Journal of Experimental Botany Advance Access published March 4, 2010

*Journal of Experimental Botany*, Page 1 of 10
doi:10.1093/jxb/erq005

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

# An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra

**Sander W. Hogewoning*, Peter Douwstra, Govert Trouwborst, Wim van Ieperen and Jeremy Harbinson**

Wageningen University, Department of Plant Sciences, Horticultural Supply Chains Group, Wageningen, The Netherlands

* To whom correspondence should be addressed. E-mail: sander.hogewoning@wur.nl

Received 20 October 2009; Revised 5 January 2010; Accepted 8 January 2010



## Abstract

**Plant responses to the light spectrum under which plants are grown affect their developmental characteristics in a complicated manner. Lamps widely used to provide growth irradiance emit spectra which are very different from natural daylight spectra. Whereas specific responses of plants to a spectrum differing from natural daylight may sometimes be predictable, the overall plant response is generally difficult to predict due to the complicated interaction of the many different responses. So far studies on plant responses to spectra either use no daylight control or, if a natural daylight control is used, it will fluctuate in intensity and spectrum. An artificial solar (AS) spectrum which closely resembles a sunlight spectrum has been engineered, and growth, morphogenesis, and photosynthetic characteristics of cucumber plants grown for 13 d under this spectrum have been compared with their performance under fluorescent tubes (FTs) and a high pressure sodium lamp (HPS). The total dry weight of the AS-grown plants was 2.3 and 1.6 times greater than that of the FT and HPS plants, respectively, and the height of the AS plants was 4–5 times greater. This striking difference appeared to be related to a more efficient light interception by the AS plants, characterized by longer petioles, a greater leaf unfolding rate, and a lower investment in leaf mass relative to leaf area. Photosynthesis per leaf area was not greater for the AS plants. The extreme differences in plant response to the AS spectrum compared with the widely used protected cultivation light sources tested highlights the importance of a more natural spectrum, such as the AS spectrum, if the aim is to produce plants representative of field conditions.**

**Key words:** Artificial solar spectrum, blue light, growth rate, leaf mass per area (LMA), light absorptance, light interception, light quality, photomorphogenesis, photosynthetic capacity.

## Introduction

The irradiance spectrum to which plants are exposed during growth has specific effects on different types of plant responses such as photosynthesis, photomorphogenesis, phototropism, and photonasty. In plant research and greenhouse horticulture, lamps (growth lamps) with different spectral outputs are widely used to provide the growth irradiance. The most commonly used lamp types are fluorescent tubes (FTs) and gas-discharge lamps, which emit a spectrum with pronounced emission lines which are characteristic for the different lamp types. More recently light-emitting diodes (LEDs), which are characterized by relatively narrow-band spectra, have become increasingly used in growth cabinets, on an experimental basis in greenhouse horticulture, and in research on growing plants in space (Hogewoning *et al.*, 2007; Massa *et al.*, 2008; Trouwborst *et al.*, 2010). A common feature of these light

Abbreviations: $A_{max}$, light-saturated assimilation; $A_{net}$, net assimilation; AS, artificial solar; DW, dry weight; FR, far-red; FT, fluorescent tube; $F_v/F_m$, ratio of variable to maximum fluorescence—the relative quantum efficiency for electron transport by photosystem II if all photosystem II reaction centres are open; HPS, high pressure sodium; LMA, leaf mass per area (g leaf $m^{-2}$ leaf area); LUR, leaf unfolding rate; PSII, photosystem II; PSS, phytochrome photostationary state; R:FR:, red to far-red ratio.

sources is that their spectrum does not resemble that of natural daylight, which has a continuous (i.e. without strong emission lines) spectrum in the PAR region (400–700 nm), even though some lamp emissions appear 'white'.

Plants have evolved under broadband spectra and are exposed to spectral differences under natural conditions dependent on weather conditions, time of day, season, and their growth environment. For example, when the sky is cloudy, daylight contains relatively more blue and less far-red (FR) between 700 nm and 750 nm than in full sunlight (Holmes and Smith, 1977). A low sun angle is associated with a low red to FR (R:FR) ratio (Franklin and Whitelam, 2007). Other factors that affect the natural spectrum are altitude, depth for aquatic plants, and, most obviously, shading by neighbouring vegetation. Inherently, leaves exposed to a shade or a sun spectrum are also exposed to a relatively low and a high irradiance, respectively, so irradiance and spectrum are often linked.

Specific parts of the spectrum are involved in sun and shade light responses of plants. Blue light and high R:FR ratios are known to induce the development of sun-type chloroplasts (Lichtenthaler, 1980; Kasperbauer and Hamilton, 1984). A low R:FR ratio is a textbook example of a spectrum inducing an overall shade-type morphology in a wide range of species, typically characterized by etiolation so that plants can reach above neighbouring plants (e.g. Grime, 1981). Other spectral responses do not overtly parallel a shade or sun spectrum response. Such responses include blue light-induced stomatal opening (e.g. Zeiger, 1990; Willmer and Fricker, 1996), which can be reversed by adding sufficient green light to the spectrum (Frechilla *et al.*, 2000; Talbott *et al.*, 2002), or reduced growth and photosynthesis when plants are grown under red light alone (e.g. Brown *et al.*, 1995; Goins *et al.*, 1997; Yorio *et al.*, 2001; Matsuda *et al.*, 2004). Many spectral responses of plants are regulated via photoreceptors, such as phytochromes, cryptochromes, and phototropins, which alter the expression of a large number of genes (Whitelam and Halliday, 2007). These numerous and complicated spectrum-regulated plant responses have been, and remain, the subject of extensive study.

Research on spectral responses of plants normally involves adding irradiance from growth lamps to daylight, modifying daylight using spectral filters, using solely growth lamps, or a combination of these methods. Whereas the specific responses of plants to a spectrum deviating from natural light may sometimes be predictable based on published research, the overall plant response is generally difficult to predict due to the complicated interaction of the many different responses. For instance, spectra enhancing the photosynthetic capacity of leaves per unit leaf area do not necessarily enhance a whole plant morphology which is favourable for light interception and therefore also do not necessarily enhance plant production.

