Life Sciences in Space Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

Opinion/Position paper

A strategic approach for investigating light recipes for ‘Outredgeous’ red romaine lettuce using white and monochromatic LEDs

M.A. Mickens[a,*], E.J. Skoog[a], L.E. Reese[a], P.L. Barnwell[a], L.E. Spencer[a,b], G.D. Massa[a], R.M. Wheeler[a]

[a] National Aeronautics and Space Administration, Kennedy Space Center, FL 32899, United States
[b] AECOM Laboratory Support Services and Operations, Kennedy Space Center, FL 32899, United States

ARTICLE INFO

Keywords:
White light-emitting diode
Broad-spectrum
Monochromatic
Light recipes
Red lettuce
Advanced Plant Habitat

ABSTRACT

To optimize crop production/quality in space, we studied various “light recipes” that could be used in the Advanced Plant Habitat currently aboard the International Space Station (ISS). Lettuce (*Lactuca sativa* cv. ‘Outredgeous’) plants were grown for 28 days under seven treatments of white (W) LEDs (control), red (635 nm) and blue (460 nm) (RB) LEDs, W + blue (B) LEDs, W + green (520 nm) (G) LEDs, W + red (R) LEDs, W + far red (745 nm) (FR) LEDs, and RGB + FR LEDs with ratios similar to natural sunlight. Total PAR was maintained near 180 µmol m^{-2} s^{-1} with an 18 h photoperiod. Lettuce grown under RGB + FR produced the greatest leaf expansion and overall shoot biomass, while leaves from WB and RB showed the highest levels of pigmentation, secondary metabolites, and elemental nutrients. All other supplemental treatments had varying impacts on morphology that were dependent on crop age. The WG treatment increased fresh mass early in the cycle, while WR increased biomass later in the cycle. The plants grown under WFR exhibited elongation of petioles, lower nutrient content, and similar shoot biomass to the W control. The findings suggest that supplementing a broad spectrum, white light background with discrete wavelengths can be used to manipulate total yield, morphology, and levels of phytonutrients in lettuce at various times during the crop cycle.

1. Introduction

The ability to control light will be vital to growing plants for food production in space or in any controlled environment built to sustain humans (Sager and McFarlane, 1997; Massa et al., 2008; Ilieva et al., 2010; Wheeler, 2017). The spectral quality of light can strongly influence how plants produce and partition various byproducts of photosynthesis (Lin et al., 2013). The rapid advancements of light-emitting diodes (LEDs) have made it possible to control and tailor the spectrum delivered to plants, providing the potential to optimize various aspects of growth and/or metabolism in crops (Stutte et al., 2009; Liu et al., 2018). LEDs have become increasingly popular light sources for controlled environment crop production due to their high conversion efficiency, long operating life, miniature size, low thermal radiation, solid-state, and absence of toxic mercury (Yorio et al., 2001; Nelson and Bugbee, 2014), and recent reviews have thoroughly evaluated the status and recent achievements of using LEDs in horticulture (Mitchell et al., 2012, 2015; Bantis et al., 2018) and in space (Zabel et al., 2016; Wheeler, 2017).

In plants, the amount of O_2 produced or CO_2 utilized per mole of photons absorbed has been shown to be dependent on the spectral regions between 400 and 700 nm (McCree, 1972; Cope et al., 2013), the most efficient being wavelengths in the red (R) (600–700 nm), and blue (B) (400–500 nm) regions (Muneer et al., 2014). However, a number of studies reporting the impacts of green (G) light (500–600 nm) on growth are attracting attention (Folta, 2004; Kim et al., 2004; Folta and Maruhnich, 2007; Zhang et al., 2011; Johkan et al., 2012). In some instances, the quantum yield of G light has been reported to be greater than that of B light due to the fraction of B light energy strongly absorbed by flavonoids and/or carotenoids and not transferred to chlorophyll reaction centers (Barnes et al., 1993; Terashima et al., 2009; Cope et al., 2014). In the study reported by Massa et al. (2015a), it was observed that the penetration of G light through leaf tissue was significantly deeper than R or B light for lettuce, radish, pepper, and canola plants. Moreover, G light is distributed more equally throughout the mesophyll, allowing deeper access to photosynthetic tissues within the leaf, penetrating through to the abaxial side (Brodersen and Vogelmann, 2010).

Broad-spectrum white LEDs (WLEDs) with correlated color temperatures (CCTs) between 3000 and 6000 K can inherently contain up

---

* Corresponding author.
E-mail address: m_mickens@outlook.com (M.A. Mickens).

https://doi.org/10.1016/j.lssr.2018.09.003
Received 3 August 2018; Received in revised form 28 August 2018; Accepted 17 September 2018
2214-5524/

Please cite this article as: Mickens, M., Life Sciences in Space Research, https://doi.org/10.1016/j.lssr.2018.09.003

to 50% of their spectral composition between 500 and 600 nm. Since WLEDs are produced by coating a B LED chip with a broad yellow (Y)-emitting phosphor, the steadily increasing efficiency of B LEDs has concomitantly improved the efficiency of WLEDs (Cope and Bugbee, 2013). Chen et al. (2016) were one of the first groups to test the WLED spectrum on the growth of 'Green Oak Leaf' lettuce, by adopting the approach of using a WLED background and adding equivalent amounts of supplemental light from B, G, Y, R and far red (FR) LEDs as separate treatments. Beneficial responses on biomass and pigment were observed for WR and WB treatments, respectively, while WY and WFR treatments exhibited negative responses on yield and morphology. However, the FR diodes ($\lambda_{max} \approx 850$ nm) used in those studies were likely too far beyond the absorption action of phytochrome (P_{fr} form) to signal the intended shade avoidance response (Butler et al., 1964; Li et al., 2011). Li and Kubota (2009) observed similar responses by supplementing white light with various monochromatic LEDs on baby 'Red Cross' lettuce, although with using fluorescent (FL) lamps instead of WLEDs, optimal biomass was observed under WFR light, while Chen et al. (2014) observed optimal yield under WR with white also being provided by a FL lamp. These studies collectively demonstrate how supplementing W with monochromatic LEDs can be a strategy to identify light recipes that can be used to enhance plant growth, morphology, and nutrition.

The efforts to investigate LED light recipes for cultivating food crops in spaceflight environments such as the International Space Station (ISS), currently revolve around utilization of NASA's Veggie and Advanced Plant Habitat (APH) growth chambers (Massa et al., 2016; Zabel et al., 2016). Veggie provides a basic capability of RGB LEDs with potential future upgrades to additional wavelengths. In terms of offering strategic light recipes, the APH is equipped with W, R, G, B, and FR LEDs and can achieve 1000 µmol m^{-2} s^{-1} total intensity (Massa et al., 2016; Morrow et al., 2016), and is the target application platform for this work.

In this study, the WLED spectrum was investigated as a control treatment on the growth of lettuce, and used as a background to evaluate the effects of supplementing W with equal amounts of monochromatic light from B, G, R, and FR LEDs. The 'Outredgeous' cultivar of red romaine lettuce was selected due to its successful and rapid growth on at least five spaceflight experiments in Veggie under basic RGB lighting, and its morphology exhibits remarkable sensitivity to light spectrum. The objective was to identify what light recipes would optimize its edible biomass, nutrient content, and secondary metabolites on the ground, and to recommend them accordingly for implementation aboard future flight experiments inside the APH. In addition to the various supplemental treatments, the 'Outredgeous' lettuce was also grown under basic RB LEDs, and a custom recipe of RGB + FR LEDs with ratios similar to sunlight for comparison.

2. Materials and methods

2.1. Growth chamber conditions

The lettuce crops were grown in environmental growth chambers (EGC M-48, Chagrin Fall, OH) located in the Space Life Sciences Laboratory (SLSL) at NASA Kennedy Space Center. The chamber air temperature, relative humidity, and CO_2 levels were maintained at 23 ± 0.4 °C, 65 ± 3%, and 1200 ± 60 ppm, respectively. Air circulation and flow within the chamber was measured and fluctuated between ~0.5 and 1.0 m/s.

