments decreased shoot fresh and dry weights and increased foliage redness of seedlings and mature plants. Compared to B₂₀R₁₆₀, B₂₀R₁₀₀FR₆₀ increased shoot fresh weight, but not dry weight, on both days. However, other phenotypic responses under static treatments changed over time. For example, leaf length under B₁₈₀ was 35% lower on day 11 but similar on day 25 compared to that under R₁₈₀. In the B₂₀ background, substituting G₆₀ for R light did not influence shoot weight on day 11 but decreased it by 19% on day 25. When plants were switched from one treatment to another on day 11, the treatments applied before day 11 influenced final shoot weight and, to a lesser extent, leaf length and foliage coloration on day 25. In comparison, effects of the treatments applied after day 11 were more pronounced. We conclude that some phenotypic responses to light quality can depend on time and that sequential light quality treatments had

cumulative effects on lettuce growth. The temporal complexity of spectral responses is critical in photobiological research and creates opportunities for time-specific spectrum delivery to optimize crop characteristics.

Keywords: controlled environment, dynamic lighting, LEDs, morphology, plant growth.

Abbreviations: B, blue; FR, far red; G, green; LED, light-emitting diode; HY5, Long Hypocotyl 5; PPE, phytochrome photoequilibrium; PPFD, photosynthetic photon flux density; R, red; TPDF, total photon flux density; WW, warm white; YPFD, yield photon flux density.

Introduction

The spectral composition of lighting in controlled environments can regulate a wide range of commercially relevant crop traits such as harvestable yield, morphology, coloration, and nutritional quality (Carvalho and Folta, 2014a). Red (R; 600–700 nm) light is typically more effective at stimulating extension growth and biomass accumulation of leafy greens than blue (B; 400–500 nm) or B+R light (Ohashi-Kaneko et al., 2007; Son and Oh, 2013; Lee et al., 2014). In contrast, B light generally suppresses extension growth (Cope et al., 2014; Wollaeger and Runkle, 2014) but stimulates production of bioactive compounds (Son and Oh, 2013; Lee et al., 2014; Kopsell et al., 2015). Green (G; 500–600 nm) light penetrates deep in the leaf and crop canopy to promote photosynthesis (Terashima et al., 2009; Brodersen and Vogelmann, 2010). Far-red (FR; 700–800 nm) light can induce shade-avoidance symptoms (Cerdán and Chory, 2003) and regulate anthocyanin production (Carvalho and Folta, 2014b). The combined effects of these wavebands on plant growth and development are often complicated by synergistic or

antagonistic interactions. Characterization of these spectral effects on various edible crops has been advanced by adjustable arrays of multicolored light-emitting diodes (LEDs) in controlled environments.

Electric lighting is substituted for sunlight to provide photosynthetic photons for indoor-grown leafy greens. It is generally static throughout the production cycle, whereas field-grown plants undergo fluctuations in light quality, intensity, and duration throughout the day and production cycle. Static lighting feeds constant energy to light-harvesting antennae of photosystem II and maintains steady electron transport and proton generation to produce NADPH and ATP, respectively, which are used in carbon fixation (Armbruster et al., 2014). In contrast, the dynamic nature of sunlight necessitates responsive and efficient photosynthetic acclimation through regulation of energy channeling and dissipation to maintain high photosynthetic efficiency (Demmig-Adams et al., 2012). In *Arabidopsis* (*Arabidopsis thaliana*), K^+ efflux antiporter 3 mediated H^+/K^+ antiport to facilitate rapid restoration of photosystem II quantum efficiency after plants were transferred from high to low light or from darkness to low light (Armbruster et al., 2014). Such mechanisms allow plants to thrive in continuously changing light environments.

Switching from static to dynamic lighting for indoor crop production adds the temporal factor in crop responses to improve crop traits. Temporal spectrum differentiation can occur in large or small segments of the crop life cycle to elicit age-dependent, desirable attributes. For example, anthocyanin accumulation in red-leaf lettuce (*Lactuca sativa*) is unnecessary for seedlings but desirable for mature plants at harvest. It can be induced rapidly by ≥ 5 d of end-of-production supplemental lighting from B and/or R LEDs (Owen and Lopez, 2015). In addition, R light induced excessive extension growth of lettuce ‘Crispa’ seedlings but increased dry weight

of mature plants compared to B or B+R light (Chen et al., 2014). Therefore, it could be potentially beneficial to produce compact seedlings under B or B+R light and then switch to R light to promote growth of mature plants. After the seedling phase, weekly progressive spectrum alternations of B and/or R light influenced shoot growth, morphology, and phytochemical accumulation of lettuce ‘Sunmang’ (Son et al., 2017). A greater dose of B light increased secondary metabolite concentrations, whereas a greater dose of R light increased shoot weight and projected leaf area (Son et al., 2017). Changing the spectrum in shorter periods of plant development can also modulate final crop phenotypes. For example, 4-d sequential B, R, and/or FR lighting treatments influenced stem elongation, anthocyanin concentration, and antioxidant capacity of kale (*Brassica napus*) seedlings, showing strong plant plasticity in response to spectral changes (Carvalho and Folta, 2014b). Furthermore, staggering B and R light within the day increased shoot weight of romaine lettuce (*Lactuca sativa* var. *longifolia*) compared to simultaneous B+R light (Jishi et al., 2016).

Under changing light conditions, a light response can be transient or persistent. Examples of a transient light response include stomatal opening and phototropism under B light as well as increasing net photosynthesis with incremental increases in photon flux densities. These rapid responses are reversible after the light condition changes. On the other hand, a spectrum applied in an early developmental phase can have persistent and irreversible influence on subsequent phenotypic responses. For example, the addition of FR light to B+R light during seedling development of snapdragon (*Antirrhinum majus*) promoted flowering when plants were finished in a greenhouse environment (Park and Runkle, 2017). In addition, B or R light applied for 7 d after emergence influenced leaf area and shoot dry weight of lettuce ‘Grand Rapids’ 16 or 42 d after emergence, irrespective of a switch to the opposite waveband on day 7 (Eskins et al., 1995).

However, such sustained spectral effects were not observed in other lettuce studies with a fixed spectrum early in seedling development and varying spectra afterwards (Johkan et al., 2010; Son and Oh, 2013). Furthermore, the influence of a spectrum on lettuce growth and morphology can vary with each developmental phase. For example, when applied day 10–17 after seed sow, B light decreased leaf area and shoot fresh weight of lettuce ‘Banchu Red Fire’ on day 17 but increased them on day 45 compared to R light (Johkan et al., 2010). The discrepancies in these studies likely result from different genetic backgrounds, light intensities, and spectral contexts.

Here, we expanded static spectral combinations to include G, FR, and warm-white (WW) light and created a wide array of lighting treatments shifted temporally between the seedling and mature phases of indoor lettuce production. The objectives of this study were 1) to investigate how spectral treatments for lettuce seedlings influence phenotypes of mature plants grown under different spectra; 2) to compare lettuce growth under single wavebands, combinations of two or three wavebands, and warm-white light; and 3) to find temporal spectral combinations for desirable lettuce growth and morphology. We postulated that 1) the spectral effects during the seedling stage would persist through the mature phase, regardless of the finishing spectral environment; 2) substituting G light for R light would increase lettuce growth during the seedling stage but have little influence on growth of mature plants; and 3) B light alone would inhibit leaf expansion and dry weight during the seedling phase but promote them during the mature phase.

Materials and methods

The propagation phase

This experiment was performed in a refrigerated walk-in growth room of the Controlled-Environment Lighting Laboratory (Michigan State University, East Lansing, MI). Seeds of red

oakleaf lettuce ‘Rouxai’ were obtained from a commercial seed producer (Johnny’s Selected Seeds, Winslow, ME) and sown in a rockwool substrate with 200 2.5-cm-wide cubes per sheet (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) on 28 Apr. and 29 Apr. 2018 for two blocks. The substrate was presoaked in deionized water supplemented with diluted (1:31) 95–98% sulfuric acid (J.Y. Baker, Inc., Phillipsburg, NJ), a water-soluble fertilizer (12N–4P₂O₅–16K₂O RO Hydro FeED; JR Peters, Inc., Allentown, PA), and magnesium sulfate (Epsom salt; Pennington Seed, Inc., Madison, GA) to achieve a pH of 3.9 and an electrical conductivity of 1.6 mS·cm⁻¹. The nutrient solution contained 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. Seed trays were covered with transparent humidity domes and placed under six different lighting treatments, each at a total photon flux density (TPFD; 400–800 nm) of 180 μmol·m⁻²·s⁻¹ with a 20-h photoperiod. Air temperature was set at 20 °C from 28 Apr. to 30 Apr. 2018 and increased to 23 °C for the remainder of the experiment. From day 1 to 11, seedlings were subirrigated as needed using the same nutrient solution with a pH of 5.8 adjusted with potassium bicarbonate. The humidity domes were removed on 3 May 2018 for both blocks.

