Stomatal Conductance of Lettuce Grown Under or Exposed to Different Light Qualities

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● *Background and Aims* The objective of this research was to examine the effects of differences in light spectrum on the stomatal conductance (G_s) and dry matter production of lettuce plants grown under a day/night cycle with different spectra, and also the effects on G_s of short-term exposure to different spectra.
● *Methods* Lettuce (*Lactuca sativa*) plants were grown with 6 h dark and 18 h light under four different spectra, red–blue (RB), red–blue–green (RBG), green (GF) and white (CWF), and G_s and plant growth were measured.
● *Key Results and Conclusions* Conductance of plants grown for 23 d under CWF rose rapidly on illumination to a maximum in the middle of the light period, then decreased again before the dark period when it was minimal. However, the maximum was smaller in plants grown under RB, RGB and GF. This demonstrates that spectral quality during growth affects the diurnal pattern of stomatal conductance. Although G_s was smaller in plants grown under RGB than CWF, dry mass accumulation was greater, suggesting that G_s did not limit carbon assimilation under these spectral conditions. Temporarily changing the spectral quality of the plants grown for 23 d under CWF, affected stomatal responses reversibly, confirming studies on epidermal strips. This study provides new information showing that G_s is responsive to spectral quality during growth and, in the short-term, is not directly coupled to dry matter accumulation. © 2004 Annals of Botany Company

Key words: *Lactuca sativa*, lettuce, light-emitting diode (LED), light quality, stomatal conductance.

INTRODUCTION

During long-term space missions, higher plant photosynthesis and transpiration could be used in artificial, controlled ecological systems to provide food for the crew as well as recycling water, and carbon dioxide and oxygen in the air (Mackowiak and Wheeler, 1996). One scenario for cultivating plants in space transit vehicles, or in settlements on a planet's surface, involves the use of electric ('artificial') light sources (Sager and Wheeler, 1992). Among the lighting technologies considered are light-emitting diodes (LEDs): they are small in mass and volume, have a solid-state construction, are safe (e.g. do not use an arc-discharge approach), and have a long operating life (Bula *et al.*, 1991; Barta *et al.*, 1992).

Before LEDs can be accepted as a light source for growing plants in space their growth and development under the light spectra produced by LEDs must be analysed (Goins, 2002). Several species of plant could be used; lettuce is favoured because of its versatility as a fresh salad crop, its adaptability to controlled-environment cultivation, and its low growth habit with a defined shoot shape (Tibbitts and Alford, 1982; Knight and Mitchell, 1988; Wheeler *et al.*, 1994). Several studies with lettuce using LEDs have been reported (Bula *et al.*, 1991; Hoenecke *et al.*, 1992; Goins *et al.*, 1998, 2001), but none measured the spectral effects on stomatal conductance, which is the focus for this study. Stomatal regulation of gas exchange by leaves is of great importance, but the long-term effects of light quality on stomata is poorly understood.

Stomatal movements can be affected by various environmental factors, including plant water status, CO_2 concentration and light (Raschke, 1975). For example, bright light and low concentrations of CO_2 stimulate opening, whilst high CO_2 concentration even in bright light, cause closure (Scarth, 1932; Raschke, 1975). Thus light has often been suggested to exert an indirect effect on stomata via a lowering of the CO_2 concentration by photosynthesis. However, both in intact leaves and in isolated epidermis (epidermal strips) stomata respond directly to light (McDonald, 2003). Stomata of leaves of *Xanthium strumarium*, *Gossypium hirsutum*, *Phaseolus vulgaris* and *Perilla frutescens* opened in light, in the absence of CO_2 fixation (Sharkey and Raschke, 1981*b*). Sharkey and Raschke (1981*a*) also measured the wavelengths of light that were most effective in opening stomata in the lower epidermis of detached leaves of *X. strumarium*. Blue light (430–460 nm) was nearly ten times more effective than red light (630–680 nm) in producing a conductance of 0·15 mol m^{-2} s^{-1}. The response spectrum of stomatal reactions to red light corresponded to that of CO_2 assimilation, and inhibitors of photosynthetic electron transport eliminated the stomatal response to red light. Also, the capacity of guard-cell protoplasts to respond to blue light provided evidence for a specific light response of stomata, independent of any other component of the leaf (Zeiger and Hepler, 1977). Thus, the red-light response is caused by light absorbed by chlorophyll, but the blue-light effect is independent of photosynthesis (Karlsson, 1986; Taiz and Zeiger, 1998; Assmann and Shimazaki, 1999).