2.2. Cultural conditions

Under each light treatment, lettuce seeds (*Lactuca sativa* cv. 'Outredgeous') were sown in 10 square plastic pots (9 cm tall, 10.2 cm width) containing 500 mL Greens Grade Arcillite (< 1 mm particle size; PROFILE Products LLC; Buffalo Grove, IL) mixed with Nutricote 18-6-8 (NPK) controlled-release fertilizer (Type 70; Florikan, Sarisota, FL) incorporated at 7.5 g L^{-1} dry medium. In each pot, four seeds were sown on the surface of moistened media in the four corners with at least 2.5 cm distance away from the walls. The pots were arranged inside 0.13 m^2 sub-irrigation trays with the water levels maintained at a depth of ~1 cm using deionized (DI) water. Trays were covered with transparent plastic covers and misted with DI water for the first 3 days to promote germination. Once germination was complete, to minimize position effects, the pots were rotated clockwise inside the tray every other day. In addition, each light treatment was rotated to new locations within the chamber after each replicated 28 day cycle (3 replications total). The water use was tracked daily by measuring water levels in the trays, and recording the amount added.

2.3. Light treatments

The seven LED light treatments were 1) white (W: B LED + phosphor, $\lambda_{max} \approx 460$ and 582 nm) LEDs as a control (CCT ~ 3150 K), 2) red and blue (RB, $\lambda_{max} \approx 635$ and 460 nm), 3) white + blue (WB, $\lambda_{max} \approx 460$ nm), 4) white + green (WG, $\lambda_{max} \approx 520$ nm), 5) white + red (WR, $\lambda_{max} \approx 635$ nm), 6) white + Far red (WFR, $\lambda_{max} \approx 745$ nm), and 7) red, green, blue, + FR (RGB + FR, $\lambda_{max} \approx 660$, 630, 520, 425 + 733 nm). Treatments 1–6 spectra were provided by 60 W SuperT LED tube panels (AIBC International, Ithaca, NY). We chose to use 635 nm red LEDs because this same wavelength is used for red in NASA's Veggie and APH plant growth chambers on International Space Station (Massa et al., 2016). Treatment 7 spectrum was customized using a 300W RX30 research fixture (Heliospectra, Göteborg, Sweden) with nine programmable channels. The light ratios of treatment 7 were influenced by Kim et al. (2004), but slightly modified with the addition of FR and a shorter wavelength of B. Table 1 summarizes the output of each treatment in terms of total

Table 1
Summary of spectral data and light recipe ratios for each treatment of white (W), red + blue (RB), white + blue (WB), white + green (WG), white + red (WR), white + far red (WFR), and red, green, blue + far red (RBG + FR) LEDs.

Parameter	Treatment						
Photon flux (µmol m^{-2} s^{-1})/light recipe ratio (%)	W	RB	WB	WG	WR	WFR	RGB + FR
Average PPFD (400–700 nm)	180 ± 33	187 ± 34	178 ± 30	180 ± 35	178 ± 32	185 ± 35	188 ± 28
Blue (400–500 nm)	37 (20%)	75 (40%)	77 (43%)	31 (17%)	28 (16%)	37 (20%)	30 (16%)
Green (500–600 nm)	86 (48%)	0 (0%)	60 (34%)	104 (57%)	68 (38%)	89 (48%)	45 (24%)
Red (600–700 nm)	58 (32%)	112 (60%)	41 (23%)	45 (25%)	82 (46%)	59 (32%)	113 (60%)
Far red (700–800 nm)	1	0	0.8	0	0	34	36
YPF[a]	165	170	158	159	166	169	163
Other ratios							
R/FR	57.0	112.0	51.2	45.0	82.0	1.7	3.1
R/B	1.54	1.5	0.53	1.45	3.0	1.60	3.8

[a] Yield photon flux (YPF) was calculated according to Sager et al. (1988).

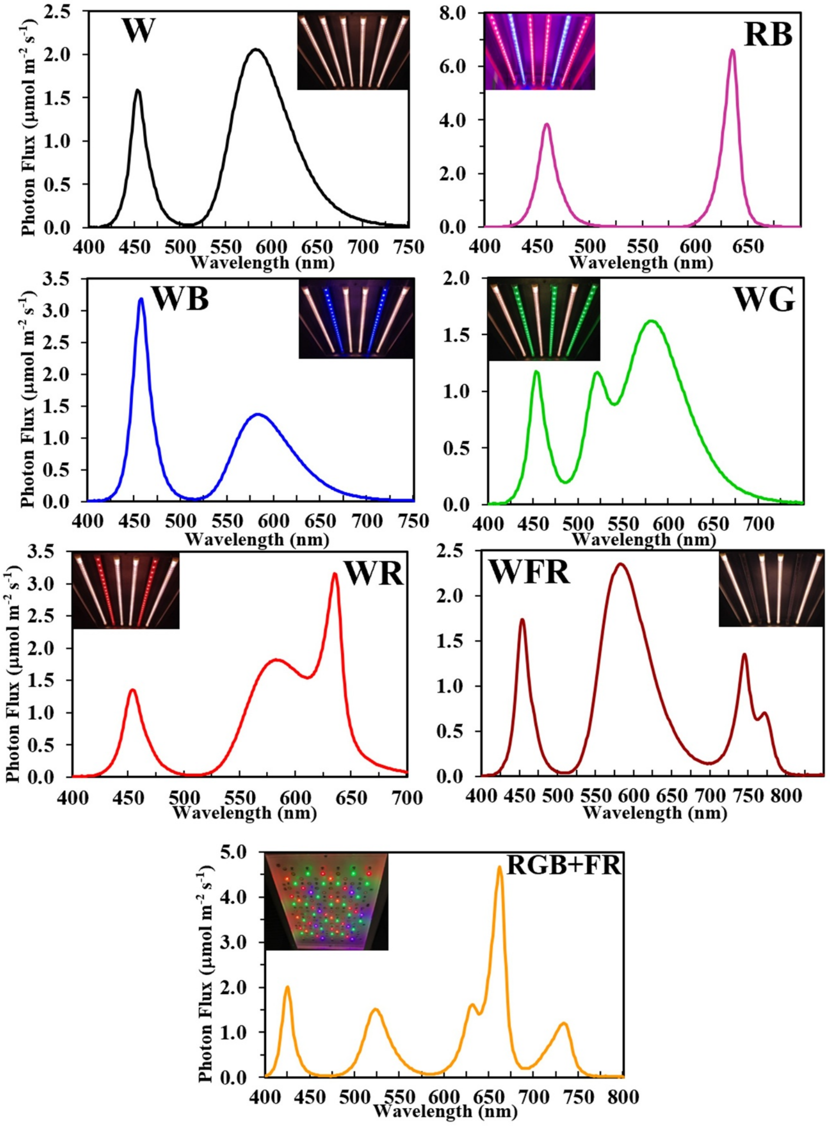


Fig. 1. Spectral distributions of white (W), red and blue (RB), white + blue (WB), white + green (WG), white + red (WR), white + far red (WFR), and RBG + FR LEDs with inset showing LED arrays. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

photosynthetic photon flux density (PPFD) and the light recipe ratios. The PPFD target was $\sim 180\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ (~ 12 $\text{mol m}^{-2}\,\text{day}^{-1}$) per treatment averaged over the entire canopy. Fig. 1 shows the spectral distribution scans of all treatments taken from 400 to 800 nm at 1 nm steps with a spectroradiometer (Model PS-100, Apogee Instruments, Logan, UT). For each supplemental treatment, the WLED background was reduced to $\sim 145\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$, while the supplemental PPFD targets were $\sim 35\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ for B, G, R, and FR. The photoperiod was 18 h (18 h light/6 h dark) in all treatments.

2.4. Plant growth measurements

At 7 days after sowing (DAS), one plant was harvested from each pot and discarded. This initial thinning removed the weakest or unsuccessfully germinated seedlings. Subsequent harvests took place on a weekly interval at 14, 21, and 28 DAS where plant growth measurements were performed. These measurements included shoot fresh mass, shoot height and diameter, leaf area, leaf number, and specific leaf mass. The specific leaf mass was calculated from the leaf area and dry mass using the formula:

$$\text{SLM} = M_{\text{dr}}/\text{LA}$$

where SLM = specific leaf mass per area (mg/cm^2), M_{dr} = shoot dry mass, and LA = crop leaf area. Relative chlorophyll estimates were made using a SPAD-502DL meter (Konica Minolta Sensing, Osaka, Japan) by clamping the sensor on three different leaves per plant and recording the average in dimensionless SPAD units. The edible biomass (shoot dry mass) was determined after plant tissues were dried in a drying oven for 48 h at 70 °C. For the 28 DAS harvest only, the plant tissues were flash frozen with liquid N_2. The samples were then vacuum freeze dried starting at –40 °C and incrementally brought up to 20 °C. After recording freeze dried weights, an analytical mill (Cole-Parmer; 110 VAC/60 Hz; Vernon Hills, IL) was used to grind the tissues to a powder in preparation for elemental and carotenoid analysis.

2.5. Elemental nutrient analysis

To analyze the elemental nutrients, 0.25 g (in duplicate) of each freeze dried and ground plant tissue sample was digested at 95 °C in 5 mL of 70% nitric acid (Trace metal grade HNO_3, Fisher Scientific, Pittsburgh, PA) and 2.5 mL of hydrogen peroxide (30% H_2O_2, certified ACS grade, Fisher Scientific) using a slight modification of the EPA Method 3050B (EPA, 1996). After digests were diluted with ultra-pure water, the samples were filtered through 0.2 µm filters, and analyzed with ICP-OES (Thermo Scientific, Asheville, NC) for Ca, Fe, K, Mg, Na, P, S, and Zn.

2.6. Secondary metabolite analysis

Lutein was the main secondary metabolite analyzed. The extraction procedure was modified from Perry et al. (2009). 100 mg (in duplicate) of freeze dried and ground plant tissue was weighed, and soaked in 5 mL of methanol (100% CH_4O, Fisher Scientific) overnight at 4 °C. The samples were then centrifuged at 3500 rpm at 4 °C for 12 min and the methanol layer was decanted off into Reacti-Vap evaporator vials leaving behind pelletized plant material in the centrifuge tubes. 5 mL of tetrahydrofuran (99% anhydrous C_4H_8O, Fisher Scientific) was added to the pellet, and centrifugation was repeated three additional times. In between each centrifugation, the decanted solvent layer was evaporated under N_2 gas leaving behind a concentrated extract in the vials. The final extract was resuspended in 1 mL of 2:1 ethanol:methyl tertiary butyl ether (MTBE), filtered through 0.2 µm filters, and analyzed via HPLC (Agilent 1260, Santa Clara, CA).

2.7. Statistical analysis

The experiment consisted of three replications of 28 day crop cycles. During each cycle, 70 plants ($n = 10$ plants per treatment × 7 treatments) were harvested on 14, 21, and 28 DAS, making a total statistical population of 630 plants (210 per cycle). Statistical analyses were performed using GraphPad Prism (Version 6.00, GraphPad Software, La Jolla, CA). Significance at the 0.05 level of significance was conducted with a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

3. Results and discussion

3.1. Effects of light recipes on growth and morphology

The impacts of the light treatments on the growth of lettuce were observed as early as 7 DAS. Fig. 2(a) shows images of the lettuce plants under all treatments at 14 DAS. The darker pigmented plants under the WB and RB treatments were expected since high B light is effective for the cryptochrome-driven production of anthocyanin (Meng et al., 2004; Vaštakaitė et al., 2015), and both contained at least 40% B light. However, there were early effects on morphology for the lettuce grown under the WG treatment that were unexpected. Compared to the other treatments, WG exhibited early hypocotyl elongation and a more rapid expansion of the initial true leaf by 7 DAS. As shown in Fig. 3(a), the early vigorous growth under WG translated into significantly higher fresh mass compared to the W control by 14 DAS. All other supplemental treatments and RGB + FR were not significantly different from the control at this stage, while RB resulted in significantly lower fresh mass. The growth under WG was also more uniform as shown by the slightly lower standard deviation (± 0.25). As for the cause of the WG response, early stem elongation has been previously reported in *Arabidopsis thaliana* seedlings grown under G light, and genetic analyses indicated that the response was independent from cryptochrome, phototropin, or phytochrome participation (Folta, 2004). These genetic analyses allude to the potential existence of a novel G light-activated photoreceptor that promotes early stem elongation in some plants, and antagonizes growth inhibition. Therefore, the WG light recipe could be used strategically as a beneficial application for young crops such as baby lettuces, or the recent rise in interest for microgreens (Samuolienė et al., 2013; Kyriacou et al., 2016), which are also being considered for use in space (Kyriacou et al., 2017).

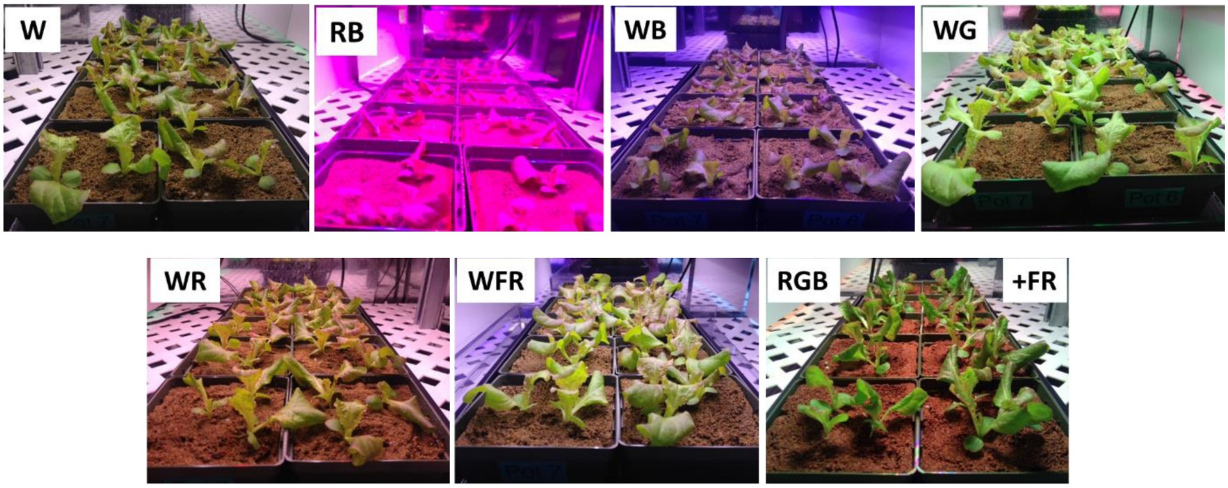
Fig. 2(b) shows images of the lettuce plants under all treatments at 21 DAS. The most notable attribute at this stage of the cycle was canopy closure and the onset of exponential growth for all treatments. Canopy closure was allowed to observe plant response to the change in spectral quality as the plants began to shade each other. In Fig. 3(b), the results for shoot diameter assess the potential for plants to encroach on neighboring plants through horizontal expansion of leaves and petioles. In noting the trends of WFR and RGB + FR going from 14 to 21 DAS, these two treatments result in shoot diameters significantly higher than the W control upon reaching 21 DAS. As expected, the WFR and RGB + FR treatments exhibit shade avoidance syndrome during this stage since FR diodes closely match the absorption action of phytochrome (P_{fr}) photoreceptors. It is well known that plants can detect the presence of neighboring plants by differences in FR radiation, which is readily transmitted through overhanging leaves (Massa et al., 2015a), triggering stem and leaf elongation responses in many species. Hence, the changes in R/FR ratio for shaded plants common to the under story of canopies can be used as a means to promote rapid shoot expansion, and increasing light interception.

The WG treatment was just as effective as the WFR and RGB + FR effects on shoot/canopy diameter since it showed no significant difference from the two. Although FR light is more influential in signaling morphological changes, it is not considered to be photosynthetically active towards the evolution of O_2 (Emerson and Lewis, 1943), but it may still work synergistically to improve the quantum efficiency of shorter wavelengths (Zhen and van Iersel, 2017). When FR is not an available option, as in the Veggie plant chamber on the International Space Station, these results support the phenomenon that deeper penetration of G light could be a tool for increasing shoot expansion, ideally for crops that contain edible stems/petioles like lettuce. However, in the days following this point in the cycle, the lettuce growth under WG began to slow down, and will be discussed further in subsequent sections. It must also be noted that visually, the WG and WFR treatments showed significant suppression of red pigmentation, potentially demonstrating that the energy partitioned to shoot elongation occurred at the expense of secondary metabolites such as anthocyanin, which has strong absorption between 500 and 570 nm (Buraidah et al., 2011).

Table 2 shows the results of the plant growth and morphology measurements from all treatments at 28 DAS. The plants with the greatest leaf area were those grown under RGB + FR and WFR, which were not statistically different from each other. This was an expected characteristic of shade avoidance triggered by FR being present in both treatments. Nonetheless, the leaf area under the RGB + FR combination was higher than all other treatments, while WFR was not statistically different from WR or WG. While plants under RB and WB trended lower, in leaf area they were not statistically different from the control. The two treatments with FR also significantly increased the overall leaf number.

As mentioned in earlier sections, the vigorous growth under WG slowed down after 21 DAS and ended up not being significantly different from the control by the final harvest. On the other hand, a considerable upward trend in growth under WR came later in the cycle between 21 and 28 DAS. Fig. 4 shows the progression of the total accumulated biomass over time, and captures these shifts in growth rate. It can be observed by following the WG line, the early dominant trend in biomass begins to diminish upon reaching 21 DAS and crosses under the trends of the WR and WFR treatments at ∼21 and 23 DAS, respectively, and meets up with the W control data point at 28 DAS. Since this observation occurred consistently for each replicated cycle, this indicates that the lettuce plants could detect the enriched G light conditions early in growth, triggering increased leaf expansion, then switching off the response when a certain maturity level is reached. This is consistent with the evolutionary possibility that seedlings growing under a shaded canopy can detect the relatively enriched G light, triggering more rapid expansion and extension to become more light-competitive, which has been referred to as “G light-induced shade avoidance” (Zhang et al., 2011; Wang et al., 2015). When a certain stage of development or biomass is accumulated, the energy expended

(a) 14 DAS



(b) 21 DAS

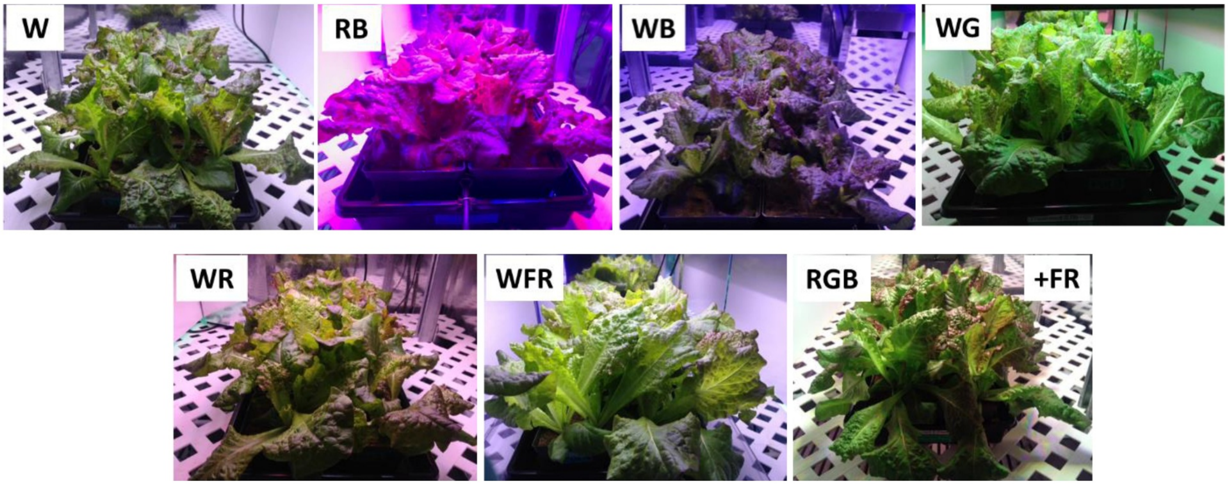


Fig. 2. Morphology of 'Outredgeous' lettuce under all light treatments at (a) 14 days after sowing (DAS) and (b) 21 DAS.

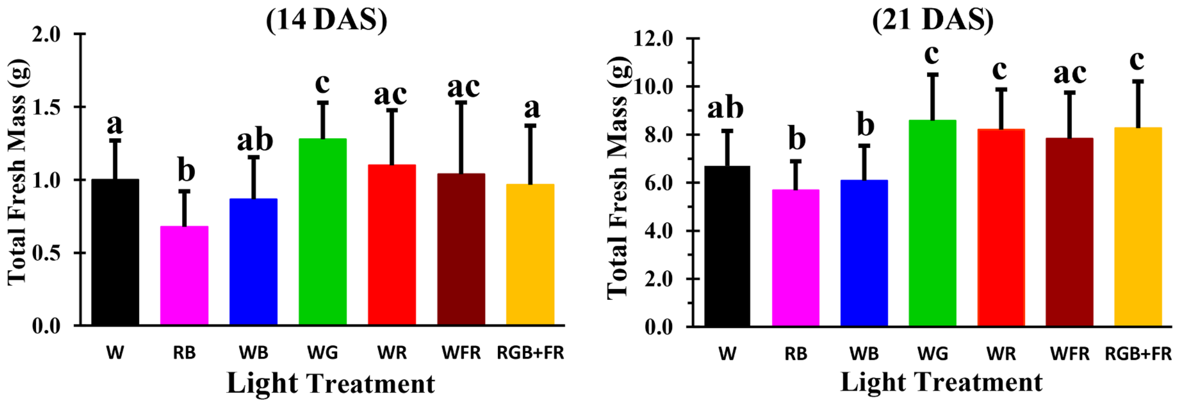
on elongation is no longer needed and growth rate adjusts (Folta and Maruhnich, 2007).

Fig. 4 shows that growth rate under the RGB + FR never declined, and sustained markedly greater biomass accumulation from 19 to 28 DAS, compared to all other treatments. The enhanced lettuce growth observed by Kim et al. (2004) under similar RGB ratios was successfully reproduced in this study, and could be improved further by the addition of FR. The addition of G was shown to be beneficial early on under WG, but the growth results under RGB + FR demonstrate that it was not the addition of G light alone that was beneficial overall, but rather the ratio of G light in conjunction with other wavelengths (ratios ≈ R > G > B). Johkan et al. (2012) demonstrated that the actual waveband of G is also equally important to consider (Liu et al., 2016, 2017). Depending on latitude and elevation, the R:G:B ratios in sunlight on a clear day around noon (peak intensity) can often be close to ~50:30:20, respectively. With this in mind, it could be possible that light recipes resembling these ratios are inherently more conducive for enhancing growth of certain types of lettuce under certain environmental conditions.