The production phase

On day 11, when the second true leaf was expanding, seedlings were transplanted into 36-cell rafts (60.9 × 121.9 × 2.5 cm; Beaver Plastics, Ltd; Acheson, AB, Canada) floating in flood tables (1.22 × 0.61 × 0.18 m; Active Aqua AAHR24W; Hydrofarm, Petaluma, CA) on three-tier racks (Indoor Harvest, Houston, TX). Plants were spaced 20 cm apart horizontally and 15 cm apart diagonally. The recirculating nutrient solution was mixed as described for seedlings to provide the following nutrients (in mg·L⁻¹): 150 N, 50 P, 200 K, 88 Ca, 58 Mg, 47 S, 2.1 Fe, 0.63 Mn, 0.68 Zn, 0.15 B, 0.56 Cu, and 0.15 Mo. It was oxygenated with a circular air stone (20.3 × 2.5

cm; Active Aqua AS8RD; Hydrofarm) connected to a 60-W air pump (Active Aqua AAPA70L; Hydrofarm). The pH, electrical conductivity, and temperature of the nutrient solution for each lighting canopy were measured daily using a portable pH and electrical conductivity meter (HI9814; Hanna Instruments, Woonsocket, RI) (Table IV-1). Potassium bicarbonate was used to increase pH when it dropped below 5.5.

Environmental conditions

Temperature in the growth room was regulated with an industrial ventilation and air-conditioning unit (HBH030A3C20CRS; Heat Controller, LLC., Jackson, MI) connected to a wireless thermostat (Honeywell International, Inc., Morris Plains, NJ). The deep-flow hydroponic system was equipped with two light quantum sensors (LI-190R; LI-COR, Inc., Lincoln, NE), two thermocouples (0.13-mm type E; Omega Engineering, Inc., Stamford, CT), two infrared sensors (OS36-01-K-80F; Omega Engineering, Inc.), a CO₂ transmitter (GMD20; Vaisala, Inc., Louisville, CO), and a relative humidity and temperature probe (HMP110; Vaisala, Inc.). All sensors were connected to a datalogger (CR1000; Campbell Scientific, Inc., Logan, UT) with a multiplexer (AM16/32B; Campbell Scientific, Inc.), which recorded environmental parameters every 10 s and logged hourly averages using computer software (LoggerNet; Campbell Scientific, Inc.). The air temperature, canopy temperature, CO₂ concentration, and relative humidity throughout the experiment (mean $\pm$ standard deviation) were 22.5 ± 1.0 °C, 24.1 ± 0.9 °C, 392 ± 31 ppm, and $44 \pm 8\%$, respectively.

Lighting treatments

Seedlings were grown under WW₁₈₀, R₁₈₀, B₂₀R₁₆₀, B₂₀G₆₀R₁₀₀, B₂₀R₁₀₀FR₆₀, or B₁₈₀ LEDs (PHYTOFY RL; OSRAM, Beverley, MA), where the subscript following each LED type indicates its photon flux density (in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The peak wavelengths of WW, B, G, R, and

FR LEDs were 639, 449, 526, 664, and 733 nm, respectively. The outputs of seven color channels, including five used in this study, in each LED fixture were independently controlled with software (Spartan Control Software; OSRAM). The specifications, layout, and positioning of the LED fixtures were as described in Chapter 2. Spectra were measured at seven locations at plant canopy of each lighting treatment using a portable spectroradiometer (PS200; Apogee Instruments, Inc., Logan, UT) (Figure IV-1). The single-band photon flux densities, photosynthetic photon flux density (PPFD; 400–700 nm), TPDF, yield photon flux density [YPFD, an integrated value based on relative quantum efficiency (McCree, 1972) and spectral data], phytochrome photoequilibrium [PPE, an estimated value based on phytochrome absorption coefficients and spectra data (Sager et al., 1988)], ratio of B to R light (B:R), and ratio of R to FR light (R:FR) for each lighting treatment were subsequently calculated (Table IV-2). To study the temporal effects of light quality, lighting treatments were switched between the propagation phase (day 0–11) and the production phase (day 11–25). Seedlings grown under each of the six lighting treatments were transferred to all six lighting treatments on day 11. This created a total of 36 unique temporal lighting combinations, six of which were static (without transfers) throughout the experiment (Table IV-3).

Data collection and analysis

Shoot fresh and dry weights, leaf morphology, and coloration data were collected on ten young lettuce plants per block grown under each of the six static lighting treatments on day 11 and on eight mature lettuce plants per block grown under each of the 36 temporal lighting combinations on day 25. Shoot fresh weight was measured with an analytical balance (GR-200; A&D Store, Inc., Wood Dale, IL) for young plants and a different one (GX-1000; A&D Store, Inc.) for mature plants based on capacities. Length of the fifth most mature true leaf was

measured to quantify extension growth. The International Commission on Illumination Lab color space analysis was conducted on a representative leaf per plant using a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc.). L^* , a^* , and b^* indicate foliage brightness (ranging from 0 for black to 100 for diffuse white), greenness–redness (corresponding to negative–positive directions), and blueness–yellowness (corresponding to negative–positive directions), respectively. Subsequently, plants were dried in an oven (Blue M, Blue Island, IL) at 60 °C for ≥ 5 d followed by dry weight measurements with the same analytical balances as for shoot fresh weight.

Data on young and mature lettuce plants were analyzed with the PROC MIXED procedure and Tukey's honestly significant difference test ($\alpha = 0.05$) in SAS (version 9.4; SAS Institute, Inc., Cary, NC). Data from static treatments were analyzed as a randomized complete block design with two blocks (using opposite racks of the growth room), six static lighting treatments, and subsampling ($n = 10$), assuming fixed block effects. Data from alternate treatments were analyzed as a strip-split-plot design with two blocks, six whole-plot levels (post-transplant lighting treatments), six subplot levels (pre-transplant lighting treatments), and subsampling ($n = 8$), assuming fixed block effects. The split-plot design included whole plots arranged in a complete randomized block design.

Results

Static lighting treatments for young and mature lettuce

On day 11, substituting $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light for R light (B₂₀R₁₆₀ versus R₁₈₀) decreased shoot dry weight by 15%, but not shoot fresh weight (Figure IV-2A). Shoot fresh (dry) weight was 40–44% (39–42%) lower under B₁₈₀ than under WW₁₈₀ and R₁₈₀. Partial substitution of R

light in B₂₀R₁₆₀ with 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light (B₂₀G₆₀R₁₀₀) or FR light (B₂₀R₁₀₀FR₆₀) did not influence shoot dry weight, whereas the substitution with FR light increased shoot fresh weight by 18%. On day 25, increasing substitution of R light with B light decreased shoot fresh and dry weights (Figure IV-2B). Shoot fresh (dry) weight under B₁₈₀ was 63–65% (52–57%) lower than under R₁₈₀ or WW₁₈₀. Substituting 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light in B₂₀R₁₆₀ decreased shoot fresh and dry weights by 19%. The same substitution with FR light increased shoot fresh weight by 22%, but not shoot dry weight.

On day 11, leaf length was the greatest under WW₁₈₀, R₁₈₀, and B₂₀R₁₀₀FR₆₀ and the lowest under B₁₈₀ (Figure IV-2C). Increasing substitution of R light with B light decreased leaf length. Substituting 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G or FR light for R light in B₂₀R₁₆₀ increased leaf length by 11% or 42%, respectively. On day 25, leaf length was the greatest under B₂₀R₁₀₀FR₆₀ and the lowest under B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀ (Figure IV-2D). Compared to WW₁₈₀, leaf length was 8% shorter under R₁₈₀ and similar under B₁₈₀. Although substituting 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B light for R light decreased leaf length by 11%, leaf length was similar under R₁₈₀ and B₁₈₀. Substituting 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light for R light in B₂₀R₁₆₀ increased leaf length by 41%, but the same substitution with G light did not influence it.

On day 11, foliage brightness (L^*) was the greatest under R₁₈₀, followed by WW₁₈₀ and B₂₀R₁₀₀FR₆₀ (Figure IV-3A). Adding B light to R₁₈₀ decreased brightness. Substituting R light in B₂₀R₁₆₀ with G or FR light increased brightness, especially with FR light. On day 25, leaves were the brightest under R₁₈₀ and WW₁₈₀ and the darkest under B₁₈₀ (Figure IV-3B). Increasing substitution of R light with B light decreased brightness. Leaves were brighter when R light in B₂₀R₁₆₀ was substituted with FR light, but not G light.

On day 11, leaves were the least red (lowest a^*) and yellowest (highest b^*) under R₁₈₀,

followed by WW₁₈₀ and B₂₀R₁₀₀FR₆₀, and the reddest and least yellow under B₁₈₀ (Figure IV-3C, IV-3E). The inclusion of B light in a R background increased redness and decreased yellowness, whereas the inclusion of G or FR light decreased redness and increased yellowness. At the same photon flux density, FR light reduced redness and increased yellowness more than G light. The a^* and b^* trends on day 25 were similar to those on day 11, except that there were no differences between R₁₈₀ and WW₁₈₀ or between B₂₀G₆₀R₁₀₀ and B₂₀R₁₀₀FR₆₀ on day 25 (Figure IV-3D, IV-3F).

Temporal lighting combinations for mature lettuce

Data on day 25 from 36 temporal lighting combinations are shown in Figure IV-4. Within each eventual treatment applied day 11–25, the initial treatments applied day 0–11 significantly influenced final shoot fresh and dry weights and leaf length on day 25, but not foliage red-green coloration. Irrespective of the eventual treatment, final shoot fresh and dry weights were generally the greatest when plants were initially grown under WW₁₈₀, R₁₈₀, or B₂₀R₁₀₀FR₆₀ and the lowest when initially grown under B₁₈₀. Responses of final shoot fresh and dry weights to initial treatments B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀ were variable within each eventual treatment. Final leaf length within each eventual treatment was mostly similar under initial treatments except B₁₈₀, under which final leaf length within each eventual treatment was slightly lower than that under some other initial treatments.

