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Spectral effects of blue and red light on growth, anatomy, and physiology of lettuce

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Abstract

Characterizing spectral effects of blue and red light ratios on plants could help expand our understanding of factors that regulate growth and development, which is becoming increasingly important as narrowband light-emitting diodes become common for sole-source lighting. Herein we report growth, physiological, and anatomical responses of two lettuce cultivars grown indoors under various blue and red ratios including monochromatic treatments. When used in combination with red, increasing the proportion of blue light generally reduced growth but increased chloroplast abundance and single-leaf photosynthetic efficiency. However, when used as single wavebands, both blue and red light increased leaf area and epidermal cell area, but reduced root dry mass, SPAD index, stomatal density, and leaf thickness compared to dichromatic light. In addition, chloroplast abundance and single-leaf physiological responses were higher in plants grown under monochromatic blue compared to red light, but the opposite trend was measured for shoot biomass. Our results show that spectral effects on morpho-anatomical leaf responses can largely influence plant growth and single-leaf physiological responses. However, a significant blue light reduction in radiation capture ultimately limits growth and productivity of lettuce plants when dichromatic blue and red light is used.

1 | INTRODUCTION

Photomorphogenesis, defined as light-mediated development (i.e., size and shape) regulated by different photoreceptors, is an important plant process heavily driven by light quality. Among typical photomorphogenic plant responses, red light (600–700 nm) tends to promote dry mass gain and leaf area expansion in plants, whereas blue light (400–500 nm) typically leads to a reduction in leaf area and stem elongation as a result of suppressed cell division and expansion (Dougher & Bugbee, 2004; Liscum et al., 1992). Blue light also regulates stomatal dynamic behavior, chloroplast development, phototropism (Akoyunoglou & Anni, 1984; Briggs et al., 2007; Muthert et al., 2020; Sakai et al., 2001; Wang et al., 2015), steady-state stomatal conductance (g_s) (Goins et al., 1997; Hernández & Kubota, 2016; Kim et al., 2004; Muneer et al., 2014; Sharkey & Raschke, 1981; Van Ieperen et al., 2012; Wang et al., 2015; Yorio et al., 2001), and water-use efficiency of indoor-grown plants (Clavijo-Herrera et al., 2018; Pennisi et al., 2019; Samuolienė et al., 2020).

Although some studies have found that blue light has a relatively small effect on single-leaf photosynthesis (Nanya et al., 2012; Ouzounis et al., 2014), others have shown that the instantaneous photosynthetic capacity of leaves increases (up to a point) in response to increasing proportion of blue light (Abidi et al., 2013; Goins et al., 1997; Graham et al., 2019; Hernández & Kubota, 2016; Hogewoning et al., 2010; Matsuda et al., 2007; Terfa et al., 2013; Wang et al., 2016; Yorio et al., 2001). However, data reported on single-leaf photosynthetic efficiency have sometimes been poorly correlated with growth and yield, partly because measurements are made in a small leaf area over short time intervals (Bugbee, 2016). In contrast, the blue light-induced reduction in plant growth is often driven by a developmental limitation in radiation capture under high blue light. Therefore, it is widely accepted that blue light impacts on photosynthetic efficiency (i.e., moles of carbon fixed per mole of photons

absorbed) are primarily driven by photomorphogenesis. Nonetheless, considering that the blue and red light ratio has been found to significantly affect single-leaf gas exchange, it likely plays a role in controlling physiological parameters that subsequently influence plant growth and development. The mechanisms underlying these effects are only poorly understood and may further elucidate intrinsic effects of using light-emitting diodes (LEDs) for plant lighting.

Characterizing how blue and red light impacts leaf anatomy could help improve our understanding of the interacting factors that regulate overall plant growth and development. For example, changes in leaf structure could help explain how gas exchange is affected by light-induced effects via CO_2 diffusion resistance and assimilation, which are dependent on leaf anatomical traits such as thickness, intercellular spaces, and chloroplast distribution/quantity within mesophyll cells (Arena et al., 2016; Evans et al., 1994; Izzo et al., 2019; Oguchi et al., 2003; Zheng & Van Labeke, 2017). Furthermore, developmental stomatal responses could help explain spectral effects on g_s and leaf transpiration rate (E), as some studies have positively correlated gas exchange with stomatal density and index under high blue light (Hogewoning et al., 2010; Jensen et al., 2018; Savvides et al., 2012; Wang et al., 2016; XiaoYing et al., 2011; Yorio et al., 2001; Zheng & Van Labeke, 2017).

The objective of this study was to characterize growth as well as physiological and anatomical responses of lettuce (*Lactuca sativa* L.) plants grown under different percentages of blue and red light provided by LEDs. We hypothesized that increasing blue light would decrease growth but increase photosynthetic efficiency per unit leaf area due to anatomical changes in response to light spectra. However, the combination of blue and red light would induce different plant responses compared to monochromatic light.

2 | MATERIALS AND METHODS

2.1 | Controlled environment set up and treatments

Plants were grown inside a walk-in growth chamber (C6 Control System with ECoSys Software, EGC) equipped with two shelving units placed on opposite sides, each with five treatment compartments (41 cm height × 50 cm width × 183 cm length) used as individual replications with a random lamp placement. The sides and back of all compartments were covered with a 0.3-mm-thick black and white polyethylene film to minimize light leakage between treatments ($\leq$5 μmol m^{-2} s^{-1}) within the experimental area. Before starting the experiment, a light map was generated using a spectroradiometer (SS-110, Apogee Instruments Inc.) placed at mid-canopy height to determine the photosynthetic photon flux density (PPFD) within each compartment. Target PPFD was achieved by controlling lamp output with dimmers (Solunar, Fluence Bioengineering) connected to a backup battery (BE425M-LM, APC).

The treatments evaluated were: 100% red (0B), 7% blue + 93% red (7B), 26% blue + 74% red (26B), 66% blue + 34% red (66B), and 100% blue (100B). All treatments provided a daily light integral (DLI) of 11.5 mol m^{-2} day^{-1} (PPFD of 200 ± 5 μmol m^{-2} s^{-1}; 16-h photoperiod from 06:00 to 22:00 $_{HR}$). The blue and red lamps (RAY66, Fluence Bioengineering) had LEDs with peak wavelengths of 446 and 664 nm, respectively (Figure 1). The phytochrome photostationary state (PPS) calculated following Sager et al. (1988) was 0.89, 0.89, 0.88, 0.86, and 0.51 for 0B, 7B, 26B, 66B, and 100B, respectively.

