Purdue University
Purdue e-Pubs

Open Access Dissertations | Theses and Dissertations

5-2018

Manipulating Light Quality, Light Intensity, and Carbon Dioxide Concentration to Optimize Indoor and Greenhouse Production of Annual Bedding Plant Seedlings

Joshua Ken Craver
Purdue University

Follow this and additional works at: https://docs.lib.purdue.edu/open_access_dissertations

Recommended Citation

Craver, Joshua Ken, "Manipulating Light Quality, Light Intensity, and Carbon Dioxide Concentration to Optimize Indoor and Greenhouse Production of Annual Bedding Plant Seedlings" (2018). *Open Access Dissertations*. 1712.
https://docs.lib.purdue.edu/open_access_dissertations/1712

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries. Please contact epubs@purdue.edu for additional information.

MANIPULATING LIGHT QUALITY, LIGHT INTENSITY, AND CARBON DIOXIDE CONCENTRATION TO OPTIMIZE INDOOR AND GREENHOUSE PRODUCTION OF ANNUAL BEDDING PLANT SEEDLINGS

by

Joshua Ken Craver

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Horticulture

West Lafayette, Indiana

May 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Roberto G. Lopez, Chair

Department of Horticulture, Michigan State University

Dr. Cary A. Mitchell, Co-chair

Department of Horticulture and Landscape Architecture, Purdue University

Dr. Jennifer K. Boldt

USDA-ARS, Greenhouse Production Research Group

Dr. Yiwei Jiang

Department of Agronomy, Purdue University

Dr. Krishna S. Nemali

Department of Horticulture and Landscape Architecture, Purdue University

Approved by:

Dr. Hazel Wetzstein

Head of the Graduate Program

This dissertation is dedicated to my lovely wife Shelby. I do not deserve a companion as loving, caring, patient, and dedicated as you have been throughout this entire graduate education. Words could never express my love for you or gratitude for all that you have sacrificed to make this degree a reality, but I promise to do my very best to show you for the rest of our life together.

ACKNOWLEDGEMENTS

The completion of this dissertation would not have been possible without the support and collaboration of so many colleagues, faculty, family, and friends. I would first like to thank my advisor, Dr. Roberto Lopez, for his continued guidance, patience, and investment in me throughout the past four years. Additionally, I would like to thank my committee members, Drs. Cary Mitchell, Yiwei Jiang, Jennifer Boldt, and Krishna Nemali, for all of their assistance and advice regarding both my research and professional development. Further, I would like to thank Rob Eddy, Michael Russell, and the rest of the greenhouse staff, without whom this research would never have been possible.

There are also so many staff, students, and faculty in the Department of Horticulture and Landscape Architecture that have supported me over the past four years. While I simply do not have space to name you all, thank you so much for your friendship and everything you have done to help me get to where I am today. Specifically, I would like to thank my fellow Lopez Lab members Garrett Owen and Madeline Olberg. From lab assistance to moral support, I feel incredibly blessed to have such dear friends seeing me through to the end of this degree.

Lastly, I would to thank all of my friends and family for their unconditional love and support through the best and worst of the last four years. My deepest gratitude goes out to my parents, Ken and Pamela Craver, who believed in me from the start and have provided nothing but love and encouragement every step of the way. Most of all, I owe this accomplishment to my amazing wife, Shelby. Serving as my most enthusiastic fan and constant motivator, I am incredibly blessed to share this life and degree with you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Craver, Joshua, K. PhD
Institution: Purdue University
Degree Received: May 2018
Title: Manipulating Light Quality, Light Intensity, and Carbon Dioxide Concentration to Optimize Indoor and Greenhouse Production of Annual Bedding Plant Seedlings
Committee Chair: Roberto G. Lopez

Annual bedding plant seedlings (plugs) are commonly produced in northern latitudes during the late winter and early spring when the natural daily light integral (DLI) in greenhouses is below recommended levels. Greenhouse supplemental lighting (SL) provides a means of increasing the DLI, with high-pressure sodium (HPS) lamps representing the current industry standard. However, low-profile and high-intensity light-emitting diode (LED) fixtures have recently emerged as a possible alternative for greenhouse SL. Additionally, due to the emission of very little radiant heat, LEDs may be used for sole-source lighting (SSL) applications where plants are produced on vertical shelving units in warehouses or shipping containers and in close proximity to the fixtures. Thus, with the development of LEDs for horticultural applications, the possibility of producing seedlings indoors using multi-layered, vertical production systems has become an increasingly realistic possibility. Therefore, the objectives of this research were to 1) compare HPS and LED SL sources in a commercial greenhouse for the propagation and finishing of annual bedding plant seedlings (Expt. 1); 2) evaluate the effects of various LED light qualities and intensities in a SSL environment on the morphology, nutrient uptake, and subsequent flowering of coreopsis (*Coreopsis grandiflora* ‘Sunfire’), pansy (*Viola* ×*wittrockiana* ‘Matrix™ Yellow’), and petunia (*Petunia* ×*hybrida* ‘Purple Wave’) seedlings (Expt. 2); and 3) determine the morphological and physiological responses of petunia ‘Dreams Midnight’ seedlings to the

interactive effect of light intensity, light quality, and carbon dioxide (CO_2) concentration under LED SSL (Expt. 3).

In Expt. 1, both seedlings and finished plants produced under LED and HPS SL were comparable in quality, while seedlings produced under no SL were of significantly lower quality. In Expt. 2, light intensity was the dominant factor in determining seedling quality, with higher light intensities generally leading to seedlings that were more compact with greater dry mass accumulation. The inclusion of far-red wavelengths during propagation was also found to reduce the time to flower for pansy 'Matrix^{TM} Yellow'. In Expt. 3, petunia 'Dreams Midnight' seedlings grown under LED SSL with a red:blue light ratio (%) of 90:10 and light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had greater dry mass accumulation and leaf area (LA) than those under the light ratio of 50:50 at the same light intensity. However, seedlings produced under a light ratio of 50:50 and light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ displayed the highest Rubisco efficiency (φ), photosynthesis at operating C_i concentration (A_{OP}), electron transport rate (ETR), and maximum net photosynthetic rate ($A_{n,max}$). A trend of increased dry mass accumulation and decreased φ for seedlings produced at a CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$ was also observed compared to 450 $\mu mol \cdot mol^{-1}$.

From results obtained in a commercial greenhouse, low-profile LEDs for greenhouse SL may be used as an alternative to traditional HPS lamps. However, the possibility of spectral manipulation in a greenhouse environment for desired growth responses appears to be limited when the relative contribution of SL from LEDs to DLI is low. For SSL production, while petunia 'Dreams Midnight' seedlings showed significantly higher φ, A_{OP}, ETR, and $A_{n,max}$ under increased intensities of blue radiation, the increased LA observed under a higher percentage of red radiation ultimately led to increased light interception and greater dry mass accumulation.

While the response is highly dependent on species and cultivar, the inclusion of far-red radiation under SSL may also be beneficial if accelerated flowering upon transplant is desired for plants with a long-day photoperiodic response. Additionally, while the CO_2-enriched environment led to higher dry mass accumulation, acclimation responses, such as reduced φ, may limit potential gains from this input. The present research provides deeper insight into the morphological and physiological responses of bedding plant seedlings to light and CO_2 in controlled environments, and establishes a foundation for future research to investigate how to best optimize these inputs.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction to Seedling Production and Lighting Applications

The production of young plants from seed for the annual bedding plant market commonly occurs during the late winter and early spring (Styer, 2003). However, in northern climates, the daily light integral (DLI) is insufficient for high-quality production in the greenhouse during this time (Fausey et al., 2005; Pramuk and Runkle, 2005). To clarify, DLI is defined as the total amount of photosynthetically active radiation (*PAR*) received by the plant each day as a function of light intensity and duration, measured as $mol \cdot m^{-2} \cdot d^{-1}$ (Torres et al., 2010). Previous research has shown that a target DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ is recommended to produce high-quality seedlings (Pramuk and Runkle, 2005; Randall and Lopez, 2014). Thus, to efficiently produce seedlings in the late winter and early spring in northern climates, where the DLI in the greenhouse can be as low as 1 to 5 $mol \cdot m^{-2} \cdot d^{-1}$, supplemental lighting (SL) must be supplied (Pramuk and Runkle, 2005). Providing SL is a means by which young plants can be grown under an optimal DLI during seasons when a lack of sufficient solar radiation may be limiting to uniform and consistent production, quality, and subsequent performance (Hernández and Kubota, 2012). Currently, high-pressure sodium (HPS) lamps are the industry standard for providing SL, with a photosynthetic photon flux density (*PPFD*) of 70 to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ commonly targeted (Lopez et al., 2017). However, older models of these fixtures are electrically inefficient compared to new technologies.

Light-emitting diodes (LEDs) are solid-state semiconductor devices that provide light with a very narrow spectrum (Stutte, 2009). For the typical commercial grower, any means of reducing energy consumption while also maintaining or improving the value of a crop is of

significant interest (Mitchell et al., 2012). Light-emitting diodes were initially a promising alternative to more traditional light sources, such as incandescent and high-intensity discharge (HID) lamps, which are generally less energy efficient and shorter-lived (Mitchell et al., 2012). However, recent studies have found that improvements in new HPS fixtures, such as the use of electronic ballasts and double-ended lamps, have led to a dramatic increase in their efficiency. Thus, the most recent HPS and LED fixtures are now relatively similar in energy efficiency (Nelson and Bugbee, 2014; Wallace and Both, 2016).

Light-emitting diodes possess many other attributes that make them desirable for a variety of production environments. One of these unique attributes is the capability to manufacture LEDs to emit a variety of narrow wave band colors (Both et al., 2017). Since specific wavelengths can be targeted using LEDs, potentially detrimental morphological or physiological plant responses can be avoided by not providing radiation that would otherwise be deemed extraneous or unnecessary (Mitchell et al., 2012). In the same way, specific wavelengths can be targeted using LEDs for their desired photomorphogenic responses. Additional advantages gained from targeting specific wavelengths using LEDs may include reduced pest and disease occurrence and increased nutritional value (Massa et al., 2008).

High-intensity discharge sources, such as HPS lamps, may increase the plant temperature by a significant amount due to the radiant heat emitted from the fixtures under high irradiance levels (Graper and Healy, 1991). Light-emitting diodes generally emit very little radiant heat, which is of particular value for growers looking at LEDs for use in sole-source lighting (SSL) applications. When producing plants under SSL conditions, the only light available to the plants is provided by electric sources. Since the waste heat from LEDs can be separated from the light that is emitted, multi-layered production in controlled environments has recently become a

possibility as plants can be placed very close to the LED fixtures (Wollaeger and Runkle, 2014). Thus, the possibility of producing plants on shelving units in the absence of sunlight has become an increasingly realistic proposition with these new lighting technologies. While SSL applications are certainly not appropriate for all crops, young plant production is one area that may benefit substantially from this technology as growers strive to achieve uniformity and high quality during months of the year when greenhouse environmental conditions are both unpredictable and unfavorable.

Research has shown that seedlings and cuttings of many annual bedding plant species can be produced at a similar or higher quality under SL from LEDs compared to HPS lamps (Currey and Lopez, 2013; Poel and Runkle, 2017; Randall and Lopez, 2014). Additionally, it has been found that SSL provided by LEDs is a viable method for the production of annual bedding plant seedlings (Randall and Lopez, 2015; Wollaeger and Runkle, 2014). For example, Randall and Lopez (2015) evaluated seedlings of vinca (*Catharanthus roseus* 'Titan Red Dark'), impatiens (*Impatiens walleriana* 'Super Elfin XP Blue Pearl'), geranium (*Pelargonium ×hortorum* 'Bullseye Red'), petunia (*Petunia ×hybrida* 'Dreams Midnight'), and French marigold (*Tagetes patula* 'Durango Yellow') under SSL using LEDs providing a red:blue light ratio (%) of either 87:13 or 70:30. It was found that, generally, seedlings produced under SSL were more compact (reduced height and leaf area), darker in foliage color (higher relative chlorophyll content), and had a higher root mass than those produced under SL or ambient lighting conditions in the greenhouse. Thus, LEDs provide an alternative for growers utilizing SL in the greenhouse while also providing a means for the production of seedlings under SSL conditions.

With production in controlled environments, managing the environment to optimize both system efficiency and plant growth is of utmost importance. With carbon dioxide (CO_2) and light

being the two primary inputs involved in photosynthesis (Tremblay and Gosselin, 1998), the following sections will aim to summarize what is currently known regarding these environmental parameters as well as deficits in our knowledge.

1.2 Light Intensity in Controlled Environments

Increases in light intensity or DLI have been found to influence the quality and time to subsequent flowering for seedlings of many bedding plant species. A quality bedding plant seedling is one that has a compact habit and reduced leaf area, a high root and shoot dry mass, a well-developed root system, and a thick stem caliper (Oh et al., 2010; Pramuk and Runkle, 2005). These qualitative parameters ultimately lead to seedlings that are more easily processed, shipped, and mechanically transplanted, which is desired by growers (Pramuk and Runkle, 2005). Part of the reason for increased seedling quality due to DLI is from the resulting increase in dry mass per unit fresh mass (Faust et al., 2005). This increased dry mass results in thicker tissues with increased carbohydrates and structural materials. In contrast, seedlings produced under lower DLIs have been found to show decreased growth rates and possess more water in the plant tissues. This effect ultimately results in softer tissues and seedlings that growers would refer to as being less "toned" (Faust et al., 2005; Graper et al., 1990).

Pramuk and Runkle (2005) found that as the DLI increased from 4.1 to 14.2 $mol \cdot m^{-2} \cdot d^{-1}$, the average number of nodes and shoot dry mass per internode increased linearly for celosia (*Celosia argentea* var. *plumosa* 'Gloria Mix'), impatiens 'Accent Red', French marigold 'Bonanza Yellow', and pansy (*Viola* ×*wittrockiana* 'Crystal Bowl Yellow') seedlings. Additionally, as the DLI increased, time to flower decreased for all species. Ultimately, according to the parameters listed previously for a quality seedling, Pramuk and Runkle (2005) stated that the quality of all species increased as the DLI increased. Additional research by Oh et

al. (2010) has shown similar results for petunia ‘Madness Red’ and pansy ‘Delta Premium Yellow’, with increased seedling quality as the DLI increased within a range from 7.6 to 17.2 $mol \cdot m^{-2} \cdot d^{-1}$. In addition to increasing seedling dry matter accumulation, the higher propagation DLI also led to a decrease in time to flower for both species (Oh et al., 2010). Similar results were also found by Hutchinson et al. (2012), as angelonia (*Angelonia angustifolia* ‘AngelMist White Cloud’), nemesia (*Nemesia fruticans* ‘Aromatica Royal’), osteospermum (*Osteospermum ecklonis* ‘Voltage Yellow’), and verbena (*Verbena* ×*hybrida* ‘Aztec Violet’) displayed a decrease in the time to flower as the DLI during propagation increased within a range from 1.2 to 12.3 $mol \cdot m^{-2} \cdot d^{-1}$.

Research conducted by Graper and Healy (1992) evaluated petunia ‘Red Flash’ seedlings under multiple DLIs, photoperiods, and photosynthetic periods. The treatments evaluated a DLI of 10 or 20 $mol \cdot m^{-2} \cdot d^{-1}$ administered as either 175 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 hours, 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 8 or 16 hours, or 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 8 hours with an additional 8 hours of day-extension photoperiodic lighting. The authors found that increased DLI was primarily responsible for increasing the growth rate and partitioning of carbohydrates into sugars for petunia seedlings. Thus, the seedlings were actively utilizing the products of photosynthesis under an increased DLI rather than partitioning these molecules into starch for storage (Graper and Healy, 1992). However, one drawback to an increased DLI during seedling production is that the shoot dry mass at flowering may be significantly reduced (Hutchinson et al., 2012; Pramuk and Runkle, 2005). Thus, while a crop may flower more quickly in the greenhouse, the finished plant will ultimately be smaller when it reaches a salable developmental stage. This earlier flowering may be beneficial when seedlings are produced with the intent for finishing in small containers, while a delay in flowering would likely be preferred for seedlings grown for larger containers as this

would encourage increased vegetative development (Mattson and Erwin, 2005). Therefore, it is important for a grower to evaluate what qualitative factors are most important for their target market as adjustments are made to light.

1.3 Light Quality in Controlled Environments

Light quality is detected by plants using photoreceptors such as phytochromes, cryptochromes, and phototropins (Cope et al., 2014; Lin, 2002; Runkle and Heins, 2001). Light-emitting diodes can be designed to emit wavelengths of light that match the absorbance peaks of these critical photoreceptors and plant pigments. The wavelengths typically deemed most important for plant growth and development are from 400 to 700 nm and are appropriately referred to as *PAR* (Cope et al., 2014). As mentioned previously, one of the benefits of LEDs is the ability to target specific wavelengths of light to elicit desired morphological or physiological responses in the plant. Chlorophylls *a* and *b* absorb light maximally in the red (663 and 642 nm, respectively) and blue (430 and 453 nm, respectively) wavebands (Kopsell et al., 2014). Thus, it has been proposed that LEDs can be selected to match the absorbance peaks of the photoreceptors involved in photosynthesis to increase plant productivity (Massa et al., 2008; Mitchell et al., 2012). As a result, LEDs providing primarily red and blue wavelengths are commonly selected in an attempt to promote increased photosynthetic activity and growth.

1.3.1 Red Wavelengths

Red wavelengths of light fall within the range of 600 to 700 nm on the visible light spectrum (Runkle and Heins, 2001). While red wavelengths are generally beneficial due to their promotion of photosynthetic activity, previous research has shown that red light alone is typically not sufficient for the optimum production of most crops. Producing plants under solely

red wavelengths of light has been found to result in responses similar to those observed in shade leaves (Buschmann et al., 1978). For example, when grown under only red wavelengths, barley (*Hordeum vulgare*) seedlings possessed chloroplasts that were more elongated and contained higher grana content and thylakoids per granum compared to chloroplasts under solely blue wavelengths. These alterations to the chloroplast are characteristic of plants grown under low light intensities (Buschmann et al., 1978).

Additionally, it has been found that many dicotyledonous crops will develop extensive hypocotyl elongation when exposed to solely red wavelengths of light (Hoenecke et al., 1992). However, a lighting combination including both red and blue wavelengths has been found to control stem elongation (Kigel and Cosgrove, 1991). Specifically, Kigel and Cosgrove (1991) found that stem elongation seemed to be regulated by both red and blue wavelengths of light in pea (*Pisum sativum* 'Alaska'). Additionally, Cope et al. (2014) found that plants exhibit a profound shade-avoidance response in the absence of blue light, which they believe is mediated by the combined activity of the photoreceptors phytochrome and cryptochrome. In addition to morphological effects, Yorio et al. (2001) found that net photosynthetic rate (A_n) in radish (*Raphanus sativus* 'Cherriette') was lowest when grown under solely red wavelengths compared to red:blue LEDs or cool white fluorescent (CWF) lamps at a *PPFD* of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These authors suggested that the decrease in A_n might be due to reduced chlorophyll content, which was also observed under solely red radiation (Yorio et al., 2001). Similarly, Goins et al. (1997) found that A_n increased in wheat (*Triticum aestivum* 'USU-Super Dwarf') under red LEDs supplemented with 10% blue radiation (blue fluorescent lamps) compared to solely red LEDs at a *PPFD* of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore, an ample discussion regarding photosynthetic and photomorphogenic responses to light quality must also include blue wavelengths.

1.3.2 Blue Wavelengths

Blue wavelengths fall within the range of 400 to 500 nm, and are generally believed to be less efficient at driving photosynthesis. One of the reasons for this loss in efficiency is due to the absorption of these wavelengths by non-photosynthetic pigments, such as anthocyanins. When blue photons are absorbed by these pigments, rather than being utilized for photosynthesis, their energy is dissipated as heat and/or fluorescence (Barnes et al., 1993). Additionally, carotenoids possess absorption maxima for blue photons, which may result in further energy losses due to their low efficiency for energy transfer to chlorophylls (Cope et al., 2014). Another reason for the loss in photosynthetic efficiency is from decreased leaf area that has been observed under high intensities of blue radiation. Specifically, this decrease in leaf area reduces the plant's ability for light capture, further decreasing the potential for photosynthesis (Cope et al., 2014).

Blue radiation has been found particularly beneficial in promoting various photomorphogenic responses (Cope et al., 2014). For example, blue wavelengths are critical for a variety of crops due to their role in growth inhibition (Cosgrove, 1981; Kigel and Cosgrove, 1991; Runkle and Heins, 2001). However, similar to observations with red wavelengths of light, subjecting plants to monochromatic blue light can result in undesirable elongation responses (Hernández and Kubota, 2016; van Ieperen et al., 2012). For example, Hernández and Kubota (2016) found that hypocotyl elongation of cucumber (*Cucumis sativus* 'Cumlaude') seedlings decreased as the percentage of blue radiation increased (up to 75%) at a light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, these authors also observed increased hypocotyl elongation under solely blue radiation compared to all other treatments. Hernández and Kubota (2016) proposed that this response might be linked to low phytochrome activation under solely blue radiation. Specifically, the authors explain that the cryptochrome mediation of hypocotyl elongation was not fully activated due to a lack of "coaction", whereby cryptochrome and phytochrome must act in

tandem to initiate the photomorphogenic response (Hernández and Kubota, 2016). Thus, the key to producing plants that are compact with reduced stem elongation appears to involve a combination of both red and blue wavelengths. Through the inclusion of a small percentage of blue radiation under a spectrum otherwise composed of red wavelengths, the excessive elongation of hypocotyls, stems, and petioles can be prevented (Goins et al., 1998; Hernández and Kubota, 2016; Hoenecke et al., 1992). As discussed previously, combinations of red and blue wavelengths provided by LEDs have also been found to produce compact bedding plant seedlings (Randall and Lopez, 2014; Wollaeger and Runkle, 2014). Thus, LEDs can be manufactured with the intent to elicit specific morphological attributes, such as the control of excessive stem elongation, during seedling production.

Blue radiation alone or in combination with red wavelengths has also been observed to affect stomatal density and aperture (Kinoshita et al., 2001; van Ieperen et al., 2012; Zeiger et al., 2002). Specifically, when blue radiation is added in small amounts alongside red radiation, it has been found that stomatal opening increases significantly compared to solely red light (Kinoshita et al., 2001; van Ieperen et al., 2012). The regulation of stomatal opening by blue radiation is believed to be mediated by phototropins (*phot1* and *phot2*) (Kinoshita et al., 2001). Increased stomatal opening ultimately leads to increased CO_2 uptake, which further increases photosynthesis (Kinoshita et al., 2001). Plants grown under high intensities of blue radiation also have higher stomatal densities. For example, Muneer et al. (2014) found that blue wavelengths were more efficient at manipulating stomatal structure and increased the total number of stomata for lettuce (*Lactuca sativa* ‘Hongyeom’) produced under a light intensity of 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to red or green wavelengths. Additionally, these same authors observed that the Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content of lettuce was highest

under blue wavelengths. Ultimately, an increase in photosynthetic enzymes appears to affect plant growth as lettuce produced under blue radiation at a light intensity of 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ displayed significantly greater leaf dry mass and A_n than plants produced under solely green or red radiation (Muneer et al., 2014).

1.3.3 Far-red Wavelengths

Stutte (2009) found that the phytochrome photostationary state could be manipulated using LEDs to either initiate earlier flowering or promote continued growth in the vegetative state. Phytochromes are the photoreceptors responsible for detecting changes in the red:far-red (R:FR) light ratio, with many species displaying shade avoidance symptoms under a low R:FR ratio (Franklin and Whitelam, 2005; Park and Runkle, 2017; Zhang and Folta, 2012). Additionally, far-red wavelengths (700 to 800 nm) have been indicated as having a significant effect on flowering (Downs and Thomas, 1982). For example, species with a long-day photoperiodic response such as campanula (*Campanula carpatica* 'Blue Clips'), coreopsis (*Coreopsis* ×*grandiflora* 'Early Sunrise'), and pansy 'Crystal Bowl Yellow' often display delayed flower initiation or development when grown under a spectrum deficient in far-red radiation (Runkle and Heins, 2001).

1.4 Carbon Dioxide in Controlled Environments

There is substantial interest in elevated CO_2 for use in controlled environments. During winter months when seedlings are often produced, CO_2 concentrations within the greenhouse can drop below what would normally be measured outdoors (Both, 2004). Specifically, CO_2 concentrations in a greenhouse without ventilation have been found to fall as low as 200 $\mu mol \cdot mol^{-1}$ (Tremblay and Gosselin, 1998). This can be detrimental to a crop, as delays can

often occur as these low CO_2 concentrations limit growth (Both, 2004). Generally, it has been found that plants respond well to elevated levels of CO_2. More specifically, environments enriched in CO_2 often lead to increased plant growth and improved water relations (Prior et al., 2011). In a review of numerous experiments regarding CO_2 enrichment, Kimball (1983) estimated that agricultural yields would increase by ~33% as a result of the earth's ambient CO_2 concentration doubling. More information on how to best manipulate and utilize CO_2 in controlled environments is essential as we strive for increased crop uniformity and quality alongside efforts for using production space more efficiently (Prior et al., 2011; Tremblay and Gosselin, 1998).

1.4.1 Greenhouse Carbon Dioxide Enrichment for Seedling Production

The enrichment of CO_2 in the greenhouse has been suggested as a means of reducing propagation time as well as the production of sturdier, higher quality seedlings (Tremblay and Gosselin, 1998). Additionally, CO_2-enriched plants have been found to possess increased water use efficiency (WUE), leading to seedlings that may overcome stress more easily during transplant (Tremblay and Gosselin, 1998). This enrichment appears to be most advantageous during seedling production due to vegetative growth being most prevalent at this stage. Due to seedlings being composed almost entirely of juvenile tissues, they are continuously expanding and able to best utilize a CO_2-enriched environment (Thomas et al., 1975; Tremblay and Gosselin, 1998). However, once the maximum potential for the formation of new tissues has been met, the increased photosynthate gained from CO_2 enrichment can no longer be utilized and is stored as starch.

As previously stated, a majority of conclusions regarding CO_2 enrichment for horticultural crops state that this input is primarily of use during seedling propagation (Thomas et

al., 1975). This conclusion has been tested in a variety of studies evaluating seedling growth in both vegetable and bedding plants. In a study evaluating the effect of elevated CO_2 on the growth of 96 genotypes of young tomato (*Lycopersicum esculentum*) plants, Lindhout and Pet (1990) found that increasing the CO_2 concentration from 320 to 750 µmol·mol^{-1} increased average overall growth by a factor of 2.3. Similarly, Krizek et al. (1974) found that cucumber 'Burpee Hybrid', lettuce 'Grand Rapids', and tomato 'Michigan-Ohio' seedlings produced in 7.5-cm pots displayed substantial increases in vegetative growth when produced under a CO_2 concentration of 2,000 µmol·mol^{-1} compared to ambient conditions. Additionally, Kaczperski et al. (1994) found that pansy 'Majestic Giant Yellow' seedlings displayed accelerated growth when produced at a CO_2 concentration of 1000 µmol·mol^{-1} compared to 500 µmol·mol^{-1}.

