Red and Blue Light Effects during Growth on Hydraulic and Stomatal Conductance in Leaves of Young Cucumber Plants

W. van Ieperen, A. Savvides and D. Fanourakis
Horticultural Supply Chains
Wageningen University
Droevendaalsesteeg 1, 6708 PB, Wageningen
The Netherlands

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Abstract

In greenhouses at high latitudes artificial lighting (AL) is applied in winter as supplementary light source to increase photosynthesis and plant growth. Additionally, AL is already solitary used in closed systems for commercial plant production. The spectral composition of AL (light quality) usually deviates from solar light, causing long-term morphological and developmental changes as well as short-term functional responses in plants. Light quality directly influences leaf photosynthesis via changes in stomatal aperture and photosynthetic quantum efficiency. Stomatal conductance (g_s), however, also depends on leaf characteristics such as the size, number, and distribution of stomata over the upper and lower surfaces of a leaf, which develop on the long-term. Besides, stomatal aperture is also influenced by leaf water status, while vice versa, leaf water status depends on transpiration and g_s. Leaf water status is also affected by its internal conductance for (liquid) water transport through the leaf (K_{leaf}). It has often been suggested that g_s and K_{leaf} should be properly dimensioned for appropriate control of g_s in relation to water stress. We investigated long-term effects of light quality on K_{leaf} and g_s in leaves of young cucumber plants, which were grown under red (R), blue (B) or combined red and blue (RB) LED (light emitting diode) light. Light quality-induced differences in g_s were largely due to differences in stomatal density, which were mainly due to differences in epidermal cell size. K_{leaf} was influenced by light quality and positively correlated with changes in g_s across the applied light qualities. Our results show that in horticultural production systems where AL is used, light quality effects on both plant photosynthesis and plant water relations are important for the efficient production of high quality plants.

INTRODUCTION

Artificial Lighting in Horticulture Influences Photosynthesis and Plant Water Relations

The introduction of artificial lighting (AL) in greenhouse horticulture enabled year round production of plants and plant products at latitudes where the low availability of solar light limits plant growth during large parts of the year (Heuvelink et al., 2006). In addition, AL started being used as a sole light source in closed production systems (Kozai and Ohyama, 2006). AL lamps, which are primarily used to increase light intensity and/or duration, have a very different spectral composition than natural solar light. Yet, photosynthesis is positively influenced by AL and increases carbohydrate production to levels sufficient for plants to grow and produce in an economically feasible way. Recently, several types of LED-based lamps became available for commercial plant production. They are expected to be more energy efficient (i.e., proportionally higher conversion of electrical energy to light than heat) than the commonly used AL lamps (e.g., high pressure sodium lamps), and allow the control over the spectral composition of light (the light quality). Most LED-based light sources for horticulture include only red and blue wavelengths. These wavelengths result in the highest photosynthetic efficiencies

on leaf basis (McCree, 1971), but still produce a light spectrum that strongly deviates from natural light. Long-term exposure to monochromatic red light can result in photosynthetic dysfunctional leaves (Hogewoning et al., 2010). Light quality can cause long-term morphological and developmental plant responses, which also influence short-term controlled properties in plants related to photosynthesis, such as stomatal conductance (g_s). Besides photosynthesis, transpiration and plant water status can also influence productivity and quality of plants. Water stress can limit photosynthesis via stomatal closure and reduce expansion growth. In horticultural research, the impact of AL on plant water relations has received far less attention than the impact on photosynthesis.

Leaf Water Relations and Photosynthesis Are Intrinsically Coupled

Photosynthesis is inextricably coupled to transpiration. CO_2 uptake by leaves for photosynthesis requires open stomata, which causes water loss by transpiration. This loss of water requires sufficient water uptake by the roots to maintain a positive leaf water balance. It also requires a long distance water transport system (xylem) with sufficient capacity to transport water from the roots towards the transpiring sites. This water transport system should also be safe to withstand the low negative pressures that might develop at low water availability and/or high leaf transpiration rates. Appropriate control of water uptake, transport through plants, and water loss are important quality aspects for young plants (i.e., in relation to hardening). While the impact of light quality on photosynthesis has lately received much attention, this was not the case for plant water relations. For successful application of AL by LEDs in horticulture both have to be taken into account.