The lack of a practical source for an irradiance whose spectrum resembles that of any kind of natural daylight means that it is difficult, or impossible, to have a controlled environment in which natural daylight-adapted plants can be grown. Plant studies using a daylight spectrum are always conducted under conditions of natural daylight which fluctuates in intensity and spectrum. This makes a clear distinction between plant responses to the intensity or the spectrum of the irradiance difficult. In the past the main criterion for an optimal growth chamber spectral irradiance was a natural plant appearance with a high production yield (e.g. Deutch and Rasmussen, 1973), rather than producing a spectrum that is inherently like that of sunlight. So though mixtures of fluorescent and incandescent lamps have been used to allow more normal plant growth and development, this spectrum is very dissimilar to that of sunlight. A spectrum which closely resembles a sunlight spectrum has now been engineered. Growth, morphogenesis, and photosynthetic characteristics of young cucumber plants grown for 2 weeks under this artificial sunlight spectrum have been compared with their performance under lamp types widely used in growth chambers or glasshouses. A growth irradiance was used in which assimilation was light-limited (or nearly so) to minimize possible effects of different assimilation rates per leaf area, caused by differences in the irradiance response of assimilation, on plant growth and development. The plants grown under the artificial sunlight developed in a strikingly different way from the plants grown under the other lamps tested. An artificial solar (AS) spectrum offers the opportunity to grow plants under controlled conditions which are far more representative of field conditions than plants grown under the current growth chamber irradiance sources.

## Materials and methods

### *Plant material and growth conditions*

Cucumber plants (*Cucumis sativus* cv. Hoffmann's Giganta) were sown in vermiculite and germinated under 100 μmol $m^{-2}$ $s^{-1}$ cool white fluorescent lamps (TLD 50 W 840 HF, Philips, The Netherlands) in a climate chamber. After 1 week, when the cotyledons had just opened, the seedlings were transplanted to a hydroponic system (Hoagland's solution, pH 5.9±0.2; EC=1.2 $mScm^{-1}$) in a climate chamber. The day/night temperature was 25 °C/23 °C, the relative humidity was 70%, and the $CO_2$ concentration was ambient.

The light treatments consisted of an irradiance provided by cool white FTs (50 W TLD 84/HF electronic, Philips, The Netherlands), a high pressure sodium lamp (HPS; 400 W SON-T agro 400, Philips, The Netherlands) and a continuous broadband spectrum, referred to as the 'artificial solar' spectrum (see below). The percentage of blue photons (i.e. in the range 400–500 nm) of the PAR (i.e. in the range 400–700 nm) was 23, 5, and 18% for the FT, HPS, and AS spectra, respectively. All plants were subjected to 100±5 μmol $m^{-2}$ $s^{-1}$ PAR and the photoperiod was 16 h. Leaf temperature during the photoperiod, which was routinely measured using an infrared thermometer (Raytek ST series, Raytek Corporation, Santa Cruz, CA, USA), was 24±0.5, 25±0.5, and 26±1 °C for FT-, HPS-, and AS-grown leaves, respectively.

### *Artificial solar spectrum*

It has been possibble to construct a light source which, except for a deficiency in the blue, produces a spectrum that closely resembles that of a standard sunlight spectrum (Fig. 1B). The reference

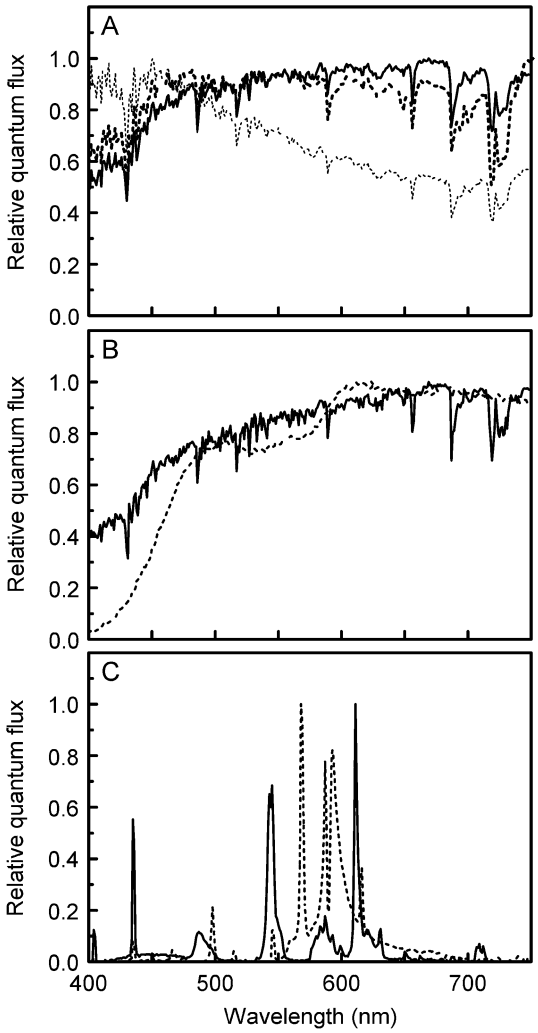


**Fig. 1.** (A) Relative spectra of direct sunlight (solid line), cloudlight (thick dotted line), and skylight (thin dotted line) measured around the autumn equinox (2009) at noon in Wageningen, The Netherlands. (B) Relative spectra of the artificial solar spectrum (dotted line) and a standard solar spectrum (solid line; ASTM, 2003). (C) Relative spectra of the high pressure sodium lamp (dotted line) and the fluorescent tubes (solid line).

spectrum for the purposes of this exercise was the ASTM G173-03 direct and circumsolar spectrum; thus it excludes skylight and takes no account of cloudlight. This is a calculated, representative direct and circumsolar irradiance spectrum for 48 contiguous states of the USA, which is available for download in a tabular form (ASTM, 2003). Cloudlight spectra are not very different from direct sunlight spectra, whereas skylight spectra are conspicuously different (e.g. Endler, 1993). The total solar irradiance is comprised of skylight, direct sunlight, and cloudlight in various proportions depending on, amongst others, the height of the sun above the horizon and weather conditions. In the absence of clouds, the total irradiance is largely dominated by direct sunlight and, under these conditions, plants will experience a predominantly direct sunlight spectrum, except under a low sun angle or when the direct sunlight is filtered by other leaves. To the best of our knowledge no comparable typical spectrum exists for other regions and therefore the ASTM spectrum is a reasonable model to use, until a better catalogue of natural spectral irradiances becomes available.