1.4.2 Prolonged Exposure to Elevated Carbon Dioxide

While there is substantial research displaying the benefits of an atmosphere enriched with CO_2, there is also evidence showing that the initial stimulation to photosynthesis may be reduced after prolonged exposure to elevated concentrations, and that suppression of plant growth may follow (Arp, 1991; Makino and Mae, 1999). The primary principle behind CO_2 enrichment is the balance between the carboxylation and oxygenation activity of Rubisco, the key enzyme involved in CO_2 fixation (Lindhout and Pet, 1990; Tremblay and Gosselin, 1998). As CO_2 concentrations are increased, carboxylation activity is favored and oxygenation is suppressed (Makino and Mae, 1999). As discussed previously, an atmosphere enriched with CO_2 allows for an increase in the carbon fixation rate, an increase in plant WUE, and may even allow for increased nitrogen use efficiency (NUE) through the reallocation of nitrogen (N) from Rubisco (Arp, 1991).

Photosynthesis stimulated under elevated CO_2 conditions is often limited by other components (Makine and Mae, 1999). Specifically, photosynthetic gains may become negated due to feedback inhibition of carbohydrate synthesis resulting from the surplus of carbohydrates produced under high CO_2 concentrations (Arp, 1991; Makino and Mae, 1999). For example, if the rate at which sucrose is synthesized surpasses the utilization rate in plant sinks, a resulting negative feedback on the enzymes involved in sucrose synthesis will likely result. Ultimately, this negative feedback will lead to decreased levels of inorganic orthophosphate (P_i) in the cytosol, resulting in higher rates of starch synthesis (Herold, 1980). Thus, it is believed that photosynthesis under these circumstances is limited by either electron-transport capacity or P_i-regeneration capacity (Makino and Mae, 1999). The accumulation of starch grains within the leaf may also inhibit photosynthetic capacity by altering normal chloroplast structure and function or by increasing diffusion resistance to CO_2 flux in the cell (Cave et al., 1981; Makino and Mae, 1999; Makino et al., 1994; Nafziger and Koller, 1976). Thus, as sink availability is saturated, the positive benefits gained under elevated CO_2 concentrations may be diminished during long-term exposure (Arp, 1991).

A decrease in Rubisco content due to prolonged exposure to elevated CO_2 concentrations has been observed in many species. This decrease in Rubisco content is typically associated with a decrease in leaf N content. Thus, photosynthetic capacity can be linked to leaf N content (Evans, 1989), with decreased photosynthesis under elevated CO_2 concentrations coinciding with a reduction in N content (Makino and Mae, 1999). Many studies have reported a 10-15% decrease in the dry mass concentration of N under elevated CO_2 concentrations (Taub and Wang, 2008). While some reports have stated that this decrease in leaf N may result from a dilution effect due to the increased assimilation of carbon from elevated CO_2 (Taub and Wang, 2008), it

has also been suggested that the decrease is actually due to a reallocation of N at the morphogenic level (Makino and Mae, 1999; Makino et al., 1997). Specifically, Makino and Mae (1999) describe that during sustained growth under an atmosphere enriched with CO_2, plants may reallocate N away from the leaf blade to the leaf sheaths and roots. Thus, plants are able to regulate photosynthesis at the whole plant level by altering their N allocation and, as a result, adjusting photosynthesis (Drake et al., 1997; Makino and Mae, 1999). Drake et al. (1997) suggest that NUE is increased under elevated CO_2 concentrations based on this increased rate of carbon assimilation per unit of N in the leaf.

1.4.3 Sink Limitations under Carbon Dioxide Enrichment

By growing plants under root-restricted conditions (i.e., small containers), the sink demand is reduced and may lead to increased starch accumulation (Robbins and Pharr, 1988). Arp (1991) found that a strong correlation exists between container volume and photosynthetic capacity. Under elevated CO_2 concentrations these effects become even more prevalent as the roots of plants become restricted more quickly, resulting in a decreased root:shoot ratio. However, it has been observed that plants containing a root storage organ, such as sugarbeet (*Beta vulgaris* 'UI 8'; Wyse, 1980) and radish ‘White Tip’ (Sionit et al., 1982), display an increased root:shoot ratio under elevated CO_2 concentrations due to the large sink for carbon present in their roots (Arp, 1991). Thus, the reduction in photosynthetic capacity observed is not inherently due to the elevated CO_2 concentrations, but rather the capacity of the plant sinks for carbohydrates (Arp, 1991). The utilization of elevated CO_2 concentrations for the production of seedlings has thus been recommended, as during this stage the plants are not typically sink-limited and can efficiently utilize the excess carbohydrates (Makino and Mae, 1999).

Frantz and Ling (2011) studied the effects of elevated CO_2 concentrations on petunia 'Madness White'. They found that increasing the CO_2 concentration from 400 to 800 µmol·mol^{-1} had no significant effect on the biomass of the plants at the final harvest. They stated that this was in contrast to many previous studies, which had shown that this same increase in the concentration of CO_2 had led to increases in growth, photosynthesis, and yield for C_3 species (Frantz and Ling, 2011). Frantz and Ling (2011) believed that the differences they noticed may have been due to restrictions to the root zone from the small container size. Specifically, they stated that the small container size might have caused the plants to become sink-limited. They believe that if this sink limitation does exist due to root restriction, only certain areas of the floriculture industry, such as young plant production, will benefit from CO_2 enrichment where the roots are not pot-bound (Frantz and Ling, 2011). However, an earlier study conducted by Niu et al. (2000) found that pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch' displayed an increase in vegetative growth, flower bud dry weight, and flower size under a CO_2-enriched atmosphere of 1000 µmol·mol^{-1}. The authors do mention that the overall magnitude of these increases was minimal. In both of these studies, pansy seedlings were transplanted into 10-cm pots (Frantz and Ling, 2011; Niu et al., 2000). These small pots may have imposed sink limitations, helping to explain the limited or nonexistent responses to elevated CO_2 observed in both studies. Therefore, bedding plant seedlings subjected to increased CO_2 concentrations may lead to significant growth increases given the lack of root restriction imposed during this stage of production.

1.4.4 Stomatal Interactions with Elevated Carbon Dioxide

For many species, it has been documented that elevated CO_2 concentrations lead to stomatal closure and decreased stomatal density (Drake et al., 1997). While an increase in the

concentration of CO_2 has been found to lead to stomatal closure, Herrick et al. (2004) found that the increased photosynthetic activity resulting from the CO_2 enrichment more than compensated for the diffusional limitation imposed by this closure. Additionally, this reduced stomatal conductivity helps to improve WUE by reducing water loss through transpiration (Drake et al., 1997). Thus, the resulting stomatal closure from elevated CO_2 concentrations appears to have no detrimental effect on the overall photosynthetic capacity of the plant.

1.5 Conclusion

Carbon dioxide has gained much publicity in recent years due to atmospheric levels continuing to rise (Tans, 2015). Regardless of the effect this increase may have on climate change, plant growth and development will certainly be influenced (Bazzaz, 1990). However, within the field of floriculture, knowledge concerning the effects of elevated CO_2 is generally lacking compared to the data on field crop and forest species (Prior et al., 2011). Additionally, in a recent economic feasibility study conducted by Banerjee and Adenaeuer (2014), the authors discuss that while vertical, indoor production applications are possible, extensive research regarding production techniques is still required to fully optimize these systems. Therefore, by determining optimal CO_2 and light parameters for production, the growing environment can be manipulated to potentially increase seedling photosynthetic rates, quality, and uniformity and decrease production time. With a better understanding of how specific light qualities, light intensities, and CO_2 concentrations affect plant morphology and physiology, more accurate guidelines and recommendations can be established concerning LED applications. The expansive list of commercially available LEDs was made evident by Stutte (2009) as this author describes how the future of this technology will see further advances in defining the emission spectra for specific applications and responses. Thus, with new LED products being introduced into the

industry annually, it is crucial that these basic guidelines and recommendations are made available to evaluate the efficacy and utility of these new introductions.

1.6 Literature Cited

Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant, Cell and Environ. 14:869−875.

Banerjee, C. and L. Adenaeuer. 2014. Up, up and away! The economics of vertical farming. J. Agr. Studies 2:40−60.

Barnes, C., T. Tibbitts, J. Sager, G. Deitzer, D. Bubenheim, G. Koerner, and B. Bugbee. 1993. Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux. HortScience 28:1197−1200.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Ann. Rev. Ecology and Systematics 21:167−196.

Both, A.J. 2004. Carbon dioxide enrichment in greenhouses, p. 47−50. In: P.R. Fisher and E. Runkle (ed.) Lighting up profits. 1st ed. Meister Media Worldwide, Willoughby, OH.

Both, A.J., B. Bugbee, C. Kubota, R.G. Lopez, C. Mitchell, E.S. Runkle, and C. Wallace. 2017. Proposed product label for electric lamps used in the plant sciences. HortTechnology 27:544−549.

Buschmann, C., D. Meier, H.K. Kleudgen, and H.K. Lichtenthaler. 1978. Regulation of chloroplast development by red and blue light. Photochemistry and Photobiology 27:195−198.

Cave, G., L.C. Tolley, and B.R. Strain. 1981. Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. Physiol. Plant. 51:171−174.

Cope, K.R., M.C. Snowden, and B. Bugbee. 2014. Photobiological interactions of blue light and photosynthetic photon flux: Effects of monochromatic and broad-spectrum light sources. Photochemistry and Photobiology 90:574−584.

Cosgrove, D.J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67:584−590.

Currey, C.J. and R.G. Lopez. 2013. Cuttings of *Impatiens*, *Pelargonium*, and *Petunia* propagated under light-emitting diodes and high-pressure sodium lamps have comparable growth, morphology, gas exchange, and post-transplant performance. HortScience 48:428−434.

Downs, R.J. and J.F. Thomas. 1982. Phytochrome regulation of flowering in the long-day plant, *Hyoscyamus niger*. Plant Physiol. 70:898−900.

Drake, B.G., M.A. González-Meler, and S.P. Long. 1997. More efficient plants: A consequence of rising atmospheric CO_2? Ann. Rev. Plant Physiol. Plant Mol. Biol. 48:609−639.

Evans, JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9−19.

Fausey, B.A., R.D. Heins, and A.C. Cameron. 2005. Daily light integral affects flowering and quality of greenhouse-grown *Achillea*, *Gaura*, and *Lavandula.* HortScience 40:114−118.

Faust, J.E., V. Holcombe, N.C. Rajapakse, and D.R. Layne. 2005. The effect of daily light integral on bedding plant growth and flowering. HortScience 40:645−649.

Franklin, K.A. and G.C. Whitelam. 2005. Phytochromes and shade-avoidance responses in plants. Ann. Bot. 96:169−175.

Frantz, J.M. and P. Ling. 2011. Growth, partitioning, and nutrient and carbohydrate concentration of *Petunia* ×*hybrida* Vilm. are influenced by altering light, CO_2, and fertility. HortScience 46:228−235.

Graper, D.F., W. Healy, and D. Lang. 1990. Supplemental irradiance control of petunia seedling growth at specific stages of development. Acta Hort. 272:153−157.

Graper, D.F. and W. Healy. 1991. High pressure sodium irradiation and infrared radiation accelerate *Petunia* seedling growth. J. Amer. Soc. Hort. Sci. 116:435−438.

Graper, D.F. and W. Healy. 1992. Modification of petunia seedling carbohydrate partitioning by irradiance. J. Amer. Soc. Hort. Sci. 117:477−480.

Goins, G.D., N.C. Yorio, M.M. Sanwo, and C.S. Brown. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Expt. Bot. 48:1407−1413.

Goins, G.D., N.C. Yorio, M.M. Sanwo-Lewandowski, and C.S. Brown. 1998. Life cycle experiments with *Arabidopsis* grown under red light-emitting diodes (LEDs). Life Support Biosph. Sci. 5:143−149.

Hernández, R. and C. Kubota. 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ. Expt. Bot. 121:66−74.

Herold, A. 1980. Regulation of photosynthesis by sink activity – the missing link. New Phytol. 86:131−144.

Hernández, R. and C. Kubota. 2012. Tomato seedling growth and morphology responses to supplemental LED lighting red:blue ratios under varied daily solar light integrals. Acta Hort. 956:187−194.

Herrick, J.D., H. Maherali, and R.B. Thomas. 2004. Reduced stomatal conductance in sweetgum (*Liquidambar styraciflua*) sustained over long-term CO_2 enrichment. New Phytol. 162:387−396.

Hoenecke, M.E., R.J. Bula, and T.W. Tibbits. 1992. Importance of ‘blue’ photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427−430.

Hutchinson, V.A., C.J. Currey, and R.G. Lopez. 2012. Photosynthetic daily light integral during root development influences subsequent growth and development of several herbaceous annual bedding plants. HortScience 47:856−860.

Kaczperski, M.P., A.M. Armitage, and P.M. Lewis. 1994. Accelerating growth of plug-grown pansies with carbon dioxide and light. HortScience 29:442 (abstr.).

Kigel, J. and D.J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049−1056.

Kimball, B.A. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agron. J. 75:779−788.

Kinoshita, T, M. Doi, N. Suetsugu, T. Kagawa, M. Wada, and K. Shimazaki. 2001. phot1 and phot2 mediate blue light regulation of stomatal opening. Nature 414:656−660.

Krizek, D.T., W.A. Bailey, H. Klueter, and R.C. Liu. 1974. Maximizing growth of vegetable seedlings in controlled environments at elevated temperature, light and CO_2. Acta Hort. 39:89−102.

Kopsell, D. A., C.E. Sams, T.C. Barickman, and R.C. Morrow. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. J. Amer. Soc. Hort. Sci. 139:469–477.

Lin, C. 2002. Blue light receptors and signal transduction. The Plant Cell 14:S207–S225.

Lindhout, P. and G. Pet. 1990. Effects of CO_2 enrichment on young plant growth of 96 genotypes of tomato (*Lycopersicum esculentum*). Euphytica 51:191–196.

Lopez, R., C. Currey, and E. Runkle. 2017. Light and young plants, p. 109−118. In: R. Lopez and E. Runkle (ed.). Light management in controlled environments. Meister Media Worldwide, Willoughby, OH.

Makino, A. and T. Mae. 1999. Photosynthesis and plant growth at elevated levels of CO_2. Plant Cell Physiol. 40:999–1006.

Makino, A., H. Nakano, and T. Mae. 1994. Effects of growth temperature on the response of ribulose-1,5-bisphosphate carboxylase, electron transport components, and sucrose synthesis enzymes to leaf nitrogen in rice, and their relationships to photosynthesis. Plant Physiol. 105:1231–1238.

Makino, A., T. Sato, H. Nakano, and T. Mae. 1997. Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. Planta 203:390–398.

Mattson, N.S. and J.E. Erwin. 2005. The impact of photoperiod and irradiance on flowering of several herbaceous ornamentals. Scientia Hort. 104:275–292.

Massa, G.D., H. Kim, R.M. Wheeler, and C.A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43:1951−1956.

Mitchell, C.A., A. Both, C.M Bourget, J.F. Burr, C. Kubota, R.G. Lopez, R.C. Morrow, and E.S. Runkle. 2012. LEDs: The future of greenhouse lighting! Chronica Hort. 52: 6–12.

Muneer, S., E.J. Kim, J.S. Park, and J.H. Lee. 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). Int. J. Mol. Sci. 15:4657–4670.

Nafziger, E.D. and H.R. Koller. 1976. Influence of leaf starch concentration on CO_2 assimilation in soybean. Plant Physiol. 57:560–563.

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS One 9:e99010. doi:10.1371/journal.pone.0099010.

Niu, G., R.D. Heins, A.C. Cameron, and W.H. Carlson. 2000. Day and night temperatures, daily light integral, and CO_2 enrichment affect growth and flower development of pansy (*Viola* ×*wittrockiana*) J. Amer. Soc. Hort. Sci. 125:436–441.

Oh, W., E.S. Runkle, and R.M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience 45:1332–1337.

Park, Y. and E.S. Runkle. 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Expt. Bot. 136:41−49.

Poel, B.R. and E.S. Runkle. 2017. Seedling growth is similar under supplemental greenhouse lighting from high-pressure sodium lamps or light-emitting diodes. HortScience 52:388–394.

Pramuk, L.A. and E.S. Runkle. 2005. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40:1336–1339.

Prior, S.A., G.B. Runion, S.C. Marble, H.H. Rogers, C.H. Gilliam, and H.A. Torbert. 2011. A review of elevated atmospheric CO_2 effects on plant growth and water relations: Implications for horticulture. HortScience 46:158–162.

Randall, W.C. and R.G. Lopez. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. HortScience 49:589–595.

Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience 50:705–713.

Robbins, N.S. and D.M. Pharr. 1988. Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. Plant Physiol. 87:409–413.

Runkle, E.S. and R.D. Heins. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Amer. Soc. Hort. Sci. 126:275−282.

Sionit, N., H. Hellmers, and B.R. Strain. 1982. Interaction of atmospheric CO_2 enrichment and irradiance on plant growth. Agron. J. 74:721–725.

Stutte, G.W. 2009. Light-emitting diodes for manipulating the phytochrome apparatus. HortScience 44:231–234.

Styer, C. 2003. Propagating seed crops, p 151–163. In: D. Hamrick (ed.). Ball redbook crop production: Volume two. 17th Ed. Ball Publishing, Batavia, IL.

Tans, P. 2015. Trends in atmospheric carbon dioxide. 1 October 2015. <http://www.esrl.noaa.gov/gmd/ccgg/trends>.

Taub, D.R. and X. Wang. 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J. Integr. Plant Biol. 50:1365–1374.

Thomas, J.F., C.D. Raper, Jr., C.E. Anderson, and R.J. Downs. 1975. Growth of young tobacco plants as affected by carbon dioxide and nutrient variables. Agron. J. 67:685–689.

Torres, A.P., C.J. Currey, R.G. Lopez, and J.E. Faust. 2010. Measuring daily light integral (DLI). Purdue Extension HO-238-B-W.

Tremblay, N. and A. Gosselin. 1998. Effect of carbon dioxide enrichment and light. HortTechnology 8:524–528.

van Ieperen, W. 2012. Plant morphological and developmental responses to light quality in a horticultural context. Acta Hort. 956:131–139.

van Ieperen, W., A. Savvides, and D. Fanourakis. 2012. Red and blue light effects during growth on hydraulic and stomatal conductance in leaves of young cucumber plants. Acta Hort. 956:223−230.

Wallace, C. and A.J. Both. 2016. Evaluating operating characteristics of light sources for horticultural applications. Acta Hort. 1134:435–444.

Wollaeger, H.M. and E.S. Runkle. 2014. Producing commercial-quality ornamental seedlings under sole-source LED lighting. Acta Hort. 1037:269–276.

Wyse, R. 1980. Growth of sugarbeet seedlings in various atmospheres of oxygen and carbon dioxide. Crop Sci. 20:456–458.

Yorio, N.C., G.D. Goins, H.R. Kagie, R.M. Wheeler, and J.C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience 36:380–383.

Zeiger, E., L.D. Talbott, S. Frechilla, A. Srivastava, and J. Zhu. 2002. The guard cell chloroplast: A perspective for the twenty-first century. New Phytol. 153:415−424.

Zhang, T. and K. Folta. 2012. Green light signaling and adaptive response. Plant Signal. Behav. 7:1–4.

CHAPTER 2. COMPARISON OF SUPPLEMENTAL LIGHTING PROVIDED BY HIGH-PRESSURE SODIUM (HPS) LAMPS OR LIGHT-EMITTING DIODES (LEDS) FOR THE PROPAGATION AND FINISHING OF BEDDING PLANTS IN A COMMERCIAL GREENHOUSE

2.1 Abstract

High-quality young plant production in the northern latitudes requires supplemental lighting (SL) to achieve a recommended daily light integral (DLI) of 10 to 12 mol·m^{-2}·d^{-1}. High-pressure sodium (HPS) lamps have been the industry standard for providing SL in greenhouses. However, low-profile and high-intensity light-emitting diode (LED) fixtures providing blue, red, white, and/ or far-red radiation have recently emerged as a possible alternative for greenhouse SL. Therefore, the objectives of this study were to 1) quantify the morphology and nutrient uptake of bedding plant seedlings under no SL, or SL from HPS lamps or LED fixtures, and 2) determine whether SL source during propagation or finishing influences finished plant quality or flowering. The experiment was conducted at a commercial greenhouse in West Lafayette, IN. Seeds of New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), French marigold (*Tagetes patula* 'Bonanza Deep Orange'), gerbera (*Gerbera jamesonii* 'Terracotta'), petunia (*Petunia ×hybrida* 'Single Dreams White'), ornamental millet (*Pennisetum glaucum* 'Jester'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), and zinnia (*Zinnia elegans* 'Zahara Fire') were sown in 128-cell trays. Upon germination, trays were placed in a double poly greenhouse under a 16-h photoperiod of ambient solar light and photoperiodic lighting of 2 µmol·m^{-2}·s^{-1} from compact fluorescent lamps, or SL of 70 µmol·m^{-2}·s^{-1} from either HPS lamps or LED fixtures with a red:blue light ratio (%) of 90:10. Seedling quality was evaluated up to four weeks after treatment initiation. Additionally, dried samples from each treatment were

analyzed for macro- and micronutrient concentration. After propagation data was collected, seedlings were transplanted and finished under SL provided by the same LED fixtures or HPS lamps in a separate greenhouse environment. Overall, seedlings produced under LED and HPS SL were comparable in quality. However, seedlings produced under SL were of significantly higher quality than those produced under no SL. Similarly, SL source during propagation and finishing had little effect on flowering and finished plant quality. While these results display that there is little difference in plant quality based on SL source, these findings further confirm the benefits gained from the use of SL for bedding plant production. Additionally, with both SL sources producing a similar finished product, growers can prioritize other factors related to SL installations such as energy savings, price of the fixtures, and fixture lifespan.

2.2 Introduction

The production of young plants (plugs) intended for spring bedding plant markets commonly begins during the late winter and early spring (Styer, 2003). For high-quality plug production, the recommended daily light integral (DLI) is 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Pramuk and Runkle, 2005; Randall and Lopez, 2014). However, in greenhouses located in northern latitudes the DLI is often insufficient during this time of the year, with DLIs as low as 1 to 5 $mol \cdot m^{-2} \cdot d^{-1}$ commonly reported (Fausey et al., 2005; Pramuk and Runkle, 2005). Supplemental lighting (SL) refers to the practice of increasing the amount of photosynthetic light made available to plants, in addition to ambient sunlight. Thus, through the provision of SL, high-quality young plants can be grown during times of the year when a lack of solar radiation may be limiting to uniform and consistent production (Hernández and Kubota, 2012).

Numerous studies have reported that increasing the DLI with SL from high-pressure sodium (HPS) lamps improves young plant quality and reduces subsequent time to flower (TTF)

of many bedding plant species (Hutchinson et al., 2012; Lopez and Runkle, 2008; Oh et al., 2010; Pramuk and Runkle, 2005). For example, Oh et al. (2010) observed increased seedling quality as the mean DLI increased within a range from 7.6 to 17.2 $mol \cdot m^{-2} \cdot d^{-1}$ for petunia (*Petunia ×hybrida* 'Madness Red') and pansy (*Viola ×wittrockiana* 'Delta Premium Yellow'). Specifically, seedling shoot dry mass (SDM) increased linearly as the propagation DLI increased. Additionally, the increased DLI during propagation hastened TTF for both species (Oh et al., 2010). Albright et al. (2000) documented a similar linear relationship between SDM and the total accumulated light from seeding to final harvest (35 d) for butterhead leaf lettuce (*Lactuca sativa* 'Ostinata'). Likewise, Graper and Healy (1992) found that an increased DLI led to increased growth rate and partitioning of carbohydrates into sugars for petunia 'Red Flash' seedlings.

High-pressure sodium lamps are the current industry standard for SL in controlled environments, commonly providing a photosynthetic photon flux density (*PPFD*; 400-700 nm) of 70 to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Lopez et al., 2017). Light-emitting diodes (LEDs) are a promising alternative to more traditional light sources, such as fluorescent and high-intensity discharge (HID) lamps, due to their energy-efficiency and long lifespans (Mitchell et al., 2012). However, advancements such as electronic ballasts and double-ended lamps have led to a competitive environment regarding the most efficient and cost-effective source for greenhouse SL. For example, recent studies have reported that double-ended HPS lamps and LED fixtures were relatively similar in terms of energy efficiency (Nelson and Bugbee, 2014; Wallace and Both, 2016).

Light-emitting diodes are solid-state semiconductor devices that are able to produce light with a very narrow spectrum (Stutte, 2009). Thus, one of the novel benefits from the utilization

of LED lighting is the ability to select wavelengths to elicit specific morphological or physiological plant responses (Morrow, 2008). For example, blue wavelengths of light (400-500 nm) have been found to serve a direct role in mediating stem extension and providing growth inhibition for a variety of crops (Cosgrove, 1981; Kigel and Cosgrove, 1991; Runkle and Heins, 2001).

Previous research has found that the use of experimental LED fixtures is a viable SL method for the production of bedding plant seedlings and cuttings (Currey and Lopez, 2013; Randall and Lopez, 2014). For example, Currey and Lopez (2013) found little difference in the growth, morphology, and subsequent flowering for cuttings of *Angelonia angustifolia* 'AngelMist White Cloud', *Nemesia fruticans* 'Aromatica Royal', *Osteospermum ecklonis* 'Voltage Yellow', and *Verbena ×hybrida* 'Aztec Violet' produced under SL providing a *PPFD* of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from either HPS lamps or LED arrays with red:blue light ratios (%) of 100:0, 85:15, or 70:30. Similarly, Randall and Lopez (2014) found that the quality of snapdragon (*Antirrhinum majus* 'Rocket Pink'), vinca (*Catharanthus roseus* 'Titan Punch'), impatiens (*Impatiens walleriana* 'Dazzler Blue Pearl'), geranium (*Pelargonium ×hortorum* 'Bullseye Scarlet'), petunia 'Plush Blue', salvia (*Salvia splendens* 'Vista Red'), French marigold (*Tagetes patula* 'Bonanza Flame'), and pansy 'Mammoth Big Red' seedlings grown under LED arrays with red:blue light ratios of 100:0, 85:15, and 70:30 providing a *PPFD* of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was greater than or similar to those produced under HPS lamps. Quality in this study was determined using the quality index (QI), an objective, integrated, and quantitative measurement by which to evaluate seedlings (Currey et al., 2013; Randall and Lopez, 2014).

