ABSTRACT

SPALHOLZ, HANS. Development of Novel Lighting Strategies for Optimal Lettuce Growth and Quality. (Under the direction of Dr. Ricardo Hernández and Dr. Brian Earl Whipker)

Advances in horticultural lighting, revolutionized by light-emitting diodes (LEDs), have created many new opportunities for the indoor horticulture industry. Static light spectrum, or fixed spectrum, technologies have dominated lighting strategies. Now, LEDs allow for tunable, narrow- or wide-spectrum emission, and convenient deployment (i.e., non-infrared cool radiation emission for close-canopy placement and longer life span) that opens new opportunities for new and novel lighting strategies. Research here explores how using dynamic light strategies, where spectrum is changed based on upon plant growing stage, to improve plant growth, morphology, and development for indoor lettuce production. Three objectives and experiments were done as follows: (1) assess the effects of different, fixed light recipe blue:red light (B:R) photon flux density (PFD) and simulated sun spectra on growth, development, and phytochemical content for three marketable stages of 'Red Oakleaf' and 'Green Oakleaf' lettuce (*Lactuca sativa* L.) (transplant, baby-leaf, and mature head-lettuce), (2) compare the effects of fixed and dynamic light recipes using B:R PFD ratios to produce 'Red Oakleaf' lettuce, and (3) using a sun-simulated LED light recipe, characterize how color components of sunlight (in terms of B, green (G), R, and far-red (Fr)) contribute to the traits of lettuce bolting, flowering, and resource partitioning between leaves and stems. In the fixed light spectrum experiment, it was found that 20B:80R, 50B:50R and 80B:20R LED treatments were optimal for transplant stage production with 13-43% lower fresh-mass:dry-mass ratio, and 62-94% greater leaf mass area than other light treatments. Although transplant lettuce grown under 100B was in the lowest rank group of dry mass production, and leaf mass area (LMA), the study also demonstrated the benefits of 100B for later lettuce stages. For example, plants in 100B had a greater leaf area and a similar fresh

mass and dry mass as compared to other B:R PFD light treatments for the head-lettuce stage (42 days old). Yet, 'Red Oakleaf' lettuce grown in 100B lacked red coloration having only 25% of the anthocyanin content of 50B:50R lettuce, a top ranked treatment in terms of anthocyanin. These results were used to design dynamic light recipes for customized growth and to optimize d plant growth and phytochemical concentration at crop stages. In the second experiment, dynamic light recipes were compared to fixed light recipes for lettuce production focused at (1) transplant stage, (2) post-transplant stage (transplant to mature harvest period), and (3) end-of-production coloration. Dynamic light recipes were found to improve dry mass content and plant canopy diameter while an end-of-production coloration treatment increased anthocyanin content using a dynamic light recipe consisting of 20B:80R (Day 3-18), 100B (day 19-39), and 50B:50R (day 40-47). In the final experiment, different colors of a sun-simulated LED light treatment were used to test their contribution to lettuce bolting and stem elongation. Here, G and a low R/Fr light had a confounding effect on stem elongation compared to low R/Fr light alone. Yet the ratio of leaf-mass:stem-mass was dependent on the R/Fr light ratio and not the inclusion of G light. Overall, this experiment demonstrated that the experimental light recipes impact the transition from the vegetative to the reproductive stage of lettuce. All of these experiments combined demonstrate the role that light quality and dynamic light recipes possess to customize plant growth and development. Applying these concepts can help improve crop productivity and nutritional components of indoor grown lettuce.

Development of Novel Lighting Strategies for Optimal Lettuce Growth and Quality

by
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DEDICATION

This thesis, and the work culminating to it, is dedicated to family and friends that have always been there to support, advise, and encourage me.

It is also dedicated to my father, who was always proud to know my horizon would be expanding. He saw me set out on my PhD journey, and was excited to see where it would take me, he did not get the chance to see the fruits of this labor in person. I am sure he is proud, I and my siblings, have never lost sight of pushing for the next achievement and always wanting the most out of life.

Hande miin awat, jango miin hawrat. Fankanta!

(Today we seed, tomorrow we harvest. Almost there!

BIOGRAPHY

Hans Spalholz was born in the rolling hills of central New York to Candes Bradbury and Hans J. Spalholz. His interest in horticulture and the natural world sprung from time spent in his local environs. He completed a bachelor's degree in Plant Science at Cornell University in 2006. From 2007 to 2009 and again in 2011 Hans served in Peace Corps/Senegal to improve food security and expand horticultural opportunities for local farmers and families dependent on their own ability to feed themselves. In 2011, Hans started a Master's degree at the University of Arizona under the guidance and direction of Dr. Chieri Kubota focusing on controlled environment agriculture and long-term cold storage of grafted vegetable seedlings. After completing his Master's in 2013 Hans went to the IGZ research Institute, near Berlin, Germany, to assist in experimentation on the impact of tomato rootstock germplasm and light quantity on fruit quality. In 2016, Hans returned to the United States to begin his doctoral studies at North Carolina State University under the direction of Dr. Ricardo Hernández.

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CHAPTER 1

Transplant lettuce response to different percent blue:red photon flux density ratios in indoor LED sole source lighting production

1.1 Abstract

As indoor growing systems play an increasing role in transplant crop production, light optimization must be developed to increase plant growth, improve morphology, and reduce electrical energy costs. Light emitting diodes (LEDs) are an increasingly used technology for the production of transplants. The objective of this experiment is to evaluate the effects of different percentages of blue (B) and red (R) photon flux density (PFD) ratios for the production of 'Red Oakleaf' and 'Green Oakleaf' lettuce transplants. Plants were grown for 16 days with LEDs in a light-quality growth chamber with 78.7 ± 1.4% RH, 19.6 ± 1.2 °C, and 812 ± 268 µmol mol -1 CO_2. Five LED light treatments consisted of different percentages of B (peak: 452 nm) and R (peak: 659 nm) PFD ratios of 0B:100R, 20B:80R, 50B:50R, 80B:20R, and 100B:0R. A fluorescent (FL) treatment was used as control. Plants were provided with 200 µmol·m^{-2}·s^{-1} photosynthetically active radiation (400-700 nm) for 18 h. Transplants in 20B:80R had greater shoot dry mass than plants in all other LED treatments. For example, transplant lettuce in 20B:80R had 15% more shoot dry mass than in the 50B:50R LED light treatment. Transplant lettuce in 100B:0R had in average 19% fewer leaves than plants in all other treatments. This preliminary study demonstrates that 20B:80R percent PFD is the preferred light quality for the production of lettuce transplant in indoor growing systems.

1.2 Introduction

Indoor production, also known as vertical farming (VF), is projected to increase at a compound annual growth rate of 25% per year (marketsandmarkets, 2017). The use of LEDs in VF offers the unique opportunity to customize the light spectrum to the plant species to maximize growth while also improving plant quality (morphology, phytochemical accumulation, hardiness, etc). Transplants or young seedlings that are grown in VF systems offer many advantages such as disease, insect, and chemical free transplants (Kozai, 2016), and a 30-40% decrease in production time has been reported when environmental conditions are optimized (Kozai, 2016).

Historically there has been a market for lettuce transplants in many countries and the interest continues to increase (Nicola & Cantliffe, 1996; Kitaya et al., 1998). The advantages of using lettuce transplants instead of direct seeding into the field include a faster production cycle, reduced plant exposure to adverse field conditions and increased flexibility with meeting market determined production windows. Additionally, bolting in the field could be delayed by producing transplants under controlled temperatures and photoperiods in VFs. For example, in VF conditions, it was found that photoperiod could impact sequential bolting of spinach (*Spinacia oleracea* L.) (Chun et al., 2000),

In commercial VF systems, the combination of blue (400-500 nm) and red (600-700 nm) LED lights is used extensively to grow an array of crops, such as leafy greens (Hoenecke et al., 1992; Massa et al., 2008). However, less research is available for spectral optimization to maximize quality and yield of lettuce transplants. Certain morphological traits are used to determine lettuce transplant quality. Those of high quality are considered to be more compact relative to their greenhouse or field direct-seeded analogs (Kitaya et al 1998; Kozai, 2016). In

addition to this preferred morphology, lettuce transplants should have a higher percent dry mass and a lowered shoot:root ratio as well as a lowered specific leaf area (SLA). These requirements differ from leafy or head lettuce production since higher fresh mass and high leaf area are preferred while compactness is considered detrimental.

Determining the proper ratio of blue to red light is critical to optimizing lettuce transplant production while also maintaining normal plant growth and development. Abnormal growth and development such as "red light syndrome" (Trouwborst et al., 2016) or excessive seedling hypocotyl elongation (Hernandez & Kubota, 2016) can occur if grown under 100% monochromatic red or blue light, respectively. Thus, finding a ratio of blue to red light that is most favorable for a specific crop is important to the transplant producer. Research has shown that light recipes that contain 5-20% blue and 80-95% red light result in the greatest biomass accumulation (Poulet et al., 2015, and Son & Oh, 2015) for both head and baby-leaf lettuce.

In this study, lettuce (*Lactuca sativa* L.) cultivars 'Red Oakleaf' and 'Green Oakleaf' transplants were grown under varied percentages of B:R photon flux PFD ratios in order to find a spectrum that will increase lettuce transplant quality and growth rate.

1.3 Materials and methods

1.3.1 Plant material and substrate

Pelletized lettuce seed 'Red Oakleaf' and 'Green Oakleaf' were sown in 1P Fafard (Conrad Fafard Inc., Agawam, MA, USA) potting mix that consisted of 77% peat, 23% perlite, and lime in 98-cell trays (725 plants m^{-2}). Seeds were then covered with a thin layer of vermiculite and germinated at 24 °C under continuous fluorescent lighting at 80 µmol m^{-2} s^{-1} for 48 h (day 0-2). After 48 h plants were then moved to the experimental chamber.

1.3.2 Chamber parameters & experimental treatments

Trays of germinated lettuce were placed in a growth chamber that consisted of seven compartments for individual light treatments. Chamber and treatment conditions are shown in Table 1. Five LED light treatments with different percentages of B:R PF ratios were evaluated: 0B:100R, 20B:80R, 50B:50R, 80B:20R, and 100B:0R. LED peaks were 452 nm for B and 659 nm for R. In addition, an array of fluorescent fixtures (T5, 6500 °K, Sunlight Supply Inc., Vancouver, WA, USA) was used as a control. From day 3-9, treatment light intensity was maintained at 100 µmol m^{-2} s^{-1} with intensity increased to 200 µmol m^{-2} s^{-1} from day 10-17 (18 h photoperiod). From day 2-9 plants were sub-fertigated as needed with hydroponic solution containing (mg L^{-1}) 45 N, 24 P, 72 K, 72 Ca, 30 Mg, 58 S, 45 Cl, 0.34 B, 0.55 Mn, 0.05 Cu, 0.05 Mo, 0.33 Zn, 2 Fe. From day 9-16, additional 50 N, 103 K, 36 Ca were added to the nutrient formulation.

1.3.3 Measurements and data collection

Morphological data was taken on 10-20 randomly selected plants per replication from each cultivar on day 18 (16 d of LED light treatment). Measurements on harvested plants included leaf count, longest leaf, leaf area, fresh mass, and dry mass (dried for 72 h at 65 °C). A threshold of 1 cm in leaf length qualified leaves for leaf count. Leaves were scanned and then processed with ImageJ 1.51g software (Schneider et al., 2012) to calculate leaf area. Statistical analysis for comparing the different treatments was done using Tukey tests ($p<0.05$) with JMP software Version Pro 12 (Cary, NC, US). Data presented here are the combined results of two repetitions conducted at two different times. No interactions were observed between the treatments and replications, and the treatments and cultivars thus results for ‘Red Oakleaf’ and ‘Green Oakleaf’ lettuce were combined.

1.4 Results and discussion

1.4.1 Longest leaf, leaf count, & leaf development

The ratio of B:R light treatment had no impact on leaf count except for when plants received 100B light which decreased transplant lettuce leaf count (Figure 1). Plants in 100B and 100R resulted in the longest and second longest leaf lengths, respectively, while treatments that included any quantity of both B and R light had no significant differences in leaf length (Figure 2). The longest leaves of plants treated with 100B and 100R light were 129% and 90% longer, respectively, than in 80B:20R which had the shortest leaves. Similarly, Son and Oh (2013) found that 2-week and 4-week old lettuce in the 100% red light treatment (peak 655 nm, 12 h photoperiod, 171 ± μmol m^{-2} s^{-1}) resulted in leaves with the greatest leaf shape index (defined as length:width) compared to all other blue to red light combinations. Son and Oh (2013) did not include a 100B treatment. Although accelerating growth is ideal, transplants with long leaf lengths are not necessarily beneficial as they may become more easily damaged during transportation or at time of transplanting. Thus, high quality lettuce transplants would have lowered leaf lengths while not reducing or penalizing dry mass. Results from this study show that any B:R light recipe that includes both blue and red light will reduce leaf length.

Visually, 100R resulted in distinct and unique plant morphology (Figure 3). 100R treatment resulted in long abnormally shaped leaves that would curl over as well as remain lighter colored when compared to transplants in different treatments (Figure 3). In the case of "Red Oakleaf" lettuce, which is a red-type lettuce, a lack of red pigmentation was noted under 100R (data not shown). The effect of monochromatic red light has been observed in previous studies (Yanagi et al., 1996) and described as "red light syndrome" (RLS) (Trouwborst et al., 2016). RLS occurs in the complete absence of blue light that is needed to activate Phototropin1

(phot1) to elucidate proper leaf orientation and promote optimal light capture (Inoue et al., 2008). Without phot1 activation, the leaf continually bends and curls as it grows and develops (Inoue et al., 2008). Also, plants with RLS lack normal anthocyanin synthesis induction which needs blue light response of the cryptochrome (cry1 and cry2) (Ohgishi et al., 2004). Furthermore, this experiment was conducted using 200 µmol m^{-2} s^{-1}, a relatively low light intensity for conventional crop cultivation, perhaps growing crops under high intensity monochromatic red light would not only result in morphological abnormalities but also potentially damage the photo-harvesting apparatus. In this instance, blue light is needed to perceive and regulate high light avoidance of the chloroplasts via NPL1 activation (Jarillo et al., 2001; Kagawa et al., 2001).

Plants in 100B light also resulted in a distinct morphology where plants had rounder, more upright leaves (Figure 3). This phenomenon was also present in previous studies (Yanagi et al., 1996) and occurs in the absence of phytochrome stimulation which prevents responses that are typical of high B ratio light recipes (Hernández and Kubota, 2016). This is known as the "coaction" effect which is evident in the absence of red light which ultimately leads to inhibition of cryptochrome activity (Hernández and Kubota, 2016).

1.4.2 Fresh and dry mass

Fresh mass and dry mass are important indicators when accessing transplant plug quality. Light treatments 100R and 20B:80R both belonged to the highest-ranking group for fresh mass of the B:R ratio treatments (Figure 4). For example, plants in 100R and 20B:80R light treatments had 45% and 32% greater fresh mass than plants under 80B:20R. In addition, the increase of B PF linearly decreased lettuce fresh mass ($P < 0.0001$) (when not including the fluorescent control and 100B treatment in the regression analysis). Meanwhile 50B:50R, 80B:20R, and 100B light

treated transplants resulted in the lowest performing group for fresh masses. Nicole et al. (2016) found a similar result upon fresh mass and also noted plants became more compact as the percent B PF increased. Similarly, dry mass decreased as B PF increased ($P < 0.0001$) (excluding plants from the 100R and 100B treatments) (Figure 5). These results, for fresh mass and dry mass, could be explained in part by the decreasing ratio of red light, which has the greatest relative quantum yield (RQY). Although red light alone is not sufficient to satisfy all qualitative aspects of normal plant growth and development it is crucial to illicit efficient photosynthetic responses using radiation with the highest action spectra (McCree 1972). Thus, as the ratio of B PFD increased, fresh mass and dry mass decreased. Interestingly, transplants treated with 100R light had 10% larger fresh mass than 20B:80R but when comparing dry mass 100R was 19% smaller than transplants under 20B:80R, contradicting the potential correlation that increased fresh mass resulted in increased dry mass. Thus 20B:80R had a larger dry mass to fresh mass ratio (DM:FM) ($P < 0.0001$, data not shown). One possible explanation for this phenomenon is that plants under RLS conditions (100R) allocate greater resources to mitigate stress rather than to biomass production. A further possible reason that could lead to a decreased DM:FM in monochromatic red light could be the absence of B PF. This qualitative aspect of B light is required to satisfy metabolic pathways to maintain the circadian rhythm. If circadian rhythm is disrupted this would subsequently impact whole plant osmotic status at the cellular level via ZTL inactivation affecting solute transport (Haydon et al., 2011). Deviations in normal ion and sugar fluxes, possibly due to the absence of blue light, at the cellular level, could decrease cell division and sequential dry mass formation in the plant. This is one possible explanation for this phenomenon but further study is needed to determine the specific mechanisms for the decreased DM:FM.

1.4.3 Leaf area and specific leaf area

Leaf area and specific leaf area (SLA) are also important parameters when accessing morphological transplant quality. In the present study, leaf area decreased as the amount of B PF increased ($P < 0.0001$) (Figure 6). Transplants under 50B:50R and 80B:20R light belonged to the lowest ranking group for leaf area while those illuminated with 100R and 100B belonged to the highest ranking group. For example, leaf area of transplants in the 80B:20R treatment was decreased by 47% when compared to transplants treated with 100R light (Figure 6). If transplants have a large SLA, or large leaf area to dry mass ratio, then the quality of the transplant is reduced (Kitaya et al., 1998). In the present study 100B and 100R transplant lettuce had the highest SLA while transplants treated with 50B:50R, 80B:20R, and 20B:80R light had the lowest SLA (data not shown). One possible explanation for this is that B PF triggers a high irradiance response and that as the percentage of B PF increases leaf area decreases (Hogewoning et al., 2010; Hernández and Kubota, 2014), yet phytochrome and cryptochrome coaction is necessary to illicit this response (Hernández and Kubota, 2016).

1.5 Conclusion

In the present study it was determined that the 20B:80R light treatment resulted in transplants with the greatest dry mass, dry mass to fresh mass ratio, and the lowest SLA and thus is recommended as the optimal B:R PF ratio of the evaluated treatments. Additional work is needed to evaluate if there is a combination of light recipes (dynamic lighting) that can be used to grow higher quality or customized transplant lettuce. Future work will be to evaluate different dynamic light recipes to improve transplant quality relative to fixed light recipes.

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Table 1.1 Treatment and environmental conditions used to grow transplant lettuce under different lighting treatments.

Parameter	Units	Day	Night
Relative Humidity	%	80.2 ± 1.4	74.1 ± 1.2
Atmospheric CO_2	µmol mol^{-1}	750 ± 278	999 ± 171
Photoperiod	h	18	6
Light Intensity (BAR)	µmol m^{-2} s^{-1}	Day 3-9: 100, Day 10-17: 200	
Daily Light Integral	mol m^{-2} d^{-1}	Day 3-9: 6.5, Day 10-17: 13	
Nutrient Solution pH, EC	n/a, dS m^{-1}	6.5 ± 0.1, 1.39 ± 0.15	
Air Velocity	m/s	1.02 ± 0.2	
Treatment	**Units**	**Day-T**	**Night-T**
0B:100R	°C	20.2 ± 1.2	18.5 ± 1.2
20B:80R		19.8 ± 1.0	17.8 ± 1.2
50B:50R		19.8 ± 1.2	17.7 ± 1.1
80B:20R		20.0 ± 1.2	17.7 ± 1.1
100B:0R		20.2 ± 1.3	18.1 ± 1.2
FL		20.7 ± 1.3	18.3 ± 1.1

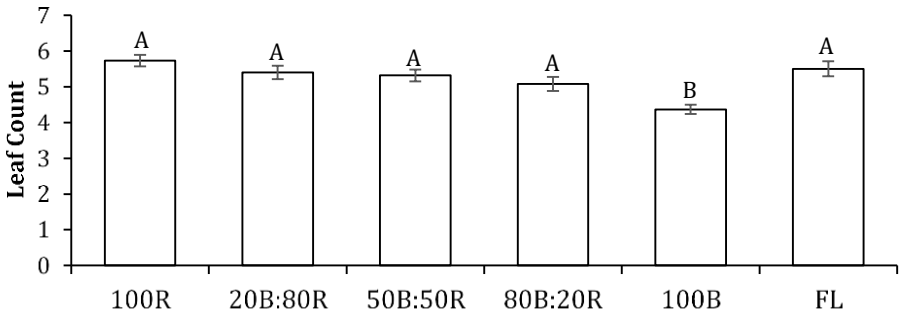

Figure 1.1 Transplant lettuce leaf count grown under 100R, 20B:80R, 50B:50R, 80B:20R, and 100B light treatments and a fluorescent (FL) control. Values are the means of individual transplants (n=40 for replication 1 and n=20 for replication 2). Significant difference was determined by Tukey Test ($P<0.05$).

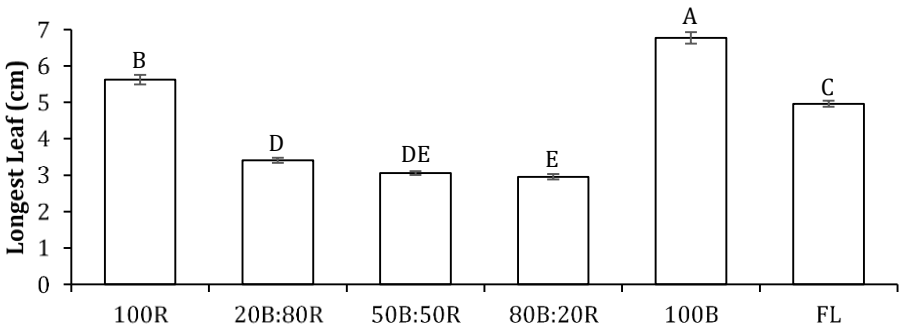

Figure 1.2 Transplant lettuce longest leaf grown under 100R, 20B:80R, 50B:50R, 80B:20R, and 100B light treatments and a fluorescent (FL) control. Values are the means of individual transplants (n=40 for replication 1 and n=20 for replication 2). Significant difference was determined by Tukey Test ($P<0.05$).

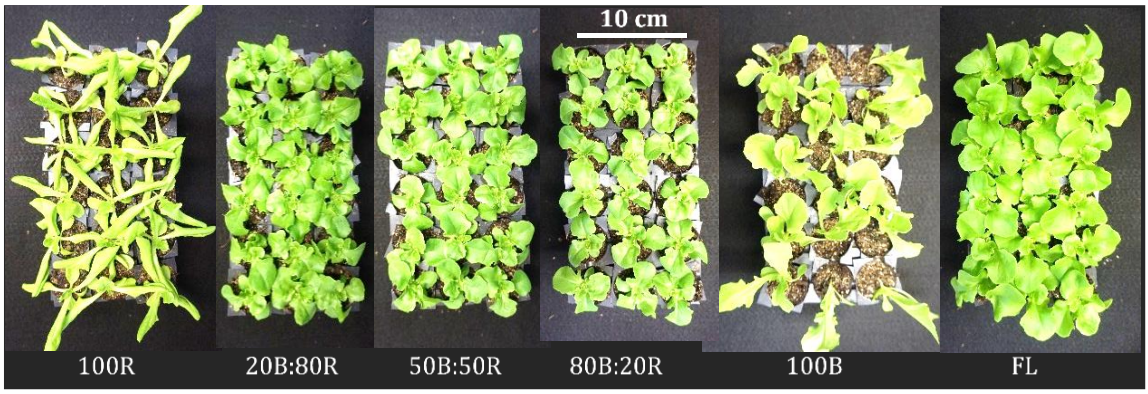

Figure 1.3 Visual appearance of ‘Green Oakleaf’ transplant lettuce grown under different lighting treatments.

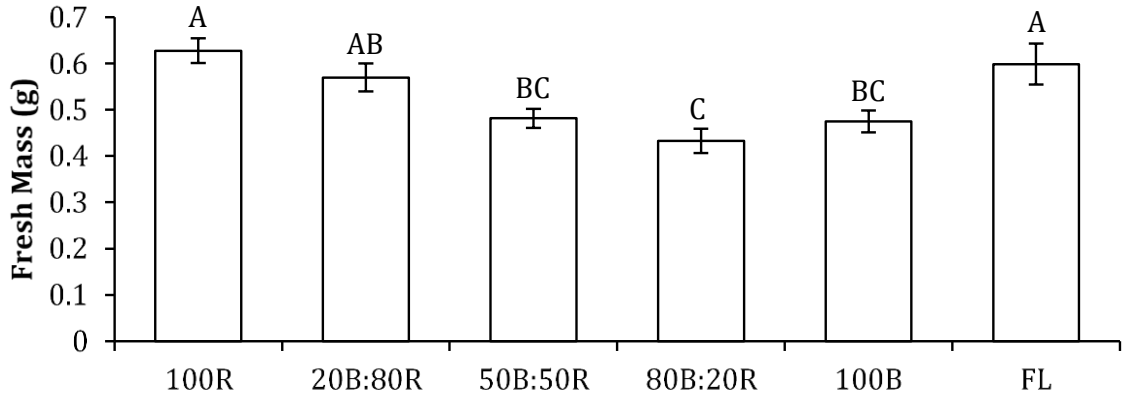

Figure 1.4 Transplant lettuce fresh mass under 100R, 20B:80R, 50B:50R, 80B:20R, and 100B light treatments and a fluorescent (FL) control. Values are the means of individual transplants (n=40 for replication 1 and n=20 for replication 2). Significant difference was determined by Tukey Test ($P<0.05$).

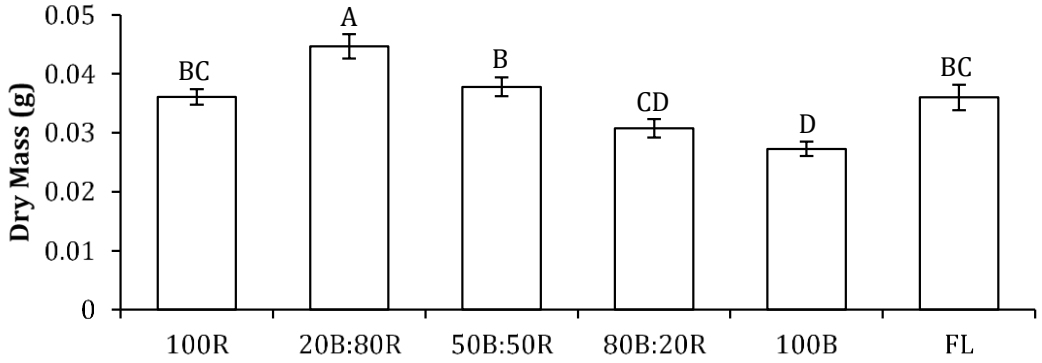

Figure 1.5 Transplant lettuce dry mass under 100R, 20B:80R, 50B:50R, 80B:20R, and 100B light treatments and a fluorescent (FL) control. Values are the means of individual transplants (n=40 for replication 1 and n=20 for replication 2). Significant difference was determined by Tukey Test ($P<0.05$).

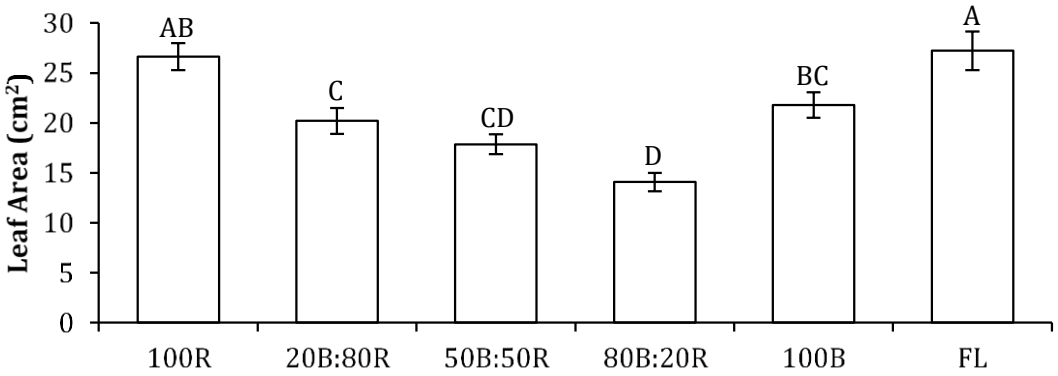

Figure 1.6 Transplant lettuce leaf area under 100R, 20B:80R, 50B:50R, 80B:20R, and 100B light treatments and a fluorescent (FL) control. Values are the means of individual transplants (n=40 for replication 1 and n=20 for replication 2). Significant difference was determined by Tukey Test ($P<0.05$).