In terms of shoot dimensions, the WFR treatment showed the greatest shoot height, while being comparable to WG and RGB + FR in shoot diameter. This indicates that the lower R/FR ratio (1.7) as presented in Table 1, increased elongation under WFR and signaled more partitioning into petiole tissues versus leaf tissue. Whereas in the RGB + FR plants, a higher R/FR ratio (3.1) resulted in increased expansion of leaf tissue without excessive elongation of petioles. Since petioles are mostly vascular tissue for transporting water and nutrients throughout the plant, the energy expended on lateral leaf expansion seemed to have a greater influence on overall biomass than that of petiole elongation (Park and Runkle, 2017). Conversely, the RB and WB treatments remained relatively dwarfed compared to the control, with the WB being comparable to the control in terms of shoot diameter, indicating that the additional wavelengths in WB increased morphological expansion slightly more than RB alone. Either way, the high flux of B light appeared to be the major influence on morphology and inhibition of elongation.

Fig. 5 shows the side-by-side treatment effects on morphology between representative lettuce plants harvested at 28 DAS. Despite the pronounced differences in morphology, the only treatments significantly different from the control in terms of yield (dry mass) were RB and RGB + FR (Table 2). This suggests that the W + supplemental treatments had a greater influence on morphology than on yield, indicating that there was greater influence on where the biomass was allocated, rather than the rate at which biomass was accumulated. It should be noted that the fresh mass of WR was comparable to RGB + FR, indicating that treatments enriched with R light coupled with suitable portions of G and lower portions of B, exhibited greater water-storage capacity in the plant tissues compared to all other

(a) Total Fresh Mass



(b) Shoot Diameter

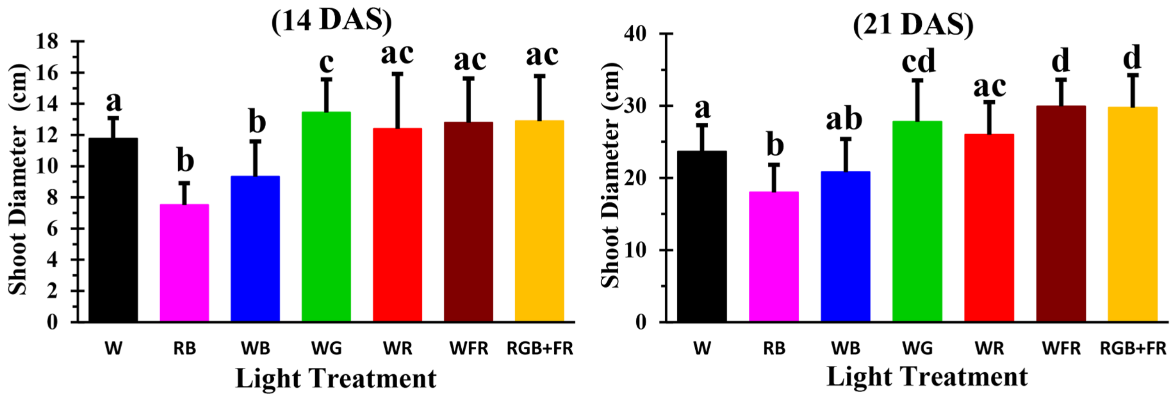


Fig. 3. Fresh mass of 'Outredgeous' lettuce plants under all light treatments at 14 and 21 DAS (a), and shoot diameter at 14 and 21 DAS (b). Means with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test (*n* = 30 per treatment).

treatments (see Table 1). Additionally, Fig. 5 also shows the differences in leaf texture between the treatments. W, WR, and RGB + FR treatments had a more wrinkled and puckered leaf texture resulting in thicker leaves, while the WG and WFR had smoother textures in which the leaves appeared thinner and were more fragile when handling. In support of this observation, the SLM results for W, WR, RB, and RGB + FR were comparable, while the WG and WFR SLM values were the lowest, despite having leaf areas considerably higher than the control (Table 2). This indicates that leaf mass values per unit area were in fact lower because the WG and WFR treatments resulted in thinner leaves. This is a plausible phenomenon in recognizing that since both G and FR light easily penetrate leaf tissue, lettuce could respond to G and FR-enriched light environments by adjusting its morphology to produce thinner leaves, which allows more radiation to reach lower canopy regions.

For the estimated chlorophyll content, Table 2 shows there was no significant difference between the W, WB, RB, WG and WR treatments, while the two treatments containing FR (WFR and RGB + FR) were significantly lower. However, the downward trend can also be observed for the WG and WR treatments. This phenomenon can be attributed to a dilution effect, and has been observed in previous studies in which supplemental FR treatments significantly increased dry mass (Li and Kubota, 2009). The biomass dilution effect has been previously described as the increased accumulation of total non-structural

Table 2
Influence of LED light recipes on leaf area, specific leaf mass (SLM), leaf number, fresh mass, dry mass, shoot height, shoot diameter, and estimated chlorophyll content at 28 DAS.

Parameter	Treatment[a]						
	W	RB	WB	WG	WR	WFR	RGB + FR
Leaf area (cm^2)	731.7 ab[b]	620.2 a	667.4 a	822.0 bc	858.4 c	935.1 cd	1006.0 d
SLM (mg/cm^2)	2.46 ae	2.37 ce	2.27 abc	2.17 cd	2.42 ae	2.06 d	2.54 e
Leaf number	14.3 a	15.3 a	14.5 a	14.0 a	14.8 a	16.6 b	17.7 b
Fresh mass (g)	22.8 ac	19.1 b	21.1 ab	25.6 cd	27.3 de	26.2 cd	30.1 e
Dry mass (g)	1.79 bc	1.47 a	1.52 ab	1.79 bc	2.08 c	1.91 c	2.55 d
Shoot height (cm)	21.1 a	19.0 b	19.1 b	24.2 cd	23.2 c	26.5 e	24.7 d
Shoot diameter (cm)	36.9 a	27.9 b	34.8 a	42.2 cd	40.8 c	43.5 d	43.9 d
Chlorophyll estimate (a.u.)	35.5 a	35.2 a	35.9 a	32.6 a	33.3 a	26.8 b	26.8 b

[a] See Fig. 1 and Table 1 for spectral characteristics.
[b] Values for the same parameter with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test (*n* = 30 per treatment).

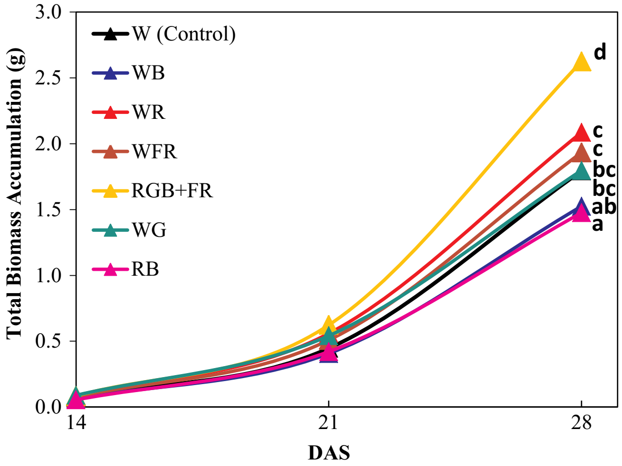


Fig. 4. Total biomass accumulated (dry mass) in ‘Outredgeous’ lettuce plants over time for all light treatments. Means with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test. All data points at each DAS are averages of 30 measurements.

carbohydrates (starch, sucrose) (TNC) relative to the content of nitrogen (N)-based proteins (Poorter et al., 1997; Taub and Wang, 2008). Hence, the dilution effect on chlorophyll content observed here indicates that the plants grown under conditions that promote increased leaf expansion undergo this expansion at the expense of lowering the ratio of total organic N occurring in the leaves.