2.2 | Plant material and growing conditions

Seeds of 'Waldmann's Green' and 'Outredgeous' lettuce (Johnny's Selected Seeds) were pre-germinated until radicle emergence under fluorescent lamps using a PPFD of 100 μmol m^{-2} s^{-1} for 24 h day^{-1} and maintained at a constant ambient temperature of 24°C. A total of 240 seedlings of each cultivar were subsequently transplanted into 48-cell plug trays (100-ml individual cell volume) filled with arcillite (Greens Grade™, Profile Products LLC), which were then cut into partial-trays of four cells with individual seedlings. Six partial-trays of each cultivar were randomly placed under each treatment compartment. Throughout the experiment, plants were sub-irrigated as necessary with tap water (EC of 0.4 mS cm^{-1}, pH of 8.3, and 40 mg L^{-1} $CaCO_3$ alkalinity). Controlled-release fertilizer (Nutricote 14N-4P-14K, 90-day release, Florikan) was top-dressed using 2.5 g L^{-1} N. Plants were grown for 18 days under a constant ambient temperature,

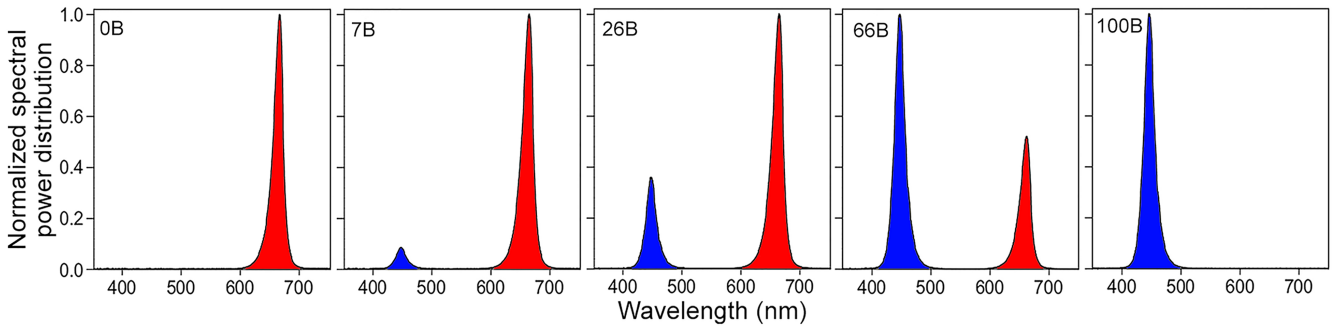

FIGURE 1 Normalized spectral power distribution for the light-emitting diode lamps used in the study. Number of photons were counted for every 1 nm wavelength. Spectral scans were recorded at mid-canopy height with a spectroradiometer

CO_2 concentration, and relative humidity (RH) of 21°C, 1000 ppm, and 65% ± 5%, respectively. Ambient temperature was uniformly maintained by installing cooling fans (AC Infinity AXIAL 1238) within the treatment compartments as needed. A data logger (DL1, ECG) was used to monitor CO_2 concentration and RH in the growth chamber throughout the experiment.

Near-canopy air temperature was monitored using fine-wire thermocouples (Type K, 5SC Series, 0.25 mm diameter, OMEGA Engineering Inc.) interfaced to a data logger (CR1000, Campbell Scientific) and placed directly under a leaf from a plant located at the center of each treatment compartment. To avoid partial shading of the plants, the thermocouples were not shielded. An additional shielded temperature and RH sensor (RC-4HA/C, Elitech Technology) was placed at the center of each treatment compartment to provide real-time data monitoring and to ensure that ambient temperature differences among treatments were <1°C. Within each compartment, plants were randomly rotated daily to minimize location effects.

2.3 | Physiological measurements

Three days prior to harvest, relative chlorophyll content was measured on fully expanded leaves from six randomly selected plants per cultivar per treatment replication using a SPAD chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc.); data were averaged based on measurements made on three different points within a leaf. Following measurements for SPAD index, a portable leaf gas exchange system (LI–6400XT, Li–Cor) was used to measure CO_2 assimilation rate (A), g_s, and E on the last fully expanded leaf from six additional plants per cultivar per treatment replication. The reference leaf temperature, RH, CO_2 concentration, and flow rate inside the cuvette were maintained at 21°C, 60% ± 5%, 1000 ppm, and 400 mol s^{-1}, respectively. A built-in light source with LEDs was used to provide 200 μmol m^{-2} s^{-1} delivered as 90% red and 10% blue light.

2.4 | Anatomical measurements

Samples used to measure anatomical leaf traits were collected 2 days prior to harvest. Epidermal imprints (2–3 cm^2) of the adaxial and abaxial leaf surfaces were made on the last fully expanded leaf from six randomly selected plants per treatment replication using the silicone-rubber impression technique (Weyers & Meidner, 1990). Imprints were observed with an optical microscope (DP71, Olympus Inc.) and images were captured with a digital camera (EOS 60D, Canon). For each sample, stomatal density (SD; number of stomata per mm^2) of the adaxial (SD_{ad}) and abaxial epidermis (SD_{ab}) was measured using ImageJ (Schneider et al., 2012). The epidermal cell area (ECA) was averaged based on measurements made on six different cells from a single leaf within each epidermal imprint using ImageJ.

Other quantitative analyses were made from leaf samples collected on six plants per cultivar per treatment replication, taken from the last fully expanded leaf per plant. Samples were fixed in fixative solution (40% formaldehyde: glacial acetic acid: 50% ethanol at 5:5:90 by volume) immediately after sampling and stored at 4°C. Each leaf sample was dissected and prepared for semi-thin sectioning. Subsamples of the leaf lamina (approximately 5 × 5 mm) were dehydrated in an ethanol series and embedded in acrylic resin (JB4, Polysciences). Semi-thin cross-sections (3 μm) of the leaf lamina were cut with a rotary microtome (RM2155, Leica) and stained with 0.025% toluidine blue in 0.1 M citrate buffer at pH 4.0. Sections of the leaf lamina were observed using a microscope (BX60, Olympus Inc.). Images were collected using a digital camera (EOS 60D, Canon) and analyzed with ImageJ. For each sample, leaf thickness, percent area occupied by intercellular spaces ($IS_{\%}$), and percent area occupied by chloroplasts (chloroplast abundance; $Chl_{\%}$) were averaged based on measurements made on three different regions from a single leaf.