To our knowledge, no research has evaluated the use of LED SL in a commercial setting. Therefore, the purpose of the study was to assess the use of LED fixtures manufactured to

provide SL as an alternative to traditional HPS lamps for the production of bedding plants in a commercial greenhouse. Specifically, the objectives of this study were to 1) evaluate the effect of SL source on the morphology and nutrient uptake of bedding plant seedlings; and 2) determine whether SL source during propagation or finishing influences finished plant quality or flowering.

2.3 Materials and Methods

2.3.1 Plant Material and Propagation Environment

Seeds of New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), French marigold 'Bonanza Deep Orange', gerbera (*Gerbera jamesonii* 'Terracotta'), petunia 'Single Dreams White', ornamental millet (*Pennisetum glaucum* 'Jester'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), and zinnia (*Zinnia elegans* 'Zahara Fire') were sown in 128-cell trays (14-mL individual cell volume) using a commercial soilless medium comprised of (by vol.) 65% peat, 20% perlite, and 15% vermiculite (Fafard Super Fine Germinating Mix; Sun Gro Horticulture, Agawam, MA). Trays were placed in a common greenhouse environment under 86% shade cloth (8635-O-FB; Ludvig Svensson, Inc., Charlotte, NC), with a constant air temperature set point of 23 °C.

Upon hypocotyl emergence, trays of each species were immediately moved to a commercial greenhouse facility (Galema's Greenhouse; West Lafayette, IN) where propagation SL treatments were established. These treatments consisted of either HPS lamps (600-watt; P.L. Light Systems, Beamsville, ON, Canada) or LED toplights (Philips 200-watt GreenPower LED toplighting modules; Philips Lighting, Rosemont, IL) with a red:blue light ratio of 90:10 (Fig. 1). Both SL sources provided a constant *PPFD* of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the course of a 16-h photoperiod (600 to 2200 HR). An ambient treatment (no SL) was also established which maintained a 16-h photoperiod through day-extension lighting supplied by compact fluorescent

lamps (CFL) providing a *PPFD* of 2 μmol·m^{-2}·s^{-1}. One tray for each species was placed under each of the SL treatments, and trays were rotated within each treatment daily to reduce any positional effects on light distribution. The propagation greenhouse was maintained at a constant air temperature set point of 23 °C. Environmental data was collected by a data logger (Model CR1000; Campbell Scientific, Inc., Logan, UT) which measured solar *PPFD* with amplified quantum sensors (LI-190; LICOR Biosciences, Lincoln, NE) and canopy air temperature using precision thermistors [fan-aspirated solar radiation shields (ST-110; Apogee Instruments, Inc.)] every 15 s within each of the treatments. The mean ± SD DLI from 4 Feb. to 30 Mar. 2015 of the ambient, HPS, and LED SL treatments was 5.4 ± 1.8, 11.1 ± 3.4, and 12.3 ± 4.0 mol·m^{-2}·d^{-1}, respectively. The mean ± SD canopy temperature from 4 Feb. to 30 Mar. 2015 under HPS and LED SL was 19.8 ± 3.6 and 20.0 ± 1.8 °C, respectively. Seedlings were irrigated with water-soluble fertilizer (Jack's Professional® 20N–0P_2O_5–20K_2O Hi Cal Peat-Lite; J.R. Peters, Inc., Allentown, PA) providing 100 mg·L^{-1} nitrogen (N).

2.3.2 Propagation Data Collection

Data was collected on seedling quality and morphology 14 (French marigold and ornamental millet), 21 (pepper, petunia, and zinnia), 28 (New Guinea impatiens) or 35 (gerbera) d after germination. Five seedlings for each species from each of the SL treatments were randomly selected for measurements and analysis. Roots and shoots of the seedlings were washed, and nondestructive measurements were taken which included stem length (cm; measured from the base of the hypocotyl to the shoot apical meristem), stem caliper (mm; measured above the lowest leaf with a digital caliper [digiMax; Wiha, Schonach, Germany]), and total number of nodes. Leaf area (LA; cm^2) was collected using a LA meter (LI-3100; LI-COR Inc., Lincoln, NE) by removing the seedling leaves at the axil. Roots and shoots (leaves and

stems) were then separated and placed in a drying oven at 70 °C for at least 4 d for the collection of root dry mass (RDM) and SDM. Based on LA and dry mass measurements, leaf area ratio [LAR; LA / (RDM + SDM)] was calculated. Additionally, stem length and caliper were used to calculate the sturdiness quotient (SQ; stem caliper/stem length) of each seedling. The quality index ([total dry mass × (shoot:root ratio + SQ)]) was then calculated according to Curry et al. (2013).

2.3.3 Nutrient Analysis

For New Guinea impatiens, pepper, petunia, and zinnia, shoots of five seedlings within each treatment were randomly collected, triple rinsed with deionized water, and placed in a drying oven at 70 °C for at least 4 d. The combined dry mass of these five seedlings provided a single sample for nutrient analysis, with a total of five samples for each species within each treatment being analyzed for each replication. Foliar N was determined using a CHN analyzer (PerkinElmer Series II CHNS/O Analyzer; PerkinElmer Instruments, Shelton, CT). For all other elements, plant tissue was digested in a microwave (MARS; CEM Corp., Matthews, NC) and nutrient concentration was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; Thermo iCAP 6300; Thermo Electron Corp., Waltham, MA) as described by Frantz (2013).

2.3.4 Finishing Environment

After propagation data collection, 10 randomly selected seedlings from each tray within the HPS and LED SL treatments were transplanted into 11.4-cm (600-mL) containers (Dillen Products, Middlefield, OH) filled with a commercial soilless medium comprised of (by vol.) 75% peat, 20% perlite, and 5% vermiculite (Fafard 2; Sun Gro Horticulture). Transplants were moved into a separate finishing greenhouse with an 18/15 ºC (day/night) air temperature set

point. Each set of ten transplants were equally distributed into one of two SL treatments for finishing which consisted of either HPS lamps (600-watt; P.L. Light Systems) or LED toplights (Philips 200-watt GreenPower LED toplighting modules; Philips Lighting) providing a constant *PPFD* of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the course of a 16-h photoperiod (600 to 2200 HR). Instantaneous *PPFD* was collected using a data logger (Model CR1000; Campbell Scientific, Inc.) with quantum sensors (LI-190; LICOR Biosciences). Additionally, mean air temperature within each SL treatment was recorded every 15 min. by a data logger (WatchDog 2800 Weather Station; Spectrum Technologies, Aurora, IL). The mean ± SD daily air temperature from 23 Mar. to 9 June 2015 under HPS and LED SL was 20.5 ± 2.4 and 20.1 ± 2.4 ºC, respectively. The mean ± SD DLI from 23 Mar. to 9 June 2015 under the HPS and LED SL treatments was 13.5 ± 4.8, and 15.0 ± 5.2 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. As necessary, plants were irrigated with acidified water alternating with fertigation using a water-soluble fertilizer (Jack's Professional® 20N–$10P_2O_5$–$20K_2O$ General Purpose; J.R. Peters, Inc.) providing 200 $mg \cdot L^{-1}$ N.

2.3.5 Finishing Data Collection

After transplant, plants were evaluated daily for first fully reflexed flower in order to calculate the TTF from the transplant date. Additionally, once the first flower on a transplant was fully reflexed, data was collected on plant height, number of nodes below the first open flower, and SDM. For ornamental millet, plants were harvested 42 d after transplant and TTF was not collected.

2.3.6 Statistical Analysis

The experiment was laid out in a complete block design, with trays assigned randomly to each SL treatment and species evaluated separately. For seedling data collection, the experiment was replicated twice over time for each of the species with morphological and nutrient data

pooled. For finishing data collection, the experiment was not repeated due to unforeseen greenhouse complications. The effect of SL treatment was compared by analysis of variance (ANOVA) using SAS (SAS version 9.3; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) and Tukey's honest significant difference (HSD) test at $P \leq 0.05$ for seedling data, while the effect of SL source during propagation (P), finishing (F), and their interaction (P×F) was compared by ANOVA for finishing data (Table 1).

2.4 Results

2.4.1 Stem Length and Caliper

The effect of SL treatment on stem length was variable among species (Fig. 2A). For New Guinea impatiens, stem length under ambient conditions was 23% and 12% greater than those produced under LED and HPS SL, respectively. Conversely, French marigold and ornamental millet displayed the greatest stem lengths under HPS SL. Specifically, stem length of French marigold was 14% greater under HPS compared to LED SL, while stem length of ornamental millet was 24% greater under HPS SL compared to ambient conditions. For the remaining four species, no significant differences in stem length were observed.

Regardless of species, stem caliper decreased for seedlings produced under ambient conditions compared to those under LED or HPS SL (Fig. 2B). For example, stem caliper was 18% and 20% (New Guinea impatiens), 36% and 35% (French marigold), 45% and 54% (ornamental millet), 15% and 22% (petunia), and 19% and 21% (zinnia) greater under LED and HPS SL, respectively, compared to ambient conditions. However, no differences in stem caliper were observed between the LED and HPS SL sources for any of the species.

2.4.2 Leaf Area and Node Number

Generally, LA was greatest for seedlings produced under SL (Fig. 2C). For example, LA was 76% and 72% (gerbera), 62% and 63% (French marigold), 115% and 116% (ornamental millet), 54% and 105% (petunia), and 94% and 102% (zinnia) greater under LED and HPS SL, respectively, compared to ambient conditions. Additionally, LA of petunia increased 33% under HPS compared to LED SL. Leaf area ratio was greatest for gerbera, New Guinea impatiens, French marigold, pepper, petunia, and zinnia produced under ambient lighting compared to both LED and HPS SL (Fig. 3). Additionally, LAR was greater under HPS compared to LED SL for pepper and petunia. Specifically, LAR of pepper and petunia increased 38% and 34%, respectively, under HPS compared to LED SL.

Generally, the number of nodes increased for seedlings produced under SL compared to ambient conditions (Fig. 2D). For example, the number of nodes increased by 33% and 33% (gerbera), 25% and 35% (French marigold), 55% and 50% (ornamental millet), 38% and 52% (petunia), and 19% and 16% (zinnia) under LED and HPS SL, respectively, compared to ambient conditions. However, differences in the number of nodes between the LED and HPS SL treatments were not observed.

2.4.3 Root and Shoot Dry Mass

The greatest accumulation of RDM and SDM occurred under LED or HPS SL for all species (Fig. 2E and 2F). For example, RDM increased 345% and 296% (gerbera), 183% and 139% (New Guinea impatiens), 392% and 340% (French marigold), 112% and 100% (ornamental millet), 455% and 381% (petunia), and 369% and 297% (zinnia) under LED and HPS SL, respectively, compared to ambient conditions. Similarly, SDM increased by 165% and 131% (gerbera), 68% and 63% (New Guinea impatiens), 162% and 119% (ornamental millet),

204% and 218% (petunia), and 195% and 195% (zinnia) under LED and HPS SL, respectively, compared to ambient conditions. No significant differences in RDM or SDM were observed between SL sources.

2.4.4 Sturdiness Quotient and Quality Index

For gerbera, New Guinea impatiens, and ornamental millet the SQ was highest under LED and HPS SL, with no significant differences observed between the two SL sources (Fig. 2G). However, the SQ of French marigold, pepper, and zinnia grown under LED SL was 15%, 23%, and 15% greater, respectively, than those produced under HPS SL.

Generally, QI values were higher under LED or HPS SL compared to ambient conditions (Fig. 2H). For example, the QI increased by 266% and 206% (gerbera), 186% and 141% (New Guinea impatiens), 422% and 355% (French marigold), 120% and 108% (ornamental millet), 412% and 322% (petunia), and 405% and 311% (zinnia) under LED and HPS SL, respectively, compared to ambient conditions. However, differences in QI between LED and HPS SL were not observed.

2.4.5 Nutrient Concentration

For many of the macronutrients, concentrations were highest under the ambient treatment for all four species evaluated (Table 2). For example, N, phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) concentrations of petunia were 69% and 41% (N), 64% and 64% (P), 40% and 22% (K), 9% and 9% (S), 22% and 9% (Ca), and 33% and 17% (Mg) higher under ambient conditions compared to LED and HPS SL, respectively. Additionally, specific macronutrient concentrations were significantly lower under LED SL for New Guinea impatiens, petunia, and zinnia compared to HPS SL. For example, concentrations of N, K, Ca, and Mg for petunia grown under HPS SL were 20%, 11%, 12%, and 14% greater, respectively,

than those produced under LED SL. Similarly, concentrations of N, K, and Mg for zinnia grown under HPS SL were 13%, 15%, and 11% greater, respectively, than those produced under LED SL.

Similar trends were observed regarding micronutrients, with greater concentrations often observed for seedlings grown under ambient conditions (Table 3). Additionally, micronutrient concentrations were often lower under LED compared to HPS SL. For example, concentrations of boron (B), iron (Fe), manganese (Mn), and zinc (Zn) for zinnia grown under HPS SL were 13%, 183%, 121%, and 23% greater, respectively, than those produced under LED SL. Similarly, concentrations of B, copper (Cu), Fe, Mn, and molybdenum (Mo) for New Guinea impatiens grown under HPS SL were 15%, 28%, 126%, 108%, and 21% greater, respectively, than those produced under LED SL.

2.4.6 Finishing

Supplemental lighting source during both propagation and finishing had little effect on TTF or finished plant quality for most species (Table 1). While no interaction between propagation and finishing SL source was observed, main effects were occasionally significant. For example, the main effect of finishing SL source on TTF was significant for zinnia, with plants finished under HPS SL flowering an average of 2 d earlier compared to LED SL (data not shown). The main effect of finishing SL source on height was significant for ornamental millet and petunia, with a 21% and 8% increase, respectively, for plants finished under HPS compared to LED SL (data not shown). Similarly, ornamental millet displayed a 78% increase in SDM when finished under HPS compared to LED SL (data not shown). When grown under HPS SL during propagation, petunia displayed an average of one additional node at flowering compared to those grown under LED SL (data not shown). The main effect of propagation SL source on

SDM was significant for gerbera and New Guinea impatiens, with a 33% and 54% increase, respectively, for plants grown under LED compared to HPS SL (data not shown).

2.5 Discussion

Desired qualities for bedding plant plugs include a compact habit, thick stem caliper, high root and shoot biomass, and a reduced LA to prevent mutual shading (Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). Plugs representing these qualities are generally more easily processed, shipped, and mechanically transplanted (Pramuk and Runkle, 2005). Generally, under a low-light environment, stem length and LA will increase through a physiological response known as shade avoidance (Franklin, 2008). In the present study, it was anticipated that plugs grown under ambient lighting would exhibit symptoms of shade avoidance due to the low DLI. While the results for stem length varied among species, LA was greatest for plugs receiving SL. Specifically, gerbera, French marigold, ornamental millet, petunia, and zinnia all displayed increases in LA under LED or HPS SL compared to ambient lighting. For all of these species, increases in node number also occurred under LED and HPS SL compared to ambient lighting. Thus, the increase in LA under SL was likely due in part to an increase in leaf number (nodes). However, seedlings grown under ambient lighting displayed symptoms of shade avoidance through increased LAR compared to LED and HPS SL. Leaf area ratio provides a measure of LA per unit of total dry mass (Hunt and Cornelissen, 1997). Thus, more resources were being used to increase LA, rather than leaf thickness, under ambient lighting conditions, to increase light interception. While LA and stem length trends were not necessarily indicative of an insufficient DLI under ambient lighting conditions, increased LAR values provide evidence for shade avoidance.

For petunia plugs, LA and LAR were reduced under LED compared to HPS SL. Leaf area ratio of pepper also decreased under LED compared to HPS SL. These responses may be due to the increased proportion of blue wavelengths supplied by the LED SL. Previous research has shown that increased percentages of blue wavelengths included in a light spectrum will inhibit the growth of bedding plant plugs (Randall and Lopez, 2014; Wollaeger and Runkle, 2015). For example, Randall and Lopez (2015) found that LA was reduced for petunia 'Dreams Midnight', impatiens 'Super Elfin XP Blue Pearl', and vinca 'Titan Red Dark' seedlings grown under sole-source LED lighting with an increased percentage of blue radiation. Similarly, Wollaeger and Runkle (2015) found that 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of blue radiation appeared to be sufficient for the stimulation of desirable growth responses, such as reduced stem length and LA, for impatiens 'SuperElfin XP Red', salvia 'Vista Red', and petunia 'Wave Pink' seedlings grown under sole-source LED lighting. However, for all other species in the present study, no differences in LA or LAR were observed between SL sources.

While the addition of blue wavelengths under a sole-source lighting environment can be beneficial for plant growth responses, the impact from the inclusion of blue radiation is likely diminished in a greenhouse environment due to ambient solar radiation. Specifically, Hernández and Kubota (2012) suggested that solar DLI provided ample blue radiation for the production of tomato (*Solanum lycopersicum* 'Komeett') seedlings. Hernández and Kubota (2014) found similar results with cucumber (*Cucumis sativus* 'Cumlaude') seedlings grown under greenhouse SL in that increases in the intensity of blue wavelengths had no significant benefit on crop growth or morphology. Poel and Runkle (2017a) evaluated HPS lamps and multiple LED fixtures, with light ratios providing 10-20% blue radiation, as a source of SL for the production of geranium 'Pinto Premium Salmon' and 'Ringo 200 Deep Scarlet', pepper 'Long Red Slim

Cayenne', petunia 'Single Dreams White' and 'Wave Misty Lilac', snapdragon 'Montego Yellow', and tomato 'Supersweet' seedlings with a target SL *PPFD* of 90 μmol·m^{-2}·s^{-1}. These authors found very little difference in seedling dry matter accumulation or morphology regardless of the SL source or percentage of blue radiation. However, Randall and Lopez (2014) found that the height of multiple bedding plant species was reduced when seedlings were grown under LED SL providing 15-30% blue radiation. Additionally, Hernández and Kubota (2014) found that under low-light conditions, with a DLI of ~5.2 mol·m^{-2}·d^{-1}, cucumber seedlings grown under LED SL with a higher percentage of blue radiation displayed decreased dry mass, leaf number, and LA. These inconsistent responses to light quality can likely be explained by the relative contributions of SL to DLI within each study. For example, Poel and Runkle (2017a) explain that SL provided 20% to 40% of the total DLI in their study, while SL in the studies by Randall and Lopez (2014, 2015) provided 40% to 70%. Poel and Runkle (2017a) conclude that ample blue wavelengths to saturate morphological responses were likely supplied from solar radiation during their study, resulting in little impact from the additional blue radiation provided by LED SL. In the present study, SL provided <33% of the average DLI for both the LED and HPS SL treatments. Thus, minimal responses to additional blue radiation from LED SL were likely observed due to contributions from solar radiation.

While differences between the SL sources were not observed, a higher stem caliper, RDM, and SDM were observed under HPS and LED SL compared to seedlings grown under ambient light. Generally, an increased DLI results in increased dry mass per unit of fresh mass, which ultimately leads to thicker tissues (Faust et al., 2005). For the seedlings produced under ambient light, the DLI was insufficient for optimal growth. Thus, dry matter accumulation was reduced which possibly resulted in softer tissues containing more water (Faust et al., 2005;

Graper et al., 1991). Multiple studies have shown that increased DLI leads to increases in the accumulation of RDM and SDM of young plants (Hernández and Kubota, 2014; Lopez and Runkle, 2008; Oh et al., 2010; Poel and Runkle, 2017b). For example, Lopez and Runkle (2008) observed that as the propagation DLI increased from 1.2 to 8.4 $mol \cdot m^{-2} \cdot d^{-1}$, RDM and SDM of petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' cuttings increased by 680% and 506%, 2395% and 106%, and 108% and 147%, respectively.

The QI provides a means of assessing young plant quality by integrating morphological parameters linked to the perception of a high-quality seedling, with increased values generally indicating higher quality (Currey et al., 2013; Randall and Lopez, 2014). Sturdiness quotient and QI values were highest under both LED and HPS SL compared to ambient light, which can be attributed to the increased stem caliper, RDM, and SDM. Additionally, higher SQ values were observed under LED compared to HPS SL for French marigold, pepper, and zinnia. While differences were not always significant, seedlings grown under LED SL for these three species displayed shorter stem lengths compared to those produced under HPS SL, ultimately resulting in increased SQ values.

The highest concentrations for both macro- and micronutrients were observed for seedlings grown under ambient light. This response is likely due to a dilution of the nutrient concentration due to the higher SDM observed under both LED and HPS SL. This dilution effect was suggested by Kuehny et al. (1991) after observing decreased foliar concentrations of nutrients under increased irradiance. These authors were able to remedy this effect though the expression of nutrient concentration on a starch-free dry weight basis (Kuehny et al., 1991). Thus, the higher nutrient concentrations observed under ambient lighting in the present study were likely due to the concurrent decrease in SDM observed.

Increased percentages of blue radiation have been linked to an increase in the concentration of many essential elements (Kopsell and Sams, 2013; Kopsell et al., 2014). However, select macro- and micronutrient concentrations were higher under HPS compared to LED SL for New Guinea impatiens, petunia, and zinnia in the present study. Thus, the increased blue radiation administered under LED SL had no effect on nutrient uptake. One possibility for the increased nutrient concentrations under HPS SL is elevated air and leaf temperature. The emission of radiant heat is commonly associated with the use of HPS lamps and has been found to increase canopy temperature (Faust and Heins, 1997). Poel and Runkle (2017a) reported that the leaf temperature relative to air temperature was 1 to 2 °C higher under HPS compared to LED SL. In the present study, leaf temperature was not measured and air temperature was similar between SL treatments. Increased leaf temperature has been found to increase stomatal opening (Urban et al., 2017), which may lead to higher nutrient uptake via increased mass flow. However, future research is required to confirm this hypothesis.

Generally, SL source during propagation or finishing had little effect on TTF or finished plant quality. However, during finishing, increased height and SDM for ornamental millet and petunia as well as a slight decrease in TTF for zinnia were observed when plants were grown under HPS SL. Increased temperatures due to the emission of radiant heat may have resulted in the increased growth and accelerated flowering for some species finished under HPS lamps. As mentioned previously, while air temperatures between the two treatments were similar, it is possible that leaf temperature was increased under HPS SL. Additionally, SL source during propagation had a limited effect on SDM at flowering, with increased values for gerbera and New Guinea impatiens when seedlings were grown under LED SL. While differences were not significant, both gerbera and New Guinea impatiens seedlings produced greater RDM and SDM

under LED compared to HPS SL during propagation. This increased dry matter accumulation may have led to accelerated establishment of transplants in the finishing environment, ultimately leading to increased SDM values at flowering.

2.6 Conclusion

The results from this study provide a practical comparison of LED and HPS SL for the production of bedding plant plugs and finished plant material in a commercial greenhouse. Based on these findings, we believe that low-profile LEDs may be used as an equivalent SL source to HPS lamps. However, when the relative contribution of SL from LEDs to DLI is low, spectral manipulation for desired growth responses appears to be limited. Through these findings, growers interested in SL installations can shift their primary focus from differences in plant quality and growth based on SL source to additional factors such as energy savings, price of the fixtures, and fixture lifespan.

2.7 Acknowledgements

I gratefully acknowledge Dr. Roberto Lopez, Dr. Jennifer Boldt, and Maria Del Rosario Del Rio for intellectual, greenhouse, and laboratory assistance; the USDA-ARS, Greenhouse Production Research Group for nutrient analysis; the USDA-NIFA SCRI grant No. 2010-51181-21369 for funding; and Philips Lighting and PL Light Systems for LED fixtures and HPS lamps, respectively. The use of trade names in this publication does not imply endorsement by Purdue University of products named nor criticism of similar ones not mentioned.

2.8 Literature Cited

Albright, L.D., A.J. Both, and A.J. Chiu. 2000. Controlling greenhouse light to a consistent daily integral. Amer. Soc. Agr. Eng. 43:421−431.

Cosgrove, D.J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67:584−590.

Currey, C.J. and R.G. Lopez. 2013. Cuttings of *Impatiens*, *Pelargonium*, and *Petunia* propagated under light-emitting diodes and high-pressure sodium lamps have comparable growth, morphology, gas exchange, and post-transplant performance. HortScience 48:428−434.

Currey, C.J., A.P. Torres, R.G. Lopez, and D.F. Jacobs. 2013. The quality index – a new tool for integrating quantitative measurements to assess quality of young floriculture plants. Acta Hort. 1000:385–391.

Fausey, B.A., R.D. Heins, and A.C. Cameron. 2005. Daily light integral affects flowering and quality of greenhouse-grown *Achillea*, *Gaura*, and *Lavandula.* HortScience 40:114−118.

Faust, J.E. and R.D. Heins. 1997. Quantifying the influence of high-pressure sodium lighting on shoot-tip temperature. Acta Hort. 418:85−91.

Faust, J.E., V. Holcombe, N.C. Rajapakse, and D.R. Layne. 2005. The effect of daily light integral on bedding plant growth and flowering. HortScience 40:645−649.

Franklin, K.A. 2008. Shade avoidance. New Phytol. 179:930−944.

Frantz, J.M. 2013. Uptake efficiency of phosphorus in different light environments by zinnia (*Zinnia elegans*) and vinca (*Catharanthus roseus*). HortScience 48:594−600.

Graper, D.F. and W. Healy. 1991. High pressure sodium irradiation and infrared radiation accelerate petunia seedling growth. J. Amer. Soc. Hort. Sci. 116:435−438.

Graper, D.F. and W. Healy. 1992. Modification of petunia seedling carbohydrate partitioning by irradiance. J. Amer. Soc. Hort. Sci. 117:477−480.

Hernández, R. and C. Kubota. 2012. Tomato seedling growth and morphology responses to supplemental LED lighting red:blue ratios under varied daily solar light integrals. Acta Hort. 956:187−194.

Hernández, R. and C. Kubota. 2014. Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. Scientia Hort. 173:92–99.

Hunt, R. and J.H.C. Cornelissen. 1997. Components of relative growth rate and interrelations in 59 temperate plant species. New Phytol. 135:395−417.

Hutchinson, V.A., C.J. Currey, and R.G. Lopez. 2012. Photosynthetic daily light integral during root development influences subsequent growth and development of several herbaceous annual bedding plants. HortScience 47:856−860.

Kigel, J. and D.J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049−1056.

Kopsell, D.A. and C.E. Sams. 2013. Increase in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light-emitting diodes. J. Amer. Soc. Hort. Sci. 138:31–37.

Kopsell, D. A., C.E. Sams, T.C. Barickman, and R.C. Morrow. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. J. Amer. Soc. Hort. Sci. 139:469–477.

Kuehny, J.S., M.M. Peet, P.V. Nelson, and D.H. Willits. 1991. Nutrient dilution by starch in CO_2-enriched chrysanthemum. J. Exp. Bot. 42:711–716.