Alternatively, the changes in morphology could potentially be influential at the cellular level. Light spectra enriched with G, R, or FR that increase biomass and tissue expansion could also increase the intracellular volume, changing chloroplast distribution and movement in a manner that decreases the probability of interacting with the light beams of a chlorophyll meter, hence yielding a lowering effect on relative chlorophyll estimates. It has previously been reported that chloroplast movement can have pronounced effects on SPAD readings (Hlavinka et al., 2013). For instance, certain light conditions encourage chloroplast migration from face position (along cell walls perpendicular to the incident light) to side position (along cell walls parallel to incident light) (Naus et al., 2010). Inevitably, changes in morphology could alter intracellular architecture to exhibit a similar response under certain low light spectra to possibly facilitate light transmission into deeper layers of leaf tissue.

3.2. Effects of light recipes on elemental nutrient accumulation

A total of eight elements were analyzed from the freeze dried plant tissues at 28 DAS, however Fig. 6 presents the results of only the four highest concentrated elements (Ca, Mg, K, and P) that are also considered as targets by the NASA Human Research Program (HRP) to supplement astronaut nutrition (Massa et al., 2015b). As observed in Fig. 6, the lettuce plants had a higher affinity to accumulate K than any other nutrient analyzed, followed by P, while Ca and Mg loads were similar. In response to light treatment, it appears the concentration trend was influenced by plant morphology and biomass, since the treatments with the highest nutrient levels can be correlated to the treatments that resulted in smaller plants with lower biomass accumulation. The lowest nutrient levels were found under the treatments that resulted in larger plants, which similar to chlorophyll, can also most likely be attributed to a biomass dilution effect.

That being the case, this is evidence that the total nutrient uptake rate remained similar under all light treatments for the entire 28 day cycle. Therefore, light recipes that are designed to promote shorter growth, allow plants to bioaccumulate greater loads of elemental nutrients over time, boosting their nutritional value. The RB and WB treatments demonstrated this effect in similar fashion, except for in K, where the WB treatment was higher compared to all treatments. The response to WG was similar to the control in all instances, while WR and WFR trended lower but were statistically comparable to the control except with K, where the WR and WFR plants had significantly lower levels. The RGB + FR treatment was statistically lower in all elements due to higher biomass accumulation and a greater dilution effect.

3.3. Effects of light recipes on secondary metabolite production

Lutein is a xanthophyllic carotenoid compound synthesized by plants either under high light intensities for photoprotection, or is triggered under certain light spectra. Since the light levels used here were considered to be low light (~180 μmol m^{-2} s^{-1}), this investigation sought to understand the production of lutein in response to light spectrum alone, keeping intensity constant for all treatments. Lutein is of interest to NASA due to its ability to reduce macular degeneration and cataract development (Brazaityte et al., 2015). In microgravity, astronauts frequently experience pressure changes in the intracranial fluid that negatively affect eye health. Meanwhile, it has been shown that consumption of more than 2.4 mg of lutein/zeaxanthin daily from foods was significantly correlated with reduced incidence of nuclear lens opacities in a study conducted over a 15-year period (Barker, 2010). Zeaxanthin, a similar compound to lutein, was also analyzed but occurred at levels not distinguishable in the HPLC chromatogram in our testing. Lutein and zeaxanthin have very close isomeric-type chemical compositions, with Lutein often being dominant (Demmig Adams and Adams, 2002). As shown in Fig. 7, the highest lutein accumulation was observed in plants grown under RB, whereas WB, WG, and WR were not significantly different from the W control. Although the significantly lower lutein under WFR and RGB + FR suggests a similar dilution effect as seen in the elemental analysis, a closer evaluation may indicate that leaf pigment combined with morphology, may have concurrent impacts on lutein accumulation. For instance, the lutein levels under WG were not significantly different from the control, but they were also not statistically different from the WFR treatment. As noted previously, the WG and WFR plants visually exhibited the greatest suppression of anthocyanin pigmentation. While B light supplementation has been commonly shown to enhance anthocyanin accumulation (Li and Kubota, 2009), these results support that spectra enriched with G or FR light can suppress anthocyanin

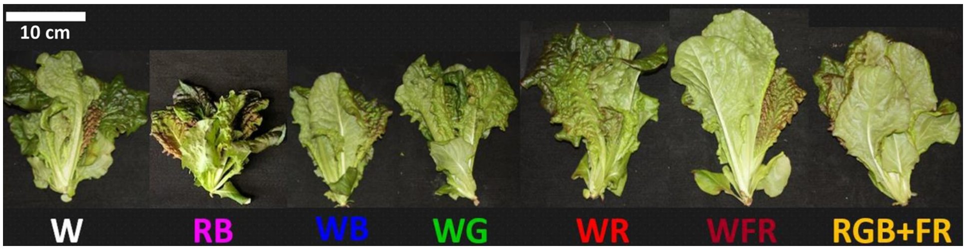


Fig. 5. Morphology of ‘Outredgeous’ lettuce grown under different light treatments of W, RB, WB, WG, WR, WFR, and RGB + FR at equal PPFD levels of ~180 μmol m^{-2} s^{-1} harvested at 28 DAS.

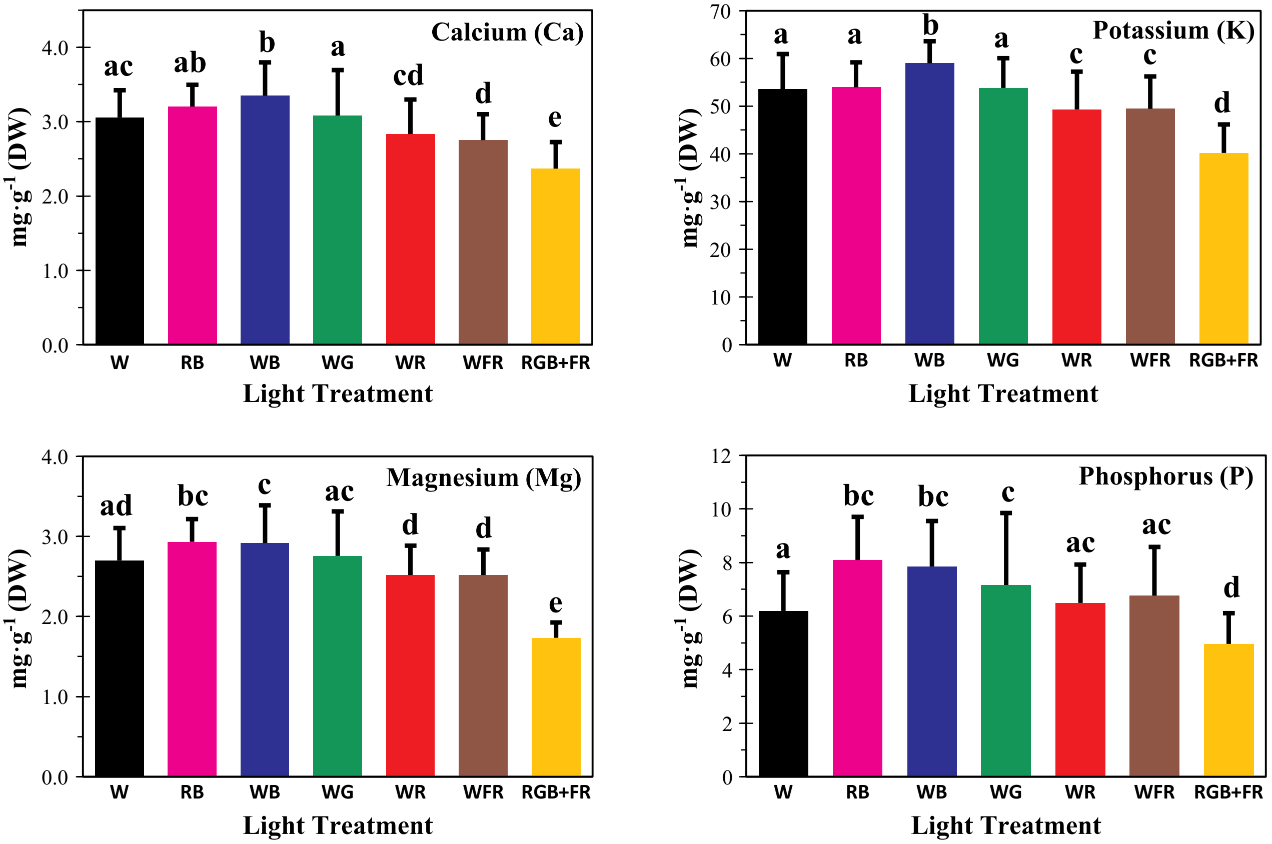


Fig. 6. Accumulation of elemental calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P) in lettuce plants harvested at 28 DAS. Means with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test (*n* = 30 per treatment).

production, which is closely linked to lutein accumulation. It must also be noted that WG, WFR, and W treatments are composed of 57%, 48%, and 48% G light, respectively, which all trended lower in lutein than RB, WB, and WR treatments. The impact of the RGB + FR on lutein was unexpected. Despite having significant visual pigmentation, it appears that dilution effects from the substantial biomass accumulation override the impact of pigment-induced lutein accumulation.

4. Conclusion

This investigation has evaluated the impacts of WLEDs on plant growth, and demonstrated a strategic approach of combining WLEDs with discrete LEDs to identify light recipes for ‘Outredgeous’ lettuce grown under ISS-relevant conditions. The impact of this approach was more influential on plant morphology than on biomass accumulation. However, as a result of the influence on morphology, this yielded secondary effects on the extent of phytonutrient accumulation and secondary metabolite production. By using this approach, it was identified that the WB and RB recipes, were best for obtaining shorter plants with higher concentrations of nutrients. In particular WB was optimal for K content in leaf tissue, and RB was optimal for lutein accumulation. Even though WG, WR, and WFR were not statistically different from the W control in terms of yield, this approach revealed they induced different impacts at various stages of the growth cycle, and the potential for

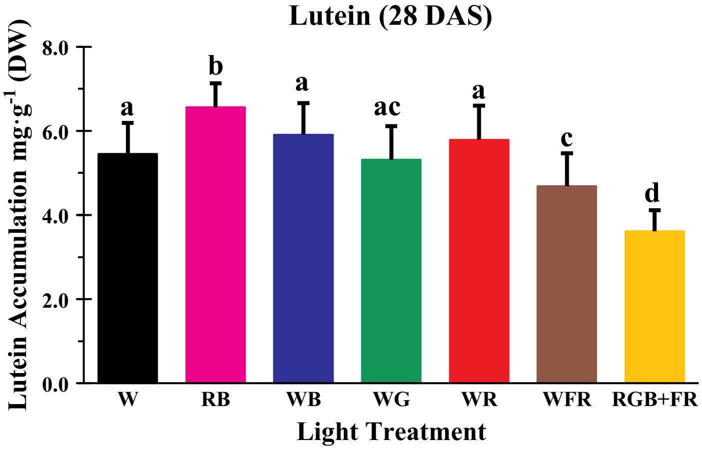


Fig. 7. Accumulation of Lutein in lettuce plants harvested at 28 DAS. Means with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test.

dynamic light management. For instance, the early rapid growth of the WG recipe could be a strategy for young crops or microgreens. It would also be informative to see if the lighting strategy could be switched from WG to WR or RGB + FR at 21 DAS to further optimize the overall yield, or switched from WB/RB to WR to provide a balance of yield and nutrients. The intention of this study was to open a new perspective of utilizing WLEDs, and serve as a call for further exploration into the effects of this lighting approach on other crops. As for the performance of the RGB + FR recipe, it was shown that these light ratios were optimal for the yield of 'Outredgeous' lettuce under our conditions. Nevertheless, this light recipe should not be perceived as an optimal lighting regime for all crops or cultivars. The PPFD and daily light integral (DLI $\approx 12\,\mathrm{mol\,m^{-2}\,day^{-1}}$) used in this study were relatively low, yet sufficient for lettuce growth, especially for spaceflight plant chambers. Still and all, responses may be different under higher light intensities. Each of the light treatments tested here had their own benefits for plant growth, and it is a matter of selecting the appropriate lighting regime to meet the needs of the grower. In this instance, the growth response of 'Outredgeous' lettuce under various light recipes has been investigated, and can be applied for use in space, or in commercial food production on Earth.

Conflict of interest statement

The authors have no potential competing interests that include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding in the submission of this manuscript.

Acknowledgments

This work was supported by funding through the Space Life and Physical Sciences Research & Applications (SLPSRA) Division of NASA's Human Exploration and Operations Mission Directorate (HEOMD), which funded the NASA Postdoctoral Fellowship of M. Mickens. We would like to thank Mr. Larry Koss, Dr. John Sager, and Dr. Mark Cui of AIBC International for technical support and guidance, and acknowledge contributions from all other NASA employees, interns, contractors, and Delaware North employees who assisted in this work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lssr.2018.09.003.

References

Bantis, F., Smirnakoub, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., Radoglou, K., 2018. Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). Sci. Hortic. 235, 437–451.

Barker, F.M., 2010. Dietary supplementation: effects on visual performance and occurrence of AMD and cataracts. Curr. Med. Res. Opin. 26 (8), 2011–2023.

Barnes, C., Tibbitts, T.W., Sager, J.C., Deitzer, G., Bubenheim, D., Koerner, G., Bugbee, B., 1993. Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux. HortScience 28, 1197–1200.

Brazaityte, A., Sakalauskiene, S., Samuoliene, G., Jankauskiene, J., Viršile, A., Novičkovas, A., Sirtautas, R., Miliauskiene, J., Vaštakaite, V., Dabašinskas, L., Duchovskis, P., 2015. The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. Food Chem. 173, 600–606.

Brodersen, C.R., Vogelmann, T.C., 2010. Do changes in light direction affect absorption profiles in leaves? Funct. Plant Biol. 37, 403–412.

Buraidah, M.H., Teo, L.P., Yusuf, S.N.F., Noor, M.M., Kufian, M.Z., Mohamed, C., Majid, S.R., Taha, R., Arof, A.K., 2011. TiO_2/chitosan-$NH_4I(+I_2)$-BMII-based dye-sensitized solar cells with anthocyanin dyes extracted from black rice and red cabbage. Int. J. Photoenergy 273683, 1–11.

Butler, W.L., Hendricks, S.B., Sielgelman, H.W., 1964. Action spectra of phytochrome in vitro. Photochem. Photobiol. 3, 521–528.

Chen, X.L., Guo, W.Z., Xue, X.Z., Wang, L.C., Qiao, X.J., 2014. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Sci. Hortic. 172, 168–175.

Chen, X., Xue, X., Guo, W., Wang, L., Qiao, X., 2016. Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental light provided by light-emitting diode. Sci. Hortic. 200, 111–118.

Cope, K., Bugbee, B.B., 2013. Spectral effects of three types of white light-emitting diodes on plant growth and development: absolute versus relative amounts of blue light. HortScience 48 (4), 504–509.

Cope, K., Snowden, M.C., Bugbee, B.B., 2014. Photobiological interactions of blue light and photosynthetic photon flux: effects of monochromatic and broad-spectrum light sources. Photochem. Photobiol. 90 (3), 574–584.

Demmig-Adams, B., Adams, W.W., 2002. Antioxidants in photosynthesis and human nutrition. Science 298, 2149–2153.

Emerson, R., Lewis, C.M., 1943. The dependence of the quantum yield of chlorella photosynthesis on wavelength of light. Am. J. Bot. 30 (3), 165–178.

Folta, K.M., 2004. Green light stimulates early stem elongation: antagonizing light-mediated growth inhibition. Plant Physiol. 135, 1407–1416.

Folta, K.M., Maruhnich, S.A., 2007. Green light: a signal to slow down or stop. J. Exp. Bot. 58 (12), 3099–3111.

Hlavinka, J., Nauš, J., Špundova, M., 2013. Anthocyanin contribution to chlorophyll meter readings and its correction. Photosynth. Res. 118, 277–295.

Ilieva, I., Ivanova, T., Naydenov, Y., Dandolov, I., Stefanov, D., 2010. Plant experiments with light-emitting diode module in Svet space greenhouse. Adv. Space Res. 46, 840–845.

Johkan, M., Shoji, K., Goto, F., Hahida, S.N., Yoshihara, T., 2012. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. Environ. Exp. Bot. 75, 128–133.

Kim, H.H., Goins, G.D., Wheeler, R.M., Sager, J.C., 2004. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. HortScience 39, 1617–1622.

Kyriacou, M.C., Rouphael, Y., Gioia, F.D., Kyratzis, A., Serio, F., Renna, M., De Pascale, S., Santamaria, P., 2016. Micro-scale vegetable production and the rise of microgreens. Trends Food Sci. Technol. 57, 103–115.

Kyriacou, M.C., De Pascale, S., Kyratzis, A., Rouphael, Y., 2017. Microgreens as a component of space life support systems: a cornucopia of functional food. Front. Plant Sci. 8 (1587), 1–4.

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64.

Li, J., Li, G., Wang, H., Denga, X.W., 2011. Phytochrome signaling mechanisms. The Arabidopsis book. American Society of Plant Biologiosts, pp. 1–26.

Lin, K.-H., Huang, M.-Y., Huang, W.-D., Hsu, M.-H., Yang, Z.-W., Yang, C.-M., 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). Sci. Hort. 150, 86–91.

Liu, H., Fu, Y., Yu, J., Liu, H., 2016. Accumulation and primary metabolism of nitrate in lettuce (*Lactuca sativa* L. var. Youmaicai) grown under three different light sources. Commun. Soil Sci. Plant Anal. 47 (17), 1994–2002.

Liu, H., Fu, Y., Wang, M., Liu, H., 2017. Green light enhances growth, photosynthetic pigments and CO_2 assimilation efficiency of lettuce as revealed by 'knock-out' of the 480–560 nm spectral waveband. Photosynthetica 55 (1), 144–152.

Liu, H., Fu, Y., Hu, D., Yu, J., Liu, H., 2018. Effect of green, yellow and purple radiation on biomass, photosynthesis, morphology and soluble sugar content of leafy lettuce via spectral wavebands "knock out. Sci. Hortic. 236, 10–17.

Massa, G.D., Kim, H.H., Wheeler, R.M., Mitchell, C.A., 2008. Plant productivity in response to LED lighting. HortScience 43, 1951–1956.

Massa, G.D., Graham, T., Haire, T., Flemming, C., Newsham, G., Wheeler, R.M., 2015a. Light- emitting diode light transmission through leaf tissue of seven different crops. HortScience 50 (3), 501–506.

Massa, G.D., Wheeler, R.M., Stutte, G.W., Richards, J.T., Spencer, L.E., Hummerick, M.E., Douglas, G.L., Sirmons, T., 2015b. Selection of leafy green vegetable varieties for a pick-and eat diet supplement on ISS. ICES-2015-252. 1–16.

Massa, G.D., Wheeler, R.M., Morrow, R.C., Levine, H.G., 2016. Growth chambers on the international space station for large plants. Acta Hortic. 1134, 215–221.

McCree, K.J., 1972. The action spectrum, absorptance, and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9, 90–98.

Meng, X.C., Xing, T., Wang, X.J., 2004. The role of light in the regulation of anthocyanin accumulation in gerbera hybrida. J. Plant Growth Regul. 44, 243–250.

Mitchell, C.A., Both, A.J., Bourget, C.M., Burr, J.F., Kubota, C., Lopez, R.G., Morrow, R.C., Runkle, E.S., 2012. LEDs: the future of greenhouse lighting!. Chron. Hortic. 52, 6–11.

Mitchell, C.A., Dzakovich, M.P., Gomez, C., Burr, J.F., Hernández, R., Kubota, C., Currey, C.J., Meng, Q., Runkle, E.S., Bourget, C.M., Morrow, R.C., Both, A.J., 2015. Light-emitting diodes in horticulture. Hortic. Rev. 1–87.

Morrow, R.C., Richter, R.C., Tellez, G., Monje, O., Wheeler, R.M., Massa, G.D., Dufour, N., Onate, B., 2016. A new plant habitat facility for the ISS. ICES-2016-320. 1–13.

Muneer, S., Kim, E.J., Park, J.S., Lee, J.H., 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L. Int. J. Mol. Sci. 15, 4657–4670.

Nauš, J., Prokopová, J., Rebíček, J., Spundova, M., 2010. SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. Photosynth. Res. 105 (3), 265–271.

Nelson, J.A., Bugbee, B., 2014. Economic analysis of greenhouse lighting: light emitting diodes vs. high intensity discharge fixtures. PLoS One 9 (6), 1–10 e99010.

Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49.

Perry, P., Rasmussen, H., Johnson, E.J., 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. J. Food Compost. Anal. 22, 9–15.

Poorter, H., van Berkel, Y., Baxter, B., den Hertog, J, Dijkstra, P., Gifford, R.M., Griffin, K.L., Roumet, C., Roy, J., Wong, S.C., 1997. The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species. Plant Cell Environ. 20, 472–482.

Sager, J.C., Smith, W.O., Edwards, J.L., Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. ASAE 31 (6), 1882–1889.

Sager, J.C., McFarlane, J.C., 1997. Chapter 1: Radiation. Growth Chamber Handbook. Committee on Controlled Environment Technology and Use (NCERA 101), pp. 1–29 Publication No. 340.

Samuolienė, G., Brazaitytė, A., Jankauskienė, J., Viršilė, A., Sirtautas, R., Novičkovas, A., Sakalauskienė, S., Sakalauskaitė, J., Duchovskis, P., 2013. LED irradiance level affects growth and nutritional quality of *Brassica* microgreens. Cent. Eur. J. Biol. 8 (12), 1241–1249.

Stutte, G.W., Edney, T., Skerritt, T., 2009. Photoregulation of bioprotectant content of red lettuce with light-emitting diodes. HortScience 44, 79–82.

Taub, D.R., Wang, X., 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J. Integr. Plant Biol. 50 (11), 1365–1374.

Terashima, I., Fujita, T., Inoue, T., Chow, W.S., Oguchi, R., 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50, 684–697.

United States Environmental Protection Agency, 1996. Method 3050B: acid digestion of sediments, sludges, and soils. Revision 2, 3050B-1-12.

Vaštakaitė, V., Viršilė, A., Brazaitytė, A., Samuolienė, A., Jankauskienė, J., Sirtautas, R., Novičkovas, A., Dabašinskas, L., Sakalauskienė, S., Miliauskienė, J., Duchovskis, P., 2015. The effect of blue light dosage on growth and antioxidant properties of microgreens. Sodininkystė ir daržininkystė 34 (1–2), 25–35.

Wang, Y., Zhang, T., Folta, K.M., 2015. Green light augments far-red-light-induced shade response. Plant Growth Regul. 77, 147–155.

Wheeler, R.M., 2017. Agriculture for space: people and places paving the way. Open Agric. 2, 14–32.

Yorio, N.C., Goins, G.D., Kagie, H.R., Wheeler, R.M., Sager, J.C., 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience 36, 380–383.

Zabel, P., Bamsey, M., Schubert, D., Tajmar, M., 2016. Review and analysis of over 40 years of space plant growth systems. Life Sci. Space Res. 10, 1–16.

Zhang, T., Maruhnich, S.A., Folta, K.M., 2011. Green light induces shade avoidance syndrome. Plant Physiol. 157, 1528–1536.

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