GROWTH AND DEVELOPMENT OF GREENHOUSE VEGETABLE SEEDLINGS UNDER SUPPLEMENTAL LED LIGHTING

by

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A Dissertation Submitted to the Faculty of the School of Plant Sciences

In Partial Fulfillment of the Requirements For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2013
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ACKNOWLEDGEMENTS

I would like to thank my advisor and mentor Dr. Chieri Kubota whose tenacious attention to detail and dedication taught me how to think as a scientist. She will always remain as my scientific role model.

My committee members Dr. Dennis T. Ray, Dr. Murat Kacira, Dr. Cary A. Mitchell, and Dr. Gene A. Giacomelli for their critical advice, revisions, and their unreserved patience. I hope that in the future, I am able to become your colleague.

Special thanks to Mark Kroggel for showering me with his knowledge on experimental design, instrumentation and his willingness to help at all times. Neal Barto for his unconditional help on programing, instrumentation set up, and revisions to this dissertation. Liliana Hernández for her revisions to this dissertation. Alex Dragotakes for his help during the experiment.
DEDICATION

TO MY WIFE

Thank you Liliana Hernández for helping me forge my academic studies and for helping me evolved from a college student to a man. Thank you for your love and impetus during tough times; and for your discipline to keep me humble.
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ABSTRACT

The greenhouse industry is interested in light emitting diodes (LEDs) as a light source supplement to solar light to improve plant growth and development. Before LEDs can be adopted as supplemental light for greenhouse crops, plant responses to LED spectral quality need to be investigated. Tomato and cucumber seedlings were grown under different supplemental blue and red photon flux ratios (B:R ratios) under high (16-19 mol m\(^{-2}\) d\(^{-1}\)) and low (5-9 mol m\(^{-2}\) d\(^{-1}\)) solar daily light integrals (DLIs). The supplemental daily light integral was 3.6 mol m\(^{-2}\) d\(^{-1}\). A treatment without supplemental light served as a control. Both tomato and cucumber seedlings had increased growth rate and improved morphology when grown under the supplemental LED light compared to the control. However, no significant differences were observed for any growth and morphological parameters measured in this study between the different B:R ratios for both cucumber and tomato transplants under high DLI conditions. Cucumber seedlings showed a tendency to decrease dry mass, leaf number and leaf area under low DLI conditions with increasing B:R ratio. Tomato seedlings did not show any differences between the different B:R ratios under low DLI conditions. Seedlings growth and morphology under supplemental LED light were compared to those under supplemental high pressure sodium (HPS) light. Cucumber seedlings under supplemental HPS light had greater shoot dry mass than those under the supplemental red LED light. Tomato shoot dry mass showed no differences between the HPS and red LED supplemental light treatments. Cucumber seedlings were also grown under supplemental LED pulsed lighting and supplemental LED continuous lighting. Cucumber seedlings showed no differences in
shoot dry mass and net photosynthetic rate between the treatments. Collectively, these studies concluded that red LED is preferred for supplemental lighting and the increase of blue light does not offer any benefits unless the efficiency of blue LEDs largely exceeds the red LEDs. The results of this research can be used for fixture development by LED manufactures and as a decision making tool for the adoption of supplemental LED lighting by greenhouse growers.
INTRODUCTION

In order to increase photosynthetic photon flux (PPF) inside greenhouses, commercial growers use high pressure sodium (HPS) lamps for supplemental lighting. The emerging LED lighting technology is a potential alternative to substitute for or supplement current HPS lighting technology. In contrast with HPS lamps, LEDs are solid-state lighting units that do not contain hazardous vapors. They have a cool diode surface and higher efficiency; and their spectral output can be selected to optimize for plant growth and development (Bourget, 2008). Before LEDs can be implemented to provide supplemental lighting for greenhouse crops, plant responses to LED light quality and LED energy consumption have to be further investigated, comparing with HPS lamps. Greenhouse vegetable transplants are considered a high-value crop ranging in price per plant between $0.75-1.50 for hydroponic greenhouses (L. Benne pers comm 2013). Vegetable transplants are commonly produced during seasons and geographical areas with low solar daily light integral (DLI). The present study aims to investigate vegetable-transplant responses to different spectral quality provided by LEDs in order to find the optimal LED light spectrum to supplement the sun under greenhouse conditions. In addition, this study compares plant responses of vegetable transplants between LED supplemental lighting and HPS supplemental lighting. Finally, this study explores further strategies for energy savings using the unique capabilities of LED lights.

The following studies have been conducted during this research:

A study evaluating cucumber seedling responses to supplemental LED spectral quality is presented in APPENDIX A. In this study, cucumber seedlings were grown in a
greenhouse with or without supplemental LED lighting at varied blue and red photon flux ratios (B:R ratios) under different solar daily light integrals. This study helped establish preferred LED spectral quality for cucumber-transplant production.

Similarly to the cucumber study, a study evaluating tomato seedling responses to supplemental LED spectral quality is presented in APPENDIX B. In this study, tomato seedlings were grown in a greenhouse with or without supplemental LED lighting at varied B:R ratios under different solar daily light integrals. This study helped establish preferred LED spectral quality for tomato-transplant production.

A study comparing the growth responses of cucumber and tomato seedlings to LED supplemental light and HPS supplemental light, at the same irradiance and photoperiod is presented in APPENDIX C.

A study where LED pulsed lighting was tested as supplemental light in the greenhouse is presented in APPENDIX D. This study aimed to explore further strategies for energy savings using unique LED light capabilities.
Plants are exposed to a variety of spectral qualities governed by geographical location, seasonality, changes in cloud patterns, and effects of surrounding vegetation. Additionally, plants under greenhouse cultivation in areas where natural light is not sufficient to grow a productive crop are exposed to significant changes in spectral quality caused by supplemental lighting with spectra dissimilar to natural light (Hogewoning, 2010). Plant responses to the light spectrum can be generally classified in two major aspects: growth responses and photomorphogenic responses. The growth responses are governed by the photosynthetically active radiation composed of wavelengths between 400-700 nm. The photomorphogenic responses are generally triggered by the blue (400-500 nm), UV (250-380 nm) and the interaction of red (600-700 nm) and far-red (700-800 nm) wavelengths.

Plant growth responses to spectral quality

Radiation with wavelengths between 400 and 700 nm is known as photosynthetically active radiation (PAR). The irradiance in this range is quantified by the photosynthetic photon flux (PPF) (number of photons per second per square meter of absorbing surface) and it is the driver of photosynthesis in higher plants.

Chlorophylls are the most important photoreceptors in photosynthesis. Higher plants have two kinds of chlorophylls, Chl a and Chl b, that vary in their abundance and absorption spectra. Chlorophyll a/b ratios in higher plants range from 3.3 to 4.2 in sun-adapted species and as low as 2.2 in shade-adapted species (Kriedemann, 2010). Blue and
red wavelengths are the absorption maxima of chlorophylls and absorption peaks vary slightly depending on extraction solvents. Chlorophyll peaks detected in ether extracts are 429 nm and 659 nm for Chl a and 455 and 642 for Chl b (Kriedemann, 2010). Chlorophyll a and b are both involved in light harvesting while only some forms of Chl a are linked into the energy-processing centers of photosynthesis (Kriedemann, 2010). Carotenoids also contribute to photosynthetic energy transduction and they absorb mainly in the blue and green parts of the PAR spectrum. Carotenoids are known as accessory pigments and they are present in higher plants at one third the abundance of chlorophylls (Kriedemann, 2010).

Light energy is mainly transferred to the reaction centers of PSI and PSII by chlorophylls and less efficiently by carotenoids (Croce et al., 2001; Hogewoning, 2010; van Amerongen and van Grondelle, 2001). McCree (1972) quantified single-leaf spectral responses using a spectral quantum-yield curve (maximum rate of photosynthesis per unit rate of absorption of quanta). From this curve it is evident that, at the single-leaf scenario, the contribution of red (600-700 nm) wavelengths to photosynthesis is higher than for other wavelengths. Unfortunately, McCree only tested single leaf responses to different PAR wavelengths and not the entire plant canopy responses. Agronomical and horticultural crops are often grown in high-density mono-cropping canopies. In these canopies, spectral quality available for the top leaves is very different than light quality available for lower leaves. Top-leaf spectral quality is abundant in the entire solar spectrum (Jones, 1992). In contrast, the PAR light quality in lower leaves is rich in green
wavelengths and poor in the red and blue wavelengths. As a consequence, photosynthesis in lower canopy leaves is manly driven by green wavelengths (Jones, 1992).

The effect of the PAR spectrum for CO$_2$ fixation is different under light-limited and light-saturated environments. Under light-limited conditions, light-use-efficiency is linear to incident light that the leaf absorbs. The amount of light absorbed is directly related to the fraction of light reflected and transmitted, which in turn is directly dependent on leaf pigment content, composition, and spatial distribution (Hogewoning, 2010). Accessory pigments such as carotenoids, flavonoids, and anthocyanins also absorb light, but this light is not transferred or partially transferred (carotenoids) to the reaction centers of photosynthesis. Plants grown under light-limited conditions have a lower quantum yield for CO$_2$ fixation if accessory pigments are abundant (Evans, 1986; Hogewoning, 2010; Inada, 1976; Nishio, 2000). Spectral quality of PAR can affect pigment composition and directly affect plant quantum yield for CO$_2$ fixation. For example, it is reported that blue light stimulates flavonoid synthesis (Hogewoning, 2010; Jackson and Jenkins, 1995; Kubasek et al., 1992) and a PAR spectrum (400-700 nm) rich in the blue fraction (400-500 nm) may stimulate the synthesis of accessory pigments that will then absorb limited available light and reduce plant quantum yield for CO$_2$ fixation.

Under light-saturated conditions, photosynthetic capacity can be affected by a variety of leaf characteristics. For example, physical restriction in intracellular space can limit the area for chloroplasts, decreasing photosynthetic rate (Hogewoning, 2010; Oguchi et al., 2003). Also, intracellular CO$_2$ concentration controlled by stomatal
conductance and mesophyll conductance can limit the rate of photosynthesis under light-saturating conditions (Hogewoning, 2010; Sharkey and Raschke, 1981; Zeiger, 1990).

Blue light is suspected to participate in leaf photosynthetic acclimation to irradiance (Anderson et al., 1995; Matsuda et al., 2008; Sanger and Bauer, 1987; Walters, 2005). Blue-light-grown plants show photosynthetic characteristics similar to those of plants grown under high irradiance, such as higher RuBisCO content (Eskin et al., 1991; López-Juez and Hughes, 1995), greater chlorophyll a/b ratio (Buschmann et al., 1978; Lichtenhaler et al., 1980; López-Juez and Hughes, 1995; Matsuda et al., 2008), and higher cytochrome f content (Leong and Anderson, 1984; López-Juez and Hughes, 1995). In addition, Matsuda et al. (2007) suggested that blue light was involved in acclimation to light at the chloroplast level in spinach.

Furthermore, extensive research has been done regarding the importance of blue light under sole-source artificial lighting conditions using LEDs. For example, Tripathy and Brown (1995) for wheat and Miyashita et al. (1997) for potato plantlets showed how blue light improved chlorophyll content of plants otherwise grown under red LEDs only. Also, wheat and Arabidopsis produced higher numbers of seeds when red LEDs were supplemented with blue LEDs (Goins et al., 1998; Goins et al., 1997).

Plant photomorphogenic and other responses to spectral quality

Higher plants have evolved a sophisticated system of photoreceptors that enable them to respond to spectral quality, quantity, and direction. The most common
photoreceptors in plant photomorphogenesis are the phytochromes, cryptochromes, and phototropins.

The first photoreceptor identified was phytochrome, a photochromic protein that can exist in two inter-convertible forms. One is Pr, which has a primary absorption peak at the red wavelength of 660 nm and a secondary absorption peak around 380 nm (blue), and the other is Pfr, with an absorption peak at 730 nm far-red wavelength and a smaller peak at 408 nm blue wavelength (Morgan and Smith, 1981). Phytochrome Pfr is considered the active phytochrome form. Phytochromes in higher plants are known to be encoded by the\textit{PHYA} – \textit{PHYE} small gene family (Fankhauser and Chory, 1997; Quail et al., 1995) and are demonstrated to control processes during the entire plant life cycle (Kendrick and Kronenberg, 1994). Early studies elucidated phytochrome-related plant responses that were present when brief pulses of red light were provided. The plant responses include activation of seed germination, inhibition of stem elongation in dark grown seedlings, initiation of leaf expansion, and flowering regulation (Briggs and Olney, 2001). Phytochromes also influence vegetative development including such responses as gravitropism, phototropism, and the shade-avoidance response (Parks et al., 1996; Smith, 1995).

Cryptochromes are commonly known as the blue-light receptors that regulate blue-light-induced responses in plants. Ahmad and Cashmore (1993) discovered cryptochrome 1 in \textit{Arabidopsis} and found sequence similarities to photolyases (flavoproteins) that are known to repair DNA damaged by UV light. However, cryptochrome does not have any photolyase activity. Cryptochromes in \textit{Arabidopsis} work
in conjunction with phytochromes to regulate light responses such as cell elongation and photoperiodic flowering. In addition, cryptochromes are also known to interact with phototropins to mediate stomatal opening (Li and Yang, 2007).

In order to optimize the photosynthetic efficiency of plants, phototropins are known to regulate light-dependent processes such as phototropism, light-induced stomatal opening, and chloroplast movement (triggered by fluctuations in light intensity).

Phototropism is the growth of plant parts towards or away from a light source. Commonly, shoots show positive phototropism (towards light) and roots negative tropism. *Arabidopsis* has two phototropins, phot1 and phot2, with overlapping functions. They are light-activated by protein kinases but with unique physiological roles (Christie, 2007).

Morphological plant responses to light are generally grouped by response to spectral quality blue (400-500 nm), red (600-700 nm), far-red (700-800 nm), and UV (280 – 380 nm).

Research reports on photomorphogenic responses to blue light are ample. Blue light is known to control guard-cell apertures, which affect CO₂ exchange and water relations. Schwartz and Zeiger (1984) studied stomatal opening under white light, blue and red light, and darkness for two plant species, and found that stomatal apertures under blue light were higher than white and red light at all photon fluxes in both species. This response is supported by other studies (Travis and Mansfield 1981, Pemadasa 1982).

Schwartz and Zeiger (1984) found that stomatal opening was correlated with the activity of two photoreceptors, a PAR-dependent receptor linked to the guard-cell chloroplast and
a second one specific to the blue-light-dependent system. They also observed that the blue-light photo-system saturated at low photon fluxes.

Blue light is also known to inhibit stem elongation. Cosgrove and Green (1981) studied the mechanism of hypocotyl-elongation inhibition by blue light in cucumber (Cucumis sativus L.) and sunflower (Helianthus annuus L.) seedlings by measuring changes in turgor. In that study, researchers demonstrated that blue light inhibited stem elongation by decreasing the yielding properties of cell walls.

Phototropism is another well-documented response to the blue-light portion of the spectrum (Blauw-Jansen, 1958; Curry, 1969; Hart et al., 1982; Macleod et al., 1986). For example, Macleod et al. (1986) researched phototropic responses of Avena coleoptiles using unilateral blue-light irradiation. In that study, they found that phototrophic responses not only depended on blue light, but also on physiological age of the plant.

End of day (EOD) spectral quality is known to affect stem extension (Blom et al., 1995; Chia and Kubota, 2010; Decoteau et al., 1988; Kasperbauer and Peaslee, 1973; Yang et al., 2012). Downs et al. (1957) were the first to show R:FR reversible control of stem extension in plants. In that study, a five minute exposure to incandescent radiation (rich in far-red) at the end of an 8-hour photoperiod provided by white fluorescent lamp increased stem length by 400% of bean (Phaseolus vulgaris), sunflower (Helianthus annuus), and morning glory (Ipomoea hederacea). The effect was fully reversed when plants were exposed to five minutes of red light. Kasperbauer (1971) showed that the effects of end-of-day far-red light under controlled-environment conditions resembled the
effects of vegetation shade in field-grown tobacco. Similarly, research showing the effects of far-red light added to the background white light for the entire photoperiod elucidated that the R:FR caused by vegetational shade modulated stem extension and that the stem-extension rate was proportional to the phytochrome photoequilibrium (Holmes and Smith, 1977; Morgan and Smith, 1976, 1981).

A common UV-light (280-380nm) response of plants is activation of the flavonoid biosynthetic pathway. Tevini et al. (1981) and Beggs and Wellman (1985) observed induction of flavonoids and anthocyanin by UV-B exposure in 14 plant species. Flavonoids generally accumulate absorbed UV radiation in the epidermis to keep it from reaching and damaging the photosynthetic system (Robberecht and Caldwell, 1978). Another response to UV radiation is the inhibition of epidermal cell elongation in sunflower seed (Tevini et al., 1981). Similarly several studies had shown that UV-B inhibited hypocotyl growth (Ballare et al., 1991).

Supplemental lighting

Supplemental lighting in greenhouse is commonly used in areas and seasons where solar radiation is not sufficient for productive plant growth. Solar radiation inside the greenhouse varies in terms of photosynthetic photon flux, daily light integral and spectral quality. The most apparent variable is geographical variation. For example, greenhouses in The Netherlands operated under an average yearly global radiation of 3650 MJ·m⁻², whereas greenhouses in the southwestern United States are under much higher global radiation (e.g., Arizona has 6687 MJ·m⁻² per year, averaged during the
same period) (Hemming et al., 2008). In addition, seasonal variation, shading from structural members and glazing, cloud patterns, aerosols, and dust molecules also limit light interception and spectral quality available for plant growth (Hemming et al., 2006; Kanniah et al., 2012). Daily light integral is a useful metric of the measured amount of PAR that plants receive daily. Daily light integral is commonly a limiting factor for greenhouse-grown plants. For example, a daily light integral of 30-35 mol m$^{-2}$ d$^{-1}$ should maximize greenhouse tomato production (Spaargaren, 2001), whereas a daily light integral of 13 mol m$^{-2}$ d$^{-1}$ is considered optimal for vegetable-seedling production (Fan et al., 2013). Spaargaren (2001) reported that the average daily light integral outside a greenhouse in The Netherlands from September to March was 12 mol m$^{-2}$ d$^{-1}$. Assuming a 30 to 40% glazing and structural-member reduction, the estimated daily light integral inside the greenhouse is 8.4 to 7.2 mol m$^{-2}$ d$^{-1}$ during the corresponding time in The Netherlands. Also, based on the outside daily light integral in Arizona (Tucson) reported by Kania and Giacomelli (2008) and assuming a 40% glazing and structural-member reduction in the greenhouse, the yearly natural light inside a greenhouse located in Arizona may range from 18 to 36 mol m$^{-2}$ d$^{-1}$ with a yearly average of 25 mol m$^{-2}$ d$^{-1}$, a suboptimal level for growing tomato plants. For this reason, supplemental lighting can be an effective tool for production of greenhouse crops around the world. A number of light fixtures are available to growers to increase average daily light integral, among those, high pressure sodium lamps and metal halide lamps are commonly used by growers, and LED lamps show potential for future adoption.
High pressure sodium lamps are one of the most energy-efficient lamps for supplemental lighting. About 27% of the electrical energy input is converted into PPF (400-700nm, 1000 W electronic ballast) (Nelson and Bugbee, 2013). Fourteen percent of the PPF emitted is in the wavelengths between 400 and 565 nm and the rest in the wavelengths up to 700 nm (Spaargaren, 2001). High pressure sodium lamps can be as efficient as 1.30 µmol J\(^{-1}\) input electric energy to PPF conversion (Nelson and Bugbee, 2013) and the useful life of the lamp is twice that for metal halide lamps (Spaargaren, 2001). High pressure sodium lamps are characterized by having a high surface temperature (max. 450 °C) and they need to be installed with enough distance above the plant to avoid causing thermal damage (Spaargaren, 2001).