Lopez, R., C. Currey, and E. Runkle. 2017. Light and young plants, p. 109−118. In: R. Lopez and E. Runkle (ed.). Light management in controlled environments. Meister Media Worldwide, Willoughby, OH.

Lopez, R.G. and E.S. Runkle. 2008. Photosynthetic daily light integral during propagation influences rooting and growth of cuttings and subsequent development of New Guinea impatiens and petunia. HortScience 43:2052−2059.

Mitchell, C.A., A. Both, C.M Bourget, J.F. Burr, C. Kubota, R.G. Lopez, R.C. Morrow, and E.S. Runkle. 2012. LEDs: The future of greenhouse lighting! Chronica Hort. 52:6−12.

Morrow, R.C. 2008. LED lighting in horticulture. HortScience 43:1947−1950.

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS One 9:e99010. doi:10.1371/journal.pone.0099010.

Oh, W., E.S. Runkle, and R.M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience 45:1332−1337.

Poel, B.R. and E.S. Runkle. 2017a. Seedling growth is similar under supplemental greenhouse lighting from high-pressure sodium lamps or light-emitting diodes. HortScience 52:388–394.

Poel, B.R. and E.S. Runkle. 2017b. Spectral effects of supplemental greenhouse radiation on growth and flowering of annual bedding plants and vegetable transplants. HortScience 52:1221–1228.

Pramuk, L.A. and E.S. Runkle. 2005. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40:1336−1339.

Randall, W.C. and R.G. Lopez. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. HortScience 49:589−595.

Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience 50:705−713.

Runkle, E.S. and R.D. Heins. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Amer. Soc. Hort. Sci. 126:275−282.

Stutte, G.W. 2009. Light-emitting diodes for manipulating the phytochrome apparatus. HortScience 44:231−234.

Styer, C. 2003. Propagating seed crops, p 151–163. In: D. Hamrick (ed.). Ball redbook crop production: Volume two. 17th Ed. Ball Publishing, Batavia, IL.

Urban, J., M.W. Ingwers, M.A. McGuire, and R.O. Teskey. 2017. Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides* ×*nigra*. J. Exp. Bot. 68:1757−1767.

Wallace, C. and A.J. Both. 2016. Evaluating operating characteristics of light sources for horticultural applications. Acta Hort. 1134:435−444.

Wollaeger, H.M. and E.S. Runkle. 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. HortScience 50:522−529.

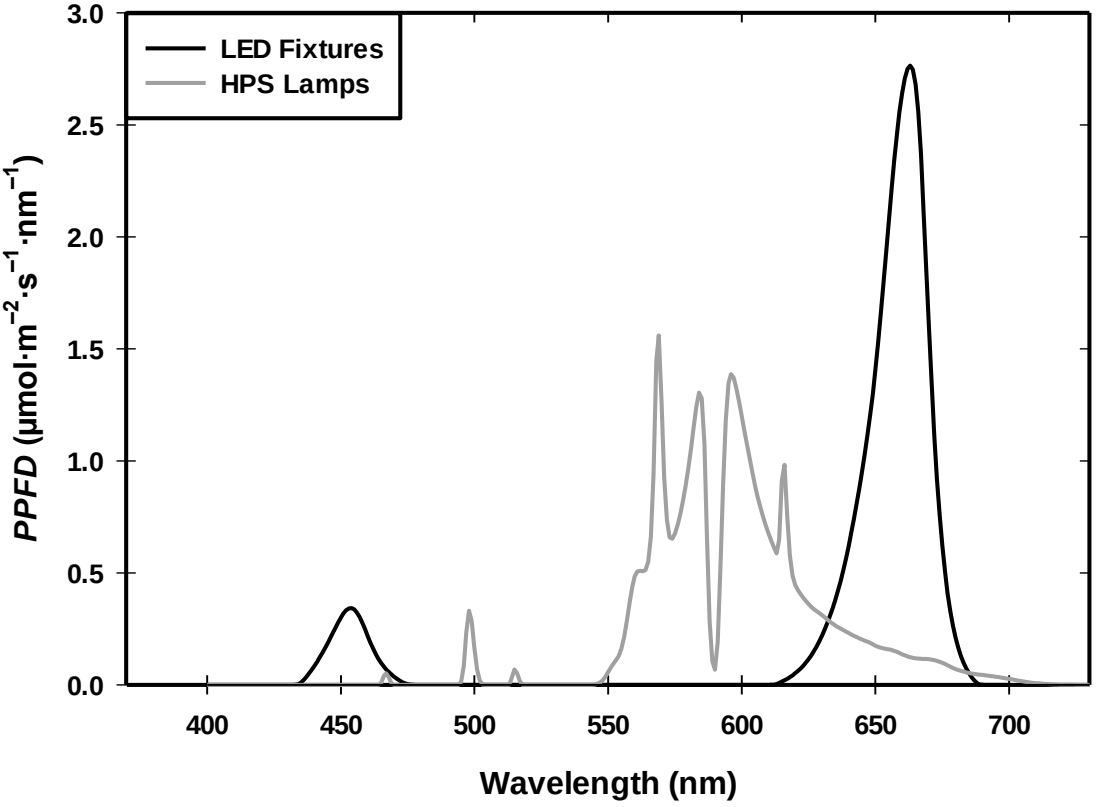


Figure 1. Spectral quality delivered from light-emitting diode (LED) fixtures or high-pressure sodium (HPS) lamps at a photosynthetic photon flux density (*PPFD*) from 400 to 700 nm of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level.

Figure 2. Propagation data for New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), French marigold (*Tagetes patula* 'Bonanza Deep Orange'), gerbera (*Gerbera jamesonii* 'Terracotta'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), petunia (*Petunia ×hybrida* 'Single Dreams White'), ornamental millet (*Pennisetum glaucum* 'Jester'), and zinnia (*Zinnia elegans* 'Zahara Fire') collected 28, 14, 35, 21, 21, 14, and 21 d after germination, respectively, grown under supplemental lighting provided by light-emitting dioide (LED) fixtures, high-pressure sodium (HPS) lamps, or no supplemental lighting (ambient). Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between supplemental lighting sources.

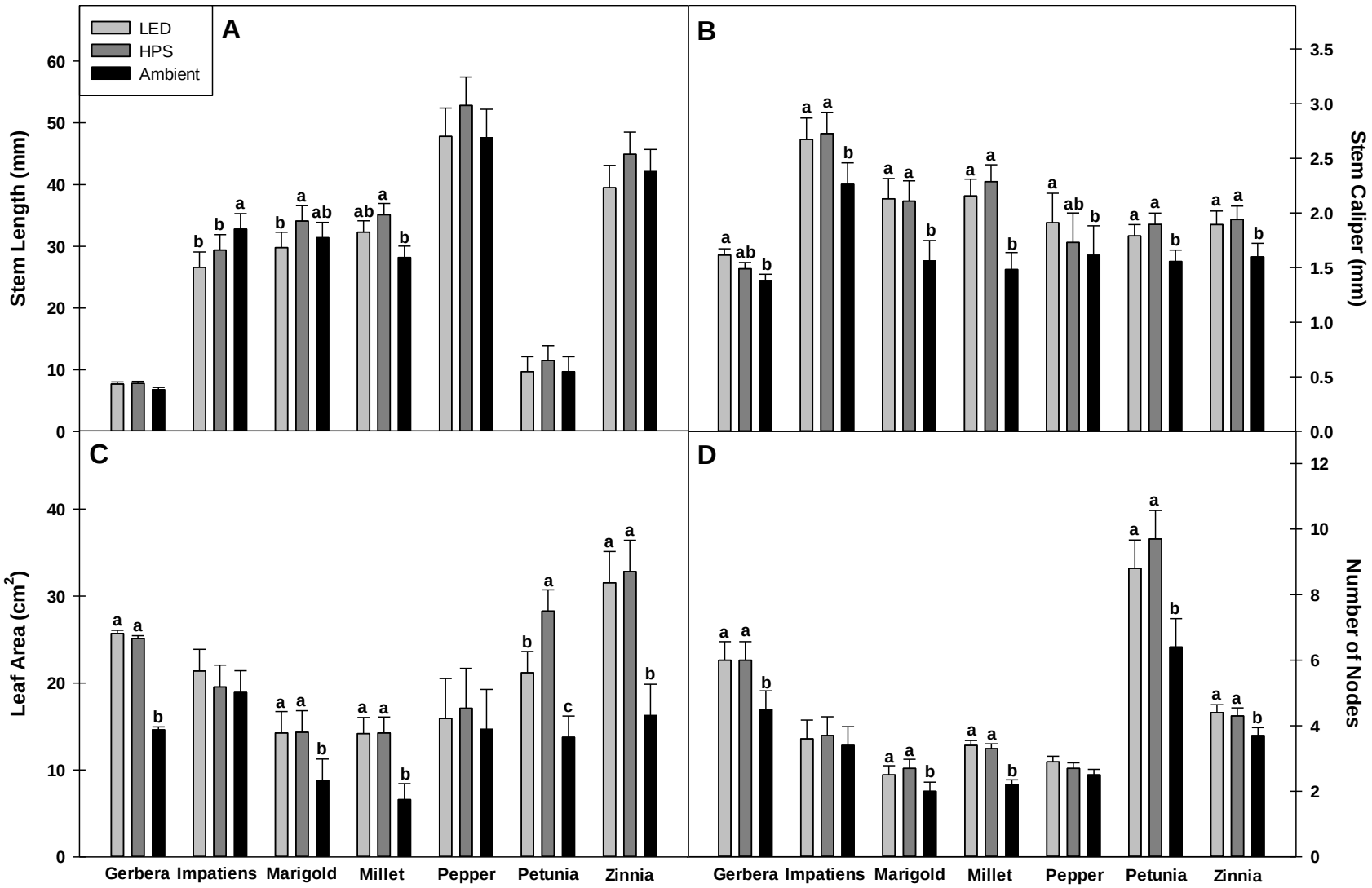
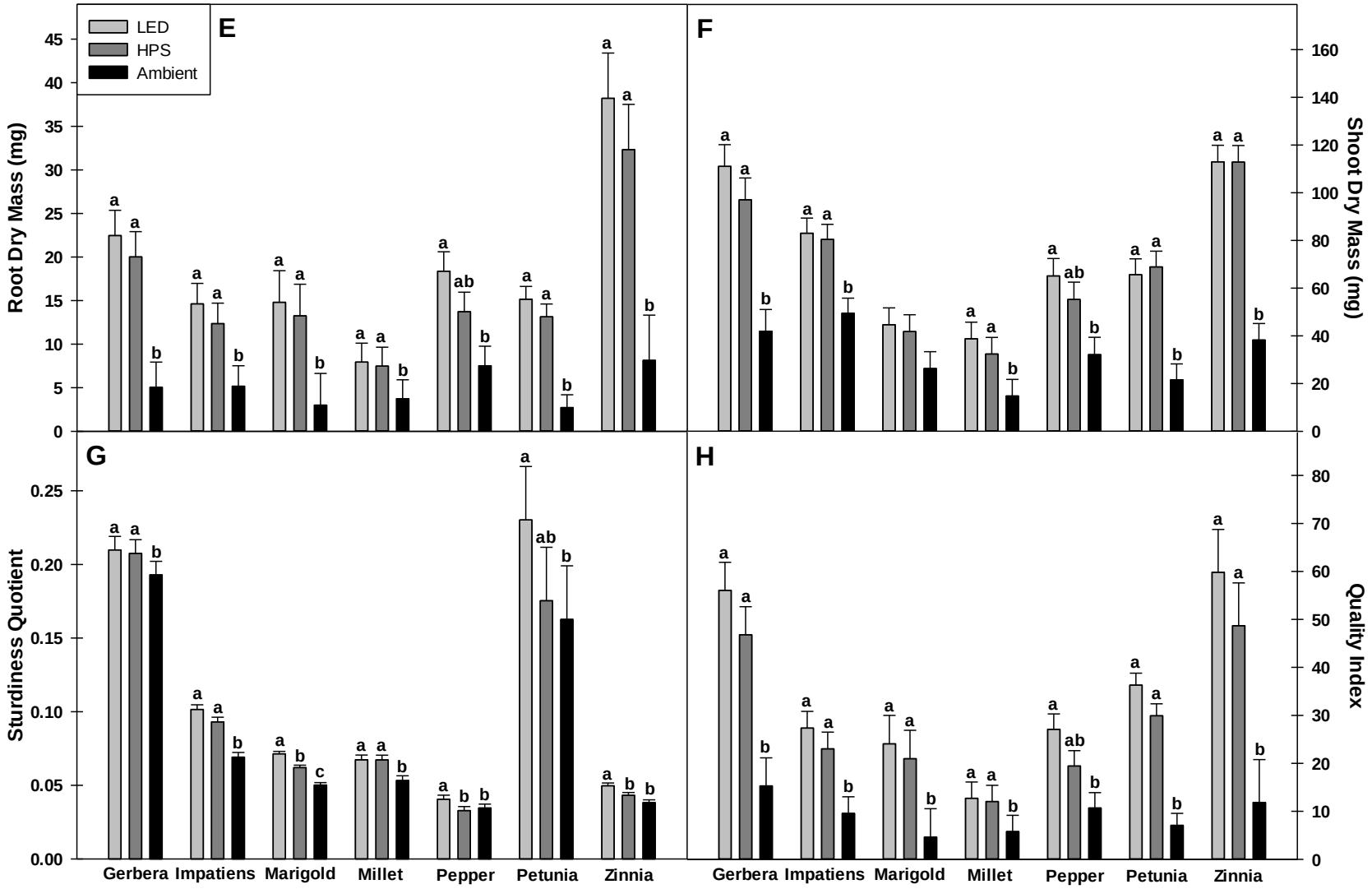

LED
HPS
Ambient
A
B
C
D
Stem Length (mm)
Stem Caliper (mm)
Leaf Area (cm2)
Number of Nodes
Gerbera
Impatiens
Marigold
Millet
Pepper
Petunia
Zinnia

Figure 2 continued



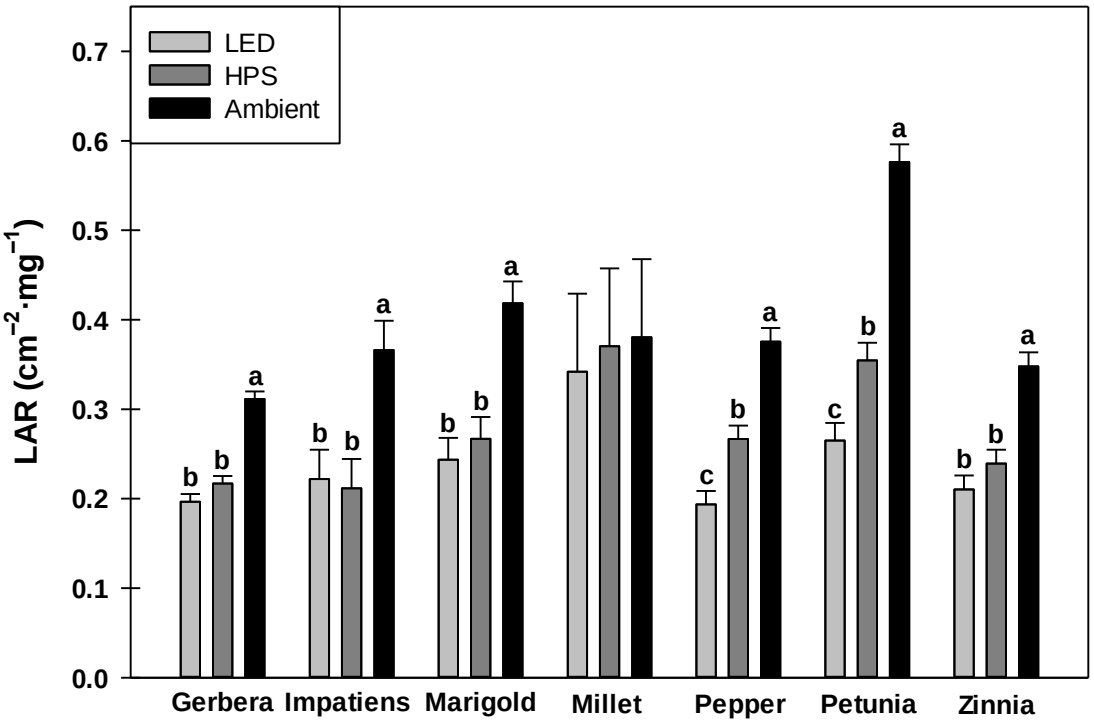


Figure 3. Leaf area ratio (LAR) for New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), French marigold (*Tagetes patula* 'Bonanza Deep Orange'), gerbera (*Gerbera jamesonii* 'Terracotta'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), petunia (*Petunia* ×*hybrida* 'Single Dreams White'), ornamental millet (*Pennisetum glaucum* 'Jester'), and zinnia (*Zinnia elegans* 'Zahara Fire') seedlings collected 28, 14, 35, 21, 21, 14, and 21 d after germination, respectively, grown under supplemental lighting provided by light-emitting dioide (LED) fixtures, high-pressure sodium (HPS) lamps, or no supplemental lighting (ambient). Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between supplemental lighting sources.

Table 1. Analysis of variance (ANOVA) for the effects of supplemental lighting source during propagation (P), finishing (F), or their interaction (P×F) on time to flower (TTF), height at flowering, number of nodes below first open flower, and shoot dry mass (SDM) at flowering for New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), French marigold (*Tagetes patula* 'Bonanza Deep Orange'), gerbera (*Gerbera jamesonii* 'Terracotta'), petunia (*Petunia ×hybrida* 'Single Dreams White'), ornamental millet (*Pennisetum glaucum* 'Jester'), and zinnia (*Zinnia elegans* 'Zahara Fire').

	TTF			Height			Nodes			SDM		
	P	**F**	**P×F**	**P**	**F**	**P×F**	**P**	**F**	**P×F**	**P**	**F**	**P×F**
Gerbera	NS[z]	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
Impatiens	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Marigold	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Millet	.[y]	.	.	NS	**	NS	NS	NS	NS	NS	*	NS
Petunia	NS	NS	NS	NS	*	NS	*	NS	NS	NS	NS	NS
Zinnia	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[y]Ornamental millet was harvested 42 d after transplant and TTF was not collected.

Table 2. Macronutrient concentration [percent dry mass (DM)] of New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), petunia (*Petunia ×hybrida* 'Single Dreams White'), and zinnia (*Zinnia elegans* 'Zahara Fire') seedlings, collected 21 to 28 d after germination, grown under supplemental lighting provided by light-emitting diode (LED) fixtures, high-pressure sodium (HPS) lamps, or no supplemental lighting (ambient).

	Macronutrients (percent DM)					
	Nitrogen (N)	**Phosphorus (P)**	**Potassium (K)**	**Sulfur (S)**	**Calcium (Ca)**	**Magnesium (Mg)**
			New Guinea Impatiens			
LED	4.58[z] b[y]	0.40 b	3.60 c	0.54 b	1.65	0.83
HPS	4.76 b	0.39 b	3.94 b	0.57 ab	1.76	0.93
Ambient	5.43 a	0.46 a	4.31 a	0.59 a	1.88	0.93
			Pepper			
LED	4.62 b	0.35 b	5.65 b	0.42 b	0.87	0.71 b
HPS	4.63 b	0.34 b	5.61 b	0.45 b	0.86	0.71 b
Ambient	5.51 a	0.44 a	7.46 a	0.58 a	0.92	0.87 a
			Petunia			
LED	4.17 c	0.33 b	5.21 c	0.54 b	0.86 c	0.51 c
HPS	5.00 b	0.33 b	5.76 b	0.54 b	0.96 b	0.58 b
Ambient	7.03 a	0.54 a	7.60 a	0.59 a	1.05 a	0.68 a
			Zinnia			
LED	4.52 c	0.33 b	4.69 c	0.41	0.96 b	0.81 c
HPS	5.09 b	0.34 b	5.41 b	0.41	1.10 a	0.90 b
Ambient	6.18 a	0.53 a	6.58 a	0.41	1.05 a	0.96 a

[z]Mean values are based on a representative sample from each treatment across two experimental replications.
[y]Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between supplemental lighting sources.

Table 3. Micronutrient concentration ($mg \cdot kg^{-1}$) of New Guinea impatiens (*Impatiens hawkeri* 'Divine Blue Pearl'), pepper (*Capsicum annuum* 'Hot Long Red Thin Cayenne'), petunia (*Petunia ×hybrida* 'Single Dreams White'), and zinnia (*Zinnia elegans* 'Zahara Fire') seedlings, collected 21 to 28 d after germination, grown under supplemental lighting provided by light-emitting diode (LED) fixtures, high-pressure sodium (HPS) lamps, or no supplemental lighting (ambient).

	Micronutrients ($mg \cdot kg^{-1}$)					
	Boron (B)	**Copper (Cu)**	**Iron (Fe)**	**Manganese (Mn)**	**Molybdenum (Mo)**	**Zinc (Zn)**
			New Guinea Impatiens			
LED	22.48[z] b[y]	8.18 b	231.6 b	97.0 b	1.10 b	57.29 b
HPS	25.81 a	10.51 a	522.3 a	202.0 a	1.33 a	60.56 b
Ambient	24.13 ab	11.45 a	391.4 a	128.8 b	1.30 a	73.64 a
			Pepper			
LED	30.53 b	9.89	175.6	58.0	1.01 b	54.90 b
HPS	32.61 b	9.50	174.3	61.4	1.18 ab	60.23 ab
Ambient	39.89 a	11.29	204.2	65.5	1.27 a	65.84 a
			Petunia			
LED	29.49 a	10.66 b	123.3 b	44.1 b	2.55 b	49.28 b
HPS	29.08 a	10.99 b	266.1 a	73.2 a	2.33 b	50.82 b
Ambient	23.63 b	15.70 a	230.6 a	58.2 ab	3.38 a	76.57 a
			Zinnia			
LED	76.94 c	12.53 b	269.1 b	107.4 b	1.58 a	31.14 c
HPS	87.21 b	12.91 b	762.4 a	237.8 a	1.43 ab	38.37 b
Ambient	98.48 a	14.73 a	541.4 a	208.6 a	1.38 b	63.74 a

[z]Mean values are based on a representative sample from each treatment across two experimental replications.
[y]Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant difference between supplemental lighting sources.

CHAPTER 3. LIGHT INTENSITY AND QUALITY FROM SOLE-SOURCE LIGHT-EMITTING DIODES (LEDS) AFFECT SEEDLING QUALITY AND SUBSEQUENT FLOWERING OF LONG-DAY PLANT SPECIES

3.1 Abstract

Light-emitting diodes (LEDs) have become an increasingly popular alternative to traditional lighting sources due to their energy efficiency, low output of radiant heat, and ability to target specific wavelengths of radiation. Previous research has shown high-quality annual bedding plant seedlings can be produced using LED sole-source lighting (SSL). However, when only red and blue radiation are used, a delay in time to flower was reported when seedlings of some long-day species were subsequently finished in a greenhouse. Thus, our objectives were to 1) evaluate the effects of light intensity and quality in a SSL environment on the morphology and nutrient uptake of annual bedding plant seedlings, and 2) determine whether an increase in light intensity or the inclusion of far-red or green radiation in a SSL environment would promote earlier flowering of long-day plants at finish. Coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') seedlings were grown at light intensities of 105, 210, or 315 µmol·m^{-2}·s^{-1}, achieved from LED arrays with light ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_{7}:B_{9}), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_{8}). Four-week old seedlings were subsequently transplanted and grown in a common greenhouse environment. Regardless of light quality, stem caliper, root dry mass, and shoot dry mass of seedlings generally increased for all three species as the light intensity increased from 105 to 315 µmol·m^{-2}·s^{-1}. Similarly, stem length of all three species generally decreased as the light intensity increased. Pansy seedlings grown under a light quality of R_{84}:FR_{7}:B_{9} flowered an average of 7 and 5 d earlier than those under R_{87}:B_{13} and R_{74}:G_{18}:B_{8},

respectively. These results provide information regarding the specific light parameters from commercially-available LEDs necessary to produce high-quality seedlings under SSL, with light intensity appearing to be the dominant factor in determining seedling quality. Furthermore, the addition of far-red wavelengths can significantly reduce time to flower after transplant and allow for a faster greenhouse turnover of some crops with a long-day photoperiodic response.

3.2 Introduction

The production of young plants from seed (plugs) for the annual bedding plant market commonly occurs during winter and early spring (Styer, 2003). However, in northern latitudes, the photosynthetic daily light integral (DLI) is not sufficient to produce high-quality young plants in the greenhouse (Lopez and Runkle, 2008; Pramuk and Runkle, 2005). Previous research has shown that a target DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ is recommended to produce high-quality young plants (Pramuk and Runkle, 2005; Randall and Lopez, 2014). Thus, to efficiently produce seedlings in northern latitudes, where the DLI in the greenhouse can be as low as 1 to 5 $mol \cdot m^{-2} \cdot d^{-1}$ during winter and early spring, supplemental lighting is recommended (Pramuk and Runkle, 2005). One alternative to traditional greenhouse production is multilayer or vertical indoor production in containers, warehouses, or chambers under sole-source lighting (SSL) provided by light-emitting diodes (LEDs). While SSL applications are not appropriate for all crops, young plant production is one area that may benefit substantially from this technology as growers strive to produce a uniform, high-quality crop during months of the year where greenhouse environmental conditions are both unpredictable and unfavorable. Additionally, young plant production may provide one of the most cost-effective applications for SSL due to the small size and high value of plugs and relatively short production cycle (Park and Runkle, 2017).

Previous research has shown that LED SSL is a viable method for the production of annual bedding plant seedlings (Randall and Lopez, 2015; Wollaeger and Runkle, 2014). Specifically, Randall and Lopez (2015) evaluated seedlings of vinca (*Catharanthus roseus* 'Titan Red Dark'), impatiens (*Impatiens walleriana* 'Super Elfin XP Blue Pearl'), geranium (*Pelargonium* ×*hortorum* 'Bullseye Red'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), and French marigold (*Tagetes patula* 'Durango Yellow') under SSL using LEDs providing a red:blue light ratio (%) of either 87:13 or 70:30. Generally, they found that seedlings produced under SSL were more compact (reduced height and leaf area), darker in foliage color (higher relative chlorophyll content), and had a higher root mass than those produced under supplemental lighting or ambient lighting conditions in the greenhouse.

Increases in light intensity and DLI have been reported to increase seedling quality and influence subsequent time to flower (TTF) for many bedding plant species (Oh et al., 2010; Pramuk and Runkle, 2005). Much of the reason for this increased seedling quality is attributed to an increase in dry mass per unit of fresh mass. Seedlings produced under lower DLIs generally show decreased growth rates and possess more water in the plant tissues, ultimately leading to softer tissues and seedlings that growers would refer to as being less "toned" (Faust et al., 2005; Graper et al., 1991). For example, Pramuk and Runkle (2005) found that as the DLI increased from 4.1 to 14.2 $mol \cdot m^{-2} \cdot d^{-1}$ during seedling production, the average shoot dry mass (SDM) per internode increased linearly for celosia (*Celosia argentea* var. *plumosa* 'Gloria Mix'), impatiens 'Accent Red', French marigold 'Bonanza Yellow', and pansy (*Viola* ×*wittrockiana* 'Crystal Bowl Yellow').