The AS spectrum was provided using a 1300 W microwave-driven sulphur plasma lamp (PI-VL1, Plasma International GmbH, Offenbach am Main, Germany), which was filtered using a colour correction filter (Gamcolor filter 1581, Los Angeles, CA, USA) in order to reduce the intensity of the green wavelengths. The resulting irradiance spectrum, lacking sufficient near-infrared wavelengths, was projected onto the plants via reflection by aluminium foil on the ceiling of the climate chamber, so that the light was well distributed over the plants. Additional quartz–halogen lamps were used to provide more near-infrared irradiance. The light output of both the plasma lamp and the quartz–halogen lamps could be adjusted without any large changes in spectral output. The desired spectrum was obtained by adjusting the light output such that 72% of the PAR was provided by the filtered plasma lamp and 28% by the quartz–halogen lamps. The spectrum and intensity of the three light sources used as growth treatment were measured using a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, The Netherlands, calibrated against a standard light source; Fig. 1B, C) and the spectra are also provided as Supplementary Table S1 available at *JXB* online. Light intensity was routinely measured using a quantum sensor (LI-COR Lincoln, NE, USA). The two devices produced comparable results. Additionally the natural spectrum of cloudlight in fully overcast conditions, direct sunlight, and skylight was measured at midday in Wageningen (52 °N 5.5 °E, The Netherlands) around the autumn equinox 2009 on the roof of a tall building (Fig. 1A and in tabular form as Supplementary Table S1).

### *Growth and morphology analysis*

For growth and morphology analysis, 10 plants per light treatment were grown for 13 d, at which point plants started shading each other. The height of the table the plants were growing on was adjusted such that the apices of the plants received 100 μmol $m^{-2}$ $s^{-1}$ irradiance throughout the experiment. The plants were dissected into different parts: leaves plus petioles, cotyledons, hypocotyls, internodes, roots, and the remainder (apex and tendrils). The different plant parts, except the roots, were imaged together with a ruler using a digital camera in order to determine the area of the leaves and cotyledons and the length of the petioles of the first two leaves, the hypocotyls, and the internodes. Image analysis was carried out using the imaging software ImageJ (http://rsbweb.nih.gov/ij/). Leaves with a length of ⩾1 cm were counted for the determination of the leaf number per plant.

After imaging, 10 leaf discs (1.28 $cm^2$) were cut from each first leaf in order to determine the leaf mass per area (LMA, g $m^{-2}$). The leaves plus petioles, cotyledons, hypocotyl, roots, discs to determine LMA, and internodes plus the remainder were oven dried at 70 °C for the first 16 h, 105 °C for the next 22 h, and held at 70 °C until weighing.

The experiment was performed in duplicate; the plants were treated as independent experimental units and the repetitions as blocks.

### *Leaf light absorptance*

Leaf light absorptance was calculated from reflectance and transmittance measurements on 12 leaf discs per light treatment, cut randomly from three first leaves per light treatment. An improved version of the system described in Soares *et al.* (2008) was used, consisting of two integrating spheres, each connected to a spectrometer and a custom-made light source. The USB-2000 spectrometers were replaced by USB-4000 spectrometers (Ocean Optics, Dunedin, FL, USA) with a custom-enlarged slit width of 100 μm to increase the signal. The spectrometers were cooled to 5 °C in order to increase the signal/noise ratio further and decrease baseline drift. Light sources consisting of two blue LEDs (405 nm and 435 nm peak wavelength) and a quartz–halogen lamp driven by a stabilized power supply were used to provide the measuring-light for the reflectance and transmittance measurements. The blue LEDs were

necessary to increase the intensity of the measuring-light in the blue region of the spectrum. Absorptance was calculated in 1 nm steps in the wavelength range 400–800 nm. The integrated absorptance of the growth light was calculated by multiplying the relative leaf absorptance spectrum by the spectrum of the growth light (spectra of the growth light are shown in Fig. 1).

### *Leaf photosynthesis measurements*

An additional set of plants was grown under the three spectra for photosynthesis measurements. The plants were grown until the second leaf, which received 100 µmol $m^{-2}$ $s^{-1}$ throughout its growth period, was fully expanded (17–22 d after planting the seedlings) and could be used for photosynthesis measurements. Leaves of different plants did not overlap and, if necessary, the second leaf was supported in a horizontal position to ensure that it received the specified irradiance.

Light–response curves were measured on six leaves per treatment using a LI-6400 photosynthesis system with a leaf chamber fluorometer (LiCor Inc., Lincoln, NE, USA). The leaf chamber is equipped with red and blue LEDs with peak wavelengths of 640 nm and 464 nm, respectively. Gas exchange was measured using a gas mix containing ambient $O_2$ and $N_2$, 22.1±1 mmol $mol^{-1}$ $H_2O$, and 380 µmol $mol^{-1}$ $CO_2$. The flow rate used was 250 µmol $s^{-1}$. After insertion into the leaf chamber, the leaf was dark adapted for 15 min and then subjected to a far-red pulse (6 µmol $m^{-2}$ $s^{-1}$ for 2 s) to oxidize the $Q_A$ pool of photosystem II (PSII), after which $F_v/F_m$ was measured. The blue light percentage of the measuring-light was set at 20%. At an irradiance of ⩾1200 µmol $m^{-2}$ $s^{-1}$ the blue light percentage was lower as the capacity for the irradiance intensity of the blue LEDs was limited to 267 µmol $m^{-2}$ $s^{-1}$. At each light intensity step the rate of photosynthesis was calculated as the mean of the last 40 s after a steady-state gas exchange was reached, which was within 10 min.

### *Curve fitting and statistics*

The photosynthesis data measured to obtain light–response curves were fitted to a non-rectangular hyperbola (Thornley, 1976) using the non-linear fitting procedure NLIN in SAS (SAS Institute Inc. 9.1, Cary, NC, USA) in order to determine the light-saturated gross assimilation ($A_{max}$).

Fisher's LSD was used to make post-hoc multiple comparisons among spectral treatment means from significant one-way analysis of variance (ANOVA) tests ($P$ <0.05; test with blocks for the growth and morphology analysis; without blocks for the photosynthesis data).

## Results

### *Plant morphology*

The difference in visual appearance of the plants growing under the three different spectra was striking (Fig. 2). The plants grown under HPS had a slightly bigger appearance than the plants grown under FTs. The AS-grown plants, however, developed considerably faster than those grown under HPS and FTs.