The objectives of this investigation were: (*a*) to characterize the pattern of stomatal conductance in lettuce plants

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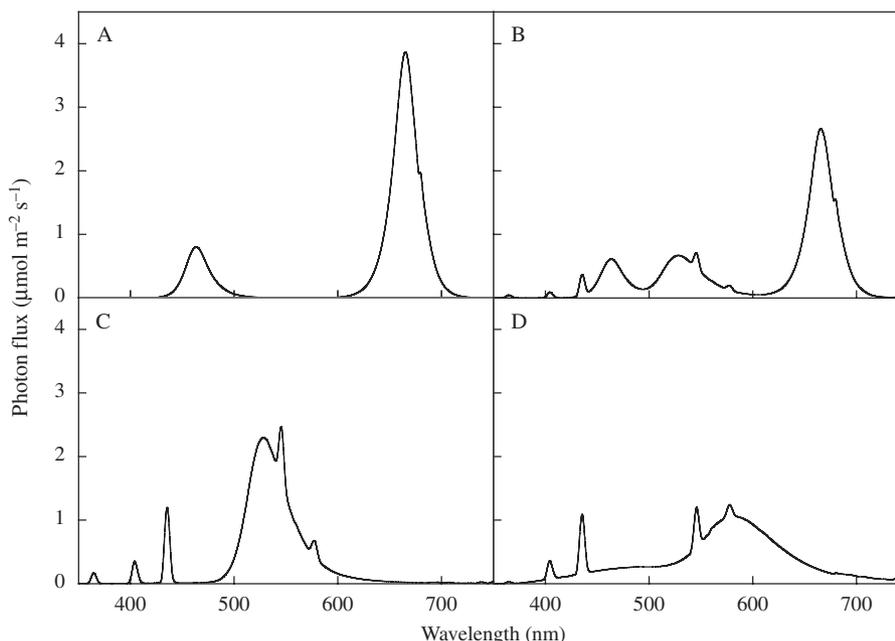
FIG. 1. Spectral distribution of light from (A) red and blue LEDs (RB), (B) red and blue LEDs with green fluorescent lamps (RGB), (C) green fluorescent lamps (GF) and (D) cool white fluorescent lamps (CWF). Spectra were recorded at the top of the plant canopy with a spectroradiometer.

developed under different spectral environments; and (*b*) to investigate the effect of changes in the spectral environment on stomatal conductance. The results will be relevant for the understanding of the responses of stomata to light spectra, and for the design of life support systems for space travel.

MATERIALS AND METHODS

Cultural conditions

Lettuce seeds (*Lactuca sativa* L. 'Waldmann's Green') were planted into plastic pots (7 cm tall, 164 mL capacity, two seeds per pot) containing horticultural vermiculite and Canadian sphagnum peat moss (Terra-Lite® Agricultural Mix, The Scotts Co., Marysville, OH, USA). Studies were done in a growth chamber (Conviron PGW-36, Pembina, ND, USA; 7·8 m³ interior plant growth volume), where 16 pots were arranged in a 0·3-m² tray under each light treatment. Lighting treatments were systematically rotated between replications to minimize edge and position effects within the growth chamber, and pots were systematically rotated every other day. Seven days after planting (DAP), the seedlings were thinned to one plant per pot. The air temperature, relative humidity, and CO_2 concentrations for all treatments were maintained at 21 ± 0.3 °C, 70 ± 4.1 % and 1200 ± 48.9 µmol mol^{-1} (0.12 kPa), respectively. Fresh half-strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950; Mackowiak *et al.*, 1989) was added as needed to the bottom of each tray to supply nutrients and replenish evapotranspiration water losses.

