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

Keywords: Cucumis sativus, leaf hydraulic conductance, light quality, stomatal conductance

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. \(\text{CO}_2\) 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 \(\mu\)mol 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; 1×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, LK12- PD12-S00, Philips), blue LEDs (B; Royal-Blue, LK12-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 CO₂ 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 gₛ. 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 (gₛ), 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 gₛ, 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 during the initial development of 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 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 ($g_{st}$) 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 $\phi_{PSII}$ (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 $\phi_{PSII}$ 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.

Literature Cited


Tables

Table 1. Leaf morphological responses to light quality.

<table>
<thead>
<tr>
<th>Light quality</th>
<th>Petiole length (cm)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monochromatic red</td>
<td>3.9±0.2 b</td>
<td>124.8±8.6 n.s.</td>
</tr>
<tr>
<td>Monochromatic blue</td>
<td>7.2±0.6 a</td>
<td>111.2±6.7 n.s.</td>
</tr>
<tr>
<td>Dichromatic red and blue</td>
<td>2.9±0.1 c</td>
<td>108.5±8.5 n.s.</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Stomatal trait</th>
<th>Leaf side</th>
<th>Light quality</th>
<th>R</th>
<th>RB</th>
<th>B</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD (# mm⁻²)</td>
<td>Adaxial</td>
<td>153</td>
<td>285</td>
<td>273</td>
<td>237</td>
<td>237b</td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>322</td>
<td>438</td>
<td>417</td>
<td>393</td>
<td>393a</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>475b</td>
<td>723a</td>
<td>690a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI (-)</td>
<td>Adaxial</td>
<td>0.10c</td>
<td>0.14b</td>
<td>0.14b</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>0.24a</td>
<td>0.26a</td>
<td>0.24a</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.17</td>
<td>0.20</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD (# mm⁻²)</td>
<td>Adaxial</td>
<td>1744</td>
<td>2216</td>
<td>2145</td>
<td>2035</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>1686</td>
<td>2152</td>
<td>2122</td>
<td>1987</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1715b</td>
<td>2184a</td>
<td>2133a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal size (µm²)</td>
<td>Adaxial</td>
<td>194</td>
<td>202</td>
<td>231</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>218</td>
<td>218</td>
<td>224</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>206</td>
<td>210</td>
<td>228</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pore area per leaf area (µm² mm⁻²)</td>
<td>Adaxial</td>
<td>1210</td>
<td>1849</td>
<td>2448</td>
<td>1836c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>2642</td>
<td>4644</td>
<td>5579</td>
<td>4288a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3852b</td>
<td>6493a</td>
<td>8027a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Light quality</th>
<th>Before osmotic stress</th>
<th>During osmotic stress</th>
<th>After osmotic stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AN</td>
<td>ϕPSII</td>
<td>AN</td>
</tr>
<tr>
<td>Monochromatic red</td>
<td>3.55±0.22</td>
<td>0.63±0.02</td>
<td>1.74±0.09</td>
</tr>
<tr>
<td>Monochromatic blue</td>
<td>3.78±0.05</td>
<td>0.72±0.01</td>
<td>3.21±0.26</td>
</tr>
<tr>
<td>Dichromatic red and blue</td>
<td>4.77±0.08</td>
<td>0.74±0.01</td>
<td>4.07±0.04</td>
</tr>
</tbody>
</table>

Means ± SEM. n=4.
Fig. 1. The effect of light quality during growth on the capacity for water transport (leaf hydraulic conductance, $K_{\text{leaf}}$) and stomatal conductance ($g_s$) of mature cucumber leaves and their correlation ($R^2=1$).