CHAPTER 2

Impact of sun-simulated and varied B:R spectrums on the growth rate, morphology, phytochemistry, and development of green and red transplant, loose leaf, and head lettuce

2.1 Abstract

Light is a critical environmental factor that drives photosynthesis and regulates plant morphology, physiology, and phytochemical content. As indoor growing systems play an increasing role in leafy green production, light optimization must be developed to increase plant growth, improve morphology, and increase nutritional content. The aim of this study was to assess the effects of different spectra on the different lettuce marketable stages (transplant, loose-leaf, and head-lettuce) growth, development, and phytochemical content over time. 'Red Oakleaf' and 'Green Oakleaf' lettuce was grown under seven spectra. A sun-simulated light treatment (SUN) was created with 5.1UV:20.0B:26.1G:26.3R:22.6Fr percent photon flux density (PFD) ratio using 388 nm, 407 nm, 424 nm, 524 nm, 625 nm, 660 nm, 733 nm and 5700K LED diodes. In addition, five treatments with different B:R percent PFD ratios were evaluated: 0B:100R (100R), 20B:80R, 50B:50R, 80B:20R, 100B:0R (100B). Fluorescent white light was used as a control (6500K). Plants were provided with 200 ± 0.7 μmol·m^{-2}·s^{-1} biologically active radiation (300-800 nm) for 18 h and grown at 20.0 ± 0.2 °C temperature. It was found that 20B:80R, 50B:50R and 80B:20R LED treatments were optimal at the transplant stage with 13-43% lower fresh-mass:dry-mass ratio, and 62-94% greater leaf mass area than other light treatments. Results from this study also demonstrated the benefits of 100B for larger lettuce stages as plants in 100B had similar fresh mass and dry mass for the head-lettuce stage (42 d) compared to other B:R PFD light treatments. In addition, plants in the SUN treatment initiated a reproductive response of bolting and rosette formation with stem lengths 2.1-4.4x longer than plants in any other treatment. Plant total phenolic content for both "Red Oakleaf' and 'Green Oakleaf' lettuce was 39-128% greater in 20B:80R, 50B:50R, 80B:20R than in other light

treatments. Anthocyanin concentration was highest in plants under 50B:50R and nitrate content increased with the increase of B PFD.

2.2 Introduction

Light is a critical environmental factor that drives photosynthesis and regulates plant morphology, physiology, and phytochemical content. The development and use of electrical lighting, especially improvements in light emitting diode (LED) fixtures, has allowed both researchers and growers to study plant-light physiology and apply this knowledge to crop production systems. One such application is vertical farming (VF), a rapidly expanding agricultural sector that relies solely on electrical lighting as source for photosynthesis. Many VF growers produce leafy greens, such as lettuce and vegetative plants of high value as their main crop. Since yield photon flux (YPF) peaks in Blue (B) and Red (R) light (McCree, 1972) LED manufacturers and growers have focused on using these respective diode colors to maximize photosynthetic activity and omit spectra that has a lower RQE. In addition, as LED technology has become more versatile in terms of efficiency and diode availability, it is possible to formulate a wide range of spectral combinations via LEDs.

Initial LED studies using either monochromatic B or R LED light have shown that selecting wavelengths that coincide only with high YPF did not result in acceptable and marketable plant morphology. On the contrary, the use of multispectral combinations of B and R light reduce abnormal growth while plant dry mass is increased when compared to monochromatic spectra. For example, early studies found that B photon flux density (PFD) was needed to prevent abnormal leaf curling and leaf orientation when R was the sole source of PF (Hoenecke et al., 1992; Yanagi et al., 1996). This abnormal morphogenesis, in addition to other traits associated with monochromatic or 100% R PFD (100R), is otherwise known as red light syndrome (RLS) as described by Trouwborst et al. (2016) in experimentation with cucumber. Similarly, the use of monochromatic B PFD (100B) results in both satisfactory and

unsatisfactory growth depending on plant developmental stage. The use of 100B on young and newly germinated plants can initially lead to “blue light syndrome” (BLS) where young plants demonstrate spindly growth that prevents normal upright plant and leaf orientation for optimal light capture (Jishi et al., 2016). Yet lettuce plants can still adapt to 100B, develop upright stature, and yield fresh mass equal to that of B:R light treatment (Amoozgar et al, 2017). For both RLS and BLS the underlying cause could be explained by the requirement of both phytochrome and cryptochrome activation needed to ensure normal morphology. Recent studies have shown that both the phytochrome and cryptochrome may rely on cross feedback in nature, providing negative feedback for one and other, for general plant functions (Wang et al., 2018). Other studies have focused on threshold requirements of certain color light to obtain normal physiology (Massa et al., 2008).

Earlier studies have shown the influence of B:R PF ratio on plant growth. Son and Oh (2013) evaluated two lettuce cultivars treated with various B:R PF ratios for 4-weeks and found that plant fresh mass decreased as the ratio of B increased. Wang et al. (2016) found similar results, with 7.7B:92.3R yielding the largest dry mass of B:R PF ratios yet interestingly 50B:50R resulted in the highest photosynthetic rate and capacity. Son and Oh (2015) also found that photosynthetic activity lacked correlation between B:R PF, dry mass accumulation, and single leaf photosynthesis. Furthermore, in B:R PF experiments there appears to be no correlation between the amount of chlorophyll content and photosynthetic rate (Amoozgar et al., 2017).

The ratio of different B:R PF also plays a large role on lettuce leaf number and leaf area. Wang et al. (2016) found that as the ratio of R PF increased so did leaf number and leaf area and were largest at 11.1B:88.9R and 7.7B:92.3R, respectively. Inclusion of white LED light of different quality with the B:R PF ratio played a large role in the amount of leaf number and leaf

area (Han et al. 2017). Han et al. (2017) deemed that a B:R PF ratio that included significant broad spectrum green and far-red was best as it resulted in higher dry mass than just a B:R PF alone. Recently, the wide availability of LED diodes has made it possible to create a LED spectrum that approaches the ratio of the 100 nm wavelength intervals for UV-A, B, G, R, and Fr of the sun spectrum; however, no research is available on comparing lettuce growth, development, and morphology under sun-simulated light spectrum to common B:R ratio spectrums.

The ratio of B and R light can also have a large role in phytochemical content of lettuce. Amoozgar et al. (2017) found that lettuce grown under 100B LED light had higher levels of vitamin C when compared to 100R, 30B:70R, or white LED light while 30B:70R enhanced chlorophyll and carotenoid content. Under monochromatic light, 100B light resulted in the highest total phenolic concentration, 2-5 times higher than 100R depending on harvest day and cultivar (Son and Oh, 2012). Total phenolic content was highest in lettuce treated with 30B:70R, 57% greater than when the lettuce was grown in 10B:90R (Son and Oh, 2015). Addition of green (G) LED light with B:R PF light did not impact total phenolic or antioxidant compound levels but decreased nitrate accumulation (Bian et al., 2016). VF growers could use this manipulation to their advantage and employ strategies that produce lettuce high in nutritional components compared to field grown lettuce.

The aim of this experiment was to assess the effects of different B:R PF ratios and white light treatments on plant growth and development over time. As B and R light can satisfy most morphological and commercial requirements of lettuce production, this study focused on how changes in B:R photon flux (PF) ratios impacted morphological changes over time in comparison to two white light treatments, involving multiple harvests. Morphological and growth rate data

were taken to identify which B:R PF ratio optimizes plant growth for each of the respective three marketable stages; 1) transplant, 2) loose-leaf, and 3) full-head stage. Furthermore, since plants have evolved under the sun and, we have the ability, to some extent, to match the sun spectrum, we compared lettuce responses between common B:R PF ratio treatments to a simulated solar irradiation treatment (SUN). The SUN treatment is used to replicate and fulfill the different ratio spectra of UV (300-399 nm), B (400-499 nm), G (500-599 nm), R (600-699 nm), and Fr (700-799 nm). This treatment provides insight as to how LED spectrum similar to solar radiation would compare to different B:R PF ratios and fluorescent white spectrum (FL) at different marketable stages.

2.3 Materials & methods

2.3.1 Plant material and growing conditions

Pelletized lettuce (*Lactuca sativa* L.) seeds 'Green Oakleaf' and 'Red Oakleaf' (Salanova®, Johnny's Selected Seed Corp., Waterville, ME, USA) were sown in 1P Fafard (Conrad Fafard Inc., Agawam, MA, USA) potting mix that consisted of 77% peat, 23% perlite, and lime, in individual cells. Each cell (3.3 cm upper diameter × 2.8 cm lower diameter × 3.9 cm deep with a volume of 21 ml) was then placed in 98-cell trays (725 plants m^{-2}). After sowing, trays were sub-irrigated with water and a thin layer of vermiculite was placed over the seed (2-5 mm thick) with each tray covered in plastic wrap to maintain moisture content. Seeds were germinated at 24 °C under continuous 5000K fluorescent lighting (F17-T8-850, TCP International Holdings Ltd., Cham, CH) at 80 µmol m^{-2} s^{-1} PPF for 48 h (day 0-2) in a growth chamber. At this point (day 2) cotyledons emerged and plants were moved to the experimental chamber. On day 18, lettuce was transplanted into larger pots (7.4 cm × 7.4 cm × 6.3 cm deep

with a volume of 173 ml) and placed at a density of 189 plants m^{-2}. On day 33, plants were spaced at a final density of 125 plants m^{-2} and kept to day 42 (D42) for final plant measurements. From day 2-9 plants were sub-fertigated as needed with hydroponic solution containing (mg L^{-1}) 45 N, 24 P, 72 K, 72 Ca, 30 Mg, 58 S, 45 Cl, 0.34 B, 0.55 Mn, 0.05 Cu, 0.05 Mo, 0.33 Zn, 2 Fe. From day 10-42 the same hydroponic nutrient solution was used and added (mg L^{-1}) 50 N, 103 K, 36 Ca.

2.3.2 Chamber parameters & LED light treatments

The experimental growth chamber consisted of seven compartments for individual light treatments. Each compartment (100 cm wide × 84.5 cm high × 61 cm deep) contained both cultivars. Environmental conditions on the compartments were measured and recorded (CR1000, Campbell Scientific Inc., Logan, UT, USA) to provide growing zone air temperature and substrate temperature (thermocouples, 0.005 gauge, T-type, Omega Inc. Stamford, CT, USA), chamber CO2 concentration (GMT222, Viasala Inc., Helsinki, FI), and chamber relative humidity (CS-215, Campbell Scientific Inc., Logan, UT, USA) (Table 1). All data was measured every five seconds, averaged and recorded every minute. For growing zone air temperature, thermocouples were placed in the center of the growing zone and located 1 cm above the top of the canopy. Treatment air velocity (Table 1) was measured at the start of the experiment, light quality and intensity (Table 2 and Figure 1) was measured at the start and end of the experiment while nutrient solution pH and electrical conductivity (EC) were measured at the time of each irrigation (HI 9813-6, Hannah Instrument Inc, Woonsocket, RI, USA) (Table 1). Treatment air velocity (laminar air flow) was provided by four equally spaced fans in the growing plane and measured at nine different points on a grid in the growing zone. Ten measurements every 30 seconds were taken for each point and averaged.

Light treatment conditions are shown in Table 2 and Figure 1. Five LED light treatments with different percentages of B:R PF ratios were evaluated: 0B:100R (100R), 20B:80R, 50B:50R, 80B:20R, 100B:0R (100B) (LX601, Heliospectra AB, Göteborg, SE). LED peaks for the B:R PF treatments were 452 nm for B (full width at half maximum (FWHM): 23 nm) and 659 nm for R (FWHM: 16 nm). A sun simulated light treatment (SUN) was created by matching the photon flux of an outdoor spectral scan (Raleigh, NC, 35° 47' 16.17" N, 78° 40' 24.22" W, June 20th, 13:20, 2016) at the different 100-nm increment wavelengths of UV (300 nm-399 nm), B (400 nm-499 nm), G (500 nm-599 nm), R (600 nm-699 nm), FR (700 nm-800 nm) using a multi-diode LED fixture (RX30, Heliospectra AB, Göteborg, SE) (Figure 1). Six LED diodes were used for the SUN treatment:, 388 nm for UV (FWHM: 11 nm), 407 nm for one peak B (FWHM: 12 nm), 424 nm for the second peak B (FWHM: 15 nm), 524 nm for G (FWHM: 30 nm), 625 nm for one R peak (FWHM: 17), 660 nm for the second R peak (FWHM: 29 nm), 733 nm for Fr (FWHM: 34 nm), and 5700K white diodes with two peaks, at 450 nm (FWHM: 24 nm) and 577 nm (FWHM: 130 nm). A fluorescent (FL) light treatments with 6500K was used as the control (Sun Blaze 21-T5 HO, Sunlight Supply Inc., Vancouver, WA, USA).

All treatments were set at equal biologically active radiation (BAR: 300-800 nm) and their respective PPF are listed in Table 2. Light measurements were taken at the start and conclusion of each repetition using a spectroradiometer (PS-200, Apogee Instruments, Inc. Logan, UT, USA). BAR is a range of radiation that can impact photosynthesis and photomorphogenesis, whereas photosynthetic active radiation (PAR) does not capture all of this range and the SUN treatment contained radiation outside PAR thus treatments were set to equal BAR (energy consumption between fixtures more similar when fixtures are set at BAR than PAR). From day 3-9, treatment BAR light intensity was maintained at 100 µmol m^{-2} s^{-1} with

intensity increased to 200 µmol m^{-2} s^{-1} at day 10 and remained at this intensity via lamp height adjustment (40 cm from top of canopy for LED fixtures and 17.5 cm for FL) for the remainder of the experiment (both intensities had 18 h photoperiod). Table 2 details the light intensity specifics of the different treatments in terms of BAR, PAR, and yield photon flux (YPF) calculated based on (Sager and McFarlane, 1997). Treated plants were positioned inside a row of border plants to prevent possible edge effects and rotated every two days to equalize light uniformity within the growing area (46 cm × 46 cm). In addition, as both cultivars occupied the same compartment, cultivar group was rotated from one side of the light field to the other at time of plant rotation. The total growing area was 56 cm × 56 cm.

2.3.3 Measurements and data collection

Morphology and growth responses

The experiment was repeated twice at two different times. Transplant stage was harvested on day 17 (D17). For morphological response 15 plants were randomly selected for repetition one while 10 plants were randomly selected for the second repetition. An intermediate harvest day, day 33 (D33) (loose-leaf stage), was also used to take morphological data on three random plants from both cultivars. On harvest day 42 (D42) (head stage), the final harvest day, five plants were randomly selected from both cultivars and repetitions. Measurements on harvested plants included leaf count, longest leaf length, leaf area, fresh mass, and dry mass (dried for 72 h at 65 °C). A threshold of 1 cm in leaf length qualified leaves for leaf count. Leaf length measurements were done using a ruler and both fresh and dry mass were measured using an electronic scale. Leaves were scanned and then processed two ways; 1) with ImageJ 1.51*g* software (Schneider et al., 2012) (D17 and D33) or 2) with a LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA) (D42). In addition to these morphological responses, stem length, stem fresh

mass, stem dry mass, marketable leaf count, marketable leaf mass (both fresh and dry), and marketable leaf area were measured on D42. Three derived responses were calculated: (1) leaf mass area (LMA) the dried shoot mass divided by leaf area (g m^{-2}), (2) ratio of fresh to dry shoot mass (FM:DM) (FM g/ DM g), (3) biomass accumulation on a mole to PPF and YPF basis (DM/mol PPF and DM/mol YPF, respectively) (mg mol^{-1} m^{-2}).

Phytochemical Concentrations

Leaf chlorophyll concentration from all three harvest plant stages (sample size is n=3) was quantified using the Moran and Porath (1980) protocol using two leaf discs (each disc area: 56.55 mm^2) from a single plant. For consistency, discs were taken 1 cm from leaf apex, avoiding the main leaf rib and margin. Two fresh plants were selected for nitrate content analysis on D42. Samples were extracted using deionized water (80 °C for 1 h). Nitrate in the extract was determined with cadmium reduction-sulfanilamide/N-1 naphthylethylenediamine using a color-automated analysis on an autoanalyzer system (Bran+Luebbe AutoAnalyzer 3, SPX Flow Technology Norderstedt GmbH, Norderstedt, DE).

Shoot anthocyanin and total phenolic content was measured on D42 from three plants. These samples were held at -80 °C. Plant shoots were individually pulverized at -80 °C using a genogrinder (SPEX, Metuchen, NJ) and extracted using acidified methanol (formic acid:methanol:deionized water, 60:37:3, v/v/v). The dilution factor of pulverized lettuce sample to extract solvent was 0.6 g to 12 ml. Samples were vortexed and centrifuged (14,000 rpm for 5-10 min at 5 °C). Supernatants were collected for respective anthocyanin and phenolic content analysis. Anthocyanin samples were assayed using the pH differential method (Giusti and Wrolstad, 1999) and phenolics by the method of Singleton et al. (1999). Absorbance was

measured using a microplate spectrophotometer (Power Wave XS-BioTek Instruments Inc., Winooski, VT, USA).

2.3.4 Statistical analysis

Statistical analysis for comparing the different treatments was done using ANOVA ($p<0.05$) with JMP software Version Pro 13.2 (SAS Institute, Cary, NC, US). If significant, Tukey HSD analysis was used for multiple comparisons between the different light treatments. When no cultivar x treatment interaction was found, results of the two cultivars 'Red Oakleaf' and 'Green Oakleaf' lettuce were combined. Where cultivar × treatment interaction was found the results are presented individually (i.e. longest leaf and dry mass only). Liner regression was used to analyze the relation between nitrate content and B photon flux. No repetition × treatment interaction was found.

2.4 Results and discussion

2.4.1 Plant fresh and dry mass responses

Light treatments had an overall effect on plant fresh and dry mass over the course of the experiment. Lettuce fresh mass did not differ among treatments on the first harvest day (D17) (Table 3). By the second harvest (D33) FL and 100R plants had 33% and 30% greater fresh mass, respectively, than 80B:20R plants (Table 3). By the final harvest (D42) FL plants had 27%, 37% and 38% greater fresh mass than 20B:80R, 50B:50R, and 80B:20R plants, respectively, but were not different from 100B (Table 3). Similarly, FL plant leaf area (Figure 2) was greater than 20B:80R, 50B:50R, and 80B:20R plants which suggests that greater leaf area causes greater fresh mass accumulation. Bian et al. (2016) also found an increase with lettuce

fresh mass as leaf area increased. In other studies, an increased ratio of R light was found to increase lettuce fresh mass, dry mass, and leaf area (Son & Oh, 2015; Wang et al., 2016). Previous studies on lettuce have shown that 100B can have no effect or reduce fresh mass. Wang et al. (2016) found that using 100B reduced shoot fresh mass by 48% when compared to 8B:92R while Amoozgar et al. (2017) found that lettuce fresh mass was the same between 30B:70R and 100B. One possible explanation for these differences is the plants ability to recover from BLS during the seedling and transplant stage and allow for proper orientation for higher light capture in later stages of growth.

Kim et al. (2004) found lettuce fresh mass was different under different white light formulations, specifically different green light qualities, and demonstrated the ability of different white light recipes to accumulate differing fresh mass. Red and green lettuce were found to differ in response to B:R PF ratios , where the increase of leaf area was correlated with an increase in fresh and dry shoot mass in red lettuce, the correlation of leaf area with fresh and dry shoot mass in green lettuce did not exist (Son & Oh, 2013).

Plant dry mass on D17 of ’Green Oakleaf’ and ‘Red Oakleaf’ grown under 20B:80R, had 15% and 27% greater dry mass respectively than those grown in 100B and 28% and 39% greater, respectively than in SUN (Table 3). D33 ‘Green Oakleaf’ lettuce had no differences among light treatments and ‘Red Oakleaf’ plants grown under 20B:80R had 39% more dry mass than SUN (Table 3). On D42, ‘Green Oakleaf’ plants in FL treatment had 24% more dry mass than in SUN, while ‘Red Oakleaf’ plants dry mass was 38% higher in 100R than in 80B:20R (Table 3).

As dry mass indicates the amount of photosynthesis and carbon fixation, variation of dry mass may be due to the respective treatment YPF (Table 2). In our experiment, comparing total dry mass accumulation to cumulative YPF demonstrated that 100B, SUN, and FL, had a 16%,

12%, and 14%, greater ability to accumulate dry mass per YPF input (mg mol^{-1} m^{-2}), than 100R respectively, in 'Green Oakleaf' lettuce (Table 4). This occurred even though 100R had a total YPF that was 21%, 25%, and 14 % greater than 100B, SUN, and FL treatments, respectively (Table 2). Strictly estimating dry mass accumulation based off of YPF would lead one to expect that 100R would be 21% and 14 % greater than 100B and FL treatments, respectively, and not just 7% greater than 100B, as well as 7% lower than FL, as results indicated (Table 2 and 3). For 'Red Oakleaf' lettuce, there was no difference in the dry mass accumulation relative to total YPF per treatment (Table 4). Cultivar effect on dry mass accumulation per YPF input was found. Reduced fresh and dry mass, as demonstrated in cucumber seedlings and lettuce plants under sole-source LEDs was found when grown with higher B:R PF ratio, which have lower YPF (Son & Oh, 2013; Hernandez & Kubota, 2016). Green leaf lettuce lacks the anthocyanin of red leaf lettuce and synthesis of anthocyanins requires resources that can result in a metabolism and production penalty, leading to reduced growth and development and biomass accumulation (Gould, 2004).

In the current study, fresh mass to dry mass (FM:DM) comparisons were also made between treatments. SUN plants had 14-38% greater FM:DM at D42 (Figure 2) when compared to all LED treatments. No difference was found between 100R, 20B:80R, 50B:50R, and 80B:20R plants for FM:DM on D42 (Table 3). Results for FM:DM were similar among all three harvest days.

2.4.2 Plant morphological responses

At D17, leaf area in 100R and FL was 84% and 93% greater than in 80B:20R plants, respectively (Figure 3). D17 and D33 results were similar for leaf area with plants in FL having the greatest leaf area and being 46% larger than in 80B:20R (Figure 3). However, for D42, plants

in 100B, SUN, and FL had 39-78% greater leaf area than plants in 20B:80R, 50B:50R, and 80B:20R (Figure 2). One possible explanation for the increased leaf area in SUN and FL plants could be due to the presence of G in these light recipes (Table 2). As G initiates a low light or shade avoidance response, leaf area is increased to compensate for low light stimuli by increasing light interception (Kim et al., 2004; Folta & Maruhnich, 2007). Also, the higher percent B PF in the B:R treatments contributed to the lower leaf area in these treatments compared to plants in SUN and FL since, in general, as high ratios of B:R light are known to decrease leaf area. Possible explanations for this can be that B light elicits a high light response that inhibits leaf expansion (Trouwborst et al., 2010; Hernandez & Kubota, 2016). Plants in 100B had 28-34% greater leaf area than all the B:R PF ratio treatments. Mixed results on the effect of 100B in plant leaf area have been found in other experiments. For example, Son et al (2013) found that leaf area response in lettuce was cultivar dependent with 100B treatments either decreasing or increasing total leaf area while Snowden et al. (2016) and Wang et al. (2016) found that 100B decreased leaf area index and leaf area in lettuce. One possible explanation for the increase in leaf area with 100B could be a novel light stimulated leaf expansion response via the acid growth pathway (Zivanovic et al., 2005) or regulating circadian rhythm pathways that promote leaf expansion (Haydon et al., 2011).

Leaf mass area (LMA) can be used to estimate leaf thickness (higher LMA equals greater thickness). Plants in treatments 20B:80R, 50B:50R, and 80B:20R had the largest LMA regardless of harvest date (Figure 4), with D42 plants in 100R having a similar LMA. For all three harvest dates, plants in 100B and SUN always belonged to the lowest LMA group and by final harvest (D42) had 35% and 45% lower LMA than 20B:80R, respectively (Figure 4). While the specific mechanisms have not been determined, cucumber had similar responses where

artificial solar spectrum decreased LMA and the decreased LMA was attributed to decreased light absorbance per leaf area (Hogewoning et al., 2010).

Plants in 100B and SUN had the least amount of leaves for all harvest days. For example, on D42 plants in 100B and SUN had 31% and 27% fewer leaves than in 20B:80R (Figure 5). Although temperature is known to affect leaf initiation, the measured temperature was no different between treatments in the current experiment (Table 1). As leaves are the main photosynthetic organs of a plant, it is crucial that leaf architecture and spacing is optimized to maximize light capture. It is interesting that plants in two distinctly different light recipes, 100B and SUN, had the same leaf count (Figure 5). This implies that molecular mechanisms of leaf number and development could be regulated by discreetly different pathways. It is still unclear exactly how light quality impacts leaf organogenesis but it appears to be related to how different colors manipulate the two hormones cytokinin and auxin (Yoshida et al., 2010). In the case of plants in SUN treatment, the decreased leaf count could be attributed to the lower YPF (Table 2) but this does not explain the decreased leaf count in 100B (Figure 5). Furthermore, artificial solar spectrum could impact species differently as Hogewoning et al. (2010) found that cucumber leaf number increased with artificial solar spectrum as compared to other white light treatments.

Both cultivars exhibited different responses to the treatments for leaf lengths. As plants in 100B and SUN had lower leaf counts, they also consistently had the longest leaves for D17 and D33 (Table 5). At D42, SUN plants had the longest leaves for both cultivars which were 114% (‘Green Oakleaf’) and 99% (‘Red Oakleaf’) longer than 50B:50R plants, respectively. Elongated leaf petioles were also found for cucumber grown with artificial solar spectrum compared to other treatments (Hogewoning et al., 2010). This could be attributed to a shade avoidance response due to the G light or decreased R:Fr ratio (or lower PSS) in these two treatments, where

plants partition dry mass differently between leaves and stems and develop long and slender leaves to minimize leaf overlap and self-shading (Morgan and Smith, 1981).

2.4.3 Light quality effect on lettuce bolting

For head lettuce (D42) plant stem length in SUN was 121-367% longer than in all other treatments (Figure 6). Lettuce stem elongation is indicative of bolting, a reproductive stage trait that precedes floral initiation, and increases bitterness. Premature bolting is detrimental to growers as it decreases overall lettuce value by decreasing flavor and appearance quality (Chen et al., 2017). In our study, lettuce stem length and stem fresh mass were highly correlated while stem length and stem dry mass were less correlated (Table 6 and Figure 6).

Certain stimuli and stresses like temperature (heat stress, lack of day-night temperature differential) (Sukprakarn, 1985; Chen et al., 2018), long-day photoperiod (Waycott, 1995), and application of exogenous gibberellin (Fukuda et al., 2011) can accelerate or induce lettuce bolting while the molecular mechanisms that regulate the transition from the vegetative to the reproductive state remain largely unknown (Chen et al., 2017). The extent of these stimuli upon bolting is cultivar dependent (Waycott, 1995; Sanchez et al., 1989). In our study, temperatures and photoperiod were the same across treatment, so the development of excessive stem elongation and sequential lettuce bolting due to light quality was not expected. Modified light treatments were found to affect bolting for lettuce where the spectrum was modified with colored shade netting (Ilic et al., 2017). Lettuce plants grown under red colored nets and pearl nets had 62% and 42%, respectively greater stem lengths than plants grown directly under the sun. In addition, lettuce plants under pearl and red nets also had greater bolting incidence (bud appearance) than plants directly under the sun (no bolting just initial stem elongation) (Ilic et al., 2017). IIic et al., 2017 did not characterize the light quality environment under the nets.

There are several possible explanations as to why stem elongation and floral initiation (Figure 6 and 7F) of plants in the SUN treatment occurred. Although SUN and FL are both considered white light treatment and contained UV, G, and Fr light, FL plants did not demonstrate a bolting response. One possible explanation for this difference, as well as the difference when SUN is compared to other treatments, is the amount of Fr light. The amount of Fr, a lower PSS, and R:Fr ratio can signal stem elongation in lettuce (Chia & Kubota, 2010) or flower initiation response in other plant species (Runkle, 2013). Lettuce is classified as a quantitative long day plant (Waycott, 1995) and the 18 hours photoperiod used in the present study is typical to elucidate a long day response (bolting and floral primordia development) that is then apparent once days begin to shorten in late summer. Yet, the only plants to bolt in this experiment were grown in the SUN treatment even though all treatments were grown under the same photoperiod and relative cool temperature (20 C°).