Light treatments

The four light sources were (1) red and blue LEDs (RB), (2) red and blue LEDs with green fluorescent lamps (RGB), (3) green fluorescent lamps (GF) and (4) cool white fluorescent lamps (CWF). Their spectra (Fig. 1) were measured from 300–1100 nm, at 2 nm steps, with a spectroradiometer (LI-1800; LI-Cor, Lincoln, NE, USA). Contributions of blue (400–500 nm), green (500–600 nm), red (600–700 nm), far-red (700–800) and total photosynthetic photon flux (PPF, 400–700 nm) were determined from bandwidth integration. From the spectrometric data for each light treatment, the yield photon flux (YPF) (Sager *et al.*, 1988), the quantum ratios of red, far-red, blue, and the calculated amount of phytochrome in P_{fr} form relative to total phytochrome at photoequilibrium (P_{fr}/P_{total}) (Sager *et al.*, 1988) were determined. Short-wave (280–2800 nm) and thermal long-wave (2800–50 000 nm) radiation were measured with Eppley PSP and PIR radiometers (Eppley Laboratories, Newport, RI, USA) (Table 1).

For RB treatments, nine LED arrays (Snap-Lite™; Quantum Devices, Inc., Barneveld, WI, USA) equipped with red gallium-aluminium-arsenide (GaAlAs) and blue gallium-nitride (GaN) LEDs were installed. Each array contained 150 red and 75 blue individual diodes. For RGB treatments, four green fluorescent lamps (F15T8/G; Interlectric Corp., Warren, PA, USA) were mounted around the nine arrays of red and blue LEDs, green light supplying 24 % to the total PPF. For GF treatments, six green fluorescent lamps (F15T8/G, Interlectric Corp., Warren, PA, USA) were used, green light providing 86 % of the total PPF. For CWF treatments, ten cool white fluorescent lamps (F15T12-CW; General Electric Co., Cleveland, OH, USA), with a 3·5-mm-thick Plexiglas heat barrier, provided 51 % of the total PPF in the green region of the spectrum. A vestibule made of black, opaque plastic prevented outside light from entering the growth area with LED arrays and fluorescent lamps.

TABLE 1. *Spectral data for red and blue LEDs (RB), red and blue LEDs with green fluorescent lamps (RGB), green fluorescent lamps (GF) and cool white fluorescent lamps (CWF)*

Parameter	Treatment			
	RB	RGB	GF	CWF
Photon flux (μmol m^{-2} s^{-1})				
PPF (400–700 nm)	150 ± 7·1	150 ± 9·2	150 ± 3·6	150 ± 12·3
Blue (400–500 nm)	24	23	15	29
Green (500–600 nm)	0	36	129	76
Red (600–700 nm)	126	92	6	45
Far-red (700–800 nm)	2	2	2	7
Yield photon flux*	130	127	122	134
Fraction (%)				
PPF	100	100	100	100
Blue	16	15	10	19
Green	0	24	86	51
Red	84	61	4	30
Ratios				
Red : far-red	63	46	3	6
Blue : red	0·2	0·3	2·5	0·6
Blue : far-red	12	12	8	4
Calculated P_{fr}/P_{total}*	0·86	0·86	0·80	0·83
Irradiance (W m^{-2})				
280–2800 nm	28	33	39	41
2800–50 000 nm	2	13	134[†]	16[y]

Spectra were recorded at the top of the plant canopy with a spectroradiometer.
*Calculated according to Sager *et al.* (1988).
[†] A clear Plexiglas barrier was placed under the CWF lamps but no barrier was used for GF lamps so as to maintain a PPF of 150 μmol m^{-2} s^{-1}.

Lighting for all treatments was 18 h photoperiod (18 h light/6 h dark) with approximately equal PPF at 150 μmol m^{-2} s^{-1} (9·7 mol m^{-2} d^{-1}). Photosynthetic photon flux was measured at the top of the plant canopy with a quantum sensor (LI-190SA; LI-Cor) calibrated with a spectroradiometer (LI-1800; LI-Cor). As the plant canopies grew closer to the light banks, PPF was maintained by adjusting the height of the pots.

Plant measurements and porometry

Stomatal conductance (G_s) measurements were taken with a steady-state porometer (LI-1600; Li-COR), calibrated by the manufacturer. Four of the youngest fully expanded leaves per treatment were used for all measurements, with readings from the abaxial side of the leaves.