The effects of initial and eventual lighting treatments on mature lettuce

To dissect the effects of initial (applied day 0–11) and eventual (applied day 11–25) lighting treatments on lettuce harvested on day 25, data of plants grown under the same initial treatments were pooled for initial treatment analysis, whereas data of plants grown under the same eventual treatments were pooled for eventual treatment analysis. The effects of the six lighting treatments

on final shoot fresh and weight weights, leaf length, and color parameters were different when applied day 0–11 versus day 11–25 (Figure IV-5, IV-6).

When the lighting treatments were applied day 0–11, final shoot fresh and dry weights (on day 25) were the greatest under WW₁₈₀, R₁₈₀, and B₂₀R₁₀₀FR₆₀, followed by B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀, and the lowest under B₁₈₀ (Figure IV-5A). In addition, final leaf length under WW₁₈₀ and R₁₈₀ was slightly greater than that under B₂₀R₁₀₀FR₆₀ and B₁₈₀ (Figure IV-5C). Leaves were slightly brighter under B₂₀G₆₀R₁₀₀ than under B₁₈₀, slightly redder under B₂₀R₁₆₀ and B₁₈₀ than under B₂₀G₆₀R₁₀₀, and slightly yellower under B₂₀G₆₀R₁₀₀ than under B₁₈₀ (Figure IV-6A, IV-6C, IV-6E). Otherwise, leaf color parameters were similar under most treatments.

In contrast, treatment effects were more pronounced when applied day 11–25. Final shoot fresh and dry weights were the greatest under R₁₈₀, followed by WW₁₈₀ and B₂₀R₁₀₀FR₆₀, and the lowest under B₁₈₀ (Figure IV-5B). Partially substituting B light for R₁₈₀ decreased shoot weight. Substituting 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G and FR light for R light in B₂₀R₁₆₀ decreased and increased shoot weight, respectively. Final leaf length was the greatest under B₂₀R₁₀₀FR₆₀, followed by WW₁₈₀ and B₁₈₀, and lowest under B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀ (Figure IV-5D). Leaf length under R₁₈₀ was between that under WW₁₈₀ and B₂₀R₁₆₀. Leaf color was the brightest under WW₁₈₀ and R₁₈₀, followed by B₂₀R₁₀₀FR₆₀, and the least bright under B₁₈₀ (Figure IV-6B). Leaf brightness under B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀ was between that under B₂₀R₁₀₀FR₆₀ and that under B₁₈₀. Leaves were the reddest under B₁₈₀, followed by B₂₀R₁₆₀, and the least red under R₁₈₀, followed by WW₁₈₀ (Figure IV-6D). Compared to B₂₀R₁₆₀, leaf redness was reduced with substitutional G and FR light, especially with the latter. The *b** trend was the opposite of the *a** trend (Figure IV-6F).

Discussion

When lettuce ‘Rouxai’ received static lighting throughout this study, phenotypic responses during the propagation and production phases were generally similar but varied under some treatments. On days 11 and 25, increasing B:R decreased shoot fresh and dry weights, increased leaf redness, and decreased leaf brightness and yellowness. In addition, increasing B:R decreased leaf length on day 11. These results are consistent with the notion that B light generally decreases extension growth and shoot weight while increasing accumulation of chlorophylls, anthocyanins, and other secondary metabolites (Son and Oh, 2013; Kopsell et al., 2015; Wollaeger and Runkle, 2015). However, compared to R₁₈₀, leaf length on day 25 was lower under B₂₀R₁₆₀ but similar under B₁₈₀. Aberrant promotion of extension growth and weight gain by B light alone was previously observed in cucumber (*Cucumis sativus*) and cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) seedlings and lettuce ‘Grand Rapids’ (Eskins et al., 1995; Liu et al., 2009; Hernández and Kubota, 2016). We showed a novel temporal shift of the B light function from growth inhibition during the seedling phase to promotion of leaf expansion, but not shoot weight, during the production phase of lettuce. Therefore, temporal specificity should be considered at least in some crops when evaluating spectral influence on plant growth.

Extension growth in arabidopsis seedlings is regulated by the activities of cryptochromes 1 and 2, which depend on the B photon flux density (Pedmale et al., 2016). Cryptochromes 1 and 2 interacted with phytochrome-interacting factors 4 and 5 in low B light to promote hypocotyl growth, whereas active repression of phytochrome-interacting factor 4 and degradation of cryptochrome 2 and phytochrome-interacting factor 5 in high B light restricted it (Pedmale et al., 2016). In the present study, all leaves of lettuce seedlings grown under high B light exhibited typical growth inhibition. However, as lettuce matured, layers of newer leaves emerged from the

central meristem and covered older ones. The newer leaves were directly exposed to abundant B light, whereas the older ones became shaded and received less B light (Franklin, 2016).

Therefore, the responses and interactions of cryptochromes and phytochrome-interacting factors likely differed in upper and lower leaves, which mostly perceived high and low B light, respectively. Because more leaves developed below the top canopy, the overall B photon flux density perceived by mature lettuce was low. This could explain the shift from inhibited extension growth of seedlings to promoted extension growth of mature plants in externally static and strong B light.

Dynamic growth responses of lettuce were also observed with substitutional G light. Substituting $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light influenced lettuce shoot weight and leaf length differently on days 11 and 25. It did not affect shoot fresh and dry weights but increased leaf length on day 11. In contrast, it decreased shoot fresh and dry weights but did not affect leaf length on day 25. In a similar study, substituting $36 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light in static B₂₄R₁₂₆ increased shoot fresh and dry weights and leaf area of lettuce ‘Waldmann’s Green’ on day 28, whereas G light alone from fluorescent lamps decreased them (Kim et al., 2004). These discrepancies can at least partly be attributed to adaptive responses to G light in photosynthetic acclimation and plant architecture, which change throughout developmental phases.

First, the efficacy of G light is comparable to that of B light and about half that of R light in the action spectrum of photosynthesis based on incident photons and instantaneous measurements (McCree, 1972). This is different from the commonly cited McCree curve, which was based on absorbed photons considering the leaf absorption spectrum. Therefore, partial substitution of R light with G light in the incident spectrum may theoretically reduce overall photosynthetic efficacy and thus weight gain in some species and cultivars. Indeed, at the same B

photon flux density of 15, 30, or 45 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, substituting 15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of G light for R light at a constant PPFD of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reduced the leaf net photosynthetic rate of lettuce ‘Green Skirt’ without affecting leaf morphology (Kang et al., 2016). In addition, the leaf net photosynthetic rate of lettuce was lower under G light alone than under R or B light alone, B+R light, or B+G+R light (Kim et al., 2004; Kang et al., 2016).

Second, when delivered at a sufficiently high photon flux density, G light can reverse B-induced growth inhibition and elicit the shade-avoidance response, such as accelerated hypocotyl and petiole elongation (Folta and Maruhnich, 2007; Zhang et al., 2011; Wang and Folta, 2013). In arabidopsis, G light reversed activation of cryptochrome 1 and degradation of cryptochrome 2 by B light (Bouly et al., 2007). Accumulation of cryptochrome 2 in substitutional G light can promote activity of phytochrome-interacting factors 4 and 5 and thus increase extension growth (Pedmale et al. 2016). Besides stem growth, partially substituting G light for R light in constant B light promoted leaf expansion of lettuce ‘Waldmann’s Green’ (Kim et al., 2004), which likely increased light capture for photosynthesis. In addition, completely substituting G light for R light in B₈₀R₈₀ increased leaf area of tomato (*Solanum lycopersicum*) seedlings but did not influence shoot fresh or dry weight (Wollaeger and Runkle, 2014), which resembles the lettuce seedling response to G light on day 11 in the present study. In other studies, the inclusion of G light generally did not influence plant growth (Hernández and Kubota, 2016; Snowden et al., 2016), indicating G light effects could depend on the genotype, spectral context, and timing of treatments.

Taken together, the varying responses to substitutional G light observed on days 11 and 25 in the present study could be attributed to a changing balance between its reduction of instantaneous photosynthesis and its enhancement of whole-plant photosynthesis through

increased leaf expansion and light interception. As lettuce grown under B+R light perceived less overall B light later in production because of leaf layering, its sensitivity to additional shade signals such as G light (when added) subsided. This could explain why leaf length under B₂₀G₆₀R₁₀₀ was initially greater than that under B₂₀R₁₆₀ on day 11 but eventually was similar to it on day 25. Increased leaf expansion likely compensated for reduced net photosynthesis in G light on day 11, leading to comparable shoot weight under B₂₀R₁₆₀ and B₂₀G₆₀R₁₀₀. The lack of such compensation on day 25 resulted in lower shoot weight under substitutional G light.