The common understanding is that phytochrome driven flower promotion depends on the conversion from the inactive P_R to the active P_{FR} form of the phytochrome that is mainly driven by R light (Craig and Runkle, 2016); our hypothesis is that, in lettuce, long day detection is present when a moderate or threshold amount of Fr is included in the spectrum (in this experiment PSS close to 0.67 and R:Fr close to 1:1.2). Several studies in ornamental crops support our findings. For example, studies in long day flowering crops have shown that a moderate R:Fr ratio during the photoperiod is more effective at promoting flowering than a high R:Fr (van Haeringen, 1998; Kim et al., 2002; Runkle & Heins, 2003). For example, Runkle & Heins (2003), showed that pansy plants grown under a Fr deficient filter with a R:Fr of 1.5 had a lower flowering percent than plants grown under a natural filter with a R:Fr ratio of 1.1. Kim et al. (2002) demonstrated that four different petunia cultivars took longer to flower when grown

under a R:Fr of 1.7 than when grown under R:Fr of 1.1. Potentially the antagonistic relationship between phytochrome A (PHYA) and phytochrome B (PHYB) could be a factor in plant long day detection. Neff and Chory (1998) found that the complex interactions between PHYA, PHYB, and CRY1, regardless of a consistent 18 h photoperiod across treatment, could induce or inhibit flowering in Arabidopsis. Another possible but less likely explanation for the bolting response is the amount and quality of G in the SUN spectrum. G light varied between the two white light treatments with G composing 26.1% and 39.1% of SUN's and FL's BAR spectrum, respectively (Table 2). Other studies have shown that under LED sole-source lighting the ratio of G light and wavelength of G light can influence stem length and flowering (Park & Runkle, 2018) and lettuce fresh weight (Johkan et al., 2011). Furthermore, there could be an additional interaction of G light and R light, Fr light, or the ratio of R:Fr. To determine which spectrum range or color ratios initiated bolting in lettuce more experimentation is needed. To our knowledge, the present research study is the first report of the critical role of light quality for lettuce flowering control.

Although the SUN-LED treatment was used to simulate solar spectrum ranges, the experimental treatment had limitations in terms of representing the solar spectrum. For example, SUN included UV radiation but did not replicate the ratio of UV-A (316-399 nm) and UV-B (280-315 nm) (Mewis et al., 2012, Jaing et al., 2012, Brown et al., 2009) typically found in outdoor growing environments. In comparison, SUN treatment only contained UV-A spectrum (Table 2). UV-A radiation activates cryptochrome response but has been demonstrated to be outside of the specific wavelengths absorbed by UVR8, which is a UV-B photoreceptor, action spectrum (peak around 270-285 nm) in *Arabidopsis thaliana* (Jaing et al., 2012, Brown et al., 2009). UVR8 response to radiation sharply declines for wavelengths greater than 300 nm,

meaning our SUN treatment lacks the necessary radiation to activate UVR8 pathways such as HY5 and CHS which are involved with anthocyanin synthesis. Although the low amount of anthocyanin and phenolic production in SUN could be explained by the lack of UVR8 activation, it does not explain why UV-A and B light activation of the cryptochrome failed to initiate anthocyanin synthesis compared to other treatments in the experiment. Reduced phenolic and anthocyanin concentrations were found by Garcia-Macias et al. (2007) in red leaf lettuce 'Lollo Rosso' when plastic film filters were used in a natural sunlight scenario that blocked UV-B and UV-A wavelengths below 380 nm.

2.4.4 Phytochemical content

Light treatment impacted phytochemical content of different compounds (Table 7). Plant total phenolic content was greater in 20B:80R, 50B:50R, and 80B:20R than in SUN, 100R, and 100B treatments (Table 7). For example the total phenolic content was between 98-118% greater in B:R (20B:80R, 50B:50R, 80B:20R) treated plants than plants in SUN. Similarly, anthocyanin content of 'Red Oakleaf' lettuce was also greater in 20B:80R, 50B:50R, and 80B:20R than in in SUN, 100R, and 100B treatments. The range of difference among plants in 20B:80R, 50B:50R, and 80B:20R was 10-11 fold greater when compared to plants in SUN (Table 7). The reason for increased phenolic and anthocyanin content in the B:R LED light treatments is due to the necessity of cryptochrome and phytochrome co-activity to promote flavonoid synthesis (Neff and Chory, 1998) and the potential explanation why plants in 100R and 100B had lower phenolic and anthocyanin content. Another plausible explanation is the percent G PF in the white light treatments. Research has shown that G light is known to inhibit B-light responses including phytochemical accumulation (Folta and Maruhnich, 2007; Sellaro et al, 2010). For example, Folta and Maruhnich (2007) described how pulsed greenlight can decrease chloroplast

transcription. For SUN, anthocyanin content could had been influenced by photosynthesis-dependent anthocyanin synthesis (Das et al., 2011), in addition to photoreceptor mechanisms. When photosynthesis is decreased, synthesis of the photo-protectant anthocyanin is decreased. This could be one potential cause as to why SUN, with sufficient B:R PF ratio for cryptochrome and phytochrome co-activity, simultaneously possessing a relatively reduced YPF, had low anthocyanin content while maintaining more total phenolics. Additionally, Li & Kubota (2009) found similar results with the inhibitory effects of Fr light on anthocyanin synthesis. By adding Fr light to a cool white fluorescent light treatment, anthocyanin, as well as xanthophylls and beta-carotene, were reduced in baby leaf lettuce compared to those plants which were just illuminated with cool white fluorescent lamps (Li & Kubota, 2009).

Leaf chlorophyll content was greatest in B:R PF light treatments that had 50% or more B light (i.e. 50B:50R and 80B:20R). Total chlorophyll concentration of plants in 50B:50R and in 80B:20R was 48-125% greater than in 100B, 100R, SUN, and FL, respectively (Table 7). One contributing factor to B:R PF treatments having higher chlorophyll content is the requirement of B to promote the chlorophyll tetrapyrrole precursor 5-aminolevulinic acid (ALA). Fan et al. (2013) found that in cabbage, monochromatic R reduced ALA but higher chlorophyll concentrations were restored with the addition of B. Similar to Fan et al. (2013), the white light treatment lettuce plants (SUN and FL) demonstrated a decreased chlorophyll content. One possible explanation for this decrease is the inclusion of G which is known to down regulate the transcription factors for chloroplast formation in multiple plant species (Folta & Maruhnich, 2007).

Nitrate content was also impacted by light treatment. As the B PF increased so did plant nitrate content ($P<0.0416$) (Figure 8). For example, nitrate levels were nearly 9-fold greater in

100B compared to 100R. Although this experiment indicated that as percent B PF increases, nitrate content increases, studies with other lettuce cultivars have not demonstrated a similar trend based on B light (Chen et al. 2014; Bian et al 2016).

2.5 Conclusion

The objective of this study was to determine the performance of two lettuce cultivars under different B:R PF light ratios and white light (SUN and FL) while examining growth at multiple harvests stages that correlate with transplant, loose-leaf, and head-lettuce stages for production. The B:R PF ratio played a large role in the morphology, phytochemical content, and visual appearance of lettuce. It is well known that utilizing LEDs that have specific B:R PF ratio can optimize and customize growth, eliciting distinct phenotypes that can be considered both advantageous or non-beneficial depending on harvest goal. Interestingly, it was found that 20B:80R of the LED treatments was optimal, when considering dry mass production and achieving normal plant morphology. Yet, results from this study also demonstrated the benefits of 100B. Although plants demonstrated BLS, D17 plants compensated for this stress establishing an upright orientation, and by D42 plants in 100B were high performer for fresh mass. Having a high FM:DM and low LMA may seem desirable to growers that are targeting a specific plant architecture for marketability. Few studies have grown lettuce using 100B and results have been contrasting. Aside from BLS, the other disadvantage of 100B was the lack of anthocyanin pigmentation which may result in quality reduction of red leaf lettuce cultivars. One potential strategy to overcome this would be to utilize 100B to achieve high fresh mass and FM:DM then using an end of production light recipe that induces anthocyanin production and coloration. Although end-of-production light recipes have been used in greenhouse sunlit settings, such as

the greenhouse optimization of end of production lighting (Owen & Lopez, 2015), LED indoor systems have yet to be optimized.

Another unique discovery observed in this experiment was that SUN simulated treatment initiated a reproductive response with stem bolting (Figure 7F) with sequential rosette formation. Although discussed earlier, further work will have to be conducted to specify which wavelengths or combination of wavelengths causes the bolting response with electrical solar spectrum.

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Table 2.1 Environmental parameters (mean ± SD) for each treatment. All parameters are combined averages from repetition 1 and 2. Temperature, CO2 concentration, and relative humidity were continuously logged while air velocity was measured at the start of the experiment and pH and EC were measured at time of fertigation.

Parameter	Treatments (photon flux ratio)						
	100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
Air temperature (°C)	20.1 ± 1.3	20.0 ± 1.2	19.8 ± 1.3	19.9 ± 1.2	20.0 ± 1.0	19.9 ± 1.3	20.3 ± 1.1
Substrate temperature (°C)	19.7 ± 1.4	19.8 ± 1.6	19.6 ± 1.5	19.8 ± 1.5	19.7 ± 1.4	20.1 ± 1.4	20.2 ± 1.3
Air Velocity (m s^{-1})	1.00 ± 0.18	1.12 ± 0.09	0.97 ± 0.11	1.07 ± 0.24	0.95 ± 0.09	0.96 ± 0.25	1.25 ± 0.05
CO_2 (µmol mol^{-1})				704.2 ± 34.0			
RH (%)				80.4 ± 2.8			
pH				6.5 ± 0.1			
EC (dS m^{-1})				1.44 ± 0.08			

Table 2.2 Spectral characterization of experimental light treatments (mean ± SD) with different blue (B) and red (R) percent photon flux, a LED simulated solar spectrum treatment (SUN), and a fluorescent control (FL). For the case of simulated solar spectrum (SUN) and fluorescence (FL) percent color is provided for biologically active radiation (BAR).

Light Parameter	Treatments (photon flux ratio)						
	100R	20B:80R	50B:50R	80B:20R	100B	SUN (5.1UV, 20.0B, 26.1G, 26.3R, 22.6Fr)	FL (1.9UV, 29.9B, 39.1G, 24.1R, 5.0Fr)
BAR*	200.9 ± 1.3	200.8 ± 1.6	200.0 ± 0.4	201.1 ± 1.7	199.8 ± 1.3	201.5 ± 0.2	199.6 ± 2.5
PPF**	199.3 ± 1.2	199.1 ± 0.5	198.8 ± 0.4	199.5 ± 1.0	198.9 ± 1.5	145.8 ± 0.4	185.8 ± 6.3
YPF†	186.2 ± 1.2	178.6 ± 0.6	166.4 ± 0.3	155.2 ± 0.9	146.7 ± 1.1	138.7 ± 0.4	160.0 ± 5.0
Cumulative YPF††	463.9	445.27	415.07	386.8	366.7	345.9	400.1
PSS†††	0.88	0.88	0.86	0.79	0.49	0.69	0.81
R:Fr††††	133.0	137.2	123.6	54.2	1.0	1.2	4.8
Photoperiod (h)	18						

*Biologically active radiation (BAR) is 300-800 nm in µmol m^{-2} s^{-1}

**Photosynthetic photon flux (PPF) is 400-700 nm in µmol m^{-2} s^{-1}

†Yield photon flux (YPF) values in µmol m^{-2} s^{-1} calculated from Sager and Mcfarlane (1997)

††Cumulative YPF (mol m^{-2}) received per treatment by through harvest day (D42)

†††Phytochrome Photoequilibrium (PSS)

†††R = 600-700 nm and Fr = 700-800 nm was used to calculate ratio R:Fr

Table 2.3 Fresh mass (combined both cultivars) and dry mass for ‘Green Oakleaf’ and ‘Red Oakleaf’ lettuce cultivars (Mean ± SE) under different LED treatment. For harvest D17-transplant, D33-loose leaf, and D42-head lettuce. Different letters represent light treatment differences for each respective harvest period (in-row) (Tukey HSD, $p<0.05$).

Parameter	Harvest (day)	Treatments (photon flux ratio)						
		100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
FM (g)	17	0.57 ± 0.11 ns	0.51 ±0.13 ns	0.43 ± 0.09 ns	0.40 ± 0.10 ns	0.42 ± 0.10 ns	0.47 ± 0.13 ns	0.55 ± 0.17 ns
	33	10.64 ± 0.63 a	9.82 ± 0.39ab	8.47 ± 0.36 ab	7.42 ± 0.66 b	9.17 ±1.16 ab	8.88 ± 1.28 ab	11.01 ± 1.34 a
	42	21.51 ±0.92 ab	20.14 ± 1.57b	18.65 ± 1.58 b	18.55 ± 1.21b	22.33 ± 0.74 ab	22.83 ± 1.36 ab	25.59 ± 1.91 a
DM (g) ‘Green Oakleaf’	17	0.03 ± 0.01 abc	0.04 ± 0.02 a	0.04 ± 0.01 abc	0.03 ± 0.01 abc	0.03 ± 0.01 bc	0.02 ± 0.01 c	0.04 ± 0.02 ab
	33	0.58 ± 0.09 a	0.63 ± 0.03 a	0.55 ± 0.03 a	0.50 ± 0.06 a	0.53 ± 0.10 a	0.46 ± 0.00 a	0.62 ± 0.05 a
	42	1.21 ± 0.14 abc	1.40 ± 0.07 ab	1.29 ± 0.02 abc	1.21 ± 0.05 bc	1.28 ± 0.08 abc	1.17 ± 0.07 c	1.45 ± 0.04 a
DM (g) ‘Red Oakleaf’	17	0.03 ± 0.01 ab	0.04 ± 0.01 a	0.03 ± 0.01 ab	0.03 ± 0.01 ab	0.02 ± 0.01 b	0.02 ± 0.01 b	0.03 ± 0.01 ab
	33	0.53 ± 0.05 ab	0.56 ± 0.04 a	0.45 ± 0.02 abc	0.39 ± 0.05 bc	0.41 ± 0.08 abc	0.34 ± 0.10 c	0.44 ± 0.07 abc
	42	1.35 ± 0.08 a	1.26 ± 0.10 ab	1.10 ± 0.01 abc	0.97 ± 0.07 c	1.16 ± 0.08 abc	1.04 ± 0.14 bc	1.17 ± 0.14 abc

Table 2.4 Dry mass accumulation relative to cumulative moles of PPF and YPF over course of treatment (Mean ± SE). Day 42 harvest (head-lettuce) found cultivar effect and is therefore separated by cultivar. Different letters represent light treatment differences for each respective harvest period (in-row) (Tukey HSD, $p<0.05$).

Parameter	Harvest	Cultivar	Treatment (photon flux ratios)						
			100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
DM/mol PPF (mg/ mol m^{-2})	42	'Green Oakleaf'	2.5 ± 0.2 c	2.8 ± 0.2 abc	2.6 ± 0.0 bc	2.4 ± 0.1 c	2.6 ± 0.2 c	3.0 ± 0.2 a	3.4 ± 0.1 ab
DM/mol YPF (mg/ mol m^{-2})	42	'Green Oakleaf'	2.7 ± 0.2 b	3.1 ± 0.0 ab	3.1 ± 1.0 ab	3.1 ± 0.1 ab	3.5 ± 0.2 a	3.4 ± 0.2 a	3.6 ± 0.0 a
DM/mol PPF (mg/ mol m^{-2})	42	'Red Oakleaf'	2.7 ± 0.2 a	2.5 ± 0.2 ab	2.2 ± 0.0 ab	2.0 ± 0.2 b	2.3 ± 0.1 ab	2.9 ± 0.4 a	2.5 ± 0.3 ab
DM/mol YPF (mg/ mol m^{-2})	42	'Red Oakleaf'	2.9 ± 0.2 a	2.8 ± 0.23 a	2.7 ± 0.0 a	2.5 ± 0.2 a	3.2 ± 0.2 a	3.0 ± 0.4 a	2.9 ± 0.3 a

Table 2.5 Respective cultivar longest-leaf-length average for each light treatment per harvest (Mean ± SE) for harvest 1 (D17-transplant stage), harvest 2 (D33-loose leaf), and harvest 3 (D42- head lettuce). Different letters represent light treatment differences for each respective harvest period (in-row) (Tukey HSD, $p<0.05$).

Parameter	Harvest	Cultivar	Treatments (photon flux ratios)						
			100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
Longest Leaf (cm)	17	'Green Oakleaf'	5.3 ± 1.0 ab	3.2 ± 0.5 b	2.9 ± 0.5 b	2.7 ± 0.5 b	6.2 ± 1.2 a	7.1 ± 1.6 a	4.7 ± 0.6 ab
	33	'Green Oakleaf'	9.3 ± 0.9 c	7.1 ± 0.5 d	7.0 ± 0.2 d	7.1 ± 0.2 d	13.3 ±0.1 ab	15.4 ± 0.2 a	11.7 ± 0.6 b
	42	'Green Oakleaf'	10.0 ± 0.7 c	8.1 ± 0.7 d	7.6 ± 0.6 d	7.9 ± 0.1 d	14.0 ±0.2 b	16.3 ± 0.4 a	13.1 ± 0.9 b
	17	'Red Oakleaf'	5.3 ± 0.9 ab	3.4 ± 0.5 b	3.0 ± 0.2 b	3.0 ± 0.4 b	6.6 ± 1.1 a	7.6 ± 1.3 a	5.1 ± 02 ab
	33	'Red Oakleaf'	9.3 ± 0.9 bc	7.5 ± 0.3 c	7.0 ± 0.0 c	7.1 ± 0.4 c	13.3 ± 0.2 a	14.6 ± 0.9 a	12.8 ± 0.6 ab
	42	'Red Oakleaf'	10.0 ± 0.4 c	8.4 ± 0.3 cd	8.1 ± 0.2 d	8.9 ±0.5 cd	14.0 ±0.1 b	16.1 ± 0.1 a	15.7 ± 0.1 ab

Table 2.6 Combined cultivar stem fresh mass and dry mass for each treatment (Mean ± SE) on harvest 3 (D42- head lettuce). Different letters represent light treatment differences (in-row) (Tukey HSD, $p<0.05$).

Parameter	Harvest	Treatment (photon flux ratios)						
		100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
Stem fresh mass(g)	42	0.89 ± 0.07 b	0.84 ± 0.06 b	0.77 ± 0.04 b	0.72 ± 0.08 b	1.3 ± 0.05 b	2.25 ± 0.39 a	1.1 ± 0.08 b
Stem dry mass (g)	42	0.09 ± 0.01 ab	0.10 ± 0.02 ab	0.09 ± 0.01 b	0.07 ± 0.01 b	0.09 ± 0.01 ab	0.12 ± 0.03 a	0.09 ± 0.01 b

Table 2.7 Average total phenolic, total anthocyanin, chlorophyll a content, chlorophyll b content, total chlorophyll content, and total nitrate content for each treatment at day 42 (final harvest) (Mean ± SD). Different letters represent light treatment differences (in-row) (Tukey HSD, $p<0.05$).

Parameter	Harvest	Treatments (photon flux ratio)						
		100R	20B:80R	50B:50R	80B:20R	100B	SUN	FL
Total Phenolics (mg kg-1)	42	91.0 ± 9.9 b	152.3 ± 18.3 a	175.6 ± 11.5 a	167.8 ± 9.4 a	109.2 ± 5.7 b	77.1 ± 3.6 b	92.8 ± 10.7 b
Total Anthocyanin (mg kg-1)	42	12.4 ± 0.6 cd	68.6 ± 22.8 abc	77.1 ± 17.6 a	70.3 ± 1.0 ab	19.5 ± 0.5 bcd	6.2 ± 0.4 d	37.8 ± 9.6 abcd
Chlorophyll Total (g m^{-2})	42	0.17 ± 0.01 d	0.26 ± 0.01 bc	0.30 ± 0.01 ab	0.33 ± 0.01 a	0.20 ± 0.01 cd	0.15 ± 0.00 d	0.20 ± 0.00 cd

*Total Anthocyanin is for ‘Red Oakleaf’ lettuce only

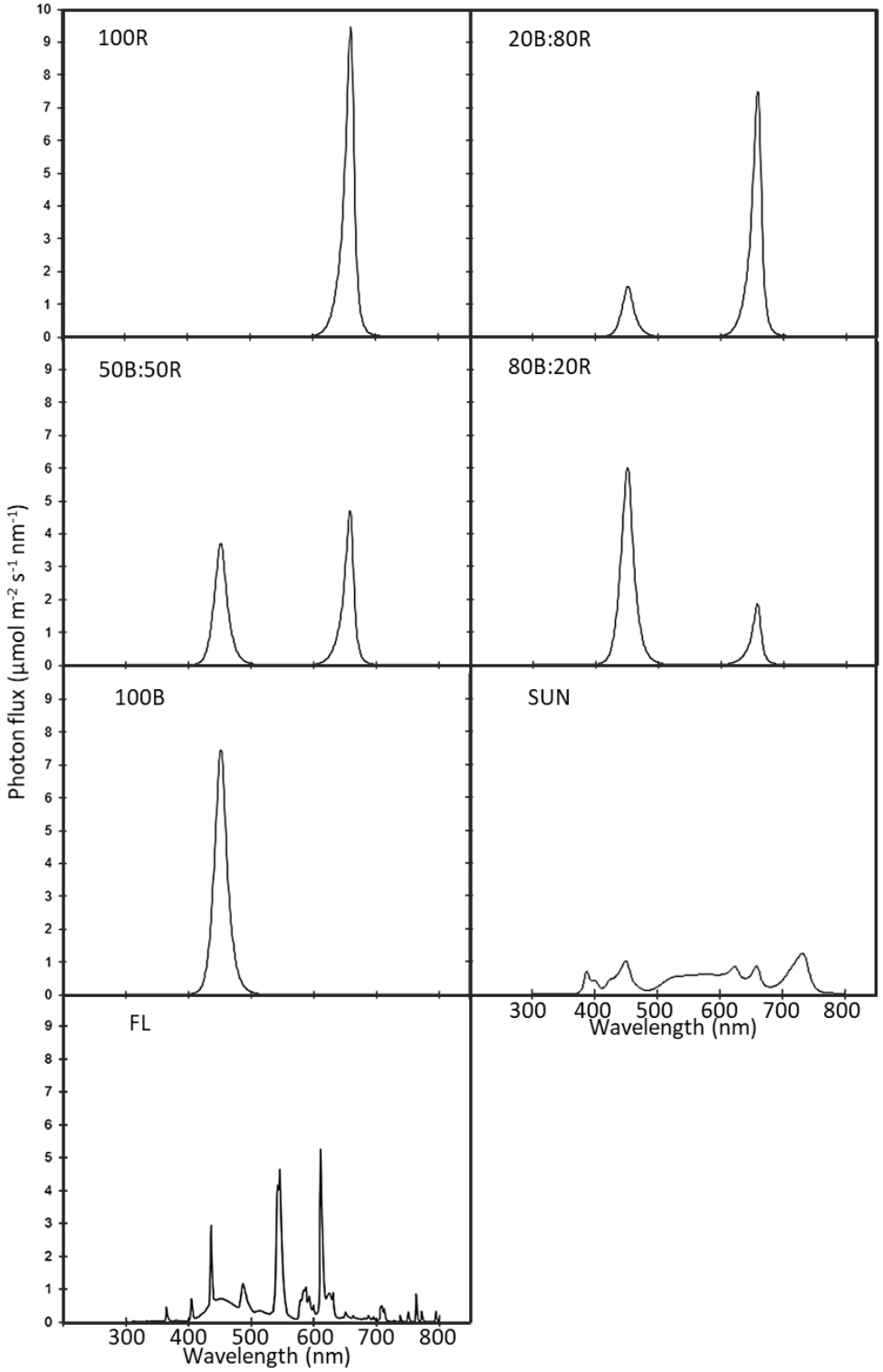

Figure 2.1 Spectral distribution of LED and Fluorescent light treatments. The spectra were recorded from a five-point light field (40 cm height for LED treatments and 17.5 cm height for fluorescent) and averaged together. Each graph is the average reading for both replications.

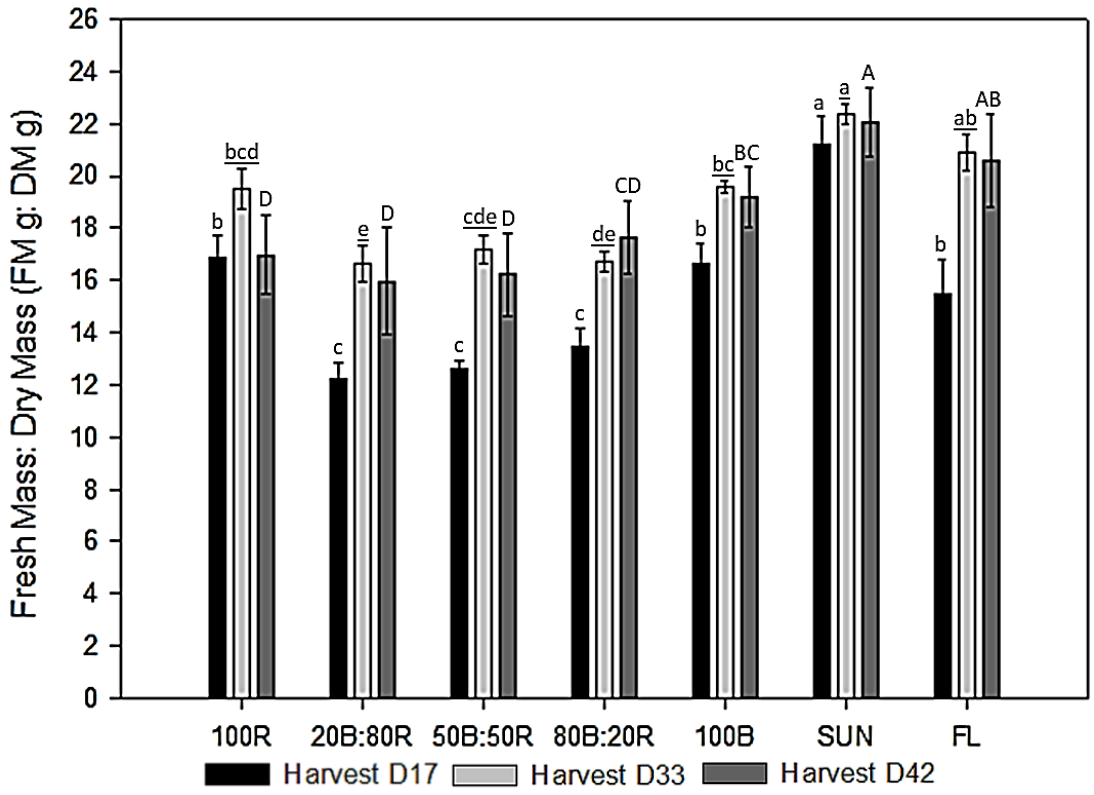

Figure 2.2 Fresh mass to dry mass (FM:DM) ratio (Mean ± SE). Different color bars represent the three different harvest times: transplant (D17), loose-leaf (D33) and head lettuce (D42). Data is combined for both cultivars, 'Red Oakleaf' and 'Green Oakleaf'. Different letters represent light treatment differences that correlate with each respective harvest period only (Tukey HSD, $p<0.05$).

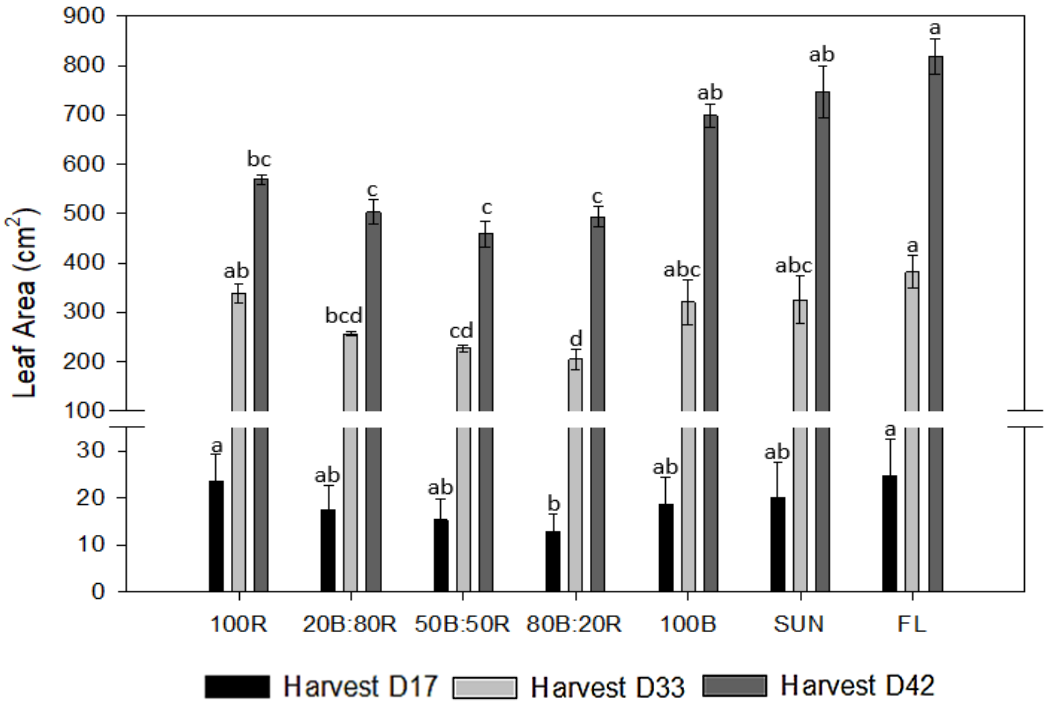

Figure 2.3 Leaf area under different LED treatments (Mean ± SE). Different color bars represent the three different harvest times: transplant (D17), loose-leaf (D33) and head lettuce (D42). Different letters represent light treatment differences that correlate with each respective harvest period only (Tukey HSD, $p<0.05$).

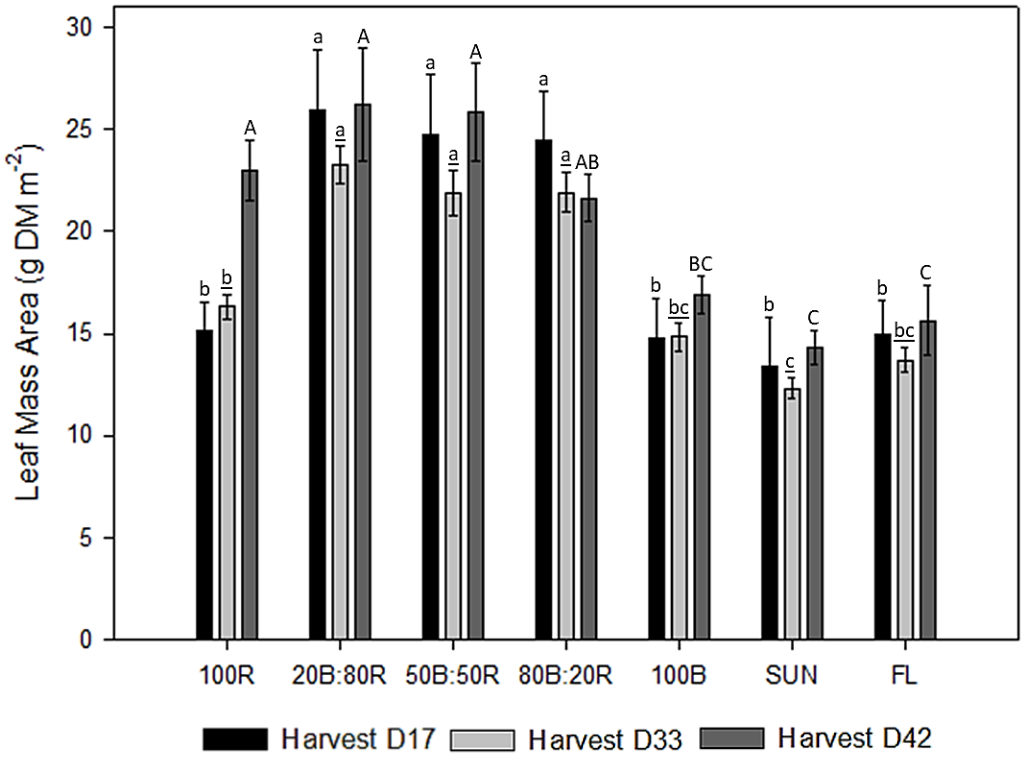

Figure 2.4 Leaf mass area under different LED treatments (Mean ± SE). Different color bars represent the three different harvest times: transplant (D17), loose-leaf (D33) and head lettuce (D42). Different letters represent light treatment differences that correlate with each respective harvest period only (Tukey HSD, $p<0.05$).

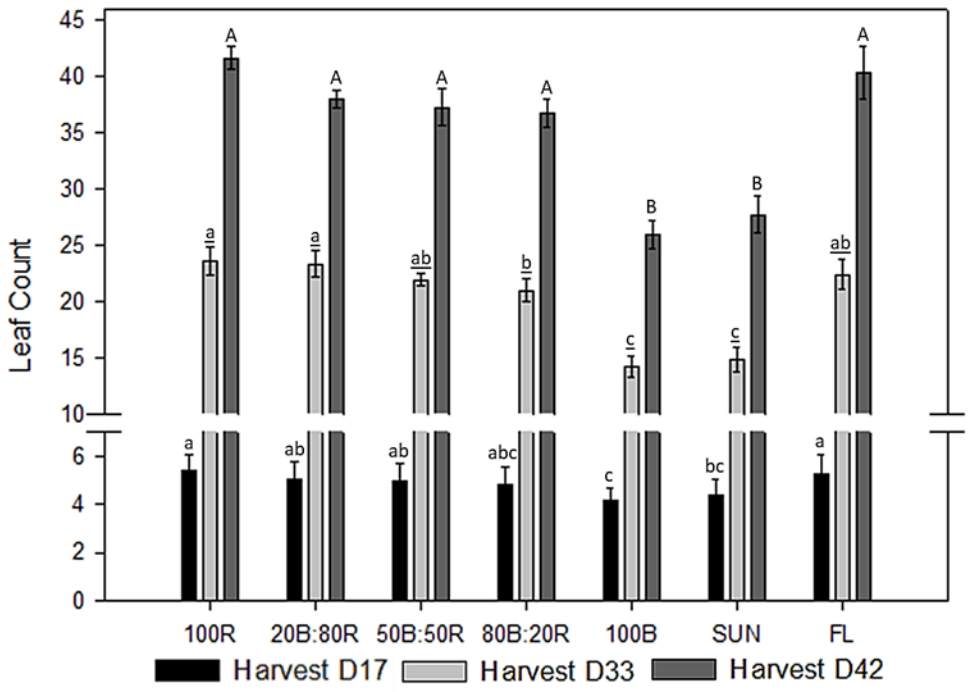

Figure 2.5 Leaf count under different LED treatments (Mean ± SE). Different color bars represent the three different harvest times: transplant (D17), loose-leaf (D33) and head lettuce (D42). Different letters represent light treatment differences correlate with each respective harvest period only (Tukey HSD, $p<0.05$).

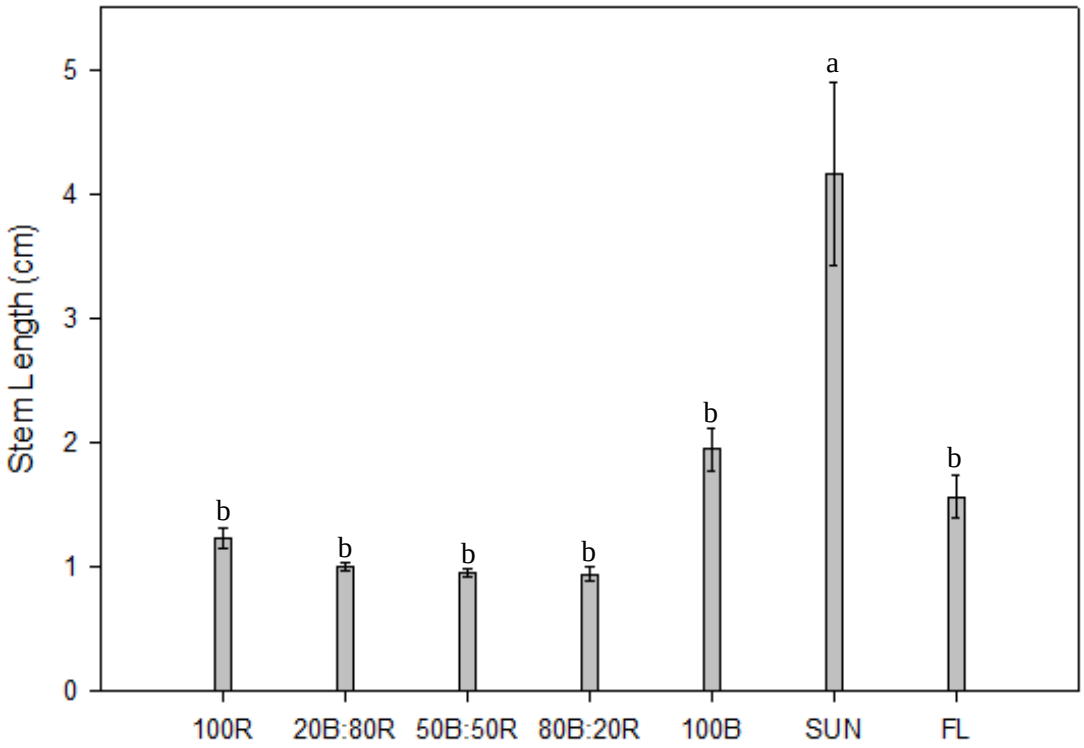

Figure 2.6 Stem length under different LED treatment (Mean ± SE) for harvest 3 (D42-head lettuce). Different letters represent light treatment differences (Tukey HSD, $p<0.05$).

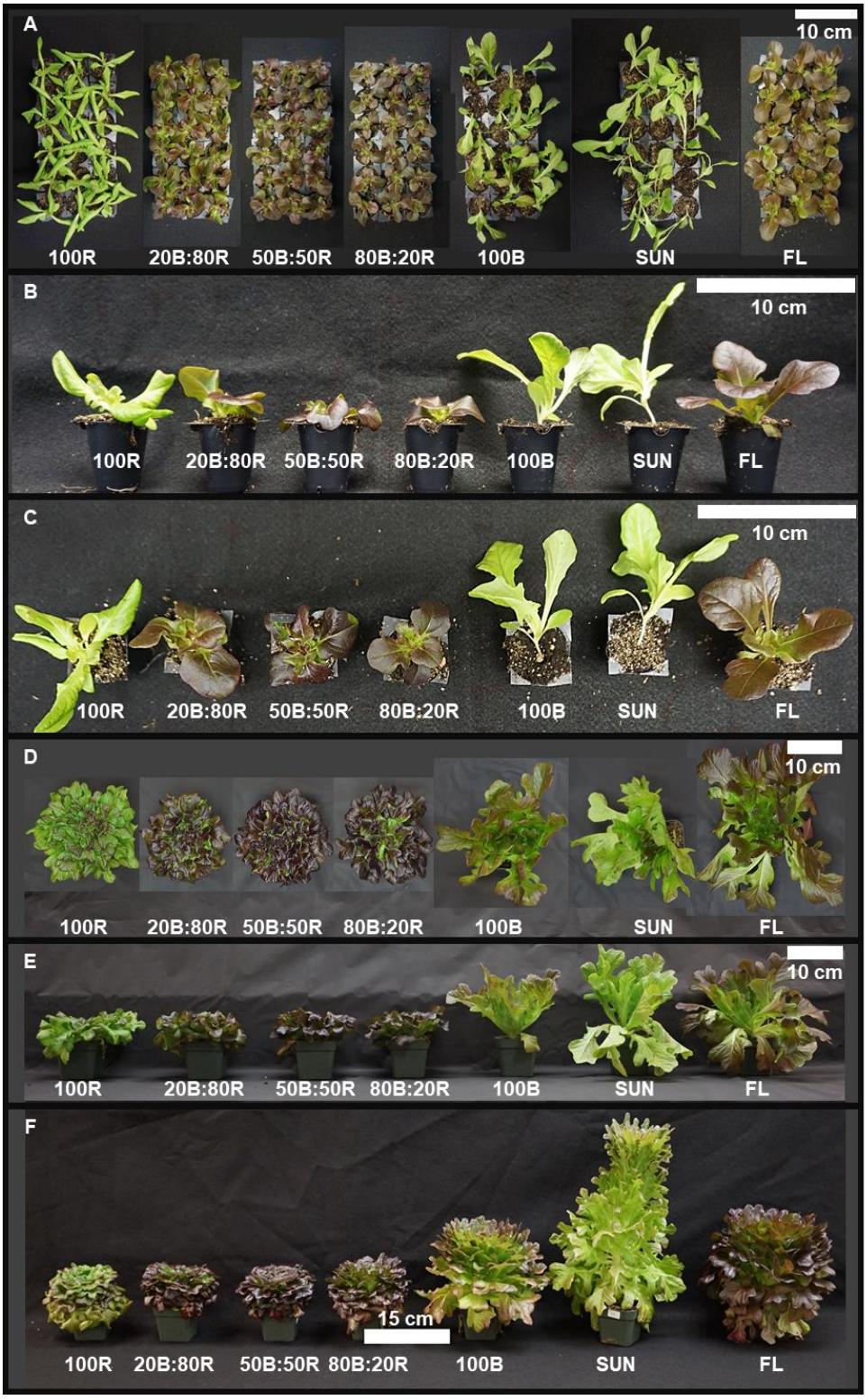

Figure 2.7 Morphology of 'Red Oakleaf' lettuce under different LED treatments. Pictures were taken on D17-transplant top view (A), D33-loose leaf side view (B) and top view (C), D42-head lettuce top view (D) and side view (E), and side view at 8 weeks (F).

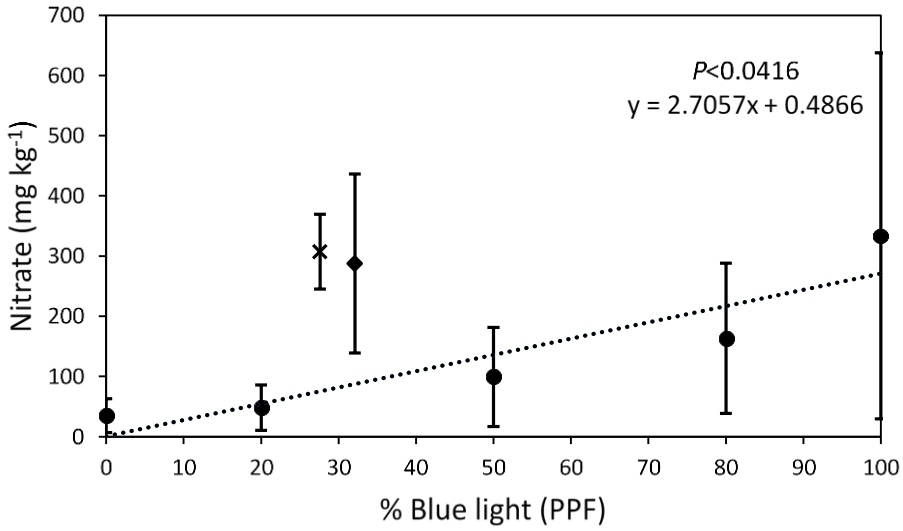

Figure 2.8 Nitrate content for both ‘Red Oakleaf’ and ‘Green Oakleaf’ (data combined) under different LED treatment (Mean ± SD) for harvest 3 (D42-head lettuce). Linear regression is for 100R, 20B:80R, 50B:50R, 80B:20R, and 100B only. Nitrate content for SUN (×) and FL (◆) lettuce are are omitted from the regression analysis but shown as reference in the graph.

CHAPTER 3

Morphological and physiological responses of red-leaf lettuce to crop stage specific dynamic light recipes in indoor production

3.1 Abstract

The use of a single light spectrum throughout the entire growth cycle (fixed spectrum) is the predominant light strategy for indoor crop production. The use of fixed spectrum overlooks the potential of dynamic light strategies, where spectrum is changed based on plant growing stage to improve plant growth, morphology, and development. The objective of this experiment is to compare the effects of fixed and dynamic light recipes using different percentages of blue (B) and red (R) photon flux density (PFD) ratios to produce 'Red Oakleaf' lettuce. Plants were grown for a total of 47 days (D47) with four treatments consisting of fixed spectrums (1) 20% blue: 80% red (20B:80R) PFD (FIXED), and (2) 20B:80R with end-of-day far-red ((FIXED+FR) and dynamic spectrums (3) 20B:80R with end-of-day far-red (end-of-day far-red for any applicable treatments consisted of 6 µmol m^{-2} s^{-1} for 30 min for a total of 10.8 mmol m^{-2} d^{-1}) (day 3-19) followed by 100% blue PFD (100B) (day 20-39) (DYNAMIC), and (4) 20B:80R with end-of-day far-red (day 3-19) followed by 100B with end-of-day far-red (day 20-39) (DYNAMIC+FR). At the end of growing cycle (day 40-47) all plants were exposed to a "finishing" light recipe consisting of 50% blue: 50% red PFD (50B:50R) to increase anthocyanin concentration and red coloration. After germination (day 0-2), plants were provided with 100 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 18 h (day 3-11) and increased to 200 µmol m^{-2} s^{-1} PPFD for 18 h from day 12-47. On day 25 FIXED and FIXED+FR plants had the same fresh mass to DYNAMIC and DYNAMIC+FR plants but had 24-40% more dry mass. On day 32 FIXED and FIXED+FR plants had 18-23% more fresh mass and 21-27% more dry mass than DYNAMIC and DYNAMIC+FR, yet by day 39, no differences in dry mass were found between any treatment. On the final day (day 47), with all plants being irradiated with the finishing light recipe of 50B:50R for 7 days, plants in all treatments had no significant

differences in shoot fresh mass; however, plants in the DYNAMIC and DYNAMIC+FR treatment had 8-12% greater dry mass than in FIXED and FIXED+FR treatment. Plants in DYNAMIC and DYNAMIC+FR had 22-64% longer stem length and 38-109% greater stem fresh mass than plants in FIXED spectrum. Plants in the DYNAMIC treatment had 26% larger canopy diameter than plants in FIXED light treatment. Plants from FIXED and FIXED+FR had 31-33% greater net photosynthetic rate than plants in DYNAMIC+FR. The 50B:50R finishing light treatment increased anthocyanin concentration by 47% for plants grown in DYNAMIC spectrum.

3.2 Introduction

Traditional horticultural lighting such as cool-white-fluorescent, high-pressure sodium, metal halide, and several light emitting diode fixtures (LED) provide a fixed spectrum that either supplement solar radiation (i.e., in greenhouses) or serves as the sole source of radiation (vertical farms) to grow plants. With the introduction of LED fixtures with multiple independently controlled channels and diodes it is possible to deploy programs to vary the spectrum and elucidate desirable plant responses. For example, specific plant response using spectral qualities to target an increase in photosynthesis, leaf area, or secondary metabolite production could be used at deliberate crop stages for more efficient production. However, even though the technology has the capability to provide dynamic changing spectrums, the scientific based information on dynamic spectral strategies is lacking. A focus now for dynamic light recipes (DLRs) should be to determine optimal light recipe formulas that promote rapid biomass accumulation and increase crop nutritional or phytochemical crop components for consumption. As previously mentioned, indoor vertical farms have relied on a static light formula while only optimizing intensity or photoperiod; however, plants have evolved in a natural environment where light quality and quantity changes throughout the photoperiod, seasons and geographical locations. Therefore, plants have evolved complex photoreceptor systems that provide spectral information that alters plant morphology, growth and development. For example, the phytochrome regulates seed germination, de-etiolation, shade avoidance, flowering and plant circadian rhythms, among other regulatory responses (Mathews, 2006; Possart et al., 2013; Wang & Wang, 2015). Other photoreceptors include: the B light-sensing cryptochromes, which regulate hypocotyl, petiole, and internode elongation, anthocyanin accumulation, and phototropism (Ahmad et al., 2002), the B light-sensing phototropins which regulate stomatal

opening, chloroplast movement, and leaf flattening (Christie et al., 2015), and the ultraviolet-B (UV-B) sensing UVR8 which regulates shade avoidance (Hayes et al., 2014), and flavonoid synthesis (Jiang et al., 2012).

Changes originating from the daily light cycle (i.e., Earth's rotation) qualitative spectral changes are due to Rayleigh scattering, an atmospheric effect that scatters shorter wavelength light (i.e., ultraviolet and blue) much more than longer wavelengths of light (ie., red and far-red), and results in a very distinct pattern and ratio of colors that change throughout the day. For example, at sunrise and sunset impinging solar radiation travels the longest distance through Earth's atmosphere due to the low sun angle (ie., maximum solar zenith distance). This event scatters blue light to a much greater extent than red and far-red which results in these longer wavelength colors to become the prevailing radiation intercepted by Earth's surface at dusk and dawn. This far-red ratio becomes 1-2 orders of magnitude greater for 20-25% of the day (Holmes and Smith, 1977). As the complete absence of light at night (before sunrise and after sunset) activates certain nocturnal responses in plants so does the qualitative shift of light at twilight to high far-red ratios, eliciting phytochrome and circadian rhythm responses. Conversely, at the minimal solar zenith distance (ie solar noon), UV and blue light reaches its highest relative amount compared to red and far-red. This prevalence of shorter wavelength radiation activates phototropins and the cryptochrome which in turn promote pigment synthesis (ie sunscreens for the plant photosynthetic machinery) and regulate leaf orientation. As current fixed spectrums used in indoor growing do not readily integrate spectral shifts, there may be an opportunity on utilizing the dynamic nature of light to improve growth and morphology.

Currently, most research has focused on DLRs that alter spectral quality and PFD changes within a short cycle or 24 h period that are repeated until harvest (Hanyu et al., 2002;

Jishi et al., 2016; Chen et al., 2017). Several studies have looked at how shifting the blue to red ratio throughout the day impact plant morphology and physiology. For example, in a series of three experiments Jishi et al. (2016), used equal daily dosages of blue and red light to employ a temporal separation approach to determine when application of blue, red, or both should be used to optimize biomass and leaf area, for multiple photoperiods. While maintaining the same daily light integral (DLI), Jishi et al. (2016) showed that when a 50B:50R light recipe was initiated by a 4 or 7 h period of 100B and a 4 or 7 h period of 100R to finish the photoperiod cos lettuce shoot fresh mass and leaf area was increased compared to using a light recipe of 50B:50R or 50B:50R light recipe with 1 h of 100B at the start and 1 h of 100R at the end. Jishi et al. (2016) also demonstrated that a photoperiod with 7 h of 100B followed by a 7 h period of 100R increased lettuce (*lactuca sativa* L.) leaf area compared to a fixed light recipe of 50B:50R but no difference was found between fresh and dry mass.

In a similar study, Chen et al. (2017) grew 'Green Oakleaf' lettuce under alternating intervals of 100B and 100R ranging from 1 h to 8 h alternation periods while using a 33B:67R light treatment as a control (with equal DLI between these treatments). They found that an alternation rate of 8 h and 1 h increased lettuce shoot fresh and dry mass as compared to the fixed light recipe of 33B:67R, and 2 other alternating light recipes (the 2 h and 4 h alternation treatments).

Both of these studies rely on balancing red and blue light. Blue light is used under low light conditions for promoting leaf expansion increasing potential light interception and harvest for photosynthesis (Wang et al., 2015). Red light is utilized to drive photosynthesis due to its greatest relative quantum efficiency (RQE). Another diurnal DLR strategy is the use of blue light at the start of the day to trigger plant stomata to open after night or darkness by activating

phototropins within the stomatal guard cells (Inoue & Kinoshita, 2017). As blue light induces a local response within the guard cell, phototropins (both phot1 and phot2) are phosphorylated by BLUE LIGHT SIGNALING1 (BLUS1) which then activates plasma membrane H^+-ATPase which controls guard cell H^+ pumping (Inoue & Kinoshita, 2017). Delayed stomatal opening could limit CO_2 for photosynthesis and reduce growth, thus early opening of stomata can be beneficial. In contrast, Chinchilla et al. (2018) found no benefit for lettuce by using 50 µmol m^{-2} s^{-1} PPFD of pre-dawn B, R, or white light. To elaborate, Chinchilla et al. (2018) tested B, R, and white pre-dawn light treatments on 'Waldmann's Green' lettuce grown under white LED light and found that pre-dawn light had no impact on plant fresh or dry mass accumulation and no difference was found between the control and any pre-dawn light treatment for leaf area.

Whereas these diurnal dynamic strategies focus on daily optimization, the current experiment focuses on varying the spectrum to optimize certain traits for the respective crop cycle stage (i.e., during germination, seedling stage, vegetative stage, or reproductive stage). We hypothesize that by employing a specific quality of light one can "push" or manipulate morphological features to gain increased dry mass accumulation or leaf area gain at critical time points. For example, high quality transplants are considered to have a high dry mass to fresh mass ratio, where the high carbohydrate reserve will withstand the stresses of transplanting and accelerate early growth (Kozai, 2016). A light recipe that increases this ratio would then be advantageous. Another example is the use of 100B to drive leaf expansion for increased light interception once lettuce plants are no longer prone to blue light syndrome.

The objective of this study is to gain insight on DLRs that optimize growth, morphology, and phytochemical content in lettuce. In our previous work (Spalholz et al., 2019) we grew lettuce under seven fixed light spectrums and collected data on growth, morphological

parameters, and phytochemical content at different growth stages in order to characterize the plant responses of each growth stage to the respective spectrum. At the transplant stage, a 20B:80R light recipe was optimal as it yielded high leaf mass area, leaf area, dry mass to fresh mass ratio, leaf count, and had shorter leaves. Although, 20B:80R transplants had 37-42% more dry mass than 100B transplants, no difference in dry mass was found at the end of the experiment while 100B plants had 28% more leaf area than 20B:80R giving them a larger appearance. Despite these positive traits in 42-day old 100B plants leaf coloring was insufficient with only 25% of the anthocyanin content of 50B:50R plants (Spalholz et al., 2019) and indicating a need for a final light treatment for color development. In addition to the selected dynamic strategy, the current dynamic light recipe experiment also included the use of end-of-day far-red light since it is known to increase cell elongation and consequently leaf expansion, and a finishing or "end-of-production" coloration to promote anthocyanin synthesis induction (Owen & Lopez, 2015). Overall, the aim of this study is to contribute to the research field of spectral optimization by using growth-stage specific DLRs to improve plant growth and morphology.

3.3 Materials & methods

3.3.1 Plant material and growing conditions

'Red Oakleaf' (Salanova®, distributed by Johnny's Selected Seed Corp., Waterville, ME, USA) lettuce (*Lactuca sativa* L.) seeds were sown into small pots filled with Sunshine Professional Growing Mix SS#1 (Sungro®, Agawam, MA, USA) potting substrate that consisted of 70-80% Sphagnum peat moss, 20-30% perlite, dolomitic lime, wetting agent, and enriched with silicon. Each pot (3.3 cm upper side width × 2.2 cm bottom side width × 5.2 cm deep with a

volume of 30 cm^3) was rotated within the tray to ensure uniform light intensity. After sowing, seeds were covered with a thin layer of vermiculite (2-4 mm thick) and trays were sub-irrigated with tap water. Trays were allowed to fully saturate before being drained and wrapped in plastic wrap. Once wrapped, the trays, consisting of 98 pots (725 plants m^{-2}), were then placed in a germination chamber. Seeds were germinated at 26 °C under continuous fluorescent lighting at 72 µmol m^{-2} s^{-1} PPFD for 60 h (day 0-3).

Once cotyledons emerged (day 3), trays were placed in their respective treatment in the experimental chamber. On day 19, plants were transplanted, using same substrate mentioned above, into larger square pots (7.4 cm wide × 6.3 cm deep with a volume of 173 cm^3) and placed at a density of 188 plants m^{-2}. Additional plant density reductions took place on day 28, 33, and 40 with densities being 67, 33, and 16.5 plants m^{-2}, respectively. A third and final transplant date occurred on day 33 into a larger pot (12.7 cm upper diameter × 9.3 cm lower diameter × 9.15 cm deep with a volume of 610 cm^3). All transplanting used the same substrate described above. Plants were sub-irrigated as needed with hydroponic solution containing (mg L^{-1}) 95 N, 24 P, 175 K, 100 Ca, 30 Mg, 58 S, 45 Cl, 0.34 B, 0.55 Mn, 0.05 Cu, 0.05 Mo, 0.33 Zn, and 2 Fe.