Lettuce grown under different light qualities. Lettuce plants were grown under RB, RGB, GF or CWF for 23 d. Four plants were harvested from each light treatment 21 DAP prior to canopy closure to minimize spectral quality changes. Measurements included leaf area, specific leaf area (SLA), shoot fresh mass (shoot FM) and shoot dry mass (shoot DM). Plant tissue samples were dried in a drying oven for 48 h at 70 °C before weighing. Canopy temperature in each light environment was measured with infrared transducers and logged (Apogee Instruments Model IRTS-P, Logan, UT, USA).

TABLE 2. *Influence of light quality on leaf area, specific leaf area (SLA), shoot fresh mass (shoot FM) and shoot dry mass (shoot DM) 21 d after planting, and canopy leaf temperature*

Parameter	Treatment*			
	RB	RGB	GF	CWF
Leaf area (cm^2)	84·5[c][†]	143·9[a]	57·8[d]	112·2[b]
SLA (m^2 kg^{-1})	47·7[b]	47·2[b]	70·9[a]	54·5[b]
Shoot FM (g)	2·40[c]	5·03[a]	1·56[c]	3·46[b]
Shoot DM (g)	0·18[b]	0·34[a]	0·09[c]	0·21[b]
Canopy leaf temp. (°C)	20·5[a]	20·4[a]	20·7[a]	20·3[a]

*See Fig. 1 and Table 1 for spectral characteristics.
[†] Mean comparison within a row by Duncan's multiple range test, $P = 0.05$. Means with same letter are not significantly different.

Stomatal conductance was measured 23 DAP on lettuce grown under each light treatment 0·5 h before lights came on, 0·5, 2·5, 5·5, 9, 12·5, 15·5 and 17 h into the light period, and then after 0·5 and 3 h of darkness. During dark measurements green light <1 μmol m^{-2} s^{-1} at approx. 15 cm from the lamp was provided by a green LED pen light (LI-Cor). It was assumed not to affect stomatal response (Wheeler and Tibbitts, 1986; Wheeler *et al.*, 1999).

Lettuce exposed to different light qualities. Lettuce plants were grown under CWF for 23 d and then given 24-h exposure to RB, RGB or GF. Stomatal conductance was measured between 2·5 and 3·5 h after lights came on, before the 24-h exposure (23 DAP), after 24-h exposure (24 DAP), and 24 h after returning to the original fluorescent lighting (25 DAP).

Statistical analysis

The experiment was repeated three times with means calculated from four plants per repetition. Using 5 % as the level of significance, statistical analysis was subjected to analysis of variance followed by Duncan's multiple range tests (SPSS Inc., Chicago, IL, USA).