The differences in plant morphology are shown quantitatively in Table 1. Leaf 1, which was fully expanded on all plants when harvested, was smaller in the FT treatment than in the HPS and AS treatments. Leaf 2 of the AS-grown plants had twice the area of that grown under HPS and four times the area of that grown under FTs. This leaf was, however, not completely expanded on all plants at the time of harvest. The number of leaves was also significantly greater for the AS plants compared with the other two treatments, and the HPS plants had a slightly, but significantly, greater number of leaves than the FT plants. Leaf number, therefore, also contributed to the significant differences in total leaf area between the treatments; the AS-grown plants had a total leaf area which was 2.5 and 1.7 times greater than that of FT and HPS plants, respectively. The petioles of leaf 1 and 2 were approximately three times longer for the AS plants than those of the other two treatments, whereas the petioles of HPS plants were slightly, but significantly, longer than those of FT plants. Due to their long petioles the leaves of individual AS-grown plants did not shade each other, whereas from leaf 3 of plants in the other treatments there was leaf shading in individual plants. Also leaf 1 and 2 of the FT and HPS plants partially shaded the cotelydons, whereas the cotyledons of the AS plants were not shaded (Fig. 2). Leaves of the FT and HPS plants were not completely horizontal and also not oriented towards the incident irradiance such that light interception would be optimal. The leaves of the AS plants were fully horizontal and better orientated for light interception. The hypocotyl was over three times longer for the AS plants than it was for the other treatments. A similar trend was found for total plant length, which was four and five times greater for the AS-grown plants than the HPS and FT plants, respectively. The total plant length was only slightly greater than the hypocotyl length for HPS and FT plants, whereas the total length of the AS plants was much greater than that of the hypocotyl. This is due to differences in internode length between the treatments. The cotyledon area of the FT plants was smaller than that of the HPS and the AS plants, despite having already been partly developed when the plants were transferred to the spectrally different irradiances, implying that the cotyledons were affected by the growth light treatment.

### *Plant dry weight and partitioning*

Overall the trends observed for the lengths and areas (Table 1) of the different plant parts of plants grown under different spectra also apply for the dry weights (DWs; Table 2). The DW differences between spectral treatments for the hypocotyl are even greater than the differences in length as the longer hypocotyls were also thicker and therefore heavier per length unit. The LMA was, in contrast to the general trend for the length, area, and DW of the plant parts, smallest for AS-grown plants and greatest for FT-grown plants. This also explains why there are no significant differences in DW of leaf 1 between AS- and FT-grown plants, whereas the differences in leaf area are significant. The DW of the cotyledons is also lower for the AS plants than for the HPS plants, whereas the area was identical. The DW of the roots and remainder (mainly internodes) was again greatest for AS-grown plants and smallest for the FT plants. The total DW of the AS plants was 2.3 and 1.6 times greater than that of the FT and HPS plants, respectively.

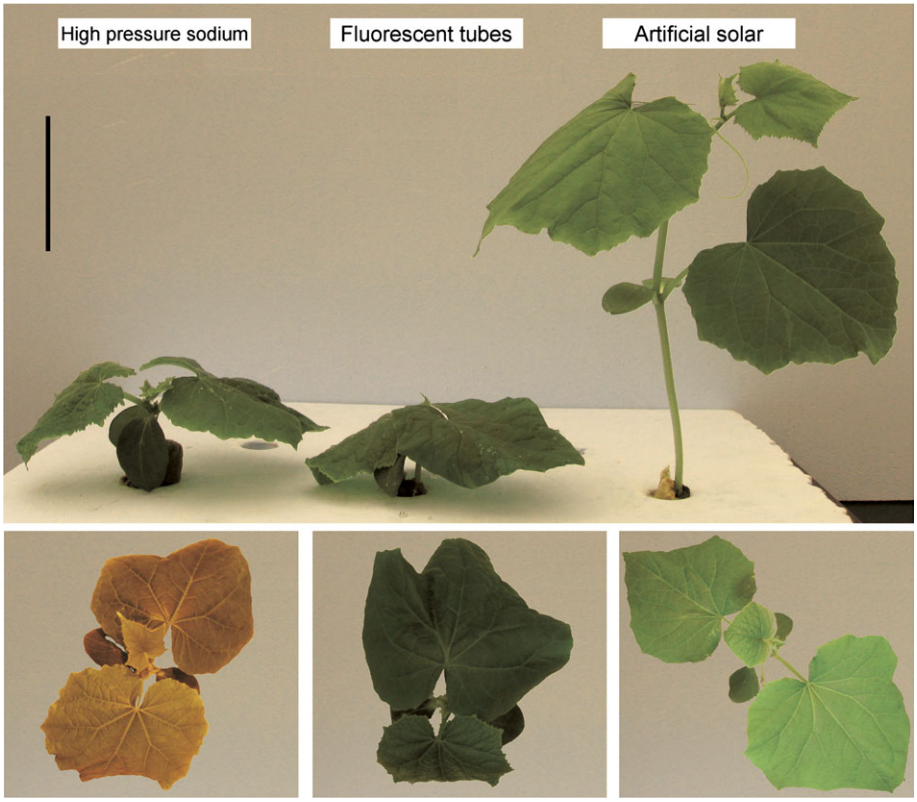


**Fig. 2.** Cucumber plants grown under a high pressure sodium lamp (left), fluorescent tubes (middle), and an artificial solar spectrum (right) 13 d after planting the seedlings. The upper image was made before the plants were dissected for growth and morphology analysis (bar=10 cm). The lower three images were made before harvest and are of plants different from those on the upper image. These three images are not scaled; the leaf colour appears unnatural due to the growth light environment.

The DW partitioning to the stem (hypocotyl, remainder) was three to four times greater for the AS-grown plants compared with the other two treatments, at the expense of partitioning to other plant parts (Table 3). Partitioning to leaf 1 and the cotyledons is lowest in the AS plants and highest in the FT plants. This result is influenced by the differences in the number of leaves per plant (Table 1). Partitioning to the roots did not differ much between the treatments and was slightly smaller for the AS-grown plants.

### *Light absorptance*

The absorptance spectra were similar for the leaves grown under FTs and HPS, whereas the absorptance of the AS-grown leaves was lower (Fig. 3). The difference in absorbed PAR between the treatments was greatest at 554 nm where FT-, HPS-, and AS-grown leaves absorbed 76, 75, and 68% of the incident irradiance, respectively. The integrated absorptance of the growth light was comparable for the three different spectra: 87, 86, and 85% for FT-, HPS-, and AS-grown leaves, respectively.