Metal halide lamps produce a more balanced light spectrum than do high pressure sodium lamps. About 50% of the PPF emitted falls in the 400-565 nm range. The highest peaks are in the green and orange/red wavelengths (495-565 nm and 590-625 nm, respectively) (Spaargaren, 2001). In the early days of commercial greenhouse production, there was much interest in metal halide lamps mainly due to their spectral distribution. However, initially these lamps were less energy efficient than high pressure sodium. Also, their lifespan was half that of high pressure sodium, and light output during their lifespan dropped quickly. The current ceramic metal halides have an energy-photon conversion efficiency of 1.34-1.44 µmol J\(^{-1}\) PPF (315 W 3100K), which is comparable to or greater than that of high pressure sodium lamps (Nelson and Bugbee, 2013).

Light emitting diodes are a promising technology to be used as supplemental lighting. LEDs have been used extensively as a sole source of lighting for plant growth in
research (Massa et al., 2008) and commercially in plant factories (Kubota and Chun, 2000). Currently, research on the use of this technology as supplemental lighting in vegetables (Gómez et al., 2013; Hernández and Kubota, 2012; Yang et al., 2012) and ornamentals (Craig and Runkle, 2013; Currey and Lopez, 2013) is revealing the potential of this technology. Bourget (2008) described LEDs as robust, solid-state semi-conductors designed to produce desirable, narrow-spectrum light of a quality that will increase quantum efficiency in plants. LEDs have increased in efficiency very rapidly. In 2008, LEDs were less efficient than high pressure sodium lamps, and now they are up to 50% for blue LEDs and 38% for red LEDs (electrical energy input converted into PPF) (Philips, 2012), with commercial fixtures ranging from 0.84 to 1.60 µmol J⁻¹ PPF efficiency (Nelson and Bugbee, 2013). Furthermore, in contrast to high pressure sodium and metal halide, LEDs are capable of frequent “on” and “off” switching, or dimming without negative impacts to diode lifetime. This capability offers the opportunity to investigate the potential benefits of pulsed lighting to plants. Pulsed lighting is characterized by frequency (number of on/off cycles that occur per second) and duty ratio (ratio of ‘on’ time to ‘off’ time in one on/off cycle). The concept is to provide pulses of light at specific frequencies in order to optimize net photosynthetic rate (Sager and Giger, 1980; Tennessen et al., 1995). If pulsed lighting can optimize net photosynthetic rate and consequently increase growth rate in greenhouse plants, then it is possible to reduce electrical energy consumption of LED supplemental lighting.

Vegetable transplants are considered as a value-added crop and are typically produced at high densities during months with low available solar radiation.
Supplemental lighting is a common practice for the production of grafted vegetable transplants. More research studying vegetable transplant morphological and growth responses to supplemental LED lights is needed.

Growth and Morphology of vegetable transplants under LED light

1. Morphology, development, photosynthesis and growth under LED lights as sole-source lighting

Morphology

Research using LEDs as a sole lighting source has focused on spectral requirements for several plant species, including spinach, lettuce, radish, wheat, and Arabidopsis (Kim et al., 2005). Similar to these plant species, vegetable transplants also had improved morphological characteristics when grown under a combination of blue and red LEDs as a sole source of lighting (Brown et al., 1995; Hogewoning et al., 2010; Kim et al., 2005; Liu et al., 2011; Massa et al., 2008; Nanya et al., 2012; van Ieperen et al., 2012). For example, stem length of pepper seedlings was larger for plants grown under red LEDs or red combined with far-red LEDs than for plants grown under a combination of red LEDs and blue fluorescent lamps or metal halide lamps (Brown et al., 1995). Also, Liu et al. (2011) tested tomato responses to different spectral colors such as red (R), blue (B), yellow (Y), green (G), and combinations of red and blue (B:R) or B:R:G. Liu et al. (2011) concluded that the combination of B:R wavelengths produced
stronger and shorter plants, whereas seedlings grown under red LEDs only had the longest hypocotyl lengths.

Research has also focused on investigating the optimal irradiance of blue light and red light to improve vegetable transplant morphology. Studies agree that the combination of B:R at a 1:1 photon flux ratio reduces hypocotyl length in tomato and cucumber seedlings (Hogewoning et al., 2010; Liu et al., 2011; Nanya et al., 2012; Savvides et al., 2012; van Ieperen et al., 2012). van Ieperen et al. (2012) and Savvides et al. (2012) tested B light, R light, and B:R 1:1 photon flux ratio in cucumber seedlings and found that petiole length was shortest under the B:R light, and longest under B light alone. Nanya et al. (2012) reported a reduction in stem length for tomato with a 1:1 B:R ratio, compared to 1:9 B:R ratio or a 3:7 B:R ratio.

The aforementioned hypocotyl extension responses to blue and red light can be explained by previous research studies. For example, photoinhibition of hypocotyl extension is regulated by phytochrome and blue light/UV-A photoreceptors (Casal, 1994). A strong synergism between the actions of these photoreceptors is observed (Attridge et al., 1984; Casal, 1994; Fernbach and Mohr, 1990; Mohr and Drumm-Herrel, 1981). The addition of blue to red background reduces Pfr levels in the internode (Casal and Smith, 1988). Casal (1994), using wildtype and aurea tomato mutants (severely reduced phytochrome levels), demonstrated that the blue-light photoreceptors and phytochrome were interdependent (Mohr, 1986). Also, Kigel and Cosgrove (1991) studied the effects of blue and red light photoinhibition on stem elongation of hydraulic parameters (osmotic pressure, hydraulic conductance) and cell-wall properties. Kigel and
Cosgrove (1991) showed that both blue and red light reduced wall relaxation (to inhibit stem extension) by different mechanisms. Red light reduces relaxation by reducing the wall yield coefficient (yield coefficient = turgor pressure - yield threshold × rate of cell expansion) together with a small increase in yield threshold (turgor pressure that must be exceeded for wall yielding to occur). On the other hand, the inhibitory effect of blue light on wall relaxation is mainly due to an increase in yield threshold. It is plausible that plants grown under red and blue light have shorter stem length because two different pathways are regulating the reduction of stem elongation. One pathway is triggered by the blue photoreceptors and the other one is triggered by the phytochrome, together creating an additive response. Under sole-source electric lighting, red and blue LEDs can be used to create the optimal B:R photon flux ratio in order to reduce stem extension and improve vegetable-transplant morphology.

Recent research has focused on vegetable seedlings responses to LED lights to improve plant morphology in commercial indoor vegetable transplant production. For example, Fan et al. (2013) tested different photon fluxes (50 to 550 µmol m\(^{-2}\) s\(^{-1}\)) using equal red and blue PF for commercial transplant production of tomato seedlings. Fan et al. (2013) found that stem diameter did not increase, and specific leaf area and plant height did not decrease, when irradiance was higher than 300 µmol m\(^{-2}\) s\(^{-1}\). When irradiance increased from 50 to 300 µmol m\(^{-2}\) s\(^{-1}\) PPF, parenchyma cells, palisade cells, and leaf-blade thickness all increased. However, under the highest PPF (550 µmol m\(^{-2}\) s\(^{-1}\)), mesophyll cells were smaller than those under 300 or 450 µmol m\(^{-2}\) s\(^{-1}\) PPF. Another study focusing on the commercial application of LEDs for indoor tomato-transplant
production showed that the first flowering truss was set at a lower (younger) node under the lowest percent blue light (10% compared to 30% and 50%) (Nanya et al., 2012). In addition, a study comparing LED lights with fluorescent lights for healing of grafted pepper plants indicated that morphological parameters were similar under LEDs compared to fluorescent lights (Jang et al., 2013).

**Stomatal conductance**

Stomatal conductance is increased with the combination of B:R at a 1:1 photon flux ratio compared to LED monochromatic red light under sole-source LED lighting growing conditions (Hogewoning et al., 2010; Liu et al., 2011; Nanya et al., 2012; Savvides et al., 2012; van Ieperen et al., 2012). Stomatal density, epidermal cell density, stomatal pore area per unit leaf area, and stomatal conductance were significantly higher for the B:R and B treatments than for the R light treatment (Savvides et al., 2012; van Ieperen et al., 2012). Hogewoning et al. (2010) reported an increase in stomatal conductance for cucumber with the increase of B:R photon flux ratio. Cherry-tomato seedlings also showed a higher stomatal conductance under a 1:1 B:R spectral energy distribution ratio compared to a 3:3:1 B:R:G ratio treatment (Liu et al., 2011).

Previous studies on blue-light effects on plants showed that blue light significantly regulated stomatal conductance by enhancing stomatal aperture (Meidner, 1968). This blue-light response is regulated by carotenoids, zeaxanthin (Zeiger et al., 2002), phototropins, and cryptochromes (Kinoshita et al., 2001; Mao et al., 2005). Also, Savvides et al. (2012) recently analyzed the effect of spectral quality on stomatal
conductance and stomatal traits of cucumber seedlings, and revealed that the strong reduction of stomatal conductance under monochromatic red was due to a decrease in pore aperture (short-term) and stomatal density (long-term). The increase in stomatal conductance in treatments containing blue was due not to an increase in number of stomata but rather to smaller epidermal cell size. In order to improve stomatal conductance under sole-source lighting, blue and red LEDs can be easily combined to produce the optimal 1:1 B:R photon flux ratio.

Photosynthesis

Vegetable-transplant research under artificial lighting generally agrees that a combination of red and blue LEDs enhances photosynthesis compared to monochromatic red or monochromatic blue alone (Brown et al., 1995; Hogewoning et al., 2010; Kim et al., 2005; Liu et al., 2011; Nanya et al., 2012; van Ieperen et al., 2012). For example, Liu et al. (2011) measured net photosynthetic rate for cherry tomato using different LED colors (red, blue, yellow, and green) compared to a combination of B:R or B:R:G. Researchers found that net photosynthetic rate was higher for the treatments containing a combination of red and blue wavelengths. This trend is corroborated by other studies with tomato (Nanya et al., 2012) and cucumber (Hogewoning et al., 2010; van Ieperen et al., 2012).

Research with cucumber seedlings has revealed that a decrease in net photosynthetic rate of red-light grown plants was due to stomatal limitations of photosynthesis. More specifically, leaves had a lower leaf internal CO₂ concentration
(restricted CO₂ diffusion into the leaf), attributed to unresponsive stomata of plants grown under red light only (Hogewoning et al., 2010; Savvides et al., 2012). Another explanation is dysfunction of the PSII system sustained by reduced values of the $F_v/F_m$ (ratio of variable fluorescence to maximal fluorescence) potentially caused by the absence of blue light (Hogewoning et al., 2010; Savvides et al., 2012). In summary, blue-light addition to red light improves stomatal traits such as stomatal conductance and prevents damage to the photosynthetic apparatus, in particular PSII.

Two recent studies show that tomato and cucumber have different photosynthetic responses to LED B:R photon flux ratios. Hogewoning et al. (2010) measured cucumber seedling net photosynthetic rate under different B:R photon flux ratios using LEDs. They showed an increase in net photosynthetic rate and photosynthetic capacity ($A_{\text{max}}$) with increase of B:R photon flux ratio (increase in the percentage of blue photon flux up to 50%), and they attributed the response to lower CO₂ diffusion into the leaf and damage to PSII in the all-red treatment. In contrast, Nanya et al. (2012) measured net photosynthetic rate with varied B:R photon flux ratios (1:1, 1:9, and 3:7) on tomato seedlings grown for 17 days under the same 1:1 B:R ratio. They showed a higher net photosynthetic rate with the decrease of the B:R photon flux ratio (lower percent of blue to red photon flux). Nanya et al. (2012) attributed this tomato response to the higher relative quantum yield at 650 nm (0.92) compared to 450 nm (0.72) previously explained by McCree (1972).

Photosynthetic responses to the PAR spectrum are wavelength specific and species specific (McCree, 1972). The combination of red and blue LEDs improves net photosynthetic rate under sole-source lighting conditions.
Hogewoning et al. (2012) used a combination of filtered 250-W quartz-halogen lamps and blue LEDs to test wavelength-dependent photosynthetic quantum yield for cucumber seedlings. He used 19 different wavebands over 400-740 nm. The highest value for single-leaf quantum yield for CO₂ fixation on an incident-light basis was found between 620 nm and 680 nm. This value was 30% larger than for 427 to 560 nm.

Net photosynthetic rate response curves vegetable transplants under different B:R ratios will help understanding of different light-quality responses between vegetable-transplant species. Such response curves will be of even greater value if they include plant dry mass and leaf-area data in addition to single-leaf net photosynthetic rate.

In addition to the physiological responses of vegetable seedlings to LED wavelengths, research in the adaptation of this technology to commercial applications is also available. For example, Fan et al. (2013) tested different irradiances from B:R LEDs in the production of tomato transplants and found that the highest net photosynthetic rate occurred at the 300 µmol m⁻² s⁻¹ PPF treatment. Similarly, Jang et al. (2013) tested spectral quality and photon-flux using LEDs and fluorescent lamps to measure pepper CO₂ exchange rate under grafting-healing conditions. They observed that CO₂ exchange rate was lowest under monochromatic red and highest under monochromatic blue compared to a fluorescent lamp control.

Dry mass

Research on dry biomass accumulation by vegetable transplants in response to LED light quality is limited. Liu et al. (2011) reported that the dry mass of cherry tomato
seedlings was higher for plants grown under B and B:R followed by those grown under B:R:G and R, respectively. In addition, Nanya et al. (2012) showed that tomato seedlings (17 days after seeding) had greater dry mass with a 1:9 B:R photon flux ratio, compared to 3:7 B:R and 1:1 B:R ratio treatments. Pepper plants grown under R light had significantly lower plant dry mass than under the 1:100 B:R ratio treatment (Brown et al., 1995). While photosynthesis of cucumber seedlings under varied B:R ratios was well studied (e.g., Hogewoning et al., 2012), the resulting seedling dry-mass as affected by B:R ratios of LED light quality have not been reported. The increased plant dry mass under B:R LEDs compared to that under monochromatic R LEDs can be attributed to higher leaf CO$_2$ concentration governed by higher stomatal conductance with the addition of blue as described in the photosynthesis section.

Other studies testing LEDs as a commercial light source for indoor cultivation have shown the potential of LEDs for vegetable-transplant production. For example, Fan et al. (2013) tested various PPF using B:R LED (1:1 ratio) for the production of tomato transplants and demonstrated that dry mass did not increase above 300 µmol m$^{-2}$ s$^{-1}$. Also, Jang et al. (2013) tested the healing of grafted pepper plants under fluorescent lamps vs. R, B, or B:R LEDs and found that dry-mass response after healing to B:R was similar to that for the fluorescent lamp control, and healing quality between the two treatments was the same.

2. Development and growth under LED lights supplementing solar light

*Plant responses to B:R photon flux ratio*
As reviewed in the previous section, a vast number of studies using LEDs as the only lighting source have shown the importance of red and blue light for healthy growth and development (Brown et al., 1995; Hogewoning et al., 2010; Kim et al., 2005; Liu et al., 2011; Massa et al., 2008; Nanya et al., 2012; van Ieperen et al., 2012). However, limited research is available on plant developmental responses to different red and blue photon flux percentages supplementing solar light. Solar radiation has approximately 31% blue and 34% red radiation (sun-facing, 37° tilted surface, energy basis W m\(^{-2}\) data representing direct and diffuse light spectrum) (ASTM, 2003). Plant responses to LED lights supplementing solar light can be different than plant responses to LEDs as a sole lighting source. Recently, research pertaining to greenhouse-transplant responses to different supplemental B:R ratios revealed that morphological responses to LED were species specific. Hernández and Kubota (2012, unpublished) tested different percentages of supplementary red and blue LED lighting for tomato (Hernández and Kubota, 2012), pepper, and cucumber transplants under varied solar daily light integrals. It was evident for all three species that the addition of LED light improved morphological parameters such as increased stem diameter and decreased final hypocotyl length compared to unsupplemented controls.

Cucumber, tomato, and pepper had the same responses to different B:R ratios of supplemental LED lighting under high solar daily light integral. However, under low daily light integral, a higher B:R ratio decreased leaf area expansion for cucumber. Hernández and Kubota (2012, unpublished) postulate that responses to LED supplemental light quality are species specific and that solar PPF fulfilled blue-light
requirements of vegetable transplants under both high and low daily light integral. They also concluded that red light alone was preferred for supplemental lighting. Similar to supplemental LED lighting conditions, plant responses to light quality under sole-source LED lighting are also species specific (Cope and Bugbee, 2013; Hogewoning et al., 2010; Nanya et al., 2012)

Studies comparing the traditionally used high pressure sodium technology to emerging LED technology have shown strong morphological changes in vegetable transplants when grown under LEDs compared to high pressure sodium lamps. Gislerød et al. (2012) reported reduced leaf expansion and stem extension for tomato, cucumber, and lettuce under LEDs compared to HPS lamps. Hernández and Kubota, (unpublished) demonstrated no differences in tomato hypocotyl length for the red-light treatment compared to a blue-light treatment, and both were 13% shorter in final hypocotyl length than those in the high pressure sodium treatment. In contrast, cucumber under red LEDs showed a 35% reduction in hypocotyl length compared to the blue treatment and high pressure sodium treatment. Also, stem diameter was increased by 7% under the red LED treatment compared to the blue and high pressure sodium treatments. In this same study, the first flowering truss of tomato was set at a lower nodal potion under the red LED treatment (Hernández and Kubota, unpublished).