RESULTS

Light quality

The LEDs used in this study had narrow spectral bands (25 nm band width at half peak height) in the red and blue, in contrast to the broad spectral bands of green and cool white fluorescent lamps (Fig. 1). The lower relative weighting of the blue (400–500 nm) and red (600–700 nm), and the higher weighting of the green (500–600 nm) reduced the YPF for the green fluorescent lamps. The red to far-red ratios of the GF and CWF were 3 and 6, respectively, whereas those of RB and RGB were 63 and 46, respectively. The calculated P_{fr}/P_{total} values for all the treatments were ≥0·80. GF produced more long-wave radiation than other sources (Table 1), attributable to the lack of heat filter. However, this did not significantly affect the average canopy leaf temperature, which was similar between light environments (Table 2).
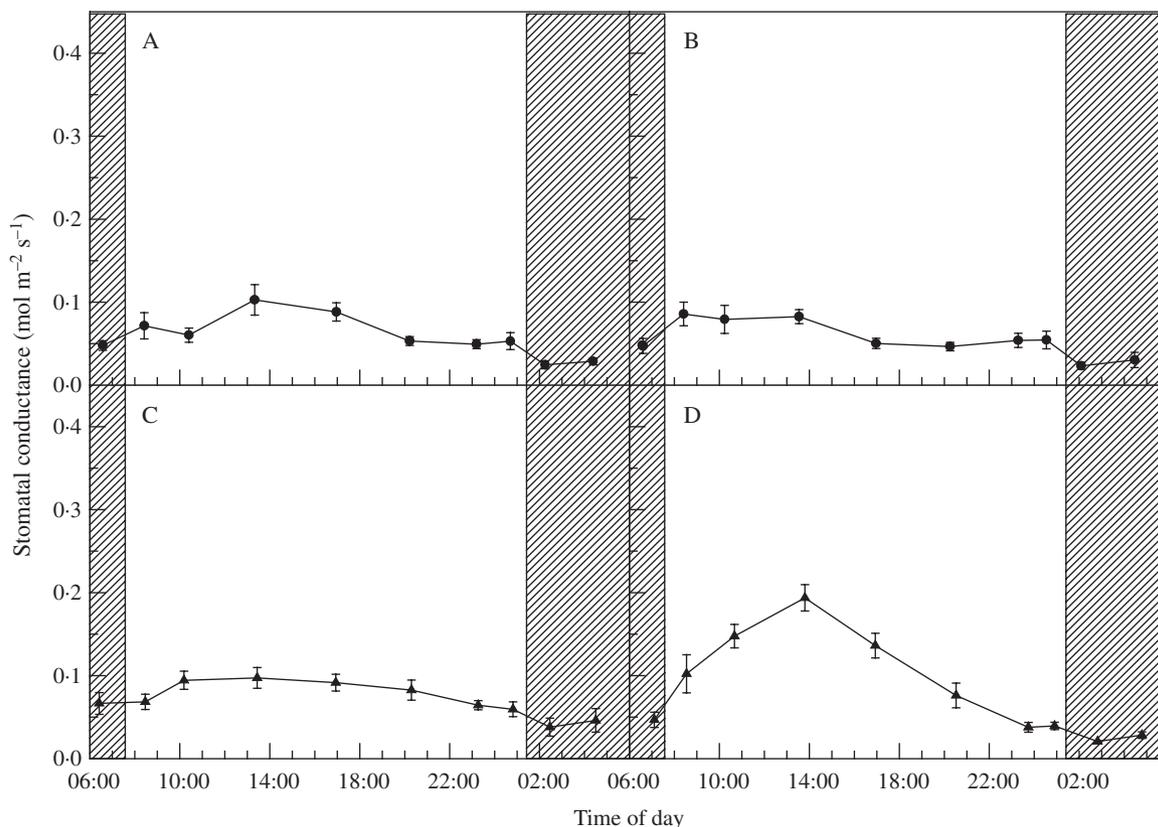
FIG. 2. Stomatal conductance of lettuce leaves grown under (A) red and blue LEDs (RB), (B) red and blue LEDs with green fluorescent lamps (RGB), (C) green fluorescent lamps (GF) and (D) cool white fluorescent lamps (CWF) during an 18-h photoperiod. Sequential measurements were taken 23 d after planting. Data represent means ± s.e. of 12 measurements. Within the photoperiod, darkness is indicated by cross-hatching.

Plant growth

Leaf area was largest in plants grown under RGB, followed by CWF, RB and then GF, whereas specific leaf area was greater under GF than RB, RGB and CWF. Shoot FM was highest in plants grown under RGB, followed by CWF, and then RB and GF. Shoot DM was highest in plants grown under RGB, followed by RB and CWF, and then GF (Table 2).

Stomatal conductance

Diurnal patterns of G_s for lettuce leaves grown under different light qualities for 23 d are shown in Fig. 2: those under RB, RGB and GF changed less than under CWF. Under CWF, conductance rose rapidly when the lights came on and then peaked near the middle of the light period, after which it dropped during the late photoperiod before darkness, and was lowest during the dark period (Fig. 2D). The minimum G_s in the dark, as a percentage of the maximum conductances during the light, was 24 % for RB, 27 % for RGB, 39 % for GF and 11 % for CWF.

Figure 3 shows G_s of lettuce leaves grown under CWF for 23 d and then exposed for 24 h to RB, RGB or GF. Initial average G_s measured before the 24-h exposure, was 0·09 ± 0·02 mol m^{-2} s^{-1}. After 24-h exposure, conductance decreased in order RB, CWF, RGB and GF, although the differences between RB and CWF and between RGB and CWF were not statistically significant. Twenty-four hours after returning plants to the fluorescent light under which they were grown, there was no significant difference between any treatments.