### *Photosynthesis*

All measured leaves had a dark-adapted $F_v/F_m$ of $\geqslant$0.8. Leaves grown under different spectra had different light–response curves (Fig. 4). The fitted light-saturated gross assimilation rate per area leaf ($A_{max}$) was significantly higher for the FT-grown leaves, compared with the two other treatments (Table 4). At growth irradiance (100 μmol $m^{-2}$ $s^{-1}$) measured net assimilation per leaf area was lowest for the AS-grown leaves and identical for the FT and HPS leaves (Table 4).

## Discussion

### *Plant growth and morphology*

The conspicuously greater size and biomass accumulation of the plants grown under an AS spectrum compared with plants grown under that of an HPS or FT appears to be related to the development by the AS plants of an architecture more favourable for light interception. The properties of the AS plants advantageous for light interception were characterized by an optimal leaf orientation (Fig. 2), long petioles preventing self-shading (Table 1), a larger total area (Table 1), and a lower LMA (Table 2). Compared with the FT plants, the HPS plants also displayed many of the features leading to improved whole plant light interception as shown by the AS plants, but in this case the extent of the differences was much smaller.

The light spectrum is known to have a strong influence on plant morphogenesis (e.g. Whitelam and Halliday, 2007).

**Table 1.** Length (cm) and area ($cm^2$) of different plant organs of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FTs), and an artificial solar spectrum (AS)
Different letters indicate significantly different means ($P$ <0.05).

| | HPS | FTs | AS |
|---|---|---|---|
| Leaf 1 ($cm^2$) | 129 a | 102 b | 131 a |
| Leaf 2 ($cm^2$) | 98 b | 55 c | 207 a |
| All leaves ($cm^2$) | 236 b | 159 c | 397 a |
| Cotyledons ($cm^2$) | 27 a | 23 b | 27 a |
| Petiole 1 (cm) | 3.4 b | 2.5 c | 9.3 a |
| Petiole 2 (cm) | 3.0 b | 2.2 c | 7.0 a |
| Hypocotyl (cm) | 4.4 b | 3.9 b | 14.0 a |
| Plant length (cm) | 5.8 b | 4.7 b | 25.8 a |
| Number of leaves | 3.4 b | 3.0 c | 4.4 a |

**Table 2.** Dry weight (DW, in mg) of plants, different plant parts, and leaf mass per area of the first leaf (LMA, in g $m^{-2}$) of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FTs), and an artificial solar spectrum (AS)
Different letters indicate significantly different means ($P$ <0.05).

| | HPS | FTs | AS |
|---|---|---|---|
| DW leaf 1 | 221 a | 190 b | 209 a,b |
| DW all leaves | 420 b | 295 c | 627 a |
| LMA | 17.1 b | 18.8 a | 15.9 c |
| DW cotyledons | 71 a | 67 a | 66 a |
| DW hypocotyl | 27 b | 17 c | 123 a |
| DW roots | 79 b | 52 c | 100 a |
| DW remainder | 15 b | 9 b | 84 a |
| DW plant | 611 b | 440 c | 1001 a |

**Table 3.** Dry weight partitioning (%) to different plant organs of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FTs), and an artificial solar spectrum (AS)
Different letters indicate significantly different means ($P$ <0.05).

| | HPS | FTs | AS |
|---|---|---|---|
| Leaf 1 | 37 b | 44 a | 22 c |
| All leaves | 68 a | 67 a | 62 b |
| Cotyledons | 12 b | 15 a | 7 c |
| Hypocotyl | 4 b | 4 b | 13 a |
| Roots | 13 a | 12 a | 10 b |
| Remainder | 2 b | 2 b | 8 a |

The three growth light spectra used (Fig. 1) are different in many respects and therefore it is difficult to attribute the differences in morphological responses to specific physiological processes mediated by the spectral environment. However, two conspicuous spectral differences between the growth light environments have been subject to extensive study. First, the AS spectrum contains a considerable amount of FR wavelengths (>700 nm), whereas FR is almost absent in the two other spectra. Secondly, the HPS spectrum contains little blue (5%), whereas the AS (17%) and FT (23%) spectra contain substantially more blue.

Studies on the effects that R:FR ratios have on plant morphogenesis (e.g. Child *et al.*, 1981; Morgan and Smith, 1981) show a general trend of taller plants, longer petioles, and a relatively greater DW partitioning to the stem at the expense of partitioning to the leaves associated with lower R:FR ratios. The R:FR ratio-induced responses are regulated via the phytochrome photostationary state (PSS) which is used as an indicator for the relative amount of active phytochrome. Sager *et al.* (1988) developed a method to estimate PSS using the complete spectrum of the plants' light environment instead of simply calculating the R:FR ratio. According to this method, the PSS of the plants in the present experiment was 0.85, 0.89, and 0.72 for FTs, HPS, and AS, respectively. The lower calculated PSS for the AS treatment may partly explain the 4–5 times greater height of the AS-grown plants and greater DW partitioning to the stem compared with the two other treatments.

A greater blue light fraction, or a higher absolute amount of blue light, is generally associated with the development of 'sun-type' leaves, which are characterized by leaves with a high LMA and a high photosynthetic capacity (e.g. Buschmann *et al.*, 1978; Lichtenthaler *et al.*, 1980; Matsuda *et al.*, 2008). Also, hypocotyl elongation is inhibited by blue light via a cryptochrome-mediated response (Ahmad *et al.*, 2002). Regarding the two lamp types containing very little FR (FT and HPS), the greater blue light fraction may explain the greater LMA and shorter stem and petioles of FT-grown plants compared with HPS-grown plants. However, the interaction of blue light fraction, R:FR ratio, and other differences in the spectrum makes it impossible to draw reliable conclusions on the mechanisms underlying the wavelength dependency of the responses of the plants grown under the three spectra used in this study. Note that the growth irradiance of 100 μmol $m^{-2}$ $s^{-1}$ in the present experiment is relatively low for a tropical crop plant like cucumber. Therefore, despite the differences in spectral output of the three lamp types used, the leaves of none of the treatments can be regarded as true 'sun-type' leaves. Cucumber leaves developing under much higher irradiances of natural sunlight usually have a considerably greater LMA than the range found here (e.g. Papadopoulos and Hao, 1997). Nonetheless the overtly greater biomass production by the plants grown under the AS spectrum, compared with the two spectra widely used in protected cultivation, shows the importance of a balanced spectral composition of growth light. The use of a growth irradiance beyond the light-limited range (e.g. ⩾300 μmol $m^{-2}$ $s^{-1}$) may well result in different assimilation rates per unit leaf area due to different irradiance–photosynthesis response curves for the different treatments (as at 100 μmol $m^{-2}$ $s^{-1}$, Fig. 4). In that case plant assimilation would be determined by the acclimation of both morphology and photosynthesis, further complicating the interpretation of the results. The AS irradiance used is in the range of intensities used in climate chambers and also, in terms of both spectral composition and intensity, representative for cloudy days in, for example, a Dutch greenhouse from autumn to spring.