In contrast, FR light was a stronger shade signal than G light at the same photon flux density and consistently increased leaf length by 41–42% on days 11 and 25 when added to B+R light. Compared to B₂₀R₁₆₀, although B₂₀R₁₀₀FR₆₀ at the same TPF_D was 32% lower in the PPFD and 26% lower in the YPFD, lettuce grown under B₂₀R₁₀₀FR₆₀ had similar shoot dry weight and 17–22% higher shoot fresh weight (partly due to increases in moisture content). The similar TPF_Ds across all lighting treatments cannot explain differences in shoot dry weight. In addition, Figure IV-7 plots shoot dry weight against the relative PPFD, YPFD, leaf length, PPFD × leaf length, or YPFD × leaf length for all lighting treatments. Only YPFD × leaf length was linearly related with shoot dry weight (Figure IV-7). Therefore, the similar dry weight with the FR light substitution (B₂₀R₁₀₀FR₆₀ versus B₂₀R₁₆₀) was likely the product of the reduced YPFD (74% of that for B₂₀R₁₆₀) and increased light interception (141–142% of that under B₂₀R₁₆₀). This suggests that changes in shoot dry weight can be predicted by multiplying percentage changes in the YPFD (to account for the changing instantaneous photosynthetic rate and quantum efficiency) and percentage changes in leaf size (to account for changing light interception due to morphological acclimation) (Figure IV-7). The YPFD is a better predictor of plant biomass than the PPFD because it accounts for relative quantum efficiency and the contribution of FR light to

net photosynthesis, albeit less significant than B, G, or R light. Lastly, light interception may be better estimated with leaf area instead of leaf length.

Increasing B:R intensified red coloration of lettuce ‘Rouxai’, whereas substitutional G or FR light decreased B-induced anthocyanin accumulation of plants treated with static lighting on days 11 and 25. Similarly, increasing the B photon flux density from 20 to 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased anthocyanin concentration of lettuce ‘Red Sails’ in a dose-dependent manner; however, the inclusion of G light reduced anthocyanin accumulation in lettuce ‘Red Sails’ and arabidopsis (Zhang and Folta, 2012). Upregulation of anthocyanin accumulation by B light is mediated by cryptochrome 1 and reversed by G light (Bouly et al., 2007). Although FR light increases anthocyanin accumulation during de-etiolation of arabidopsis seedlings through phytochrome A, which stabilizes Long Hypocotyl 5 (HY5) to promote expression of anthocyanin biosynthetic genes (Li et al., 2014; Liu et al., 2015), it can also decrease anthocyanin accumulation through phytochrome B (Zheng et al., 2013). In addition, partial substitution of white light with FR light decreased anthocyanin concentration of lettuce ‘Red Cross’ (Li and Kubota, 2009). Therefore, G and FR light likely antagonize B light in regulation of anthocyanin accumulation of red-leaf lettuce through cryptochromes and phytochromes, respectively. Alternatively, with similar total anthocyanin content per leaf, anthocyanin concentration can decrease as leaf area increases with G or FR light. Direct biosynthetic regulation and the “dilution” effect may occur concurrently and warrant further investigation.

When lettuce ‘Rouxai’ was grown under different initial treatments day 0–11 but the same eventual treatments day 11–25, initial light quality had a residual effect on final shoot fresh and dry weights, responses of which generally resembled those under static treatments. For example, for plants transferred to the same R₁₈₀ or B₁₈₀ treatment on day 11, final shoot dry weight was

greater when initially grown under R₁₈₀ than under B₁₈₀. In a similar study, when lettuce ‘Grand Rapids’ was transferred from R₁₀₀ to B₁₀₀ or from B₁₀₀ to R₁₀₀ on day 7, shoot dry weight of mature lettuce was primarily influenced by light quality applied before, rather than after, the transfer (Eskins et al., 1995). Contrary to typical B-induced growth inhibition, shoot dry weight and leaf area were consistently greater under B₁₀₀ than under R₁₀₀ applied during seedling development or throughout the experiment (Eskins et al., 1995). Such unique B light responses may be species- and cultivar-specific. In addition, a temporal shift of B light responses was previously reported in lettuce ‘Banchu Red Fire’, which was grown under fluorescent lamps day 0–10; R₁₀₀, B₅₀R₅₀, or B₁₀₀ day 10–17; and then sunlight with supplemental fluorescent lamps day 17–45 (Johkan et al., 2010). Increasing B:R during the seedling phase decreased leaf area and fresh weight on day 17 but increased them on day 45 (Johkan et al., 2010). Although spectral effects varied in these and our studies, they all showed lasting influences of light quality applied during the seedling phase on subsequent plant growth. A sustained environmental treatment delivered early in seedling development could persist into the mature phase possibly by DNA methylation or irreversible activation or suppression of growth-related genes (Bird, 1993; Eskins et al., 1995). The latency of early light signals was also evident in accelerated flowering of mature snapdragon and petunia (*Petunia ×hybrida*) by additional FR light applied during the seedling phase (Park and Runkle, 2017, 2018).

Although light quality during the seedling phase modified shoot fresh and dry weights of mature lettuce, the magnitude of this modification was less pronounced than that by light quality in the mature phase. As the plant underwent the exponential growing phase, light interception increased drastically with leaf development, which likely led to greater impacts of eventual treatments on photosynthesis and morphology. In addition, leaf length and coloration of mature

lettuce were primarily controlled by eventual lighting treatments and negligibly affected by initial ones. The greater influence of eventual light quality on foliage coloration could at least partly be attributed to rapid anthocyanin accumulation in lettuce under light stresses within days (Owen and Lopez, 2015). In general, final lettuce shoot weight, leaf length, and coloration were similar under lighting treatments applied day 0–25 and day 11–25, further highlighting the predominant role of eventual light quality. Nonetheless, the lasting initial spectral effects exerted significant influence on final shoot weight and thus should be considered for growth of both seedlings and mature plants. In another treatment-switching experiment, spectral effects during the seedling (day 0–14) and mature (day 14–28) phases on final growth of lettuce ‘Crispa’ depended on specific lighting combinations (Chang and Chang, 2014). Therefore, dynamic lighting strategies should be based on specific cultivars and potentially interactive environmental factors such as light quality, the PPFD, and temperature.

In general, antagonistic B and FR light decreased and increased lettuce shoot weight and leaf expansion, respectively, and had opposite effects on foliage coloration. Static B light alone elicited similar biomass accumulation but different leaf expansion during propagation and production. Substituting substantial G light for R light did not influence growth of seedlings but decreased growth of mature lettuce. Plants grown under a consistently R-rich environment (i.e., R or WW light) had the greatest growth at the expense of pigmentation. Temporally alternating light quality improved precision of phenotype control over static lighting. Phenotypic responses to static lighting provided insights into spectral influence. Subsequently, differential lighting treatments could be delivered at various developmental stages to optimize crop growth and quality attributes. Our results suggest that lettuce biomass can be maximized with WW, R, or B+R+FR light during propagation, followed by R light during production. End-of-production B

light can be used to induce anthocyanin accumulation. Finally, effects of light quality applied during the seedling phase persisted into the mature phase, although they were less pronounced than those applied during the mature phase.

Acknowledgements

We thank David Hamby, Rodrigo Pereyra, Charles Brunault, Alan Sarkisian, Steve Graves, and Dorian Spero from OSRAM Innovation for lighting support; Nathan Kelly for experimental assistance; Steve Brooks for technical assistance; Randy Beaudry, Dan Brainard, Roberto Lopez, and Emily Merewitz for instruments; and material donations from Grodan and JR Peters, Inc. This work was supported by Michigan State University AgBioResearch Project GREEN GR17-072 and the USDA National Institute of Food and Agriculture, Hatch project 192266.

APPENDIX

Table IV-1. The pH, electrical conductivity, and water temperature (mean $\pm$ standard deviation) of nutrient solutions for six lighting treatment plots in two blocks during the lettuce production phase. Plants were grown under warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Lighting treatment	pH		Electrical conductivity ($\text{mS}\cdot\text{cm}^{-1}$)		Water temperature ($^{\circ}\text{C}$)	
	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
WW ₁₈₀	6.0 $\pm$ 1.0	6.2 $\pm$ 0.9	1.8 $\pm$ 0.0	1.8 $\pm$ 0.1	23.7 $\pm$ 0.3	23.3 $\pm$ 0.3
R ₁₈₀	6.1 $\pm$ 1.0	6.3 $\pm$ 0.9	1.9 $\pm$ 0.1	1.7 $\pm$ 0.1	22.9 $\pm$ 0.2	23.9 $\pm$ 0.4
B ₂₀ R ₁₆₀	6.1 $\pm$ 1.0	6.3 $\pm$ 0.9	1.9 $\pm$ 0.1	1.7 $\pm$ 0.1	22.9 $\pm$ 0.2	23.9 $\pm$ 0.4
B ₂₀ G ₆₀ R ₁₀₀	6.1 $\pm$ 1.0	6.3 $\pm$ 0.9	1.9 $\pm$ 0.1	1.7 $\pm$ 0.1	22.9 $\pm$ 0.2	23.9 $\pm$ 0.4
B ₂₀ R ₁₀₀ FR ₆₀	6.0 $\pm$ 1.0	6.2 $\pm$ 0.9	1.8 $\pm$ 0.0	1.8 $\pm$ 0.1	23.7 $\pm$ 0.3	23.3 $\pm$ 0.3
B ₁₈₀	6.0 $\pm$ 1.0	6.2 $\pm$ 0.9	1.8 $\pm$ 0.0	1.8 $\pm$ 0.1	23.7 $\pm$ 0.3	23.3 $\pm$ 0.3

Table IV-2. Spectral characteristics of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Integrated parameters include the photosynthetic photon flux density (PPFD; 400–700 nm), the total photon flux density (TPFD; 400–800 nm), and the yield photon flux density [YPFD; the product of relative quantum efficiency (McCree, 1972) and spectral data from 300 to 800 nm]. The estimated phytochrome photoequilibrium (PPE) was calculated as described by Sager et al. (1988).