From these studies, we can conclude that the complex morphological responses of vegetable transplants to LED supplemental lighting are species specific and solar-daily light integral specific. It appears that tomato and pepper have similar responses to B:R supplemental LED lighting, and no differences are detected between different B:R ratios
under varied solar background daily light integrals. It also appears that cucumber 
transplants are more sensitive to LED light qualities than tomato and pepper transplants, 
and that addition of blue light under low solar daily light integral conditions reduces leaf 
expansion that can consequently reduce plant growth (Hernández and Kubota, 
unpublished). Also under supplemental lighting conditions, cucumber-transplant 
hypocotyl elongation is suppressed by red LED light compared to blue LED and high 
pressure sodium lights. Future research will identify species-specific morphological and 
developmental responses of vegetable transplants with an LED supplemental spectrum. 
Further examination of mature plant responses, including fruit yield and fruit quality, is 
warranted.

End-of-day plant responses

The recent technological development of high-intensity far-red LEDs has 
promoted research on vegetable-transplant morphological responses to red and far-red 
ratios, especially with regard to stem extension.

Adequate hypocotyl elongation is important for grafted vegetable-seedling 
rootstocks. Short hypocotyls can lead to scions contacting the soil, thereby rooting and 
defeating the purpose of the graft. Recent studies using far-red LEDs on tomato and 
cucurbit rootstocks indicate two key parameters that optimize hypocotyl length: 1) 
species-specific end-of-day (EOD) far-red-light response curve and 2) minimum dose 
(irradiance x duration) requirements of EOD far-red light. For example, Chia and Kubota 
(2010) and Yang et al. (2012) examined the EOD treatments as a non-chemical means to
increase hypocotyl length. Tomato and cucurbit rootstocks were exposed to far-red LEDs (peak wavelength 735 and 742 nm, respectively), and incandescent lamps. The authors concluded that 1) light blends higher in far-red (i.e., low R/FR) were more effective promoting hypocotyl elongation, 2) a minimum R:FR dose of 4 mmol m$^{-2}$ d$^{-1}$ is sufficient to enhance hypocotyl length, and 3) the photon flux of light (µmol m$^{-2}$ s$^{-1}$) and amount of time the lights are energized are flexible as long as the minimum dose is achieved.

Additional research in this area is needed to find rootstock species-specific EOD far-red-light minimum dosage to enhance hypocotyl length.

Research on practical means to transfer EOD research to commercial propagators will further increase LED adoption. For example, Yang et al. (2012) pioneered the idea of installing far-red LEDs on irrigation booms to apply EOD far-red light to growing transplants in propagation greenhouses.

**Plant growth responses**

The recent development of high-intensity LED lighting has generated interest in its application for supplemental greenhouse lighting for vegetable transplants. Vegetable transplant light-quality responses have to be re-evaluated for supplemental lighting conditions due to solar light’s broad spectrum. Recent research has shown that growth response to LED supplemental lighting was species specific and that morphological responses to different spectra contributed to the differences in growth response. For example, Hernández and Kubota (unpublished) tested tomato, pepper, and cucumber transplants grown under different percentages of supplemental blue and red LEDs at
varied solar daily light integrals. Their results showed an increase in dry mass under LED supplemental light compared to no supplemental light for both high and low solar daily light integrals for all three vegetables. When comparing growth parameters between the different proportions of blue-to-red LED light, tomato and pepper did not exhibit differences between the treatments. However, cucumber exhibited a significant decrease in number of leaves, a decrease in dry mass, and an increase of chlorophyll with the increase of B:R ratio. Net photosynthetic rate did not show any differences between any of the LED treatments in tomato, cucumber and pepper.

Hernández and Kubota (unpublished) compared the growth of tomato and cucumber transplants under supplemental red LEDs, blue LEDs, and high pressure sodium lamps. Tomato exhibited no differences in net photosynthetic rate between spectral-quality treatments when measured under 1000 µmol m$^{-2}$ s$^{-1}$, and ambient CO$_2$ concentration. In contrast, a significant increase in net photosynthetic rate was observed for the blue treatment compared to high pressure sodium treatment in cucumber. Also, total chlorophyll per unit leaf area of tomato was higher for the high pressure sodium treatment compared to the blue LED treatment. This is in contrast to cucumber, for which total chlorophyll per unit leaf area was higher under the blue LED treatment compared to the high pressure sodium control. Similarly, the addition of intra-canopy blue light supplementing high pressure sodium light had positive effects on development and photosynthetic pigment accumulation by cucumber plants (Menard et al., 2006; Samuoliene et al., 2012) but not for tomato (Menard et al., 2006; Samuoliene et al., 2012) or pepper (Samuoliene et al., 2012) plants.
PRESENT STUDY

The research for this dissertation is presented as four manuscripts in separate appendices. Each manuscript contains an introduction, methods, results, discussion, and conclusions. The following are summaries of important findings from each of the manuscripts.

GROWTH AND MORPHOLOGICAL RESPONSE OF CUCUMBER SEEDLINGS TO SUPPLEMENTAL RED AND BLUE PHOTON FLUX RATIOS UNDER VARIED SOLAR DAILY LIGHT INTEGRALS (APPENDIX A)

The objective of this study was to find the optimal supplemental LED spectral quality for greenhouse cucumber in order to enhance plant growth and improve plant development. In order to find the optimal blue (B) and red (R) photon-flux ratio, cucumber seedlings (cv. Cumlaude) were grown in a greenhouse with and without supplemental LED lighting (DLI: 3.5 mol m\(^{-2}\) d\(^{-1}\)) at varied blue and red photon-flux ratios under different solar daily light integrals. The treatments were 0B:100R%, 4B:96R%, and 16B:84R% photon flux, and a control without supplemental lighting. The two contrasting daily light integrals were 5.2 and 16.2 mol m\(^{-2}\) d\(^{-1}\). Growth and morphological parameters were all improved under supplemental LED lighting compared to the no supplemental light control. Under high daily light integral, no significant differences were observed for any of the parameters between the different B:R ratios. Under low daily light integral, chlorophyll concentration increased with increasing blue photon flux (without increasing PPF) of the supplemental lighting. Reduction in dry mass, leaf count, and leaf area were observed with increasing blue photon flux (without
increasing PPF) under low-daily light integral. The reductions in dry mass and leaf count were attributed to the reduction in leaf area. No differences in net photosynthetic rate among the B:R ratios was measured. From this study, we concluded that the use of 100% red LED is preferred for cucumber seedlings, and additional blue LED was not beneficial.

This manuscript was prepared for submission to: Scientia Horticulturae

TOMATO SEEDLING GROWTH AND MORPHOLOGICAL RESPONSES TO SUPPLEMENTAL LED LIGHTING RED:BLUE RATIOS UNDER VARIED DAILY SOLAR LIGHT INTEGRALS (APPENDIX B)

The objective of this study was to find the preferred supplemental LED spectral quality for greenhouse tomato in order to increase plant growth and improve plant development. Similarly to the cucumber study, tomato seedlings (cv. Komeett) were grown in a greenhouse with and without supplemental LED lighting. The supplemental LED B:R photon-flux-ratio treatments were 0B:100R%, 4B:96R%, 16B:84R% PF and a control without supplementary lighting. The supplemental LED B:R ratio lighting treatments provided 55.5 ± 1.4 μmol m\(^{-2}\) s\(^{-1}\) PF for 18 hours and were evaluated under low and high daily solar light integrals (8.9 ± 0.9 and 19.4 ± 1.9 mol m\(^{-2}\) d\(^{-1}\), respectively). Growth and morphological parameters including shoot dry mass, leaf count, stem diameter, hypocotyl length, leaf area, and chlorophyll concentration indicated the benefit of supplemental light; especially under low daily light integral. However, there were no significant differences among different B:R photon flux ratios regardless of
daily light integral. These results suggest that for ‘Komeett’ tomato seedlings grown in a greenhouse, the use of 100 % red LED supplemental lighting was sufficient and no additional blue light was required regardless of daily light integral.

This study was published:

LED SUPPLEMENTAL LIGHTING FOR VEGETABLE TRANSPLANT PRODUCTION: SPECTRAL EVALUATION AND COMPARISONS WITH HID TECHNOLOGY (APPENDIX C)

The objective of this study was to investigate cucumber and tomato responses to different supplemental LED B:R photon-flux ratios and to compare side-by-side supplemental LED-preferred spectrum to current high pressure sodium supplemental lighting spectrum. Results showed that shoot dry mass under red LEDs was not significantly different from that under high pressure sodium for tomato but was 25% lower under red LEDs than high pressure sodium for cucumber. It appears that growth of tomato seedlings (2nd true leaf stage) under 100% red LED was comparable to that under high pressure sodium light, but the growth of cucumber seedlings was greater under the high pressure sodium treatment than for the 100% red LED treatment.

This study was submitted for publication to: Acta Horticulturae 30/July/2013

PULSING EFFECTS OF SUPPLEMENTAL LED LIGHTING ON CUCUMBER SEEDLING GROWTH AND MORPHOLOGY IN GREENHOUSES (APPENDIX D)
The objective of this study was to evaluate plant responses to supplemental LED pulsed lighting (turn on and off high photon fluxes with rapid frequency). Cucumber (cv. Cumlaude) seedlings were grown to the second true leaf stage. Sunlight was supplemented with red LEDs for 18 hours with an average photon flux of 60 μmol m\(^{-2}\) s\(^{-1}\). The treatments consisted of a no supplemental lighting (control), continuous red-LED lighting, and pulsed red-LED lighting at 50% duty ratio (2.5 kHz). Results showed that both the continuous and the pulsed supplemental lighting improved the growth and morphology of cucumber seedlings compared with those in the control. Plant hypocotyl length was greater in the pulsed-lighting treatment than that of the continuous lighting treatment by 7.5%. However, there were no significant differences in number of leaves, fresh and dry mass, leaf area, and chlorophyll concentration between the two treatments. We concluded that pulsed lighting at 50% duty ratio and 2.5 kHz could not substitute for continuous lighting as supplemental light for cucumber because no benefits were observed and compact plants are preferred by nursery propagators.

This study was submitted for publication to: Acta Horticulturae 30/July/2013

APPLICATION OF RESULTS

The results of this research can be directly applied to the vegetable transplant industry. This research showed an increase in vegetable transplant growth using supplemental LED lighting, even under relatively high light conditions (high DLI). If LED efficiencies continue to increase while the costs per fixture decrease, the adoption of LEDs as supplemental lighting in high light areas may be justifiable. This research also showed that at current LED efficiencies, supplemental red LED light is preferred for
cucumber seedlings and sufficient for tomato seedlings growth and development. LED manufactures looking to optimize LED fixtures for horticultural applications may use this information for product development. For example, LED fixtures for supplemental lighting can be constructed with only red LEDs until blue LEDs efficiencies increase. This research showed that even though LEDs are a promising technology as supplemental lighting for the production of vegetable transplants, high pressure sodium lamps are still more efficient at producing dry mass per kilowatt-hour. Vegetable transplant growers looking to adopt supplemental LED technology can use this information to help decide when doing so will maximize the returns on their investment. And finally, this research presented data from a preliminary study using LED pulsed lighting as supplemental lighting. Scientists looking to further investigate the potential benefits of LED pulse lighting can use these results as basis to design new research.
REFERENCES


APPENDIX A: GROWTH AND MORPHOLOGICAL RESPONSE OF CUCUMBER
SEEDLINGS TO SUPPLEMENTAL RED AND BLUE PHOTON FLUX RATIO
UNDER VARIED SOLAR DAILY LIGHT INTEGRALS

To be submitted to Scientia Horticulturae
Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals

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ABSTRACT

High intensity light-emitting diodes (LEDs) have the potential to be used as supplemental lighting technology in greenhouses. However, LED light quality requirements of greenhouse crops grown when supplementing the solar spectrum are unknown. In this study, to find the requirements, cucumber (Cucumis sativus L. cv. Cumlaude) seedlings were grown in a greenhouse with and without supplemental LED lighting (PPF: 54 ± 1.1 μmol·m²·s⁻¹) at varied blue (400–500 nm with the peak at 455 nm) and red (600-700 nm with the peak at 661 nm) photon flux (PF) ratios (B:R ratios) under different solar daily light integrals (DLI). The treatments were 0B:100R% (54 μmol·m²·s⁻¹ red PF), 4B:96R% (2.3 and 52 μmol·m²·s⁻¹ blue and red PF, respectively), 16B:84R% (8.5 and 46.2 μmol·m²·s⁻¹ blue and red PF, respectively), and a control without supplemental lighting. The solar DLIs during the experiment were 5.2 ± 1.2 and 16.2 ± 5.3 mol·m²·d⁻¹ created inside a greenhouse using shade screen. Regardless of B:R ratio, morphological and growth parameters were all improved under supplemental LED
lighting compared to the no-supplemental-light control. Under high DLI conditions, no significant differences were measured for any parameters between the different B:R ratios. Under low DLI, chlorophyll concentration increased with increasing B:R ratio (i.e., increasing blue PF without increasing photosynthetic photon flux PPF) of the supplemental lighting. Reduction in dry mass, leaf count, and leaf area were measured with increasing B:R ratio under low DLI conditions. The reduction in dry mass and leaf count were attributed to the reduction in leaf area. Leaf net photosynthetic rate measured under ambient CO₂, ambient temperature, and 1000 μmol·m⁻²·s⁻¹ PPF (light source: tungsten halogen lamp) also showed no difference among treatments of B:R ratios, indicating that B:R ratio treatments did not cause any changes in plant photosynthetic apparatus. When used for supplemental lighting in the greenhouse, use of 100% red LED is preferred for cucumber seedlings, and additional blue LED was not beneficial.

*Keywords*: DLI, greenhouse, light-emitting diode, *Cucumis sativus*, spectral quality, B:R ratio.

1. Introduction

Solar radiation inside the greenhouse varies in terms of photosynthetic photon flux (PPF), daily light integral (DLI), and quality. The most apparent variable is geographical location. Greenhouses in The Netherlands operate under an outdoor yearly global radiation of 3650 MJ·m⁻² (average of one year) while greenhouses in the southwestern United States are under much higher global radiation (e.g., Arizona has
6687 MJ·m⁻²·year⁻¹, averaged over the same period) (Hemming et al., 2008). In addition, seasonal variation, structural shading, cloud patterns, atmospheric aerosols and dust molecules also affect irradiance and light quality available for plant growth (Hemming et al., 2006; Kanniah et al., 2012). Light is commonly a limiting factor for greenhouse-grown plants. For example, 30-35 mol·m⁻²·d⁻¹ is reportedly an optimal light level to maximize greenhouse tomato production (Spaargaren, 2001), and similarly 13 mol·m⁻²·d⁻¹ is considered optimal for vegetable-seedling production (Fan et al., 2013). Spaargaren (2001) reported that DLI outside the greenhouse in The Netherlands from September to March was 12 mol·m⁻²·d⁻¹ on average. Assuming a 30 to 40% glazing and structural reduction, the estimated DLI inside the greenhouse is 7.2 to 8.4 mol·m⁻²·d⁻¹ during the corresponding time in The Netherlands. Also, based on the outside DLI in Arizona (Tucson) reported by Kania and Giacomelli (2008) and assuming 40% glazing and structural reduction, the yearly natural light inside a greenhouse located in Arizona may range from 18 to 36 mol·m⁻²·d⁻¹ with a yearly average of 25 mol·m⁻²·d⁻¹, a suboptimal level for growing tomato plants. For this reason, supplemental lighting can be an effective tool for production of greenhouse crops around the world. However, due to high energy costs, use of supplemental lighting in high-light regions such as Arizona is not a common practice in commercial greenhouses.

High pressure sodium (HPS) lamps and metal halide (MH) lamps are most commonly used for supplemental lighting in greenhouses, whereas LED lamps show potential for future adoption. The efficiency of LEDs has increased very rapidly. Today, the electric-energy-to-light conversion of LEDs is up to 49 - 50 % efficiency for blue
LEDs and 32 - 38% for red LEDs (Cope and Bugbee, 2013; Philips, 2012) compared to 19-27% efficiency of HPS lamps (Cope and Bugbee, 2013; Spaargaren, 2001). Also, LEDs have a relatively cool diode surface that permits its installation closer to the canopy than HPS and MH lamps, and they are also robust and durable (Bourget, 2008). In addition to the higher efficiency, longer durability, and cooler diode surface than HPS lamps, one of the most important advantages of LEDs is the capacity of adding multiple narrow-spectrum diodes to manipulate light quality. The light quality provided by multiple LEDs can be optimized to match crop-specific light requirements in order to increase plant quantum efficiency, promote growth, and/or improve morphology.

LEDs have been studied extensively as the sole source of light for plant growth (Massa et al., 2008) and commercially in plant factories to a limited extent (Kubota and Chun, 2000). Research using LEDs as the sole light source generally agree that blue (400-500 nm) and red (600-700 nm) light are optimal wavelengths to promote plant growth and maintain normal plant development (Brown et al., 1995; Hogewoning et al., 2010b; Kim et al., 2005; Liu et al., 2011; Massa et al., 2008; Nanya et al., 2012; van Ieperen et al., 2012). In vegetable seedlings such as pepper, tomato and cucumber, the combination of blue and red wavelengths promotes growth and improves morphology of plants when grown under artificial light only. For example, Liu et al. (2011) grew cherry tomato seedlings using different LED colors and found that tomato plants had shorter height and exhibited higher net photosynthetic rate (NPR) in the blue plus red, or in the blue plus red plus green treatments than for monochromatic red, blue, yellow, or green. Also, van Ieperen et al. (2012) and Savvides et al. (2012) showed that cucumber
seedlings grown under blue plus red lights were more compact and had higher stomatal conductance than did monochromatic red or monochromatic blue treatments. Likewise, Hogewoning et al. (2010b) showed an increase of NPR with the increase of blue-light percentage at the same PPF.

Recently, more research is available on using high intensity LEDs for supplemental lighting of greenhouse ornamental crops (Currey and Lopez, 2013) and mature vegetable plants (Gómez et al., 2013; Trouwborst et al., 2010). Most research on LEDs for supplemental lighting in greenhouses is focused on comparisons with conventional HID technology. These research reports employed red and blue LEDs mixed at a ratio between 5 - 20% blue PF (80-95% red PF) (Gómez et al., 2013; Trouwborst et al., 2010), presumably based on previous studies conducted under LEDs as the sole source of light.