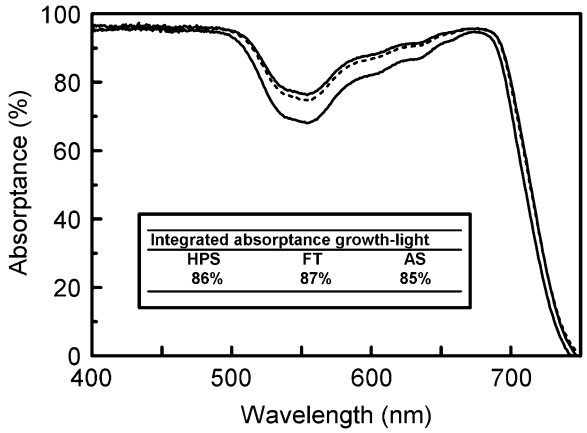


**Fig. 3.** Absorptance spectra for cucumber leaves grown under 100 μmol $m^{-2}$ $s^{-1}$ incident irradiance provided by fluorescent tubes (FTs; upper solid line), a high pressure sodium lamp (HPS; dashed line), and an artificial solar spectrum (AS; lower solid line). The table indicates the integrated absorptance (%) of the three different growth light sources, the relative spectra of which are given in Fig. 1.

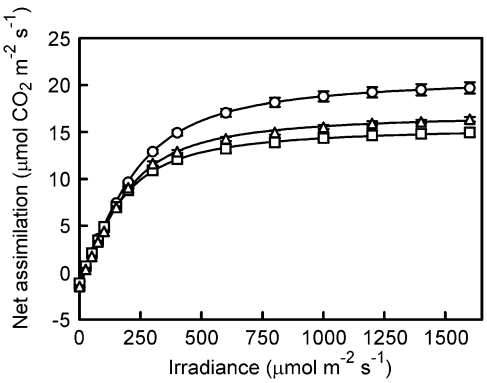


**Fig. 4.** Irradiance–$CO_2$ fixation response curves for leaves grown under 100 μmol $m^{-2}$ $s^{-1}$ incident irradiance provided by fluorescent tubes (circles), a high pressure sodium lamp (squares), and an artificial solar spectrum (triangles). Lines through the data points represent the fit to the non-rectangular hyperbola. Error bars represent the SEM.

**Table 4.** Net assimilation at growth irradiance ($A_{net}$ at 100 μmol $m^{-2}$ $s^{-1}$) and fitted light-saturated gross assimilation ($A_{max}$) of cucumber plants grown under a high pressure sodium lamp (HPS), fluorescent tubes (FTs), and an artificial solar spectrum (AS)
Different letters indicate significantly different means ($P$ <0.05).

| | HPS | FTs | AS |
|---|---|---|---|
| $A_{net}$ at 100 μmol $m^{-2}$ $s^{-1}$ | 4.9 a | 4.9 a | 4.5 b |
| $A_{max}$ (fitted) | 16.9 b | 22.5 a | 18.7 b |

Beside the morphological responses leading to better light interception by the AS-grown plants, the leaf unfolding rate (LUR, leaves per day) was also greatest for these plants, enhancing light interception even further by increasing leaf number per plant. Both assimilate supply and temperature have been identified as factors affecting LUR (Kiniry *et al.*, 1991; Marcelis, 1993). Although the AS plants had the best light interception and would therefore be expected to produce the most assimilates, leaf temperature of the AS leaves was also slightly higher. In some species, for example tomato and sweet pepper, LUR is mainly dependent upon temperature, with assimilate supply having little effect (Heuvelink and Marcelis, 1996). However, in cucumber, assimilate supply has been reported to have a strong effect on LUR (Marcelis, 1993). Challa and van de Vooren (1980) developed a mathematical model describing the dependency of the leaf development rate per week on light intensity and temperature for cucumber. According to that model, the influence of the differences in leaf temperature (<3 °C) between our treatments on LUR was negligible at the light intensity and temperature used in the present experiment, suggesting that the differences in LUR were mainly dependent on assimilate supply. Nonetheless, effects on LUR mediated via spectrum-induced signals cannot be excluded.

### *Leaf light absorptance and photosynthesis*

The lower light absorptance per leaf area of AS-grown leaves (Fig. 3) may be attributed to the lower LMA (Table 2) of these leaves. Nevertheless, despite the different absorptance spectra, the integrated absorptance of the growth light was only 2% and 1% greater for FTs and HPS, respectively, compared with AS.

The $A_{max}$ values were higher for leaves grown under spectra containing more blue light (Table 4). Blue light has been reported to increase the photosynthetic capacity of leaves (e.g. Buschmann *et al.*, 1978; Lichtenthaler *et al.*, 1980), and leaves developed under blue or mixed red/blue light have a greater $A_{max}$ than leaves grown under red light alone (e.g. Bukhov *et al.*, 1995; Matsuda *et al.*, 2004). In studies on leaf responses to irradiance, a higher irradiance was usually reported to lead to both a higher LMA and $A_{max}$, as recently reviewed by Poorter *et al.* (2009). Blue light deficiency was associated with a lower LMA in soybean (Britz and Sager, 1990), and the LMA of cucumber leaves grown under a range of different red/blue ratios correlated positively with $A_{max}$ (SWH, unpublished results). Though a trend of increasing $A_{max}$ with increasing blue fraction of the growth irradiance was found, LMA showed no clear dependency on the blue light fraction during growth. Notably, the AS- (18% blue) grown leaves had a (not significantly) greater $A_{max}$, but a smaller LMA, than the HPS- (5% blue) grown leaves (Tables 2, 4). R:FR ratios do not have a strong effect on LMA (Poorter *et al.*, 2009). It is significant that the generally reported relationship between LMA and $A_{max}$ can be broken, presumably due to effects of wavelengths in the broadband AS spectrum other than the relatively well studied blue, red, and FR effects on plant development. The change in the relationship between LMA and $A_{max}$ also indicates that the large differences in morphology between the AS plants and the HPS and FT

plants cannot be simply attributed to the considerable presence of FR wavelengths in the AS spectrum whereas the HPS and FT spectra contain very little FR (Fig. 1).