3.3.2 Chamber parameters & LED light treatments

Plants were grown in an experimental growth chamber that contained eight compartments. Compartments measured 100 cm wide × 84.5 cm high × 61 cm deep. Environmental conditions for each compartment were measured and recorded using a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA). Logged data consisted of air temperature measured closed to the leaf boundary layer and substrate temperature (thermocouples, 0.005 gauge, T-type, Omega Inc. Stamford, CT, USA), chamber CO_2 concentration (GMT222, Viasala Inc., Helsinki, FI), and chamber relative humidity (CS-215, Campbell Scientific Inc., Logan, UT,

USA) (Table 1). Environmental data was measured every five seconds and then averaged and recorded every 5 minutes. Air velocity was measured prior to experiment initiation (10 repeated measures were taken 30 seconds apart and then averaged for each of the 9 different locations in a grid arrangement within the experimental growing area) with a hotwire omni-directional anemometer (Table 1). Laminar air flow was provided by four equally spaced fans within the growing area at plant level. Both light quality and quantity were measured at the start and end of the experiment. Nutrient solution pH and electrical conductivity (EC) were measured every irrigation session (HI 9813-6, Hannah Instrument Inc, Woonsocket, RI, USA) (Table 1). CO_2 concentration and relative humidity was logged every 5 minutes using the method described above for temperature (Table 1).

LED light treatment

LED fixtures (LX601C/G, Heliospectra AB, Göteborg, SE) were used for the four light treatments. Four light treatments were all described as blue (B) or red (R) percent PFD: (1) a fixed 20% blue and 80% red (20B:80R) (FIXED) (Fig. 1a), (2) a fixed 20B:80R with end of day far-red light recipe (FIXED+FR) (Fig. 1b), (3) a dynamic light recipe that was grown with 20B:80R with EOD+FR from day 3-18 and 100% blue (100B) from day 19-39 (DYNAMIC) (Fig. 1c), and (4) a dynamic light recipe grown with 20B:80R+FR from day 3-18 and 100B+FR from day 19-39 (DYNAMIC+FR) (Fig. 1d). From day 40-47 all treatments were provided a 50% blue and 50% red (50B:50R) light treatment (Fig. 1). LED peaks were 452 nm for B with full width at half maximum (FWHM) at 23 nm, 659 nm for R (FWHM: 16 nm), and 732 nm for FR (FWHM: 20 nm). Light measurements were taken at the start and conclusion of the experiment. All treatments were set at equal photosynthetic active radiation (PAR: 400-700 nm). For LED fixture spectral qualities measured by a spectroradiometer (PS-200, Apogee Instruments Inc.,

Logan, UT, USA) see Figure 2. From day 3-10, treatment PPFD light intensity was maintained at 100 µmol m^{-2} s^{-1} with intensity increased to 200 µmol m^{-2} s^{-1} at day 10 and remained at this intensity until conclusion of the experiment. Both intensities had an 18 h photoperiod. End-of-day far-red consisted of a 30-minute period at 5.8 ± 0.13 µmol m^{-2} s^{-1} applied immediately after the daily photoperiod (10.4 mmol m^{-2} d^{-1}). Fixture height was adjusted to maintain equal intensity as the plants grew (constant 40 cm from top of canopy). Table 3 details the light intensity specifics of the different treatments in terms of PPFD, yield photon flux (YPF), and phytochrome photostationary state (PSS) based from Sager and McFarlane (1997). Experimental plants were positioned inside a row of border plants to prevent boundary effects and rotated every two days to ensure light uniformity within the experimental growing area (46 cm × 46 cm) from day 3-32 and rotated every day from day 33-47. The total growing area was 56 cm × 56 cm.

3.3.3 Measurements and data collection

Morphology and growth responses

Transplant stage was harvested on day 18 for the only two treatments at this stage, FIXED and FIXED+FR treatments. For morphological response 15 plants were randomly selected per repetition on the first harvest day (day 18). Ten plants were sampled on the second harvest day (day 25), and six, three, and three plants were harvested on days 32, 39, and 47, respectively, for morphological traits. Different sample sizes were in part dictated by the necessity to achieve normal density of lettuce plants that simulate commercial density levels, which is why more plants were sampled on day 25 than on days 39 or 47. Measurements on harvested plants included leaf count (2 cm minimum), longest leaf length, leaf area, fresh mass, and dry mass (dried for 72 h at 65 °C). Additionally, stem measurements were taken on day 32, 39, and 47 including stem length, stem fresh mass, and stem dry mass. Leaf and stem length

measurements were done using a ruler with excised plant material, dry and fresh mass was measured with an electronic scale. Leaves were scanned at every harvest and then processed with ImageJ 1.51g software (Schneider et al., 2012). On day 47, plant diameter and height were measured using a vernier caliper and ruler, respectively. In addition to the listed morphological responses, derived responses were calculated: (1) leaf mass area (LMA) the dried shoot mass divided by leaf area (g/cm^{-2}), (2) ratio of fresh to dry shoot mass (FM:DM) (FM g/ DM g), (3) leaf dry mass to stem dry mass partitioning (LDM:SDM), and (4) biomass accumulation on a mole YPF basis (FM/mol YPF and DM/mol YPF, respectively) (g/ mol).

Phytochemical concentrations

Leaf chlorophyll concentration from all five harvest points (sample size is n=5 for day 18 and 25, n=6 for day 32, and n=3 for day 39 and 42) was quantified using the Moran and Porath (1980) protocol using two leaf discs (each disc area: 56.55 mm^2) from a single plant. For consistency, discs were taken 1 cm from the leaf apex, avoiding the main leaf rib. Shoot anthocyanin concentration was measured on day 18, 25, 39, and 47 (sample size was n=5 for day 18, n=4 for day 25, and n=3 for both day 39 and 47). Whole shoot samples were held at -80 °C until freeze dried at -20 °C and pulverized after drying. Day 18 samples required 2 minutes of grinding at 800 rpm while all other samples required 6 minutes at 800 rpm using a 1600 miniG tissue homogenizer (SPEX SamplePrep, Metuchen, NJ, USA). For anthocyanin concentration analysis the whole shoot was used for days 18 and 25 while only leaf material was used for days 39 and 4. Anthocyanin concentration was quantified using the protocol from Li and Kubota (2009). Both anthocyanin and chlorophyll concentration were measured using a spectrophotometer (GeneSys 10S UV-VIS, Thermo Fisher Scientific, Waltham, MA, USA).

Net photosynthetic rate, transpiration rate, and stomatal conductance

Leaf photosynthetic CO_2 assimilation rate (Pn), transpiration (E), and stomatal conductance (gs) were measured with a gas exchange system (LI-6800, LI-COR, Lincoln, NE, USA) on day 47. Chamber conditions were 24 °C, 400 µmol mol-1 CO2, and 200 µmol m^{-2} s^{-1} of 50B:50R light while whole plants remained *in situ* under experimental conditions.

3.3.4 Statistical analysis

Statistical analysis for comparing the different treatments was done using an ANOVA Students tests ($P<0.05$) with JMP software Version Pro 13.2 (SAS Institute, Cary, NC, USA). For the case of comparisons between two different harvest days, for a single treatment, a T-test ($P<0.05$) was performed using the same software. Repetition was considered a fixed variable during statistical analysis.

3.4 Results & discussion

3.4.1 Harvest day 18

Plants were grown only under the light treatments FIXED and FIXED+FR until day 18 (D18). Plant dry mass or fresh mass did not differ with light treatment and FIXED plants had a higher FM:DM (4%) on D18 (Table 3 & 4). Anthocyanin and chlorophyll did not differ between light treatments (Table 4).

3.4.2 Plant biomass and biomass partition response

For harvest D25, FIXED had 15% greater fresh mass than DYNAMIC plants (Table 5). Meanwhile, no difference in fresh mass was found among any other treated plants. For plant dry mass on D25, FIXED and FIXED+FR plants had a 24-40% larger dry mass than DYNAMIC and DYNAMIC+FR plants (Table 5). In other words, photoperiod light recipe (i.e., 20B:80R or

100B) had a greater effect on plant dry mass than EODFR. By D32, FIXED and FIXED+FR were 18-22% and 17-27 % larger in fresh mass and dry mass, respectively, than DYNAMIC or DYNAMIC+FR (Table 5). Yet by D39 no difference in plant fresh or dry mass among treatments was found (Table 5). On D47, 7 days after all light treatments were switched to 50B:50R for the coloration phase of the experiment, no difference in plant fresh mass was observed among treatments (Table 6). Yet, by D47 DYNAMIC and DYNAMIC+FR had 9-12% greater dry mass than FIXED and FIXED+FR when illuminated by 50B:50R for 7 days (Table 6). Although all plants had the same fresh mass at D39 and D47 after receiving the same photosynthetic photon flux (PPF), DYNAMIC and DYNAMIC+FR had a 19-24% higher DM/molYPF than FIXED and FIXED+FR (Figure 3) on D47, meaning plants were capable of more efficient biomass production on a photon basis. Similarly, DYNAMIC and DYNAMIC+FR had a 15-18% higher FM/molYPF than FIXED and FIXED+FR (Figure 4).

There are different possibilities as to why this occurred based on plant architecture and physiology. Steep leaf angle and increased node lengths (not measured) could improve light penetration within the canopy to allow for more photosynthesis. In terms of light quality studies have found similar results where the ratio of R light increases lettuce fresh mass, dry mass, and leaf area increase (Son & Oh, 2015; Wang et al., 2016). As for 100B having the same fresh mass, previous studies on lettuce have shown the benefit of 100B, but also the penalty of 100B, in terms of fresh mass. Wang et al. (2016) found that using 100B reduced shoot fresh mass by 48% when compared to 8B:92R while Amoozgar et al. (2017) found that lettuce fresh mass was the same between 30B:70R and 100B. One possible explanation for the differences is the plants ability to recover from blue light syndrome (BLS) during seedling and transplant stage and allow

for proper orientation for higher light capture. In the current experiment, seedlings were not grown under 100B and thus excluded from any artifact of BLS.

When comparing the ratio of plant fresh mass to dry mass (FM:DM) the only harvest day to show a difference was the first (D18) which showed a difference among treatments, with FIXED plants having a 4% greater FM:DM than FIXED+FR plants (Table 4). Fresh and dry mass for plant leaf and stem were measured on D47 to determine the effect of light quality on biomass partition between plant leaf and stem. When comparing the ratio of leaf fresh mass to stem fresh mass (LFM:SFM) FIXED plants had the greatest LFM:SFM being 64% larger than DYNAMIC+FR plants (Table 6). A similar trend corresponded with leaf dry mass to stem dry mass (LDM:LSM) with FIXED plants having a LDM:SDM 59% larger than DYNAMIC+FR plants. As a high LFM:SFM and LDM:SDM ratio is preferred by growers, since their ultimate goal is to produce saleable leaves and not stems, the FIXED treatment is ideal (Table 6). Yet further analysis has shown that this ratio, nor light treatment, ultimately decreased leaf fresh and dry mass content (data only showed for Harvest day 47).

3.4.3 Plant morphology

Leaf area measurements taken across every harvest day indicated there was no difference in plant leaf area regardless of light treatment (Tables 4, 7, and 8). Mixed results on the effect of 100B in plant leaf area have been found in other experiments. For example, Son et al. (2013) found that leaf area response in lettuce was cultivar dependent with 100B light treatments either decreasing or increasing total leaf area while Snowden et al. (2016) and Wang et al. (2016) found that 100B light decreased leaf area index and leaf area, respectively, in lettuce. One possible explanation for the increase in leaf area with B light could be a novel light stimulated leaf expansion response via the acid growth pathway (Zivanovic et al., 2005) or regulating circadian

rhythm pathways that promote leaf expansion (Haydon et al., 2011). The fact that no leaf area differences were found between treatments given 100B and 20B:80R light in our experiment could be due to the fact that 100B promotes leaf expansion but as its overall YPF is lower than 20B:80R then it is limited by a decreased ability to convert light energy into further growth.

By the second harvest (D25) FIXED and FIXED+FR plants had 25-33% greater LMA than the two dynamic treatments (Table 7). A similar trend continued through D32 which showed that plants in the fixed treatments had 14 % greater LMA than plants in the DYNAMIC and DYNAMIC+FR treatment (Table 7). In a previous fixed light recipe study on cucumber plants, a similar trend was found with decreased LMA being attributed to decreased light absorptance per leaf area (Hogewoning et al., 2010. Interestingly, plants harvested on D39 (Table 7) and D47 (Table 8) demonstrated no difference in LMA among any treatments, indicating that (1) plants illuminated with 100B light eventually increased in thickness from D32 to D39, more so than 20B:80R, therefore matching LMA and (2) subsequent illumination of all plants with 50B:50R did not alter LMA based of off previous light treatment. As for plant-canopy diameter, DYNAMIC and DYNAMIC+FR were 15-20% larger than FIXED and FIXED+FR (Figure 5 and 6) while no difference was found in plant height among treatment (Table 6).

Leaf count differences changed with harvest time (Table 8 and 9). No difference between plant leaf count was found on D18 (Table 4) while on D25 plants in FIXED treatment had 14-18% more leaves than plants in FIXED+FR, DYNAMIC, and DYNAMIC+FR (Table 9). A similar trend was found for the remaining harvests (D32, D39, and D47) with plants in FIXED treatment having 8-26% more leaves than plants in DYNAMIC and DYNAMIC+FR (Table 8 and 9). As leaves are the main light capturing and photosynthetic organs of a plant it is crucial

that leaf architecture and spacing is optimized to maximize light capture. It is still unclear exactly how light quality impacts leaf organogenesis but this ability appears to be related to how different colors manipulate the two hormones cytokinin and auxin via photoreceptor activation (Yoshida et al., 2010).

For the first harvest (D18), plants in the FIXED+FR treatment had leaves 16% longer than plants in FIXED (Table 4). By D25, plants in FIXED treatment had the 10-15% shorter leaves than FIXED+FR, DYNAMIC, and DYNAMIC+FR plants (Table 9). For the remainder of the harvest days (D32, D39, and D47), plants in treatments DYNAMIC and DYNAMIC+FR had longest-leaves that were 9-20% longer than plants in FIXED and in FIXED+FR treatments (Table 8 and 9). Two factors could have contributed to these results: (1) the impact of B lights role in leaf expansion and (2) a decreased R:Fr ratio inducing longer leaves, a plant response to minimize leaf overlap and self-shading (Morgan and Smith, 1981).

Plant stem length was taken on D25, D32, D39, and D47 (Table 8 and 9). On D25 plant s in DYNAMIC and DYNAMIC+FR 19-35% longer stems than FIXED and FIXED+FR (Table 9). On day 32, DYNAMIC+FR plants had stems that were 22% longer than FIXED plants while no difference was found between all other treatments (Table 9). On D39 and D47, DYNAMIC and DYNAMIC+FR had 40-63% longer stem length than FIXED and FIXED+FR plants for both harvest days (Table 8 and 9). This, similar to longest leaf length, could be attributed to a shade avoidance response due to decreased R:Fr ratio in these two treatments where plants partition dry mass differently between leaves and stems and develop long and slender leaves to minimize leaf overlap and self-shading (Morgan and Smith, 1981).

3.4.4 Plant phytochemical concentration

Plant phytochemical concentration response was impacted by the different light treatments. On the first harvest (D18) chlorophyll concentration was similar between FIXED and FIXED+FR (Table 4). By the second harvest (D25) FIXED and DYNAMIC+FR plants both had 15-19% greater chlorophyll concentration than DYNAMIC and DYNAMIC+FR plants (Table 7). Results were similar for D32; for example, plants in FIXED had 19% higher chlorophyll concentration than in DYNAMIC+FR (Table7). The impact of light quality has been shown to have a great impact on chlorophyll concentration across different crop species. More specifically, it has been demonstrated by Li et al (2017) that an increased ratio of B light can decrease chlorophyll concentration in cotton, similar to lettuce in the current study. Furthermore, Li et al (2017) demonstrate that B light can increase spongy palisade tissue, the leaf tissue where most photosynthesis occurs, which maximizes holding capability for plant chlorophyll. Beyond photosynthetically active radiation the presence of FR light has been shown to decrease overall chlorophyll concentration. Hereaut-Bron et al. (1999) found that the presence of far-red light decreases clover chlorophyll concentration. Earlier light studies have confirmed that FR light decreases chlorophyll synthesis (Glick et al. 1985; Chow et al., 1990). This effect could have attributed to DYNAMIC+FR having the lowest chlorophyll concentration in the current study. Hereaut-Bron et al. (1999) suggested that as FR promotes efficient capture of light energy in the PSII center compared to when Fr is absent, results in a lower requirement for chlorophyll in the presence of Fr. In turn, this improved efficiency of photosynthesis expresses a reduced need for chlorophyll and chlorophyll synthesis within the plant. Interestingly the previous separation of plant chlorophyll concentration between treatments no longer existed by D39; when all treatments resulted in the same chlorophyll concentration. The trend of similar chlorophyll

concentration remained the same for the remainder of the experiment, even after 7 days of 50B:50R (Table 8).

Anthocyanin is a water-soluble red pigment that provides red coloration in leaves and is an anti-oxidant component in the human diet. Light quality plays a large role in plant anthocyanin biosynthesis. More specifically the B:R light ratio can impact concentration with high B photon flux increasing anthocyanin concentration (Spalholz et al., 2019) while FR light decreases its level (Li & Kubota, 2008) in lettuce. While anthocyanin concentration was similar between FIXED and FIXED+FR on D18, D25 plants in FIXED and FIXED+FR had 45-84% greater anthocyanin concentration than plants in DYNAMIC and DYNAMIC+FR (data not shown). Similar results were found for D39 (no anthocyanin samples were taken on D32) with plants in FIXED and FIXED+FR had 39-69% greater anthocyanin concentration than plants in DYNAMIC and DYNAMIC+FR (Figure 7). By the final harvest day (D47), after 7 days of 50B:50R for all plants, plant anthocyanin concentration was the same for plants previously grown in FIXED, FIXED+FR, and DYNAMIC plants while plants previously grown in DYNAMIC+FR had 30-41% lower anthocyanin concentration than in FIXED and FIXED+FR (Figure 7). These results indicate that a light recipe, like 50B:50R, can induce anthocyanin synthesis and increase anthocyanin concentration for DYNAMIC by 47% but not for other experimental treatments when comparing anthocyanin concentration of DYNAMIC between D39 and D47 (Figure 7). These results confirm the necessity to activate both the cryptochrome and phytochrome for optimal anthocyanin development. It has been demonstrated that crosstalk or “co-activity” of these two photoreceptors are required to fully promote flavonoid synthesis (Neff and Chory, 1998) and the reason why plants in 100% R light or 100% B light have decreased flavonoid concentration, and more specifically minimal anthocyanin synthesis

expression. A previous study has demonstrated the merit of "end-of-production" light supplementation strategies to promote anthocyanin synthesis in a greenhouse setting in order to color lettuce (using increased B light ratios) (Owen & Lopez, 2015).

3.4.5 Photosynthetic capacity, transpiration, and stomatal conductance

Photosynthetic measurements taken on D47 indicated that FIXED and FIXED+FR plants had 31-33% greater net photosynthetic rates (Pn) than DYNAMIC plants (Figure 8). DYNAMIC+FR plants shared similar Pn with all other treatments. A decreased Pn typically results in an overall decrease of plant biomass. Heraut-Bron et al. (1999) demonstrated this correlation in white clover with treatments characterized by lower Pn resulting in a lower final biomass. In our study plants with lower Pn (DYNAMIC) had larger dry mass than plants in treatments with high Pn (ie FIXED and FIXED+FR). The most likely cause for the discrepancy in results is light capture by the canopy. As leaves in DYNAMIC and DYNAMIC+FR presented themselves in an upright angle (leaf angle or orientation was not specifically characterized in this study) compared to the FIXED and FIXED+FR (see Fig. 7), it is possible that with an upright leaf stature, abaxial leaf tissues contributed to an overall increased A, as characterized in other studies when partitioned between adaxial and abaxial surfaces (Tsuyama et al., 2003; Terashima et al., 2009). As the LI-COR 6800 only irradiates the adaxial side of the leaf for Pn, transpiration (E), and stomatal conductance (gs) measurement, and not the abaxial side, the abaxial leaf surface contribution to overall leaf Pn is omitted as photosynthesis of the abaxial leaf surface is temporarily not activated while in the LI-COR chamber. No differences in plant E (Figure 9) and gs (Figure 10) were found.

3.5 Conclusion

The objective of this study was to optimize plant growth and phytochemical content using a novel B:R PFD DLR, applying production strategies already known from B:R PFD FLRs. The use of a DLR in this study demonstrated the benefits of: (1) early stage 20B:80R illumination (Day 3-19) to overcome BLS, (2) the benefit of 100B on the final dry mass content and overall plant diameter when followed by a (3) 50B:50R light recipe that could increase anthocyanin content in DYNAMIC plants. Further work should be conducted to see what other light formulations can be used to optimize plant growth and decrease the coloration time of 100B treated lettuce.

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Table 3.1 Experimental conditions for each treatment. Datalogged parameters are air temperature (1 cm above tallest leaf), substrate temperature, CO_2 concentration, relative humidity (RH), pH, and electrical conductivity (EC). Air velocity was measured at the start and end of the experiment. Selected parameters with standard deviation for each treatment according to photoperiod (ie, day or night). All parameters are combined averages from repetition 1 and 2.

Treatments	FIXED		FIXED+FR		DYNAMIC		DYNAMIC+FR	
Parameters	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
Air temperature (°C)	23.1 ± 0.0	17.8 ± 0.1	23.1 ± 0.0	17.6 ± 0.0	23.6 ± 0.2	18.3 ± 0.3	23.2 ± 0.2	18.1 ± 0.4
Substrate temperature (°C)	21.7 ± 0.1	17.4 ± 0.0	21.5 ± 0.1	16.7 ± 0.4	21.8 ± 0.1	17.3±.1	22.0 ± 0.7	17.5 ± 0.8
Air velocity (m s^{-1})	0.50 ± 0.15		0.55 ± 0.20		0.49 ± 0.28		0.64 ± 0.19	
CO_2 (µmol mol^{-1})	DAY: 543 ± 75; NIGHT: 592 ± 38							
RH (%)	DAY: 73.9 ± 8.5; NIGHT: 72.3 ± 7.7							
pH	6.4 ± 0.12							
EC (dS m^{-1})	1.3 ± 0.1							

Table 3.2 Light treatment qualities of experimental light recipes. Spectral characterization of experimental light treatments with different light recipes consisting of blue (B) and red (R), and 30 minutes of end-of-day far-red (EOD-FR) photon flux density (PFD). Values in the table represent averages of all initial- and end-of-experiment light measurements for respective light recipe.

Light Recipe	**PFD***	**YPF****	**PSS†**
20B:80R	198.8 ± 0.7	178.5 ± 0.7	0.882 ± 0.0
50B:50R	199.3 ± 0.5	166.8 ± 0.3	0.863 ± 0.0
100B	199.2 ± 0.4	147.0 ± 0.2	0.497 ± 0.0
EOD-FR	5.8 ± 0.13	1.31 ± 0.04	0.150 ± 0.0

*PFD is 400-700 nm for 20B:80R, 50B:50R, and 100B or 700-800 nm EOD-FR (µmol m^{-2} s^{-1})
**Yield photon flux (YPF) values in µmol m^{-2} s^{-1} calculated from Sager and Mcfarlane (1997)
†Phytochrome photostationary state (PSS) calculated from Sager and Mcfarlane (1997)

Table 3.3 Harvest day 18 morphology for 'Red Oakleaf' lettuce under 20B:80R (FIXED) and 20B:80R plus end-of-day far-red (FIXED+FR) illumination. Each treatment had a sample size of 10 plants (for replication 1 and 2) except for chlorophyll content (n=5). Fresh mass to dry mass ratio (FM:DM), Leaf mass area (LMA), and curled leaf width:flat leaf width (CW:FW) were calculated.

	D18 Harvest								
Treatment	Fresh Mass (g)	Dry Mass (g)	FM:DM	Leaf Count	Longest Leaf (cm)	Leaf Area (cm^2)	LMA ($g\ m^{-2}$)	CW:FW	Chl Content ($g\ m^{-2}$)
FIXED	1.6 ± 0.1 a	0.084 ± 0.004 a	18.6 ± 0.0 a	9.4 ± 0.4 a	7.5 ± 0.1 b	64.9 ± 1.4 a	13.1 ± 0.3 a	0.84 ± 0.01 a	0.24 ± 0.02a
FIXED+FR	1.6 ± 0.1 a	0.093 ± 0.006 a	17.9 ± 0.3 a	8.9 ± 0.3 a	8.7 ± 0.0 a	71.2 ± 1.7 a	13.1 ± 0.6 a	0.76 ± 0.01 a	0.23 ± 0.01 a
p-value	0.7001	0.5508	0.2768	0.6392	0.0181	0.2877	0.9928	0.2032	0.8952

Table 3.4. Harvest day 18 chlorophyll and anthocyanin content for ‘Red Oakleaf’ lettuce under 20B:80R (FIXED) and 20B:80R plus end-of-day far-red (FIXED+FR) radiation. Each treatment had a sample size of 5 for each analysis

	D18 Harvest	
Treatment	Chlorophyll Content (g m^{-2})	Anthocyanin Content (mg g^{-2})
FIXED	0.24 ± 0.02 a	2.21 ± 0.7 a
FIXED+FR	0.23 ± 0.01 a	1.3 ± 0.1 a
p-value	0.2032	0.3425

Table 3.5 Harvest day 25, 32, and 39 biomass for ‘Red Oakleaf’ lettuce under 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). Each treatment had a sample size of 10, 6, and 3 plants for harvest day 25, 32, and 39, respectively (for replication 1 and 2) except for chlorophyll content (n=5 for harvest day 25, n=6 for harvest day 32, and n=3 for harvest day 39).

	D25 Harvest			D32 Harvest			D39 Harvest				
Treatment	Fresh Mass (g)	Dry Mass (mg)	FM:DM	Fresh Mass (g)	Dry Mass (g)	FM:DM	Fresh Mass (g)	Dry Mass (g)	FM:DM	LFM:SFM	LDM:SDM
FIXED	7.3 ± 0.0 a	0.407 ± 0.012 a	17.9 ± 0.6 a	27.1 ± 0.9 a	1.3 ± 0.1 a	21.1 ± 0.6 a	70.2 ± 2.6 a	3.6 ± 0.2 a	19.8 ± 0.1 a	22.9 ± 3.9 a	21.2 ± 0.1 a
FIXED+FR	6.8 ± 0.5 ab	0.380 ± 0.027 a	18.0 ± 0.0 a	26.8 ± 0.1 a	1.4 ± 0.0 a	20.0 ± 0.7 a	71.6 ± 2.3 a	3.6 ± 0.2 a	19.8 ± 0.3 a	25.2 ± 0.2 ab	20.6 ± 0.5 a
DYNAMIC	6.5 ± 0.2 ab	0.306 ± 0.039 b	21.7 ± 2.2 a	22.7 ± 0.3 b	1.1 ± 0.1 b	21.3 ± 1.4 a	65.3 ± 0.4 a	3.3 ± 0.0 a	20.0 ± 0.0 a	17.6 ± 0.0 ab	16.1 ± 0.2 b
DYNAMIC+FR	6.2 ± 0.2 b	0.290 ± 0.018 b	21.6 ± 1.0 a	22.0 ± 0.1 b	1.1 ± 0.0 b	20.6 ± 0.0 a	63.4 ± 5.3 a	3.2 ± 0.2 a	19.7 ± 0.5 a	15.6 ± 0.4 b	15.0 ± 0.1 b
p-value	0.1069	0.0144	0.1002	0.0082	0.0331	0.5146	0.4565	0.3613	0.7123	0.1176	0.0011

Table 3.6 Harvest day 47 biomass and plant height measurements for ‘Red Oakleaf’ lettuce under 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). Each treatment had a sample size of 3 plants for harvest day 47 (for replication 1 and 2) except for chlorophyll content (n=3).

