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Growth, Water-Use Efficiency, Stomatal Conductance, and Nitrogen Uptake of Two Lettuce Cultivars Grown under Different Percentages of Blue and Red Light

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Abstract: The objective of this study was to characterize growth, water-use efficiency (WUE), stomatal conductance (g_s), SPAD index values, and shoot nitrogen uptake of two lettuce cultivars grown under different percentages of blue and red light. The treatments evaluated were 100% red; 7% blue + 93% red; 26% blue + 74% red; 42% blue + 58% red; 66% blue + 34% red; and 100% blue. Broad-spectrum (19% blue, 43% green, and 38% red) light was used to observe the effects of wavelength interactions. All of the treatments provided an average daily light integral (DLI) of 17.5 mol·m^{-2}·d^{-1} (270 ± 5 µmol·m^{-2}·s^{-1} over an 18-h photoperiod). The experiment was replicated three times over time; each terminated 21 days after treatment initiation. Leaf area, specific leaf area (SLA), and SPAD index had a similar response in that all of the parameters increased with up to 66% blue light, and slightly decreased or remained constant with 100% blue light. In contrast, leaf number, shoot dry mass, and WUE generally decreased in response to blue light. Conversely, for every 10% increase in blue light, g_s increased by 10 mmol·m^{-2}·s^{-1}. Nitrogen uptake was unaffected by light quality. Our findings indicate that when grown under different blue and red photon flux ratios, the WUE of lettuce significantly decreases under higher blue light, which could be attributed to a reduction in plant growth (leaf number and dry mass), and an increase in g_s. However, green light within broad-spectrum lamps might counteract blue-light mediated effects on g_s and WUE in lettuce.

Keywords: controlled environments; *Lactuca sativa*; blue light; red light; water consumption

1. Introduction

Continuous technological improvements, low lamp-surface temperature, and high energy-use efficiency, along with decreasing capital costs, are making light-emitting diodes (LEDs) the light source of choice to grow plants in controlled (growth chamber) and semi-controlled (greenhouse) environments. Numerous studies have shown that the production advantages of using LEDs range from the reduction of electrical energy inputs to increased growth and flowering, and improved quality of plant products [1]. Furthermore, spectral control of electric lighting has facilitated research evaluating biochemical and physiological plant responses to narrow-spectrum radiation [2].

Increasing blue light (400 nm to 500 nm) often inhibits cell division and expansion, reducing leaf area and stem elongation, and increasing leaf thickness in most plant species; compact plants with smaller, thicker leaves typically result in higher photosynthetic rate per unit of leaf area, but reduced radiation capture [3]. This reduction in radiation capture is believed to be the primary reason for reduced growth (dry mass gain) in response to higher blue light [4]. In addition, blue light is less

efficient at driving photosynthesis than other wavebands of photosynthetically active radiation (400 nm to 700 nm), which can be attributed to (1) significant energy losses due to radiation capture by non-photosynthetic pigments (e.g., anthocyanins); and (2) inefficient energy transfer by accessory pigments (e.g., carotenoids) [5]. Blue light is also known to regulate chloroplast development, as well as to control photomorphogenic and phototrophic plant responses [6,7]. In contrast, red light (600 nm to 700 nm) typically promotes dry mass gain and leaf area expansion in many plant species, and is the waveband with the highest instantaneous quantum efficiency for driving photosynthesis, with a broad peak from 620 nm to 660 nm [8].

A general conclusion from sole-source light-quality research suggests that plant responses to LEDs are species and sometimes cultivar-specific, and greatly depend on light intensity, duration of treatment, and other environmental interactions [9]. However, several studies indicate that 5% to 20% of blue light within the total photosynthetic photon flux (*PPF*) is typically needed to improve growth and development and to minimize shade-avoidance responses (e.g., elongated internodes, petioles, and hypocotyls, larger, thinner leaves, and decreased chlorophyll production) in controlled environments [9–15].

Blue Light Effects on Stomatal Conductance (g_s) and the Potential Role with Water Relations

An area that has been largely overlooked in LED plant-lighting research is the impact that light quality has on water-use efficiency (WUE) and nutrient uptake. In contrast, stomatal responses to blue light have been widely studied, and indicate that stomatal aperture is a blue-light induced response in the guard cell that promotes g_s by enhancing the aperture of the stomatal pore through the action of cryptochrome and phototropin photoreceptors [16–18]. Stomatal conductance refers to the capacity of stomata to allow CO_2 into the leaf or to lose water. A number of studies have demonstrated that blue light increases steady-state g_s of plants [15,19–25]. Although there is a general consensus that stomata affect plant–water relations and gas exchange, a definitive regulatory mechanism that explains how blue light-induced stomatal responses control g_s, transpiration, and WUE is unclear due to the existence of complex feedback loops, where changes in transpiration rate or plant–water relations resulting from changes in g_s can themselves affect conductance [26].