Light Quality Influences Stomatal Conductance and Leaf Hydraulic Conductance

The almost instantaneous effect of light quality on stomatal aperture is well known: red and blue light stimulate stomatal opening and therefore stomatal conductance (g_s) via different mechanisms that influence turgor pressure in the guard cells (Zeiger et al., 2002; Lawson, 2009). However, long-term effects of light quality might also be important as g_s also relies on size, number and distribution of stomata over the upper and lower leaf surfaces (Franks and Beerling, 2009). Turgor in the guard cells is, among many other factors, influenced by leaf water relations (Comstock, 2002; Buckley, 2005). Vice versa, leaf water relations also depend on g_s, because g_s has a strong effect on leaf water loss. Leaf water status is also influenced by the ability of leaves to import and transport water. In many plant species, an important part (at least 25%) of the resistance for water transport between roots and stomatal cavities is located in the leaf (Sack and Holbrook, 2006). This relatively high hydraulic resistance in the leaf (often described by its reciprocal, the leaf hydraulic conductance per leaf area (K_{leaf})) importantly influences leaf water status, considering the limited length of the total transport path for water through a plant. It consists of apoplastic (xylem vessels, intercellular spaces and cell walls) and symplastic components (cell to cell water transport). There are strong indications that a higher light intensity and longer duration induce a higher K_{leaf} (Scoffoni et al., 2008; Sellin et al., 2008; Voicu et al., 2008) while there are also indications that light quality can influence K_{leaf}, as short-term exposure (not during growth) of sun- and shade leaves to different light qualities resulted in different values of K_{leaf} (Sellin et al., 2011).

Here we show and discuss the importance of light quality during growth on both K_{leaf} and g_s of leaves of young cucumber plants, and their combined effect on leaf photosynthesis.

MATERIAL AND METHODS

Plant Material and Growth Conditions

Cucumber (*Cucumis sativus* 'Hoffmann giganta') seedlings were sown in rockwool and left to grow under 16 h photoperiod provided by white fluorescent tubes (100 µmol m^{-2} s^{-1} PPFD; Philips TLD 58W/84, Eindhoven, The Netherlands). Before the

appearance of the first leaf, plants were transferred in three growth units (four plants per growth unit; l×w×h=0.8×0.8×1.6 m; HomeboxS, EastSide-Impex., Berlin, Germany) under the same PPFD but three different light qualities using red LEDs (R; Red, LXK2-PD12-S00, Philips), blue LEDs (B; Royal-Blue, LXK2-PR14-R00, Philips), and a combination of red and blue LEDs (RB; R:B, 70:30). The plants, from sowing, were grown under constant day and night temperature (21±1°C), relative humidity (75%), and CO_2 concentration (~400 ppm), and after transfer they were watered by half-strength Hoagland solution (pH=5.4±0.2, EC=1 mS cm^{-1}). All the measurements were performed on the first fully expanded leaf.

Leaf Hydraulic Conductance

K_{leaf} was measured using the vacuum pump method (Sack et al., 2002) with adaptations (Savvides et al., 2012). This method determines K_{leaf} independently from the g_s. All K_{leaf} measurements were done under dim fluorescent light (PPFD ~5 μmol m^{-2} s^{-1}). K_{leaf} was then determined by dividing the absolute conductance of the leaf by its area (determined on spatial calibrated images of flattened leaves by ImageJ open access software, version 1.44p).

Stomatal Features

Stomatal features were determined using the silicon rubber impression technique (Weyers and Meidner, 1990) as described in detail in Fanourakis et al. (2011). The measurements were made on four leaves (one leaf per plant) per light quality treatment on both leaf surfaces. Stomatal features were assessed at both adaxial and abaxial leaf epidermes, between major veins, midway between the tip and the base and away from the edge. Stomatal densities and epidermal cell size (SD and ECS, respectively) were determined using light microscopy (×250 magnification) on five fields in each sampling area. Stomatal index (SI) was determined as described in Salisbury (1928). ECD was estimated as the guard cell density (2× SD) plus the non-stomatal epidermal cell density. Individual stomatal parameters (width, length, pore length and width) were measured on 20 randomly selected stomata per sampling area (at ×1000). To calculate stomatal and pore area, both stomata and pore were assumed to be elliptical. Pore area per leaf area was calculated as the sum of the pore area per stoma × stomatal density of the two leaf surfaces.

Leaf Gas Exchange

Stomatal conductance (g_s), net photosynthesis (A_N) and operating efficiency of photosystem II (Φ_{PSII}=F'$_q$/F'$_m$; Baker, 2008) measurements were performed using the portable gas exchange system and leaf fluorometer (LI-6400F; Li-Cor Biosciences, Lincoln, NE). The microclimate within the chamber was adjusted to be the same with the growth conditions. All measurements were carried out inside the growth-units. In total, eight plants were used for gas exchange measurements per light quality treatment. Water stress was then applied and gas exchange measurements were repeated as described in Savvides et al. (2012).

Data Analysis

The data analysis was performed using the package IBM SPSS Statistics 19 (IBM Corporation, NY). The parameters investigated were statistically tested by one-way ANOVA and the means were compared by the least significant difference (LSD) multiple comparison test (P≤0.05). The effects of light quality on leaf stomatal traits were tested on both leaf sides (abaxial and adaxial) and statistically tested by two-way ANOVA. (Linear regression analysis (P≤0.05) was used to test the significance of the correlation between K_{leaf} and g_s, between K_{leaf} and Φ_{PSII} and between K_{leaf} and A_N of leaves developed under the three different light qualities).

RESULTS AND DISCUSSION

Light Quality during Growth Influences K_{leaf} and g_s

K_{leaf} of the first true leaf significantly varied between light qualities with by far lowest values in leaves of plants grown at monochromatic R. K_{leaf} in leaves that were raised under monochromatic B and dichromatic RB LEDs was approximately similar (Fig. 1). We previously showed that growth of young cucumber plants under monochromatic R resulted in a dysfunctional photosynthetic apparatus (Hogewoning et al., 2010), chlorosis and (in tomato seedlings) curled leaves. Present results add that monochromatic R also reduces the water transport capacity in leaves, which might lead to a suboptimal leaf water- and nutrient-status. In line with the current results, the presence of blue light enhanced secondary xylem thickness in stems and the spongy and palisade mesophyll thickness in leaves of *Capsicum annuum* (Schuerger et al., 1997). Present results show that the simple fact that R light has the highest photosynthetic efficiency in leaves, which developed under broadband AL (McCree, 1971), does not automatically make monochromatic R a good choice for AL. Leaf lamina area did not significantly differ between the light treatments, but petiole length was significantly higher under monochromatic B (Table 1). The difference in petiole length, however, can by no means explain the observed differences in K_{leaf} as no differences in K_{leaf} were observed between RB and B, and the lower K_{leaf} in leaves grown under R than under B was accompanied with shorter petioles, while the reverse should have been expected if petiole length would have mattered.

Stomatal conductance (g_s) differed between the applied light qualities with a trend comparable to K_{leaf} (Fig. 1) resulting in a strong positive correlation between K_{leaf} and g_s. Positive correlations between K_{leaf} and g_s were also observed with leaves grown under different light intensities or humidities (Brodribb and Jordan, 2008; Sellin et al., 2008).

Light Quality during Growth Influences g_s via Stomatal Density

We investigated the impact of R, B, and RB on stomatal density (SD) and stomatal index (SI) at adaxial and abaxial leaf surfaces. In all light qualities more stomata were located at the abaxial than at the adaxial leaf surface (Table 2). Adaxial SI was slightly lower in leaves grown under R than under B and RB light, but this was not the case at the abaxial leaf surface, where most stomata reside. Light quality can influence stomatal development mediated through cryptochromes and phytochromes (Kang et al., 2009). Our results show that at low light intensity, light quality has hardly any effect on stomatal development in cucumber. Stomatal conductance (g_s), however, was strongly influenced by light quality, but this could also not be explained by structural differences in stomatal size (Table 2). Stomatal density (SD), however, was strongly influenced by light quality, with highest numbers in leaves grown under B and RB LED light. This was clearly caused by a strong effect on epidermal cell size: in leaves grown under R light, epidermal cells were much larger (Table 2), which automatically reduced the number of stomata per leaf area and thus g_s. Effects on SD are structural. It can therefore be concluded that light quality has an important permanent effect on g_s during leaf development, which in our case, i.e., at low light in cucumber, is basically mediated by an effect on epidermal cell size.

Stomatal pore area per leaf area, calculated from stomatal aperture and SD, was twice as large in leaves grown under B and RB than under R LED light (Table 2). This long-term effect structurally decreases the potential (maximal) stomatal conductance (g_s) in R-grown leaves, but cannot fully explain the fourfold lower g_s that was observed under R light (Fig. 1). Maybe also differences in cuticular and/or boundary layer resistance between R- and RB & B-grown leaves attributed to the observed differences in g_s.

Light Quality during Leaf Growth Influences the Impact of (Osmotic) Water Stress on Photosynthesis

Leaf net photosynthesis (A_N) showed a slightly different pattern among the

applied light qualities than K_{leaf} and g_s: lowest net photosynthesis rate was observed in the leaves grown under monochromatic R light (Table 3) and highest in leaves grown under RB light. The low A_N in R-grown leaves was largely due to dysfunctional photosynthetic systems, here indicated by the lower ϕ_{PSII} (Table 3). The lower A_N in R-grown leaves was only to a small extent caused by the lower g_s, as was earlier shown, because the internal CO_2 concentration in the leaves only slightly differed between R, RB and B-grown leaves (Hogewoning et al., 2010; Savvides et al., 2012). Savvides et al. (2012) showed that in R-grown leaves g_s was not completely unresponsive to environmental triggers, as osmotic stress in the root environment reduced g_s to the same relative extent in R-, B- and RB-grown leaves. This indicates that light and drought related signals can independently influence g_s. However, the osmotic induced drought stress reduced ϕ_{PSII} only in R-grown leaves, and this reduction was, at least partially, permanent (Savvides et al., 2012). This indicates that temporary water stress caused permanent damage to the photosynthetic machinery in R- but not in B- and RB-grown leaves. Leaves grown under monochromatic R did not only reduce photosynthesis, but were also more vulnerable to water stress. The impact of osmotic stress on K_{leaf} and leaf water status were not measured but it can easily be argued that the much lower K_{leaf} in R-grown leaves more severely reduced leaf water status in R- than in B- and RB-grown leaves. This, in combination with the already less healthy photosynthetic systems due to light quality only in R-grown leaves, might have caused this permanent damage.

CONCLUSIONS

Light quality can cause long-term effects on leaf water relations. Young cucumber plants grown under monochromatic red light, develop leaves with a decreased internal water transport capacity, a decreased g_s, decreased photosynthetic rates and an increased sensitivity for permanent damage due to osmotic induced water stress.

With different combination of R and/or B growth light, K_{leaf} positively correlated g_s, which supports speculations about a possible involvement of photoreceptors in the assumed well-coordinated development of the internal water transport system and water loss properties of leaves.

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Tables

Table 1. Leaf morphological responses to light quality.