One of the benefits LEDs provide is the ability to select specific wavelengths of light to elicit desired morphological or physiological plant responses. Red wavelengths are most

commonly associated with their role in photosynthesis, while blue wavelengths are believed to be less efficient due to their absorption by pigments other than chlorophyll (Barnes et al., 1993; Cope et al., 2014; Franklin, 2008; Massa et al., 2008). Another reason for the loss in photosynthetic activity may be due to decreased leaf area (LA), which has been observed under high percentages of blue radiation (Cope et al., 2014). However, this inhibition response to blue wavelengths is desirable for many crops as a means of controlling excessive growth (Cope et al., 2014; Cosgrove, 1981; Kigel and Cosgrove, 1991; Runkle and Heins, 2001). Thus, LEDs can be manufactured with a variety of plant responses in mind, such as the control of stem elongation or matching the absorbance peaks of photoreceptors involved in photosynthesis (Mitchell et al., 2012).

Additionally, Stutte (2009) found that the phytochrome photostationary state could be manipulated using LEDs to either initiate earlier flowering or promote continued growth in the vegetative state. Far-red radiation has a significant effect in the plant processes of stem elongation and flowering (Downs and Thomas, 1982). For example, a deficiency in far-red radiation has often been found to delay flower initiation or development in species with a long-day photoperiodic response such as campanula (*Campanula carpatica* 'Blue Clips'), coreopsis (*Coreopsis* ×*grandiflora* 'Early Sunrise'), and pansy 'Crystal Bowl Yellow' (Runkle and Heins, 2001). Thus, reductions in light intensity as well as the lack of critical wavelengths in environments utilizing SSL may lead to delays in flowering and a reduction in seedling quality for some species.

While limited research has been conducted on the effects of light quality for young-plant production under SSL, to our knowledge, no research to date has evaluated how the manipulation of light intensity within various light qualities might further influence seedling quality and TTF

under SSL conditions. Additionally, by furthering our understanding regarding the impacts of LED SSL on nutrient uptake, a more thorough outlook on how to optimize production within these environments may be provided. Thus, our objectives were to: 1) evaluate the effects of various light qualities and intensities in a SSL environment on the morphology and nutrient uptake of annual bedding plant seedlings, and 2) determine whether an increase in light intensity or the inclusion of far-red or green wavelengths during seedling production in a SSL environment would promote earlier flowering of long-day plant species.

3.3 Materials and Methods

3.3.1 Plant Material and Germination Environment

Seeds of coreopsis 'Sunfire', pansy 'Matrix^{TM} Yellow', and petunia 'Purple Wave' were sown in 288-cell trays (6-mL individual cell volume) using a commercial soilless medium comprised of (by vol.) 65% peat, 20% perlite, and 15% vermiculite (Fafard Super Fine Germinating Mix; Sun Gro Horticulture, Agawam, MA) and germinated under a 16-h photoperiod (0600 to 2200 HR) in a glass-glazed greenhouse at Purdue University, West Lafayette, IN (lat. 40 °N). An environmental control system (Maximizer Precision 10; Priva Computers Inc., Vineland Station, Ontario, Canada) was used to adjust and measure the greenhouse air temperature. Solar photosynthetic photon flux density (*PPFD*; 400-700 nm) was measured by quantum sensors (SQ-110; Apogee Instruments, Inc., Logan, UT) every 15 s and the average was logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc., Logan, UT). The mean ± SD DLI and average daily temperature (ADT) from sowing to hypocotyl emergence were 7.5 ± 1.7 $mol \cdot m^{-2} \cdot d^{-1}$ and 22.7 ± 0.3 °C, respectively. Trays were regularly misted using clear water to maintain high humidity and soil moisture until germination occurred.

3.3.2 Growth Chamber Environment

Upon hypocotyl emergence, plug trays were placed under SSL treatments with a 16-h photoperiod (0600 to 2200 HR) in a walk-in growth chamber (C5 Control System; Environmental Growth Chambers, Chagrin Falls, OH). The air temperature, relative humidity, and CO_2 set points were 21 °C, 70/80% day/night (D/N; 16 h/8 h), and 500 μmol·mol^{-1}, respectively. A data logger (DL1 Datalogger; Environmental Growth Chambers) was used to record average air temperature, D/N relative humidity, and CO_2 concentration every 15 min, with a mean ± SD of 21.0 ± 0.1 °C, 69.8 ± 0.5% D/79.5 ± 0.5% N, and 499.6 ± 33.1 μmol·mol^{-1}, respectively. Seedlings were irrigated with water-soluble fertilizer (Jack's LX 16N–0.94P_2O_5–12.3K_2O Plug Formula for High Alkalinity Water; J.R. Peters, Inc., Allentown, PA) providing (in mg·L^{-1}): 100 nitrogen (N), 10 phosphorus (P), 78 potassium (K), 18 calcium (Ca), 9.4 magnesium (Mg), 0.10 boron (B), 0.05 copper (Cu), 0.50 iron (Fe), 0.25 manganese (Mn), 0.05 molybdenum (Mo), and 0.25 zinc (Zn).

3.3.3 Sole-source Lighting Treatments

A multilayer production system was utilized in the growth chamber for the establishment of SSL treatments. Light-emitting diode arrays providing light ratios of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8) (Philips GreenPower LED production modules; Koninklijke Philips Electronics, N.V., Netherlands) were mounted to nine stainless steel shelves (123-cm long and 61-cm wide). Non-reflective blackout cloth was used to prevent light pollution between treatments. Light intensity treatments were established by mounting 2, 4, or 6 modules, spaced 20.3, 12.2, or 8.6 cm apart and 38 cm above the crop canopy to achieve an average *PPFD* of 105, 210, or 315 μmol·m^{-2}·s^{-1}, respectively. A 16-h (0600 to 2200 HR) photoperiod provided plants with a DLI of 6, 12, or 18 mol·m^{-2}·d^{-1},

respectively. Light quality and *PPFD* were measured at the beginning and confirmed at the end of each experimental replication by taking nine individual spectral scans per treatment using a spectrometer (PS-100; StellarNet, Inc., Tampa, FL). Average *PPFD* and spectral qualities for each treatment are reported in Table 4 and Figure 4, respectively. Trays were rotated within each treatment daily to reduce any positional effects on light distribution.

3.3.4 Seedling Data Collection

After 28 d under the SSL treatments, five seedlings from each treatment were randomly selected for measurements and analysis. Roots and shoots of the seedlings were washed, and nondestructive measurements were taken for stem length (cm; measured from the base of the hypocotyl to the shoot apical meristem) and stem caliper (mm; measured above the lowest leaf with a digital caliper [digiMax; Wiha, Schonach, Germany]). Leaf area (cm^2) was recorded using a LA meter (LI-3100; LI-COR Inc., Lincoln, NE) by removing the seedling leaves at the axil. Roots and shoots (leaves and stems) were then separated and placed in a drying oven at 70 °C for at least 4 d for the collection of root dry mass (RDM) and SDM. Additionally, stem length and caliper were used to calculate the sturdiness quotient (SQ; stem caliper/stem length) of each seedling. The quality index (QI; [total dry mass × (shoot:root ratio + sturdiness quotient)]) was then calculated according to Curry et al. (2013).

3.3.5 Nutrient Analysis

After 28 d, shoots of eight seedlings within each treatment were randomly collected, triple rinsed with deionized water, and placed in a drying oven at 70 °C for at least 4 d. The combined dry mass of these eight seedlings provided a single sample for nutrient analysis, and a total of five samples for each species within each treatment was analyzed. Foliar N was determined using a CHN analyzer (PerkinElmer Series II CHNS/O Analyzer; PerkinElmer

Instruments, Shelton, CT). For all other elements, plant tissue was digested in a microwave (MARS6; CEM Corp., Matthews, NC) and nutrient concentration was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; Thermo iCAP 6300; Thermo Electron Corp., Waltham, MA) as described by Frantz (2013).

3.3.6 Finishing Environment

After 28 d, five randomly selected seedlings from each tray were transplanted into 11.4-cm (600-mL) containers (Dillen Products, Middlefield, OH) filled with a commercial soilless medium comprised of (by vol.) 75% peat, 20% perlite, and 5% vermiculite (Fafard 2; Sun Gro Horticulture) on 23 Dec. 2014 (replication 1) and 11 Feb. 2015 (replication 2). Plants were placed in a common finishing environment with an air temperature set point of 20 °C. An environmental control system (Maximizer Precision 10; Priva Computers Inc.) managed exhaust fan and evaporative-pad cooling, radiant hot water heating, and retractable shade curtains for the greenhouse. Solar *PPFD* was measured by quantum sensors (SQ-110; Apogee Instruments, Inc.) every 15 s and the average was logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc.). Supplemental lighting was provided by 1000-W high-pressure sodium (HPS) lamps to assist in achieving a minimum DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$. Average daily temperature and DLI were 20.3 ± 0.5 °C and 12.5 ± 3.9 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. When necessary, plants were irrigated with clear water alternating with fertigation using a combination of two water-soluble fertilizers (3:1 mixture of 15N–2.2P_2O_5–12.5K_2O and 21N–2.2P_2O_5–16.6K_2O; Everris, Marysville, OH) to provide the following (in $mg \cdot L^{-1}$): 200 N, 26 P, 163 K, 50 Ca, 20 Mg, 1.0 Fe, 0.5 Mn and Zn, 0.24 Cu and B, and 0.1 Mo.

3.3.7 Finishing Environment Data Collection

After transplant, plants were evaluated daily for first fully reflexed flower in order to calculate the TTF from the transplant date. Additionally, once the first flower on a transplant was fully reflexed, data was collected on the number of nodes below the first open flower and SDM.

3.3.8 Statistical Analysis

The experiment was a completely randomized design with light quality (three levels) and light intensity (three levels) as factors and species evaluated separately. The experiment was replicated three times over time for the seedling data collection and twice over time for the finishing data collection. The effects of light intensity and quality were compared by analysis of variance (ANOVA) using SAS (SAS version 9.3; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) and Tukey's honest significant difference (HSD) test at $P \leq 0.05$. With the majority of variables displaying no significant interaction between light intensity and quality (Table 5), main effect means were reported for morphological and finishing data (Tables 6 and 7) while the interaction was reported for nutrient data (Tables 8 and 9). Additionally, the factors of light intensity and quality were evaluated separately for morphological and finishing data. The effect of light intensity was compared within light qualities, while the effect of light quality was compared within light intensities for each species (Tables 10 and 11).

3.4 Results

3.4.1 Stem Length and Caliper

For all species, stem length decreased as light intensity increased (Table 6). Stem length was 9%, 16%, and 21% shorter as light intensity increased from 105 to 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for coreopsis, pansy, and petunia, respectively. Stem caliper increased as light intensity increased for

all three species (Table 6). When light intensity increased from 105 to 315 μmol·m^{-2}·s^{-1}, stem caliper increased 14%, 14%, and 10% for coreopsis, pansy, and petunia, respectively.

Regarding light quality, stem length was greatest for pansy and petunia under the ratio of R_{84}:FR_7:B_9 (Table 7). For example, stem length of pansy was 7% and 13% shorter under the light qualities of R_{87}:B_{13} and R_{74}:G_{18}:B_8, respectively, compared to R_{84}:FR_7:B_9. Likewise, stem length of petunia was 15% shorter under the light quality of R_{87}:B_{13} compared to R_{84}:FR_7:B_9. For coreopsis and petunia, stem caliper was greatest under the light quality of R_{84}:FR_7:B_9 (Table 7). Stem caliper of coreopsis increased 12% and 9% under the light quality of R_{84}:FR_7:B_9 compared to R_{87}:B_{13} and R_{74}:G_{18}:B_8, respectively. Additionally, stem caliper of petunia increased 13% and 11% under the light quality of R_{84}:FR_7:B_9 compared to R_{87}:B_{13} and R_{74}:G_{18}:B_8, respectively.

3.4.2 Leaf Area

For petunia, LA decreased as light intensity increased (Table 6). As light intensity increased from 105 to 315 μmol·m^{-2}·s^{-1}, LA of petunia decreased 23%. Conversely, as light intensity increased, LA of pansy increased. As light intensity increased from 105 to 315 μmol·m^{-2}·s^{-1}, LA of pansy increased 16%. Increases in LA were also observed under the light quality of R_{84}:FR_7:B_9 for all three species (Table 7). Leaf area of pansy increased 18% under the light quality of R_{84}:FR_7:B_9 compared to R_{87}:B_{13}, and LA of petunia increased 27% and 14% compared to R_{87}:B_{13} and R_{74}:G_{18}:B_8, respectively.

3.4.3 Root and Shoot Dry Mass

As light intensity increased, both RDM and SDM increased for all three species (Table 6). For example, as light intensity increased from 105 to 315 μmol·m^{-2}·s^{-1}, RDM of coreopsis, pansy, and petunia increased 269%, 245%, and 212%, respectively. Likewise, SDM of coreopsis,

pansy, and petunia increased 90%, 131%, and 93%, respectively, as light intensity increased from 105 to 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

For coreopsis, the greatest RDM accumulation was observed under the light quality of R_{84}:FR_{7}:B_{9} (Table 7). For example, RDM of coreopsis increased 26% and 19% under the light quality of R_{84}:FR_{7}:B_{9} compared to R_{87}:B_{13} and R_{74}:G_{18}:B_{8}, respectively. Shoot dry mass was greatest under the light quality of R_{84}:FR_{7}:B_{9} for coreopsis and petunia (Table 7). For coreopsis, SDM increased 33% and 22% under the light quality of R_{84}:FR_{7}:B_{9} compared to R_{87}:B_{13} and R_{74}:G_{18}:B_{8}, respectively. Likewise, SDM of petunia increased 23% under the light quality of R_{84}:FR_{7}:B_{9} compared to R_{87}:B_{13}.

3.4.4 Quality Parameters

While light quality had very little effect, seedlings grown under higher light intensities displayed higher SQ and QI values for all three species (Table 6). For example, SQ values for coreopsis, pansy, and petunia increased 24%, 38%, and 41% as light intensity increased from 105 to 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Similarly, as light intensity increased from 105 to 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$, QI values for coreopsis, pansy, and petunia were 255%, 231%, and 236% greater, respectively. In terms of light quality, QI values for coreopsis were 29% and 23% greater under the light quality of R_{84}:FR_{7}:B_{9} compared to R_{87}:B_{13} and R_{74}:G_{18}:B_{8}, respectively (Table 7).

3.4.5 Nutrient Concentration

For all three species, both macro- and micronutrient concentration generally decreased as light intensity increased (Tables 8 and 9). In petunia, nutrient concentration was often highest under the light quality of R_{84}:FR_{7}:B_{9} for both macro- and micronutrients. For example, at a light intensity of 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$, petunia accumulated 30%, 19%, 18%, 34%, and 25% more sulfur (S), Ca, Mg, Cu, and Zn, respectively, under the light quality of R_{84}:FR_{7}:B_{9} compared to

R_{74}:G_{18}:B_8. However, in pansy, the highest nutrient concentrations were often observed under the light quality of R_{87}:B_{13}. Specifically, at a light intensity of 105 µmol·m^{-2}·s^{-1}, pansy accumulated 12% more S and Mg under the light quality of R_{87}:B_{13} compared to R_{84}:FR_7:B_9. Additionally, at a light intensity of 315 µmol·m^{-2}·s^{-1}, pansy accumulated 19% more S under the light quality of R_{87}:B_{13} compared to R_{74}:G_{18}:B_8. Coreopsis displayed a similar trend to pansy at a light intensity of 105 µmol·m^{-2}·s^{-1}, with 9% more P and 20% more Mn for seedlings under the light quality of R_{87}:B_{13} compared to R_{84}:FR_7:B_9 and R_{74}:G_{18}:B_8, respectively.

3.4.6 Finishing

A significant decrease in TTF was observed in pansy and petunia seedlings grown under the light quality of R_{84}:FR_7:B_9 (Table 7). Specifically, pansy seedlings grown under the light quality of R_{84}:FR_7:B_9 flowered an average of 7 and 5 d earlier compared to R_{87}:B_{13} and R_{74}:G_{18}:B_8, respectively. Additionally, high light intensities led to a decrease in TTF for coreopsis and pansy (Table 6). For example, pansy flowered an average of 5 and 4 d earlier when seedlings were grown at a light intensity of 210 or 315 µmol·m^{-2}·s^{-1}, respectively, compared to 105 µmol·m^{-2}·s^{-1}. Likewise, coreopsis flowered an average of 6 d earlier as the propagation light intensity increased from 105 to 315 µmol·m^{-2}·s^{-1}.

For pansy and petunia, the number of nodes at first flower was lower when seedlings were grown under the light quality of R_{84}:FR_7:B_9 (Table 7). For example, when pansy seedlings were grown under the light quality of R_{84}:FR_7:B_9, three fewer nodes were present at first flower compared to the other two light quality treatments. Similar results were observed for dry mass in pansy, with seedlings grown under the light quality of R_{84}:FR_7:B_9 possessing a decreased SDM at flower (Table 7). Specifically, pansy displayed a 30% decrease in SDM at flower when seedlings were grown under the light quality of R_{84}:FR_7:B_9 compared to R_{87}:B_{13}. Regarding light

intensity, SDM at flower decreased as light intensity increased for coreopsis and pansy (Table 6). For example, SDM of coreopsis and pansy decreased 16% and 27%, respectively, as the light intensity increased from 105 to 315 μmol·m^{-2}·s^{-1}.

3.5 Discussion

A high-quality bedding plant seedling is one that has a compact habit and reduced LA, a high RDM and SDM, a well-developed root system, and a thick stem diameter (Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). These qualitative parameters ultimately lead to seedlings that are more easily processed, shipped, and mechanically transplanted, which is desired by growers (Pramuk and Runkle, 2005). Under SSL, we found that high light intensities and light qualities of R_{87}:B_{13} and R_{74}:G_{18}:B_8 generally led to compact seedlings with a shorter stem length and smaller LA. High light intensities have been found to reduce the level of endogenous gibberellins (GAs) within higher plants, ultimately leading to reduced stem elongation and a more compact habit (Graebe, 1987; Potter et al., 1999). Thus, the compact seedling growth observed under higher light intensities was likely due to decreased GA levels. In the greenhouse, Pramuk and Runkle (2005) reported comparable results, with seedlings of celosia ‘Gloria Mix’, impatiens ‘Accent Red’, and salvia (*Salvia splendens* ‘Vista Red’) becoming more compact as the greenhouse DLI increased. Similarly, Lopez and Runkle (2008) found shoot height of petunia ‘Tiny Tunia Violet Ice’, ‘Double Wave Spreading Rose’, and ‘Supertunia Mini Purple’ cuttings increased by 40%, 34%, and 55%, respectively, as the DLI decreased from 5.9 to 1.2 mol·m^{-2}·d^{-1}.

Far-red radiation is known to have a significant effect on promoting extension growth and leaf expansion (Downs and Thomas, 1982). Light signals in the plant are perceived by photoreceptors, which include phytochromes, cryptochromes, and phototropins (Franklin, 2008;

Runkle and Heins, 2001). Phytochromes are the photoreceptors responsible for detecting changes in the red:far-red (R:FR) light ratio. In response to a lower R:FR ratio, many plants will display morphological changes such as increased stem elongation and LA and reduced leaf thickness, a response commonly referred to as shade avoidance (Franklin and Whitelam, 2005; Park and Runkle, 2017; Zhang and Folta, 2012). Park and Runkle (2017) found that stem length of geranium 'Pinto Premium Orange Bicolor', petunia 'Wave Blue', snapdragon 'Trailing Candy Showers Yellow', and impatiens 'Super Elfin XP Red' seedlings displayed an inverse linear relationship with estimated phytochrome photoequilibrium, which serves as an indicator of the relative amount of active phytochrome in plants. Specifically, as the estimated phytochrome photoequilibrium increased, stem length decreased. Additionally, they found that leaf expansion was promoted for some species under a low R:FR ratio, as long as the light intensity was sufficient for growth. In the present study, the addition of FR radiation reduced the R:FR ratio in the light quality treatment of R_{84}:FR_{7}:B_{9}, and seedlings exhibited increased stem elongation and LA as a result.

Blue radiation has been shown to result in growth inhibition for a variety of species (Cosgrove, 1981; Runkle and Heins, 2001). This is likely due to the blue light photoreceptor, cryptochrome, acting on one or more steps in the process of cell enlargement (Cosgrove, 1981; Kigel and Cosgrove, 1991; Runkle and Heins, 2001). Excessive hypocotyl elongation of seedlings has been reported under LED SSL containing high proportions of red radiation and little to no blue radiation (Hoenecke et al., 1992). Thus, blue radiation may be essential under SSL to minimize stem elongation and produce compact seedlings (Hoenecke et al., 1992; Wollaeger and Runkle, 2014).

Reductions in LA under an increased percentage of blue radiation have previously been observed for petunia ‘Dreams Midnight’, impatiens ‘Super Elfin XP Blue Pearl’, and vinca ‘Titan Red Dark’ seedlings (Randall and Lopez, 2014). Wollaeger and Runkle (2015) found that approximately 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of blue radiation, in a spectrum of predominately red radiation, was sufficient to inhibit extension growth and LA expansion for impatiens ‘SuperElfin XP Red’, petunia ‘Wave Pink’, salvia ‘ Vista Red’, and tomato (*Solanum lycopersicum* ‘Early Girl’) seedlings. This coincides with our findings in the present study, where petunia seedlings grown under lower light intensities displayed increased LA and stem elongation under the light qualities of R_{84}:FR_{7}:B_{9} and R_{74}:G_{18}:B_{8} compared to R_{87}:B_{13} (Table 10). Under the light qualities of R_{84}:FR_{7}:B_{9} and R_{74}:G_{18}:B_{8} at a light intensity of 105 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the intensity of blue light was approximately 8 and 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. However, the intensity of blue light under the light quality of R_{87}:B_{13}, where differences in stem elongation and LA amongst light intensities were not observed, was approximately 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Franklin (2008) found that reductions in light intensity, specifically blue radiation, can elicit physiological responses characteristic of a low R:FR ratio. Therefore, it is likely that under these lower light intensities, seedlings grown under light qualities with lower percentages of blue radiation were not exposed to a sufficient quantity to inhibit responses connected to shade avoidance. In addition, green radiation absorbed by cryptochrome can stimulate a response similar to shade avoidance, as these wavelengths can reverse the effects of blue light-inhibited hypocotyl elongation (Zhang and Folta, 2012). While this mechanism is not fully understood, the addition of green radiation may have also resulted in the increased LA and stem elongation observed in petunia under lower light intensities.

For all three species in the present study, both RDM and SDM increased under higher light intensities. This observation is well documented, with many greenhouse studies reporting

an increased DLI led to increased biomass accumulation and growth rate (Graper and Healy, 1991; Graper and Healy, 1992; Lopez and Runkle, 2008; Pramuk and Runkle, 2005). For example, Oh et al. (2010) found that pansy 'Delta Premium Yellow' and petunia 'Madness Red' seedlings displayed a linear increase in SDM under increasing DLIs. In the present study, both RDM and SDM for all three species continued to significantly increase up to the highest light intensity of 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Thus, while it is generally recommended that seedlings be grown at a minimum DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ (175 to 210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod), potential increases in seedling quality and a decrease in production time may be possible under higher light intensities.

Root dry mass and SDM of seedlings grown under R_{84}:FR_7:B_9 were often greater than seedlings grown under R_{87}:B_{13} or R_{74}:G_{18}:B_8. Wollaeger and Runkle (2014) suggest the primary role of light quality on biomass accumulation in tomato 'Early Girl', salvia 'Vista Red', impatiens 'SuperElfin XP Red', and petunia 'Wave Pink' seedlings can be attributed to an increase in LA. As LA increases, the potential for biomass accumulation also increases due to a greater potential for light interception. Leaf area was greatest under the light quality of R_{84}:FR_7:B_9 for all three species in the present study. Therefore, it is likely that the addition of far-red wavelengths allowed for an increase in LA by lowering the R:FR ratio, ultimately leading to increased light interception and SDM accumulation. In addition to increasing LA, the inclusion of far-red radiation may have increased the total photosynthetically active radiation (*PAR*) available to the seedlings. Li and Kubota (2009) found that fluorescent white light supplemented with far-red LEDs led to increased fresh and dry weight of lettuce (*Lactuca sativa* 'Red Cross') compared to fluorescent light alone. Increasing the quantum yield with far-red light has also been shown to increase whole-plant net assimilation for multiple bedding plant species

(Park and Runkle, 2016; Park and Runkle, 2017). Recent studies have also shown that photosynthetic activity in photosystem II (PSII) can be stimulated by far-red radiation (Pettai et al., 2005; Thapper et al., 2009). For example, Zhen and van Iersel (2017) found that the inclusion of far-red radiation to a red:blue or warm-white LED spectrum resulted in increased photosynthetic activity in PSII, decreased non-photochemical quenching, and enhanced net photosynthetic rate in lettuce 'Green Towers'. In the present study, target light intensities were achieved by accounting for *PAR* rather than total photon flux (*TPF*; 400-800 nm). Thus, the light intensities established using the R_{84}:FR_7:B_9 LEDs did not account for the additional 7% far-red radiation (~7-22 $\mu mol \cdot m^{-2} \cdot s^{-1}$). As the impacts of far-red radiation continue to be researched, future studies may need to utilize *TPF* rather than *PPFD* to measure the amount of light available for photosynthetic activity.

The QI provides an objective, integrated, and quantitative measurement for further evaluation of seedling quality, with higher values indicating higher quality (Currey et al., 2013; Randall and Lopez, 2014). The highest quality seedlings were consistently produced under higher light intensities, with little to no effect from light quality. This increased quality was primarily due to seedlings grown under higher light intensities exhibiting reduced stem elongation, increased stem caliper, and increased RDM and SDM.

Both macro- and micronutrient concentrations were generally lowest in seedlings grown under high light intensities. Similar to observations made by Gerovac et al. (2016), this trend may be the result of a dilution of nutrients due to the higher SDM consistently found at higher light intensities (Table 6). Kuehny et al. (1991) previously investigated this effect of nutrient dilution in chrysanthemum (*Chrysanthemum* ×*morifolium* 'Fiesta') and found that foliar concentrations of nutrients were lower under increased irradiance and an elevated CO_2

concentration. However, when data were expressed on a starch-free dry weight basis, the authors found that most of the differences observed between treatments were no longer apparent (Kuehny et al., 1991). Therefore, it is plausible that seedlings grown under the lower light intensities in the present study were more nutrient dense simply due to reduced biomass accumulation.