	D47 Harvest					
Treatment	Fresh Mass (g)	Dry Mass (g)	FM:DM	LFM:SFM	LDM:SDM	Height (cm)
FIXED	118.7 ± 0.9 a	6.5 ± 0.1 b	18.2 ± 0.1 a	19.5 ± 0.1 a	17.5 ± 0.3 a	12.6 ± 0.6 a
FIXED+FR	121.7 ± 4.7 a	6.6 ± 0.1 b	18.4 ± 0.4 a	16.0 ± 0.5 b	14.5 ± 0.2 b	12.8 ± 0.3 a
DYNAMIC	126.6 ± 0.5 a	7.3 ± 0.1 a	17.2 ± 0.2 a	11.9 ± 0.0 c	11.0 ± 0.2 c	13.2 ± 0.2 a
DYNAMIC+FR	126.4 ± 6.0 a	7.1 ± 0.3 a	17.7 ± 0.1 a	9.5 ± 0.1 d	9.8 ± 0.1 d	14.15 ± 0.2 a
p-value	0.2942	0.0167	0.1798	0.0003	0.0003	0.2164

Table 3.7 Harvest day 25, 32, 39, and 47 leaf area and leaf mass area (LMA) for 'Red Oakleaf' lettuce 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). Each treatment had a sample size of 10, 6, 3, and 3 plants for harvest day 25, 32, 39, and 47 respectively (for replication 1 and 2) except for chlorophyll content (n=5 for harvest day 25, n=6 for harvest day 32, and n=3 for harvest day 39).

	D25 Harvest		D32 Harvest		D39 Harvest		D47 Harvest	
Treatment	Leaf Area (cm^2)	LMA ($g\ m^{-2}$)	Leaf Area (cm^2)	LMA ($g\ m^{-2}$)	Leaf Area (cm^2)	LMA ($g\ m^{-2}$)	Leaf Area (cm^2)	LMA ($g\ m^{-2}$)
FIXED	262.8 ± 13.5 a	15.5 ± 1.2 a	809.1 ± 33.6 a	16.1 ± 0.3 a	1930.0 ± 97.1 a	18.0 ± 2.0 a	0.34 ± 0.02 a	18.5 ± 1.2 a
FIXED+FR	249.6 ± 14.9 a	15.1 ± 0.1 a	816.9 ± 13.4 a	16.6 ± 0.6 a	1947.3 ± 47.6 a	17.8 ± 0.4 a	0.34 ± 0.01 a	18.4 ± 0.1 a
DYNAMIC	258.9 ± 2.5 a	11.8 ± 1.6 b	762.9 ± 19.1 a	14.1 ± 0.9 b	1978.9 ± 5.4 a	15.5 ± 0.0 a	0.36 ± 0.00 a	18.5 ± 0.1 a
DYNAMIC+FR	248.4 ± 6.9 a	11.6 ± 0.4 b	738.7 ± 39.3 a	14.5 ± 0.9 b	1955.8 ± 7.4 a	15.5 ± 0.8 a	0.36 ± 0.02 a	18.1 ± 0.3 a
p-value	0.8055	0.0507	0.4169	0.0189	0.9479	0.377	0.5606	0.9809

Table 3.8 Harvest day 25, 32, 39, and 47 leaf morphology and stem length for 'Red Oakleaf' lettuce under 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Each treatment had a sample size of 10, 6, and 3 plants for harvest day 25, 32, 39, and 47 respectively (for replication 1 and 2) except for chlorophyll content (n=5 for harvest day 25, n=6 for harvest day 32, and n=3 for harvest day 39).

	D25 Harvest			D32 Harvest			D39 Harvest			D47 Harvest		
Treatment	Leaf Count	Longest Leaf (cm)	Stem Length (cm)	Leaf Count	Longest Leaf (cm)	Stem Length (cm)	Leaf Count	Longest Leaf (cm)	Stem Length (cm)	Leaf Count	Longest Leaf (cm)	Stem Length (cm)
FIXED	17.1 ± 0.4 a	10.5 ± 0.1b	0.7 ± 0.0 b	35.4 ± 1.2 a	12.5 ± 0.2 c	1.2 ± 0.1 b	60.5 ± 0.8 a	13.6 ± 0.3 b	1.7±0.1 b	96.3 ± 0.3 a	14.0 ± 0.3 b	2.8 ± 0.0 c
FIXED+FR	15.0 ± 0.4 b	11.6 ± 0.3 a	0.8 ± 0.0 b	31.3 ± 0.8 ab	13.4 ± 0.4 bc	1.3 ± 0.1 ab	56.0 ± 1.3 ab	14.0 ± 0.2 b	1.9±0.1 b	89.3 ± 0.3 ab	14.1 ± 0.0 b	3.2 ± 0.3 c
DYNAMIC	14.8 ± 0.4 b	12.2 ± 0.1 a	1.0 ± 0.0 a	29.5 ± 1.1 b	14.6 ± 0.2 ab	1.5 ± 0.0 ab	51.0 ± 1.0 bc	16.3 ± 0.5 a	2.7±0.1 a	83.5 ± 3.5 b	16.2 ± 0.1 a	3.9 ± 0.0 b
DYNAMIC+FR	14.5 ± 0.4 b	12.3 ± 0.2 a	1.0 ± 0.0 a	28.8 ± 0.5 b	15.0 ± 0.2 a	1.5 ± 0.0 a	48.2 ± 1.2 c	16.3 ± 0.3 a	2.8±0.1 a	80.2 ± 2.2 b	16.6 ± 0.2 a	4.6 ± 0.1 a
p-value	0.0514	0.0196	0.0174	0.0604	0.0224	0.0952	0.0195	0.0227	0.0028	0.0532	0.005	0.0087

Table 3.9 Harvest day 25, 32, 39, and 47 chlorophyll content (g m^{-2}) for 'Red Oakleaf' lettuce under 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Sample size was: n=5 for harvest day 25, n=6 for harvest day 32, and n=3 for harvest day 39, n=3 for harvest day 47.

Treatment	D25 Harvest	D32 Harvest	D39 Harvest	D47 Harvest
FIXED	0.28 ± 0.00 a	0.33 ± 0.01 a	0.34 ± 0.01 a	0.33 ± 0.03 a
FIXED+FR	0.28 ± 0.01 a	0.31 ± 0.01 b	0.31 ± 0.01 a	0.38 ± 0.02 a
DYNAMIC	0.24 ± 0.01 b	0.30 ± 0.00 c	0.32 ± 0.00 a	0.40 ± 0.01 a
DYNAMIC+FR	0.24 ± 0.00 b	0.28 ± 0.01 d	0.31 ± 0.02 a	0.36 ± 0.02 a
p-value	0.0258	0.0006	0.2848	0.2677

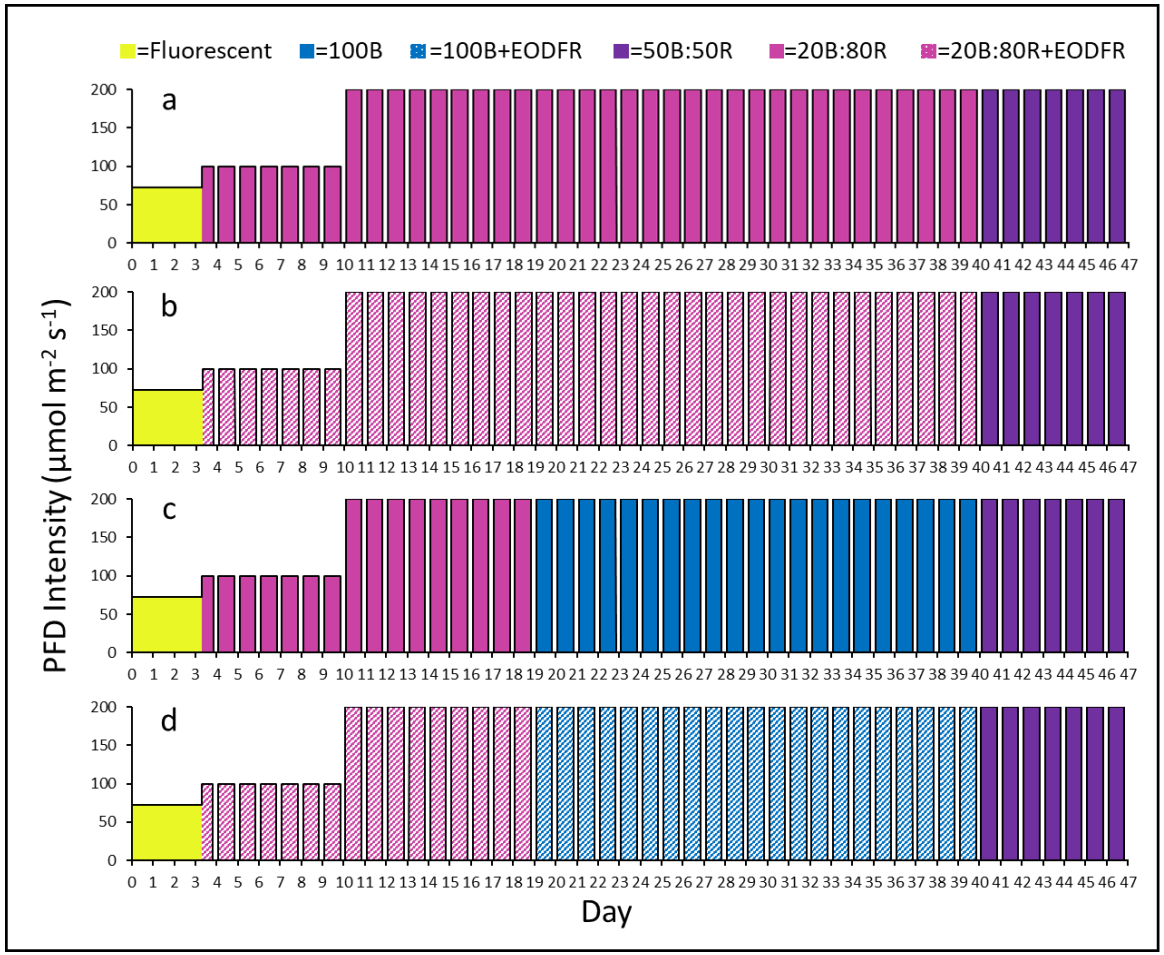

Figure 3.1 Experimental light treatment diagram. The four experimental light treatments of 20B:80R (FIXED) (a), 20B:80R plus end-of-day far-red (FIXED+FR) (b), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC) (c), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR) (d). 5.8 µmol m^{-2} s^{-1} of end of day far-red was applied for 30 minutes after regular.

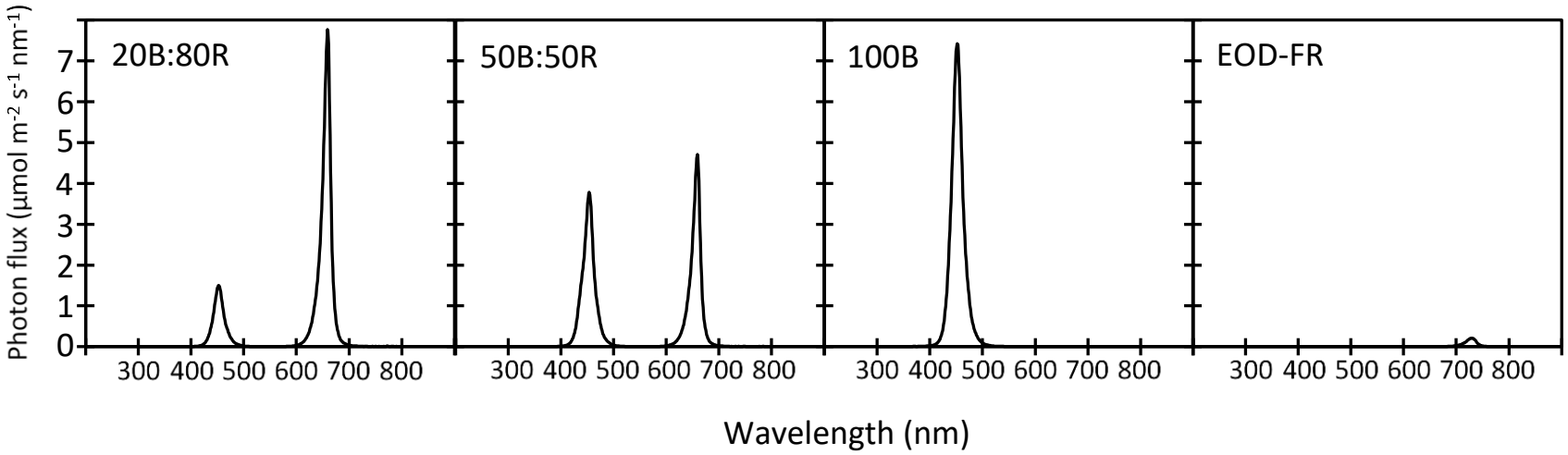

Figure 3.2 Spectral distribution of LED light treatments. Spectra recorded from a five point light field (40 cm height) averaged together. Each graph is the average reading for both replications and all respective light treatments; 20B:80R, 50B:50R, 100B, and far-red (EOD-FR).

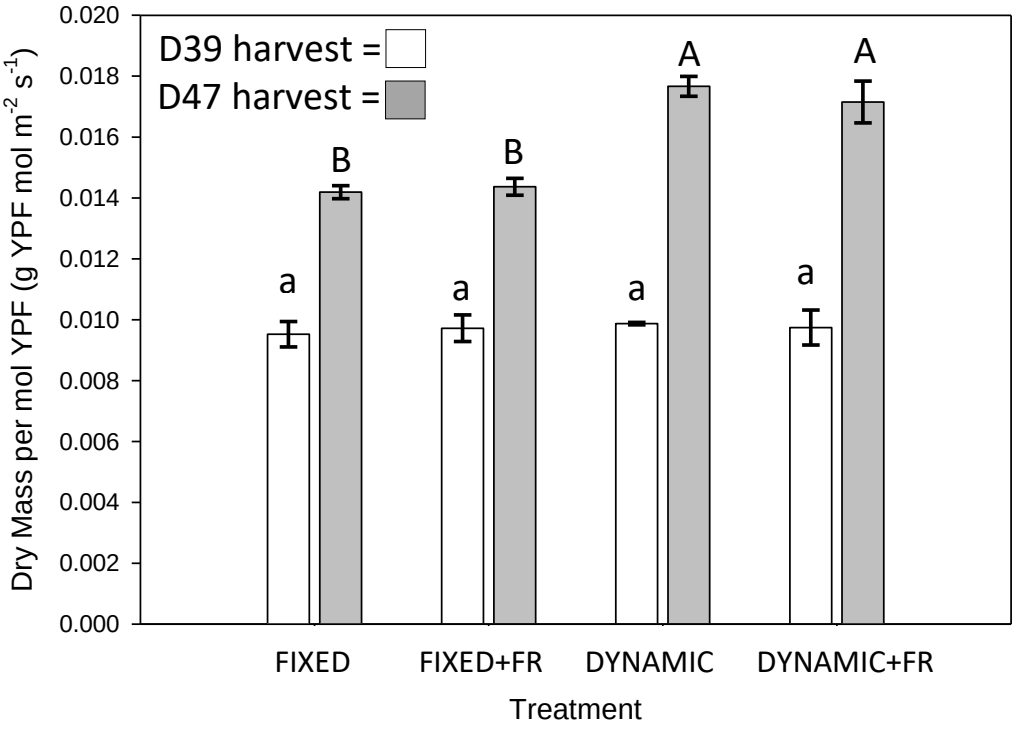

Figure 3.3 Dry mass per mole yield photon flux (YPF) under different LED treatment for harvest day 39 (white) and day 47 (grey). LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. STUDENTS test score correlate with each respective harvest period (D39 or D47). Sample size is n=3.

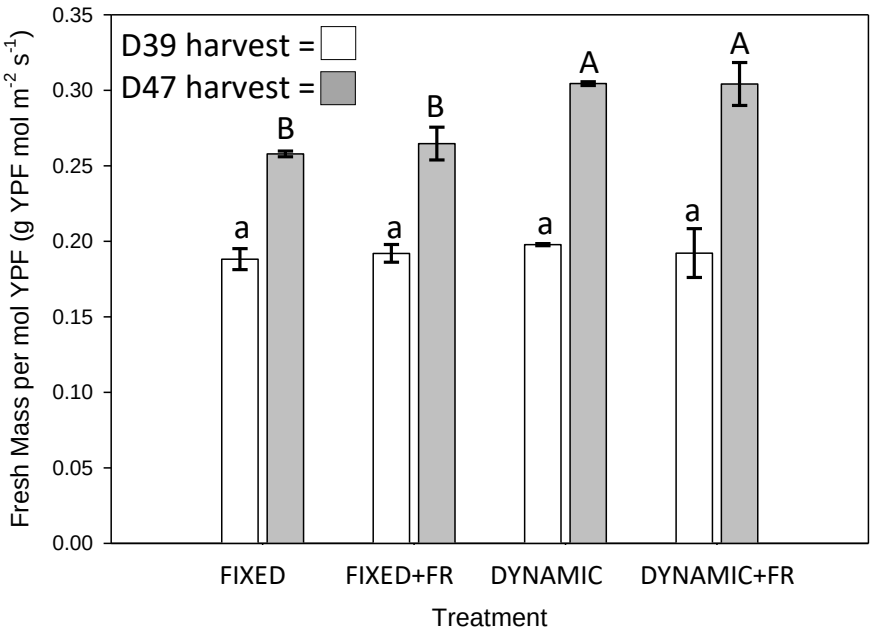

Figure 3.4 Fresh mass per mole YPF under different LED treatment for harvest day 39 (white) and day 47 (grey). LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. STUDENTS test score correlate with each respective harvest period only. Sample size is n=3.

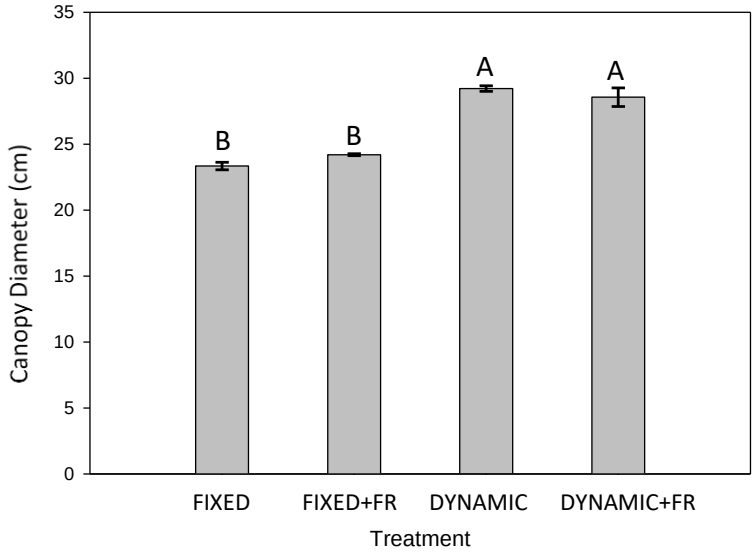

Figure 3.5 Plant diameter under different LED treatment for harvest day 39 (white) and day 47 (grey). LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. STUDENTS test score correlate with each respective harvest period only. Sample size is n=3.

Figure 3.6 Morphology of 'Red Oakleaf' lettuce under LED treatment. LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Pictures were taken harvest day 18 (a), 25 (b), 32 (c), 39 (d), and 47 (e). The white bar for each respective harvest day is equal to 10 cm.

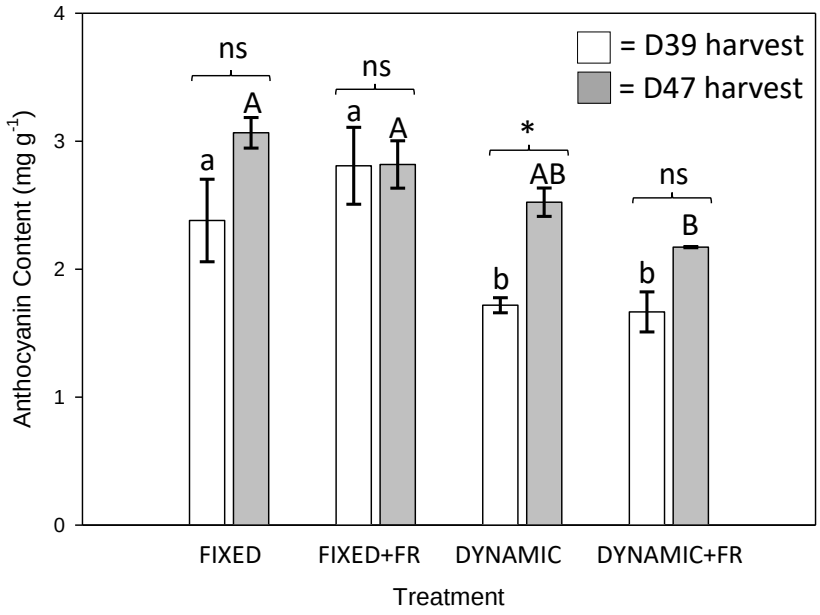

Figure 3.7 Plant anthocyanin concentration under different LED treatment for harvest day 39 (white) and day 47 (grey). LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. STUDENTS test score correlate with each respective harvest period only. A T-test compared results of each treatment between sampling days (indicated by bracket, ns= non-significant and *= significant difference $p > 0.05$). Sample size is n=3.

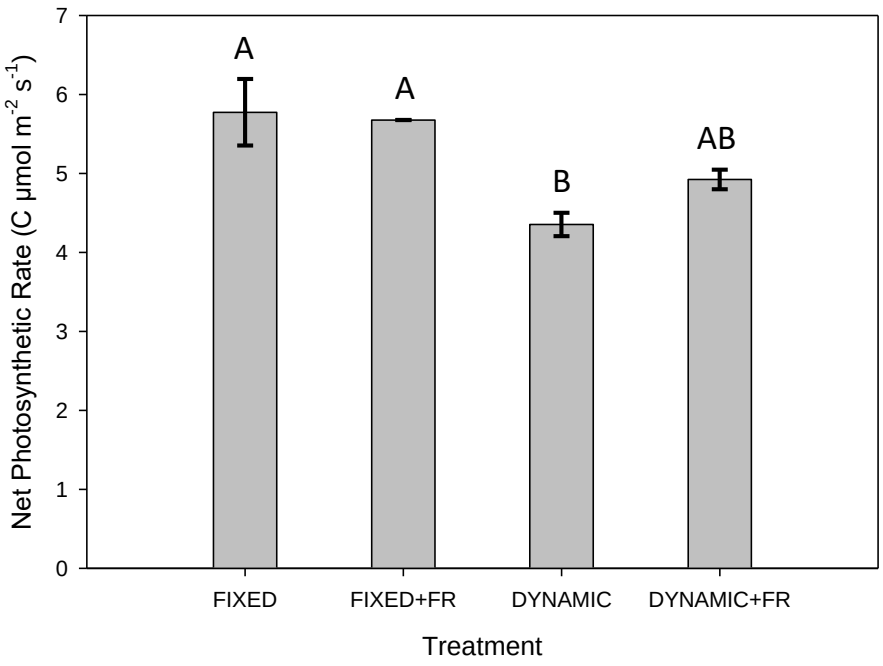

Figure 3.8 Leaf photosynthetic CO_2 assimilation rate on lettuce leaves grown with four different LED treatments taken on day 47. LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Measuring chamber conditions were 24 °C, 400 µmol mol^{-1} CO_2, and 200 µmol m^{-2} s^{-1} of 50B:50R light. Sample size is n=3 with youngest, fully mature leaves measured.

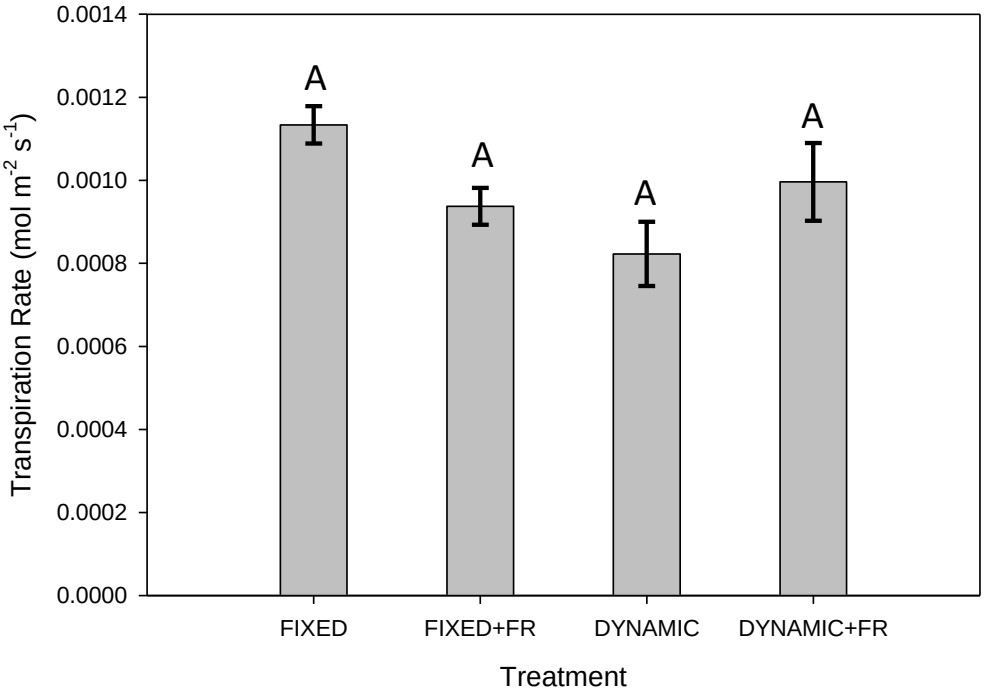

Figure 3.9 Transpiration rate of lettuce leaves grown under four different LED treatment taken on day 47. LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Measuring chamber conditions were 24 °C, 400 µmol mol-1 CO2, and 200 µmol m-2 s-1 of 50B:50R light. Sample size is n=3 with youngest, fully mature leaves measured.

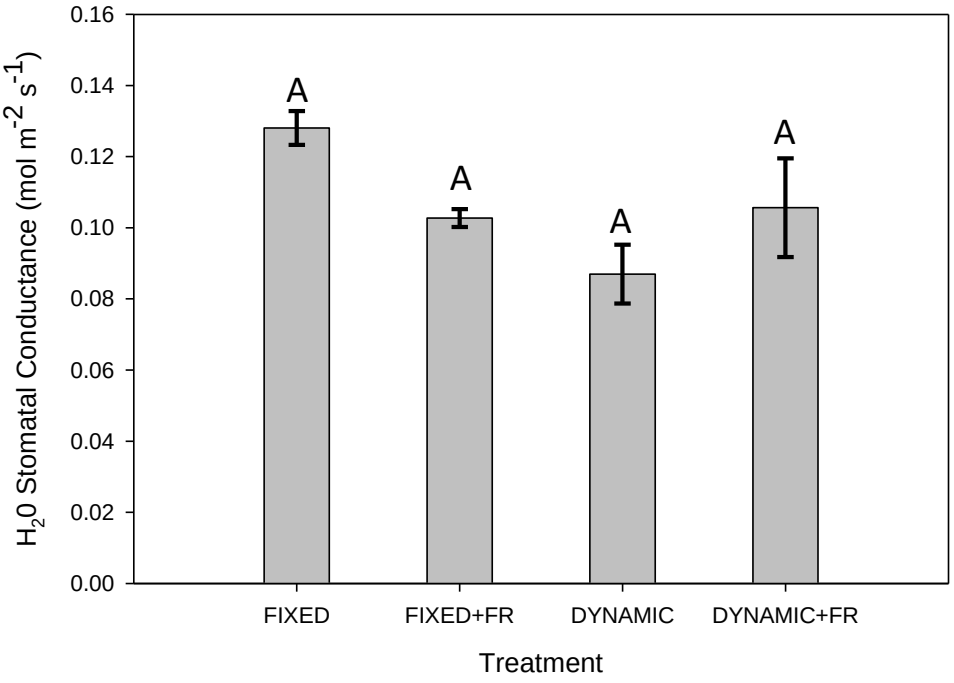

Figure 3.10 Stomatal conductance of lettuce leaves grown under four different LED treatment taken on day 47. LED treatments were: 20B:80R (FIXED), 20B:80R plus end-of-day far-red (FIXED+FR), 20B:80R plus end-of-day far-red that is changed to 100B on day 18 (DYNAMIC), and 20B:80R plus end-of-day far-red that is changed to 100B plus end-of-day far-red on day 18 (DYNAMIC+FR). All treatments were switched to 50B:50R on day 40. Measuring chamber conditions were 24 °C, 400 µmol mol-1 CO2, and 200 µmol m-2 s-1 of 50B:50R light. Sample size is n=3 with youngest, fully mature leaves measured.