	LED lighting treatment					
	WW ₁₈₀	R ₁₈₀	B ₂₀ R ₁₆₀	B ₂₀ G ₆₀ R ₁₀₀	B ₂₀ R ₁₀₀ FR ₆₀	B ₁₈₀
	Single-band photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
B	12.5	0.3	19.2	22.9	18.8	178.4
G	52.8	0.7	0.7	59.7	0.8	0.9
R	98.1	176.9	158.5	99.4	102.2	0.5
FR	18.1	2.1	1.9	1.3	60.7	0.1
	Integrated photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					
PPFD	163.3	177.9	178.4	181.9	121.8	179.7
TPFD	181.4	180.0	180.3	183.2	182.4	179.9
YPFD	149.5	165.0	162.1	156.5	119.3	134.4
	Light ratio					
B:R	0.13	0.00	0.12	0.23	0.18	386.80
R:FR	5.42	83.96	82.98	76.19	1.68	3.49
PPE	0.829	0.882	0.880	0.878	0.764	0.480

Table IV-3. Temporal lighting combinations during lettuce propagation and production. Plants were grown under static or alternate lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Day 0–11 (propagation)	Day 11–25 (production)
WW ₁₈₀	WW ₁₈₀
	R ₁₈₀
	B ₂₀ R ₁₆₀
	B ₂₀ G ₆₀ R ₁₀₀
	B ₂₀ R ₁₀₀ FR ₆₀
R ₁₈₀	B ₁₈₀
	WW ₁₈₀
	R ₁₈₀
	B ₂₀ R ₁₆₀
	B ₂₀ G ₆₀ R ₁₀₀
B ₂₀ R ₁₆₀	B ₂₀ R ₁₀₀ FR ₆₀
	B ₁₈₀
	WW ₁₈₀
	R ₁₈₀
	B ₂₀ R ₁₆₀
B ₂₀ G ₆₀ R ₁₀₀	B ₂₀ G ₆₀ R ₁₀₀
	B ₂₀ R ₁₀₀ FR ₆₀
	B ₁₈₀
	WW ₁₈₀
	R ₁₈₀
B ₂₀ R ₁₀₀ FR ₆₀	B ₂₀ R ₁₆₀
	B ₂₀ G ₆₀ R ₁₀₀
	B ₂₀ R ₁₀₀ FR ₆₀
	B ₁₈₀
	WW ₁₈₀
B ₁₈₀	R ₁₈₀
	B ₂₀ R ₁₆₀
	B ₂₀ G ₆₀ R ₁₀₀
	B ₂₀ R ₁₀₀ FR ₆₀
	B ₁₈₀

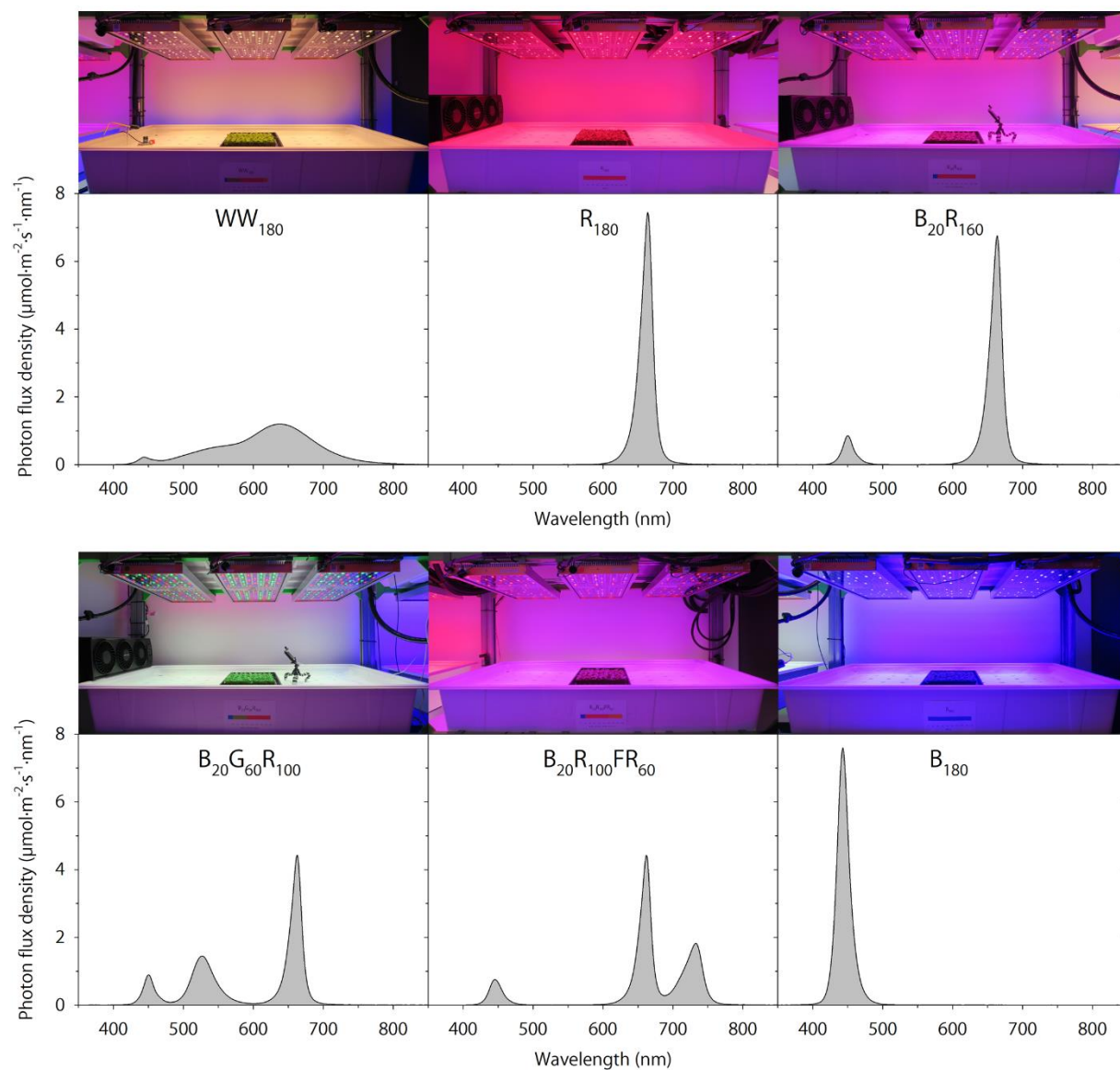


Figure IV-1. Spectral distributions of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number following each LED type is its respective photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

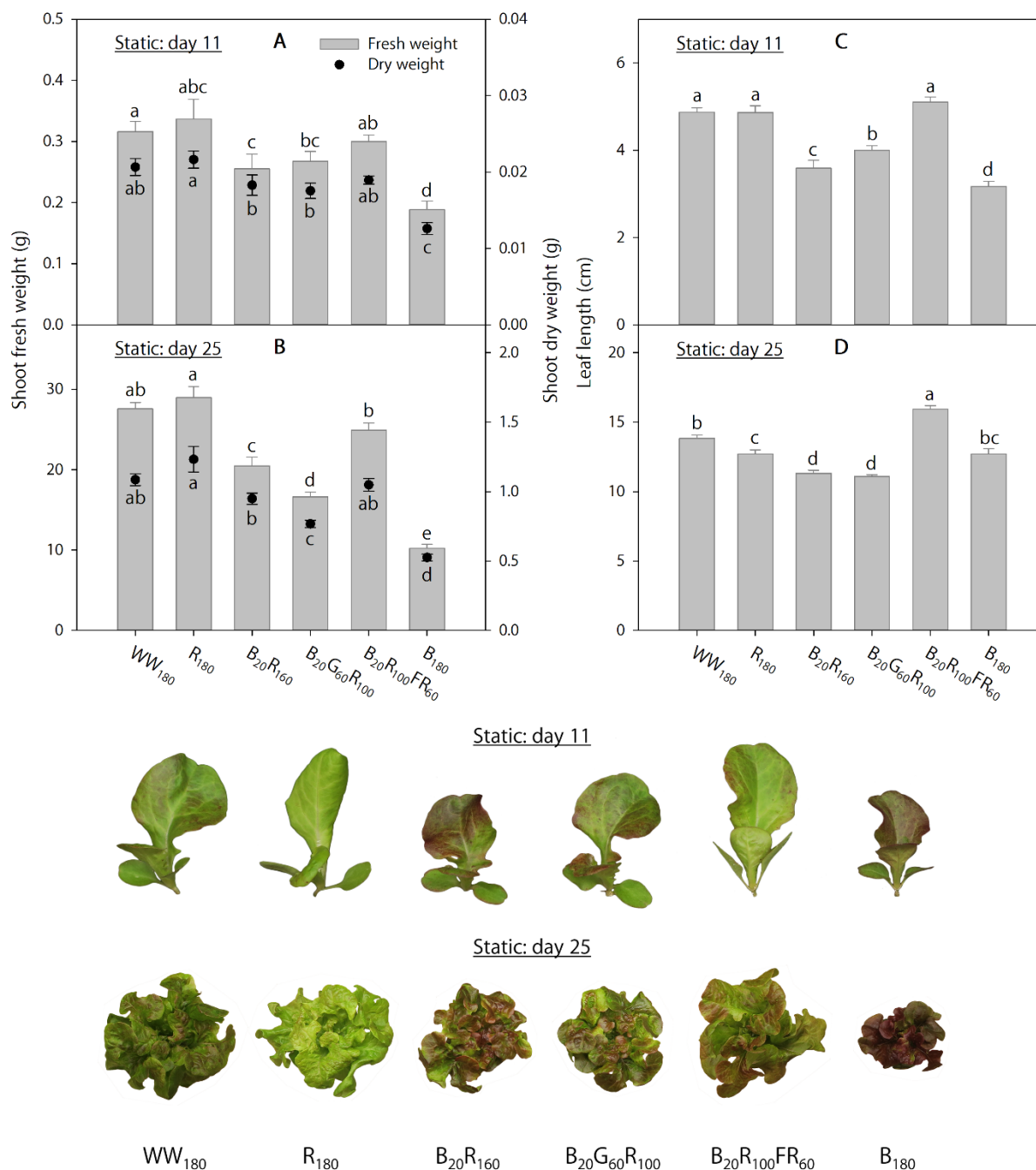


Figure IV-2. Shoot fresh and dry weights and leaf length on days 11 and 25 of lettuce 'Rouxai' grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

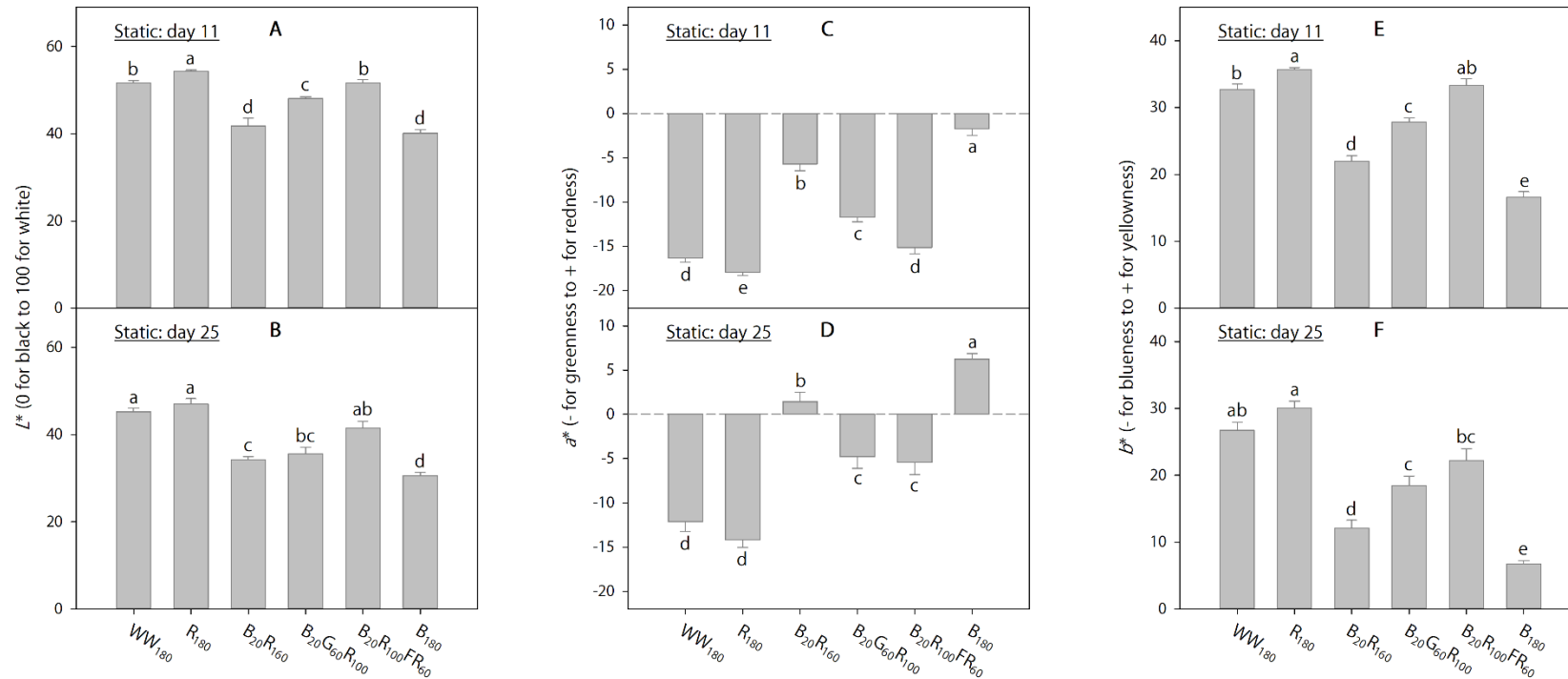


Figure IV-3. Lab color space parameters on days 11 and 25 of lettuce ‘Rouxai’ grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters in each graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

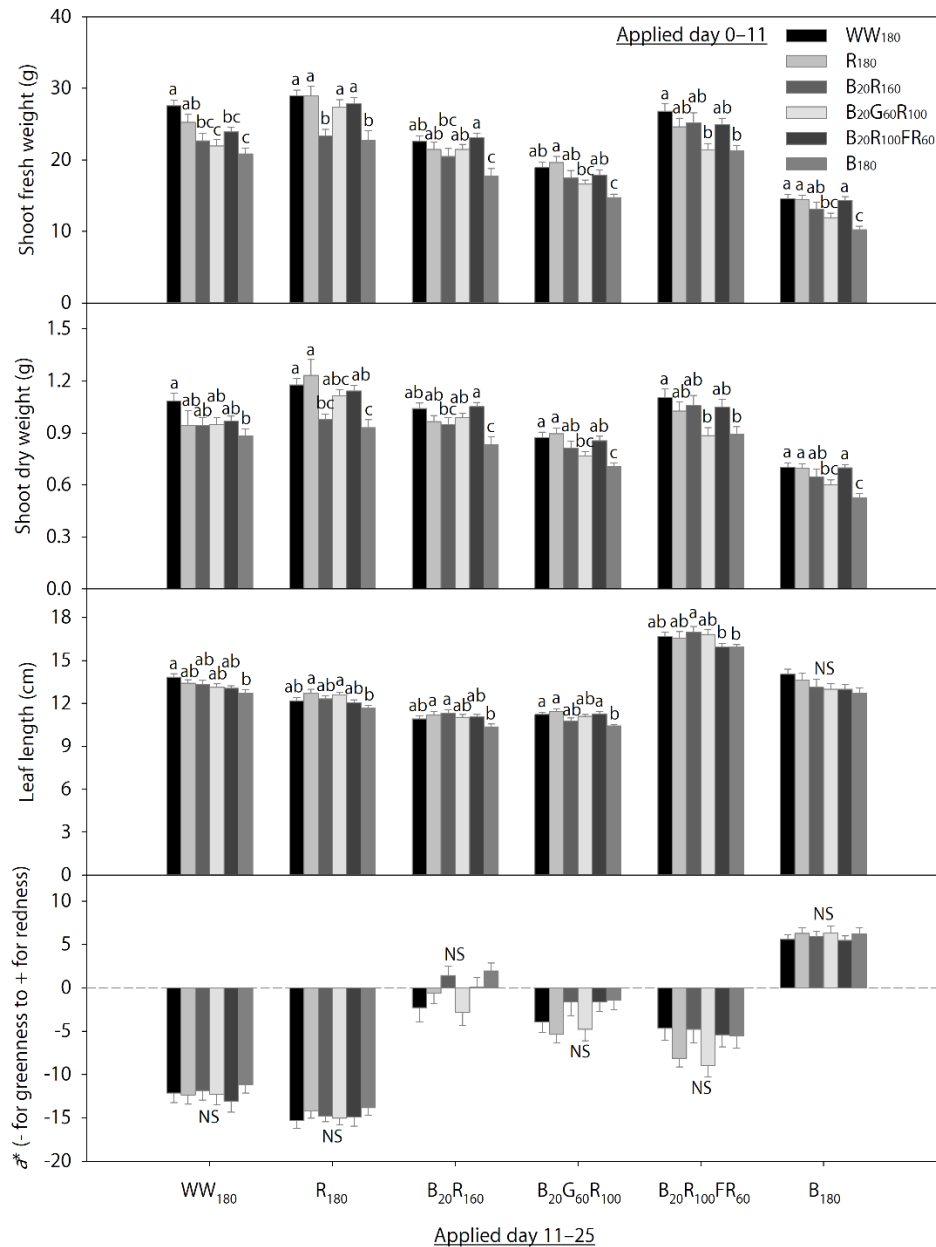


Figure IV-4. Shoot fresh and dry weights, leaf length, and the a^* color space coordinate of lettuce 'Rouxai' on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and treatment applied during day 11–25 are significantly different based on Tukey's honestly significant difference test ($\alpha = 0.05$). NS, non-significant. Error bars show standard errors.

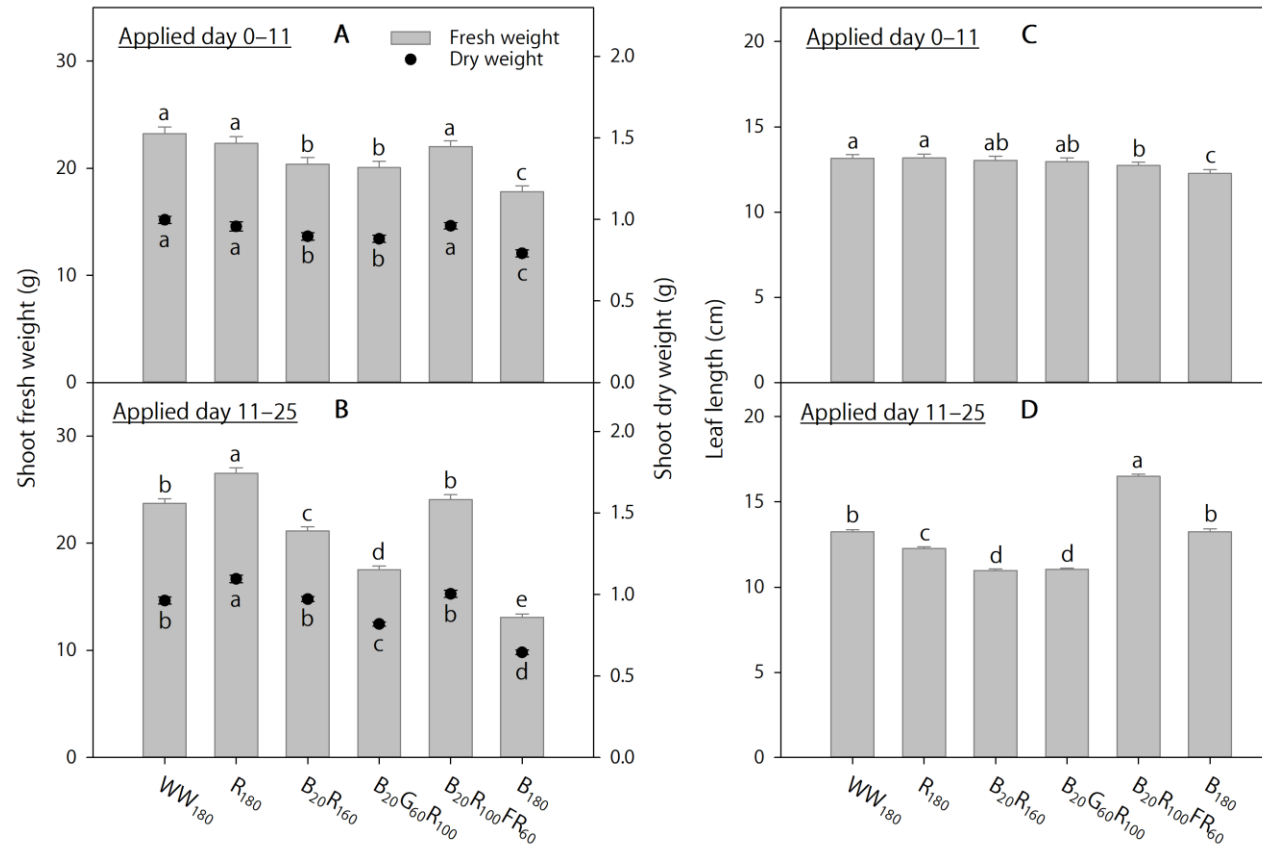


Figure IV-5. The effects of initial (applied day 0–11) and eventual (applied day 11–25) lighting treatments on pooled final shoot fresh and dry weights and leaf length of lettuce ‘Rouxai’ on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each parameter and graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

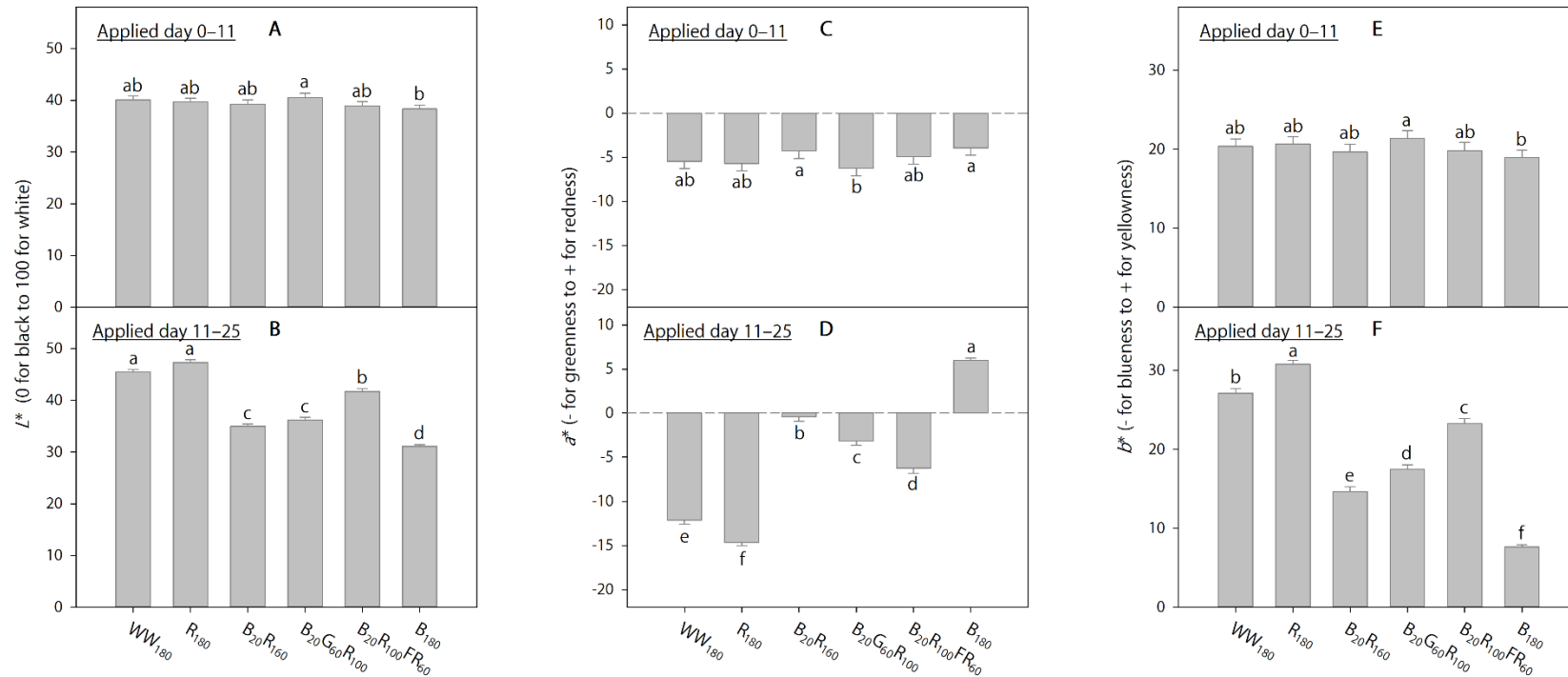


Figure IV-6. The effects of initial (applied day 0–11) and eventual (applied day 11–25) lighting treatments on pooled final Lab color space parameters of lettuce ‘Rouxai’ on day 25. Plants were grown under each of six lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs) during day 0–11, transferred to all six treatments on day 11, and grown until day 25. The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Means followed by different letters within each graph are significantly different based on Tukey’s honestly significant difference test ($\alpha = 0.05$). Error bars show standard errors.