2.5 | Growth measurements

For each cultivar, six plants per treatment replication were destructively harvested 18 days after treatment initiation by cutting the shoots at substrate level. A leaf area meter (LI-3000A, Li–Cor) was used to measure total leaf area. Shoot fresh mass (SFM) was measured using an electronic balance. Subsequently, shoots and washed roots were oven-dried to a constant mass at 70°C for shoot and root dry mass (SDM and RDM, respectively) determination.

2.6 | Data analyses

The five treatments were replicated two times for each cultivar, and data from all plants in the six partial trays were averaged and treated as a single data point per replication. The influence of the two different categorical independent variables (i.e., cultivar and treatment) and their possible interaction on each of the dependent variables were analyzed using a two-way analysis of variance. A linear and quadratic regression analysis was used to evaluate the quantitative response of each dependent variable to blue light (% of PPFD). For RDM, monochromatic red light was dropped from the analysis as its trend differed drastically from that of other treatments; this is similar to the approach used by Hernández and Kubota (2016) and Clavijo-Herrera et al. (2018). Bivariate correlation coefficient analyses with the Spearman's rank correlation test were used to determine the strength and direction of relationships between growth, physiological, and anatomical variables. All data were processed and analyzed using Excel version 16 (Microsoft Corp.) and SPSS Statistics version 21 (IBM Corp.).

3 | RESULTS

3.1 | Growth responses

Leaf area and SFM were higher in 'Waldmann's Green' compared to 'Outredgeous' lettuce, but no cultivar differences were measured

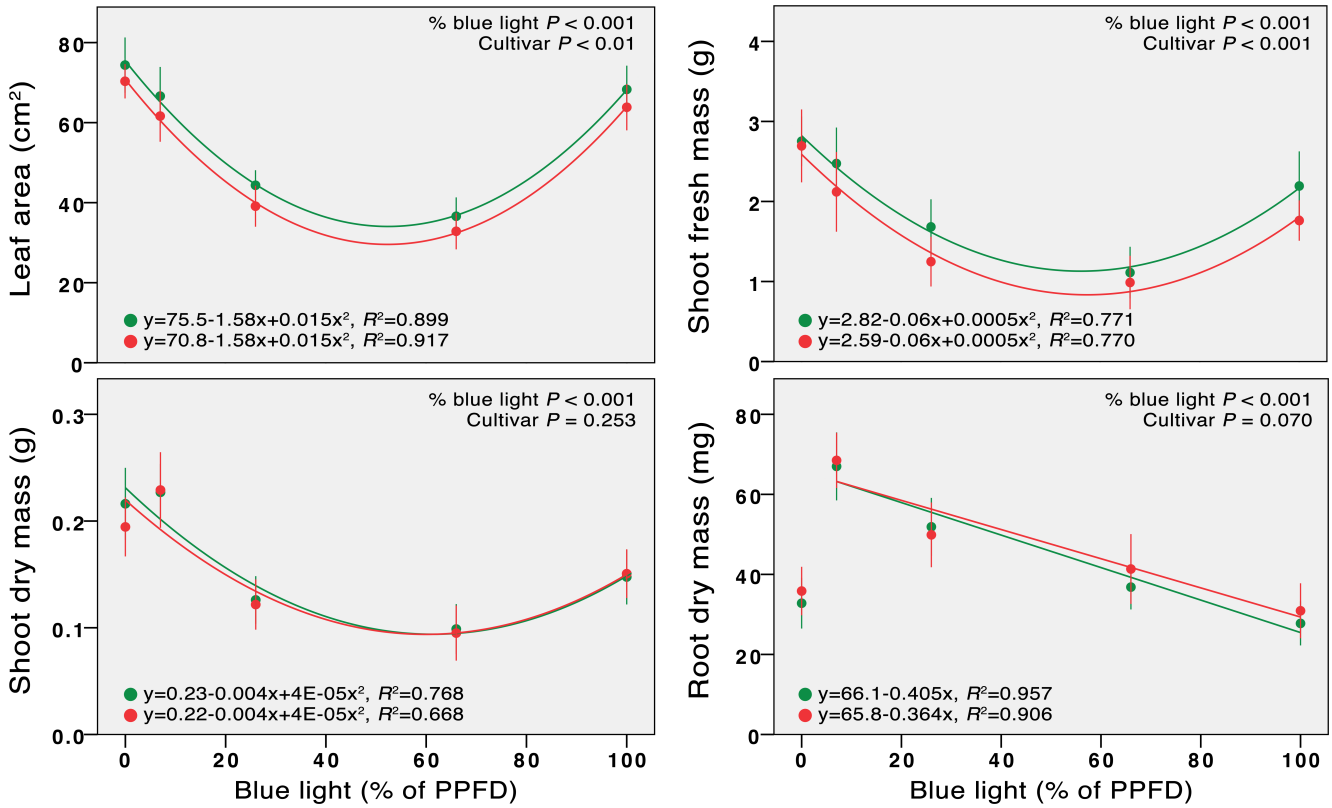

FIGURE 2 Effect of percent of blue light on growth responses of 'Waldmann's Green' (green symbols) and 'Outredgeous' (red symbols) lettuce. Each data point shows the mean and standard error of two replications with six samples per replication

for SDM and RDM (Figure 2). For both cultivars, leaf area, SFM, and SDM decreased with increasing blue light up to 66%, followed by an increase with 100B. In addition, values for leaf area and RDM were similar between plants grown under 0B and 100B (74 and 68 cm^2 and 33 and 28 mg for 'Waldmann's Green' lettuce and 70 and 64 cm^2 and 36 and 31 mg for 'Outredgeous' lettuce, respectively). Both monochromatic treatments produced the largest leaves, which were more than double the size of those from plants grown under 66B, regardless of the cultivar. The highest SFM was measured in plants grown under 0B (2.75 and 2.69 g for 'Waldmann's Green' and 'Outredgeous,' respectively), while both SDM and RDM were highest under 7B. Conversely, both SFM and SDM were lowest under 66B.

3.2 | Physiological responses

Overall, the SPAD index was higher in 'Waldmann's Green' compared to 'Outredgeous' lettuce, whereas the opposite cultivar trend was measured for g_s and E (Figure 3). Increasing blue light from 0% to 66% increased SPAD index from 21 to 31, A from 12 to 21 μmol m^{-2} s^{-1}, g_s from 0.1 to 0.3 mol m^{-2} s^{-1}, and E from 1.4 to 2.4 mmol m^{-2} s^{-1} in 'Waldmann's Green.' Similar responses were measured in 'Outredgeous' lettuce plants, where the SPAD index increased from 18 to 26, A from 13 to 21 μmol m^{-2} s^{-1}, g_s from 0.2 to 0.4 mol m^{-2} s^{-1}, and E from 1.6 to 2.6 mmol m^{-2} s^{-1}.

3.3 | Leaf anatomical responses

Regardless of the cultivar, the general dorsiventral structure of the leaf lamina was not affected by the spectral treatments based on the presence of distinct upper and lower surfaces with amphistomatic epidermis and bi- or multi-seriate palisade and spongy parenchyma (Figure 4). However, quantitative anatomic analyses showed significant treatment effects (Figure 5). For both cultivars, SD_{ad} and SD_{ab} followed a quadratic response to blue light, with an increase up to 26B. In addition, plants grown under 100B had the lowest values for SD_{ad} and SD_{ab}, followed by 0B. Both ECA and $IS_{\%}$ decreased with increasing blue light up to 66%, characteristic of a reduced cell size and a denser mesophyll structure (Figures 4 and 6); however, values for both variables were similar between 0B and 100B. In contrast, $Chl_{\%}$ and leaf thickness increased with increasing blue light up to 66B, and values for leaf thickness were similar in plants grown under 0B and 100B (245 and 243 μm in 'Waldmann's Green,' and 201 and 202 μm in 'Outredgeous' lettuce, respectively). Overall, leaves of plants grown under monochromatic light were thinner than those under blue and red LEDs. In addition, leaves of 'Outredgeous' were thinner than those of 'Waldmann's Green,' but the opposite cultivar trend was measured for $Chl_{\%}$.

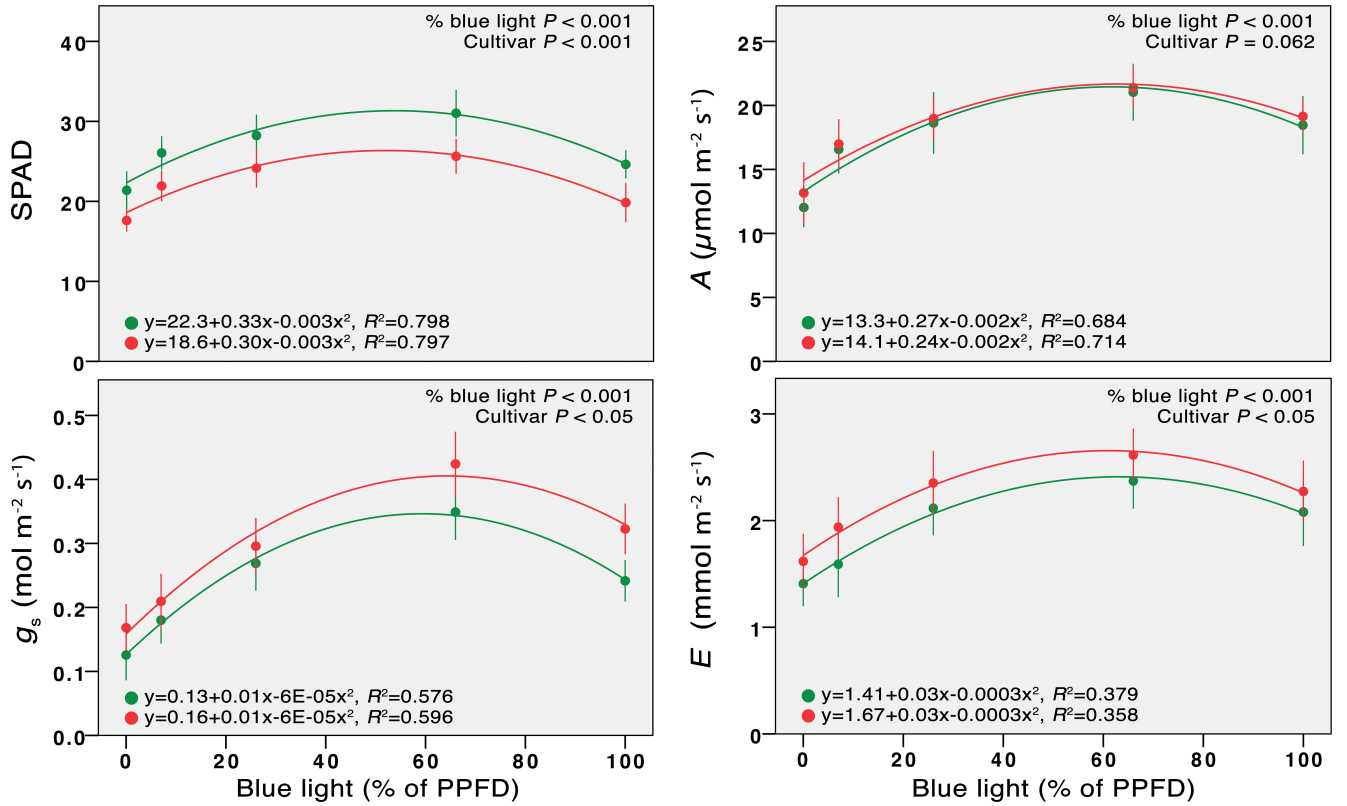

FIGURE 3 Effect of percent of blue light on single-leaf physiological responses of 'Waldmann's Green' (green symbols) and 'Outredgeous' (red symbols) lettuce. Each data point shows the mean and standard error of two replications with six samples per replication

3.4 | Bivariate correlations

Several correlations were observed among the growth, physiological, and anatomical variables measured in our study (Table 1). For example, leaf area, SFM, and SDM were positively correlated to ECA (0.79, 0.67, and 0.62 for 'Waldmann's Green' lettuce and 0.80, 0.70, and 0.58 for 'Outredgeous' lettuce, respectively). Similarly, the SPAD index was positively correlated to leaf thickness (0.56 and 0.58 for 'Waldmann's Green' and 'Outredgeous' lettuce, respectively). In addition, all physiological parameters were positively correlated to $Chl_{\%}$, with correlation coefficients of 0.78, 0.75, 0.63, and 0.51 for SPAD index, A, g_s and E, respectively, in 'Waldmann's Green' lettuce, and 0.74, 0.61, 0.75, and 0.57 for those same variables in 'Outredgeous' lettuce. Furthermore, A was negatively correlated to $IS_{\%}$ (–0.45 for both cultivars).

4 | DISCUSSION

4.1 | Responses of lettuce plants grown under dichromatic blue and red light

Increasing blue light up to 66% reduced leaf area and biomass of lettuce, regardless of the cultivar (Figure 2). Similar findings have been reported by others for lettuce (Clavijo-Herrera et al., 2018; Spalholz et al., 2020; Wang et al., 2016) and for other plant species (Hernández & Kubota, 2016; Pennisi et al., 2019). However, higher blue light up to 66% also led to a general increase in SPAD index and single-leaf photosynthetic efficiency (Figure 3), which is also similar to the findings of others (Clavijo-Herrera et al., 2018; Hernández & Kubota, 2016; Hogewoning et al., 2010; Matsuda et al., 2007; Shengxin et al., 2016; Terfa et al., 2013; Wang et al., 2016; Yorio et al., 2001). These apparently contrasting trends are likely attributed to interacting spectral factors affecting morpho-anatomical development, which are known to influence both instantaneous (e.g., SPAD and gas exchange) and developmental (e.g., SD and leaf area expansion) plant responses (Lehmeier et al., 2017; Oguchi et al., 2003; Son & Oh, 2015; Vogelman et al., 1996).

Several studies have shown that leaf thickness, number of chloroplasts, stomatal density, and mesophyll and/or epidermal cells increase with higher blue light (Dougher & Bugbee, 2004; O'Carrigan et al., 2014; Schuerger et al., 1997; Shengxin et al., 2016; Zheng & Van Labeke, 2017). Our results show a significant increase in leaf thickness and $Chl_{\%}$ with increasing blue light up to 66%, whereas SD generally increased up to 26B, followed by a decrease with 66B and 100B (Figure 5). The higher $Chl_{\%}$ in response to blue light indicates that plants had more chloroplasts per unit leaf area, which ultimately determined the number of photosynthetic enzymes including Rubisco (Björkman, 1981; Boardman, 1977; Oguchi et al., 2003; Von Caemmerer & Farquhar, 1981). This could help explain the blue

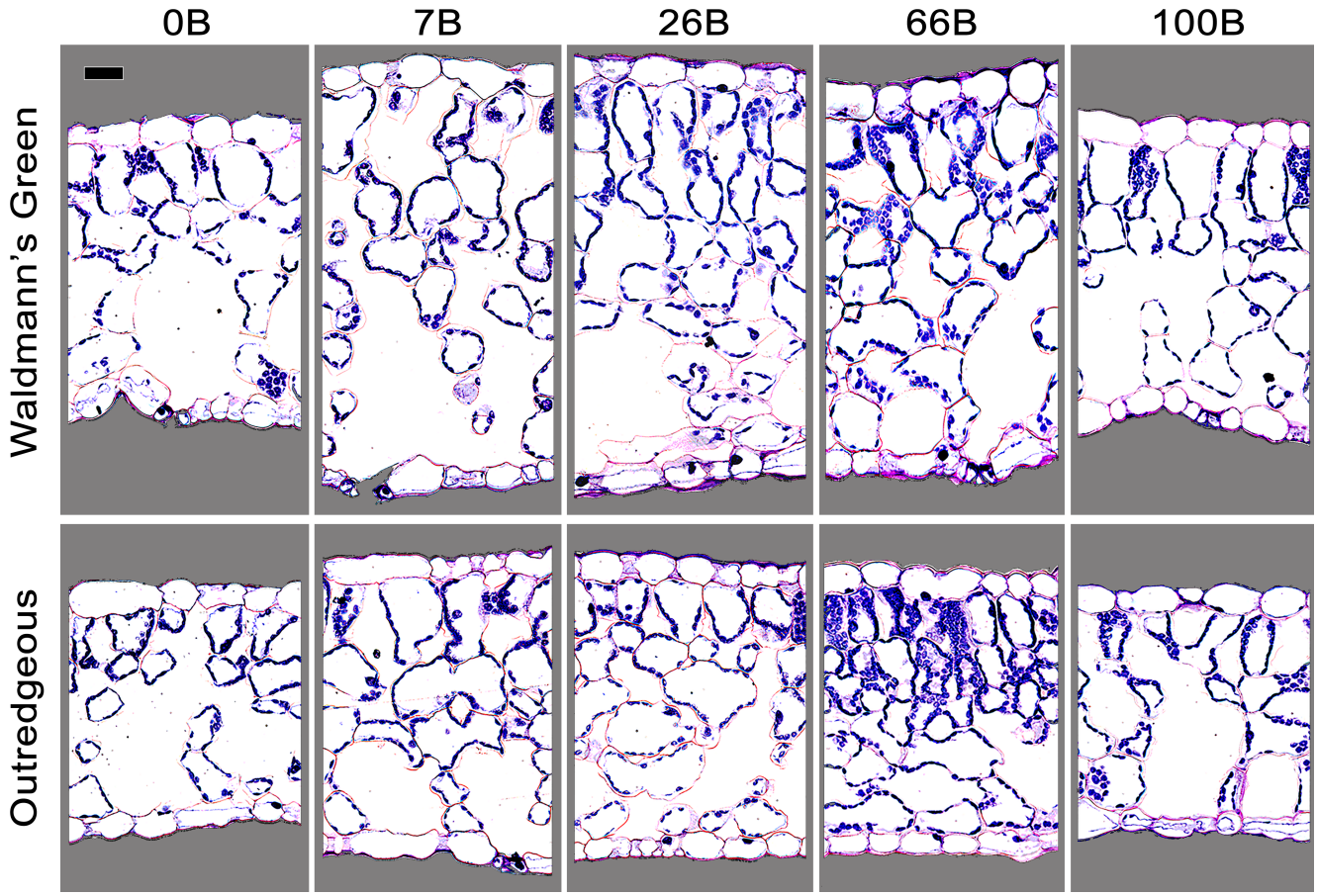

FIGURE 4 Leaf lamina cross sections of 'Waldmann's Green' and 'Outredgeous' lettuce grown under different spectral qualities (0B = 0% blue + 100% red; 7B = 7% blue + 93% red; 26B = 26% blue + 74% red; 66B = 66% blue + 34% red; 100B = 100% blue + 0% red). Images are at the same magnification. Bar = 25 μm

light-induced increase in leaf-level photosynthesis, as supported by the positive correlation between $Chl_{\%}$ and *A* (Table 1). However, the enhancement of g_s and *E* under 66B was not driven by SD as both SD_{ad} and SD_{ab} generally increased up to 26B. Nonetheless, the different light-quality treatments likely played a role at regulating stomatal dynamic behavior. Matthews et al. (2020) explained that stomatal responses to light spectra can be divided into mesophyll/photosynthetic red-light responses, which are responsible for the close correlation between stomatal dynamic behavior and leaf-level gas exchange, as well as and blue-light responses, which are thought to be independent of mesophyll photosynthesis. Furthermore, the denser mesophyll developed with increasing blue light, as indicated by a decrease in $IS_{\%}$ up to 66%, likely enhanced *E* and CO_2 diffusion through the leaves, possibly assisting the chloroplast-driven increase in single-leaf photosynthetic efficiency measured with higher blue light. This is also supported by the negative correlation between $IS_{\%}$ and *A*.

In agreement with our findings, others have shown that changes in leaf cell density and airspace patterning can increase leaf photosynthetic capacity (Lehmeier et al., 2017), which, as highlighted in the present study, can be regulated with blue and red light (Figures 3 and 5). It appears that although blue light has potential to increase the photosynthetic efficiency per unit leaf area, a blue light-induced reduction in radiation capture will ultimately limit growth and productivity of lettuce grown under dichromatic blue and red light (Bugbee, 2016). However, strategies such as increasing plant density or producing baby-leaf lettuce might help maintain productivity when growing smaller plants under high blue-light percentages, which could also increase the edible quality of some lettuce cultivars (Carvalho & Folta, 2014).

4.2 | Responses of lettuce plants grown under monochromatic blue and red light

For both cultivars, lettuce plants grown under monochromatic light had considerable differences compared to those grown under dichromatic blue and red light, but plants grown under 0B or 100B had several morphological, physiological, and anatomical similarities (Figures 2, 3, and 5). In agreement with our results, others have shown that, when grown under monochromatic red light, many plant species develop abnormal characteristics, including excessive hypocotyl elongation (Cosgrove, 1981; Goins et al., 1998; Hoenecke et al., 1992; Izzo et al., 2020; Kigel & Cosgrove, 1991), lower stomatal density (Savvides et al., 2012), curled leaves (Spalholz & Hernández, 2017;

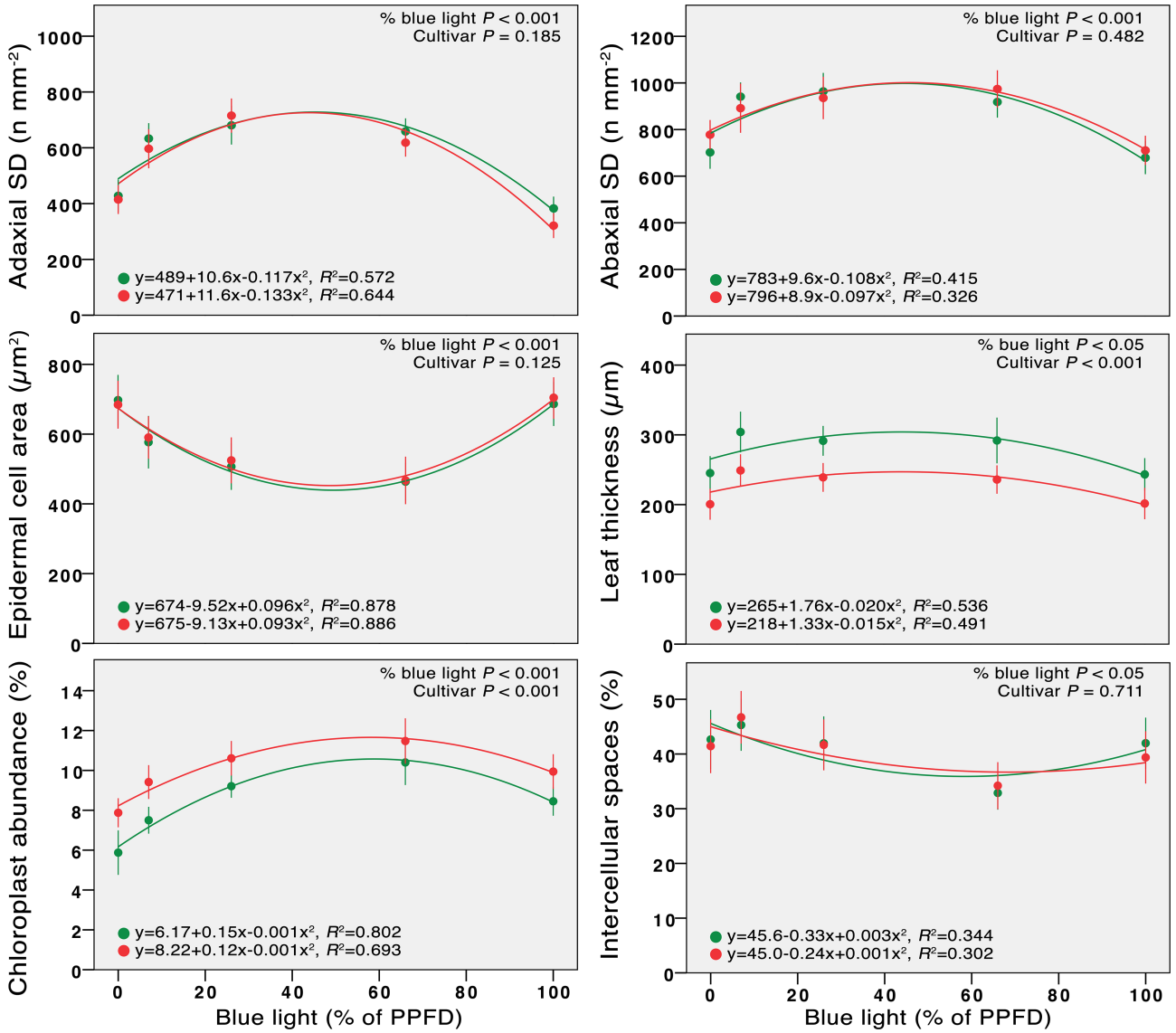

FIGURE 5 Effect of percent of blue light on anatomical responses of 'Waldmann's Green' (green symbols) and 'Outredgeous' (red symbols) lettuce. Each data point shows the mean and standard error of two replications with six samples per replication

Yanagi et al., 1996), and lower photosynthetic rates (Goins et al., 1997; Matsuda et al., 2004; Yorio et al., 2001), which are thought to be the result of a dysfunctional photosynthetic machinery sometimes referred to as the 'red light syndrome' (Hogewoning et al., 2010; Miao et al., 2019; Trouwborst et al., 2016). Similarly, research has shown that plants grown under monochromatic blue light have longer stems (Liu et al. 2011, Nanya et al., 2012, Wollaeger & Runkle, 2014, Kong et al., 2018) and may produce less biomass than those grown under blue-enriched light supplemented with other wavebands (Hernández et al., 2016; Hernández & Kubota, 2016). However, when used as part of dynamic spectral lighting within a 24-h period (Chinchilla et al., 2018; Jishi et al., 2016) or during the cropping cycle (H. Spalholz 2019. Thesis, North Carolina State University, Raleigh, NC, USA), monochromatic blue light has been shown to promote lettuce growth by increasing leaf area expansion. There is significant potential to customize stage-specific growth, morphology, and development of indoor-grown plants by furthering our understanding on how spectral dynamic lighting can be used in controlled environments. Further studies could evaluate the potential application of monochromatic light during certain developmental stages that are less susceptible to the anomalies observed when using monochromatic blue and red light during the entire photoperiod and cropping cycle.

Overall, plants of both cultivars grown under monochromatic blue or red light had a higher leaf area and ECA, but a lower RDM, SPAD, SD, and leaf thickness compared to those grown under combinations of blue and red light (Figures 2, 3, and 5). Most of these effects are common shade-avoidance responses typically observed under low red:far-red ratios (Franklin & Whitelam, 2018; Smith & Whitelam, 1997). Similar to our findings, Keuskamp et al. (2011) reported an increase in cell growth and elongation in *Arabidopsis* grown under <1 μmol m^{-2} s^{-1} of blue light. The authors suggested

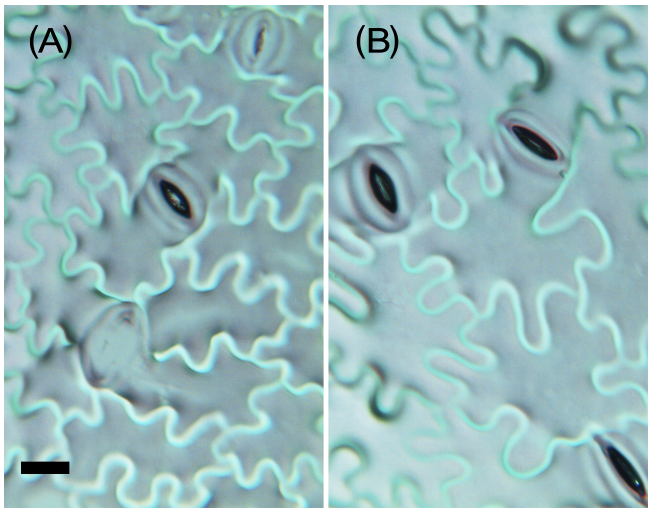

FIGURE 6 Microscope photograph of leaf epidermal cells of lettuce plants grown under 66% blue light (A) and 100% blue light (B). Images are at the same magnification. Bar = 10 μm

TABLE 1 Significant bivariate correlations between morphological, physiological, and anatomical responses of 'Waldmann's Green' and 'Outredgeous' lettuce plants grown indoors under different blue and red photon flux ratios

Cultivar		LT	$Chl_{\%}$	$IS_{\%}$	ECA
'Waldmann's Green'	LA				0.79**
	SFM				0.67**
	SDM			0.59*	0.62**
	SPAD	0.55*	0.78**		
	A		0.75**	−0.45*	
	g_s		0.63**		
	E		0.51*		
'Outredgeous'	LA				0.80**
	SFM			0.49*	0.70**
	SDM			0.59*	0.58*
	SPAD	0.58*	0.74**		
	A		0.61**	−0.45*	
	g_s		0.75**		
	E		0.57*		

Note: ** and * indicate significant correlation at the 0.01 and 0.05 $P \leq$ level, respectively.
Abbreviations: *A*, CO_2 assimilation rate; $Chl_{\%}$, percent area occupied by chloroplasts; *E*, transpiration rate; ECA, epidermal cell area; g_s, stomatal conductance; $IS_{\%}$, percent area occupied by intercellular spaces; LA, leaf area, LT, leaf thickness; SDM, shoot dry mass; SFM, shoot fresh mass.

that low blue light leads to shade-avoidance responses regulated by the action of auxin and brassinosteroids. Kong et al. (2018) later found similar results and concluded that low blue light-mediated shade-avoidance responses are associated with a low PPS, which corresponds with our findings. As indicated in their study and in agreement with our observations, reductions in radiation capture and growth appear to only occur when blue light is used in combination with other wavebands (Kong et al., 2018). Mickens et al. (2018) found similar results when comparing growth of red romaine lettuce using broadband white or monochromatic LEDs. It is also plausible that different responses among plants grown under monochromatic light and those grown under blue and red dichromatic light are attributed to changes in phytochrome-cryptochrome coactions, which ultimately regulate genome expression and plant growth and development (Wang et al., 2018).

Overall, values for leaf area, RDM, leaf thickness, ECA, and $IS_{\%}$ were similar between both monochromatic treatments (Figures 2 and 5). However, regardless of the cultivar, values for all single-leaf physiological responses and $Chl_{\%}$ were higher in plants grown under monochromatic blue compared to red light, further highlighting the importance of blue light for adequate photosynthetic function (Figure 3). For example, SPAD, *A*, g_s, *E*, and $Chl_{\%}$ were 15, 53, 92, 48, and 44% higher, respectively, in leaves of 'Waldmann's Green' lettuce plants grown under 100B compared to 0B. However, opposite trends were measured for shoot biomass, where for 'Waldmann's Green,' 0B resulted in 26 and 46% more SFM and SDM, respectively, compared to 100B, which corresponds with the results of Wollaeger and Runkle (2014) and Dieleman et al. (2019).

As described by others, physiological responses of plants grown under 0B could have been affected by disruptions in the development and functioning of the photosynthetic machinery (Hogewoning et al., 2010; Izzo et al., 2020; Miao et al., 2019; Trouwborst et al., 2016; Wang et al., 2015). However, despite the measured limitations in photosynthetic efficiency, growth under monochromatic red light must have been compensated by other factors that ultimately drove a higher biomass production (Figures 2 and 5). While some studies have shown that leaf area can be higher under monochromatic red compared to blue light (Dieleman et al., 2019), others have reported opposite (Hernández & Kubota, 2016; Wollaeger & Runkle, 2014) or similar trends (Hernández et al., 2016; Spalholz et al., 2020; Wollaeger & Runkle, 2014). The fact that we found similar responses in leaf area, ECA, and leaf thickness between both monochromatic treatments suggests that other factors, in addition to radiation and photosynthetic capture as a function of leaf area, are likely responsible for the contrasting trends measured in our study between single-leaf physiological responses and biomass production.

4.3 | Spectral effects in single-leaf photosynthesis

Although studies with LEDs have shown that spectral quality responses vary widely among species, cultivars, and environmental conditions (Graham et al., 2019; Mitchell & Sheibani, 2020; Olle & Viršile, 2013; Snowden et al., 2016), it is well established that blue light above 5%–10% typically results in compact plants with a limited ability to intercept light, ultimately affecting whole-plant photosynthesis and growth (Bugbee, 2016; Gómez & Izzo, 2018). In addition, blue photons are partly absorbed by inactive (e.g., anthocyanin) and accessory (e.g., carotenoids) pigments that do not participate in the

energy transfer to chlorophyll reaction centers and thus are commonly considered to have a lower photosynthetic efficiency than red photons (Barnes et al., 1993). This is in agreement with the spectral efficiency curves described by Hoover (1937), McCree (1972), and Inada (1976), who characterized the effects of individual wavebands of light on single-leaf photosynthesis and showed that blue photons are used less efficiently than red photons. In contrast, our findings (and those of others) show an increase in photosynthetic efficiency per unit leaf area from higher blue light up to 66% (Figure 3), which is generally attributed to more chloroplasts and overall photosynthetic machinery per unit leaf area (Abidi et al., 2013; Goins et al., 1997; Graham et al., 2019; Hernández & Kubota, 2016; Hogewoning et al., 2010; Izzo et al., 2020; Matsuda et al., 2007; Terfa et al., 2013; Wang et al., 2016; Yorio et al., 2001). As described by Bugbee (2016), plant growth and development under mixed colors of light may not be well predicted using these curves, and instead, spectral effects on radiation capture can be more closely related to whole-plant photosynthesis and growth.

Wu et al. (2019) stated that potential synergistic effects of different wavebands are hard to interpret from these curves. Accordingly, others have shown that different photosynthetic rates could be obtained when combining two or more wavebands of light (Emerson, 1957; Emerson & Rabinowitch, 1960; Hogewoning et al., 2012; Murakami et al., 2018; Terashima et al., 2009; Zhen & Bugbee, 2020; Zhen & van Iersel, 2017). In the studies published by Hoover (1937), McCree (1972), and Inada (1976), plants were grown under broadband white light and photosynthesis was measured using single wavebands. In contrast, plants in our study were grown under different combinations of blue and red light and thus had specific morpho-anatomical characteristics that affected photosynthetic efficiency and growth (Figures 2, 3, and 5). Furthermore, gas exchange was measured using a light source with blue and red LEDs at a fixed ratio that differed from all the treatments used during growth and development.

As shown by Elings et al. (2016) and Dieleman et al. (2019), spectral composition effects from the light-source used to measure gas exchange can be significant. Partly in agreement with the spectral efficiency curves reported by Hoover (1937), McCree (1972), and Inada (1976), the two aforementioned studies showed that leaves grown and measured under monochromatic red light had a higher photosynthetic rate than those grown and measured under monochromatic blue light. However, both studies showed that overall, photosynthesis was higher when blue and red LEDs were used as a light source compared to ambient light. In addition, both studies showed that plants grown under monochromatic blue light and measured with blue and red LEDs had the highest instantaneous photosynthetic rate, followed by plants grown under white light and measured under blue and red LEDs. These findings suggest that spectral effects of blue and red light on morpho-anatomical responses can significantly affect single-leaf gas exchange, likely due to differences in leaf structure and accumulation of chloroplasts within mesophyll cells. However, as stated above, those responses may not always be good predictors of growth and productivity, especially when considering the effect that spectral quality has on the production of secondary metabolites that increase the nutritional quality of lettuce (Carvalho & Folta, 2014). Hogewoning et al. (2010) explained that there are fundamental differences between leaf adaptation and instantaneous effects in response to spectral quality. Nevertheless, the interacting factors driving the dichotomy of increasing single-leaf photosynthesis and decreasing growth must be further investigated to properly identify how blue and red light interact in shaping plant growth and development. Studies evaluating spectral effects on photoreceptor activation and phytohormonal responses might provide further insights to better understand plant responses when using sole-source lighting from LEDs.

5 | CONCLUSIONS

When used in combination with red, increasing the proportion of blue light generally reduced growth but increased single-leaf photosynthetic efficiency of 'Waldmann's Green' and 'Outredgeous' lettuce plants compared to monochromatic light. However, when used as single wavebands, both blue and red light led to a higher leaf area and ECA, but a lower RDM, SPAD, SD, and leaf thickness than dichromatic light. In addition, values for RDM, ECA, leaf thickness, and $IS_{\%}$ were similar between both monochromatic treatments. However, values for $Chl_{\%}$ and single-leaf physiological responses were higher in plants grown under monochromatic blue compared to red light, but the opposite trend was measured for shoot biomass. Our results show that for both cultivars, spectral effects on morpho-anatomical leaf responses can largely influence plant growth and single-leaf gas exchange. However, a significant blue-light reduction in radiation capture ultimately limits growth and productivity when blue and red light are combined, despite increases in single-leaf photosynthesis. Clearly, the use of LEDs has enormous potential to regulate plant growth and productivity by controlling morpho-anatomical and physiological responses to spectral quality. Using dynamic spectral changes within a 24–h period or during the cropping cycle has the potential to enhance radiation capture, photosynthetic capacity, and photosynthetic performance of plants, which is just one of the many potential applications of LEDs still to be investigated for the advancement of sole-source lighting in controlled environments.

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AUTHOR CONTRIBUTIONS

Luigi G. Izzo: conceptualization, methodology, investigation, data curation, writing—original draft, writing—review and editing. Matthew A. Mickens: conceptualization, writing—review and editing. Giovanna Aronne: methodology, resources, writing—review and editing. Celina

Gómez: conceptualization, resources, writing—original draft, writing—review and editing, supervision.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the first author (Luigi Gennaro Izzo, luigigennaro.izzo@unina.it), upon request.

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