Measured net assimilation per area ($A_{net}$) at 100 μmol $m^{-2}$ $s^{-1}$ irradiance was slightly lower for the AS-grown leaves, compared with that of the two other treatments (Table 4). This measured difference may be due to the spectrum of the measuring-light, instead of a real *in situ* difference in $A_{net}$. The AS leaves developed under a spectrum containing both wavelengths exciting preferentially PSI (>680 nm) and PSII (<680 nm), whereas the HPS- and FT-grown leaves developed under a spectrum preferentially exciting PSII (Evans, 1986, 1987). The measuring-light spectrum, provided by red and blue LEDs, slightly over-excites PSII. Leaves have been shown to be able to tune their photosytem stoichiometry to the growth light spectrum in order to optimize the excitation balance between the photosystems (Chow *et al.*, 1990; Walters and Horton, 1995). Therefore, the PSII antennae of the AS-grown leaves may have been relatively greater than those in the leaves grown under FT and HPS, which would lead to a decrease in light use efficiency of the measuring-light spectrum. Nonetheless, a possible relative decrease in light use efficiency of red and blue wavelengths due to acclimation to the AS spectrum is not expected to be so large that it could outweigh the 10% lower $A_{net}$ measured on the AS leaves at 100 μmol $m^{-2}$ $s^{-1}$ irradiance.

### *Implications of the plant responses to an artificial solar spectrum*

Whereas photosynthesis per leaf area at growth irradiance was not markedly different for the leaves grown under the different spectra, plant development and biomass accumulation were. The differences are attributed to spectrum-induced differences in morphogenesis, which led to a DW of the AS-grown plants which was as much as 2.3 times greater than that of FT-grown plants after only 13 d growing at a light-limiting irradiance. The use of an artificial solar spectrum is the only method allowing a reliable comparison between a 'natural spectrum' and the spectrum of different lamp types or combinations, as under real daylight conditions the light intensity cannot be kept stable or be caused to change reliably in a predictable fashion. So far, to the best of our knowledge, no plant research studies have been published using an AS spectrum resembling a realistic solar spectrum as closely as the one used in the present experiment. Fujiwara and Sawada (2006) described a prototype of an LED-based solar lamp which seems promising, and Krizek *et al.* (1998) have compared the performance of cucumber grown for 14 d under a microwave-powered sulphur lamp and a metal–halide lamp. Although the spectrum of the sulphur lamp was not adjusted in that study so that it fitted a solar spectrum more closely and the plants were allowed to shade each other during growth, the sulphur lamp-grown plants showed a greater DW, total leaf area, petiole length, and total height than the metal–halide lamp-grown plants, as did the AS-grown plants compared with the FT- and HPS-grown plants in the present experiment.

Even in the 1950s it was recognized that FTs alone resulted in 'short plants' (Wassink and Stolwijk, 1956). Growth cabinet lighting was therefore sometimes adjusted (e.g. FTs in combination with incandescent lamps). The aim of such lighting modifications was to produce morphologically normal appearing plants rather than to produce plants using a normal (i.e. similar to sunlight) spectral irradiance (see, for example, Deutch and Rasmussen, 1973). Despite the importance of these earlier observations, it is currently uncommon for plants to be grown with the addition of FR light from incandescent lamps. Even then the extent to which plants grown under these conditions resemble field-grown plants in ways other than their appearance is unclear. A light source spectrally resembling natural sunlight should allow the production of plants under controlled environment conditions that more closely resemble their field-grown counterparts, or at least to discover for which purposes conventional light sources are unsuitable. Further, the extra productivity of the AS-grown plants in comparison with the HPS plants (1.6 times greater) points to the strong possibility that assimilation lighting in glasshouses could be made more productive. Especially in winter at northern latitudes when the natural photoperiod is short and the natural irradiance intensity is low, a considerable part of the daily irradiance is supplied by HPS lamps. Early in the production cycle when plants are small, crops could be made more productive by developing light sources that stimulate better the development of leaf area at the expense of LMA to increase light interception, and longer internodes and petioles to reduce self-shading.

## Supplementary data

The relative spectra of cloudlight in fully overcast conditions, direct sunlight, and skylight at midday in Wageningen (The Netherlands) around the autumn equinox 2009, and the spectra of the three light sources used (artificial solar, high pressure sodium, and fluorescent tube light) are available in a tabular form (Table S1) as supplementary data at *JXB* online.

## Acknowledgements

This research is supported by the Dutch Technology Foundation STW (WPB.6662). We gratefully acknowledge Jan Snel (Wageningen UR Greenhouse Horticulture) for kindly lending us their sulphur plasma lamp, and the Unifarm staff (Wageningen University) for technical assistance. We also thank two anonymous referees for valuable comments.

## References

**Ahmad M, Grancher N, Heil M, Black RC, Giovani B, Galland P, Lardemer D.** 2002. Action spectrum for cryptochrome-dependent

hypocotyl growth inhibition in Arabidopsis. *Plant Physiology* **129,** 774–785.

**ASTM.** 2003. Standard tables for reference solar spectral irradiances: direct normal and hemispherical on 37° tilted surface. Standard G173-03, American Society for Testing and Materials, West Conshohocken, PA, available from: http://www.astm.org/Standards/G173.htm.

**Britz SJ, Sager JC.** 1990. Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad-spectrum or blue-deficient light-sources. *Plant Physiology* **94,** 448–454.

**Brown CS, Schuerger AC, Sager JC.** 1995. Growth and photomorphogenesis of pepper plants under red light-emitting-diodes with supplemental blue or far-red lighting. *Journal of the American Society for Horticultural Science* **120,** 808–813.

**Bukhov NG, Drozdova IS, Bondar VV.** 1995. Light response curves of photosynthesis in leaves of sun-type and shade-type plants grown in blue or red-light. *Journal of Photochemistry and Photobiology B—Biology* **30,** 39–41.

**Buschmann C, Meier D, Kleudgen HK, Lichtenthaler HK.** 1978. Regulation of chloroplast development by red and blue-light. *Photochemistry and Photobiology* **27,** 195–198.

**Challa H, van de Vooren J.** 1980. A strategy for climate control in greenhouses in early winter production. *Acta Horticulturae (ISHS)* **106,** 159–164.

**Child R, Morgan DC, Smith H.** 1981. Control of development in Chenopodium album L. by shadelight: the effect of light quality (red far-red ratio) on morphogenesis. *New Phytologist* **89,** 545–555.