Light quality	Petiole length (cm)	Leaf area (cm^2)
Monochromatic red	3.9±0.2 b	124.8 ±8.6 n.s.
Monochromatic blue	7.2±0.6 a	111.2±6.7 n.s.
Dichromatic red and blue	2.9±0.1 c	108.5±8.5 n.s.

Means ± SEM. Different letters indicate significant differences (P=0.05; n=10).

Table 2. Stomatal features on adaxial and abaxial leaf surfaces of plants grown under different light qualities[1].

Stomatal trait	Leaf side	Light quality			
		R	RB	B	Mean
SD (# mm^{-2})	Adaxial	153	285	273	237[b]
	Abaxial	322	438	417	393[a]
	Total	475[b]	723[a]	690[a]	
SI (-)	Adaxial	0.10[c]	0.14[b]	0.14[b]	0.13
	Abaxial	0.24[a]	0.26[a]	0.24[a]	0.25
	Mean	0.17	0.20	0.19	
ECD (# mm^{-2})	Adaxial	1744	2216	2145	2035
	Abaxial	1686	2152	2122	1987
	Mean	1715[b]	2184[a]	2133[a]	
Stomatal size (μm^2)	Adaxial	194	202	231	209
	Abaxial	218	218	224	220
	Mean	206	210	228	
Pore area per leaf area (μm^2 mm^{-2})	Adaxial	1210	1849	2448	1836[b]
	Abaxial	2642	4644	5579	4288[a]
	Total	3852[b]	6493[a]	8027[a]	

[1] R: monochromatic red, B: monochromatic blue, RB dichromatic red and blue (red:blue, 70:30).
Different letters indicate significant differences (P=0.05; n=4). SD: stomatal density; Total SD: the sum of SDs on both leaf surfaces; ECD: epidermal cell density; SI: stomatal index.

Table 3. Net leaf photosynthesis (AN) and PSII efficiency (ϕPSII) measured in leaves of plants grown at different light qualities, before (-0.05 MPa), during (-0.15 MPa) and after (-0.05 MPa) osmotic stress in the root environment.

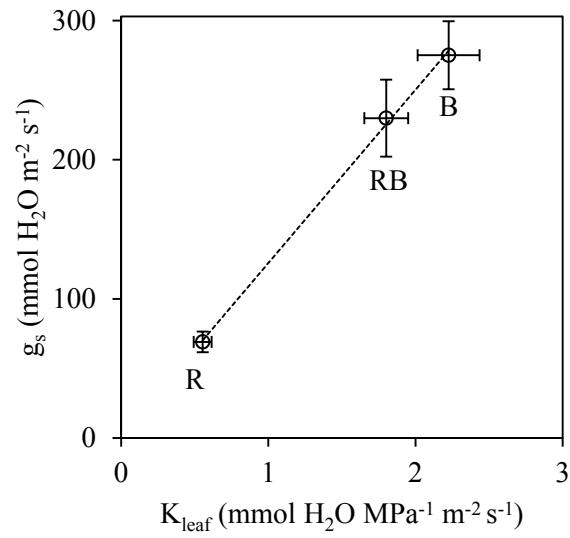| Light quality	Before osmotic stress		During osmotic stress		After osmotic stress	
	A_N	ϕ_{PSII}	A_N	ϕ_{PSII}	A_N	ϕ_{PSII}
Monochromatic red	3.55±0.22	0.63±0.02	1.74±0.09	0.53±0.02	2.83±0.25	0.55±0.03
Monochromatic blue	3.78±0.05	0.72±0.01	3.21±0.26	0.74±0.00	3.78±0.05	0.75±0.00
Dichromatic red and blue	4.77±0.08	0.74±0.01	4.07±0.04	0.75±0.00	4.63±0.11	0.76±0.00

Means ± SEM. n=4.

Figures

Fig. 1. The effect of light quality during growth on the capacity for water transport (leaf hydraulic conductance, K_{leaf}) and stomatal conductance (g_s) of mature cucumber leaves and their correlation ($R^2=1$).