DISCUSSION

The DM accumulation for plants grown under RB and CWF was similar. This indicated that normal growth for lettuce could be achieved with only red and blue photons. Yorio *et al.* (2001) reported similar results for lettuce 'Waldmann's Green' when plants were grown with red LEDs and blue fluorescent lamps. Hoenecke *et al.* (1992) suggested that a blue-photon flux between 15 and 30 µmol m^{-2} s^{-1} for 12 h each day would be acceptable for lettuce growth. In the present study, the blue photon flux was 15–29 µmol m^{-2} s^{-1}, which suggested that the difference in blue photon flux among treatments was negligible.

The major difference between the spectra in the PPF region was the relative ratio of green (500–600 nm) or red (600–700 nm) light rather than that of blue (400–500 nm) light. The P_{fr}/P_{total} values, which provide an indicator of the expected morphogenic responses common to a wide variety of plants (Sager *et al.*, 1988), were between 0·80 and 0·86 for all treatments. This indicates that
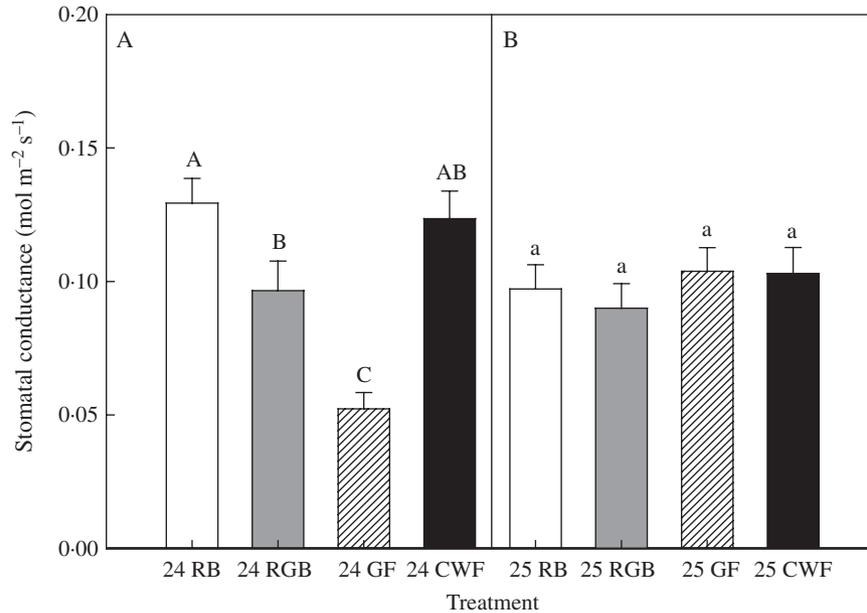
FIG. 3. Stomatal conductance of lettuce leaves grown under cool white fluorescent lamps (CWF) for 23 d and then exposed for 24 h to red and blue LEDs (RB), red and blue LEDs with green fluorescent lamps (RGB) or green fluorescent lamps (GF). Measurements were taken after (A) 24-h exposure (24 DAP) and (B) 24 h after returning to the initial fluorescent lighting (25 DAP). Data represent means ± s.e. of 12 measurements.

the phytochrome photostationary state would not differ significantly between treatments, so it is unlikely that differences in the phytochrome system were responsible for the observed effects. There was very little far-red radiation in the spectra; the greatest amount was from CWF (7 μmol $m^{-2} s^{-1}$). Hence, the differences in plant growth appeared to originate from the difference in the amount of green light rather than a change in red light.

Stomatal movement is controlled by various environmental and endogenous factors (Gorton *et al.*, 1993; McDonald, 2003). Light is of major importance, with opening in the light and closing in the dark. However, although the growth chamber lighting in this study was either on or off, under CWF conductance increased gradually in the morning and decreased gradually later in the day. This trend was less marked under RB, RGB and GF.