Plant responses to supplemental blue PF in greenhouses can be different than those to supplemental blue PF under sole-source artificial lighting, as the background solar radiation has much blue PF, possibly meeting the requirement of blue PF for plant growth. According to ASTM (2003), solar radiation contains approximately 31% blue and 34 % red radiation over photosynthetically active radiation (sun-facing, 37° tilted surface, energy basis W·m⁻² data representing direct and diffuse light spectrum). However, limited research is available on plant responses to different blue and red percentages of greenhouse supplemental lighting. In a previous study, we examined different percentages of red and blue PF in supplemental lighting to grow tomato seedlings under varied solar DLIs (Hernández and Kubota, 2012). Tomato seedlings did
not show any responses to the varied B:R ratios of supplemental lighting regardless of 
solar DLI. Therefore, solar radiation seemed to fulfill blue-light requirements of tomato 
seedlings under the experimental conditions, and red light alone was sufficient for 
supplemental lighting of tomato seedlings.

To further investigate the light-quality requirement, especially the blue PF 
requirement in supplemental lighting, we conducted a similar experiment using a 
different plant species, cucumber (Cucumis sativus L.). Research under sole-source 
artificial light suggested that plant responses to light quality are species specific (Cope 
and Bugbee, 2013; Hogewoning et al., 2010b; Nanya et al., 2012), and this may be true 
for supplemental lighting under greenhouse conditions. In order to take advantage of the 
potential of LEDs as photosynthetic supplemental light technology, it is imperative to 
investigate plant responses under varied B:R ratios, varied solar DLIs, and for other 
important greenhouse transplant species.

Cucumber is the second-most produced vegetable in hydroponic greenhouses in 
the USA. Around 23% of all vegetables grown hydroponically in the USA are 
cucumbers (Nanfelt, 2011). In addition, cucumbers are known to be more sensitive to 
light quality and irradiance treatments than other greenhouse crops such as tomato and 
Therefore, while we did not see any significant differences in plant growth and 
development as affected by supplemental light quality (B:R ratios) for tomato, we 
hypothesized that cucumber seedlings may respond to different B:R ratios under low 
solar-DLI conditions. Our objectives were to find the optimal light quality of
supplemental LED light for cucumber transplants, to identify any interactions caused by background solar DLI, and consequently to advance the adoption of LED fixtures with optimal light quality by greenhouse-production areas.

2. Materials and methods

2.1. Plant materials and growing conditions

Greenhouse cucumber ‘Cumlaude’ seeds (Rijk Zwaan, Bergschenhoek, The Netherlands) were sown in rockwool plugs (plug size: 2.5 cm L x 2.5 cm W x 4.0 cm H) (Grodan, Delta, Canada) then covered with a layer of vermiculite. Seeded trays were kept in darkness for 24 hours and the substrate temperature was maintained at 28 °C. Plugs were then transferred to rockwool cubes (cube size: 7 cm L x 7 cm W x 6.5 cm H) (Grodan, Delta, Canada) and then moved into the greenhouse. The greenhouse (Tucson, AZ) was covered with double-layer acrylic glazing, oriented north to south, and equipped with pad-and-fan cooling system, under-bench misting system for humidification, and natural-gas-forced hot-air heating system. The greenhouse had a floor area of 108 m², with 2.5-m gutter height and 4.3-m peak height. When cotyledons of seedlings were expanded, uniform seedlings were selected and subjected to the treatments. The plants were sub-irrigated as needed with nutrient solution containing (mg/L) 90 N, 47 P, 144 K, 160 Ca, 60 Mg, 113 S, 105 Cl, as well as micro-nutrients.
2.2. LED light source

The LED fixtures (CCS Inc., Kyoto, Japan) used in the present study were 35 x 34 cm in area and built with 24 blue LEDs (peak wavelength 455 nm, full width at half maximum (FWHM): 15 nm) and 510 red LEDs (peak wavelength 661 nm, FWHM: 20 nm) with a digital controller (ISC-101-4, CCS Inc., Kyoto, Japan) capable of controlling output of blue and red LEDs independently. Six LED fixtures were mounted in the greenhouse at 1.3 m above a bench uniformly irradiating six 0.3-m² plant-growth areas. Before starting the experiment, PF and light distribution from all fixtures was measured using a spectroradiometer (PAR-NIR, Apogee Instruments Inc., UT, USA) to ensure light quality and distribution consistencies among the fixtures.

2.3. Environmental conditions: Solar DLI, temperature, atmospheric moisture

The greenhouse floor area was divided in two sections from north to south. Inside the greenhouse the top (1.5 m above the bench) and the side of one half (west section) were covered with two layers of shade cloth (XLS55F harmony revolux) with manufacturer specifications of 55% light transmission (Ludvig Svensson Inc., Charlotte, NC, USA). The two layers of shade cloth were placed on top of each other to achieve ~ 25 % radiation transmittance and create low solar DLI conditions. The other half (east section) of the greenhouse was left without shade cloth to create high solar DLI conditions. DLIs were recorded everyday throughout the experiment using a quantum sensor (LI-190, LI-
COR Inc., Lincoln, NE) placed at 1.35 m height from the bench in the middle of the each section of greenhouse, in order to avoid contamination by supplemental lighting. In order to maintain similar air temperatures between the two DLIs, a set of fans were placed under the benches inside the greenhouse. Air temperature measured 60 cm above the canopy and air temperature measured directly under the leaves (near-canopy air temperature) were recorded for each treatment with two fine-wire thermocouples (type T, gauge 24, Omega Inc., Stamford, CT, USA) (16 thermocouples in the greenhouse). Atmospheric moisture was measured in the middle of the greenhouse using a humidity probe (HMP110, Vaisala Inc., Helsinki, FI.). Atmospheric moisture was maintained by a misting system (Orbit Irrigation Products Inc., Bountiful, Utah) installed on each bench misting to the air (plants were not in contact with misting water). All sensors were connected to a CR-1000 datalogger with a multiplexer (Campbell Scientific, Logan, UT, USA) scanned every minute and recorded at 10-minute intervals.

2.4. Supplemental light treatments

Three different B:R ratios were applied to LED supplemental lighting under two DLI conditions. Percentages of blue and red PF were adjusted independently by the input voltage of blue and red LEDs using the controller. Photon fluxes over the bench surface were measured on five locations in the plant-growth area using a spectroradiometer (PAR-NIR, Apogee Instruments Inc., Logan, UT, USA) (Table 1). Additionally, a no-supplemental light treatment (control) was included under both DLI conditions. In order
to have similar shading patterns in the LED treatments and the control treatment, a mock plywood panel of identical size to the LED fixture was installed at the corresponding location above the control treatment. There were a total of four treatments within the high or low DLI sections of the greenhouse, separated approximately 3.3 m each to avoid light contamination between treatments. The location of the light-quality treatments within the same DLI section in the greenhouse was rotated daily from north to south in order to minimize environmental differences between treatments. Additionally, individual experimental plants were systematically rotated daily inside the small growing area in order to ensure even light exposure to all plants.

2.5. Plant measurements and experimental design

Sixteen to 18 days after sowing, when plants reached the transplant stage, plant height, hypocotyl length, and epicotyl length were measured using a ruler. Stem diameter was measured using a digital caliper. Plant shoots were then removed from the roots by cutting hypocotyl at the substrate surface line, and the shoot fresh mass was recorded using an electronic balance. Two 2.9 cm$^2$ leaf circles were cut in order to quantify chlorophyll concentration based on Moran and Porath (1980). Number of leaves per plant greater than one centimeter were counted and recorded. Images of leaves and cotyledons were then scanned and leaf area per plant was calculated using a LIA 32 software for leaf area analysis (K. Yamamoto, Nagoya, Japan). Plant shoots were placed in paper envelopes and transferred into a drying oven at 80 °C for at least 48 hours to
obtain the shoot dry mass. Net photosynthetic rate was measured with a portable photosynthesis system (CIRAS-2, PP System, MA, USA) with a 1000 μmol·m⁻²·s⁻¹ PPF (halogen lamp), under greenhouse temperature of 25 ± 2 °C and ambient CO₂ concentration. The experiment was repeated three times: 1) 27-September-2011 to 15-October-2011; 2) 18-October-2011 to 4-November-2011; and 3) 7-October-2011 to 22-October-2011. Each repetition had 20 cucumber plants (experimental units) per treatment for a total of 80 plants per repetition. Experimental plants were surrounded by a row of “border” plants to prevent edge effects. Analysis of variance (P = 0.05) was performed to identify any difference among treatments, considering each plant as an experimental unit (n=20) repeated three times. Mean separations were analyzed using Tukey-Kramer HSD (P = 0.05). In addition, the correlation coefficient and linear regression were applied to identify the quantitative response of percent blue light of the supplemental lighting. Analysis of variance confirmed that there were no significant interactions between the treatment and replication in time. The statistical analysis was performed using JMP software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Environmental conditions

Environmental conditions inside the greenhouse during the three repetitions are shown in Table 2. High DLI conditions had at least three times higher DLI than low DLI conditions.
conditions. Supplemental LED lighting contributed 18% and 40% of total DLI (supplemental + solar) for high and low DLI conditions, respectively (Fig 1). Greenhouse air temperature was maintained the same under both DLI conditions in order to minimize temperature effects on plants. However, canopy air temperature was 0.7 °C higher under the high DLI conditions during the day, due to the higher short-wave radiation in the high DLI conditions. Actual leaf temperature could have been higher than canopy air temperature under the high DLI conditions.

3.2. Effects of supplemental LED lighting

Dry mass, fresh mass, and leaf number were increased by the addition of supplemental LED light compared to the no-supplemental-light treatment (control) by 21%, 13%, and 4%, respectively for high DLI conditions, and 32%, 22%, and 10%, respectively, for low DLI conditions (Table 3). Chlorophyll concentration was also increased by the addition of supplemental LED light compared to control by 26% and 21% for high and low DLI conditions, respectively (Table 3). There was no significant difference in NPR between supplemental LED light treatment and control treatment under high DLI conditions. However, under low-DLI conditions the supplemental LED light treatment had 17% higher NPR than the control.

Morphological parameters were also improved by the supplemental lighting (Table 4). Hypocotyl length and epicotyl length were decreased by 32% and 12%, respectively under high-DLI conditions and by 30% and 12%, respectively under low-
DLI conditions by supplemental lighting. Stem diameter and leaf area were increased by supplemental lighting by 11 % and 8 %, respectively under high DLI conditions and by 20 % and 15 %, respectively under low DLI conditions.

3.3. Effects of percent blue photon flux of the supplemental LED lighting

Growth, NPR, chlorophyll concentration, and morphology as affected by the B:R ratios and the results of pairwise comparisons between individual treatment and the control (no supplemental lighting) are shown in Tables 5 and 6, respectively.

Under high-DLI conditions, shoot dry mass for 0B-100R%, 4B-96R%, and 16B-84R% was 21, 22, and 18 % higher than the control, respectively. The 0B-100R%, 4B-96R%, and 16B-84R% treatments had 14, 11, and 12 % higher shoot fresh mass than the control, respectively. Leaf number per plant of the 0B-100R% treatment was 7.4% greater than the control. Chlorophyll concentration for 0B-100R%, 4B-96R%, and 16B-84R% was 23, 23, and 28 % higher than the control, respectively. NPR in the control treatment was no significantly different from any of the B:R ratio treatments. Under high-DLI conditions, the increase of blue photon flux did not significantly affect shoot dry mass, shoot fresh mass, leaf number, chlorophyll concentration, or NPR in the B:R ratio treatments.

Under low-DLI conditions, shoot dry mass for 0B-100R%, 4B-96R%, and 16B-84R% was 36, 33, and 27 % higher than for the control, respectively. The increase of blue photon flux in the B:R ratio treatments showed a weak effect on decreasing dry mass in the regression analysis (P = 0.0536). The 0B-100R%, 4B-96R%, and 16B-84R%
treatments had 24, 21, and 19% higher shoot fresh mass than the control treatment, and there was no significant linear response found by percent blue photon flux. Leaf number per plant of the 0B-100R% treatment was 12% greater than the control, and plant leaf number significantly decreased with the increase of blue photon flux in the B:R ratio treatments (P = 0.0029). Chlorophyll concentration increased with the increase of blue photon flux in the B:R ratio treatments (P = 0.0012), and chlorophyll concentration for the 0B-100R%, 4B-96R%, and 16B-84R% treatments was 15, 24, and 27% larger than the control, respectively. NPR for 0B-100R%, 4B-96R%, and 16B-84R% was 16, 16, and 17% higher than the control, respectively, and there was no significant linear response associated with percent blue photon flux.

Under high-DLI conditions, hypocotyl length was 30, 35, and 31% longer in the control treatment than for the 0B-100R%, 4B-96R%, and 16B-84R% treatments, respectively. The control treatment had 14% longer epicotyl length than the 4B-96R% and 16B-84R% treatments. B:R ratio treatments had 11 - 12% significantly greater stem diameter than did the control. The 0B-100%R, 4B-96R%, and 16B-84R% treatments had 13, 8, and 3% larger leaf area per plant than the control treatment, respectively. Under high-DLI conditions, the increase of blue photon flux did not significantly affect hypocotyl length, epicotyl length, stem diameter, and leaf area in the B:R ratio treatments.

Under low-DLI conditions, the control treatment had 30, 28, and 31% longer hypocotyl length than the 0B-100R%, 4B-96R%, and 16B-84R% treatments, respectively, and hypocotyl length was not significantly affected by the B:R ratios of
supplemental LED lighting. Epicotyl length of the control was 14 % and 16 % longer than 4B-96R%, and 16B-84R% treatments, respectively and it was not affected by B:R ratios of supplemental lighting. The supplemental LED light treatments had 20 % larger stem diameter than the control, and stem diameter was not significantly affected by the B:R ratios of supplemental LED lighting. The 0B-100R%, 4B-96R%, and 16B-84R% treatments had 21, 15, and 10 % larger leaf area per plant than the control, respectively. Leaf area per plant significantly decreased with the increase of blue photon flux in the B:R ratio treatments (P = 0.0163).

4. Discussion

4.1 Effects of supplemental LED lighting

Plant dry mass is known to increase linearly with increase in incident irradiance (Acock et al., 1971), and an increase of DLI will consequently increase dry mass linearly. In the present study, plant shoot dry mass was increased by 1.5 % (low DLI) and 1.2 % (high DLI) per 1 % increase of DLI by supplemental lighting. These numbers are within the range reported by Hao and Papadopoulos (1999), who studied the effects of supplemental lighting on cucumber and found a 0.8 to 2.3 % increase in total plant dry mass per 1% increase of DLI by supplemental lighting. While 1% increase in dry mass is expected for a 1% increase in DLI, in the present experiment the shoot dry mass exhibited a 1.2 - 1.5% increase by a 1% increase in DLI from supplemental lighting. This may be explained by examining root mass, which was not measured in the present experiment. In order to increase radiation interception and consequently growth rate at a
seedling stage, it is important for young plants to partition more dry mass to the shoot than the roots (Marcelis et al., 1998). In our experiment, a potential greater dry mass allocation to the shoot may explain the higher than expected increase in dry mass by increase in DLI from supplemental lighting.

Supplemental lighting is commonly used in areas and during seasons with low solar DLI (Hao and Papadopoulos, 1999; Hemming et al., 2008; Hemming et al., 2006), although in areas with relatively high DLIs, supplemental radiation is not widely adopted. Supplemental radiation is known to improve plant morphology and increase plant growth (Dorais et al., 1991; Grimstad, 1987; McCall, 1992). In this experiment, plant growth and morphology were improved even under high-DLI conditions (16.2±5.3 mol·m⁻²·d⁻¹).

Similar results were found for tomato seedlings under high-DLI conditions (19.4 ± 1.9 mol·m⁻²·d⁻¹) (Hernández and Kubota, 2012). Cucumber originates from tropical climates and grows well under high DLI. For example, cucumber at the seedling stage is known to grow well under 24 h photoperiods; however, this practice is not common due to the high energy cost of current supplemental lighting technology (Spaargaren, 2001). If LED technology keeps increasing in efficiency and decreasing in cost, the adoption of supplemental lighting technology in areas with relative high DLI may increase.

4.2. Effects of supplemental light quality provided by LEDs

Research on plant responses to blue and red light using LEDs is ample. Red light is known to have the highest relative quantum yield from the PAR spectrum (single leaf
measurements) (McCree, 1972), and chlorophyll absorption peaks are also closer to the red spectrum (Kriedemann, 2010; Sager and McFarlane, 1997) and blue light is a key wavelength for normal plant development (Blaauw and Blaauw-Jansen, 1970; Cosgrove, 1981; Hogewoning et al., 2010b; Schwartz and Zeiger, 1984).

Furthermore, vegetable seedlings grown under artificial lighting showed increased growth (Liu et al., 2011) and net photosynthetic rate (Hogewoning et al., 2010b) with the increase of blue light to the red-light-dominant spectrum. In the present study, the increased percent of blue PF to supplemental lighting did not provide any benefits, and rather limited the growth and development of cucumber plants.

Earlier studies have shown that plants require a minimum threshold of blue light for normal plant photosynthesis and development. For example, Mochizuky et al. (2004) showed that in order to produce essential proteins for the PSII system, dark-adapted Arabidopsis requires a minimum of 5 μmol·m⁻²·s⁻¹ blue light. Similarly, cucumber plants grown under monochromatic red develop morphological leaf disorders that can be prevented with the additions of 7% blue light (7 μmol·m⁻²·s⁻¹) (Hogewoning et al., 2010b).

Sunlight PAR spectrum (400-700nm) contains around 31% blue light on the energy basis (400-500 nm) out of photosynthetically active radiation (sun-facing, 37° tilted surface, energy basis W·m⁻² data representing direct and diffuse light) (ASTM, 2003). Furthermore, in our experiment, blue PF accounted for 22% of PAR of solar radiation (measured inside the experimental greenhouse around noon on a sunny day in Tucson, AZ, USA). In this experiment, blue-light threshold for normal plant development
and photosynthesis for cucumber was fulfilled by the solar light under both high and low DLI conditions.

In this study, under low DLI conditions, an increase of chlorophyll concentration per unit leaf area was observed with increasing percent blue PF. The chlorophyll concentration reportedly increased with the increase of blue PF in algae (Jeffrey, 1980; Jeffrey and Vesk, 1981; Vesk and Jeffrey, 1977). Also, Tripathy and Brown (1995) showed that wheat seedlings germinated under red light alone (500 μmol·m⁻²·s⁻¹ red PF) failed to produce chlorophyll but blue light supplementation (30 μmol·m⁻²·s⁻¹) restored chlorophyll synthesis. More recently, Hogewoning et al. (2010b) examined the effect of increasing the percentage of blue PF (red and blue light spectrum) on chlorophyll content per unit leaf area of cucumber plants. Hogewoning et al. (2010b) showed that chlorophyll content per unit leaf area increased with the increased percent blue PF up to 50%. In addition, using green algae Oh-Hama and Hase (1981) demonstrated that the first steps for chlorophyll biosynthesis were regulated by blue light. In the present study, considering the solar and LED spectra, the blue PF percentages of the treatments were 19.6 %, 14.8 %, and 13.2%, when measured at around solar noon. During the period when LEDs provide the sole light source (5:30 pm - 8:00 pm and 2:00 am - 6:00 am), the percentages were 16%, 4% and 0% blue (Table 1). Similar to Hogewoning et al. (2010b) in the present study, plants showed an increase in chlorophyll concentration per unit leaf area with the increase in blue photon flux. We postulate that plants grown under lower percentages of blue light had decreased capability to biosynthesize chlorophyll.
Under low-DLI conditions, plants showed a decrease in shoot dry mass, leaf count, and leaf area with an increase of percent blue light (Table 5). McCree (1972) quantified quantum yield of photosynthesis (initial slope of photosynthetic light response curve) as affected by selected wavelengths of light for 22 different crop species and found that for cucumber, quantum yield of 600-700 nm (red light) was higher than that of 400-500 nm (blue light) by 31%. The single-leaf spectral quantum yield reported by McCree (1972) may well represent plant spectral quantum yield under transplant production. During transplant production, mutual plant-canopy shading is minimal due to low leaf area index (LAI) (LAI = 0.2 in the present study), and single-leaf measurements of NPR can predict quantum yield responses of the crop. In theory, due to the higher quantum efficiency of red light, increasing blue PF would decrease NPR and thereby the relative plant dry mass.

In the present experiment, plants grown under different percentages of blue and red PF had the same photosynthetic capacity (no differences on NPR between treatments) under near-saturating PPF of white light, suggesting that B:R ratio treatments did not cause any changes in plant photosynthetic apparatus. However, it is possible that NPR of plants under treatment light quality (in-situ) may have a lower NPR under higher blue PF. This is supported by the lower NPR per chlorophyll concentration observed in this experiment with the increase of blue PF (increase in chlorophyll per leaf area and no effect in NPR by the increase in blue PF). Hogewoning et al. (2012) grew cucumber seedlings under different light sources and recorded their photosynthetic quantum yield. They showed that leaves tune their photosystem stoichiometry to their growth-
environment spectrum in order to increase light-use efficiency. Therefore, in the present experiment, it is likely that plants grown under higher red PF (or lower blue PF) had a higher NPR especially during the time when LEDs were the only light source (5:30 pm – 8:00 pm and 2:00 am- 6:00 am). However, the difference between the relative quantum yields of the treatments is likely marginal. For example, if we average the quantum yield for blue wavelengths (400-500 nm) and red wavelengths (600-700 nm) from data reported by McCree (1972), we find that the relative quantum yield is 0.646 for blue wavelengths and is 0.846 for red wavelengths (31% greater than blue) for cucumber. From these numbers we can calculate the relative quantum yield for the three supplemental LED treatments, which is 0.846, 0.838, and 0.814 for the 0B-100R%, 4B:96R%, and 16B-84R% treatments, respectively. When supplemental lighting increased the DLI by 40% under low-DLI conditions, then the 4B:96R% and 16B-84R% treatments have only a 0.4% and 1.5% lower relative quantum yield than the 0B-100R% treatment, respectively. Therefore, the reduced relative quantum yield of the treatments with increased blue PF cannot fully explain the 12 % lower plant dry mass observed in the 16% blue treatments (16B-84R%) treatment compared to the all-red treatment (0B-100R%) under low DLI conditions.

Another more plausible explanation for the reduction in growth under low-DLI conditions is the reduction of leaf area caused by the increase of B:R ratio (increased blue PF). In this experiment, the increase of blue PF reduced the leaf area of plants and consequently the light intercept per plant. The reduction in leaf expansion rate by increasing blue PF was as much as 13 % compared to 0B-100R% treatment, a similar
range of reduction in dry mass (12%). Research using artificial-light only has shown the decrease in leaf area with the addition of blue light for different crops, including cucumber. For example, Hogewoning et al. (2010a) tested cucumber plant responses to different artificial lights including fluorescent lamps (FL), high pressure sodium (HPS) lamps and an artificial light with spectrum close to the sun (AL). The treatments varied in their percentage of blue light to the total spectrum with 23%, 5% and 18% for FL, HPS and AL, respectively. Leaf area under the highest percentage of blue in the FL treatment was significantly smaller than the other two treatments. Similarly, Li and Kubota (2009) showed a significant decrease of leaf length in lettuce plants grown under white light supplemented with blue light (50% more blue light) compared to the white light treatment alone. In addition, Hogewoning et al. (2010b) measured leaf mass area (LMA, the leaf mass per unit leaf area) of cucumber plants under different percentages of blue PF and found an increase in LMA with the increase of blue light. Trouwborst et al. (2010) found a lower total leaf area, and higher LMA under the combination of overhead HPS and blue-red LED inter-lighting compared to that under overhead HPS only (less blue light). In this present study, LMA increased with the increase of blue PF under both high (P = 0.0381) and low (P = 0.005) DLIs (data not shown). This, in conjunction with the decrease of leaf area, shows an increase of leaf thickness with the increase of blue PF. Leaf thickness has been reported to increase with the increase of blue light and decrease with the increase of red light (Sæbø et al., 1995; Schuerger et al., 1997). For example, Schuerger et al. (1997) tested leaf anatomical characteristics of pepper plants under metal halide spectrum, B:R spectrum, red spectrum, and R:Fr spectrum and found increased
leaf thickness with the addition of blue light (leaf area was not reported in this experiment). Leaf responses (decrease of leaf area and increase of leaf thickness) to blue PF increase (under a constant PPF) are similar to leaf responses to irradiance increase even under very low irradiances (Hogewoning et al., 2010b; Poorter et al., 2009; Poorter et al., 2010). High irradiance responses include the increase of leaf thickness and decrease of leaf area. In this study, we postulate that the addition of blue light triggered the high irradiance response of reducing leaf area that consequently resulted in less plant dry mass under low DLI. In other words, under low DLI conditions (5.2 ± 1.2 mol·m⁻²·d⁻¹), light interception by leaves is critical. The reduction of leaf area can significantly reduce the amount of light intercepted by the plant, and less light interception, under light-limited conditions, will significantly reduce plant growth.

Plant responses to light quality are species specific. The findings of this study showed a reduction of cucumber growth with the addition of blue light. In contrast, also under supplemental lighting conditions, Hernández and Kubota (2012) showed no effect of different B:R ratios on tomatoes when grown under varied DLI. Furthermore, under artificial-light growing conditions, Hogewoning et al. (2010b), measured cucumber seedling net photosynthetic rate (NPR) under different percentages (0-50% and 100% blue) of blue PF when used in combination with red LEDs. Researchers showed an increase in NPR and photosynthesis capacity with the increase of the percentage of blue PF. In contrast, in tomatoes, Nanya et al. (2012) measured net photosynthetic rate with varied blue percentages (10%, 30%, 50% blue) and showed a higher NPR with the decrease of the percent blue light and increase of percent red light. Also, in other crops,
Cope and Bugbee (2013) showed differences in stem length and leaf area between radish, soybean and wheat to similar PAR spectrum.

4.3 LED red and blue electrical efficiencies

Blue LEDs have increased in efficiency and surpassed red LEDs. The current energy-to-PF conversion efficiencies of commercially available red LEDs and blue LEDs are 1.7 µmol·J⁻¹ and 1.9 µmol·J⁻¹, respectively, which is equivalent to 32 and 49% electric to light energy conversion efficiency, respectively (Cope and Bugbee, 2013). A higher efficiency translates to less power consumption. For example, an all-red LED fixture with a 626 µmol·s⁻¹ photon flux emission rate (effective flux) will require 368 W of power. In contrast, a fixture with the same emission rate but with all blue LEDs will required 329 W of power (not including power consumed by controller and cooling system). By using the linear regression equation of plant dry mass to percent blue generated in the present study (plant dry mass in grams = 0.50 - 0.0039 × %blue), in conjunction with the LED’s emission rate, and the LED’s power draw, it is possible to estimate the optimal B:R ratio to obtain the largest grams per kilowatt-hour (g·kWh⁻¹). At the current red and blue LED efficiencies (1.7 and 1.9 µmol·J⁻¹), the largest g·kWh⁻¹ was still obtained by using all-red LEDs. By further simulating the electric-energy-to-plant-dry-mass conversion efficiency, we found that blue LED efficiency needs to be 1.8 times higher than the red LED efficiency to show any benefits for energy savings. For example, if blue LED efficiency is 3.1µmol·J⁻¹ and red is 1.7 µmol·J⁻¹, the optimal B:R ratio to maximize g·kWh⁻¹ would be 5B:95R%.
5. Conclusion

In this study, cucumber transplants had improved morphology and increased growth under LED supplemental light in both high- and low-DLI conditions. Supplemental lighting for vegetable transplants in areas where solar DLI is considered high may become feasible using more efficient LEDs. Additional research has to be performed comparing LED supplemental lighting with current HPS supplemental lighting technology in the basis of plant responses, energy efficiency, and capital cost.

Cucumber transplants grown under different B:R photon flux ratios did not show benefits in crop growth and morphology with the increase of blue PF. Moreover, cucumber showed a decrease in dry mass, leaf count and leaf area with the increase of blue PF under low-DLI conditions. In this study, the increase in blue PF did not affect the plant growth under high-DLI conditions but suppressed the plant growth under low-DLI conditions. Under supplemental-lighting conditions with DLI in a range of 5 – 16 mol m\(^{-2}\) d\(^{-1}\), sun spectrum fulfills the blue-light- quality requirement for cucumber transplants, and red supplementary light is adequate to promote plant growth and improve plant morphology. LED manufacturers can apply these findings for development of greenhouse supplemental lighting fixtures. Future research needs to examine the effect supplemental LED lighting at the seedling stage has on mature plants in commercial growing conditions.
Acknowledgements

The authors would like to thank Mark Kroggel, Neal Barto, Dr. Murat Kacira, at the University of Arizona (CEAC) for technical advice; CCS Inc. Kyoto, Japan for providing LED units. This project was funded by USDA NIFA SCRI grant No: 2010-51181-21369.

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Table 1. LED Supplemental light treatments, percent blue photon flux, blue photon flux, red photon flux, phytochrome photostationary state (Pfr/P_total), and average photosynthetic photon flux (PPF) per treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% blue</th>
<th>Blue photon flux (400-500 nm)</th>
<th>Red photon flux (600-700 nm)</th>
<th>Pfr/P_total b</th>
<th>PPF c (400-700 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0B-100R%</td>
<td>0</td>
<td>0.0 µmol·m⁻²·s⁻¹</td>
<td>54.8 µmol·m⁻²·s⁻¹</td>
<td>0.891</td>
<td>53.7 ± 1.2</td>
</tr>
<tr>
<td>4B-96R%</td>
<td>4</td>
<td>2.3 µmol·m⁻²·s⁻¹</td>
<td>52.0 µmol·m⁻²·s⁻¹</td>
<td>0.890</td>
<td>54.0 ± 1.1</td>
</tr>
<tr>
<td>16B-84R%</td>
<td>16</td>
<td>8.5 µmol·m⁻²·s⁻¹</td>
<td>46.2 µmol·m⁻²·s⁻¹</td>
<td>0.886</td>
<td>54.2 ± 1.0</td>
</tr>
</tbody>
</table>

a Measured at the beginning of experiment, average of five measurements at plant height in the growing area
b Phytochrome photostationary state (Sager et al., 1988)
c Average and standard deviation of six measurements, two measurements per repetition per treatment at the beginning and end of experiment.
Table 2. Environmental parameters measured inside the greenhouse. Average and standard deviations of the three repetitions.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Units</th>
<th>Low DLI treatment</th>
<th>High DLI treatment</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar DLI (^a)</td>
<td>mol·m(^{-2})·d(^{-1})</td>
<td>5.2 ± 1.2</td>
<td>16.2 ± 5.3</td>
<td>4 locations at plant height</td>
</tr>
<tr>
<td>Supplemental DLI</td>
<td>mol·m(^{-2})·d(^{-1})</td>
<td>3.50 ± 0.1</td>
<td></td>
<td>Calculation from initial and final measurements of instantaneous PPF</td>
</tr>
<tr>
<td>Greenhouse Air temperature</td>
<td>°C</td>
<td>Day: 22.4 ± 0.7</td>
<td>Day: 22.4 ± 0.7</td>
<td>Eight locations above canopy for each treatment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Night: 17.5 ± 0.6</td>
<td>Night: 17.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Canopy Air temperature</td>
<td>°C</td>
<td>Day: 21.5 ± 0.6</td>
<td>Day: 22.2 ± 0.1</td>
<td>Eight locations inside canopy for each treatment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Night: 17.5 ± 0.6</td>
<td>Night: 17.6 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Photoperiod</td>
<td>hours</td>
<td>18 (2AM-8PM)</td>
<td></td>
<td>Solar(^b) + supplemental light</td>
</tr>
<tr>
<td>Vapor pressure deficit (VPD)</td>
<td>kPa</td>
<td>Day: 1.40 ± 0.3</td>
<td></td>
<td>One location, canopy height</td>
</tr>
<tr>
<td></td>
<td>kPa</td>
<td>Night: 0.56 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atmospheric CO(_2)</td>
<td>µmol·mol(^{-1})</td>
<td></td>
<td></td>
<td>Natural ventilation</td>
</tr>
<tr>
<td>Nutrient solution pH</td>
<td></td>
<td>5.74 ± 0.1</td>
<td></td>
<td>Measured each fertigation</td>
</tr>
<tr>
<td>Nutrient solution EC</td>
<td>dS·m(^{-1})</td>
<td>1.94 ± 0.1</td>
<td></td>
<td>Measured each fertigation</td>
</tr>
</tbody>
</table>

\(^{a}\) Daily light integral

\(^{b}\) Sunset were at 6:00, 5:40, and 5:25 pm for three replicated experiments, respectively.
Table 3. Effects of supplemental LED lighting on plant growth, chlorophyll concentration and net photosynthetic rate of cucumber seedlings under high and low solar DLI conditions.

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>DLI</th>
<th>Dry mass (g)</th>
<th>Fresh mass (g)</th>
<th>Leaf number (per plant)</th>
<th>Chlorophyll (g/m²)</th>
<th>NPR (µmol·m⁻²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No supplemental light</td>
<td>high</td>
<td>0.49 ± 0.029b</td>
<td>8.3 ± 0.35b</td>
<td>2.5 ± 0.07b</td>
<td>0.23 ± 0.007b</td>
<td>15.2 ± 0.33a</td>
</tr>
<tr>
<td>LED supplemental light</td>
<td>high</td>
<td>0.62 ± 0.018a</td>
<td>9.5 ± 0.24a</td>
<td>2.6 ± 0.04a</td>
<td>0.31 ± 0.004a</td>
<td>15.9 ± 0.23a</td>
</tr>
<tr>
<td>No supplemental light</td>
<td>low</td>
<td>0.32 ± 0.018B</td>
<td>6.4 ± 0.27B</td>
<td>1.9 ± 0.06B</td>
<td>0.22 ± 0.006B</td>
<td>10.6 ± 0.39B</td>
</tr>
<tr>
<td>LED supplemental light</td>
<td>low</td>
<td>0.47 ± 0.014A</td>
<td>8.2 ± 0.18A</td>
<td>2.1 ± 0.02A</td>
<td>0.28 ± 0.004A</td>
<td>12.7 ± 0.19A</td>
</tr>
</tbody>
</table>

*High Daily Light Integral = 16.2 ± 5.3, Low Daily Light Integral = 5.2 ± 1.2 mol·m⁻²·d⁻¹*

Total chlorophyll (a and b) per plant leaf area

Net Photosynthetic rate (rate of CO₂ exchange µmol·m⁻²·s⁻¹) measured for fully expanded leaves under 1000 µmol m⁻² s⁻¹ white light.

Means followed by different lower or upper case letters are significantly different at P < 0.05.

Table 4. Effects of supplemental LED lighting on morphological parameters of cucumber seedlings under high and low solar DLI conditions

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>DLI</th>
<th>hypocotyl length (cm)</th>
<th>epicotyl length (cm)</th>
<th>stem diameter (mm)</th>
<th>leaf area (cm² per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No supplemental light</td>
<td>high</td>
<td>10.5 ± 0.139a</td>
<td>3.03 ± 0.212a</td>
<td>5.28 ± 0.107b</td>
<td>179 ± 7.495b</td>
</tr>
<tr>
<td>LED supplemental light</td>
<td>high</td>
<td>7.17 ± 0.018b</td>
<td>2.68 ± 0.118b</td>
<td>5.96 ± 0.066a</td>
<td>194 ± 4.397a</td>
</tr>
<tr>
<td>No supplemental light</td>
<td>low</td>
<td>13.6 ± 0.213A</td>
<td>2.89 ± 0.231A</td>
<td>4.46 ± 0.103B</td>
<td>146 ± 6.058B</td>
</tr>
<tr>
<td>LED supplemental light</td>
<td>low</td>
<td>9.58 ± 0.092B</td>
<td>2.54 ± 0.108B</td>
<td>5.57 ± 0.058A</td>
<td>172 ± 3.362A</td>
</tr>
</tbody>
</table>

*High Daily Light Integral = 16.2 ± 5.3, Low Daily Light Integral = 5.2 ± 1.2 mol·m⁻²·d⁻¹*

Means followed by different lower or upper case letters are significantly different at P < 0.05.
### Table 5: Effects of supplemental LED light quality on growth, chlorophyll concentration and net photosynthetic rate of cucumber seedlings under high and low solar DLI conditions

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>DLI(^a)</th>
<th>Shoot dry mass (g)</th>
<th>Shoot fresh mass (g)</th>
<th>leaf number</th>
<th>Chlorophyl(^b) (g/m(^2))</th>
<th>NPR(^c) (µmol·m(^{-2})·s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0B-100R%</td>
<td>high</td>
<td>0.62 ± 0.032*</td>
<td>9.7 ± 0.42*</td>
<td>2.7 ± 0.07*</td>
<td>0.30 ± 0.005*</td>
<td>15.9 ± 0.38</td>
</tr>
<tr>
<td>4B-96R%</td>
<td>high</td>
<td>0.63 ± 0.032*</td>
<td>9.3 ± 0.41*</td>
<td>2.6 ± 0.07</td>
<td>0.30 ± 0.006*</td>
<td>15.5 ± 0.48</td>
</tr>
<tr>
<td>16B-84R%</td>
<td>high</td>
<td>0.60 ± 0.032*</td>
<td>9.4 ± 0.42*</td>
<td>2.6 ± 0.06</td>
<td>0.32 ± 0.009*</td>
<td>16.2 ± 0.33</td>
</tr>
<tr>
<td>Correlation coefficient(^d)</td>
<td>-0.0626</td>
<td>-0.0335</td>
<td>-0.0931</td>
<td>0.196</td>
<td>0.0988</td>
<td></td>
</tr>
<tr>
<td>Significance(^e)</td>
<td>0.5199</td>
<td>0.6586</td>
<td>0.7646</td>
<td>0.0977</td>
<td>0.4091</td>
<td></td>
</tr>
<tr>
<td>0B-100R%</td>
<td>low</td>
<td>0.50 ± 0.022*</td>
<td>8.4 ± 0.29*</td>
<td>2.17 ± 0.05*</td>
<td>0.26 ± 0.006*</td>
<td>12.6 ± 0.26*</td>
</tr>
<tr>
<td>4B-96R%</td>
<td>low</td>
<td>0.48 ± 0.027*</td>
<td>8.1 ± 0.31*</td>
<td>2.03 ± 0.03</td>
<td>0.29 ± 0.005*</td>
<td>12.6 ± 0.35*</td>
</tr>
<tr>
<td>16B-84R%</td>
<td>low</td>
<td>0.44 ± 0.021*</td>
<td>7.9 ± 0.31*</td>
<td>1.98 ± 0.03</td>
<td>0.30 ± 0.008*</td>
<td>12.8 ± 0.38*</td>
</tr>
<tr>
<td>Correlation coefficient(^d)</td>
<td>-0.1862</td>
<td>-0.0916</td>
<td>-0.2207</td>
<td>0.379</td>
<td>0.0457</td>
<td></td>
</tr>
<tr>
<td>Significance(^e)</td>
<td>0.0536</td>
<td>0.2214</td>
<td>0.0029</td>
<td>0.0012</td>
<td>0.7029</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) High Daily Light Integral = 16.2 ± 5.3, Low Daily Light Integral = 5.2 ± 1.2 mol·m\(^{-2}\)·d\(^{-1}\)

\(^b\) Total chlorophyll (a and b) per plant leaf area

\(^c\) Net Photosynthetic rate (rate of CO\(_2\) exchange µmol m\(^{-2}\)·s\(^{-1}\)) measured for fully expanded leaves

\(^d\) Linear correlations between percent blue photons (0 – 16%) and each parameter

Means followed by asterisk (*) are significantly different from that in the no supplemental light control (t-test, P≤0.05) Table 3 shows the mean values of the control.
Table 6. Effects of supplemental LED light quality on morphological parameters of cucumber seedlings under high and low solar DLI conditions

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>DLIa</th>
<th>hypocotyl length (cm)</th>
<th>epicotyl length (cm)</th>
<th>stem diameter (mm)</th>
<th>leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0B-100R%</td>
<td>high</td>
<td>7.37 ± 0.130*</td>
<td>2.83 ± 0.219</td>
<td>5.96 ± 0.117*</td>
<td>203 ± 8.10*</td>
</tr>
<tr>
<td>4B-96R%</td>
<td>high</td>
<td>6.86 ± 0.100*</td>
<td>2.61 ± 0.196*</td>
<td>5.97 ± 0.110*</td>
<td>194 ± 7.50*</td>
</tr>
<tr>
<td>16B-84R%</td>
<td>high</td>
<td>7.29 ± 0.143*</td>
<td>2.61 ± 0.199*</td>
<td>5.96 ± 0.118*</td>
<td>184 ± 7.15*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficientd</td>
<td>0.0312</td>
<td>-0.0446</td>
<td>0.0014</td>
<td>-0.0931</td>
<td></td>
</tr>
<tr>
<td>Significancee</td>
<td>0.6769</td>
<td>0.5553</td>
<td>0.9819</td>
<td>0.2138</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0B-100R%</td>
<td>low</td>
<td>9.58 ± 0.158*</td>
<td>2.78 ± 0.190</td>
<td>5.57 ± 0.093*</td>
<td>184 ± 5.50*</td>
</tr>
<tr>
<td>4B-96R%</td>
<td>low</td>
<td>9.84 ± 0.183*</td>
<td>2.49 ± 0.190*</td>
<td>5.56 ± 0.103*</td>
<td>172 ± 6.05*</td>
</tr>
<tr>
<td>16B-84R%</td>
<td>low</td>
<td>9.32 ± 0.131*</td>
<td>2.37 ± 0.181*</td>
<td>5.58 ± 0.108*</td>
<td>161 ± 5.61*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficientd</td>
<td>-0.1226</td>
<td>-0.1032</td>
<td>0.0047</td>
<td>-0.1789</td>
<td></td>
</tr>
<tr>
<td>Significancee</td>
<td>0.101</td>
<td>0.1676</td>
<td>0.9501</td>
<td>0.0163</td>
<td></td>
</tr>
</tbody>
</table>

a High Daily Light Integral = 16.2 ± 5.3, Low Daily Light Integral = 5.2 ± 1.2 mol·m⁻²·d⁻¹

b Linear correlations between percent blue photons (0 – 16%) and each parameter

Means followed by asterisk (*) are significantly different from that in the no supplemental light control (t-test, P<0.05) Table 3 shows the mean values of the control.
Fig 1. Photosynthetic photon flux of the supplemental LED light treatments in conjunction with solar PPF under low (A) and high (B) DLI growing conditions. The control treatment is composed of only solar PPF. Data represents an average of 3 spectroradiometer measurements on 7/8/2011, 2/10/2012, 3/28/2012 and 4/1/2012 inside the greenhouse under conditions similar to the present experiment.
APPENDIX B: TOMATO SEEDLING GROWTH AND MORPHOLOGICAL RESPONSES TO SUPPLEMENTAL LED LIGHTING RED:BLUE RATIOS UNDER VARIED DAILY SOLAR LIGHT INTEGRALS

Published on Acta Horticulturae
Tomato Seedling Growth and Morphological Responses to Supplemental LED Lighting Red:Blue Ratios under Varied Daily Solar Light Integrals

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School of Plant Sciences, The University of Arizona, Tucson, Arizona, USA.

Keywords: PAR, DLI, greenhouse, light-emitting diode, Solanum lycopersicum, spectral quality.

Abstract

Supplemental lighting is proven to increase transplant growth and quality in vegetable-nursery greenhouses. To evaluate plant responses to supplemental LED light quality, tomato seedlings (cv. Komeett) were grown in a greenhouse (Tucson, AZ, USA) until the second true-leaf stage with $55.5 \pm 1.4 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetic photon flux of supplemental LED lighting (18-hour photoperiod). Treatments consisted of different red : blue photon-flux ratios (1) 100 % red : 0 % blue, (2) 96 % red : 4 % blue, (3) 84 % red : 16% blue, and a control without supplemental lighting. These ratios were evaluated under low and high daily solar light integrals (DLI) ($8.9 \pm 0.9$ and $19.4 \pm 1.9 \, \text{mol m}^{-2} \, \text{d}^{-1}$, respectively) created by different shade screens deployed in the greenhouse. Growth and morphological parameters including dry shoot mass, leaf count, stem diameter, hypocotyl length, leaf area, and chlorophyll concentration indicated the benefit of supplemental light, especially under low DLI, but there were no significant differences among different red : blue ratios regardless of DLI. The seedlings also exhibited the same high photosynthetic capacity measured under $1000 \, \mu\text{mol m}^{-2} \, \text{s}^{-1}$ PPF, ambient temperature, and CO$_2$ concentration regardless of the red : blue ratios. From this preliminary study, it seems that for ‘Komeett’ tomato seedlings grown in the greenhouse, use of 100 % red
LED supplemental lighting was sufficient and no additional blue light was required regardless of DLI.

INTRODUCTION

Greenhouse supplemental lighting typically is used in geographical areas and seasons in which sunlight is the limiting factor for production. Vegetable transplants of tomato, cucumber, and pepper seedlings show increased growth and quality when photosynthetically active radiation is supplemented with conventional high-pressure sodium (HPS) lamps. The emerging high-intensity light-emitting diode (LED) technology presents a potential alternative to current lighting technology due to its long functional lifespan, low operating temperature, low energy consumption, and selective spectral output. Nevertheless, before being introduced to the commercial greenhouse market, this technology must be properly evaluated in terms of economic feasibility, lamp placement within the greenhouse/canopy, irradiance intensity, and spectral quality required for plant growth.

Early research on space life-support systems focused on using LED lamps as a sole source of light to grow plants (Bula et al., 1991; Barta et al., 1992; Hoenecke et al., 1992; Morrow et al., 1995; Croxdale et al., 1997; Stankovic et al., 2002; Zhou, 2005; Massa et al., 2008). Two ranges of wavelengths that have been widely accepted are red (600 to 700 nm) and blue (400 to 500 nm). Addition of blue is generally recommended to maintain plant morphology and to maximize plant growth (Massa et al., 2008). In contrast, there is limited research on LED light-quality requirements when used in
greenhouses as a supplement to the sunlight PAR spectrum (400-700 nm), which already contains around 31 % blue on the energy basis (400-500 nm) (data representing direct and diffuse light spectrum for 48 states in USA) (ASTM, 2003). Typically, North American greenhouse nurseries for vegetable seedlings use supplemental lighting for ~18 hours a day, from the middle of night to soon after the sunset (L. Benne, personal communication). Following this conventional practice of photoperiod and intensity, we have examined red and blue LED light qualities at varied ratios. In order to determine whether the light-quality requirement for supplemental lighting may be affected by solar daily light integrals (DLIs), an experiment was designed to compare plant responses to light qualities under two different levels of DLI created inside a greenhouse using shade screens.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

The tomato cultivar ‘Komeett’ was used for the present study. Seeds were placed in a dark growth chamber on a moist filter paper inside a Petri dish under 29 °C for 2 days on 7/20/2011 until radical emergence. Germinated seeds were transferred to a 98-cell seedling tray filled with commercial substrate (SunGro Sunshine Mix #3, Bellevue, WA, USA). Trays were placed inside a greenhouse (Tucson, AZ, USA) covered by double-layered acrylic glazing. Daytime and nighttime temperatures were 25.4 ± 0.4 °C and 23.1 ± 0.4 °C respectively, controlled by a pad-and-fan evaporative-cooling system.
Solar DLIs and LED Treatments

The greenhouse was divided into north-south compartments to create DLIs of 8.9 ± 0.9 mol m\(^{-2}\)d\(^{-1}\) (low) in one half and 19.4 ± 1.9 mol m\(^{-2}\)d\(^{-1}\) (high) in the other half using selected shade screens (Ludvig Svensson, Inc, Charlotte, NC, USA). Three light-quality treatments and a ‘no-light’ control were set up in both compartments. The three treatments consisted of (1) 100 % red : 0 % blue, (2) 96 % red : 4 % blue, and (3) 84 % red : 16 % blue. Blue and red LEDs had peak wavelengths of 455 nm and 661 nm (FWHM: 20 nm and 15 nm), respectively. All LED treatments provided PPF of 55.5 ± 1.4 μmol m\(^{-2}\)s\(^{-1}\) at canopy level (18-hour photoperiod from 02:00 to 20:00 HR) from 8 days (cotyledon expansion) to 19 days (second true-leaf stage) after seeding. This increased the average DLI by 3.6 mol m\(^{-2}\)d\(^{-1}\) in both compartments of the greenhouse.

Plant Measurements

One day before destructive plant measurements (18 days after seeding, when plants reached two-true-leaf stage), photosynthesis measurements were performed with a portable photosynthesis system (CIRAS-2, PP System, MA, USA) with 1000 μmol m\(^{-2}\)s\(^{-1}\) PPF (halogen lamp), under greenhouse temperature of 25 ± 2 °C and ambient CO\(_2\) concentration. After photosynthesis measurements, aerial parts of plants (shoots) were excised from the base in order to measure number of leaves (leaf length > 1 cm), hypocotyl length, stem diameter, and leaf area. The shoots were then either processed for chlorophyll extraction or dried at 80 °C for at least 48 hours to obtain shoot dry mass.
Extraction and quantification of total chlorophylls were based on Moran and Porath (1980).

**Experimental Design and Data Analysis**

The central sections of 98-cell trays were cut into eight sections containing 9 cells (one experimental unit). Treatments consisted of 8 experimental units with 9 plants per experimental unit. Four experimental units were grouped together as a block (two blocks per treatment). Within each block, the four experimental units were rotated in position every day to minimize potential location effects within the tray. In addition, location of the three LED treatments and the control were changed every day inside the same greenhouse to improve the uniformity of environmental conditions to which plants of each treatment were exposed. Analysis of variance (P = 0.05) was performed to identify potential differences among treatments, considering the 9-cell section as an experimental unit (n=8). Mean separations were analyzed using Tukey-Kramer HSD (P = 0.05).

**RESULTS**

Number leaves, leaf area, and dry mass data are presented in Fig 1. Number of leaves (Fig 1a) did not differ among treatments, including the control (no-LED) under high DLI (P = 0.391) or low-DLI conditions (P=0.215). LED treatments drove an increase (55 - 57 %) in shoot dry mass (Fig 1b) compared to control under low DLI conditions (P = 0.001) but no significant differences compared to control under high-DLI conditions (P = 0.657). No differences were detected among LED treatments.
Morphological characteristics (hypocotyl length, stem diameter, and leaf area) are shown in Fig 2. No differences were found compared to control for hypocotyl length (Fig 2a) under high DLI (P = 0.093) or Low DLI (P = 0.063). No significant differences in hypocotyl length occurred among LED treatments. High DLI stem diameter (Fig 2b) resulted in no differences among treatments and control (P = 0.569). In contrast, low-DLI LED treatments led to an increase (18 – 19 %) in stem diameter compared to control (P < 0.0001). No significant differences in stem diameter were present among LED treatments under low DLI. Leaf area (Fig 2c) did not show any statistical differences among treatments under high (P = 0.993) and low (P = 0.232) DLI conditions.

Chlorophyll concentration and net photosynthetic rate (NPR) are shown in Fig 3. Chlorophyll concentration (Fig 3a) showed an increase under 100 % red : 0 % blue and 96 % red : 4 % blue LED treatments over the no-light control (20 and 24 % respectively), and 100 % red : 0 % blue gave an increase (13 %) compared to 84 % red 16 % blue treatment under high DLI (P = 0.0002). Under low-DLI, chlorophyll concentration showed an increase (17 – 21 %) under LED lights compared to control (P = 0.0004). No significant differences among LED treatments were detected under low-DLI conditions. No significant differences among treatments, including the control group, were detected for NPR (Fig 3b) under high (P = 0.703) or low (P = 0.630) DLI conditions.

DISCUSSION

Research focusing on light requirements for several crops has shown a need for blue light to have normally developed plants (Blaauw et al., 1970; Cosgrove, 1981;
Schwartz et al., 1984; Hogewoning et al., 2010a). More recently, research has shown the benefit of blue-light addition to increase growth and net photosynthetic rate of cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*) seedlings (Liu et al., 2011).

Mochizuki et al. (2004) demonstrated that a minimum amount of blue light (5 μmol m$^{-2}$ s$^{-1}$) was required in order to produce the D2 protein of the PSII system in *Arabidopsis*. In addition, Hogewoning et al. (2010a) determined that physiological leaf disorders in cucumbers caused by red light alone were eliminated with 7% blue light (7 μmol m$^{-2}$ s$^{-1}$). However, these requirements have not been confirmed for supplemental light quality used in the presence of solar radiation in greenhouses. In the United States, commercial greenhouse DLI may vary from a low of 2.5 mol m$^{-2}$ d$^{-1}$ to a high of 35 mol m$^{-2}$ d$^{-1}$ depending on latitude and month of the year (Faust, 2004). Out of PAR, approximately 31% (energy basis) is in the blue spectrum (400-500 nm) (ASTM, 2003). If tomato plants also require a minimum amount of blue light for normal development, 25% blue light (photon flux units) (our measurement data, Tucson, AZ, USA) clearly meets the qualitative threshold for normal plant development.

The results of this preliminary study demonstrated that tomato seedling growth, morphology, and photosynthesis did not change with the addition of blue LED light at either blue ratio, compared to 100% red LED light. This suggests that solar DLI provided the blue-light requirements for tomato seedlings. This preliminary study is the first to report that supplemental red LED is sufficient for tomato seedlings and no additional blue light is required regardless of DLI when grown under solar background light.
In this study, plants grown under supplemental lighting were exposed to the unique light quality as the sole source of light some part of the day. The duration of the period having LEDs as the sole source was around 4 hours before sunrise and 20 - 30 minutes after sunset in this experiment. Therefore, the blue-light requirement with regard to plant photosynthesis and morphology need to be further evaluated for various lighting patterns in the future.

Additionally, recent research indicates the quantitative impact of blue light in plants. Hogewoning et al. (2010a) showed an increase in maximum photosynthetic rate ($A_{\text{max}}$) upon increasing the blue-light percentage up to 50% for cucumber. This work also showed that the increase in $A_{\text{max}}$ was associated with an increase in leaf mass / area (LMA). In other words, Hogewoning et al. (2010a) found an increase in $A_{\text{max}}$ and LMA by varying the blue percentage while maintaining constant irradiance. Also, Hogewoning et al. (2010b) showed lower $A_{\text{max}}$ per unit LMA for plants grown under 5% blue compared to plants grown under 23 % blue or 18 % blue. Hogewoning et al. (2010a) mentioned that responses to blue percentages higher than 22 % were similar to high irradiance leaf responses. Also, more recently, Liu et al. (2011) showed that a 100 % blue LED treatment in cherry-tomato seedlings had an increase in dry mass compared to 100 % red LED treatment. Therefore, it will be of interest to examine an extremely high percent blue LED treatment in our future work. Furthermore, blue LED efficiency is improving rapidly (Philips data sheet #DS68), due to the demand for white LEDs and it is prominent that blue LED will quickly surpass red LED efficiency. Supplemental light
quality must be decided not only from plant responses, but also by energy saving and overall costs in the future.

CONCLUSIONS

Tomato seedlings grown using supplemental LED lighting of different B:R ratios showed no differences in growth and morphology regardless of background DLI. It is postulated that background solar light fulfills blue light qualitative and quantitative requirements. This preliminary study is the first to report that red LED is sufficient for supplemental lighting and no blue light is required for tomato seedlings production.

ACKNOWLEDGEMENTS

The authors would like to thank Mark Kroggel, Neal Barto, Dr. Murat Kacira, at the University of Arizona (CEAC) for technical advise; Dr. Cary Mitchell at Purdue University for valuable abstract revisions; and CCS Inc. Kyoto, Japan providing LED units. This project was funded by USDA SCRI grant No: 2010-51181-21369.

Literature Cited


Fig 1. Effects of different LED light-quality treatments (red : blue) on tomato seedlings (a) number of leaves (> 1 cm), and (b) shoot dry mass per plant 18 days after seeding (11 days after the start of LED treatment). White bars show results under high background DLI conditions (19.4 ± 1.9 mol m\(^{-2}\) d\(^{-1}\)) and dotted bars under low background DLI conditions (8.9 ± 0.9 mol m\(^{-2}\) d\(^{-1}\)). Different letters represent statistical significant differences (P < 0.05). Bars indicate SE (n=8). High DLI and Low DLI were analyzed separately.
Fig 2. Effects of different LED light-quality treatments (red : blue) on tomato seedlings (a) hypocotyl length, (b) stem diameter, and (c) leaf area per plant 18 days after seeding (11 days after the start of LED treatment). White bars show results under high background DLI conditions (19.4 ± 1.9 mol m⁻² d⁻¹) and dotted bars under low background DLI conditions (8.9 ± 0.9 mol m⁻² d⁻¹). Different letters represent statistical significant differences (P < 0.05). Bars indicate SE (n=8). High DLI and Low DLI were analyzed separately.
Fig 3. Effects of different LED light quality treatments (red : blue) on the (a) chlorophyll concentration per unit area and (b) net photosynthetic rate (NPR) (1000 μmol m$^{-2}$ s$^{-1}$ PPF, ambient temperature and CO$_2$ concentration) of tomato seedlings 18 days after seeding (11 days after the start of LED treatment). White bars show results under high background DLI conditions (19.4 ± 1.9 mol m$^{-2}$ d$^{-1}$) and dotted bars under low background DLI conditions (8.9 ± 0.9 mol m$^{-2}$ d$^{-1}$). Different letters represent statistical significant differences (P < 0.05). Bars indicate SE (n=8). High DLI and Low DLI were analyzed separately.
APPENDIX C: LED SUPPLEMENTAL LIGHTING FOR VEGETABLE
TRANSPLANT PRODUCTION: SPECTRAL EVALUATION AND COMPARISONS
WITH HID TECHNOLOGY

To be submitted to Acta Horticulturae
LED Supplemental Lighting for Vegetable Transplant Production: Spectral Evaluation and Comparisons with HID Technology

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Keywords: greenhouse, light-emitting diode, Solanum lycopersicum, Cucumis sativus, spectral quality, high pressure sodium

Abstract

Supplemental lighting is a key technology to improve transplant growth and quality in vegetable-nursery greenhouses. Light emitting diodes (LEDs) are a recent addition to current supplemental greenhouse-lighting technology. However, LED lighting technology must be evaluated in terms of economic feasibility and plant responses. At the University of Arizona, a two-phase study was conducted to evaluate supplemental LED lighting technology for vegetable-transplant production in greenhouses. Tomato (Solanum lycopersicum cv. Komeett) and cucumber (Cucumis sativus cv. Cumlaude) were examined. The first phase study evaluated blue-light-supplemental-lighting requirement under daily solar light integrals of 6–9 mol m$^{-2}$ d$^{-1}$ created by shade screens deployed in the greenhouse. The LEDs used for supplemental lighting were a mix of red and blue providing an average of 55 μmol m$^{-2}$ s$^{-1}$ PP$F$ for 18 hours (2 AM – 8 PM) or 3.5 mol m$^{-2}$ d$^{-1}$ over the plant canopy surface. The three treatments were 0% blue (0 μmol m$^{-2}$ s$^{-1}$), 4% blue (2.2 μmol m$^{-2}$ s$^{-1}$), or 16% blue (8.8 μmol m$^{-2}$ s$^{-1}$) and a control with no supplemental lighting. Blue and red LEDs had peak wavelengths of 455 nm and 661 nm, respectively. Plant growth of the young seedlings were largely improved by supplemental LED lighting for both species compared to the control (no supplemental lighting). For example, dry mass was increased by 39% for tomato and 47% for cucumber. For tomato,
no significant differences in dry mass were measured among different % blue treatments. However, cucumber plants showed significant reduction in dry mass when the % blue photon flux was increased. The second-phase study consisted of a side-by-side comparison of 100% red LED (632 nm peak wavelength) and a conventional high pressure sodium (HPS) lamp at the same PPF and photoperiod used in the first phase study. Plant dry mass under red LEDs was not different from that under HPS for tomato but was 25% lower under red LEDs than HPS for cucumber. Based on the results from phase one of the study, it is clear that for ‘Komeett’ tomato and ‘Cumlaude’ cucumber, 100% red supplemental LED is sufficient to improve growth of the seedlings. Phase two results indicate that growth of tomato seedlings under 100% red LEDs was comparable to that under HPS, but the growth of cucumber seedlings was greater under the HPS than the 100% red LED lighting. From this study it is evident that plant species respond differently to supplemental lighting and in order to improve growth and morphology in greenhouse crops the selection of supplemental lighting should be species specific.

INTRODUCTION

Supplemental lighting can improve photosynthetic rates, fruit yield, plant morphology, and plant growth in greenhouse crops (Blom and Ingratta, 1984; Blain et al., 1987; Hendricks, 1992; Hao and Papadopoulos, 1998). Supplemental lighting is widely used in geographical areas and seasons where sunlight is the limiting factor for crop production. High pressure sodium lamps (HPS) have a relatively high photon-emission rate (also known as effective flux: μmol s⁻¹) per fixture, and they can attain good light
distribution with the appropriate fixture reflector. These lamps are commonly used in pot-plant, cut-flower, and vegetable-transplant production as supplemental lighting (Masson et al., 1991; Hendriks, 1992; McCall, 1992; Fierro et al., 1994; Hao and Papadopoulos, 1998). However, the disadvantages of HPS lamps are a yellow-biased spectral output with high infrared radiation and they are built with hazardous mercury vapor. For example, HPS light quality in the photosynthetically active radiation (PAR) is composed of 5.2% blue (400-500 nm), 52.4% green (500-600 nm), and 42.4% red (600-700 nm) wavelengths (spectroradiometer scan of a 600-W HPS lamp). Also, infrared radiation is 64% of total lamp irradiance (W m$^{-2}$ 280-2800 nm) (Graper and Healy, 1991). An alternative to the current HPS technology is the emerging high-intensity light-emitting diodes (LEDs). In comparison to the current technology, LEDs can be optimized in spectral quality, they have more flexibility with installation (low infrared radiation), and they are a solid state emitter (no mercury vapor). Previous research that used LEDs as the sole source of light concluded that wavelengths in the red (600 to 700 nm) supplemented with blue (400 to 500 nm) were sufficient for plant growth and development (Bula et al., 1991; Barta et al., 1992; Hoenecke et al., 1992; Morrow et al., 1995; Croxdale et al., 1997; Stankovic et al., 2002; Zhou, 2005; Massa et al., 2008). In contrast, there is limited research on light-quality requirements using LEDs when they are supplemental to solar radiation for greenhouse crops. This study examined growth responses of young seedlings of greenhouse tomato, pepper, and cucumber to different red:blue photon ratios of supplemental photosynthetic lighting (400-700 nm) in order to
find the optimal light-quality spectrum using LEDs. This optimal LED spectrum was then compared side-by-side with conventional HPS technology.

**MATERIALS AND METHODS**

**Plant material**

Greenhouse tomato (cv Komeett) and cucumber (cv Cumlaude) were used in this experiment. Tomato and cucumber seeds were placed in a dark growth chamber, on a moist filter paper inside a petri dish at 29 °C for 1-2 days until radicle emergence. Tomato seeds were then transferred into a 98-cell seedling tray filled with commercial substrate (SunGro Sunshine Mix #3, Bellevue, WA, USA) (phase 1) or 7 cm L x 7 cm W x 6.5 cm H rockwool cubes (Grodan Delta, Canada) (phase 2). Cucumber seeds were transferred into the rockwool cubes. After cotyledon expansion (4 – 5 days from seeding), seedlings were given supplemental light treatments until they reached second true leaf stage. Seedlings were fertigated as needed using a nutrient solution (90 mg/l N, 47 mg/l P, 144 mg/l K, 160 mg/l Ca, 60 mg/l Mg, 113 mg/l S, 105 mg/l Cl).

**Supplemental light treatments**

The procedures of using supplemental lighting followed the commercial practices of a collaborating industry with minor modifications. The greenhouse solar DLI was reduced (see environmental parameters) by using selected shade screens (Ludvig Svensson, Inc, Charlotte, NC, USA) to achieve the target typical DLIs in fall to winter seasons in Northern latitudes.
Phase 1: For the light-quality experiment (phase 1), cucumber and tomato seedlings were grown with supplemental lighting of 54.5 ± 0.6 and 54.9 ± 0.4 μmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux (PPF) over the canopy (18-hour photoperiod from 2 AM to 8 PM) of LED light, respectively. A non-supplemental light treatment was included as the control. The supplemental lighting provided an average of 3.54 mol m$^{-2}$ d$^{-1}$ daily light integral (DLI). The three treatments consisted of (1) 100 % red and 0 % blue, (2) 96 % red and 4 % blue, and (3) 84 % red and 16 % blue. Blue and red LEDs had peak wavelengths of 455 nm and 661 nm, respectively.

Phase 2: For the side-by-side comparison experiment (phase 2), cucumber and tomato seedlings were grown with supplemental lighting of 54.5 ± 1.0 μmol m$^{-2}$ s$^{-1}$ PPF over the canopy (18-hour photoperiod from 2 AM to 8 PM) of red-LED (632 nm peak wavelength) and a 600-W high pressure sodium (HPS) lamp. The supplemental lighting provided an average of 3.53 mol m$^{-2}$ d$^{-1}$ daily light integral (DLI).

Plant measurements

Aerial parts of plants (shoots) were excised from the base for measuring shoot fresh mass. The shoots were then dried at 80 °C for at least 48 hours to obtain the shoot dry mass. Plants were excised at the second-true-leaf stage 17 days after seeding for phase 1 and 25 days after seeding for phase 2.
Experimental Design and Data Analysis

Both experimental phases were carried out in the same greenhouse in Tucson, AZ with a north–south orientation, 108 m² floor area, 2.5-m gutter height, 4.3-m peak height, double-layer acrylic glazing, and with a pad-fan evaporative-cooling system.

Phase 1: Experiments for tomato and cucumber were conducted separately. For tomatoes, 8 experimental units comprising 9 plants each were measured per treatment (n=8). This experiment was conducted from 8/8/2011 to 8/25/2011. For cucumber, each plant was considered an experimental unit (n=20). This experiment was conducted from 9/25/2011 to 10/14/2011. The location of the three LED treatments and the no-light control were changed every day under the lamps to improve the uniformity of environmental conditions among treatments. Analysis of variance (P = 0.05) was performed to identify any difference among treatments. Linear regression (P = 0.05) was performed to test the effect of the percent blue light on seedling growth.

Phase 2: The experiment was carried out from 1/04/2013 to 1/28/2013. Tomato and cucumber seedlings were grown at the same time. Twenty tomato plants and twenty cucumber plants were equally spaced under the two treatments (11 plants per m²) to minimize mutual shading. Each plant was considered an experimental unit (n=20 per crop). Analysis of variance (P = 0.05) was performed to identify any difference among treatments.
RESULTS AND DISCUSSION

Environmental parameters

Phase 1: Solar daily light integral (DLI) were 8.8 ± 2.0 and 6.1 ± 0.9 mol m$^{-2}$ d$^{-1}$ measured in two locations at plant height for tomato and cucumber, respectively. Sunrise and sunset times during the experiments were 6:00 AM and 7:30 PM for tomato; 6:30 AM and 6:30 PM for cucumber. The photoperiod including natural and supplemental lighting was 18 hours (2 AM to 8 PM) for both crops. The canopy air temperature measured directly under the plant canopy in 4 locations was 26.2 °C ± 2.3 and 21.5 °C ± 1.0 during the day and 23.3 °C ± 1.0 and 16.9 °C ± 0.3 during the night for tomato and cucumber, respectively. The atmospheric moisture measured as percent relative humidity was 70.6 % ± 9.5 and 60 % ± 15 during the day and 83.1 % ± 6.7 and 79 % ± 13 during the night for tomato and cucumber, respectively.

Phase 2: The solar DLI was 3.9 ± 0.04 mol m$^{-2}$ d$^{-1}$ measured in two locations at plant height. Sunrise and sunset times during the experiment were 7:30 AM and 5:40 PM. The photoperiod including natural and supplemental lighting was 18 hours (2 AM to 8 PM). The canopy air temperature measured in 2 locations in center under plant canopy was 22.7 °C ± 0.5 during the day and 14.5 °C ± 1.0 during the night for the red-LED system compared to 23.7 °C ± 0.6 day and 15.1 °C ± 1.0 for the HPS treatment. Greenhouse air temperature away from the plant canopy was 24.3 °C ± 0.9 during the day and 14.8 °C ± 1.0 during the night for the red LED treatment and 24.4 °C ± 0.9 during the day and 15.3 °C ± 1.0 during the night for the HPS treatment. Therefore, this difference in
temperatures under the canopy observed between the treatments was caused by radiant heat coming from the HPS fixture. The atmospheric moisture measured in percent relative humidity was 55.2 % ± 7.5 during the day and 70.4 % ± 8.2 during the night.

**LED supplemental light benefits**

Shoot dry mass of tomato and cucumber was significantly increased (P = 0.0001) by 47 % and 39 % respectively, in the LED treatments compared to the no-light control. LED supplemental lighting composed 29 % of the total light (solar + LED) for tomatoes and 37 % for cucumber (Fig 1). The increase of dry mass under supplemental lighting is an expected response. Research has shown that plant canopy photosynthesis is linear to instantaneous PPF (Acock et al., 1978) and consequently, the increase of plant dry mass per day is linear to the increase in DLI until saturation. In our experiment, tomato dry mass was increased by 1.6 % per 1 % of supplemental light and cucumber dry mass was increased by 1.1% per 1% of supplemental light. These numbers are within the range found by Hao and Papadopoulos (1998) of 0.9 to 2.8 % marketable yield increase per 1 % of supplemental light under low-DLI conditions (supplemental lighting was 10-30% of natural light). The higher increase of dry mass in tomato compared to cucumber can be caused by the warmer night temperatures. During the tomato experiment, the pad-and-fan evaporative cooling system was only able to reduce night temperature to 21.5 °C instead of the 16 °C set point do to the outside environmental conditions. Of the 10.5 hours of night period (sunset to sunrise), supplemental lighting was operated for four hours. During these hours, photosynthetic capacity of tomato at 54.5 ± 1.0 μmol m⁻² s⁻¹ PPF and
21.5 °C ± 1.0 temperature might be higher than those of cucumber at the same PPF with a lower temperature of 16.9 °C ± 0.3, and consequently the dry mass increase per supplemental light is higher.

**LED supplemental light quality**

Using red light (600 – 700 nm) as the main supplemental light quality is justified by the McCree curves (McCree, 1972) that indicate that red wavelengths have a higher relative quantum yield than other PAR wavelengths (leaf measurement). In addition, red wavelengths are closer to the absorption peak of chlorophyll (660 nm) (Sager and McFarlane, 1997). The addition of blue (400 – 500 nm) was included in the experiment because sole source artificial light research showed that blue light is necessary for normal plant development (Blaauw et al., 1970; Cosgrove, 1981; Schwartz et al., 1984; Hogewoning et al., 2010). Also, in some reports, the increase of blue light increased growth (Liu et al., 2011), and net photosynthetic rate (Hogewoning et al., 2010; Liu et al., 2011) of cucumber and tomato seedlings.

In this study, tomato plants showed no statistical differences in shoot dry mass (P=0.5594) between treatments with different percents of blue light (data not shown). In contrast to tomato, cucumber seedlings had a reduction in shoot dry mass (P=0.0028), with the increase of blue-light percentage (Fig 2). Literature states that a minimum amount of blue light is required for normal plant development. This threshold varies between crops. For example, in *Arabidopsis* only 5 μmol m⁻² s⁻¹ are required in order to produce essential proteins for the PSII system (Mochizuki et al., 2004). Similarly,
cucumbers grown under red light alone developed physiological leaf disorders, and those disorders were prevented by the addition of 7% blue light (Hogewoning et al., 2010). In our experiment, the light-quality scan of solar radiation inside the greenhouse showed a profile of 25% blue light (photon flux units - Tucson, AZ, USA). This study concludes that tomato and cucumber fulfill their blue-light threshold from the solar radiation and that additional blue supplemental light contributes as photosynthetic radiation and no additional morphological benefits are observed compared to 100% red treatment.

To our knowledge, no research reports are available on the responses of plants to blue light supplementing solar light. Research on sole-source artificial lighting suggests that the addition of blue light is critical to maintain normal plant morphology and to maximize plant growth (Massa et al., 2008; Hogewoning et al., 2010). In this study, there was no increase in growth under higher percentage of blue light. In contrast, under treatments with 4% or 16% blue light, cucumber seedlings had less growth and were more compact (less leaves and smaller leaf area) than under 0% blue treatment (100% red treatment). Further analysis is currently underway to understand the mechanism of growth reduction in cucumber by increased percent of supplemental blue photons.

**LED and HPS side-by-side comparison**

No significant difference was found for shoot dry mass between the red-LED treatment and the HPS treatment for tomato plants. In contrast, shoot dry mass of the HPS treatment was 25% ($P = 0.0001$) higher than the red treatment for cucumber (Fig 3). The increase in dry mass for the HPS treatment can be attributed to the increase in
canopy temperature caused by infrared radiation from the HPS treatment. Temperature is an important parameter for plant growth. Under limiting temperature ranges leading up to optimum, plant growth and development respond linearly to temperature increase, and the rate of the response is species specific (Lieth and Pasian, 1990; Adams et al., 200). In this experiment, the day-time air temperature and night-time air temperature measured under the canopy were 1 °C and 0.5 °C, respectively, higher in the HPS treatment compared to control. The actual leaf surface temperature was potentially higher than 1 °C for the HPS treatment due to infrared radiation from the lamp. Further experiments to elucidate the difference in response between tomato and cucumber are being conducted.

CONCLUSION

Both tomato and cucumber seedling shoots increased in dry weight under supplemental lighting. However, the response to different supplemental light quality was different between them. Tomato seedlings grown until the second true leaf stage showed no difference in growth between red LED treatments and the red:blue LED treatments. Tomato plants fulfill their qualitative blue-light threshold with natural light, and additional blue light does not provide additional benefits. Cucumber plants grown to the second true-leaf stage showed a decrease in growth with increased percentage of blue light. Cucumbers are more sensitive to the light quality differences. Red-LED supplemental lighting is sufficient for the growth of tomato and cucumber seedlings.

In the side-by-side comparison of red-LED and HPS supplemental lighting, an increase in growth was observed for cucumber under the HPS treatment. However,
tomato did not show such growth improvement at this young seedling stage. The increase in growth observed for cucumber can be explained by the higher leaf temperature in the HPS treatment caused by infrared radiation from the lamp.

ACKNOWLEDGEMENTS

The authors would like to thank Mark Kroggel, Neal Barto, Dr. Murat Kacira, at the University of Arizona (CEAC) for technical advice; CCS Inc. Kyoto, Japan and Orbitec Corp. Madison, USA for providing LED units. This project was funded by USDA SCRI grant No: 2010-51181-21369.
Literature Cited


Figures

**Fig 1.** Tomato ‘Komeett’ and cucumber ‘Cumlaude’ shoot dry mass under supplemental LED lighting or no supplemental light. Asterisk (*) between the graphs represents statistically significant differences between treatments based on ANOVA (P = 0.05).

**Fig 2.** Cucumber ‘Cumlaude’ shoot dry mass with increase of the blue percentage in the red:blue ratio. 0% blue light equals to 100% red light. Lines through data points represent the fit of a linear regression (n=20 for cucumber).
Fig 3. Cucumber ‘Cumlaude’ and tomato ‘Komeet’ grown under supplemental high pressure sodium lamps (HPS) and red LED lamps (Red-LED). Asterik (*) between the graphs represents statistically significant differences between treatments based on ANOVA (P = 0.05).
APPENDIX D: PULSING EFFECTS OF GREENHOUSE SUPPLEMENTAL LED LIGHTING ON CUCUMBER SEEDLING GROWTH AND MORPHOLOGY

To be submitted to Acta Horticulturae
Pulsing Effects of Greenhouse Supplemental LED Lighting on Cucumber Seedling Growth and Morphology

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Keywords: greenhouse, light-emitting diode, *Cucumis sativus*, duty ratio, frequency, vegetable transplants

Abstract

Light Emitting Diodes (LEDs) are an alternative to current greenhouse supplemental lighting technology due to their advertised durable construction, improved energy efficiency, and selective spectral output. In addition, LEDs have the capacity to turn on and off high photon fluxes with rapid frequency (pulsed lighting). At the University of Arizona, LED pulsed lighting was tested as supplemental light in greenhouse. Cucumber (*Cucumis sativus* cv. Cumlaude) seedlings were grown until the second true leaf stage. Sunlight DLI (daily light integral, 7.6 ± 0.7 mol m\(^{-2}\) d\(^{-1}\)) was supplemented with red LEDs (661 nm) for 18 hours (2 AM – 8 PM) with an average intensity of 60 μmol m\(^{-2}\) s\(^{-1}\) PPF. This provided an additional 3.89 mol m\(^{-2}\) d\(^{-1}\) of red supplemental light to DLI. The treatments consisted of (1) no supplemental lighting (control), (2) continuous red-LED lighting, and (3) pulsed red-LED lighting at 50% duty ratio (2.5 kHz). The experiment was repeated twice. Results showed that both the continuous and the pulsed supplemental lighting improved the growth and morphology of cucumber seedlings, compared with those in the control. Significant differences between the continuous and the pulsed lighting treatments were observed for hypocotyl length. Plant hypocotyl length was greater in the pulsed lighting treatment than that of the continuous lighting treatment by 7.5%. However, there were no significant differences in
number of leaves, fresh and dry mass, leaf area, and chlorophyll concentration between the two treatments. We concluded that the pulsed lighting examined in this study (50% duty ratio, 2.5 kHz) could not substitute for continuous lighting in greenhouse cucumber transplants (cv. Cumlaude) as supplemental lighting since no benefits were observed and nursery propagators prefer compact cucumber transplants.

INTRODUCTION

Photosynthetic processes such as photochemistry, electron transport, and carbon metabolism have reaction speeds that vary in reaction time (Tennessen et al. 1995). For example, photochemistry reactions occur in picoseconds and nanoseconds (Diner, 1986), electron shuttling occurs in microseconds to milliseconds, and carbon metabolism occurs in seconds (Hardison and Hedley 1988, Kischbaum and Pearcy 1988). Pulsed lighting is characterized by frequency (on/off cycles per second) and duty ratio (on:off time ratio per on/off cycle). The concept behind using pulsed lighting is to provide pulses of light at specific frequencies in order to optimize the light reactions (photochemistry) that are limited by the dark reactions (electron transport, carbon metabolism) (Tennessen et al. 1995, Sager and Giger 1980).

Research reported that plants under pulsed light have higher photosynthesis than plants under continuous light (McCree and Loomis 1969, Kriedemann et al. 1973). However, other research stated that photosynthesis under pulsed light can never exceed the rate of photosynthesis under continuous lighting (Weller and Franck 1941, Rabinowitch 1956). Sager and Giger (1980) re-evaluated these studies following two
fundamental concepts: 1) identical integrated photon flux (IPF) should be used when comparing pulsed and continuous lighting and 2) identical time bases should be used for plant response rate and IPF. Sager and Giger (1980) concluded that most studies claiming that pulsed lighting was more efficient than continuous lighting did not follow the two fundamental concepts, or they did not provide enough data to re-evaluate their findings.

Pulsed lighting research continued by using primarily LEDs as a lighting source. For example, Tennessen et al. (1995) measured efficiency of photosynthesis in continuous and pulsed lighting on tomato (Lycopersicon esculentum). Continuous light was compared to pulsed lighting treatments that varied from 0 to 500 μmol m⁻² s⁻¹, frequencies of 5 kHz and 6.7 kHz and duty ratios of 1%, 5% and 10%. The researchers found that the net photosynthetic rate in pulsed light was the same as in continuous light.

In contrast, Mori et al. (2002) compared the growth of lettuce (Lactuca sativa) under continuous white LEDs to that under pulsed white LED light at varied frequencies ranging between 0.1 and 5 kHz and varied duty ratios (10 to 100 % duty ratio). Researchers found that the combination of 50% duty ratio and 2.5 kHz had the highest relative growth rate and net photosynthetic rate.

Light emitting diodes (LEDs) with pulsed lighting capacity are an alternative to current greenhouse supplemental lighting technology. The objective of this study is to evaluate LED pulsed lighting technology in greenhouses as supplemental lighting in order to potentially reduce electrical consumption and/or improve growth and morphology. To our knowledge, this is the first study evaluating LED pulsed lighting as a supplemental lighting technology for greenhouse nursery crops.
MATERIALS AND METHODS

Location: The experiment was performed inside a greenhouse (Tucson, AZ, US) covered with double-layer acrylic glazing, oriented north to south, and equipped with pad-and-fan cooling system, and natural-gas-forced hot-air heating system. The greenhouse had a floor area of 108 m\(^2\), with 2.5-m gutter height and 4.3-m peak height. The experiment was conducted from 6/7/2012 to 8/12/2012.

Plant material: Greenhouse cucumber (Cucumis sativus L.) cultivar Cumlaude (Rijk Zwaan, Bergschenhoek, Netherlands) was used. Seeds were planted in rockwool plugs (2.5 L x 2.5 W x 4.0 H cm, Grodan Delta, Canada) and placed in the dark in a germination chamber until radicle emergence (28 °C temperature) for 24 hours. The seedling plugs were then moved to a greenhouse and inserted into 7 cm L x 7 cm W x 6.5 cm H rockwool cubes (Grodan Delta, Canada). After cotyledon expansion (4 – 5 days from seeding), seedlings were given light treatments until they reached the second true leaf stage 12 ± 2 days after seeding. Seedlings were fertigated as needed using a nutrient solution (90 mg/l N, 47 mg/l P, 144 mg/l K, 160 mg/l Ca, 60 mg/l Mg, 113 mg/l S, 105 mg/l Cl in addition to micro nutrients).

Supplemental light treatments: The supplemental light treatments were 1) 60 μmol m\(^{-2}\) s\(^{-1}\) PPF of continuous 100% duty ratio red LED light and 2) 60 μmol m\(^{-2}\) s\(^{-1}\) PPF of pulsed (2.5 kHz) 50% duty ratio red LED light, where peak light intensity was twice as
great as the continuous light. A computer-controlled red-LED system (CCS Inc., Kyoto, Japan) 661 nm; FWHM:15 nm) was used for both treatments. To achieve the 60 μmol m\(^{-2}\) s\(^{-1}\) PPF at 50% duty ratio, first we adjusted the LED system to provide 120 μmol m\(^{-2}\) s\(^{-1}\) PPF at 100% duty ratio at 2.5 kHz, and then reduced the duty ratio to 50% without changing the input power and the frequency. The photoperiod of the supplemental light treatments was 18 hours (2 AM – 8 PM) providing an additional 3.89 mol m\(^{-2}\) d\(^{-1}\) of red supplemental light to solar daily light integral (DLI). Plants under solar light only, served as the control treatment.

**Plant measurements:** Photosynthesis measurements were performed one day before destructive plant measurements using a portable photosynthesis system (CIRAS-2, PP System, MA, USA) with 1000 μmol m\(^{-2}\) s\(^{-1}\) PPF (halogen lamp) under greenhouse temperature of 25.9 ± 1.4 °C and ambient CO\(_2\). Destructive measurements consisted of plant height, number of leaves (length > 1 cm), hypocotyl length, stem diameter, leaf area, shoot fresh mass, chlorophyll extraction (Moran and Porath, 1980) and shoot dry mass (80 °C for at least 48 hours).

**Experimental design and data analysis:** Plant measurements were done on 20 cucumber seedlings per treatment (experimental units). Experimental units were rotated every day to minimize the location effect inside the tray. In addition, the locations for the two LED treatments were switched every day inside the same greenhouse to improve the uniformity of environmental conditions. Analysis of variance (P = 0.05) was performed
to identify any difference among treatments, considering each of the 20 plants as an experimental unit (n=20). Mean separations were analyzed using student’s t-test (P = 0.05). The experiment was conducted twice.

RESULTS AND DISCUSSION

Environmental parameters in the greenhouse

The recorded DLI measured in 2 locations in the greenhouse at plant height was 7.5 ± 0.7 mol m⁻² d⁻¹. The canopy air temperature measured at two locations directly under the plant canopy was an average of 25.9 ± 1.4 °C and 21.4 ± 9.3 °C during the day and night, respectively. Atmospheric moisture measured as % relative humidity was 61.2 ± 6.4 and 72 ± 9.3 during the day and night, respectively.

Growth and photosynthesis parameters

The LED supplemental lighting at 50% duty ratio pulsed at 2.5 kHz increased dry mass, fresh mass, and chlorophyll concentration by 48, 18, and 32 % respectively, compared to the no-light control (Table 1). However, there was no significant difference in these growth-related parameters nor net photosynthetic rate between supplemental 2.5 kHz, 50% duty ratio pulsed-lighting and continuous supplemental lighting (Table 2).

These results support the findings by Tennessen et al. (1995), Sager and Giger (1980), Weller and Frank (1941), and Rabinowitch (1956), which explained that the rate of photosynthesis under pulsed lighting at the identical IPF and equal application times can only approach, and never exceed, the photosynthesis rate of continuous lighting.
Under low IPF, the photosynthetic apparatus is light limited, and a linear increase of photosynthesis will occur with an increase of IPF. In contrast, at high IPF, the limiting factor is the rate by which enzymes activate processes (enzyme limited). Under high light conditions (enzyme-limited), the increase of light reaction activity does not lead to an increase of net photosynthetic rate. Rabinowitch (1956) found that under high IPF of either pulsed or continuous lighting, the rate of photosynthesis was maximized when one oxygen molecule was released per chlorophyll molecule every 50 seconds and that high IPF pulsed lighting can only approach continuous lighting photosynthetic rates but never exceed them. Another reason that we did not demonstrate the positive effect of pulsed lighting is that the frequency and duty ratios were not optimized for cucumber photosynthesis, if such a combination ever exists. Previous researches have tested a limited range of combinations. For example, Tenesse et al (1995) only used 5 and 6.7 kHz frequencies and 1, 5, and 10% duty ratios for tomato. Research has to focus on finding the optimized, species specific frequency and duty-ratio recipes, in order to potentially save energy by using pulsed lighting technology. For instance, Mori et al. (2002) tested 8 different frequencies ranging from 0.1 to 500 kHz and 5 different duty ratios ranging from 10 to 70% in lettuce. After testing all these combinations he found that the optimal combination of 2.5 kHz frequency and 50% duty ratio increased photosynthetic rate and relative growth rate compared to continuous light.

**Morphological parameters**
Morphological parameters were significantly improved by the pulsed supplemental lighting treatment (2.5 kHz, 50% duty ratio) compared to the no-light control. Hypocotyl and epicotyl length were reduced to 61 and 77% of those of the control, respectively (Table 3). Similarly, stem diameter and leaf-area were increased by 33 and 17%, respectively (Table 3). These morphological parameters of the pulsed supplemental lighting treatment were statistically the same as those of the continuous supplemental lighting, except for hypocotyl length (Table 4). Hypocotyl length in the pulsed lighting treatment was 8% longer than in the continuous light treatment.

The inhibition of hypocotyl elongation is controlled by multiple photoreceptors (Arnim and Deng 1996). For example, cucumber hypocotyl cell elongation is inhibited by blue light (Cosgrove 1994, Spalding and Cosgrove 1989, 1992) and in Arabidopsis, phytochromes A and B, CRY1/HY4 (blue light receptor), and a genetically isolated UV-A receptor have been shown to inhibit hypocotyl cell elongation (Goto et al. 1993, Koornneef and Kendrick 1994, Whitelam and Harberd 1994, Young et al. 1992).

In this study, we postulate that the reduction of hypocotyl elongation by continuous light compared to pulsed light is governed by phytochrome-mediated responses. Two phytochrome genes are phyA and phyB. phyA is down regulated under continuous light and phyB is not significantly regulated by light. phyA gene expression leads to an increase of phytochrome A which is more stable in the Pfr form and phyB gene expression leads to an increase in phytochrome B which is more stable in the Pr form (Arnim and Deng 1996, Mancinelli, 1994). The decrease in the amount of phyA mRNA caused by continuous lights is correlated with the degradation of the Pfr form
(Koorneef and Kendrick, 1994). Seedlings under the pulsed-lighting treatment are exposed to 50% dark periods between light pulses. Hypocotyl elongation in the pulsed treatment can be caused by the potentially higher expression of phyA, leading to more Pr form available. In contrast, under continuous lighting (100% duty ratio), more Pfr form is available due to the constant expression of phyB and low expression of phyA. In other words, the continuous lighting treatment has more phytochrome in the Pfr than the Pr form, leading to the inhibition of hypocotyl elongation.

**CONCLUSION**

We concluded that supplemental pulsed lighting (50% duty ratio, 2.5 kHz) cannot substitute for supplemental continuous lighting in greenhouse cucumber transplants (cv. Cumlaude) as no benefits were observed. However, additional research must be performed to find the specific frequency/duty ratio recipe that can improve growth of greenhouse cucumber under sole-source artificial light. If an optimal recipe is found under sole-source artificial light, then this recipe can be evaluated as a supplemental lighting technology under greenhouse conditions.

**ACKNOWLEDGEMENTS**

The authors would like to thank CCS Inc. Kyoto, Japan. This project was funded by USDA SCRI grant No: 2010-51181-21369.
Literature Cited


Tables

Table 1. Effects of pulsed supplemental lighting on growth parameters of cucumber ‘Cumlaude’.

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>dry mass (g)</th>
<th>leaf number</th>
<th>Chlorophyl$^1$ (mg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No supplemental light</td>
<td>0.25 ± 0.006b</td>
<td>3</td>
<td>32.4 ± 0.945b</td>
</tr>
<tr>
<td>50% Duty Ratio (2.5 kHz)</td>
<td>0.37 ± 0.012a</td>
<td>3</td>
<td>42.7 ± 0.524a</td>
</tr>
</tbody>
</table>

$^1$Total chlorophyll (a and b) per plant leaf area

Table 2. Effects of pulsed and continuous supplemental lighting on growth and photosynthesis parameters of cucumber ‘Cumlaude’.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>dry mass (g)</th>
<th>leaf number</th>
<th>Chlorophyl$^1$ (mg/m$^2$)</th>
<th>NPR$^2$ (μmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Duty Ratio (2.5 kHz)</td>
<td>0.44 ± 0.019a</td>
<td>3</td>
<td>28.7 ± 0.719a</td>
<td>12.3 ± 0.439a</td>
</tr>
<tr>
<td>Continuous light</td>
<td>0.48 ± 0.022a</td>
<td>3</td>
<td>30.4 ± 0.515a</td>
<td>13.0 ± 0.479a</td>
</tr>
</tbody>
</table>

$^1$Total chlorophyll (a and b) per plant leaf area
$^2$Net Photosynthetic rate (rate of CO$_2$ exchange μmol m$^{-2}$ s$^{-1}$)

Table 3. Effects of pulsed supplemental lighting on morphological parameters of cucumber ‘Cumlaude’.

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>hypocotyl length (mm)</th>
<th>epicotyl length (mm)</th>
<th>stem diameter (mm)</th>
<th>leaf area (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No supplemental light</td>
<td>149 ± 0.125a</td>
<td>21.9 ± 0.81a</td>
<td>3.74 ± 0.09b</td>
<td>124 ± 2.98b</td>
</tr>
<tr>
<td>50% Duty Ratio (2.5 kHz)</td>
<td>90.4 ± 0.144b</td>
<td>16.8 ± 0.70b</td>
<td>4.97 ± 0.06a</td>
<td>145 ± 4.14a</td>
</tr>
</tbody>
</table>

Table 4. Effects of pulsed and continuous supplemental lighting on morphological parameters of cucumber ‘Cumlaude’.

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>hypocotyl length (mm)</th>
<th>epicotyl length (mm)</th>
<th>stem diameter (mm)</th>
<th>leaf area (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Duty Ratio (2.5 kHz)</td>
<td>95.7 ± 1.44a</td>
<td>22.4 ± 1.16a</td>
<td>5.27 ± 0.082a</td>
<td>181 ± 6.10a</td>
</tr>
<tr>
<td>Continuous light</td>
<td>88.6 ± 1.24b</td>
<td>24.0 ± 1.13a</td>
<td>5.38 ± 0.105a</td>
<td>188 ± 6.67a</td>
</tr>
</tbody>
</table>