Light quality also influenced nutrient concentration. Specifically, for both coreopsis and pansy, select macro- and micronutrients were significantly higher under the light quality of R_{87}:B_{13} compared to R_{84}:FR_{7}:B_{9} and R_{74}:G_{18}:B_{8}. An increased percentage of blue radiation has been found to result in higher concentrations of essential elements in microgreens (Kopsell and Sams, 2013; Kopsell et al., 2014). Kopsell et al. (2014) proposed that blue radiation serves a dominant role in regulating processes linked to nutrient content, including membrane permeability, proton pumping, and ion channel activities. Additionally, blue radiation has been found to play a primary role in the regulation of stomatal opening (Kinoshita et al., 2001; van Ieperen et al., 2012), which may directly affect mass flow and uptake of nutrients. Therefore, the increase in select macro- and micronutrients for coreopsis and pansy under R_{87}:B_{13} LEDs may have resulted from the 4-5% increase in blue radiation compared to the other two treatments.

The effects of DLI on flowering in greenhouse-grown bedding plant species is well documented (Faust et al., 2005; Oh et al., 2010; Pramuk and Runkle, 2005). Pramuk and Runkle (2005) found that TTF for celosia ‘Gloria Mix’, impatiens ‘Accent Red’, salvia ‘Vista Red’, marigold ‘Bonanza Yellow’, and pansy ‘Crystal Bowl Yellow’ decreased as the greenhouse DLI increased during propagation. Additionally, these authors reported that the percentage of impatiens ‘Accent Red’ and marigold ‘Bonanza Yellow’ seedlings with visible bud at transplant also increased as the DLI increased (Pramuk and Runkle, 2005). Lopez and Runkle (2008) found

a similar decrease in TTF for petunia ‘Tiny Tunia Violet’ and ‘Supertunia Mini Purple’ as the greenhouse DLI increased from 1.4 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$ during cutting propagation. Plants exhibiting a facultative irradiance response tend to flower earlier and develop fewer nodes prior to flower initiation when exposed to higher light environments (Erwin et al., 2017). In the present study, both coreopsis and pansy possessed a facultative irradiance response as exhibited by their earlier flowering and decreased number of nodes at flower.

With coreopsis and pansy, reduced SDM at first flower was observed as the light intensity increased. Hutchinson et al. (2012) found similar results; TTF for *Angelonia angustifolia* ‘AngelMist White Cloud’ and *Osteospermum ecklonis* ‘Voltage Yellow’ decreased linearly as the DLI during propagation increased, with lower SDM values observed alongside this decrease in days to flower. This earlier flowering may be beneficial when seedlings are produced with the intent for finishing in small containers, while a delay in flowering would likely be preferred for seedlings intended for large containers, as this would encourage increased vegetative development (Hutchinson et al., 2012; Mattson and Erwin, 2005).

For pansy, TTF significantly decreased when seedlings were grown under the light quality of R_{84}:FR_{7}:B_{9} compared to the other light quality treatments. Far-red radiation has been shown to have a significant effect on the promotion of flowering for plants with a long-day photoperiodic response (Downs and Thomas, 1982). However, species with a long-day photoperiodic response may respond differently to the inclusion/exclusion of far-red radiation. For some species, the response to far-red radiation is specific to post-inductive flower development, while in others the effect is specific to flower induction (Thomas and Vince-Prue, 1997; Runkle and Heins, 2001). For coreopsis and petunia, it is likely that far-red radiation was unnecessary for flower induction, leading to only minor differences observed in TTF with the

inclusion of these wavelengths. However, in pansy the inclusion of far-red radiation led to a significant decrease in TTF, leading to the assumption that flower induction was accelerated during propagation under this light ratio. Park and Runkle (2017) found similar results in that only one of two species evaluated with a long-day photoperiodic response was responsive to the inclusion of far-red radiation during seedling production for earlier flowering. However, both species displayed increased growth and characteristic photomorphogenic responses to the inclusion of far-red radiation. Thus, these authors concluded that the regulation of flowering and photomorphogenic responses from far-red radiation are independent within the plant (Park and Runkle, 2017). While all three species displayed photomorphogenic responses to far-red radiation in the present study, pansy was the only species that possessed a flowering response. Thus, the characterization and selection of species and cultivar responses to the inclusion or exclusion of various wavelengths of light is critical when designing SSL applications.

3.6 Conclusion

Based on our results, light intensity appears to be the dominant factor influencing seedling quality under SSL. While light quality can induce a variety of photomorphogenic responses, the highest quality seedlings for all three species were consistently produced under the light intensity of 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, far-red wavelengths included in the spectrum may be beneficial if accelerated flowering upon transplant is desired for long-day plants, but this response is highly dependent on the species and cultivar. Therefore, these results provide further information regarding the specific light parameters from commercially available LEDs necessary to produce high-quality bedding plant seedlings under SSL.

3.7 Acknowledgements

I gratefully acknowledge Dr. Roberto Lopez, Dr. Jennifer Boldt, and Maria Del Rosario Del Rio for intellectual, greenhouse, growth chamber, and laboratory assistance; the USDA-ARS, Greenhouse Production Research Group for nutrient analysis; the USDA-NIFA SCRI grant No. 2010-51181-21369 for funding; and Philips Lighting and Hort Americas for LED lighting assistance and donations. The use of trade names in this publication does not imply endorsement by Purdue University of products named nor criticism of similar ones not mentioned.

3.8 Literature Cited

Barnes, C., T. Tibbitts, J. Sager, G. Deitzer, D. Bubenheim, G. Koerner, and B. Bugbee. 1993. Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux. HortScience 28:1197−1200.

Cope, K.R., M.C. Snowden, and B. Bugbee. 2014. Photobiological interactions of blue light and photosynthetic photon flux: Effects of monochromatic and broad-spectrum light sources. Photochemistry and Photobiology 90:574−584.

Cosgrove, D.J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67:584−590.

Currey, C.J., A.P. Torres, R.G. Lopez, and D.F. Jacobs. 2013. The quality index – a new tool for integrating quantitative measurements to assess quality of young floriculture plants. Acta Hort. 1000:385–391.

Downs, R.J. and J.F. Thomas. 1982. Phytochrome regulation of flowering in the long-day plant, *Hyoscyamus niger*. Plant Physiol. 70:898−900.

Erwin, J., N. Mattson, and R. Warner. 2017. Light effects on bedding plants, p. 119−134. In: R. Lopez and E. Runkle (eds.). Light management in controlled environments. Meister Media Worldwide, Willoughby, OH.

Faust, J.E., V. Holcombe, N.C. Rajapakse, and D.R. Layne. 2005. The effect of daily light integral on bedding plant growth and flowering. HortScience 40:645−649.

Franklin, K.A. 2008. Shade avoidance. New Phytol. 179:930−944.

Franklin, K.A. and G.C. Whitelam. 2005. Phytochromes and shade-avoidance responses in plants. Ann. Bot. 96:169−175.

Frantz, J.M. 2013. Uptake efficiency of phosphorus in different light environments by zinnia (*Zinnia elegans*) and vinca (*Catharanthus roseus*). HortScience 48:594−600.

Gerovac, J.R., J.K. Craver, J.K. Boldt, and R.G. Lopez. 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of *Brassica* microgreens. HortScience 5:497−503.

Graebe, J.E. 1987. Gibberellin biosynthesis and control. Ann. Rev. Plant Physiol. 38:419−465.

Graper, D.F. and W. Healy. 1991. High pressure sodium irradiation and infrared radiation accelerate petunia seedling growth. J. Amer. Soc. Hort. Sci. 116:435−438.

Graper, D.F. and W. Healy. 1992. Modification of petunia seedling carbohydrate partitioning by irradiance. J. Amer. Soc. Hort. Sci. 117:477−480.

Hoenecke, M.E., R.J. Bula, and T.W. Tibbits. 1992. Importance of 'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427−430.

Hutchinson, V.A., C.J. Currey, and R.G. Lopez. 2012. Photosynthetic daily light integral during root development influences subsequent growth and development of several herbaceous annual bedding plants. HortScience 47:856−860.

Kigel, J. and D.J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049−1056.

Kinoshita, T, M. Doi, N. Suetsugu, T. Kagawa, M. Wada, and K. Shimazaki. 2001. Phot1 and phot2 mediate blue light regulation of stomatal opening. Nature 414:656−660.

Kopsell, D.A. and C.E. Sams. 2013. Increase in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light-emitting diodes. J. Amer. Soc. Hort. Sci. 138:31–37.

Kopsell, D. A., C.E. Sams, T.C. Barickman, and R.C. Morrow. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. J. Amer. Soc. Hort. Sci. 139:469–477.

Kuehny, J.S., M.M. Peet, P.V. Nelson, and D.H. Willits. 1991. Nutrient dilution by starch in CO_2-enriched chrysanthemum. J. Exp. Bot. 42:711–716.

Li, Q. and C. Kubota. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67:59–64.

Lopez, R.G. and E.S. Runkle. 2008. Photosynthetic daily light integral during propagation influences rooting and growth of cuttings and subsequent development of New Guinea impatiens and petunia. HortScience 43:2052−2059.

Massa, G.D., H. Kim, R.M. Wheeler, and C.A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43:1951−1956.

Mattson, N.S. and J.E. Erwin. 2005. The impact of photoperiod and irradiance on flowering of several herbaceous ornamentals. Scientia Hort. 104:275−292.

Mitchell, C.A., A. Both, C.M Bourget, J.F. Burr, C. Kubota, R.G. Lopez, R.C. Morrow, and E.S. Runkle. 2012. LEDs: The future of greenhouse lighting! Chronica Hort. 52:6−12.

Oh, W., E.S. Runkle, and R.M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience 45:1332−1337.

Park, Y. and E.S. Runkle. 2016. Investigating the merit of including far-red radiation in the production of ornamental seedlings grown under sole-source lighting. Acta Hort. 1134:259−266.

Park, Y. and E.S. Runkle. 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Expt. Bot. 136:41−49.

Pettai, H., V. Oja, A. Freiberg, and A. Lasik. 2005. Photosynthetic activity of far-red light in green plants. Biochim. Biophys. Acta 1708:311–321.

Potter, T.I, S.B. Rood, and K.P. Zanewich. 1999. Light intensity, gibberellin content and the resolution of shoot growth in *Brassica*. Planta 207:505–511.

Pramuk, L.A. and E.S. Runkle. 2005. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40:1336−1339.

Randall, W.C. and R.G. Lopez. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. HortScience 49:589−595.

Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience 50:705−713.

Runkle, E.S. and R.D. Heins. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Amer. Soc. Hort. Sci. 126:275−282.

Stutte, G.W. 2009. Light-emitting diodes for manipulating the phytochrome apparatus. HortScience 44:231−234.

Styer, C. 2003. Propagating seed crops, p 151–163. In: D. Hamrick (ed.). Ball redbook crop production: Volume two. 17th Ed. Ball Publishing, Batavia, IL.

Thapper, A., F. Mamedov, F. Mokvist, L. Hammarstrom, and S. Styring. 2009. Defining the far-red limit of photosystem II in spinach. Plant Cell 21:2391–2401.

Thomas, B. and D. Vince-Prue. 1997. Photoperiodism in plants. 2nd ed. Academic Press, London.

van Ieperen, W., A. Savvides, and D. Fanourakis. 2012. Red and blue light effects during growth on hydraulic and stomatal conductance in leaves of young cucumber plants. Acta Hort. 956:223−230.

Wollaeger, H.M. and E.S. Runkle. 2014. Producing commercial-quality ornamental seedlings under sole-source LED lighting. Acta Hort. 1037:269−276.

Wollaeger, H.M. and E.S. Runkle. 2015. Growth and acclimation of impatiens, salvia, petunia, and tomato seedlings to blue and red light. HortScience 50:522−529.

Zhang, T. and K. Folta. 2012. Green light signaling and adaptive response. Plant Signal. Behav. 7:1–4.

Zhen, S. and M.W. van Iersel. 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209:115–122.

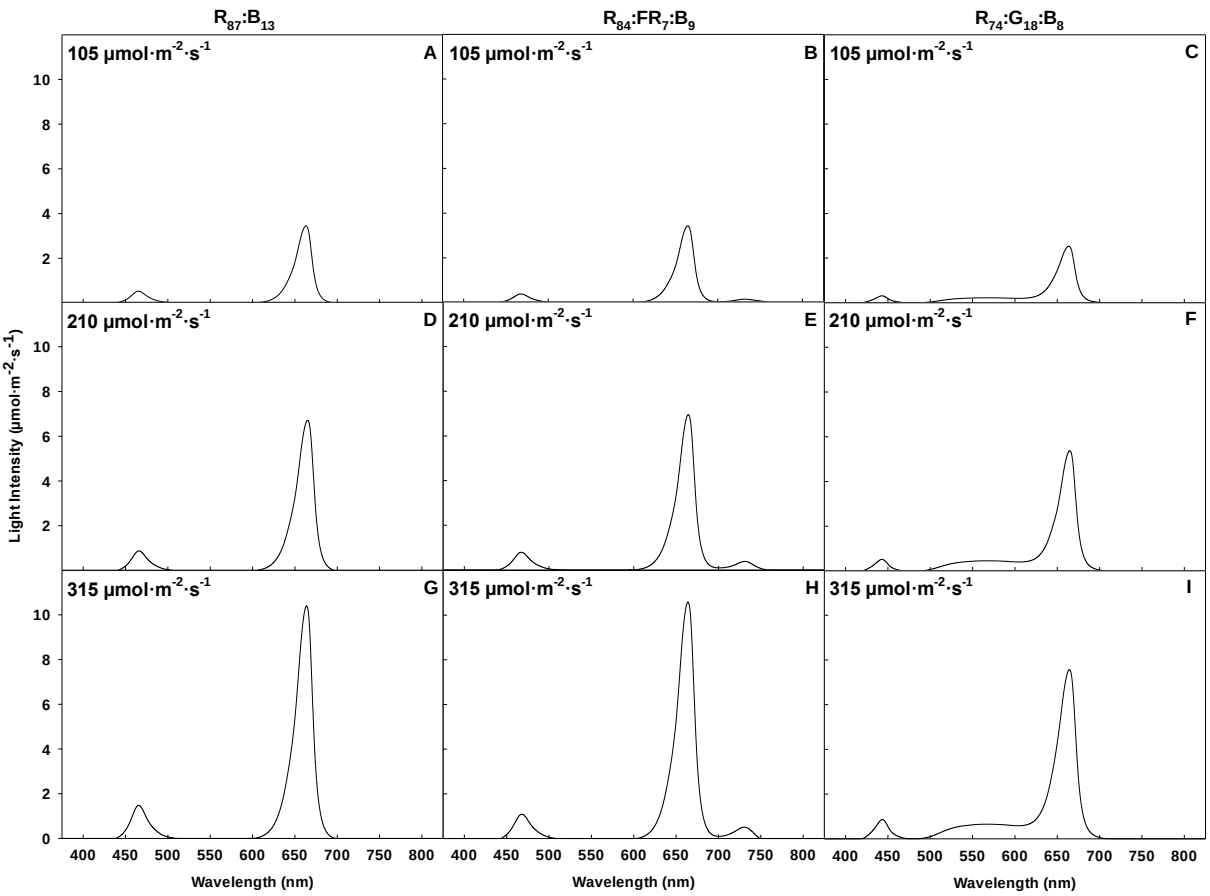


Figure 4. Spectral quality delivered from sole-source light-emitting diode (LED) arrays with light qualities (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_{7}:B_{9}), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_{8}) at a photosynthetic photon flux density (*PPFD*) from 400 to 700 nm of 105, 210, or 315 µmol·m^{-2}·s^{-1} at canopy level.

Table 4. Average photosynthetic photon flux density (*PPFD*) from 400 to 700 nm ± SD delivered from sole-source light-emitting diodes (LEDs) with light ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_{7}:B_{9}),or red:green:blue 74:18:8 (R_{74}:G_{18}:B_{8}) to achieve target light intensities of 105, 210, and 315 µmol·m^{-2}·s^{-1}. The average daily light integrals (DLIs), measured from 400 to 700 nm, under a 16-h photoperiod (0600 to 2200 HR) are also reported. Mean values reported are the average of nine spectral scans across three experimental replications.

Light intensity treatment (µmol·m^{-2}·s^{-1})	**Light quality treatment (%)**	**Avg *PPFD* (µmol·m^{-2}·s^{-1})**	**Avg DLI (mol·m^{-2}·d^{-1})**
105	**R_{87}:B_{13}**	102.9 ± 19.3	5.9 ± 1.1
	R_{84}:FR_{7}:B_{9}	103.2 ± 18.0	5.9 ± 1.0
	R_{74}:G_{18}:B_{8}	103.2 ± 18.1	5.9 ± 1.0
210	**R_{87}:B_{13}**	205.0 ± 32.7	11.8 ± 1.9
	R_{84}:FR_{7}:B_{9}	208.1 ± 34.0	12.0 ± 2.0
	R_{74}:G_{18}:B_{8}	206.7 ± 28.6	11.9 ± 1.6
315	**R_{87}:B_{13}**	311.9 ± 52.7	18.0 ± 3.0
	R_{84}:FR_{7}:B_{9}	310.2 ± 48.3	17.9 ± 2.8
	R_{74}:G_{18}:B_{8}	311.0 ± 52.0	17.9 ± 3.0

Table 5. Analysis of variance (ANOVA) for the effects of light quality (LQ), light intensity (LI), or LQ×LI from sole-source light-emitting diodes (LEDs) on propagation (28 d after germination) and finishing (transplanted 28 d after germination) for coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola ×wittrockiana* 'Matrix™ Yellow'), and petunia (*Petunia ×hybrida* 'Purple Wave').

	Coreopsis			**Pansy**			**Petunia**		
	LQ	**LI**	**LQ×LI**	**LQ**	**LI**	**LQ×LI**	**LQ**	**LI**	**LQ×LI**
				Propagation					
Stem length (mm)	NS[z]	*	NS	***	***	NS	**	***	NS
Stem caliper (mm)	***	***	NS	NS	***	NS	***	**	NS
Root dry mass (mg)	**	***	NS	NS	***	NS	NS	***	NS
Shoot dry mass (mg)	***	***	NS	NS	***	*	**	***	NS
Leaf area (cm^2)	***	NS	NS	**	**	NS	**	**	NS
Sturdiness quotient	NS	***	NS	**	***	NS	NS	***	NS
Quality index	**	***	NS	NS	***	NS	NS	***	NS
				Finishing					
Time to flower (d)	NS	***	NS	***	***	NS	*	NS	*
Number of nodes	NS	NS	NS	*	**	NS	***	NS	**
Shoot dry mass (mg)	NS	*	NS	**	***	NS	NS	NS	NS

[z]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 6. Propagation (28 d after germination) and finishing (transplanted 28 d after germination) data for the main effect of light intensity including stem length, stem caliper, leaf area, root (RDM) and shoot dry mass (SDM), sturdiness quotient, quality index, time to flower, number of nodes below the first open flower, and SDM at flowering for coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') seedlings grown under light intensities of 105, 210, or 315 µmol·m^{-2}·s^{-1} delivered from sole-source light-emitting diodes (LEDs) during propagation.

	Coreopsis			**Pansy**			**Petunia**		
	Light Intensity (µmol·m^{-2}·s^{-1})								
	105	**210**	**315**	**105**	**210**	**315**	**105**	**210**	**315**
Stem length (mm)	7.4 a[z]	7.1 ab	6.7 b	8.6 a	7.4 b	7.2 b	5.8 a	4.9 a	4.6 b
Stem caliper (mm)	1.18 c	1.26 b	1.34 a	1.08 b	1.22 a	1.23 a	1.24 b	1.33 a	1.37 a
RDM (mg)	6.2 c	16.3 b	22.9 a	5.1 c	12.7 b	17.6 a	6.7 c	16.1 b	20.9 a
SDM (mg)	30.1 c	46.0 b	57.2 a	29.3 c	51.5 b	67.7 a	30.0 c	48.6 b	58.0 a
Leaf area (cm^2)	10.0	10.1	9.5	9.8 b	11.2 a	11.4 a	16.6 a	14.5 b	12.8 b
Sturdiness quotient	0.17 b	0.18 b	0.21 a	0.13 b	0.17 a	0.18 a	0.22 c	0.28 b	0.31 a
Quality index	13.9 c	33.9 b	49.3 a	11.3 c	27.3 b	37.4 a	16.3 c	40.8 b	54.7 a
Time to flower (d)	59 a	57 a	53 b	39 a	34 b	35 b	45	45	45
Number of nodes	9	8	8	7 a	6 b	6 b	16	16	15
SDM at flower (g)	13.3 a	12.4 ab	11.2 b	3.3 a	2.1 b	2.4 b	11.6	11.6	11.0

[z]Means sharing a letter within a species are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were not significant for the main effect of light intensity.

Table 7. Propagation (28 d after germination) and finishing (transplanted 28 d after germination) data for the main effect of light quality including stem length, stem caliper, leaf area, root (RDM) and shoot dry mass (SDM), sturdiness quotient, quality index, time to flower, number of nodes below the first open flower, and SDM at flowering for coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') seedlings grown under light quality ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8) delivered from sole-source light-emitting diodes (LEDs) during propagation.

	Coreopsis			**Pansy**			**Petunia**		
	Light Quality								
	R_{87}:B_{13}	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
Stem length (mm)	6.8	7.4	7.0	7.7 b	8.3 a	7.2 b	4.7 b	5.5 a	5.1 ab
Stem caliper (mm)	1.20 b[z]	1.34 a	1.23 b	1.16	1.20	1.17	1.25 b	1.41 a	1.27 b
RDM (mg)	13.7 b	17.2 a	14.5 b	11.5	11.2	12.6	15.1	14.6	14.1
SDM (mg)	38.9 b	52.0 a	42.4 b	46.4	53.2	49.0	41.0 b	50.6 a	45.0 ab
Leaf area (cm^2)	8.7 c	11.2 a	9.7 b	9.9 b	11.7 a	10.7 ab	13.0 b	16.5 a	14.5 b
Sturdiness quotient	0.18	0.19	0.18	0.16 ab	0.15 b	0.17 a	0.28	0.27	0.26
Quality index	29.1 b	37.5 a	30.4 b	25.0	24.0	27.0	38.2	37.9	35.8
Time to flower (d)	57	55	57	39 a	32 b	37 a	46 a	44 b	45 ab
Number of nodes	8	9	8	7 ab	6 b	7 a	17 a	14 b	17 a
SDM at flower (g)	12.5	11.7	12.8	3.0 a	2.1 b	2.6 ab	11.5	10.8	12.0

[z]Means sharing a letter within a species are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were not significant for the main effect of light quality.

Table 8. Macronutrient concentration [percent dry mass (DM)] of coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola ×wittrockiana* 'Matrix™ Yellow'), and petunia (*Petunia ×hybrida* 'Purple Wave') seedlings 28 d after germination grown under light intensities (LIs) of 105, 210, or 315 µmol·m^{-2}·s^{-1} delivered from sole-source light-emitting diodes (LEDs) with light quality (LQ) ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8).

		Macronutrients (percent DM)					
LI	**LQ**	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium (K)**	**Sulfur (S)**	**Calcium (Ca)**	**Magnesium (Mg)**
				Coreopsis			
105	**R_{87}:B_{13}**	4.32[z]	0.37 a[x]	6.64	0.66 ab	1.21	1.14
	R_{84}:FR_7:B_9	4.01	0.34 b	6.49	0.62 ab	1.22	1.12
	R_{74}:G_{18}:B_8	4.19	0.36 ab	6.39	0.61 b	1.18	1.09
210	**R_{87}:B_{13}**	2.81	0.24 cd	4.82	0.65 ab	1.12	0.98
	R_{84}:FR_7:B_9	3.09	0.24 c	5.01	0.69 a	1.21	1.05
	R_{74}:G_{18}:B_8	2.84	0.22 cde	4.56	0.60 b	1.15	0.95
315	**R_{87}:B_{13}**	2.76	0.20 e	4.21	0.66 ab	1.09	0.96
	R_{84}:FR_7:B_9	2.68	0.21 de	4.22	0.69 a	1.11	0.96
	R_{74}:G_{18}:B_8	2.70	0.23 cde	4.15	0.64 ab	1.10	1.00
LQ		NS[y]	NS	*	***	*	NS
LI		***	***	***	NS	***	***
LQ×LI		NS	***	NS	*	NS	NS
				Pansy			
105	**R_{87}:B_{13}**	4.81	0.52	4.91	0.47 a	0.88	1.00 a
	R_{84}:FR_7:B_9	4.67	0.51	5.52	0.42 b	0.87	0.89 b
	R_{74}:G_{18}:B_8	4.73	0.53	4.95	0.42 b	0.80	0.91 ab
210	**R_{87}:B_{13}**	3.18	0.31	3.15	0.38 b	0.63	0.73 c
	R_{84}:FR_7:B_9	3.07	0.31	3.41	0.39 b	0.68	0.76 c
	R_{74}:G_{18}:B_8	2.96	0.29	3.12	0.37 bc	0.60	0.73 cd
315	**R_{87}:B_{13}**	2.74	0.25	2.60	0.38 b	0.57	0.69 cd
	R_{84}:FR_7:B_9	2.53	0.23	2.68	0.37 bc	0.61	0.71 cd
	R_{74}:G_{18}:B_8	2.47	0.22	2.49	0.32 c	0.51	0.64 d

Table 8 continued

LQ		*	NS	***	***	***	*
LI		***	***	***	***	***	***
LQ×LI		NS	NS	NS	*	NS	**
					Petunia		
105	**R_{87}:B_{13}**	4.76 a	0.42	6.62	0.91 cd	1.20 a	1.18 a
	R_{84}:FR_{7}:B_{9}	4.46 a	0.41	6.78	0.90 d	1.24 a	1.15 a
	R_{74}:G_{18}:B_{8}	4.54 a	0.41	6.76	0.87 d	1.22 a	1.20 a
210	**R_{87}:B_{13}**	2.52 bc	0.21	4.12	1.01 bcd	1.10 b	0.94 bc
	R_{84}:FR_{7}:B_{9}	2.94 b	0.24	4.56	1.05 abc	1.10 b	1.00 b
	R_{74}:G_{18}:B_{8}	2.52 bc	0.23	4.26	1.10 ab	1.08 b	1.00 b
315	**R_{87}:B_{13}**	2.22 c	0.18	3.53	0.93 cd	0.93 c	0.86 cd
	R_{84}:FR_{7}:B_{9}	2.23 c	0.19	3.76	1.16 a	1.07 b	0.94 bc
	R_{74}:G_{18}:B_{8}	2.01 c	0.17	3.37	0.89 d	0.90 c	0.80 d
LQ		NS	NS	**	**	***	NS
LI		***	***	***	***	***	***
LQ×LI		*	NS	NS	***	**	**

[z]Mean values are based on a representative sample from each treatment across three experimental replications.