While the aperture and closing of stomata is a near-instantaneous, short-term response to a variety of environmental and endogenous signals, anatomical characteristics such as stomatal index and density are generally considered to be long-term developmental adaptations to the environment, which also influence the conductance of gases through the leaf mesophyll and stomata. High g_s under high blue light has been positively correlated with an increase in stomatal index and density [12,22,23,25,27,28]. It is likely that the upregulation of stomatal density with blue light affects plant–water relations due to increases in g_s and transpiration.

As the fundamental shift of incorporating LEDs in the horticulture industry continues to expand, important questions must be addressed regarding the production outcomes of using narrow-spectrum sole-source lighting. The objective of this study was to characterize the growth, WUE (based on shoot dry mass and water used), g_s, SPAD index, and shoot nitrogen uptake of two lettuce cultivars grown under different percentages of blue and red sole-source lighting from LEDs. Furthermore, broad-spectrum light was used to observe the effects of wavelength interactions. We hypothesized that a higher percentage of blue light would result in more compact growth, lead to a higher SPAD index, and increase g_s, water-, and nitrogen uptake, but decrease WUE.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Seeds of 'Cherokee' and 'Waldmann's Green' lettuce (Johnny's Selected Seeds, Winslow, MN, USA) were pre-germinated until radicle emergence (24 h) and subsequently transplanted into 48-cell plug trays (100-mL individual cell volume) filled with horticultural grade

substrate composed (by volume) of 60% peat and 40% perlite (Sunshine Mix #4; Sun Gro Horticulture, Agawam, MA, USA). Seedlings were propagated inside a growth room with an average *PPF* of 180 ± 5 μmol·m^{-2}·s^{-1} (24-h photoperiod) provided by broad-spectrum LED lamps (168 × 5.6 cm, RAY66; Fluence Bioengineering, Austin, TX, USA). Room temperature, CO_2 concentration, and relative humidity (RH) were set at 25 °C, 400 ppm, and 60% to 80%, respectively. Seedlings were sub-irrigated as necessary with a water-soluble fertilizer solution (20N-4.4P-16.6K with micronutrient; Jack's Professional® General Purpose, J.R. Peters Inc., Allentown, PA, USA) at a concentration of 100 mg·L^{-1} N.

The experiment was initiated two weeks after sowing, when 35 uniform seedlings per cultivar were selected to receive lighting treatments, and were each subsequently transplanted into 11-cm diameter (620-mL) containers filled with 185 g of moist substrate (Sunshine Mix #4) amended with 1 g of fertilizer (10N-4.4P-8.3K; Scotts®, Marysville, OH, USA; 1–2 months release). Each seedling (with a partially saturated substrate) weighed 35 g before transplanting. A double layer of plastic grip liner (12 cm × 12 cm) was placed at the bottom of each container to minimize loss of substrate through the drainage holes. All of the container surfaces were covered with a white plastic film (17 cm × 17 cm) to minimize water evaporation from the substrate surface. A small cross was cut in the middle of the film to fit a single plant. Combined shoots from extra seedlings were oven-dried, weighed, and ground for nutrient analysis at week zero.

Throughout the experiment, plants were sub-irrigated daily for 1 h using 18-cm diameter (600-mL) black plastic reservoirs covered with an opaque lid immediately after each irrigation event. Containers with plants were placed on top of the reservoir lids following sub-irrigation. Each lid had four drainage holes to allow excess water to passively drain back into the reservoir. Reservoirs were refilled with tap water every three days to return to a pre-set water volume of 400 mL.

2.2. Lighting Treatments

All of the treatments provided a daily light integral (DLI) of 17.5 mol·m^{-2}·d^{-1} (270 ± 5 μmol·m^{-2}·s^{-1}; 18-h photoperiod from 05:00 to 23:00 $_{HR}$), which was measured with a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT, USA) at mid-canopy height. The blue and red LED lamps (RAY66; Fluence Bioengineering) had peak wavelengths of 446 nm and 664 nm, respectively (as measured with a spectroradiometer; see Figure 1 for spectral characteristics). Five randomly selected plants per cultivar were placed within each treatment. Seven light treatments were evaluated in the study: 100% red (0B); 7% blue + 93% red (7B); 26% blue + 74% red (26B); 42% blue + 58% red (42B); 66% blue + 34% red (66B); 100% blue (100B), and broad-spectrum light, which provided 19% blue, 43% green (500 nm to 600 nm), and 38% red.