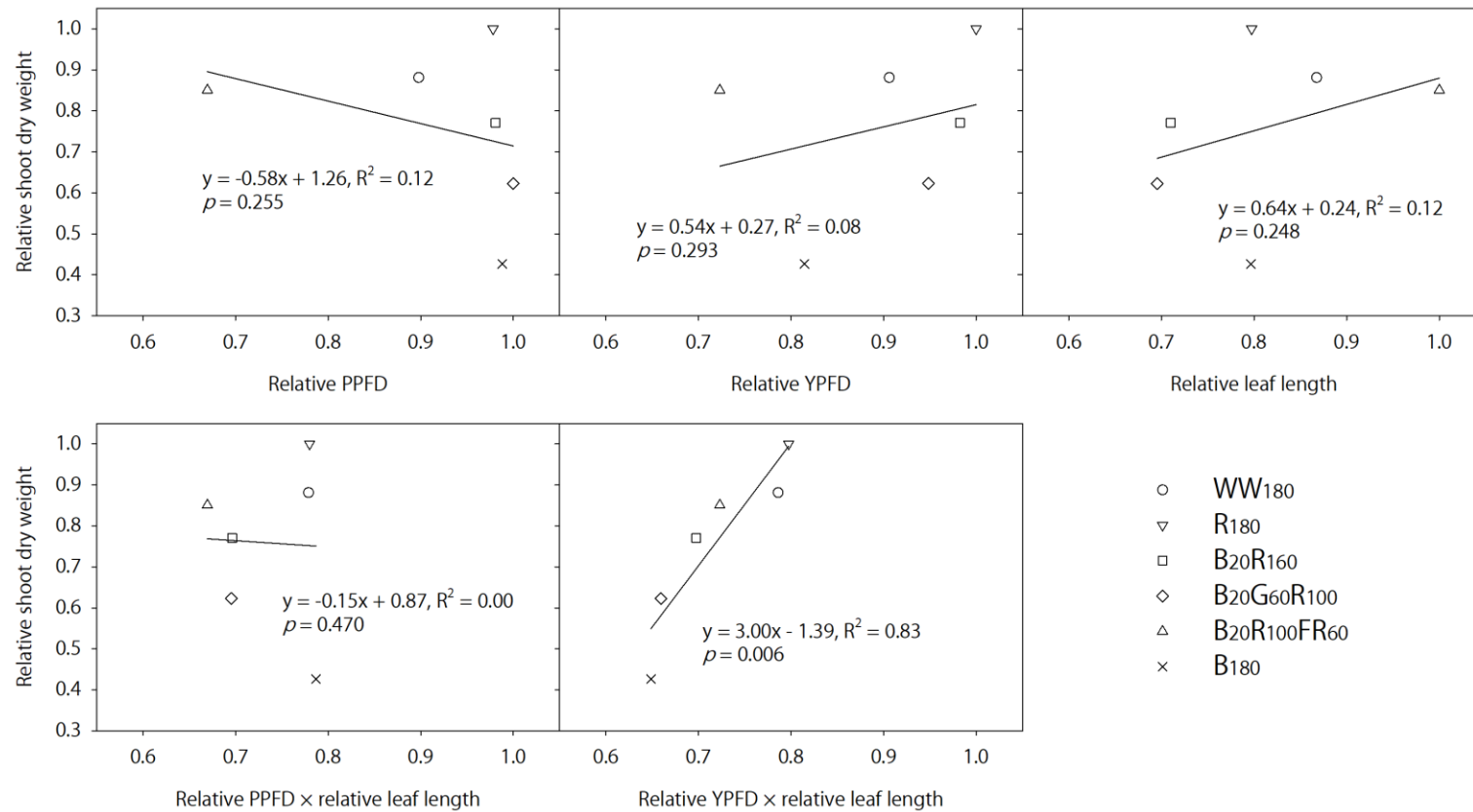


Figure IV-7. Relative shoot dry weight of lettuce 'Rouxai' on day 25 plotted against the relative photosynthetic photon flux density (PPFD), relative yield photon flux density (YPFD), relative leaf length, relative PPFD × relative leaf length, and relative YPFD × relative leaf length. Plants were grown under six static lighting treatments consisting of warm-white (WW) or mixed blue (B; 400–500 nm), green (G; 500–600 nm), red (R; 600–700 nm), and far-red (FR; 700–800 nm) light-emitting diodes (LEDs). The number for each LED type is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Data were averaged for each lighting treatment from two blocks. Linear regression equations, coefficients of determination, and p-values for slopes are provided. The only significant linear relationship occurs between relative YPFD × relative leaf length and relative shoot dry weight ($\alpha = 0.05$).

LITERATURE CITED

LITERATURE CITED

- Armbruster, U., Carrillo, L.R., Venema, K., Pavlovic, L., Schmidtman, E., Kornfeld, A., Jahns, P., Berry, J.A., Kramer, D.M. and Jonikas, M.C., 2014. Ion antiport accelerates photosynthetic acclimation in fluctuating light environments. *Nat. Commun.* 5:5439 doi:10.1038/ncomms6439.
- Bird, A.P., 1993. Imprints on islands. *Curr. Biol.* 3:275–277.
- Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R. and Ahmad, M., 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J. Biol. Chem.* 282:9383–9391.
- Brodersen, C.R. and Vogelmann, T.C., 2010. Do changes in light direction affect absorption profiles in leaves?. *Funct. Plant Biol.* 37:403–412.
- Carvalho, S.D. and K.M. Folta. 2014a. Environmentally modified organisms—expanding genetic potential with light. *Critical Rev. Plant Sci.* 33:486–508.
- Carvalho, S.D. and Folta, K.M., 2014b. Sequential light programs shape kale (*Brassica napus*) sprout appearance and alter metabolic and nutrient content. *Hortic. Res.* 1:8 doi:10.1038/hortres.2014.8.
- Cerdán, P.D. and J. Chory. 2003. Regulation of flowering time by light quality. *Nature* 423:881–885.
- Chang, C.L. and Chang, K.P., 2014. The growth response of leaf lettuce at different stages to multiple wavelength-band light-emitting diode lighting. *Sci. Hortic.* 179:78–84.
- Chen, X.L., Guo, W.Z., Xue, X.Z., Wang, L.C. and Qiao, X.J., 2014. Growth and quality responses of ‘Green Oak Leaf’ lettuce as affected by monochromatic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). *Sci. Hortic.* 172:168–175.
- Cope, K.R., M.C. Snowden, B. Bugbee. 2014. Photobiological interactions of blue light and photosynthetic photon flux: Effects of monochromatic and broad-spectrum light sources. *Photochem. Photobiol.* 90:574–584.
- Demmig-Adams, B., Cohu, C.M., Muller, O. and Adams, W.W., 2012. Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynth. Res.* 113:75–88.
- Eskins, K., Warner, K. and Felker, F.C., 1995. Light quality during early seedling development influences the morphology and bitter taste intensity of mature lettuce (*Lactuca sativa*) leaves. *J. Plant Physiol.* 147:709–713.

- Folta, K.M. and Maruhnich, S.A., 2007. Green light: a signal to slow down or stop. *J. Exp. Bot.* 58:3099–3111.
- Franklin, K.A., 2016. Photomorphogenesis: Plants feel blue in the shade. *Curr. Biol.* 26:R1275–R1276.
- Hernández, R. and Kubota, C., 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot.* 121:66–74.
- Jishi, T., Kimura, K., Matsuda, R. and Fujiwara, K., 2016. Effects of temporally shifted irradiation of blue and red LED light on cos lettuce growth and morphology. *Sci. Hortic.* 198:227–232.
- Johkan, M., Shoji, K., Goto, F., Hashida, S.N. and 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.
- Kang, W.H., Park, J.S., Park, K.S. and Son, J.E., 2016. Leaf photosynthetic rate, growth, and morphology of lettuce under different fractions of red, blue, and green light from light-emitting diodes (LEDs). *Hortic. Environ. Biotechnol.* 57:573–579.
- Kim, H.H., Goins, G.D., Wheeler, R.M. and Sager, J.C., 2004. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. *HortScience* 39:1617–1622.
- Kopsell, D.A., Sams, C.E. and Morrow, R.C., 2015. Blue wavelengths from LED lighting increase nutritionally important metabolites in specialty crops. *HortScience* 50:1285–1288.
- Lee, J.S., T.G. Lim, and Y.H. Kim. 2014. Growth and phytochemicals in lettuce as affected by different ratios of blue to red LED radiation. *Acta Hortic.* 1037:843–848.
- Li, T., Jia, K.P., Lian, H.L., Yang, X., Li, L. and 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.
- Li, Q. and Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67:59–64.
- Liu, X.Y., Chang, T.T., Guo, S.R., Xu, Z.G. and Li, J., 2009. Effect of different light quality of LED on growth and photosynthetic character in cherry tomato seedling. *Acta. Hortic.* 907:325–330.
- Liu, Z., Zhang, Y., Wang, J., Li, P., Zhao, C., Chen, Y. and Bi, Y., 2015. Phytochrome-interacting factors PIF4 and PIF5 negatively regulate anthocyanin biosynthesis under red light in *Arabidopsis* seedlings. *Plant Sci.* 238:64–72.
- McCree, K.J., 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agric. Meteorol.* 9:191–216.

- Ohashi-Kaneko, K., M. Takase, N. Kon, K. Fujiwara, and K. Kurata. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environ. Control Biol.* 45:189–198.
- Owen, W.G. and 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.
- Park, Y. and 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.
- Park, Y. and Runkle, E.S., 2018. Far-red radiation and photosynthetic photon flux density independently regulate seedling growth but interactively regulate flowering. *Environ. Exp. Bot.* <https://doi.org/10.1016/j.envexpbot.2018.06.033>.
- Pedmale, U.V., Huang, S.S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A., Sridevi, P., Nito, K., Nery, J.R. and Ecker, J.R., 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* 164:233–245.
- Sager, J.C., Smith, W.O., Edwards, J.L. and Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. Am. Soc. Agric. Eng.* 31:1882–1889.
- Snowden, M.C., Cope, K.R. and Bugbee, B., 2016. Sensitivity of seven diverse species to blue and green light: interactions with photon flux. *PloS ONE* 11(10):e0163121.
- Son, K.H., Lee, J.H., Oh, Y., Kim, D., Oh, M.M. and In, B.C., 2017. Growth and bioactive compound synthesis in cultivated lettuce subject to light-quality changes. *HortScience* 52:584–591.
- Son, K.H. and 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.
- Terashima, I., Fujita, T., Inoue, T., Chow, W.S. and Oguchi, R., 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. *Plant Cell Physiol.* 50:684–697.
- Wang, Y. and Folta, K.M., 2013. Contributions of green light to plant growth and development. *Am. J. Bot.* 100:70–78.
- Wollaeger, H.M. and Runkle, E.S., 2014. Growth of impatiens, petunia, salvia, and tomato seedlings under blue, green, and red light-emitting diodes. *HortScience* 49:734–740.
- Wollaeger, H.M. and Runkle, E.S., 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. *HortScience* 50:522–529.
- Zhang, T. and Folta, K.M., 2012. Green light signaling and adaptive response. *Plant Signal.*

Behav. 7:75–78.

Zhang, T., Maruhnich, S.A. and Folta, K.M., 2011. Green light induces shade avoidance symptoms. *Plant Physiol.* 111.180661.

Zheng, X., Wu, S., Zhai, H., Zhou, P., Song, M., Su, L., Xi, Y., Li, Z., Cai, Y., Meng, F. and Yang, L., 2013. *Arabidopsis* phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. *Plant Cell tpc.*112.107086.