**Chow WS, Melis A, Anderson JM.** 1990. Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proceedings of the National Academy of Sciences, USA* **87,** 7502–7506.

**Deutch B, Rasmusse O.** 1974. Growth chamber illumination and photomorphogenetic efficacy I. Physiological action of infrared radiation beyond 750 nm. *Physiologia Plantarum* **30,** 64–71.

**Endler JA.** 1993. The color of light in forests and its implications. *Ecological Monographs* **63,** 1–27.

**Evans JR.** 1986. A quantitative-analysis of light-distribution between the two photosystems, considering variation in both the relative amounts of the chlorophyll–protein complexes and the spectral quality of light. *Photobiochemistry and Photobiophysics* **10,** 135–147.

**Evans JR.** 1987. The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal of Plant Physiology* **14,** 69–79.

**Franklin K, Whitelam G.** 2007. Red:far-red ratio perception and shade avoidance. In: Whitelam G, Halliday K, eds. *Light and plant development*. Oxford: Blackwell Publishing, 211–234.

**Frechilla S, Talbott LD, Bogomolni RA, Zeiger E.** 2000. Reversal of blue light-stimulated stomatal opening by green light. *Plant and Cell Physiology* **41,** 171–176.

**Fujiwara K, Sawada T.** 2006. Design and development of an LED–artificial sunlight source system prototype capable of controlling relative spectral power distribution. *Journal of Light and Visual Environment* **30,** 170–176.

**Goins GD, Yorio NC, Sanwo MM, Brown CS.** 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. *Journal of Experimental Botany* **48,** 1407–1413.

**Grime JP.** 1981. Plant strategies in shade. In: Smith H, ed. *Plants and the daylight spectrum*. London: Academic Press, 159–186.

**Heuvelink E, Marcelis LFM.** 1996. Influence of assimilate supply on leaf formation in sweet pepper and tomato. *Journal of Horticultural Science* **71,** 405–414.

**Hogewoning SW, Trouwborst G, Engbers GJ, Harbinson J, Van Ieperen W, Ruijsch J, Schapendonk AHCM, Pot SC, Van Kooten O.** 2007. Plant physiological acclimation to irradiation by light emitting diodes (LEDs). *Acta Horticulturae (ISHS)* **761,** 183–191.

**Holmes MG, Smith H.** 1977. Function of phytochrome in natural-environment. 1. Characterization of daylight for studies in photomorphogenesis and photoperiodism. *Photochemistry and Photobiology* **25,** 533–538.

**Kasperbauer MJ, Hamilton JL.** 1984. Chloroplast structure and starch grain accumulation in leaves that received different red and far-red levels during development. *Plant Physiology* **74,** 967–970.

**Kiniry J, Rosenthal W, Jackson B, Hoogenboom G.** 1991. Predicting leaf development in crop plants. In: Hodges T, ed. *Predicting crop phenology*. Boca Raton, FL: CRC Press, 30–42.

**Krizek D, Mirecki R, Bailey W.** 1998. Uniformity of photosynthetic photon flux and growth of 'poinsett' cucumber plants under metal halide and microwave-powered sulfur lamps. *Biotronics* **27,** 81–92.

**Lichtenthaler H, Buschmann C, Rahmsdorf U.** 1980. The importance of blue light for the development of sun-type chloroplasts. In: Senger H, ed. *The blue light syndrome*. Berlin: Springer-Verlag, 485–494.

**Marcelis LFM.** 1993. Leaf formation in cucumber (*Cucumis sativus* L.) as influenced by fruit load, light and temperature. *Gartenbauwissenschaft* **58,** 124–129.

**Massa GD, Kim H-H, Wheeler RM, Mitchell CA.** 2008. Plant productivity in response to LED lighting. *Hortscience* **43,** 1951–1956.

**Matsuda R, Ohashi-Kaneko K, Fujiwara K, Goto E, Kurata K.** 2004. Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. *Plant and Cell Physiology* **45,** 1870–1874.

**Matsuda R, Ohashi-Kaneko K, Fujiwara K, Kurata K.** 2008. Effects of blue light deficiency on acclimation of light energy partitioning in PSII and $CO_2$assimilation capacity to high irradiance in spinach leaves. *Plant and Cell Physiology* **49,** 664–670.

**Morgan DC, Smith H.** 1981. Control of development in *Chenopodium album* L. by shadelight: the effect of light quantity (total fluence rate) and light quality (red–far-red ratio). *New Phytologist* **88,** 239–248.

**Papadopoulos AP, Hao XM.** 1997. Effects of three greenhouse cover materials on tomato growth, productivity, and energy use. *Scientia Horticulturae* **70,** 165–178.

**Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R.** 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182,** 565–588.

**Sager JC, Smith WO, Edwards JL, Cyr KL.** 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Transactions of the ASAE* **31,** 1882–1889.

**Soares AS, Driscoll SP, Olmos E, Harbinson J, Arrabaca MC, Foyer CH.** 2008. Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with $CO_2$enrichment in the C-4 species Paspalum dilatatum. *New Phytologist* **177,** 186–198.

**Talbott LD, Nikolova G, Ortiz A, Shmayevich I, Zeiger E.** 2002. Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. *American Journal of Botany* **89,** 366–368.

**Thornley JHM.** 1976. *Mathematical models in plant physiology: a quantitative approach to problems in plant and crop physiology*. London: Academic Press.

**Trouwborst G, Oosterkamp J, Hogewoning SW, Harbinson J, van Ieperen W.** 2010. The responses of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy. *Physiologia Plantarum* 1399–3054 10.1111/j..2009.01333.x.

**Walters RG, Horton P.** 1995. Acclimation of *Arabidopsis thaliana* to the light environment—changes in photosynthetic function. *Planta* **197,** 306–312.

**Wassink EC, Stolwijk JAJ.** 1956. Effects of light quality on plant growth. *Annual Review of Plant Physiology* **7,** 373–400.

**Whitelam G, Halliday K.** 2007. *Light and plant development*. Oxford: Blackwell Publishing.

**Wilmer C, Fricker M.** 1996. *Stomata*. London: Chapmann & Hall.

**Yorio NC, Goins GD, Kagie HR, Wheeler RM, Sager JC.** 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *Hortscience* **36,** 380–383.

**Zeiger E.** 1990. Light perception in guard cells. *Plant, Cell and Environment* **13,** 739–744.