Plants were grown on compartments (183 cm × 41 cm × 61 cm) within multilayer shelves placed inside a walk-in growth chamber (C6 Control System with ECoSys Software; EGC, Chagrin Falls, OH, USA). Each compartment was a replicate of a treatment comprised of two lamps with a 25-cm separation distance to ensure light uniformity. Before starting the experiment, a light map was generated to determine the maximum *PPF* for each treatment (no plants present). The light output to achieve our target *PPF* was controlled with a dimmer (Solunar; Fluence Bioengineering) that was connected to a backup battery (BE425M-LM; APC, West Kingston, RI, USA). Light pollution ($\leq$5 μmol·m^{-2}·s^{-1}) within treatments was minimized by covering the sides and back of the shelves with a double layer of 0.3-mm thick black and white polyethylene film (white side facing the plants). A black and white polyethylene film curtain (215 cm × 200 cm) was used to prevent light pollution between the two opposite shelves (black side facing the plants). Within each treatment, plants were randomly rotated daily to minimize the location effects within the experimental area.

The average ambient day (from 05:00 to 23:00 $_{HR}$) and night (from 23:00 to 05:00 $_{HR}$) air temperature of the chamber were set at 21 °C and 20 °C, respectively. However, radiation from the lamps raised the ambient temperature during the photoperiod, which was uniformly maintained at ~22 °C by installing cooling fans (AC Infinity AXIAL 1238; City of Industry, CA, USA) as needed.

The set point for ambient CO_2 and RH were 405 ppm and 70%, respectively. Near-canopy air temperature was monitored using fine-wire thermocouples (Type K, 5SC Series, 0.25 mm diameter; OMEGA Engineering Inc., Norwalk, CT, USA) that were placed directly under a leaf from a plant located at the center of each treatment and interfaced to a data logger (CR1000; Campbell Scientific, Logan, UT, USA) (Table 1). To avoid partial shading of the plants, the thermocouples were not shielded. An additional shielded temperature and RH sensor (RC-4HA/C; Elitech, Milpitas, CA, USA) 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 $\leq$1 °C. A data logger (DL1; ECG) was used to record average CO_2 concentration and RH every 15 min. The average mean $\pm$ SD for CO_2 concentration and RH were 504.6 $\pm$ 31.2 µmol·mol^{-1} and 66.3 $\pm$ 8.0%, respectively.

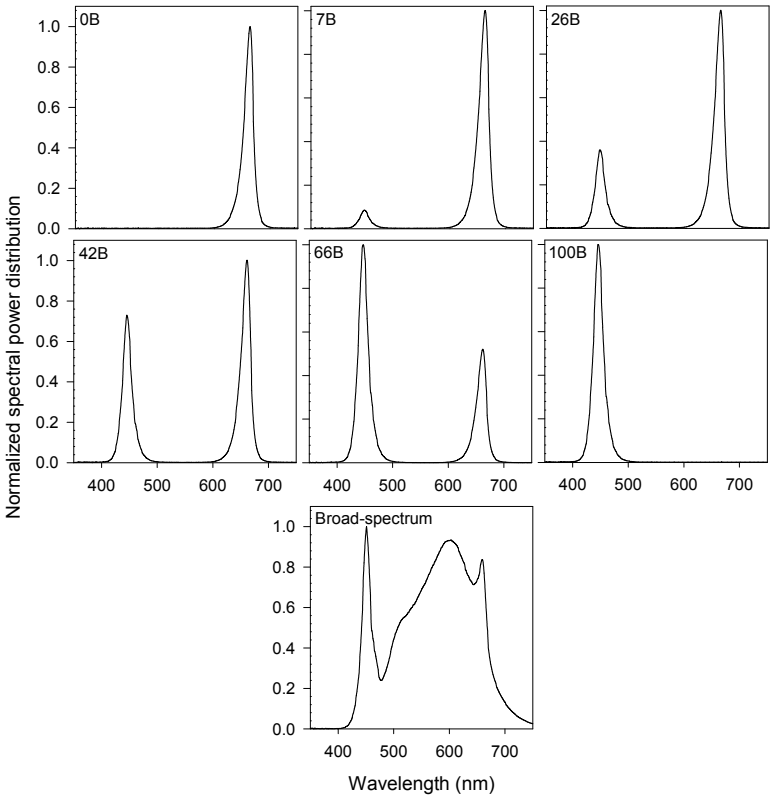

Figure 1. Normalized spectral power distribution for the light-emitting diode (LED) lamps used in the experiment. Number of photons was counted for every 1 nm. B = blue light percentage, with the remaining light provided by red LEDs.