CHAPTER 4

Characterization of solar radiation spectral contribution in lettuce bolting and flowering using LEDs in an indoor setting

4.1 Abstract

Lettuce bolting, induced by heat stress and plant age, is a detrimental trait to the lettuce grower as it reduces crop marketability and limits field production windows to avoid bolting conditions. Yet the impact of light quality on bolting is not fully understood. The objective of this experiment is to characterize how different sunlight spectral components blue (B), green (G), red (R), and far-red (FR) light, contribute to bolting initiation. To examine the role of each color on bolting four light formulas were used in the following ratios (percent relative to PAR): (1) 28B:35G:38R:34FR (B-G-R-Fr), (2) 27B:35G:38R:0FR (B-G-R), 43B:0G:57R:57FR (B-R-Fr), and (4) 43B:0G:57R:0FR (B-R-lowFr). From days 3-11 plants were provided with 75 µmol m^{-2} s^{-1} photosynthetic photon flux density (400-700nm) (PPFD) for 18 h and increased to 150 µmol m^{-2} s^{-1} PPFD for 18 h from days 12 to 70. Morphological data was collected on day 18 (D18), 25 (D25), 32 (D32), 46 (46), and 70 (D70). Differences in stem length, which measures initial bolting response, were found on the first harvest day and were evident for each remaining harvest day. On D18, B-G-R-Fr plants had the longest stems being 282% and 261% longer than B-G-R and B-R-lowFr plant stems, respectfully, with both treatments resulting in the shortest stemmed plants. B-G-R-Fr plants had the longest stems for all other harvests days and by D70 were 33% longer than B-R-Fr plants, the second longest stemmed plants, and 381-424% longer than B-R-Fr and B-G-R plants, the shortest stemmed plants. No differences were found for fresh and dry mass by D70 among light treatments. Interestingly, by D70 the ratio of fresh leaf mass to fresh stem mass (LFM:SFM) was 403-448% higher in B-G-R and B-R plants compared to B-G-R-Fr and B-R-Fr plants. B-G-R treatment plants had the smallest leaf area, being 8-11% smaller than B-R-Fr, B-R-lowFr, and B-G-R-Fr plants by D70. Meanwhile, no differences were found among treatments for plant leaf mass area (LMA) by D70. These results demonstrate how

individual colors can both regulate and accelerate the physiological transition toward the lettuce reproductive stage and impact overall plant resource partitioning relative to organ development.

4.2 Introduction

Lettuce is a widely consumed crop cultivated in outdoor fields, greenhouses, and indoor vertical farm (VF) systems. Lettuce bolting, the transition from the vegetative to the reproductive phase of growth, is marked by stem elongation and flower formation which drastically reduces lettuce eating quality (Chen et al., 2017). Lettuce bolting can be induced by high ambient temperatures (ie summer season and the tropics), plant age, exogenous gibberellin application, and day length (Waycot, 1995). As an avoidance strategy for heat induced bolting, cultivation is restricted to cooler seasons to prevent bolting which poses two types of losses: (1) losses where fields are deliberately kept fallow during the hot seasonand (2) losses due to unexpected and unseasonal warm periods that cause lettuce bolting. Yet, in addition to the previous listed causes, the impact of light quality on lettuce bolting has not been thoroughly evaluated to determine how certain solar spectral ranges (ie B, G, R, Fr) contribute to this phenomenon. By understanding how light quality contributes to lettuce bolting light spectrum manipulation could be utilized to induce and accelerate bolting or conversely, in commercial production, repress or delay the on-set of bolting.

Light quality and flowering

Light is the main energy driving photosynthesis in plants and light quality (ie, the types and ratios of colors in a given light recipe) provides information to the plants environment which triggers plant response. The qualitative aspect of light is perceived by multiple families of photoreceptors. These include the red and far-red light absorbing phytochrome proteins (the

earliest identified photoreceptor), the UV-A and blue light absorbing cryptochromes, blue light absorbing phototropins, ZEITLUPE (perception of blue light for maintaining circadian rhythm entrainment), and the most recently discovered, UVR8 protein (perception of near UV-C and UV-B). In addition to these different photoreceptor families, it is known that within a given family multiple photoreceptor types exist due to mutation of ancient gene homologs which led to functional divergence. For example, in angiosperms there can be five phytochrome types (PHYA, PHYB, PHYC, PHYD, and PHYE), two cryptochromes (CRY1 and CRY2), and two phototropins (PHOT1 and PHOT2). In the case of the PHY family, different roles can be played be each PHY while the duplicate CRY and PHOT types are currently known only to be redundant within each family. Furthermore, photoreceptor homolog genes (i.e., multiple encoding gene insertions) of a photoreceptor type exist to ensure utility and genetic redundancy via multiple copies being present.

Photoreceptor types can play distinct and specific roles in plant photomorphogenesis that can be dissimilar to their related photoreceptor type. For example, PHYA is present in seeds and dark grown seedlings (i.e., germination activities) and is quickly degraded in the presence of continuous red and white light, while PHYB-E are found and functioning in the presence of light (i.e., starting at germination and the remainder of the plants life cycle). While PHYA plays no role with flowering, PHYB is influential, being the phytochrome modeled as opposed to PHYA, and PHYC-E. In addition, antagonistic roles between PHYA and PHYB will render each other nonfunctional when simultaneously expressed. The roles of each distinct phytochrome type, well characterized in Arabidopsis, have been found to be highly conserved among all higher plants (Mathews, 2006).

Lettuce, a facultative long day plant like Arabidopsis, requires a long day photoperiod (12 or more hours of day light) to flower. In Arabidopsis, this photoperiodism required for flowering is perceived in the leaf where florigen is synthesized (Song et al., 2014; Golembeski & Imaizumi, 2015) then transported to plant vasculature where the florigen is then relocated and accumulated. In the leaf, PHY proteins are converted between the P_{fr} (active form) and P_r (inactive form) depending on the ratio of red to far red light. Regardless of intensity all phytochromes function fundamentally the same by acting as a switch that exists in two forms; either the red absorbing form P_r (peak absorption of 660 nm) or the far-red absorbing form P_{fr} (peak absorption 730 nm). In the presence of high red light or a high R:Fr ratio the red absorbing P_r form is converted into P_{fr} and vice versa in the presence of high Fr intensity.

There is evidence that there are additional or potential conformational changes that can cause an additional different phytochrome state than either the P_r and P_{fr} form (Ulijasz et al., 2010) as well as the unknown impact of phytochrome nuclear body sizing (Wang and Wang, 2015) that could have further implications. Once in the P_{fr} form, the phytochrome can then "switch" back to its original P_r configuration by either a slower "dark" (ie at night or in darkness) reversion, when light is absent, or in the presence of far-red light. The dark reversion is also known as thermal reversion and is described as having 2 steps (each step consists of the reversion of a single homodimer, hence a third form phytochrome type candidate) that at both stages are temperature rate dependent (higher temperatures increase reversion to P_r form) (Legris et al., 2016). The trait of temperature relevancy, found only in PHYB, also makes PHYB a thermosensor perhaps exacerbating temperature bolting effects in the presence of low R:FR light (ie sun light). In terms of flowering, as day length increases the amount of night time phytochrome P_{fr} accumulates to a greater degree, with a shorter time span given to revert back to

the P_{fr} state during the night is increased while the shortened period to dawn reinforces the balance in favor of said P_{fr} levels.

Although the molecular mechanisms have not been established, it is clear that PHYB regulates the circadian rhythm which sequentially regulates flowering (Wang and Wang, 2015). It is known that PHYB (Specifically the P_{fr} form) can directly interact with PHYTOCHROME INTERACTING FACTORS (PIFs) which then determine CONSTANS (CO) gene expression levels, a central circadian rhythm entrainment gene, which then initiates FLOWERING LOCUS T (FT) expression. The expression of FT in the leaf is the final step before florigen synthesis and transport to the shoot apical meristem, upstream of local floral morphogenesis genes such as APETALA1 (AP1) and LEAFT (LFY). The FT homolog gene for lettuce, LsFT, was first described in 2011 (Fukuda et al. 2011).

The influence of other colors on circadian rhythm entrainment have also been documented. Fraser et al. (2016) outlined the synergistic and, in some instances, inhibiting roles of photoreceptor crosstalk between the phytochrome and cryptochrome in plant shade avoidance strategies (SAS). In an earlier study, the dynamics of phytochrome and cryptochrome circadian rhythm entrainment, under different light recipes, outlined synergistic effects of blue and red light (Somers et al., 1998). Interestingly, although green (G) light (500-599 nm) favors the P_r status of PHYB over the P_{fr} state, it has the phenotypic and functional effect of contributing to the P_{fr} state. A potential explanation for this property is the characteristic of green light to inactivate cryptochrome signaling, masking blue light signals that could otherwise down regulate far-red light responses of the phytochrome (Bouly et al., 2007).

The objective of the current experiment was to gather baseline information on the impact of light quality on lettuce bolting using morphological data and determine if the absence of

spectral components of sunlight have differential impacts on plant biomass partitioning as well as influencing the rate of transition from the vegetative to the reproductive stage.

4.3 Materials & methods

4.3.1 Plant material and growing conditions

'Red Oakleaf' (Salanova®, distributed by Johnny's Selected Seed Corp., Waterville, ME, USA) lettuce (*Lactuca sativa* L.) seeds were sown into square pots filled with Sunshine Professional Growing Mix #1 N&O RSi (Sungro® Horticulture, Lot code: AND18 066 135130, Agawam, MA, USA) potting substrate that consisted of 70-80% Sphagnum peat moss, 20-30% perlite, dolomitic lime, and 0.0001% silicon dioxide from calcium silicate. Each square pot (3.3 cm upper side width × 2.2 cm bottom side width × 5.2 cm deep with a volume of 30 cm^3) could be independently moved within the tray to allow for rotation to ensure uniform light dosage within the light field for each plant each tray had 98 pots with a plant density of 725 plants m^{-2}. Single seeds were sown into each cell and covered with a thin layer of vermiculite (2-4 mm thick). Trays were then sub-irrigated with tap water, allowed to saturate and then drained before being wrapped in plastic wrap. Once wrapped, the trays were then placed in a germination chamber. Seeds were germinated at 25.5 °C under continuous fluorescent lighting at 69 µmol m^{-2} s^{-1} PPFD (photosynthetic photon flux density) for 51 h (days 1-3).

Once cotyledons emerged (day 3), plastic wrap was removed, and trays were placed in their respective treatment in the experimental chamber. On day 20, plants were transplanted into larger square pots (7.4 cm wide × 6.3 cm deep with a volume of 173 cm^3) and placed at a density of 188 plants m^{-2}. Additional plant density reductions took place on day 26, 33, and 40 with densities being 89, 45, and 22.5 plants m^{-2}, respectively. A third and final transplant date

occurred on day 40 into a larger pot (12.7 cm upper diameter × 9.3 cm lower diameter × 9.15 cm deep with a volume of 610 cm^3). All transplanting used the same substrate described above. Plants were sub-fertigated as needed with hydroponic solution containing (mg L^{-1}) 95 N, 24 P, 175 K, 100 Ca, 30 Mg, 58 S, 45 Cl, 0.34 B, 0.55 Mn, 0.05 Cu, 0.05 Mo, 0.33 Zn, and 2 Fe.

4.3.2 Chamber parameters & LED light treatments

Plants were grown in an experimental growth chamber that contained eight compartments. Compartments measured 100 cm wide × 84.5 cm high × 61 cm deep. Environmental conditions for each compartment were measured and recorded using a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA). Logged data consisted of leaf boundary layer and substrate temperature (thermocouples, 0.005 gauge, T-type, Omega Inc. Stamford, CT, USA), chamber CO_2 concentration (GMT222, Viasala Inc., Helsinki, FI), and chamber relative humidity (CS-215, Campbell Scientific Inc., Logan, UT, USA). Environmental data was measured every five seconds and then averaged and recorded every five minutes. Air velocity was measured prior to experiment initiation (10 repeated measures were taken 30 seconds apart and then averaged for each of the 9 different locations in a grid arrangement within the experimental growing area) with a hotwire omni-directional anemometer. Laminar air flow was provided by four equally spaced fans within the growing area at plant level. Both light quality and quantity were measured at the start and end of the experiment. Nutrient solution pH and electrical conductivity (EC) were measured every irrigation (HI 9813-6, Hannah Instrument Inc, Woonsocket, RI, USA). Table 1 contains the environmental conditions for compartment temperature, air velocity, CO_2 concentration, relative humidity, and fertigation data.

LED light treatment

LED fixtures (LX601C and RX30, Heliospectra AB, Göteborg, SE) were used for the four light treatments. Light treatments, on an absolute µmol m^{-2} s^{-1} ratio, were (1) a balanced white light treatment (42Blue: 52.5Green: 55.5Red: 48.5Far-red) (B-G-R-Fr), (2) a balanced white light treatment omitting Far-red (42Blue: 52.5Green: 55.5Red: 0Far-red) (B-G-R), (3) a balanced light treatment omitting Green (42Blue: 55.5Red: 48.5Far-red) (B-R-Fr) and (4) a blue and red treatment omitting Green and Far-red radiation (42Blue: 55.5Red) (B-R-lowFr). LED monochromatic peaks were 452 nm for B with full width at half maximum (FWHM) of 23nm, 524 nm for G (FWHM: 37nm), 659 nm for R (FWHM: 16 nm), and 733 nm for FR (FWHM: 20 nm). Light measurements were taken at the start and conclusion of the experiment and averaged together for the light field summary (consisted of 5 points within light field).

All treatments were set at equal photosynthetic active radiation (PAR: 400-700 nm) with Far-red radiation (700-800nm, outside of PAR) added to the light recipe in respective quantity listed above. See Figure 1 for LED fixture spectral qualities measured by a spectroradiometer (PS-200, Apogee Instruments Inc., Logan, UT, USA). From day 3-10, treatment PAR light intensity was maintained at 75 µmol m^{-2} s^{-1} with intensity increased to 150 µmol m^{-2} s^{-1} at day 11 and remained at this intensity until conclusion of the experiment (60 days in total). Both intensities had an 18 h photoperiod. Fixture height was adjusted to maintain equal intensity as the plants grew (constant 35 cm from top of canopy). Table 2 also details the light intensity specifics of the different treatments in terms of PAR, yield photon flux (YPF), and phytochrome photostationary state (PSS) based from the Sager and McFarlane (1997) value table. Experimental plants were enclosed by a row of perimeter border plants to prevent possible boundary effects and rotated every two days to equalize light uniformity within the experimental

growing area (46 cm × 46 cm) from day 3-20 and rotated every day from day 21-70. The total growing area was 56 cm × 56 cm.

4.3.3 Measurements and data collection

The first harvest was taken on day 18 (D18). For morphological response 10 plants were randomly selected per repetition on the first harvest day (D18). The second harvest day, day 25 (D25), had a sample size of 10 while harvest days on day 32, 46, and 70 (D32, D46, and D70, respectively) had a sample size of 6, 6, and 5, respectively, for morphological traits. Measurements on harvested plants included leaf count (1 cm minimum), longest leaf length, stem length (D25, D32, D46 and D70 only as stems were immeasurable at D18), leaf area, stem and leaf fresh mass, and stem and leaf dry mass (dried for 72 h at 65 °C). Leaf and stem length measurements used a ruler while mass used an electronic scale. Leaf area data was measured via an LI-COR 3100 (Li-cor, Lincoln, NE, USA). In addition to the listed morphological responses, derived responses were calculated: (1) leaf mass area (LMA) which is the dried leaf mass divided by leaf area (g/m^{-2}), (2) ratio of fresh to dry shoot mass (FM:DM) (FM g/ DM g), (3) fresh leaf mass to fresh stem mass ratio (LFM:SFM) and dry leaf mass to dry stem mass (LDM:SDM).

Plant histology

Histological dissection was performed on D70 plant stems to determine the impact of light quality on the transition of vegetative to floral stage growth. A longitudinal cut through the shoot or floral apex was performed using a scalpel and photos were taken of the cross section using a dissecting microscope. Five plants were sampled from each treatment for floral transition assessment, recorded as either being in the reproductive or vegetative state.

4.3.4 Statistical analysis

Statistical analysis for comparing the different treatments was done using an ANOVA Students tests ($p<0.05$) with JMP software Version Pro 13.2 (SAS Institute, Cary, NC, USA). Repetition was considered a fixed variable during statistical analysis.

4.4 Results & discussion

4.4.1 Stem morphology

Plants in B-G-R-Fr had 261-282%, 234%, 181-198%, 182-202% and 381-424% longer stem lengths than plants in B-G-R and B-R-lowFr for D18, D25, D32, D46 and D70, respectively (Figure 2). In addition, plants in B-G-R-Fr had 36%, 34%, 19%, 20%, and 48% longer stem lengths than plants in B-R-Fr for D18, D25, D32, D46 and D70, respectively (Figure 2). Plants in B-R-Fr had 166-181%, 150%, 136-149%, 135-151%, and 224-253% longer stem lengths than plants in B-G-R and B-R-lowFr for day 18, 25, 32, 45 and 70, respectively (Figure 2). Apical meristem dissections on day 70 showed that 80%-100% of plants in the B-G-R-Fr and B-R-Fr treatment, respectively, produced capitulum meristems (transitioned to reproductive-flowering stage) while plants in B-G-R and B-R-lowFr had no capitulum meristems (vegetative stage, leaf primordia) (Figure 3).

Plants in the B-G-R-Fr treatment and in B-R-Fr were exposed to 8-98x and 15-171x more far-red PFD than plants in the B-G-R and B-R-lowFr treatments, respectively. Consequently, plants in the B-G-R-Fr and B-R-Fr treatments were exposed to a PSS of 0.71 and 0.69 and plants in B-G-R and B-R-Fr treatments to a PSS of 0.87 and 0.89, respectively. Similarly, in an outdoor experiment using colored nets to alter light quality for lettuce cultivation, colored netting that increased the relative amount of Fr also increased lettuce stem length compared to the control

group planted in an open field (Ilić et al, 2017). In cucumber, Hogewoning et al. found that a simulated sun treatment increased plant length (ie stem elongation) by 4.5 and 5.4 times longer than high pressure sodium lamp and fluorescent lamps, respectively (Hogewoning et al., 2010). More specifically, Kalaitzoglou et al. (2019) relate tomato plant height to phytochrome stationary state (PSS) and found that as PSS decreased, plant height increased. In the current study, B-G-R-Fr and B-R-Fr plants with a PSS treatment value of 0.71 and 0.69, respectively, had longer stems than B-G-R and B-R-lowFr plants, which had a PSS treatment value of 0.87 and 0.86, respectively. There are several mechanisms that may cause or contribute to stem elongation due to light quality. Typically, a light environment with a reduced PSS value is interpreted and corresponded, regardless to a low light or shaded environment to the plant, via phytochrome activation and status.

In response to low light, shade intolerant plants increase stem length, develop narrower leaves, suppress branching, and flower earlier (Mathews, 2006). This is otherwise known as a shade avoidance syndrome (SAS) and is initiated by the plant in low light environments or recognizing a low R:Fr light ratio. Beyond the ratio of R:Fr, the ratio of blue to green light (B:G), like heat and photoperiod, may initiate bolting, a response that indicates a transition from the vegetative to the reproductive stage in lettuce. This may explain the increase in stem length of B-G-R-Fr over B-R-Fr although both these treatments having similar R:Fr ratios and PSS values. It has been shown that a decreased B:G ratio (meaning a higher component of G light, as in B-G-R-Fr) can exacerbate the SAS, due to G light partially inactivating cryptochrome1 and cryptochrome 2 activity, photoreceptors that would otherwise inhibit SAS in the presence of B light (Fraser et al., 2016). This mechanism can be a backup SAS response or synergistic response that allows the plant to overcome shading. B light is an indication to the plant that it is exposed

to unshaded open sky where a SAS response would be unnecessary and unbeneficial use of resources. Since levels of G light increases the deeper one goes into shaded canopies (B and R light is more readily absorbed while G light is reflected by and transmitted through the leaf) this would be an indication to the plant so stretch, via stem elongation, to reach open skies.

Plant apical meristem dissections on D70 showed that 80% of B-G-R-Fr and 100% of B-R-Fr produced capitulum meristems while B-G-R and B-R-lowFr had no capitulum meristems, only showing continued growth and development of leaf primordia. Capitulum meristems are meristems that have transitioned from the vegetative stage (ie producing leaf primordia) to the floral (reproductive) stage (Figure 3 for representative visual), indicated by the presence of a flower rosette and a reduction in leaf primordia at the growing tip. In a study that aimed to examine the impact of heat treatment on lettuce bolting and expression levels of FLOWERING LOCUS T (LsFT), the gene that initiates floral development in lettuce (synthesized in the leaves), heat treatment induced lettuce bolting and floral stage transitioning similar to results in this experiment in B-G-R-Fr and B-R-Fr plants that transitioned from vegetative to floral stage (Chen et al., 2017).

4.4.2 Whole plant fresh and dry mass responses

Light treatment had an overall effect on plant fresh mass for Day 18, 25, 32, and 46 but had no impact on plants by Day 70. Plants treated with B-G-R and B-R-lowFr were 32-44% larger in fresh mass than B-G-R, which was consistently the lowest performer for D18, D25, and D32 (Figure 4). Interestingly on D46 B-G-R-Fr and B-R-Fr plants had the largest fresh mass, being 22-24% larger than B-G-R plants, the treatment with the smallest fresh mass (Figure 4). By D70, no differences were found for plant fresh mass among any of the treatments. Similarly

for dry mass, B-G-R and B-R-lowFr plants had the largest dry mass for D18, D25, and D32, being 40-52% larger than B-G-R-Fr treated plants, which were consistently the lowest ranked for dry mass on those harvest days (Figure 5). As leaves in B-G-R-Fr and B-R-Fr presented themselves in an upright manner (leaf angle and arrangement on an elongated stem) compared to the B-G-R and B-R-lowFr treatment plants (see Figure 3) that had higher leaf overlap, B-G-R-Fr and B-R-Fr plants had the ability to photosynthesize more and thus assimilate CO_2 faster, which leads to a higher biomass (Terashima et al. 2009; Tsuyuma et al. 2003). More specifically, as the leaf angle and exposure to light increases, abaxial leaf tissues contributed to an overall carbon sequestration. This could be one possible explanation why B-G-R-Fr and B-R-Fr matched dry mass with B-G-R and B-R-lowFr for the last two harvests. Yet, as B-G-R-Fr and B-R-Fr had a higher yield photon flux (YPF), the increased YPF value and simultaneous increased ability to photosynthesize may have nullified any light qualitative aspect that was hindering earlier biomass accumulation. This is made evident by D46 and D70 where no difference was found in plant dry mass among treatment.

As for the ratio of plant fresh mass: dry mass (FM:DM) plants from B-G-R-Fr and B-R-Fr had a larger FM:DM ratio for D18, D25, and D46, containing 16-20% more FM:DM than B-G-R and B-R-lowFr, while showing no differences on D32 and D70 (Table 3). As FM:DM increases it indicates that the plant has an increased ability to retain and hold water for a given amount of dry mass or cell count. Cell elongation, triggered by a SAS response, does not increase dry mass but rather water content as the water is needed to maintain turgor and rigidity of stretched cells (Sasidharan et al., 2010; Briadwood et al., 2013). This SAS mechanism is the likely cause of an increased FM:DM as the plant leaves, petioles, and stems are stretched and,

this in turn, would result in an increase of FM:DM as the plant leaves and stems are stretching in B-G-R-Fr and B-R-Fr.

4.4.3 Plant biomass partitioning and morphological responses

Plants treated with B-G-R and B-R always had a larger leaf to stem dry mass (LDM:SDM) than those treated with B-G-R-Fr and B-R-Fr, allocating 48-61% more biomass to leaves than stem (Table 3). The same trend was found for leaf to stem fresh mass (Table 3). Furthermore, when comparing the stems ability to retain water (again, an indication of stem growth and elongation) B-G-R-Fr plant stems were able to hold 33-38% more water than B-G-R and B-R-lowFr across all harvest days except D32 (Table 3).

The number of plant leaves was also impacted by treatment light quality. B-G-R and B-R-lowFr plants always had the most amount of leaves while B-G-R-Fr plants always had the least amount, having 22-41% more leaves than B-G-R-Fr for all harvest days (Table 3). B-G-R-Fr and B-R-Fr plants shared similar results for leaf number D18, D25, D32, and D46 except on D70 when B-R-Fr plants had 18% more leaves than B-G-R-Fr. Previous studies in lettuce have demonstrated similar results of low PSS values and leaf count (Ilić et al., 2017; Spalholz et al, 2019). As B-G-R-Fr plants had the lowest leaf count throughout the experiment at the same time B-G-R-Fr plants also had the longest leaves. Meanwhile the treatment of B-G-R resulted in plants with the shortest leaf, being 22-42% shorter than B-G-R-Fr across all harvest days (Table 3). Elongating leaves in a low R/Fr light environment is typical where plants exhibit development of long and slender leaves to minimize leaf overlap and self-shading while optimizing light penetration (Morgan and Smith, 1981) similar to what was found in B-G-R-Fr and B-R-Fr.

Light treatment also played a role in total plant leaf area. On D18 and D25, the leaf area of B-G-R and B-R-lowFr plants were 22-26% larger than B-G-R-Fr plants (Figure 6). While no treatment difference in leaf area was found on D32, interestingly for the last two harvests (D46 and D70) B-G-R-Fr and B-R-Fr plants had the largest leaf area, being 11-19% larger than B-G-R (Figure 6). Light treatment also impacted leaf mass area (LMA), a measure of leaf thickness. For D18, D25, and D46 plants in B-G-R and in B-R-lowFr had 21-36% larger LMA than plants in B-G-R-Fr and in B-R-Fr (Table 3), while on D32 only B-G-R plants had a higher LMA than B-G-R-Fr and B-R-Fr, being 32-34% larger than plants in both of those treatments (Table 3). No treatment difference for LMA was found on D70 (Table 3). By the conclusion of the experiment there was no difference in plant LMA differences that were demonstrated at the seedling stage. This is supported by findings in another study for 28-day old lettuce (Kim et al., 2004) where an increased ratio of G light decreased LMA. Kim et al (2004), described in terms specific leaf area (the inverse of LMA), found that a 10B:86G:4R light recipe had a decreased LMA compared to 15B:24G:61R, 16B:84R, and 19B:51G:30R light recipe.

4.5 Conclusion

The objective of this study was to determine the role of individual colors in LED simulated sun light (B, G, R, and Fr) on lettuce bolting and resource partitioning between plant organs. Although by D70 the four treatments had no impact on fresh mass and dry mass, results from the experiment demonstrate how light quality impacts bolting response and plant resource partitioning. This study suggests that Fr and G light have a synergistic effect on stem elongation, a prominent lettuce bolting response, as compared to Fr light alone. Meanwhile, the synergism of G and Fr was not found in FLM:FSM or DLM:DSM with no difference being found between B-

G-R-Fr and B-R-Fr (both low R:Fr treatments), meaning that G light did not impact these ratios under low R:Fr. In terms of apical meristem histology, high R:Fr treatments (B-G-R and B-R-lowFr) inhibited floral development, while the low R:Fr B-G-R-Fr and B-R-Fr treatments resulted in the formation of a floral meristem capitulum. This study provides baseline information about the role of G and Fr but further research is needed to address color quantity and G:Fr ratio to determine if responses observed in this study are facultative or met by a certain threshold. In addition, the role of overall light intensity with the given color ratios to determine should be evaluated to see how responses change since plants in this study were grown under 150 µmol m^{-2} s^{-1} PPFD. Furthermore, as this experiment demonstrated that high R/Fr can reduces bolting response it would be valuable to a producer if using high R/Fr lettuce transplants improves or confers bolting resistance to high field scenarios reducing the risk of crop loss.

4.6 Literature cited

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Table 4.1 Environmental parameters with standard deviation for each light treatment. Light treatments are described as 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). All parameters are combined averages from repetition 1 and 2. Temperature, CO_2 concentration, and relative humidity were continuously logged while air velocity was measured at the start of the experiment and pH and EC measurements were taken at each fertigation.

Parameter	Treatments							
	B-G-R-Fr		B-G-R		B-R-Fr		B-R-lowFr	
	Day	Night	Day	Night	Day	Night	Day	Night
Air temperature (°C)	22.0 ± 0.5	17.2 ± 0.4	22.1 ± 0.5	17.2 ± 0.3	21.9 ± 0.5	17.2 ±0.3	21.9 ± 0.5	17.3 ± 0.4
Substrate temperature (°C)	20.4 ± 0.8	16.1 ± 0.7	20.2 ± 0.6	16.1 ± 0.6	20.6 ± 0.8	16.3 ± 0.8	20.7 ± 0.9	16.3 ±0.8
Air Velocity (m s^{-1})	0.51 ± 0.14		0.44 ± 0.21		0.38 ± 0.07		0.50 ± 0.12	
			Day				Night	
CO_2 (µmol mol^{-1})			486 ± 15				565 ± 45	
RH (%)			69.4 ± 4.4				65.8 ± 5.2	
pH			6.2 ± 0.2					
EC (dS m^{-1})			1.24 ± 0.06					

Table 4.2 Spectral characterization of four experimental LED light treatments with the following ratios: 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr).

Light Parameter	Treatments (photon flux ratio)			
	B-G-R-Fr	B-G-R	B-R-Fr	B-R-lowFr
PPF*	150.2 ± 0.5	149.9 ± 0.3	150.5 ± 1.0	151.4 ± 0.2
YPF**	131.2 ± 0.6	123.5 ± 0.1	142.5 ± 0.3	129.8 ± 0.2
PSS†	0.71 ± 0.00	0.87 ± 0.00	0.69 ± 0.00	0.86 ± 0.00
Blue (400-500 nm)	42.5 ± 0.3	42.1 ± 0.0	64.3 ± 0.2	64.3 ± 0.2
Green (500-600 nm)	52.3 ± 0.3	52.4 ± 0.0	0.61 ± 0.0	0.61 ± 0.0
Red (600-700 nm)	56.2 ± 0.6	55.6 ± 0.1	86.0 ± 0.5	86.2 ± 0.1
Far-red (700-800 nm)	49.1 ± 0.5	0.5 ± 0.1	85.6 ± 0.1	5.9 ± 0.0
Red:Far-red	1.14 ± 0.00	126.33 ± 9.59	1.01 ± 0.01	15.01 ± 0.08
Blue:Red	0.76 ± 0.00	0.76 ± 0.00	0.75 ± 0.00	0.75 ± 0.00
Blue:Green	0.81 ± 0.00	0.80 ± 0.00	105.86 ± 1.57	105.45 ± 2.57
Photoperiod (h)	18			

*Photosynthetic photon flux (PPF) is 400-700 nm in µmol m^{-2} s^{-1}
**Yield photon flux (YPF) values in µmol m^{-2} s^{-1} calculated from Sager and Mcfarlane (1997)
†Phytochrome photostationary state (PSS)

Table 4.3 Morphology results of 'Green Oakleaf' lettuce with SE by each harvest. D25 has a sample size of 10 (n=10), D32 (n=6), D46 (n=6), D70 (n=5). Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). Leaf fresh mass (LFM), stem fresh mass (SFM), leaf fresh mass to stem fresh mass ratio (LFM:SFM), total leaf dry mass (LDM), stem dry mass (SDM), leaf dry mass to stem dry mass (LDM:SDM), stem fresh mass to stem dry mass (SFM:SDM), total fresh mass to dry mass ratio (FM:DM), and leaf mass area (LMA) were measured.

	Parameter (units)	Treatments			
		B-G-R-Fr	B-G-R	B-R-Fr	B-R-lowFr
D25	LFM (g)	2.81 ± 0.08 b	4.80 ± 0.10 a	3.50 ± 0.40 b	5.04 ± 0.03 a
	SFM (g)	0.26 ± 0.01 a	0.17 ± 0.01 b	0.26 ± 0.02 a	0.18 ± 0.00 b
	LFM:SFM	10.72 ± 0.77 b	28.72 ± 1.22 a	13.76 ± 0.54 b	27.4 ± 0.42 a
	LDM (g)	0.13 ± 0.01 b	0.24 ± 0.00 a	0.16 ± 0.02 b	0.26 ± 0.00 a
	SDM (g)	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a
	LDM:SDM	11.52 ± 0.84 c	22.03 ± 0.83 a	13.65 ± 0.8 b	21.70 ± 0.23 a
	SFM:SDM	23.97 ± 0.65 a	15.25 ± 0.35 c	21.51 ± 0.31 b	15.19 ± 0.37 c
	FM:DM	22.5 ± 07 a	19.6 ± 0.3 b	21.7 ± 0.2 a	19.0 ± 0.0 b
	Leaf count	9.9 ± 0.1 b	16.7 ± 0.5 a	10.5 ± 0.5 b	16.4 ± 0.8 a
	Longest leaf (cm)	12.9 ± 0.1 a	8.2 ± 0.2 b	13.4 ± 0.2 a	8.9 ± 0.0 b
	LMA (g m^{-2})	10.8 ± 0.2 b	16.0 ± 0.3 a	11.9 ± 0.3 b	17.0 ± 0.2 a
D32	LFM (g)	10.68 ± 0.80 c	15.08 ± 0.07 ab	13.32 ± 0.47 b	16.47 ± 0.55 a
	SFM (g)	0.87 ± 0.04 b	0.45 ± 0.03 c	0.98 ± 0.03 a	0.54 ± 0.01 c
	LFM:SFM	12.25 ± 0.37 b	33.38 ± 2.18 a	13.70 ± 0.01b	30.86 ± 0.43 a
	LDM (g)	0.47 ± 0.04 b	0.80 ± 0.04 a	0.59 ± 0.02 b	0.77 ± 0.03 a
	SDM (g)	0.04 ± 0.00 ab	0.03 ± 0.00 b	0.04 ± 0.00 a	0.03 ± 0.00 b
	LDM:SDM	12.61 ± 0.41 b	25.67 ± 0.63 a	13.32 ± 0.25 b	25.58 ± 1.74 a
	SFM:SDM	23.70 ± 0.02 a	14.75 ± 2.11 b	22.13 ± 0.35 ab	18.01 ± 2.41 ab
	FM:DM	23.0 ± 0.1 a	18.8 ± 1.0 a	22.7 ± 0.1 a	21.5 ± 1.7 a
	Leaf count	19.9 ± 0.6 b	33.2 ± 0.0 a	20.5 ± 0.2 b	33.1 ± 1.4 a
	Longest leaf (cm)	16.1 ± 0.4 a	10.3 ± 0.0 c	16.5 ± 0.1 a	11.2 ± 0.0 b
	LMA (g m^{-2})	11.8 ± 0.2 b	17.9 ± 08 a	12.3 ± 0.1 b	15.6 ± 1.4 ab
D46	LFM (g)	42.43 ± 0.83 a	34.66 ± 0.94 c	41.73 ± 0.79 a	38.45 ± 0.12 b
	SFM (g)	5.26 ± 0.44 a	1.39 ± 0.03 b	4.77 ± 0.32 a	1.64 ± 0.04 b
	LFM:SFM	8.23 ± 0.92 b	25.11 ± 0.28 a	8.90 ± 0.72 b	23.51 ± 0.55 a
	LDM (g)	1.87 ± 0.02 b	1.89 ± 0.01 b	1.89 ± 0.09 b	2.08 ± 0.06 a
	SDM (g)	0.23 ± 0.01 a	0.09 ± 0.00 b	0.23 ± 0.00 a	0.10 ± 0.00 b
	LDM:SDM	8.33 ± 0.27 c	21.54 ± 0.22 a	8.35 ± 0.20 c	20.38 ± 0.53 b
	SFM:SDM	23.52 ± 1.77 a	15.72 ± 0.35 b	21.00 ± 1.79 a	16.02 ± 0.47 b
	FM:DM	22.9 ± 0.0 a	18.3 ± 0.4 b	22.1 ± 0.8 a	18.4 ± 0.6 b
	Leaf count	50.9 ± 0.1 b	73.0 ± 3.2 a	48.8 ± 0.4 b	73.5 ± 0.9 a
	Longest leaf (cm)	17.1 ± 0.1 a	11.3 ± 0.2 b	16.7 ± 0.3 a	12.2 ± 0.3 b
	LMA (g m^{-2})	15.1 ± 0.5 b	18.8 ± 0.1 a	16.1 ± 1.1 b	19.2 ± 0.8 a
D70	LFM (g)	75.55 ± 8.71 c	109.59 ± 3.64 ab	89.07 ± 1.83 bc	118.26 ± 0.72 a
	SFM (g)	49.56 ± 3.01 a	9.99 ± 0.80 b	43.19 ± 4.68 a	11.22 ± 0.75 b
	LFM:SFM	1.56 ± 0.28 b	11.1 ± 0.50 a	2.13 ± 0.24 b	10.72 ± 0.88 a
	LDM (g)	5.3 ± 0.39 a	5.14 ± 0.03 a	5.90 ± 0.24 a	5.84 ± 0.30 a
	SDM (g)	1.53 ± 0.15 a	0.47 ± 0.00 b	1.52 ± 0.31 a	0.55 ± 0.05 b
	LDM:SDM	3.63 ± 0.69 b	11.49 ± 0.37 a	4.06 ± 0.67 b	10.86 ± 0.72 a
	SFM:SDM	33.17 ± 1.71 a	21.95 ± 2.28 b	29.11 ± 3.05 ab	20.61 ± 0.85 b
	FM:DM	18.4 ± 0.3 a	21.4 ± 0.9 a	17.9 ± 0.9 a	20.4 ± 1.1 a
	Leaf count	159.4 ± 5.2 b	204.3 ± 0.5 a	185.0 ± 2.4 a	193.3 ± 9.5 a
	Longest leaf (cm)	15.6 ± 0.3 a	12.1 ± 0.5 c	14.5 ± 0.1 ab	13.1 ± 0.3 bc
	LMA (g m^{-2})	15.5 ± 1.1 a	16.7 ± 0.3 a	17.1 ± 1.1 a	17.5 ± 0.6 a

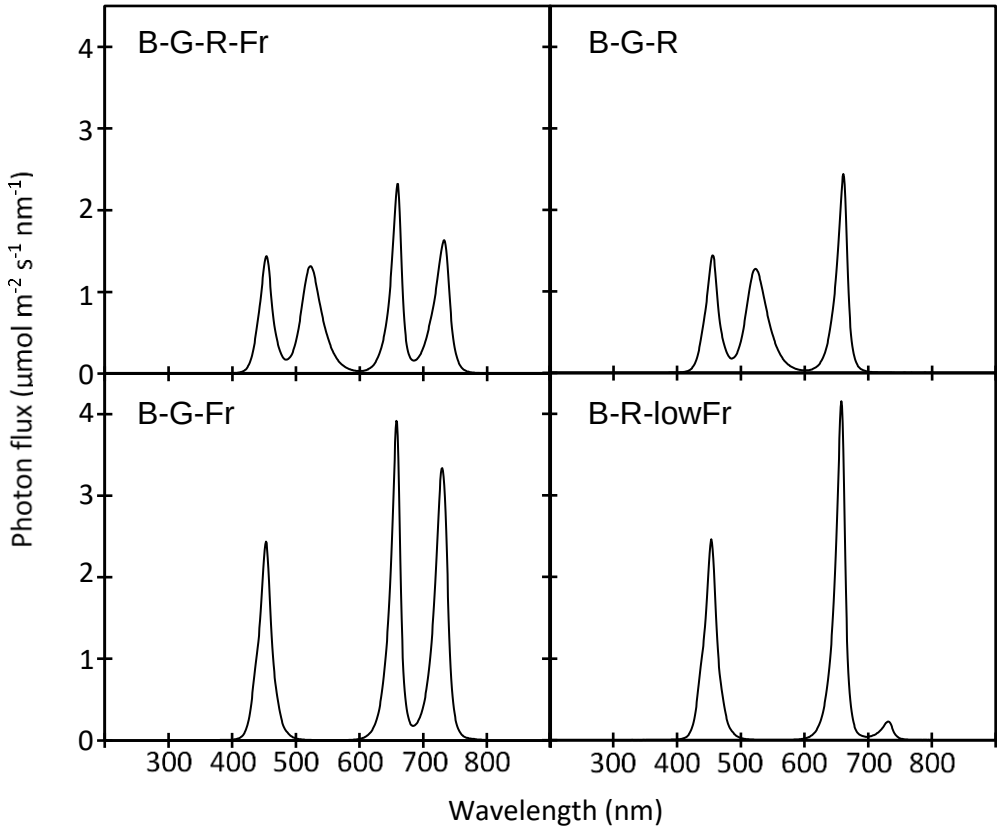

Figure 4.1 Spectral quality of each respective light treatment. These values are the repetition averages of the initial and final light measurements for each 2 repetitions. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr).

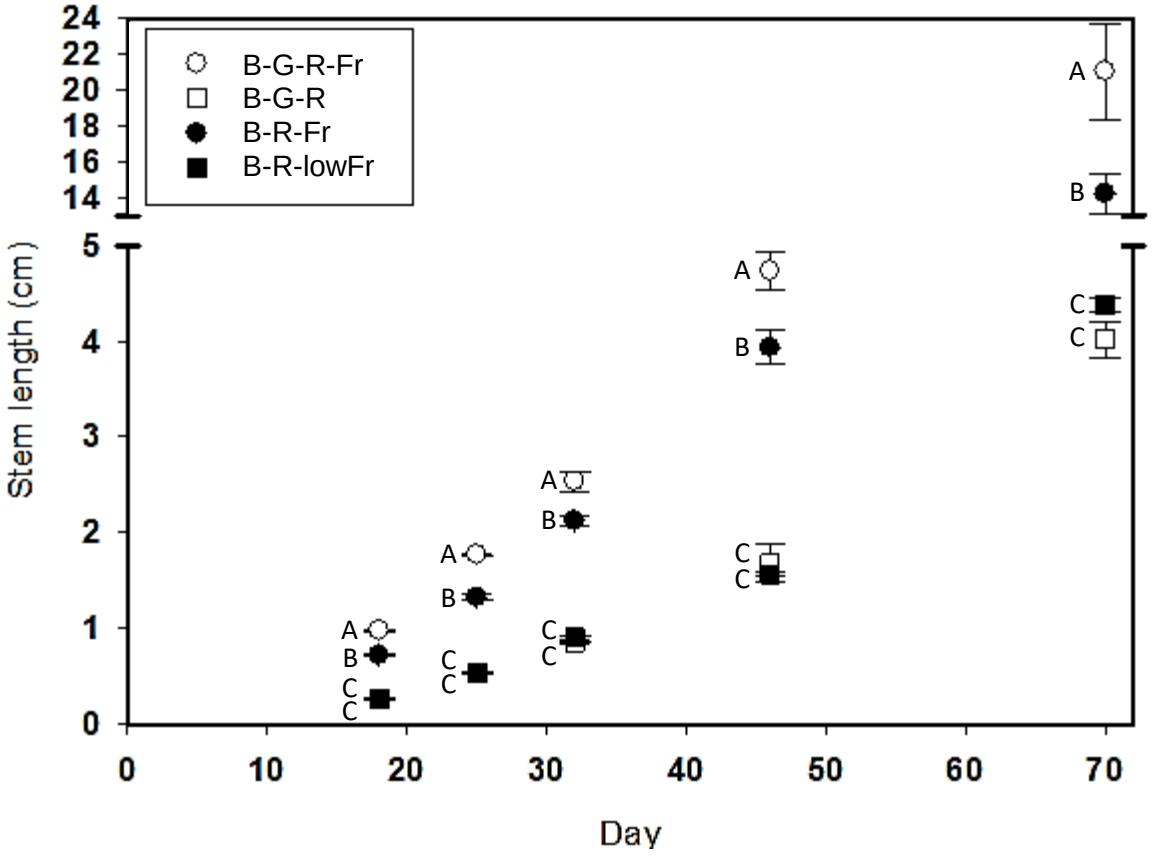

Figure 4.2 'Green Oakleaf' lettuce stem length for 2 repetitions with SE by each harvest. D25 has a sample size of 10 (n=10), D32 (n=6), D46 (n=6), D70 (n=5). Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). ANOVA score levels correlate with each respective harvest period only.

Figure 4.3 'Green Oakleaf' lettuce under different light treatments. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). Figure 3A is D18, 3B is D25, 3C is D32, 3D is D46 and 3E is D70. The white rectangle in the photograph for each respective harvest day is 10 cm.

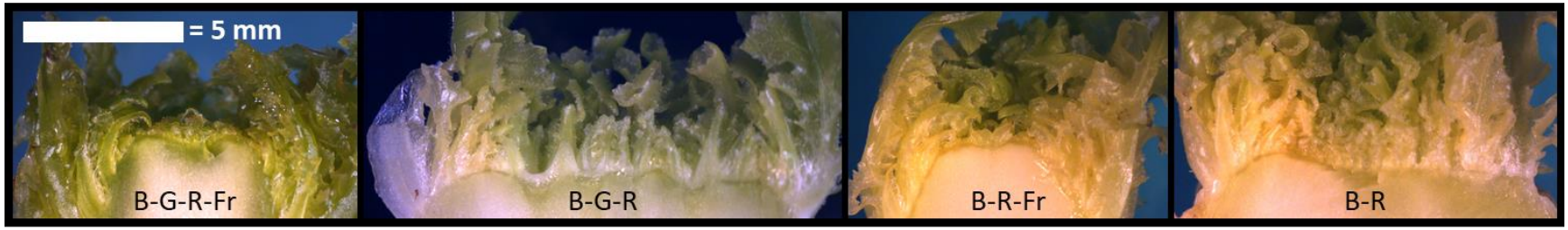

Figure 4.4 ‘Green Oakleaf’ lettuce apical meristem cross-sections at 70 days under four light treatments. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr).

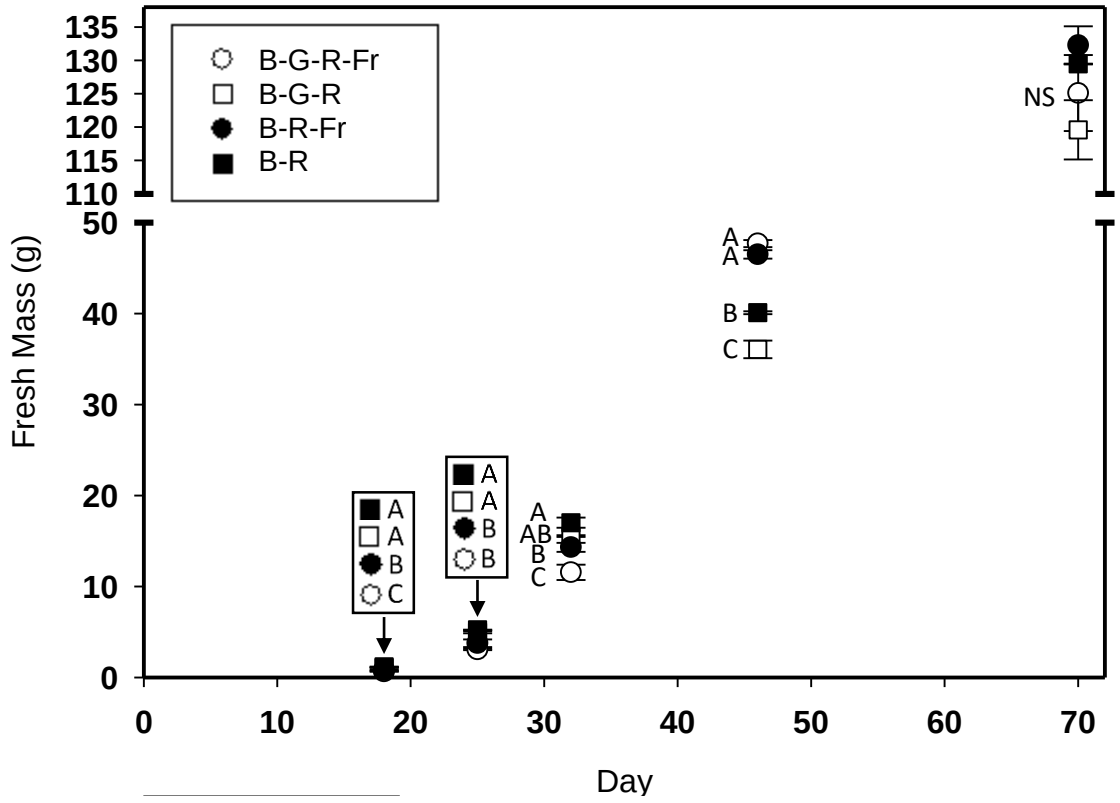

Figure 4.5 'Green Oakleaf' lettuce fresh mass for 2 repetitions with SE by each harvest. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). D25 has a sample size of 10 (n=10), D32 (n=6), D46 (n=6), D70 (n=5). ANOVA score levels correlate with each respective harvest period only. Boxes set in graph indicate ANOVA score levels on harvest days at which the arrow is pointing.

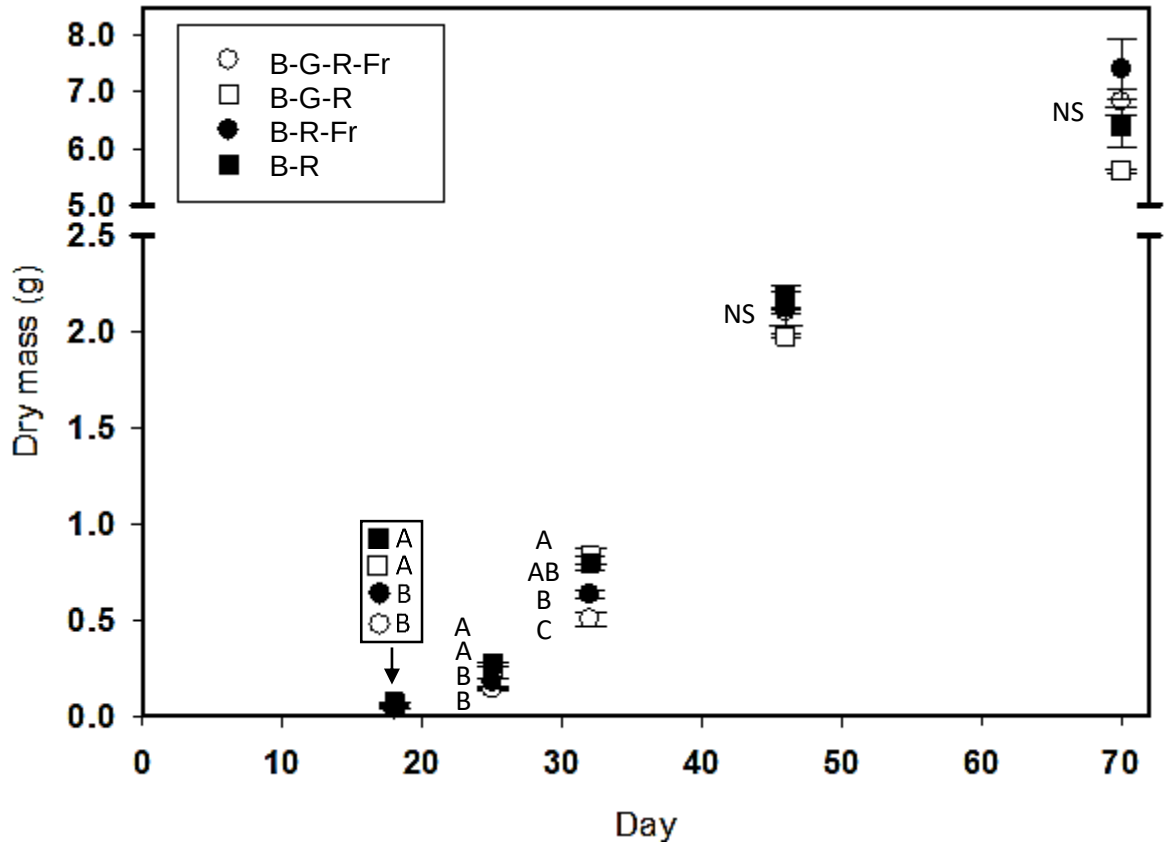

Figure 4.6 'Green Oakleaf' lettuce dry mass for 2 repetitions with SE by each harvest. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). D25 has a sample size of 10 (n=10), D32 (n=6), D46 (n=6), D70 (n=5). ANOVA score levels correlate with each respective harvest period only. The box set in graph indicate ANOVA score levels on harvest days at which the arrow is pointing.

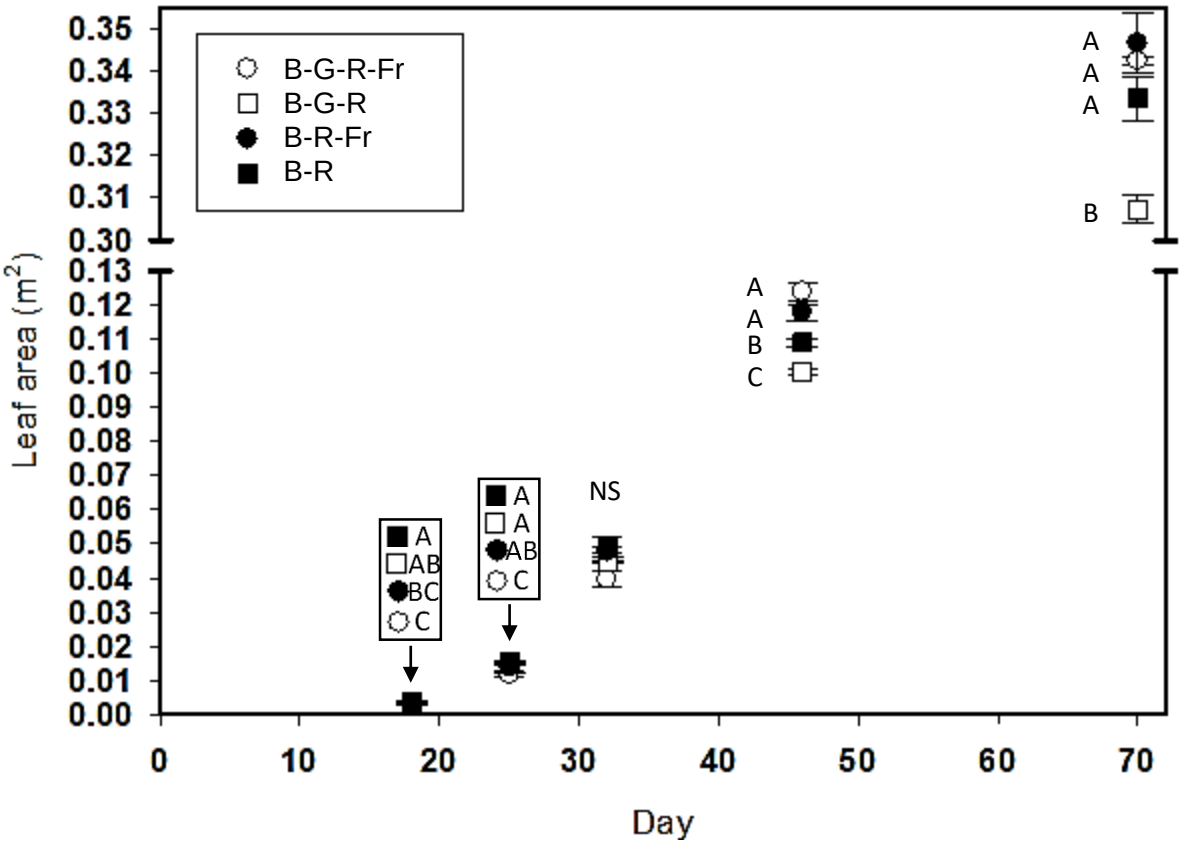

Figure 4.7 'Green Oakleaf' lettuce leaf area for 2 repetitions with SE by each harvest. Light treatments are 42blue: 52.5green: 55.5red: 48.5far-red (B-G-R-Fr), 42blue: 52.5green: 55.5red: 0far-red (B-G-R), 42blue: 55.5red: 48.5far-red (B-R-Fr) and 42blue: 55.5red (B-R-lowFr). D25 has a sample size of 10 (n=10), D32 (n=6), D46 (n=6), D70 (n=5). ANOVA score levels correlate with each respective harvest period only. Boxes set in graph indicate ANOVA score levels on harvest days at which the arrow is pointing.