A possible explanation for the lower G_s in plants grown under RB and RGB, is the relatively monochromatic light. Stomatal conductance is generally smaller with such light, compared with broad-spectrum light source such as CWF, e.g. of lettuce plants grown under red LEDs (Yorio *et al.*, 2001) and of *Beta vulgaris* grown under red and blue LEDs (Goins, 2002). In addition, since leaf adaptation to spectral quality involved changes in SLA and overall leaf structure, variation in stomatal density per unit leaf area could also have contributed to differences in measured G_s (Schoch *et al.*, 1980). In this study, SLA was greatest in plants grown under GF, i.e. they had the thinnest leaves. Consequently, photomorphogenic responses to light quality could influence overall G_s through altering light capture by leaves, without directly affecting stomatal movement.

Such modifications in leaf morphology in response to different spectral qualities during growth confound the interpretation of the spectral effects. Investigating the

effects of spectral quality on stomatal conductance without the morphological modifications is possible by growing plants under the same lighting conditions before temporarily exposing the plants to different light treatments. This was done by growing lettuce plants under CWF for 23 d, then exposing them for 24 h to RB, RGB or GF, and then returning to the initial CWF lighting. The conductance was different after 24-h exposures to different spectral qualities, with maximum conductance under RB and minimum under GF. However, these effects were reversible, since G_s was the same 24 h after returning to the original CWF lighting regardless of previous treatments. It is possible that the temporary changes in spectral quality affected guard cells through photosynthesis.

There are several reports of light quality affecting stomatal movement. Light is perceived by cellular photoreceptor systems stimulating two distinct photosensory pathways. One is the transduction of photosynthetically active radiation by photosynthetic reactions in the guard cell chloroplasts, the other is induced by blue light (Gorton *et al.*, 1993; Taiz and Zeiger, 1998). Experimentally, stomata, which have achieved their steady-state aperture under red light irradiation, open wider when exposed to additional weak blue light (Ogawa *et al.*, 1978; Assmann, 1988). It has been suggested that zeaxanthin, a part of the xanthophyll cycle, may act as the blue light guard cell photoreceptor (Srivastava and Zeiger, 1995; Niyogi *et al.*, 1998; Quiñones *et al.*, 1998; Zeiger and Zhu, 1998; Frechilla *et al.*, 1999).

After 24-h exposure, conductance in plants under RB, with no green light, was higher than under RGB, with a larger proportion of green light, and smallest under GF with the highest fraction of green and the lowest fraction of red light (86 % and 4 % of total PPF, respectively). Therefore, the large G_s under RB may have resulted from lack of green

light. Green light reversal of blue-light-stimulated stomatal opening occurs in a number of species, including *Vicia faba*, *Commelina communis*, *Pisum sativum*, *Nicotiana glauca*, *Arabidopsis thaliana*, *Nicotiana tabacum*, *Allium cepa* and *Hordeum vulgare* (Frechilla *et al*., 2000; Talbott *et al*., 2002). Simultaneous exposure to equal fluxes of blue and green light resulted in approx. 50 % reversal of normal blue light opening. Complete reversal occurred when the flux of green light was approximately twice that of blue light. These results suggested that blue-green reversibility of stomatal opening is a basic photobiological property of guard cells (Talbott *et al*., 2002). In the present study, the experimental approach was different from Frechilla *et al*. (2000) and Talbott *et al*. (2002), who studied stomatal responses of epidermal strips; however, the stomatal responses to spectral quality were similar.

In conclusion, this investigation demonstrated that spectral quality during growth affected the pattern of diurnal stomatal conductance. However, morphological or physiological adaptations probably occurred, since DM accumulation was greatest in plants grown under RGB, although their conductance was smaller than in plants grown under CWF. The observed diurnal fluctuations in G_s caution against the single measurements of photosynthetic rates and/or G_s made during the day, when comparing treatments. In addition, temporary changes in spectral quality affected G_s, in agreement with stomatal responses of epidermal strips. The effects of the different light treatments on G_s reversed after returning to the initial light source. The effects of light spectrum observed on plant production, must be incorporated into the design of spectrally balanced LED systems for supporting plant growth, especially for very specialized applications, such as long-term space missions.

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