Table 1. Average daily near-canopy air temperature (ADT) measured during each experimental replication. The treatments evaluated were: 100% red (0B); 7% blue + 93% red (7B); 26% blue + 74% red (26B); 42% blue + 58% red (42B); 66% blue + 34% red (66B); 100% blue (100B); and broad-spectrum light, which provided 19% blue, 43% green, and 38% red.

Treatment	ADT (°C)		
	Rep. 1	Rep. 2	Rep. 3
0B	21.7 [z]	22.1	21.9
7B	21.9	21.6	22.7
26B	21.6	22.2	21.6
42B	21.9	22.3	21.9
66B	21.9	21.7	21.9
100B	21.4	21.5	22.2
Broad-spectrum	21.1	21.5	21.7

[z] The standard deviation for all data points ranged from 0.8 °C to 2.4 °C.

2.3. Data Collection and Plant Measurements

Water use was measured for all of the plants with a digital balance prior to refilling the reservoir after each irrigation event. Stomatal conductance was measured 19 days after treatment initiation with a leaf porometer (SC-1; Decagon Devices Inc., Pullman, WA, USA). Additionally, prior to harvest, SPAD index was measured with a chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan) on three different points on the same leaf. Data for g_s and average SPAD index were collected for the youngest fully expanded leaf of each plant.

The experiment was terminated 21 days after treatment initiation. Immediately following harvest, the number of leaves (>1 cm) per plant was counted, and total leaf area was measured using ImageJ (National Institute of Health, Bethesda, MD, USA), making sure that all of the leaves were flat on a surface. Leaf tissue was oven-dried to a constant mass at 70 °C for dry mass determination. Specific leaf area (SLA) was calculated by dividing the leaf area by dry mass; root mass was not measured. Samples for dry mass were ground, and concentrations of total Kjeldahl nitogen were measured by semi-automated colorimetry following procedures described by O'Dell [29] to compare the total shoot nitrogen content at the end of the experiment. Nitrogen uptake was calculated by multiplying the total shoot dry mass by tissue nitrogen content; the initial nitrogen content of the plants at week zero was subtracted from the final nitrogen content to get the total nitrogen uptake over the course of the experiment. Five samples of moist and dry substrate were weighed at the beginning and end of the experiment to calculate the water in the substrate, which was then subtracted from the total volume of water applied to calculate the total water used (mL). Water-use efficiency was calculated by dividing the total shoot dry mass by the total amount of water used per plant after transplanting. The substrate surface evaporative water flux was assumed to be zero.

2.4. Data Analysis

Three replications over time were conducted following the same procedures as previously described; all of the lighting treatments were re-randomized within the chamber before the start of each replication. All of the response variables were analyzed using generalized linear mixed models procedures, as implemented in SAS PROC GLIMMIX (SAS/STAT version 14.1; SAS Institute, Cary, NC, USA). Random effects for the model were experimental replication and its interaction with cultivar and treatment. Depending on the response variable, we used the normal (WUE, leaf area, and shoot dry mass), lognormal (g_s, SPAD index, SLA, and nitrogen uptake), or Poisson (leaf number) distribution function, each with its own canonical link function. The percent of blue light was modeled as a quantitative effect nested within each cultivar. Broad-spectrum light was dropped from this analysis in order to minimize confounding spectral effects from green light. In addition, because of the very different plant response to 0B compared to treatments from 7B to 100B, 0B was also excluded from this analysis; this is similar to the approach used by Hernández and Kubota and Hernández [20,30]. For each response variable, we evaluated both a linear and a quadratic fit; the latter was chosen as the appropriate model for leaf area, SLA, shoot dry mass, and SPAD index. Contrasts were then used to compare slopes for the cultivars. No contrast was significant at $P = 0.05$, and a model with a separate intercept for each cultivar and a common slope that was either linear or quadratic was chosen for most response variables, except for leaf area, for which a model with a single intercept and a single slope was deemed to be sufficient. To identify differences among individual treatments (including broad-band light and 0B), a two-way analysis of variance (ANOVA) was performed. Because the cultivar × treatment interaction was not significant ($P > 0.07$), pairwise comparisons for the main effect treatment means were completed using Tukey's test ($P = 0.05$).

3. Results

The regression line in all figures excludes data from broad-spectrum light and the 0B treatment (Figures 2 and 3). However, means for all treatments were included on all figures to provide a

reference for the response to blue light and to facilitate the development of an alternative hypothesis for the response.