[y]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

[x]Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

Table 9. Micronutrient concentrations (mg·kg^{-1}) of coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') seedlings 28 d after germination grown under light intensities (LIs) of 105, 210, or 315 µmol·m^{-2}·s^{-1} delivered from sole-source light-emitting diodes (LEDs) with light quality (LQ) ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8).

		Micronutrients (mg·kg^{-1})				
DLI	**LED**	**Copper (Cu)**	**Iron (Fe)**	**Manganese (Mn)**	**Molybdenum (Mo)**	**Zinc (Zn)**
				Coreopsis		
105	**R_{87}:B_{13}**	58.69^{z}	222.03	44.54 a^{x}	1.13	49.79
	R_{84}:FR_7:B_9	54.95	254.84	40.63 ab	1.05	47.04
	R_{74}:G_{18}:B_8	60.92	202.90	37.01 bcd	1.06	48.38
210	**R_{87}:B_{13}**	43.76	240.50	35.62 bcd	0.87	36.05
	R_{84}:FR_7:B_9	46.80	162.85	36.70 bcd	0.87	35.14
	R_{74}:G_{18}:B_8	40.54	246.57	38.19 abc	0.75	34.11
315	**R_{87}:B_{13}**	37.44	235.88	30.83 cd	0.71	30.37
	R_{84}:FR_7:B_9	37.80	146.03	30.35 d	0.71	31.41
	R_{74}:G_{18}:B_8	39.95	191.04	38.49 ab	0.87	31.53
LQ		NS^{y}	NS	NS	NS	NS
LI		***	NS	***	***	***
LQ×LI		NS	NS	***	NS	NS
				Pansy		
105	**R_{87}:B_{13}**	33.65 a	187.97	66.18	1.64	68.92
	R_{84}:FR_7:B_9	35.21 a	141.47	61.89	1.82	65.31
	R_{74}:G_{18}:B_8	37.99 a	146.00	68.16	1.47	69.28
210	**R_{87}:B_{13}**	24.93 b	104.55	50.53	1.70	51.67
	R_{84}:FR_7:B_9	24.91 b	111.78	49.24	1.57	50.00
	R_{74}:G_{18}:B_8	23.20 b	94.40	51.99	1.58	48.06
315	**R_{87}:B_{13}**	21.58 b	96.19	44.32	1.67	41.21
	R_{84}:FR_7:B_9	22.06 b	89.94	37.65	1.51	43.02
	R_{74}:G_{18}:B_8	20.44 b	111.65	37.51	1.42	40.62

Table 9 continued

LQ		NS	NS	NS	*	NS
LI		***	***	***	NS	***
LQ×LI		*	NS	NS	NS	NS
				Petunia		
105	**R_{87}:B_{13}**	54.92 a	155.72	42.63	3.35	74.13 ab
	R_{84}:FR_{7}:B_{9}	57.94 a	154.34	43.94	3.34	67.35 b
	R_{74}:G_{18}:B_{8}	63.40 a	148.19	46.72	3.41	76.43 a
210	**R_{87}:B_{13}**	41.34 bcd	110.60	33.67	2.99	56.85 c
	R_{84}:FR_{7}:B_{9}	44.79 b	108.96	31.22	2.76	56.21 c
	R_{74}:G_{18}:B_{8}	43.19 bc	106.39	33.23	2.75	56.14 c
315	**R_{87}:B_{13}**	34.95 cd	96.41	26.21	2.28	49.64 cd
	R_{84}:FR_{7}:B_{9}	43.19 bc	97.85	23.74	2.39	56.11 c
	R_{74}:G_{18}:B_{8}	32.26 d	86.49	24.79	1.98	44.75 d
LQ		*	NS	NS	NS	NS
LI		***	***	***	***	***
LQ×LI		**	NS	NS	NS	***

[z]Mean values are based on a representative sample from each treatment across three experimental replications.
[y]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[x]Means sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

Table 10. Comparisons for the effect of light quality within light intensities and light intensity within light qualities for stem length, stem caliper, leaf area, root and shoot dry mass, number of nodes, sturdiness quotient, and quality index for coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola ×wittrockiana* 'Matrix™ Yellow'), and petunia (*Petunia ×hybrida* 'Purple Wave') seedlings 28 d after germination grown under light intensities (LIs) of 105, 210, or 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$ delivered from sole-source light-emitting diodes (LEDs) with light quality (LQ) ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8).

Stem Length (mm)

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	7.3 a[z]	7.7	7.1	8.5 B[y]a	9.6 Aa	7.6 B	5.0 B	6.4 Aa	5.9 Aa
210	7.1 a	7.3	6.9	7.4 ab	7.5 b	7.2	4.7	5.2 b	4.8 b
315	6.1 b	7.1	6.9	7.1 ABb	7.9 Ab	6.8 B	4.4	4.9 b	4.6 b

Stem Caliper (mm)

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	1.09 Bb	1.28 Ab	1.16 Bb	1.07 b	1.10 b	1.07 b	1.15 Bb	1.39 A	1.18 B
210	1.23 Ba	1.34 Aab	1.22 Bb	1.21 a	1.22 a	1.22 a	1.27 ab	1.40	1.31
315	1.28 Ba	1.43 Aa	1.31 Ba	1.20 a	1.27 a	1.21 a	1.33 Ba	1.46 A	1.31 B

Leaf Area (cm^2)

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	8.6 B	11.7 A	9.7 B	8.8	10.7 b	9.9 b	13.9 B	19.6 Aa	16.3 ABa
210	9.5 B	10.9 A	9.9 AB	10.4	11.3 ab	11.9 a	12.7	15.4 b	15.5 a
315	8.1 B	11.0 A	9.4 AB	10.6 B	13.1 Aa	10.3 Bab	12.4 AB	14.6 Ab	11.5 Bb

Table 10 continued

Root Dry Mass (mg)

	Coreopsis			Pansy LQ			Petunia		
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	5.4 c	6.8 c	6.3 c	5.9 c	4.7 c	4.6 b	5.7 c	7.8 b	6.5 c
210	15.4 b	17.9 b	15.7 b	12.0 b	10.8 b	15.3 a	17.2 b	16.2 a	14.9 b
315	20.2 Ba	27.0 Aa	21.4 Ba	16.6 a	18.1 a	18.1 a	22.2 a	19.7 a	20.8 a

Shoot Dry Mass (mg)

	Coreopsis			Pansy LQ			Petunia		
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	25.8 Bb	35.9 Ac	28.5 Bc	28.1 c	30.4 c	29.4 b	24.2 Bc	36.7 Ac	29.2 ABb
210	41.9 Ba	53.1 Ab	43.1 Bb	47.1 b	50.5 b	57.1 a	43.4 b	50.9 b	51.3 a
315	48.9 Ba	67.0 Aa	55.7 ABa	64.0 a	78.7 a	60.5 a	55.3 a	64.1 a	54.6 a

Sturdiness Quotient

	Coreopsis			Pansy LQ			Petunia		
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	0.15 b	0.18	0.17	0.14 ABb	0.12 Bb	0.15 Ab	0.23 b	0.22 b	0.20 b
210	0.18 b	0.18	0.18	0.17 a	0.17 a	0.17 a	0.28 ab	0.28 a	0.28 a
315	0.21 a	0.21	0.19	0.18 a	0.17 a	0.18 a	0.32 a	0.31 a	0.31 a

Quality Index

	Coreopsis			Pansy LQ			Petunia		
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	11.8 Bc	16.1 Ac	13.8 ABc	13.6 b	9.8 c	10.3 b	14.4 c	19.2 c	15.3 c
210	31.8 b	37.5 b	32.3 b	25.8 a	23.7 b	32.4 a	42.3 b	41.8 b	38.5 b
315	43.7 Ba	59.0 Aa	45.2 Ba	35.6 a	38.5 a	38.2 a	58.0 a	52.7 a	53.5 a

Table 10 continued

[z]Means sharing a lowercase letter within a species and light quality are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lowercase lettering were not significant for the effect of light intensity.
[y]Means sharing a uppercase letter within a species and light intensity are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no uppercase lettering were not significant for the effect of light quality.

Table 11. Comparisons for the effect of light quality within light intensities and light intensity within light qualities for time to flower, number of nodes below the first open flower, and shoot dry mass at flowering for coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix™ Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') propagated under light intensities (LIs) of 105, 210, or 315 µmol·m^{-2}·s^{-1} delivered from sole-source light-emitting diodes (LEDs) with light quality (LQ) ratios (%) of red:blue 87:13 (R_{87}:B_{13}), red:far-red:blue 84:7:9 (R_{84}:FR_7:B_9), or red:green:blue 74:18:8 (R_{74}:G_{18}:B_8) and transplanted 28 d after germination into a common greenhouse environment.

Time to Flower (d)

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	60 a[z]	58	59	42 a	37 a	39	47	44	45
210	58 a	55	58	35 b	30 b	35	47	43	45
315	52 b	52	55	40 A[y]ab	29 Bb	36 A	43	44	46

Number of Nodes

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	9	9	8	7	7 a	7	18 A	13 B	18 A
210	9	8	8	6	6 b	7	16	15	17
315	8	9	8	7 AB	6 Bb	7 A	16	15	15

Shoot Dry Mass (g)

	Coreopsis			Pansy			Petunia		
				LQ					
LI	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**	**R_{87}:B_{13}**	**R_{84}:FR_7:B_9**	**R_{74}:G_{18}:B_8**
105	13.6	12.2	13.9	3.8 a	3.0 a	3.1	12.4	10.5	11.8
210	13.3	11.7	12.3	2.1 b	1.6 ab	2.4	11.5	10.9	12.4
315	10.2	11.0	12.3	3.2 Aab	1.6 Cb	2.4 B	10.5	10.9	11.7

Table 11 continued

[z]Means sharing a lowercase letter within a species and light quality are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lowercase lettering were not significant for the effect of light intensity.
[y]Means sharing a uppercase letter within a species and light intensity are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no uppercase lettering were not significant for the effect of light quality.

CHAPTER 4. PHYSIOLOGICAL ACCLIMATION OF PETUNIA SEEDLINGS TO VARYING LIGHT QUALITY, LIGHT INTENSITY, AND CARBON DIOXIDE CONCENTRATION FOR INDOOR PRODUCTION

4.1 Abstract

Indoor production of bedding plant seedlings (plugs) using sole-source lighting (SSL) may present value in increasing uniformity and consistency compared to greenhouse production. However, there is currently limited information on physiological responses of seedlings to varying light intensities, light qualities, and carbon dioxide (CO_2) concentrations under SSL. Seeds of petunia (*Petunia ×hybrida* 'Dreams Midnight') were sown in 128- and 288-cell trays and placed on multi-layer shelves in walk-in growth chambers. Light treatments were established using light-emitting diode (LED) arrays providing red:blue light ratios (%) of 50:50 or 90:10 and light intensities of 150 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Carbon dioxide treatments were conducted using two growth chambers with set points of 450 or 900 $\mu mol \cdot mol^{-1}$. Morphological measurements such as leaf area (LA) and dry mass were measured weekly. Additionally, photosynthesis (A) response to increasing light (A-*PPFD*) and leaf internal CO_2 concentration (A-C_i) were measured using a portable leaf photosynthesis system. Regardless of CO_2 concentration, seedlings grown under the light ratio of 90:10 and light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ produced greater total dry mass (TDM) and LA than those grown under the light ratio of 50:50. However, seedlings grown under the light ratio of 50:50 at a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ displayed the highest maximum net photosynthetic rate ($A_{n,max}$), Rubisco efficiency (φ), photosynthesis at operating C_i concentration (A_{OP}), and electron transport rate (ETR). Even though photosynthesis per unit area was highest for seedlings produced under the light ratio of 50:50, the increase in LA observed under the light ratio of 90:10 ultimately led to greater TDM. A trend of increased dry

mass accumulation and decreased φ for seedlings produced at an elevated CO_2 concentration was also observed. Based on these results, further increases in A were likely limited by a suboptimal light intensity, resulting in insufficient Ribulose-1,5-bisphosphate (RuBP)-regeneration under operating conditions. Therefore, an increase in CO_2 concentration should coincide with higher light intensities to prevent a RuBP-limited state due to insufficient ETR during seedling production. Additionally, although there is potential for the stimulation of "sun-type" responses from increased intensities of blue radiation, future research is needed to elucidate how these responses can be fully utilized in controlled environments to optimize seedling production.

4.2 Introduction

With the development of light-emitting diodes (LEDs) and advancements in environmental control technologies, indoor production utilizing sole-source lighting (SSL) has become a potential alternative to traditional greenhouse production for many crops. Specifically, the production of annual bedding plant seedlings has been proposed for indoor production due to the high value of the crop, relatively short production cycle, and high production density in small tray sizes (Park and Runkle, 2017; Randall and Lopez, 2015). Additionally, production of bedding plant seedlings generally occurs during the late winter and early spring, which presents substantial limitations for greenhouse growers in northern latitudes due to the insufficient daily light integral (DLI) often observed during these periods. For example, while a target DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ has been recommended for the production of high-quality seedlings, the greenhouse DLI is often measured as low as 1 to 5 $mol \cdot m^{-2} \cdot d^{-1}$ during winter months (Pramuk and Runkle, 2005; Randall and Lopez, 2014). Thus, vertical indoor systems may provide a cost-effective alternative to greenhouse production, as previous research has found LED SSL as a

viable method for the production of bedding plant seedlings (Randall and Lopez, 2015; Wollaeger and Runkle, 2014).

While substantial research has been conducted regarding the effects of light intensity on plant morphological and physiological responses, research regarding the effects of light quality is limited (Hernández and Kubota, 2016). Specific to seedlings, increased light intensities generally lead to increased quality and dry mass accumulation (Faust et al., 2005; Pramuk and Runkle, 2005; Oh et al., 2010). For example, Graper and Healy (1992) grew petunia (*Petunia ×hybrida* 'Red Flash') seedlings under multiple greenhouse lighting scenarios and found that DLI was primarily responsible for increasing the growth rate and the partitioning of carbohydrates into sugars. While light quality also has a direct effect on plant morphology and physiology, these responses are generally less understood and are often species specific. Chlorophylls *a* and *b* absorb light maximally in the red (663 and 642 nm, respectively) and blue (430 and 453 nm, respectively) wavebands, with a strong correlation between absorption peaks and maximum photosynthetic efficiency (Kopsell et al., 2014). Therefore, many LED SSL applications have focused on red and blue wavelengths in an attempt to maximize chlorophyll absorption. For example, Randall and Lopez (2015) evaluated morphological responses of vinca (*Catharanthus roseus* 'Titan Red Dark'), impatiens (*Impatiens walleriana* 'Super Elfin XP Blue Pearl'), geranium (*Pelargonium ×hortorum* 'Bullseye Red'), petunia 'Dreams Midnight', and French marigold (*Tagetes patula* 'Durango Yellow') seedlings produced under SSL using LEDs providing a red:blue light ratio (%) of either 87:13 or 70:30. These authors found that seedlings produced under SSL were generally of higher quality, possessed increased chlorophyll content, and accumulated greater root dry mass (RDM) than those produced in a traditional greenhouse environment with supplemental lighting.

In addition to light, carbon dioxide (CO_2) is a limiting input of photosynthesis (Tremblay and Gosselin, 1998). While elevated CO_2 concentrations have generally been viewed as beneficial for plant growth, limited information is available regarding how to best utilize CO_2 in controlled environments (Prior et al., 2011). Frantz and Ling (2011) studied the effects of elevated CO_2 on petunia 'Madness White' and found that increasing the CO_2 concentration from 400 to 800 $\mu mol \cdot mol^{-1}$ had no significant effect on biomass accumulation. These authors concluded that the absence of increased biomass under elevated CO_2 might be due to sink-limited conditions imposed by the small container size used for the study. Therefore, based on this finding, Frantz and Ling (2011) proposed that only certain areas of the greenhouse industry, such as seedling production, would likely benefit from an increased CO_2 concentration. While previous research has found CO_2 enrichment beneficial, knowledge concerning the effects of CO_2 is lacking for floriculture crops compared to data on field crop and forest species (Prior et al., 2011).

In a recent study conducted by Banerjee and Adenaeuer (2014), the authors discuss that while vertical, indoor production applications are possible, extensive research regarding production techniques is still required to optimize these systems. With an increased understanding of seedling physiological responses to light intensity, light quality, and CO_2 concentration, informed adjustments to these inputs can be made to optimize production for indoor environments. Specifically, because CO_2 enrichment has been linked to increased plant growth (Prior et al., 2011), by optimizing these environmental inputs, we can manipulate the growing environment to increase seedling quality and decrease the length of time necessary to produce a crop. Therefore, the objective of the present study was to evaluate the morphological and physiological responses of petunia seedlings in two plug tray sizes to the interactive effect of

light intensity, light quality, and CO_2 concentration under LED SSL in an indoor production environment.

4.3 Materials and Methods

4.3.1 Plant Material and Growth Chamber Environment

Seeds of petunia ‘Dreams Midnight’ were sown in 128-cell and 288-cell trays using a commercial soilless medium comprised of (by vol.) 65% peat, 20% perlite, and 15% vermiculite (Fafard Super Fine Germinating Mix; Sun Gro Horticulture, Agawam, MA). Trays were divided into 40-cell (128-cell trays) or 144-cell (288-cell trays) sections to facilitate data collection. Trays were immediately placed under treatment conditions with a 16-h photoperiod (0600 to 2200 HR) in walk-in growth chambers (C5 Control System; Environmental Growth Chambers, Chagrin Falls, OH). The air temperature and relative humidity set points for both chambers were 22 ˚C and 55/60% (day/night), respectively, and were measured and logged every 15 min by a data logger (DL1 Datalogger; Environmental Growth Chambers). Average temperature and relative humidity for the two chambers are reported in Table 12. Seedlings were misted manually to maintain soil moisture until germination occurred. Upon hypocotyl emergence, seedlings were irrigated with water-soluble fertilizer (Jack’s LX 16N–0.94P_2O_5–12.3K_2O Plug Formula for High Alkalinity Water; J.R. Peters, Inc., Allentown, PA) providing (in $mg \cdot L^{-1}$): 100 nitrogen (N), 10 phosphorus (P), 78 potassium (K), 18 calcium (Ca), 9.4 magnesium (Mg), 0.10 boron (B), 0.05 copper (Cu), 0.50 iron (Fe), 0.25 manganese (Mn), 0.05 molybdenum (Mo), and 0.25 zinc (Zn).

4.3.2 Treatment Conditions

A multi-layer production system was utilized in the growth chambers for the establishment of light treatments. Light quality treatments consisted of ten red and six blue LED arrays providing red:blue light ratios of 50:50 (R_{50}:B_{50}) or 90:10 (R_{90}:B_{10}) (Philips GreenPower LED research modules; Koninklijke Philips Electronics, N.V., Netherlands) mounted to stainless steel shelves (123-cm long and 61-cm wide) 40 cm above the crop canopy. Light intensity treatments consisted of two levels, 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [average photosynthetic photon flux density (*PPFD*); 400-700 nm], achieved using dimming units. Thus, a 16-h photoperiod provided plants with a DLI of 8.6 or 17.3 $mol \cdot m^{-2} \cdot d^{-1}$. Light quality and intensity were measured at the beginning of each experimental replication by taking nine individual spectral scans per treatment using a spectrometer (BLACK-Comet UV-VIS Spectrometer; StellarNet, Inc., Tampa, FL). Average *PPFD* and spectral quality for each treatment are reported in Table 13 and Fig. 5, respectively. Each growth chamber maintained a separate CO_2 concentration set point of 450 or 900 $\mu mol \cdot mol^{-1}$. Thus, eight treatment combinations were established with two levels of light quality, two levels of light intensity, and two levels of CO_2 concentration.

Each treatment environment contained four 40-cell and two 144-cell trays for each species. Fixed mounted infrared thermocouples with ABS plastic housing (OS36-01-T-80F, Apogee Instruments, Inc., Logan, UT) were installed on each shelf to measure leaf temperature, and precision thermistors (ST-100; Apogee Instruments, Inc.) measured air temperature within each treatment. Air and leaf temperature were measured every 15 s, and the average was logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc., Logan, UT) (Table 14).

4.3.3 Morphological Data Collection

At 14, 21, and 28 d after hypocotyl emergence, one tray of each size (40-cell and 144-cell) was randomly selected from each treatment. Ten seedlings were randomly sampled from each tray for morphological measurements. Nondestructive measurements included stem length (mm; measured from the base of the hypocotyl to the shoot apical meristem) and stem caliper [mm; measured below the lowest leaf with a digital caliper (digiMax; Wiha, Schonach, Germany)]. Relative chlorophyll content (RCC) was measured on the youngest fully-expanded leaf using a SPAD chlorophyll meter (SPAD-502; Konica Minolta Sensing Americas, Inc., Ramsey, NJ). Leaf area (LA; cm^2) was collected using a LA meter (LI-3100; LI-COR Inc., Lincoln, NE) by removing the seedling leaves at the axil. Seedling roots, stems, and leaves were washed and separated to determine RDM (mg), stem (SDM; mg), leaf (LDM; mg), and total dry mass (TDM = RDM + SDM + LDM). Leaf mass area (LMA = LDM / LA; $mg \cdot cm^{-2}$) was calculated based on LA and dry mass measurements. Additionally, stem caliper and length were used to calculate the sturdiness quotient (SQ = stem caliper / stem length) of each seedling. The quality index (QI = [TDM × (root:shoot ratio + SQ)]) of each seedling was also calculated according to Currey et al. (2013).

4.3.4 Physiological Data Collection

Gas exchange measurements were collected on seedlings grown in 128-cell trays using a portable photosynthesis meter (LI-6400XT; LI-COR Inc.). Photosynthetic responses to increasing light (A-*PPFD*) were conducted using a whole plant chamber attachment (6400-17 Whole Plant Arabidopsis Chamber; LI-COR Inc.). Measurements were taken on four seedlings from each treatment 22 d after germination to determine maximum gross photosynthetic rate ($A_{g,max}$), maximum net photosynthetic rate ($A_{n,max}$), light compensation point (LCP), light

saturation point (LSP), and quantum yield (α). Measurements were conducted using an LED light source providing a descending *PPFD* of 1500, 1250, 1000, 500, 250, 100, 50, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with three minutes of acclimation at each step. The light quality, CO_2 concentration, and leaf temperature reflected the environmental set points established for the experiment.

Photosynthetic responses to leaf internal CO_2 concentration (A-C_i) were conducted using a leaf chamber fluorometer attachment (6400-40 Leaf Chamber Fluorometer; LI-COR Inc.). Measurements were taken on four seedlings from each treatment 29 d after germination to determine the CO_2 compensation point (Γ), Rubisco efficiency (φ), internal CO_2 concentration at the operating point (C_{iOP}), photosynthetic rate at the operating point (A_{OP}), stomatal conductance to CO_2 (g_s), mesophyll conductance to CO_2 (g_m), and electron transport rate (ETR). The CO_2 concentration within the leaf chamber was decreased from the ambient level for the given treatment (450 or 900 $\mu mol \cdot mol^{-1}$) to 25 $\mu mol \cdot mol^{-1}$, returned to ambient, and then increased to a maximum of 1000 $\mu mol \cdot mol^{-1}$ in steps of 100 $\mu mol \cdot mol^{-1}$ to prevent feedback inhibition during measurements. Three minutes of acclimation were allowed at each step before measuring. Additionally, at the end of each step, the fluorescence signal from the leaf under ambient *PPFD* (F_S) and the maximum fluorescence signal from a subsequent saturating flash of light (F_M') were collected. The light quality, light intensity, and leaf temperature reflected the environmental set points established for the experiment.

4.3.5 Calculations and Statistical Analyses

For the A-*PPFD* analysis, a nonlinear regression was fitted using SigmaPlot (SigmaPlot version 12.5; Systat Software, Inc., San Jose, CA) and calculations from the fitted equation (Fig. 6) were made using the model described by Nemali and van Iersel (2004):

$$A_n = A_{g,max} (1 - e^{-\alpha \times PPFD / Ag,max}) - R_d,$$

where R_d is dark respiration and $A_{n,max} = A_{g,max} - R_d$. Quantum yield was calculated as the slope of the response curve at a *PPFD* of zero, and refers to the maximum efficiency at which plants can use incident radiation to fix CO_2. The LCP and LSP were calculated by solving the equation for $A_n = R_d$ and $A_n = A_{g,max} \times 0.95$, respectively.

A nonlinear regression {$A_n = A_0 + [(a \times C_i) / (b + C_i)]$; where A_0 is the estimated photosynthetic rate when C_i is 0, $A_0 + a$ is the maximum attainable A at saturating C_i, and b is a regression coefficient} was fitted using SigmaPlot (SigmaPlot version 12.5; Systat Software, Inc.) for the A-C_i analysis. Calculations from the fitted hyperbolic equation (Fig. 7) were made according to Nemali and van Iersel (2008).

Carbon dioxide compensation point was calculated as the C_i when A = 0 as:

$$\Gamma = - (A_0 \times b) / (A_0 + a)$$

Rubisco carboxylation efficiency was calculated as the slope of the response curve at Γ:

$$\varphi = [(a \times b) / (b \times \Gamma)]^2$$

Assimilation rate at the operating point [the C_i at ambient CO_2 concentration (C_{iOP})] was calculated as:

$$A_{OP} = A_0 + [(a \times C_{iOP}) / (b + C_{iOP})]$$

Stomatal conductance was calculated according to Long and Bernacchi (2003) as:

$$g_s = [A_{OP} / (C_a - C_{iOP})]$$

Mesophyll conductance to CO_2 was calculated as the slope of the response curve at C_{iOP}:

$$g_m = [(a \times b) / (b + C_{iOP})]^2$$

Based on the fluorescence signals obtained during A-C_i analysis, the quantum efficiency of Photosystem II (Φ_{PSII}) was calculated as:

$$\Phi_{PSII} = (F_M' - F_S) / F_M'$$

Electron transport rate could then be calculated as:

$$ETR = \Phi_{PSII} \times f_{II} \times \alpha_L \times PPFD_A$$

where f_{II} is the fraction of absorbed quanta used by PSII (assumed to be 0.5), α_L is leaf fractional absorptance, and $PPFD_A$ is the ambient *PPFD*. A nonlinear regression [$ETR = Y_0 + a \times (1 - e^{-b \times Ci})$] was also fitted for ETR using SigmaPlot (SigmaPlot version 12.5; Systat Software, Inc.) to determine ETR at C_{iOP}.

Due to unforeseen limitations, a 450 µmol·mol^{-1} CO_2 concentration was not attainable in one of the walk-in growth chambers used for this experiment. Thus, the two CO_2 concentrations were confined to individual growth chamber environments, limiting the ability to randomize this variable in the experimental design. Therefore, CO_2 concentrations were evaluated separately for this analysis. For the factors of light quality (2 levels) and light intensity (2 levels) within each growth chamber environment, a completely randomized design with a factorial arrangement was utilized. The experiment was replicated three times over time for both morphological and gas exchange measurements in both growth chamber environments. The effects of light intensity and quality on morphology and the physiological parameters obtained from the A-*PPFD* and A-C_i responses were compared by analysis of variance (ANOVA) using SAS (SAS version 9.3; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) and Tukey's honest significant difference (HSD) test at $P \leq 0.05$ (Tables 15-18).

4.4 Results

4.4.1 Morphology and Growth

The interaction between light intensity and quality for stem length was significant at both CO_2 concentrations on day 14, with the shortest stem length observed under the light intensity of

150 μmol·m^{-2}·s^{-1} and light quality of R_{50}:B_{50} (150_R_{50}:B_{50}; light intensity_light quality) (Table 15). For example, stem length of seedlings grown at the CO_2 concentration of 450 μmol·mol^{-1} under 150_R_{50}:B_{50} was 21%, 19%, and 21% shorter compared to 150_R_{90}:B_{10}, 300_R_{50}:B_{50}, and 300_R_{90}:B_{10}, respectively. The interaction between light intensity and quality was also significant at the CO_2 concentration of 450 μmol·mol^{-1} on day 21 (Table 15). While 150_R_{50}:B_{50} still yielded the shortest results, stem length was also 7% and 11% shorter under 300_R_{50}:B_{50} compared to 150_R_{90}:B_{10} and 300_R_{90}:B_{10}, respectively. When no interaction was observed, the main effects of light intensity and quality were both significant, with shorter stem lengths observed under the light quality of R_{50}:B_{50} compared to R_{90}:B_{10} and light intensity of 150 compared to 300 μmol·m^{-2}·s^{-1} (Table 15).

For stem caliper, the interaction between light intensity and quality was only significant at the CO_2 concentration of 450 μmol·mol^{-1}, with the lowest values observed under 150_R_{50}:B_{50} on all harvest dates (Table 15). For example, stem caliper under 150_R_{90}:B_{10}, 300_R_{50}:B_{50}, and 300_R_{90}:B_{10} on day 28 was 14%, 29%, and 36% greater, respectively, compared to 150_R_{50}:B_{50}. Stem caliper under 300_R_{50}:B_{50} and 300_R_{90}:B_{10} was also 13% and 19% greater, respectively, compared to 150_R_{90}:B_{10} on day 28. At the CO_2 concentration of 900 μmol·mol^{-1}, the main effects of light intensity and quality were significant for all harvest dates, with increased stem caliper observed under the light quality of R_{90}:B_{10} compared to R_{50}:B_{50} and light intensity of 300 compared to 150 μmol·m^{-2}·s^{-1} (Table 15).

For LA, the interaction between light intensity and quality was significant on days 14 and 21 at the CO_2 concentration of 450 μmol·mol^{-1} (Table 15). On day 14, LA under 150_R_{90}:B_{10}, 300_R_{50}:B_{50}, and 300_R_{90}:B_{10} was 86%, 136%, and 164% greater, respectively, compared to 150_R_{50}:B_{50}. Leaf area was also 27% and 42% greater under 300_R_{50}:B_{50} and 300_R_{90}:B_{10},

respectively, compared to 150_R_{90}:B_{10}. Similarly, on day 21, LA was lowest under 150_R_{50}:B_{50} (Table 15). Additionally, LA was 21% and 29% greater under 150_R_{90}:B_{10} and 300_R_{90}:B_{10}, respectively, compared to 300_R_{50}:B_{50}. Similar results were observed with the interaction between light intensity and quality at the CO_2 concentration of 900 μmol·mol^{-1} on day 21 as LA was lowest under 150_R_{50}:B_{50} (Table 15). Additionally, with the same conditions, LA under 300_R_{90}:B_{10} increased 11% compared to 300_R_{50}:B_{50}. While no interaction was observed on day 28 at both CO_2 concentrations, the main effect of light quality was significant for LA with the greatest values observed under the light quality of R_{90}:B_{10} compared to R_{50}:B_{50} (Table 15).

For RCC, the interaction between light intensity and quality was significant for all treatment combinations except at the CO_2 concentrations of 900 μmol·mol^{-1} on day 14 (Table 15). Generally, the highest RCC was observed under 300_R_{50}:B_{50}. For example, on day 28 at the CO_2 concentrations of 450 μmol·mol^{-1}, RCC under 300_R_{50}:B_{50} was 21%, 19%, and 8% greater compared to 150_R_{50}:B_{50}, 150_R_{90}:B_{10}, and 300_R_{90}:B_{10}, respectively. Likewise, on day 28 at the CO_2 concentrations of 900 μmol·mol^{-1}, RCC under 300_R_{50}:B_{50} was 17%, 16%, and 5% greater compared to 150_R_{50}:B_{50}, 150_R_{90}:B_{10}, and 300_R_{90}:B_{10}, respectively.

The interaction between light intensity and quality for RDM was only significant on day 28 at the CO_2 concentration of 900 μmol·mol^{-1}, with the greatest RDM values observed under both 300_R_{50}:B_{50} and 300_R_{90}:B_{10} (Table 15). Root dry mass also was 41% higher under 150_R_{90}:B_{10} compared to 150_R_{50}:B_{50}. When no interaction was observed, the main effects of light intensity and quality were generally both significant, with increased RDM observed under the light intensity of 300 compared to 150 μmol·m^{-2}·s^{-1} and light quality of R_{90}:B_{10} compared to R_{50}:B_{50} (Table 15).

For both LDM and TDM, the interaction between light intensity and quality was significant at the CO_2 concentration of 450 µmol·mol^{-1} on day 14 (Table 15). Increased LDM and TDM were observed at the higher light intensity, with seedlings under both 300_R_{50}:B_{50} and 300_R_{90}:B_{10} possessing significantly greater values than both 150_R_{50}:B_{50} and 150_R_{90}:B_{10}. Additionally, LDM and TDM increased 96% and 84%, respectively, under 150_R_{90}:B_{10} compared to 150_R_{50}:B_{50}. When no interaction was present, the main effects of light intensity and quality were significant with increased LDM and TDM under the light intensity of 300 compared to 150 µmol·m^{-2}·s^{-1} and light quality of R_{90}:B_{10} compared to R_{50}:B_{50} (Table 15). Additionally, in general, the main effect of light intensity for LMA was significant for both CO_2 concentrations on all harvest dates, with higher values observed under the light intensity of 300 compared to 150 µmol·m^{-2}·s^{-1} (Table 15).

The main effect of light intensity was significant for QI at both CO_2 concentrations on all harvest dates, with higher values observed under the light intensity of 300 compared to 150 µmol·m^{-2}·s^{-1} (Table 15). Additionally, the main effect of light quality was significant for all treatment combinations except at the CO_2 concentration of 450 µmol·mol^{-1} on day 14, with higher QI values observed under R_{90}:B_{10} compared to R_{50}:B_{50} (Table 15). The interaction between light intensity and quality was only significant for QI at the CO_2 concentration of 450 µmol·mol^{-1} on day 28 (Table 15). Specifically, QI decreased 38%, 62%, and 66% under 150_R_{50}:B_{50} compared to 150_R_{90}:B_{10}, 300_R_{50}:B_{50}, and 300_R_{90}:B_{10}, respectively.

Similar morphological responses were observed for seedlings produced in 288-cell trays (Table 16). Therefore, results regarding production in 128-cell trays were the primary focus for discussion.

4.4.2 A-*PPFD* Analysis

While there was no interaction, the main effect of light quality was significant for $A_{g,max}$, $A_{n,max}$, LCP, and α at both CO_2 concentrations, with higher values observed under the light quality of R_{50}:B_{50} compared to R_{90}:B_{10} (Table 17). For example, $A_{g,max}$ and $A_{n,max}$ were 30% and 33% greater under the light quality of R_{50}:B_{50} (28.4 and 26.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively) compared to R_{90}:B_{10} (21.9 and 19.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively), at the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$. Similarly, $A_{g,max}$ and $A_{n,max}$ were 23% and 25% greater under the light quality of R_{50}:B_{50} (22.2 and 20.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to R_{90}:B_{10} (18.0 and 16.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$), respectively, at the CO_2 concentration of 450 $\mu mol \cdot mol^{-1}$. The interaction between light intensity and quality was significant for LSP at the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$, with the highest LSP observed under 300_ R_{50}:B_{50} (Table 17). Additionally, the main effect of light quality was significant for LSP at the CO_2 concentration of 450 $\mu mol \cdot mol^{-1}$, as the LSP under the light quality of R_{50}:B_{50} (1294 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was higher than R_{90}:B_{10} (779 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The main effect of light intensity was significant for LCP at both CO_2 concentrations, with significantly higher values under the light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 17). Additionally, the main effect of light intensity was significant for α at the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$, with higher values observed under the light intensity of 150 compared to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 17).

4.4.3 A-C_i Analysis

The interaction between light intensity and quality was significant for φ, C_{iOP}, A_{OP}, g_s, and ETR at both CO_2 concentrations (Table 18). The highest values for φ**,** A_{OP}, g_s, and ETR were observed under 300_R_{50}:B_{50}. While C_{iOP} was relatively similar among light treatments, the lowest values were observed under 300_R_{90}:B_{10} at both CO_2 concentrations. Although there was

no interaction between light intensity and quality for g_m, the main effect of light intensity was significant (Table 18). Specifically, g_m increased under the light intensity of 300 compared to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at both CO_2 concentrations.

4.5 Discussion

4.5.1 Morphology and Growth

Desirable qualities in a bedding plant seedling include reduced stem length, large stem caliper, limited LA, and high dry mass (Oh et al., 2010; Pramuk and Runkle, 2005). These attributes increase the durability of seedlings during shipment, while also facilitating mechanical transplant and establishment in the production environment. In the present study at both CO_2 concentrations, petunia seedlings grown under the light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ possessed increased stem caliper and dry mass compared to the light intensity of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. High light intensities have commonly been found to increase seedling dry mass accumulation for multiple bedding plant species (Graper and Healy, 1991; Pramuk and Runkle, 2005; Oh et al., 2010). This increase in light intensity (or DLI) ultimately leads to thicker tissues with increased carbohydrates and structural carbon content for growth (Faust et al., 2005). The QI provides an objective and quantitative means by which to evaluate seedling quality, incorporating morphological parameters crucial to durability and transplant success (Currey et al., 2013; Randall and Lopez, 2014). Quality index values were highest under the light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which can be attributed to the increase in stem caliper and dry mass which was also observed under this light intensity.

At both CO_2 concentrations, increased dry mass accumulation and LA were observed under the light quality of R_{90}:B_{10}. A strong correlation between LA and the accumulation of biomass exists in many plant species due to the increased surface area available for light

interception (van Ieperen, 2012; Wollaeger and Runkle, 2014). Wollaeger and Runkle (2014) evaluated tomato (*Solanum lycopersicum* 'Early Girl'), salvia (*Salvia splendens* 'Vista Red'), impatiens 'SuperElfin XP Red', and petunia 'Wave Pink' seedlings grown under LED SSL with red:blue light ratios of 100:0, 94:6, 88:12, 75:25, 50:50, or 0:100 at a light intensity of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Similar to our findings, they observed that both LA and dry mass decreased as the percentage of blue radiation increased. Based on this observation, Wollaeger and Runkle (2014) proposed that the primary role of light quality in the accumulation of biomass was due to increased LA. Similar results were found by Hogewoning et al. (2012b) with greenhouse-grown tomato 'Mecano' and cucumber (*Cucumis sativus* 'Venice'). These authors attributed differences in dry mass primarily to changes in petiole and/or internode length resulting from the spectral quality of supplemental lighting (Hogewoning et al., 2012b). Park and Runkle (2017) likewise attributed increased seedling growth indirectly to leaf expansion promoted by the inclusion of far-red radiation. Therefore, we propose that the increased LA under the light quality of R_{90}:B_{10} led to increased dry mass accumulation for petunia seedlings.

The differences in LA between light qualities in the present study was likely due to the increased percentage of blue radiation under the light ratio of R_{50}:B_{50}. Photomorphogenic responses are commonly observed under blue radiation (Cope et al., 2014). In accordance with our findings, blue wavelengths are often utilized for the production of a variety of crops due to their role in growth inhibition responses such as reduced stem extension (Cosgrove, 1981; Kigel and Cosgrove, 1991; Runkle and Heins, 2001). Additionally, LA expansion may be restricted by blue radiation (Hernández and Kubota, 2016; Ohashi-Kaneko et al., 2007). Hernández and Kubota (2016) found that increased percentages of blue radiation (up to 75%) provided to cucumber 'Cumlaude' seedlings resulted in decreased LA and increased LMA at a light intensity

of 100 µmol·m^{-2}·s^{-1}. These authors concluded that the addition of blue radiation in their study resulted in a response similar to observations under high irradiance, regardless of the low light intensity utilized.

Leaf mass area generally increases under high light intensities and increased percentages of blue radiation (Hernández and Kubota, 2016; Poorter et al., 2009). In accordance with previous research, the LMA of petunia seedlings in the present study increased under the light intensity of 300 µmol·m^{-2}·s^{-1} compared to 150 µmol·m^{-2}·s^{-1}. However, LMA did not differ between the light qualities of R_{50}:B_{50} and R_{90}:B_{10} at either CO_2 concentration. Matsuda et al. (2007) found similar results in that LMA for spinach (*Spinacia oleracea* 'Megaton') did not vary among LED red:blue light ratios of 90:10, 70:30, or 50:50 at a light intensity of 300 µmol·m^{-2}·s^{-1}. These authors also found that leaf N content per unit LA and A increased as the intensity of blue radiation increased (up to 100 µmol·m^{-2}·s^{-1}). Based on these results, Matsuda et al. (2007) concluded that the response to blue radiation observed was independent of an acclimation response to irradiance. Rather, these authors suggested that increasing blue radiation plays a direct role in chloroplast acclimation by altering N partitioning to thylakoid components (Matsuda et al., 2007).

Generally, as the percentage of blue radiation increases within a spectrum, chlorophyll content increases (Hogewoning et al., 2010; Kopsell et al., 2014; Matsuda et al., 2007). For example, Kopsell et al. (2014) found that total chlorophyll content of sprouting broccoli (*Brassica oleacea* var. *italica*) increased under LED lighting with up to 20% blue radiation at a light intensity of 250 µmol·m^{-2}·s^{-1}. While RCC values obtained via SPAD are unitless, they provide an accepted estimation of leaf chlorophyll content (Ruiz-Espinoza et al., 2010). Additionally, a strong correlation between leaf N and RCC, measured with a SPAD chlorophyll

meter, has been observed for multiple horticultural crops (Shaahan et al., 1999; Wang et al., 2012; Zanin and Sambo, 2006). In the present study, RCC was highest under 300_ R_{50}:B_{50}, indicating potential increases in both leaf chlorophyll concentration and N content. Thus, the increased RCC observed under 300_ R_{50}:B_{50} at both CO_2 concentrations may indicate an acclimation response similar to that proposed by Matsuda et al. (2007).

4.5.2 A-*PPFD* Analysis

Red wavelengths of light have commonly been associated with dry mass accumulation due to their action at the absorption peaks of chlorophylls (Massa et al., 2008). However, plants grown under solely red wavelengths generally possess a lower dry mass and develop responses similar to those observed during shade avoidance (Buschmann et al., 1978; Hoenecke et al., 1992; Yorio et al., 2001). For example, Yorio et al. (2001) found that the dry weight of lettuce (*Lactuca sativa* 'Waldmann's Green'), radish (*Raphanus sativus* 'Cherriette'), and spinach 'Nordic IV' decreased under solely red LEDs compared to red LEDs supplemented with ~8% blue radiation (blue fluorescent lamps) or cool-white fluorescent (CWF) lamps providing a light intensity of ~300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Thus, a spectral ratio providing both red and blue wavelengths is recommended to prevent undesirable morphological responses and optimize growth. However, blue radiation has generally been labeled as less efficient at driving A than other wavelengths, primarily due to decreased LA and low quantum efficiency (Cope et al., 2014). Thus, recommendations are commonly made to minimize blue radiation in production environments and focus on wavelengths that are more photosynthetically efficient.

Part of the reason for this low quantum efficiency is due to the absorption of blue wavelengths by pigments other than chlorophylls (Barnes et al., 1993; Hogewoning et al., 2012a). Specifically, while greater than 90% of blue photons are absorbed, it has been estimated

that 20% of these photons are absorbed by photosynthetic carotenoids and inactive pigments (Barnes et al., 1993). Photosynthetic carotenoids have an absorption maxima for blue wavelengths, but only a fraction of this energy is transferred to chlorophylls (Croce et al., 2001; Hogewoning et al., 2012a). Additionally, when inactive pigments, such as anthocyanins, absorb blue photons, the energy is lost as heat or fluorescence rather than being transferred to the reaction centers (Barnes et al., 1993). As a result, blue radiation generally has a lower quantum yield compared to red radiation (Evans, 1987; Hogewoning et al., 2012a; McCree, 1972). Results from the present study support these findings, as quantum yield was significantly lower under the light ratio of R_{50}:B_{50} compared to R_{90}:B_{10} at both CO_2 concentrations (Table 17). Additionally, LCP was highest under the light ratio of R_{50}:B_{50}, which suggests that more radiation may have been necessary to offset losses from R_d with increased percentages of blue light. Based on whole-plant measurements with wax begonia (*Begonia semperflorens-cultorum* 'Cocktail Vodka'), Nemali and van Iersel (2004) proposed that LCP decreased with increasing quantum yield, further validating the observations made in the present study.

Regardless of low quantum yield, increasing blue radiation has been found to increase A in multiple species (Hogewoning et al., 2010; Huché-Thélier et al., 2016; Matsuda et al., 2007; Yorio et al., 2001). For example, Hogewoning et al. (2010) grew cucumber 'Hoffmann's Giganta' with red:blue light ratios of 100:0, 93:7, 88:12, 85:15, 70:30, 50:50, and 0:100 at a light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and found that as the percentage of blue radiation increased, $A_{g,max}$, chlorophyll content, and N per unit LA increased. The authors concluded that $A_{g,max}$ in cucumber responds quantitatively to increased blue radiation, stimulating a "sun-type" response in leaves even under the relatively low light intensity utilized for the study. This "sun-type" response was outlined by Poorter et al. (2009), who stated that increased irradiance generally leads to a higher

photosynthetic capacity and LMA. However, they describe that when photosynthetic capacity is expressed on a per unit LDM basis, values are similar between plants grown under high and low light intensities. Thus, these authors concluded that leaf anatomy is the primary driver of light-saturated A due to the linear scaling of photosynthetic capacity with leaf biomass (Poorter et al., 2009).

In the present study, both $A_{g,max}$ and $A_{n,max}$ (on a LA basis) were highest under the light quality of R_{50}:B_{50} at both CO_2 concentrations. However, significant differences in LMA were not observed between light qualities. As discussed previously, Matsuda et al. (2007) observed increased N content and A under an increased percentage of blue radiation. Murchie and Horton (1998) proposed that two levels of acclimation, chloroplast and leaf, exist regarding changes in A. Therefore, similar to observations made by Matsuda et al. (2007), the increased $A_{n,max}$ observed under the higher percentage of blue radiation was likely not a morphological "sun-type" irradiance response, as LMA was unaffected. Rather, we hypothesize that increased $A_{n,max}$ was due to acclimation at the chloroplast level, with the possibility of increased N partitioning to electron transport and light-harvesting components.

Net photosynthesis of petunia seedlings reached light saturation at a much higher light intensity under R_{50}:B_{50} compared to R_{90}:B_{10} at both CO_2 concentrations. This increase in LSP is likely linked to an increase in the Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content under higher percentages of blue radiation. Previous research has found that plants grown under high light intensities generally possess a higher LSP, which can mainly be attributed to increased Rubisco (Callan and Kennedy, 1995; Sukenik et al., 1987). Rubisco carboxylation efficiency provides an indicator of total Rubisco content or the number of Rubisco active sites. In the present study, φ was highest under 300_R_{50}:B_{50}. Thus, the higher LSP

observed under increased percentages of blue radiation was likely due to an increase in Rubisco content.

4.5.3 A-C_i Analysis

Under high light intensities, A is generally limited based on φ at low CO_2 concentrations, ETR at high CO_2 concentrations, and triose phosphate utilization at a maximum CO_2 concentration (Bunce, 2016; Sharkey et al., 2007). Under low light intensities, limitations to A are generally the result of reduced ETR (Bunce, 2016; Farquhar et al., 1980). In the present study, A_{OP} increased under 300_ R_{50}:B_{50}, which was the direct result of concurrent increases in both φ and ETR. Due to A_{OP} being co-limited by φ and ETR, increases in both Rubisco content and Ribulose-1,5-bisphosphate (RuBP)-regeneration were necessary for the increase in A_{OP} observed. Sharkey et al. (2007) proposed that an increased ETR alongside increased CO_2 indicates Rubisco-limited conditions, while stagnant ETR with increased CO_2 indicates RuBP-regeneration limitation. For our results, while ETR increased with increasing C_i, limitations were present under both CO_2 concentrations. Specifically, at the CO_2 concentration of 450 μmol·mol^{-1}, ETR became saturated at a C_i of 137-357 μmol·mol^{-1}; while at the CO_2 concentration of 900 μmol·mol^{-1}, saturation occurred at a C_i of 288-555 μmol·mol^{-1}. For all light treatments at both CO_2 concentrations, C_{iOP} was greater than C_i for maximum ETR (data not shown). Thus, further increases in A_{OP} were likely limited by a suboptimal light intensity, resulting in insufficient RuBP-regeneration under operating conditions. A similar conclusion was made by Ainsworth and Rogers (2007), who found that the operating point at elevated CO_2 was limited by RuBP-regeneration for a variety of crops. Thus, these authors concluded that as the CO_2 concentration rises, A will generally be limited by RuBP regeneration. While A will continue to rise past this point of RuBP limitation, these increases are merely the result of repressed photorespiration

(Ainsworth and Rogers; 2007). Similarly, Drake et al. (1997) found that increased A_n would occur to some degree with increasing C_a regardless of whether conditions were Rubisco- or RuBP-limiting due to the repression of photorespiration. Although A continued to rise as C_i increased based on A-C_i analyses in the present study, these increases past the point of RuBP-limited conditions were likely due to decreased photorespiration. Therefore, given the conditions of the present study, seedling production under elevated CO_2 should prioritize increased light intensities to prevent an RuBP-limited state due to insufficient ETR.

Blue radiation is often associated with the accumulation of Rubisco in the chloroplast (Weston et al., 2000). Thus, the increase in φ observed under 300_ R_{50}:B_{50} was likely due to the increased intensity of blue radiation. Muneer et al. (2014) evaluated lettuce 'Hongyeom' seedlings under high (238 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light intensities provided by red or blue LEDs and found that Rubisco content was highest under high intensities of blue radiation. Similarly, Matsuda et al. (2004) found that larger amounts of photosynthetic components, including Rubisco, increased in rice (*Oryza sativa* 'Sasanishiki') as a result of increased N content for plants grown under both red and blue radiation compared to solely red radiation. This relationship between leaf N content and Rubisco is important to consider, as Rubisco has been found to constitute 25% of leaf N content in many C_3 plants (Bainbridge et al., 1995; Drake et al., 1997). Results in the present study were similar, as increased φ coincided with increased RCC under 300_ R_{50}:B_{50}, indicating an increase in leaf N content. The increase in ETR observed under 300_ R_{50}:B_{50} was likely due to the chloroplast acclimation response described previously (Matsuda et al., 2007), with increased N partitioned to components involved in electron transfer, such as Cyt *f*. Additionally, with the increase in RCC, seedlings

under 300_ R_{50}:B_{50} may have been capable of increased radiation absorption compared to the other light treatments, ultimately leading to increased ETR.

For the process of photosynthesis, CO_2 must move from the air surrounding a leaf, through stomata, and into the sub-stomatal internal cavities (Flexas et al., 2008). After entering through the stomata, CO_2 must then diffuse through the leaf mesophyll to the site of carboxylation inside the chloroplast stroma. In the present study, g_s was highest under 300_ R_{50}:B_{50} at both CO_2 concentrations. This increase was likely due to the increased intensity of blue radiation under this treatment. Blue wavelengths have been found to increase g_s in many previous studies (Kinoshita et al., 2001; van Ieperen et al., 2012; Zeiger et al., 2002). Kinoshita et al. (2001) proposed that increased stomatal opening from blue radiation ultimately leads to increased CO_2 uptake, which further increases assimilation within the plant. Additionally, both g_m and g_s have been shown to respond positively to increased light intensity for many species (Flexas et al., 2007; Long et al., 2006; Piel et al., 2002). Specifically, Piel et al. (2002) found that both g_s and g_m increased in leaves of hybrid walnut trees (*Juglans nigra* ×*regia*) exposed to full sun compared to shaded conditions. Increased g_s may have contributed to the increased A_{OP} observed under 300_ R_{50}:B_{50}, as more CO_2 would have been available to coincide with the increased φ observed under this treatment. According to Piel et al. (2002), a positive relationship also exists between A and g_m for many plant species. Similar to previous findings (Flexas et al., 2007; Piel et al., 2002), g_m increased under the light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at both CO_2 concentrations in the present study. While the interaction between light intensity and quality was not significant, g_m was highest under 300_ R_{50}:B_{50} at both CO_2 concentrations where the highest A_{OP} was also observed. Thus, increased g_s and g_m under 300_ R_{50}:B_{50} likely contributed to the increase in A_{OP} also observed under this light treatment.

4.5.4 Carbon Dioxide Concentration

The activity of Rubisco depends greatly on the ratio of CO_2 and O_2 concentration (Lindhout and Pet, 1990). Under elevated CO_2, a shift in balance occurs where the carboxylation activity of Rubisco is favored while oxygenation is generally suppressed (Lindhout and Pet, 1990; Makino and Mae, 1999; Tremblay and Gosselin, 1998). Thus, increased CO_2 concentrations often lead to increased plant growth, with seedling production of primary interest due to plant tissue juvenility leading to primarily vegetative growth (Prior et al., 2011; Thomas et al., 1975; Tremblay and Gosselin, 1998). While statistical comparisons were not conducted, a trend of increased dry mass accumulation was observed in the present study for petunia seedlings grown at a CO_2 concentration of 900 compared to 450 µmol·mol^{-1} (Tables 15 and 16). Lindhout and Pet (1990) found similar results in that increasing the CO_2 concentration from 320 to 750 µmol·mol^{-1} led to an increase in average overall growth by a factor of 2.3 for multiple genotypes of tomato. Likewise, