Minor Spectral Shifts Modulate Morphology, Stomatal Physiology and Nutrient Yield in Culinary Herbs under Controlled Environment Agriculture

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ABSTRACT

Controlled environment agriculture (CEA) offers an unprecedented opportunity to adjust and custom light spectra for intentional enhanced growth and nutrient quality. In a 49-day experiment based on the NASA Growing Beyond Earth program, our hypothesis tested whether a slight increase in the blue and green fractions of light, an unintentional factor attributed to calibration error, would affect morphology, stomatal conductance, and estimated nutritional yield of three widely cultivated and medicated culinary herbs: Sweet Basil (*Ocimum basilicum 'Genovese'*), Cilantro (*Coriandrum sativum 'Cilantro Cruiser'*), and Chervil (*Anthriscus cerefolium 'Vertissimo'*). All plants were grown in five identically controlled environment chambers - four experimental chambers with slightly more blue (≈62.5 PPFD) and green (≈23.5 PPFD) photons and one control with a white light setting (≈121.1 PPFD green; ≈45.8 PPFD blue).

Plant height, width, and depth were measured over seven timepoints, and stomatal conductance (GSW) was measured for sweet basil and cilantro, however, not chervil due to leaf sensitivity to struct GSW was calculated as the mean of three fully expanded leaves sampled from the mid-canopy. In basil, measurements were taken from the central opposite leaf pair and one additional fully expanded leaf at the same nodal position. In cilantro, measurements were taken from the central leaves within the primary leaf cluster. GSW was measured at different times of day (day 46 at ~11:30 AM; days 44 and 49 at ~15:30), reflecting typical morning–afternoon changes in stomatal opening. Extreme outliers were addressed through winsorisation, and no additional GSW measurements were excluded. Following this, two replicate GSW readings per leaf were averaged, and values were subsequently averaged at the plant level. Using these processed data, significant species-specific differences in experimental response were observed. Sweet Basil showed modest reduction in height in the experimental spectral conditions, however, maintained constant values in canopy width and depth relative to the control. Cilantro experienced significant reductions across all three dimensions in experimental chambers, while chervil experienced a reduction in height, however, a relative increase in width across conditions in only one replicate. Morning stomatal conductance averaged 0.044–0.13 mol m^{-2} s^{-1} for basil and 0.13–0.28 mol m^{-2} s^{-1} for cilantro, exceeding afternoon values by 60–120 %. These values fell within the published ranges of reported values for both well-watered basil and cilantro under optimal conditions. Therefore, minor variations in the environment have the potential to lead to significant plant physiological changes. The modest spectral shift therefore influenced plant architecture and stomatal physiology in a species-dependent manner, with potential implications for nutrient yield and water-use efficiency. Our findings underscore the importance of routine spectral calibration in CEA systems and highlight the need to account for diurnal variation

when interpreting physiological measurements.

INTRODUCTION

Importance of light quality in controlled environment agriculture. Controlled environment agriculture (CEA) regulates temperature, humidity, nutrient supply, and light quality using complex systems, enabling year-round production of high-value crops (Matthews et al., 2019). One of the key characteristics influencing plant development is light quality, defined as the proportion of photons at different wavelengths within the photosynthetically active radiation spectrum (Bernardo et al., 2023). Blue light (400–500 nm) triggers phototropin-mediated stomatal opening and plays a key role in photomorphogenesis (Shimazaki et al., 2007), while red light (600–700 nm) is central to photosynthetic electron transport and carbon fixation (Matthews et al., 2019). Green light penetrates deeper into plant canopies than red or blue light, influencing leaf expansion and shade-avoidance responses (Larsen et al., 2020). Although many LED studies evaluate large contrasts in red-to-blue ratios, minor spectral shifts can occur due to calibration drift or fixture aging (Nelson and Bugbee, 2014). Such subtle changes may have disproportionate effects on plant physiology, and understanding these effects is critical for optimizing energy efficiency and nutrient yield in CEA systems (Bugbee, 2016).

Medicinal significance of the studied herbs. While culinary herbs are valued for their aroma and flavor, many are also recognized for the presence of pharmacologically active compounds. This study focused on three culinary herbs because each is now being grown indoors using CEA.

Sweet basil (Ocimum basilicum 'Genovese'). Basil contains abundant phenolic compounds and essential oils that exhibit antibacterial, anti-inflammatory, and antioxidant properties (Carović-Stanko et al., 2010). The phytochemical composition of basil is sensitive to environmental conditions, including light quality, with spectral composition influencing concentrations of health-relevant compounds (Lee and Scagel, 2009). As these chemical changes may alter both nutritional and therapeutic value, understanding basil's response to subtle spectral variation is of particular interest.

Cilantro (Coriandrum sativum 'Cilantro Cruiser'). Cilantro leaves and seeds are rich in antioxidants and essential oils (Wangensteen et al., 2004). The bioactivity of cilantro is attributed to compounds such as phenolic acids, flavonoids, and linalool, which contribute to antimicrobial and anti-inflammatory effects (Laribi et al., 2015). Due to the plant's delicate leaf structure and high surface-area-to-volume ratio, cilantro may be especially sensitive to changes in light spectrum that affect stomatal behavior and transpiration.

Chervil (Anthriscus cerefolium 'Vertissimo'). Chervil is a lesser-studied herb related to parsley and is nutritionally rich in vitamins A, C, and K, as well as calcium, iron, and potassium (Vyas et al., 2012). Polyphenols and flavonoids in chervil exhibit antioxidant activity, contributing to reduced oxidative stress. Because chervil has not been widely studied under controlled spectral treatments, investigating the plant's response to spectral variation provides novel insight into its cultivation for nutritional benefit.

Previous research and knowledge gaps. Previous controlled environment studies have largely examined large changes in red and blue light fractions to optimize biomass or manipulate morphology. Red-dominant spectra tend to increase biomass accumulation, whereas higher blue fractions reduce stem elongation while increasing leaf thickness and width (Hogewoning et al., 2010). However, less is known about the physiological effects of small spectral adjustments. Even modest increases in blue light can influence stomatal conductance and water-use efficiency due to the sensitivity of guard-cell photoreceptors (Kalaitzoglou et al., 2021). Similarly, green light, though less efficiently absorbed, can promote growth by penetrating the canopy and modifying internal light distribution (Terashima et al., 2009).

Because plant morphology and gas-exchange responses are species-specific, limited research has examined how subtle spectral rebalancing affects widely cultivated culinary herbs. This pilot study begins to address this gap by evaluating the morphological and gas-exchange responses of three ethnobotanically significant herbs to small increases in blue and green light under controlled conditions (Bugbee, 2016).

MATERIALS AND METHODS

Chamber configuration and spectral treatments. The experiment was conducted in five identical controlled environment chambers (internal dimensions 60 × 45 × 55 cm) lined with reflective Mylar to maximize uniform light distribution. Each chamber contained a programmable LED panel with independently adjustable red, blue, green, and white diodes spanning 400–700 nm. Experimental chambers were set to deliver approximately 100 μmol m^{-2} s^{-1} red, 100 μmol m^{-2} s^{-1} blue, and 90 μmol m^{-2} s^{-1} white photons, while the control chamber delivered approximately 157 μmol m^{-2} s^{-1} white photons. Quantum sensor measurements (LI-180, LI-COR Biosciences) taken at canopy level at the end of the experiment confirmed that experimental chambers received 62.51 μmol m^{-2} s^{-1} blue and 23.54 μmol m^{-2} s^{-1} green photons, while the control chamber emitted 45.8 μmol m^{-2} s^{-1} blue and 121.1 μmol m^{-2} s^{-1} green photons. Red photon flux was slightly lower than intended in both treatments. A 12-h photoperiod (7:00 AM–7:00 PM) was maintained.

Plant material and cultivation. Seeds were planted in 10 cm plastic pots filled with a 3:1 mixture of compressed coconut coir and Nutricote T180 (18–6–8 NPK) slow-release fertilizer. For each species, three experimental pots and one control pot were used, yielding twelve pots total. Pots were distributed across chambers such that each chamber contained one pot of each species to minimize chamber effects. Ten seeds were initially planted per pot; following germination, seedlings were thinned via randomized culling to three plants per pot for basil and cilantro and five for chervil. Pots were placed on a capillary mat wicking system and supplied with reverse-osmosis water as needed. Ambient temperature was maintained at 24–25 °C with relative humidity between 50–65%.

Phenotypic data collection. Plant height, canopy width, and canopy depth were measured weekly using a digital caliper (±1 mm) at 4:00 PM to standardize diurnal variation. Height was defined as the distance from the substrate surface to the tallest leaf tip, width as the maximum horizontal spread, and depth as the maximum front-to-back extension. Measurements were recorded on days 7, 14, 21, 35, 42, and 49. Day 0 measurements were recorded as 0 cm. Final fresh biomass was harvested on day 50 by cutting aboveground tissue at the substrate line and weighing on a precision balance (±0.01 g).

Gas-exchange and Stomatal Conductance Measurements. Stomatal conductance to water vapor (GSW) was measured on days 44, 46, and 49 using an LI-600 Portable Photosynthesis System. Measurements were taken on fully expanded leaves, with readings on day 46 collected in the morning and the other two dates in the afternoon to provide a temporal snapshot of conductance. In addition to GSW, the second derivative GSW^2/s was recorded. Environmental and instrument parameters, including flow rate (μmol s^{-1}), ambient CO_2 concentration (Qamb), leaf temperature (Tleaf), reference temperature (Tref), and relative humidity at the leaf surface and in the chamber (RH_r and RH_s), were also documented for each measurement. All raw GSW values are reported in mol m^{-2} s^{-1}.

Data cleaning and statistical analysis. Negative GSW values and values exceeding physiologically plausible limits were removed or winsorized based on manufacturer guidance (< 1 mol m^{-2} s^{-1}; "LI-6400/XT Portable...", n.d.). Outliers were treated using IQR-based winsorisation to retain biologically reasonable maxima (cilantro control = 0.2405 mol m^{-2} s^{-1}; basil control = 0.132 mol m^{-2} s^{-1}; basil experimental 1 = 0.1345 mol m^{-2} s^{-1}) (Wilcox, 2013).

Replicate trimming. After winsorisation was applied, two values per plant per date were kept reducing intra-plant variability. For each plant on each date, the three leaf measurements were arranged in order of absolute deviation from that plant's median GSW, and the two values closest to the median were kept. This procedure yields six GSW values per species–treatment group per date (two leaves × three plants). For cilantro, which had only two leaves per plant, all winsorised values were retained. The retained GSW values used for statistical analyses (Table 1).

Table 1: Retained GSW values (mol m^{-2} s^{-1}) after winsorisation and trimming. Up to six values were retained for each species-treatment-date combination (two leaves per plant, three plants), however, fewer values are shown when measurements were missing or excluded. Values exceeding predetermined thresholds were capped (winsorised) at 0.132 mol m^{-2} s^{-1} for basil control, 0.1345 mol m^{-2} s^{-1} for basil experimental 1, 0.20325 mol m^{-2} s^{-1} for basil experimental 2 and 0.2405 mol m^{-2} s^{-1} for all cilantro conditions.

Species	Treatment	Date	Time of Day	Retained GSW Values (mol m^{-2} s^{-1})
Cilantro	Control	12/6	Afternoon	0.098, 0.095, 0.101, 0.083, 0.118, 0.075
		12/8	Morning	0.2405, 0.2405, 0.061, 0.165, 0.099, 0.075
		12/11	Afternoon	0.059, 0.2405, 0.052, 0.108, 0.079, 0.024
Cilantro	Experimental 1	12/6	Afternoon	0.141, 0.154, 0.155, 0.134, 0.175, 0.191
		12/8	Morning	0.2405, 0.109, 0.2405, 0.2405, 0.2405, 0.2405
		12/11	Afternoon	0.093, 0.203, 0.198, 0.221, 0.2405, 0.2405
Cilantro	Experimental 2	12/6	Afternoon	0.112, 0.126, 0.134, 0.142, 0.150, 0.161
		12/8	Morning	0.189, 0.2405, 0.2405
		12/11	Afternoon	0.156, 0.224, 0.134, 0.131
Sweet Basil	Control	12/6	Afternoon	0.055, 0.046, 0.046, 0.045, 0.045, 0.039
		12/8	Morning	0.042, 0.043, 0.038, 0.034, 0.033, 0.075
		12/11	Afternoon	0.049, 0.049, 0.050, 0.045, 0.058, 0.062
Sweet Basil	Experimental 1	-	-	Data excluded due to anomalously low and variable values
Sweet Basil	Experimental 2	12/6	Afternoon	0.075, 0.075, 0.070, 0.080, 0.064, 0.063
		12/8	Morning	0.129, 0.113, 0.151, 0.164, 0.094, 0.174
		12/11	Afternoon	0.076, 0.065, 0.057, 0.097, 0.110, 0.039

Morphological traits. Morphological traits were analyzed using two-way ANOVA with treatment and week as actors for each species independently (Quinn and Keough, 2002). Where significant effects were detected, Tukey's HSD was applied (Tukey, 1949). Gas-exchange data were analyzed using two-way ANOVA where assumptions were met, verified by Shapiro–Wilk and Levene's tests (Levene, 1960; Shapiro and Wilk, 1965). Non-parametric Kruskal–Wallis tests were used when assumptions were violated (McDonald, 2014). All analyses were conducted in Python 3.11 using pandas for data manipulation and SciPy/statsmodels for ANOVA. Graphs were generated with matplotlib and seaborn.

RESULTS AND DISCUSSION

Baseline growth. The overall germination time of all species was 5-7 days and there was a strong growth of seedlings. The height, width and depth of the control plants were virtually zero in the first week since day 0 is the baseline as determined by the protocol. The control basil (week 2) had a height of 1.95 ± 0.11 cm, width of 3.15 ± 0.22 cm and depth of 2.30 ± 0.12 cm (mean ± SD) and the cilantro and chervil were quite similar. At week 2, no significant difference was realized between the treatments.

Sweet basil. Basil grew steadily for seven weeks; however, the spectral shift decreased plant growth. Control chervil reached a maximum height of 8.63 cm, while Experimental 1 and 2 reached heights of 7.63 cm and 7.19 cm, respectively (Table 2). Experimental 1 was shorter than the control by about 6%, and Experimental 2 was shorter by 16%. Canopy width was also reduced, with the control at 10.91 cm compared to 10.56 cm (-3.2%) in Experimental 1 and 10.63 cm (-3.1%) in Experimental 2. Canopy depth showed smaller differences, with 10.56 cm (+47.8%) in Experimental 1 and 8.56 cm (+19.8%) in Experimental 2. These data indicate that the blue/green light reduced vertical growth while allowing for lateral expansion, potentially due to leaf curling or petiole bending (Hogewoning et al., 2010; Matthews et al., 2019).

Cilantro cruiser. Cilantro was most responsive to spectral change. The control averaged final height of 12.15 cm, while experimental plants were 7.63 cm (-37.2%) and 7.32 cm (-39.8%), respectively. Experimental canopy width decreased from 13.44 cm in the control to 8.68 cm and 8.81 cm in experimental 1 and experimental 2, respectively (-35.4% and –34.4%). Depth also decreased from 11.58 cm in the control to 7.27 cm (-37.2%) and 8.81 cm (-23.9%). These reductions translate into visibly smaller, more compact canopies (Figure 1). Cilantro stems are thinner, so this reduction in growth is likely due to a reduction of cell expansion taking place during low red:green ratios, however, blue light integrity allows for non-elongation via cryptochrome signaling (Azizi et al., 2025).

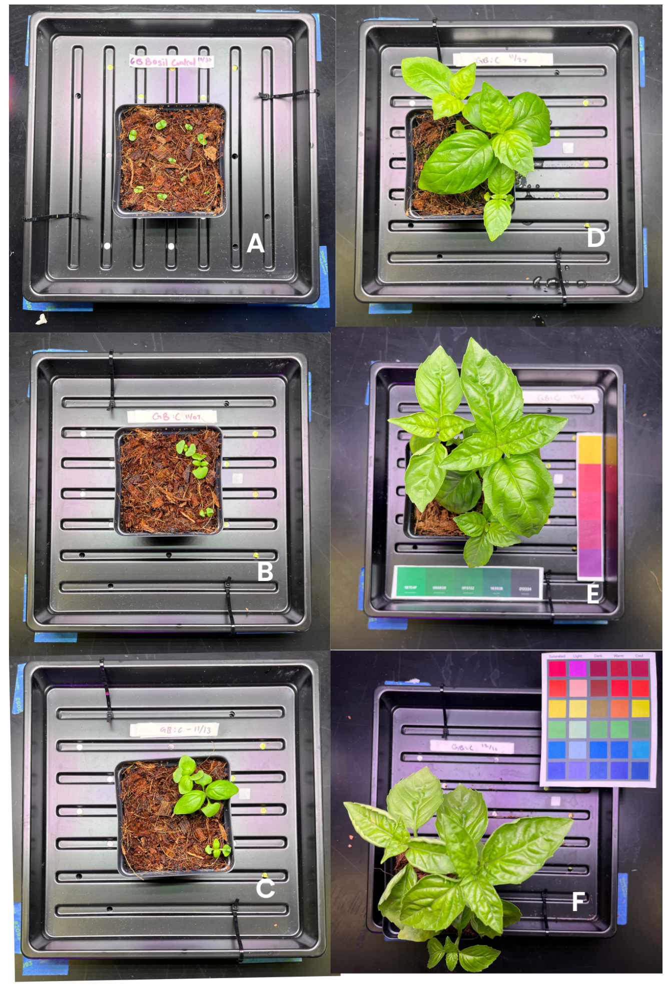

Figure 1: Growth progression of a single sweet basil control pot over the experimental period. Panels A–F show weekly images from week 1 through week 7, with week 4 (day 28) omitted due to facility recess as mentioned. The images illustrate plant development under the growth apparatus used in the study.

Chervil vertissimo. Chervil exhibited a mixed response to the spectral shift. Control plants reached 8.63 cm in height, 10.91 cm in width, and 13.02 cm in depth. Experimental 1 plants were shorter at 6.40 cm (−25.9%) but were notably wider at 13.31 cm (+22.0%), suggesting that increased green photons promoted horizontal leaf expansion. Experimental 2 plants reached 7.19 cm (−16.7%) in height and 10.56

cm (−3.2%) in width. Depth decreased to 10.37 cm (−20.4%) in Experimental 1 and 10.83 cm (−16.8%) in Experimental 2. The response of chervil varied due to the plant's feathered leaves and phototropism; green photons penetrated the canopy better than blue photons, allowing for an increase in lateral development (Azizi et al., 2025).

Weekly growth dynamics and relative growth rates. To analyze growth patterns more integrally, we measured growth in height, width, and depth week to week. Between days 0 and 14, cilantro control increased by an additional 3.11 cm (days 0 to 7) and an additional 1.61 cm (days 7–14), while the basil control increased 1.06 cm and 0.89 cm, respectively. After day 21, growth patterns significantly decrease for all plants, with the greatest increases occurring during the recovery period from holiday (days 21–35); for cilantro and basil, with a spectral change, growth occurred at even lower levels relative to the control in the final 2 weeks, as expected based on compounded growth inhibition. Relative growth rates (RGR) between measurements were assessed by measuring growth by the natural log of relative size per day; for example, the cilantro control had an RGR of 0.06 day^{-1} measured between measurements (days 7 and 14) and an RGR of 0.019 day^{-1} between measurements (days 42 and 49) (Hay and Porter, 2006). This measurement shows a decrease in gradually measured growth; this pattern also occurred for basil experimental 2, which had an early measurement RGR of 0.065 day^{-1} and a late measurement RGR of 0.006 day^{-1}, strongly inhibited. These measurements show how over time, consistent measurement patterns suggest small differences when combined over cumulative time create significant differences in endpoints.

Canopy architecture and shape ratios. In addition to measurements of space, relative plant shape can be determined by canopy shape, in which width/height and depth/height ratios correspond to potential light acquisition, shape efficiency, and growth favorability (Matthews et al., 2019).

For cilantro, the control treatment width:height ratio was approximately 1.1, however, the experimental 2 appeared wider, at 1.20, which suggests experimental plants had the same shape, however, gained width relatively; for basil, the control had a width:height ratio of 1.13 as anticipated, however, for experimental 1, basil plants had a decreased ratio (0.82), which is typical as width to height is decreased to a greater extent relative to width (Table 3). Depth:height ratios showed that cilantro experimental 2 had a value of 1.20, nearly a perfect cube, while basil control plants had a ratio of 0.58, reflecting their columnar habit. Chervil experimental 1 displayed the most extreme architecture, with a width:height ratio of 2.08 and depth:height ratio of 1.62, indicating a sprawling, shallow canopy (Table 3). These differences in canopy shape have implications for intra-canopy light distribution, microclimate and nutrient allocation (Azizi et al., 2025).

Stomatal conductance. Average GSW differed across measurement times (Table 4). Conductance measured in the morning on Day 46 was 60–120% higher than measurements taken in the afternoon on Days 44 and 49. Because measurements were collected at single time points on separate days rather than multiple time points within the same day, these data serve as a baseline comparison of stomatal conductance across treatments rather than a direct evaluation of diurnal dynamics. The observed differences align with established physiological patterns of higher morning stomatal opening, which typically facilitates greater carbon assimilation early in the day. However, to explicitly quantify diurnal variation and temporal stomatal behavior, controlled measurements taken multiple times within the same day would be required (Pou et al., 2014).

Sweet basil. Stomatal conductance readings for basil in Experimental 1 were near zero with high standard deviation, likely due to sensor re-sealing or removal from leaf injury. Because these values were far below the baseline and literature range (0.05–0.20 mol m^{-2} s^{-1}), they were excluded from the findings (Matthews et al., 2019). The morning GSW values were 0.044 mol m^{-2} s^{-1} in the control and 0.138 mol m^{-2} s^{-1} in Experimental 2 (Table 4), with afternoon

Table 2. Final week canopy dimensions (day 49) for each species and treatment. Values are means for three pots; standard deviations were not calculated because individual pot measurements were averaged in the original dataset. Percentage differences relative to the control illustrate the magnitude of change.

Species	Treatment	Height (cm)	Height Δ (%)	Width (cm)	Width Δ (%)	Depth (cm)	Depth Δ (%)
Sweet Basil	Control	12.40	-	13.99	-	7.14	-
	Experimental 1	11.61	–6.4%	9.52	–31.9 %	10.56	+47.8 %
	Experimental 2	9.92	–20.0 %	11.37	–18.7 %	8.56	+19.8 %
Cilantro	Control	12.15	-	13.44	-	11.58	-
	Experimental 1	7.63	–37.2 %	8.68	–35.4 %	7.27	–37.2 %
	Experimental 2	7.32	–39.8 %	8.81	–34.4 %	8.81	–23.9 %
Chervil	Control	8.63	-	10.91	-	13.02	-
	Experimental 1	6.40	–25.9 %	13.31	+22.0 %	10.37	–20.4 %
	Experimental 2	7.19	–16.7 %	10.56	–3.2 %	10.83	–16.8 %

means of 0.049 and 0.073 mol m^{-2} s^{-1}, respectively. The spectral shift effectively increased morning stomatal opening in basil, with little impact in the afternoon (Shimazaki et al., 2007).

Table 3. Canopy architecture ratios at final harvest (day 49). Exp. 1 refers to Experimental 1 while Exp. 2 refers to Experimental 2. Ratios greater than 1.0 indicate that the canopy is wider or deeper than canopy height.

Species	Treatment	Width/Height (cm:cm)	Depth/Height (cm:cm)
Sweet Basil	Control	1.13	0.58
	Exp. 1	0.82	0.91
	Exp. 2	1.15	0.86
Cilantro	Control	1.11	0.95
	Exp. 1	1.14	0.95
	Exp. 2	1.20	1.20
Chervil	Control	1.26	1.51
	Exp. 1	2.08	1.62
	Exp. 2	1.47	1.51

Cilantro cruiser. The means for cilantro were as follows: the control was 0.147 mol m^{-2} s^{-1} in the morning; experimental 1 was 0.219 mol m^{-2} s^{-1}; experimental 2 was 0.223 mol m^{-2} s^{-1}. In the afternoon, means dropped to 0.094 mol m^{-2} s^{-1} for control; 0.179 mol m^{-2} s^{-1} for experimental 1; and 0.103 mol m^{-2} s^{-1} for experimental 2. The treatment in question was significant ($p < 0.01$) irrespective of time of day. The expected GSW range for cilantro is 0.206–0.219 mol m^{-2} s^{-1} (Matthews et al., 2019). Thus, the GSW for the expected range supports the findings against expected literature numbers. The experimental treatments were higher than expected, likely due to increased blue light intensity at the treatment level increasing phototropin stimulation (Shimazaki et al., 2007; Kalaitzoglou et al., 2021).

Table 4. Mean GSW (mol m^{-2} s^{-1}) of each species and treatment group after trimming. Exp. 1 refers to Experimental 1 while Exp. 2 refers to Experimental 2. Each bar represents six leaves per species of each treatment group (two leaves per plant, three plants). Morning data were taken at 11:30 AM (day 46) while afternoon data were taken at 3:30 PM (days 44 and 49).

Species	Treatment	Morning GSW (mol m^{-2} s^{-1})	Afternoon GSW (mol m^{-2} s^{-1})
Sweet Basil	Control	0.044	0.049
	Exp. 2	0.138	0.073
Cilantro	Control	0.147	0.094
	Exp. 1	0.219	0.179
	Exp. 2	0.223	0.149

Chervil. Gas-exchange measurements were not possible for chervil due to the plant's finely dissected leaves, which could not be secured in the LI-600 chamber.

Final week biomass results. The plants were harvested on day 50 for biomass collection, and leaf mass per pot was recorded for each treatment group (Table 5). For Sweet basil, the control pots yielded an average of 3.53 g of leaves, while Experimental 1 pots produced 4.40 g (+24.5%) and Experimental 2 pots produced 2.80 g (-20.8%). Cilantro control pots averaged 2.87 g, compared with 1.10 g (-61.6%) in Experimental 1 and 1.93 g (-32.6%) in Experimental 2. Chervil control pots had 1.24 g, Experimental 1 reached 1.48 g (+19.0%), and Experimental 2 had 1.30 g (+4.6%). These adjusted biomass values indicate that the blue/green spectral shift promoted weight accumulation in basil and chervil under Experimental 1 conditions, while decreasing leaf biomass in basil Experimental 2 and in both cilantro experimental groups. Overall, the observed differences reflect species-specific tolerance and adaptive responses to the spectral treatments, providing insight into how these herbs acclimate to changes in light quality (Hogewoning et al., 2010).

Table 5. The table shows the mean biomass of each species-treatment group with the percentage change relative to the control. Exp. 1 refers to Experimental 1 while Exp. 2 refers to Experimental 2. Positive change indicates an increase relative to the control; negative values indicate a decrease.

Species	Treatment	Biomass (g)	Percent Change (%)
Sweet Basil	Control	3.53	-
	Exp. 1	4.40	+24.65%
	Exp. 2	3.03	−14.16%
Cilantro	Control	2.87	-
	Exp. 1	1.40	−51.22%
	Exp. 2	1.93	−32.75%
Chervil	Control	1.24	-
	Exp. 1	1.48	+19.35%
	Exp. 2	1.38	+11.29%

Interpretation of significance. Repeated-measures ANOVA revealed that treatment significantly affected Sweet basil and cilantro morphology, however, had little effect on chervil. For Sweet basil, treatment influenced height ($F(2,30)=15.05$, $p<0.001$), width ($F(2,30)=4.50$, $p=0.020$), and depth ($F(2,30)=4.99$, $p=0.013$). Cilantro exhibited strong treatment effects on all traits, with height ($F(2,30)=24.89$, $p<0.001$), width ($F(2,30)=37.98$, $p<0.001$), and depth ($F(2,30)=17.42$, $p<0.001$) significantly altered by the spectral treatments. In contrast, chervil showed no significant treatment effects for height ($F(2,30)=2.79$, $p=0.078$), width ($F(2,30)=0.73$, $p=0.489$), or depth ($F(2,29)=0.016$, $p=0.901$), indicating that the plant's morphology was largely unaffected by the experimental light conditions.

Stomatal conductance (GSW) was analyzed using a repeated-measures mixed model with plant as a random effect and Treatment and Time of Day as fixed effects. For cilantro, treatment significantly affected GSW ($p=0.0415$), and time of day also had a strong effect ($p=0.00625$), while the Treatment × Time-of-Day interaction was not significant ($p=0.41$), indicating consistent diurnal variation, while treatment responses remained similar across morning and afternoon measurements. For Sweet basil (Control vs. Experimental 2; Experimental 1 excluded due to outlier issues), treatment significantly increased GSW ($p=0.00011$), time of day had a strong effect ($p=0.00009$), and the Treatment × Time-of-Day interaction was also significant ($p=0.00045$), demonstrating that the spectral shift induced higher stomatal opening in the morning compared with the afternoon. These results support the presence of strong diurnal differences and treatment-linked stomatal responses, particularly in basil.

Species-specific morphological responses. Our results indicate that an increase in blue and green light has real effects on herb morphology, yet subsequent magnitude and directional response is species-specific (Hogewoning et al., 2010). Cilantro had a considerable dwarfing phenotype from the spectral shift, with reductions of 35–40% in height, width, and depth (Table 2). Basil showed only a mild reduction in height (up to 28%) with stabilized or increased canopy depth under experiment 1, meaning leaf curling or lateral expansion. Chervil reduced height from baseline yet achieved wider canopies in one replicate. This is likely due to the presence and sensitivity of photoreceptors (Matthews et al., 2019).

Both basil and cilantro are C3 herbs with larger leaves, however, basil has more substantial stem tissue which may counteract the avoidance of stem elongation. Cilantro's finer stem structure and greater surface-area-to-volume ratio of leaves may be more conducive to blue-light–induced inhibition of expansion (Hogewoning et al., 2010). This is also true for chervil, since with leaves that are feathered, the additional stimulation of green light penetrated the canopy more than vertical elongation would facilitate control; thus, increased experimental results for width compared to control (Azizi et al., 2025).

Our results align with previous findings that high fractions of blue light effectively inhibit stem elongation while enhancing leaf thickness (Hogewoning et al., 2010). However, previous investigations assessed most results under major shifts of spectrum; our investigation shows that an increase of ≈17 µmol m^{-2} s^{-1} blue compounded with ≈−100 µmol m^{-2} s^{-1} green (relative to control) constitutes major morphological differences for sensitive plants (namely cilantro) (Kalaitzoglou et al., 2021). Thus, this substantiates calibrated LED fixtures needing frequent monitoring and adjustment - what may seem like slight shifts can drastically alter what remains of yield.

Stomatal physiology and diurnal variation. Stomatal conductance is known to respond rapidly to changes in light quality and intensity (Matthews et al., 2019). In particular, phototropin receptors activated by blue light promote stomatal opening through H^+-ATPase stimulation and guard cell swelling (Shimazaki et al., 2007). Accordingly, the higher GSW values observed during morning measurements are consistent with established physiological responses reported for basil and cilantro under higher blue-light availability (Pou et al., 2014).

Pre-treatment stomatal conductance for the control plants was 0.0491 mol m^{-2} s^{-1} for basil and 0.0827 mol m^{-2} s^{-1} for cilantro were lower than reported ranges for hydroponic sweet basil (0.052–0.151 mol m^{-2} s^{-1}) and average coriander (0.206 mol m^{-2} s^{-1}), potentially reflecting differences in lighting exposure (Matthews et al., 2019). However, morning GSW readings approached the upper range of literature-reported values, with cilantro in Experiment 1 reaching 0.2567 mol m^{-2} s^{-1}. While these observations cannot be used to directly quantify diurnal stomatal regulation, they suggest that the blue–green spectral shift was associated with elevated stomatal conductance under morning measurement conditions.

Increased stomatal opening would facilitate greater CO_2 uptake and increase transpiration and water loss (Kalaitzoglou et al., 2021). Because plants were continuously irrigated throughout the experiment, this increase did not impose water limitation; however, under low-resource conditions, similar responses could substantially reduce water-use efficiency (Matthews et al., 2019).

Notably, substantial variation in GSW was observed between morning and afternoon measurement days. Because measurements were collected at single time points on different days rather than repeatedly within the same day, time-of-day effects could not be isolated from day-to-day variability. Future studies should standardize measurement timing or implement same-day time-course sampling to prevent temporal effects from obscuring treatment responses.

Nutrient yield implications. Fresh biomass is associated with nutrient yield, which was reduced in both cases under spectral shift, primarily with cilantro (Hay and Porter, 2006). Considering that herbs are grown predominantly for foliage, reduced canopies equate to reduced edible yield. Thus, while reducing basil (very marginal, as hypothesized) in means was observed, potential increases in secondary metabolite exposure from blue light could offset reductions in biomass; exposure to blue light has been linked to essential oil percentage increase and phenolic concentration (Peng et al., 2024). Cilantro was reduced by almost 40% in means, which likely reduces phytochemical and nutrient yield as well; yield for chervil could not be determined, though morphological reduction suggests decreased biomass. Therefore, minor changes in spectral setup could inhibit commercial systems where nutritional yield and biomass for sale are critical.

Managerial contributions: winsorisation, cleaning up of the data. Our pipeline demonstrates a good base of variable data regarding gas-exchange. By removing negative values and winsorising extreme high outliers at physiologically realistic values (to be taken as the IQR), we avoided discarding entire replicates while reducing the undue influence of sensor artefacts. Refining the list of the leaf replicas to the two values closest to the median of each plant further smoothed the variability without any valuable data being lost. Notably here, we retained six measurements per species-treatment-date and maintained replication. Such an approach may be applicable in determining future gas exchange of herbs or other small leaf crops.

ANOVA. Species showed distinct responses to the spectral treatments. Cilantro exhibited the strongest treatment effects on height, width, and depth, while basil showed moderate effects, especially in height and depth. Chervil was largely unaffected, except for depth, which responded subtly. Stomatal conductance mirrored these patterns: cilantro showed significant treatment and time-of-day effects ($p = 0.0415$ and $p = 0.0063$, respectively), while basil had highly significant effects for treatment, time of day, and their interaction ($p < 0.001$ for all), indicating enhanced morning stomatal opening under the blue/green shift. These results highlight species-specific morphological and physiological sensitivity to light quality, and the importance of accounting for diurnal variation in controlled environment experiments.

Future directions and limitations. First, the spectral shift amplified both blue and green photons, and because blue light is expected to drive stronger responses, the simultaneous reduction in the green might have also contributed to photosynthesis and the shape of plants. In future, blue and green fractions need to be tweaked separately, de-correlating the effects between them. Second, we had a small sample (three pots per treatment) and we could not measure chervil GSW. Sampler sizes would enable us to have more statistical power with bigger sample sizes and improve the variability of GSW sensors of tiny leaves. Third, we did not measure secondary metabolites or nutrition on a direct basis. As sweet basil and cilantro have been used for health considerations, the quantification of essential oils and flavonoids as they impact taste as well as vitamins in various spectra should be done in future studies to observe the changes brought by the shift in the phytochemical composition. Lastly, we did the experiment over a 49-day period, exploring the effects of shorter term, micro-green like plants; longer periods involving flowering and reproductive phases may show more effects. These limitations emphasize the need for multi-factor, longer-term CEA studies that integrate morphology, physiology, and biochemical outcomes.

Direct implications in the controlled environment agriculture and medicinal plants production. Controlled-environment agriculture allows us to finely manipulate variables; however, our results indicate that even small spectral variations may substantially influence plant morphology, stomatal behavior, and yield. In high-value medicinal herbs, such as basil, cilantro, and chervil, both biomass and phytochemicals define value, making it therefore important to maintain correct spectra. The findings highlight the importance of regular calibration on LED devices and of spectral sensors that monitor real-time PPFD at specific wavelengths. In addition, it is important to take into consideration the time of day at which gas-exchange measurements are performed so that the interpretation of these measurements is accurate. This work provides a roadmap on how to probe the subtle spectral properties in CEA systems.

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