3.1. Growth Parameters

The response of leaf area was similar to the response of SLA in that both parameters increased up to 66B, and slightly decreased or remained constant with 100B (Figure 2A,B). Pairwise comparisons indicated that lettuce plants produced from 25% to 55% larger leaves under 0B compared to the other treatments; no other treatment differences were measured for leaf area. Similarly, plants grown under 0B produced significantly thinner leaves (higher SLA) than those grown under 7B or 26B; no other treatment differences were measured for SLA. One less leaf was produced per plant for every 10% increase in blue light (Figure 2C). Pairwise comparisons indicated that plants grown under 7B or 26B produced from four to six more leaves than those grown under 66B or 100B; no significant differences in leaf number were measured among treatments with 42% or less blue light. Shoot dry mass decreased as blue levels increased up to 66%, which was followed by a slight increase in the 100B treatment (Figure 2D). Pairwise comparisons indicated that plants grown under 7B produced 46% or 43% more dry mass than those grown under 66B or 100B, respectively.

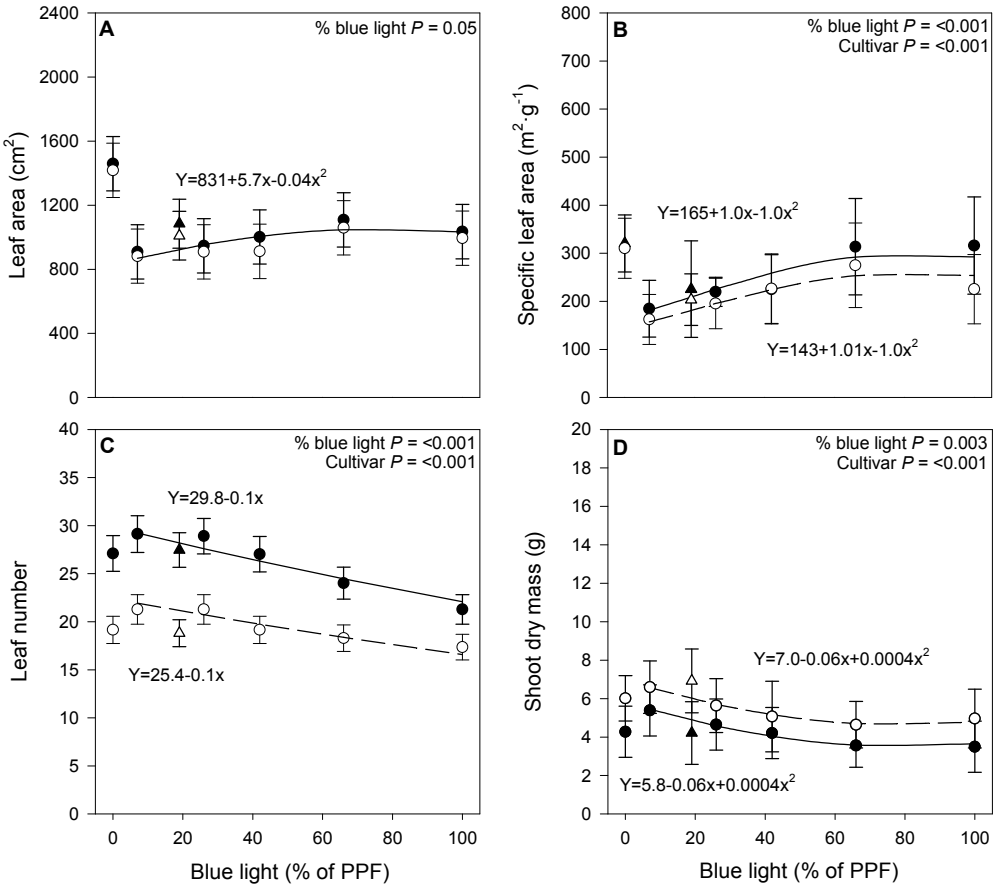

Figure 2. Effect of percent blue light on growth and morphology of 'Cherokee' (black symbols) and 'Waldmann's Green' (white symbols) lettuce grown under one of seven sole-source light-emitting diode (LED) treatments: 100% red (0% blue); 7% blue + 93% red; 26% blue + 74% red; 42% blue + 58% red; 66% blue + 34% red; 100% blue; or broad-spectrum light, which provided 19% blue, 43% green, and 38% red. Each data point shows the mean of three replicate studies for each cultivar (n = 3) ± SE. Regression lines exclude broad-spectrum light (triangles) in order to minimize confounding spectral effects; 0% blue light was also excluded from the regression analysis.

3.2. Physiological Responses

Water-use efficiency decreased in response to increasing blue light (Figure 3A). In addition, pairwise comparisons indicated that WUE was higher in plants grown under 0B or broad-spectrum light compared to 66B or 100B; no other treatment differences were measured for WUE. For every 10% increase in blue light, g_s increased by 10 mmol·m^{-2}·s^{-1} (Figure 3B). Pairwise comparisons indicated that g_s in plants grown under 100B was up to 87% higher relative to 0B or broad-spectrum light. Similarly, g_s in plants grown under 66B was 62% or 48% higher compared to 0B or broad-spectrum light, respectively; no differences in g_s were measured among treatments with 42% or less blue light. The response of SPAD index indicated an increase up to 66B, followed by a slight decrease with 100B (Figure 3C). Pairwise comparisons indicated that SPAD index was significantly higher in plants grown under 66B compared to 0B, 7B, and broad-spectrum light; among those three treatments, 0B had the lowest SPAD index. The response for nitrogen uptake was not significant, and no differences were measured among treatments (Figure 3D).

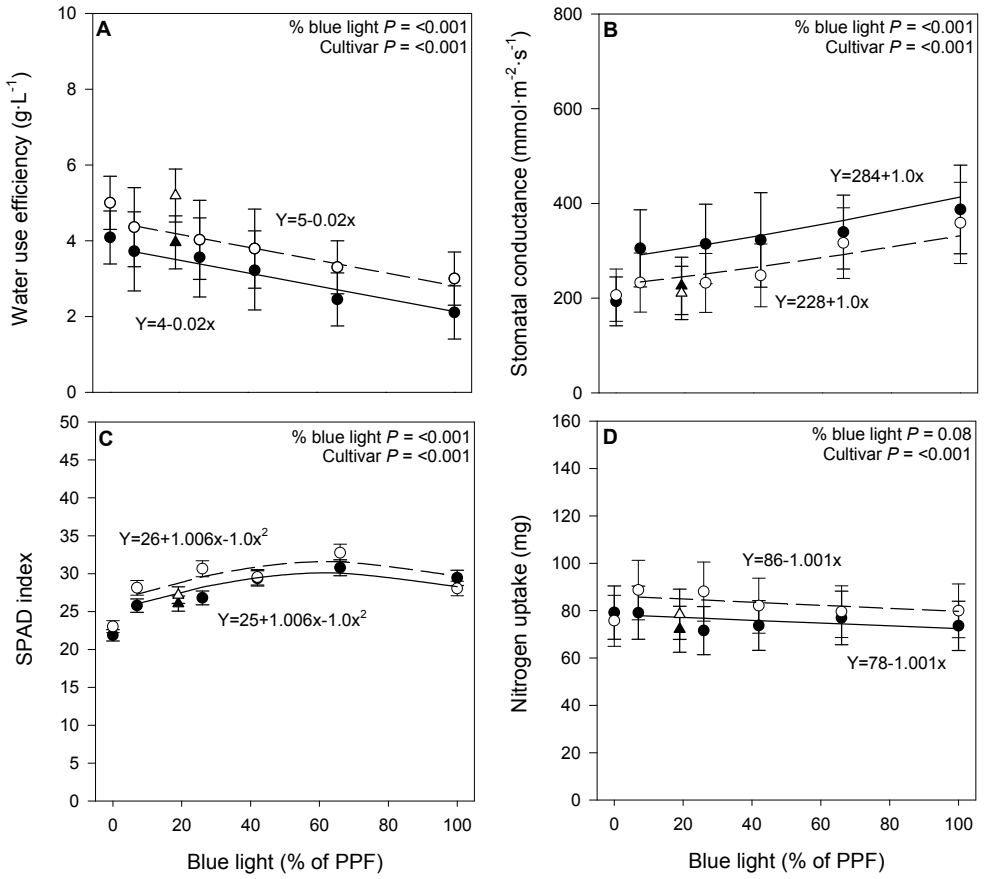

Figure 3. Effect of percent of blue light on physiological responses of 'Cherokee' (black symbols) and 'Waldmann's Green' (white symbols) lettuce grown under one of seven sole-source light-emitting diode (LED) treatments: 100% red (0% blue); 7% blue + 93% red; 26% blue + 74% red; 42% blue + 58% red; 66% blue + 34% red; 100% blue; or broad-spectrum light, which provided 19% blue, 43% green, and 38% red. Each data point shows the mean of three replicate studies for each cultivar (n = 3) ± SE. Regression lines exclude broad-spectrum light (triangles) in order to minimize confounding spectral effects; 0% blue light was also excluded from the regression analysis.

4. Discussion

4.1. Growth Parameters

Except for 0B, leaf area and SLA increased up to 66% blue light (Figure 2A,B). However, these responses to higher blue light do not correspond with the literature, which reports a blue light-induced reduction in lettuce leaf area and SLA [5,9]. Smaller, thicker leaves under higher blue light have been associated with a decrease in the size and number of leaf epidermal cells, which tend to reduce overall plant growth due to a reduction in radiation capture [3]. Although we did find a general growth (shoot dry mass) reduction in response to blue light, it cannot be attributed to a decrease in light interception from smaller, thicker leaver (Figure 2D). Instead, the linear reduction in leaf number in response to higher blue light could, to some extent, explain the response to shoot dry mass (Figure 2C). Others have reported similar reductions in leaf number in response to higher blue light [20,30–32].

Leaves were significantly larger under 0B (100% red light) compared to all other treatments (Figure 2A). Similar to our results, Son and Oh [33] reported larger lettuce leaves in plants grown under 100% red light compared to treatments with various red-to-blue photon flux ratios. Ohashi-Kaneko et al. [31] also found a higher leaf area and SLA of lettuce plants grown under red compared to blue light. Although larger, thinner leaves developed under 100% red light might increase radiation capture in some plants, monochromatic red light can induce a shade-avoidance response that has been shown to reduce photosynthetic rates in many plant species [10,12,19,34]. This shade-avoidance response has been associated with a lack of cryptochrome activation from the absence of blue light [35]. Accordingly, even when 100% red light resulted in the highest leaf area and shoot dry mass, Son and Oh [33] described lettuce plants to be chlorotic and etiolated. The suboptimal growth and development that is typically reported for plants grown under monochromatic red light might explain why in our study, although 0B produced the largest leaves, it did not result in the highest biomass production (Figure 2D).

4.2. Physiological Responses

We found a general decrease in WUE and increase in g_s in response to higher blue light (Figure 3A,B). To our knowledge, no other studies have quantified WUE in response to blue and red percentages based on harvested dry mass (as opposed to calculating WUE based on single-leaf gas exchange measurements). However, anecdotally, plants grown under blue-enriched light have been found to require more irrigation events than those grown under higher red light (C.A. Mitchell, personal communication).

Similar to our results, numerous studies have reported increases in steady-state g_s under high blue light (Figure 3B). Borowski et al. [36] reported higher g_s in lettuce grown under 12% to 21% blue compared to 100% red light. Wang et al. [37] also measured higher g_s in lettuce grown under low red-to-blue-light ratios compared to plants grown under red light only. Stomatal conductance is primarily determined by the number, size, and aperture of stomata [38]. Because stomatal development and behavior facilitate water vapor release via transpiration, changes in steady-state g_s in response to blue light might affect the trade-off between transpirational water loss and carbon gain in plants and therefore, could play a key role regulating WUE. Both blue and red light are known to stimulate stomatal opening, but the photoreceptors that control stomatal movements and turgor pressure in the guard cells depend on the stimulating waveband. Wang et al. [39] reported that phototropins and cryptochromes are responsible for blue light-induced stomatal opening, whereas phytochrome B drives the red light-induced response. Moreover, the higher quantum efficiency of blue over red light in stimulating stomatal aperture suggests that blue is the driving waveband to regulate the intracellular guard cell signaling that controls stomatal aperture [16]. Since aperture is one of the primary factors regulating g_s, blue light responses in stomatal aperture are likely to control the capacity of stomata to regulate conductance.

High g_s under high blue light has been positively correlated with an increase in stomatal index and density [12,22,23,25,27,28]. However, some studies have shown that developmental stomatal responses (e.g., index or density), thought to be regulated by cryptochrome-mediated changes, do not always correspond with g_s [17,40,41]. We did not measure stomatal development. Nonetheless, it is likely that the increase in g_s that was measured in our study is partially the result of an increase in the number of stomata, in addition to an increase in stomatal aperture in response to higher blue light.

When applied with blue light, green light has been shown to counteract blue-light mediated effects on g_s [42]. Therefore, the proportion of green light within broad-spectrum light might explain why g_s was significantly lower under broad-spectrum light relative to 66B or 100B, and correspondingly, WUE was significantly higher under broad-spectrum light relative to 66B or 100B (Figure 2A). In addition, monochromatic red light has been reported to result in dysfunctional stomata that are unresponsive to light quality, which might explain why the lowest g_s was measured on 0B [12].

Although the controlling mechanisms are unclear, stomata are water-conserving pores that regulate WUE through g_s. While stomatal control is not the only factor to regulate water flux through plants, short-term (and possibly long-term) stomatal responses to blue light are most likely responsible for the decreasing trend in WUE measured in our study under higher blue light (Figure 3A,B). When water availability and boundary layer resistance are not limiting, water loss by transpiration significantly increases under low leaf stomatal resistance, such as that present when stomatal pores are wide open [43,44]. This loss of water requires sufficient water uptake by plant roots to maintain a positive leaf water balance [23]. Furthermore, although we did not quantify leaf hydraulic conductance (K_{leaf}), which reflects the water flow through the leaf veins, across the mesophyll tissue, and through the stomatal pore, others have shown its relationship with blue light. Zheng and Van Labeke [25] showed that higher blue light increased K_{leaf} of ficus (*Ficus benjamina*) and gloxinia (*Sinningia speciosa*) compared to plants grown under 100% red light or broad-spectrum LEDs (7% blue). This is in agreement with van Ieperen et al. [23] and Savvides et al. [28], who reported that blue light increases K_{leaf} and g_s of cucumber (*Cucumis sativus*). Moreover, Schuerger et al. [45] reported an increase in the secondary xylem formation of pepper (*Capsicum annum*) plants in response to blue light, which directly affects the water flux through a plant and further indicates the impact that blue light has on plant–water relations. Finally, relative quantum efficiency curves indicate that blue light is up to 35% less efficient than red light in driving photosynthesis [8]. Therefore, it is likely that photosynthesis per unit of radiation capture decreased with increasing blue light, which could have negatively affected the WUE measured in our study.

An alternative hypothesis that could plausibly explain our measured increase of g_s and decrease in WUE in response to blue light could be related to the role that g_s and transpiration play in regulating leaf temperature (Figure 3B,D) [26]. Cope et al. [5] suggested that 20% of blue photons are absorbed by inactive pigments, possibly resulting in leaf warming without carbon gain. Urban et al. [46] recently found that the evaporative cooling of transpiring leaves increases with stomatal aperture and conductance in response to higher leaf temperature. Therefore, potential internal increases in leaf temperature driven by the inefficiency of leaves to use all of the absorbed blue photons for photochemistry might lead to excess water loss (relative to carbon gained) and effectively decrease WUE.

Our results show opposite trends between shoot dry mass and SPAD index (Figures 2D and 3C). Similar to our findings, others have reported a reduction in chlorophyll concentration with higher lettuce growth based on either non-destructive measurements of leaf greenness (e.g., SPAD index) or chlorophyll extraction [47–49]. The increase in SPAD index with higher blue light could be attributed to a reduction in plant growth, which has been shown to lead to an accumulation of higher chlorophyll content on a per-unit leaf mass basis and/or a potential increase in chlorophyll biosynthesis [9]. Sood et al. [50] reported a blue-light enhancement of chlorophyll biosynthesis via an increase in the tetrapyrrole precursor 5-aminolevulinic acid. In contrast, red light has been shown to downregulate the protein and gene expression of enzymes involved in chlorophyll biosynthesis [50,51]. Similar to

our findings for SPAD index, Son and Oh [49] reported a higher total chlorophyll content in 'Sunmang' and 'Grand Rapid TBR' lettuce grown under 7:3 or 8:2 compared to 9:1 red:blue ratios. The authors had also measured a significantly higher SPAD index in lettuce grown under 59%, 47%, or 35% blue light compared to 100% red [33]. However, Snowden et al. [9] reported minimal changes in chlorophyll content index (CCI) of lettuce leaves, but significant increases in CCI with higher blue light for tomato, cucumber, radish (*Raphanus sativus*), and pepper. They suggested that the increase in CCI with higher blue light can be attributed to the shade-avoidance syndrome, where plants exposed to higher blue light accumulate more chlorophyll in the leaves in order to optimize photosynthetic efficiency at low light intensities [9].

A number of studies have demonstrated that narrow-band LEDs can induce significant changes in the nutritional attributes of plants [1,2]. However, to our knowledge, no studies have referred to the effect that light quality has on nutrient uptake from a production–input perspective. Although shoot nitrogen content significantly increased in response to blue light (data not shown), there was no treatment effect on lettuce nitrogen uptake (Figure 3D). Hepworth et al. [52] reported a positive correlation between g_s (from an increase in stomatal density) and phosphate uptake capacity (from an increase in root growth). The authors had previously proposed that stomatal density upregulates nutrient accumulation via mass flow [53]. While it is likely that plants grown under higher blue light had higher stomatal density, our results do not indicate higher nitrogen uptake from an increase in g_s in response to blue light.

Our findings indicate that when all other environmental parameters are equal, the spectral quality from LEDs can significantly affect the volume of water that is required to produce lettuce plants in controlled environments. In addition, our findings suggest that lettuce grown under broad-spectrum LEDs with 19% blue and 43% green light have a higher WUE compared to plants grown under sole-source light sources with $\geq$66% blue light and no green light. It is possible that green light within broad-spectrum LEDs might counteract the potential increases in water uptake from increasing g_s in response to blue light. While searching for the optimal spectral quality for plant growth and development, careful consideration needs to be placed for balancing biomass production, plant morphology, product quality, and resource inputs.

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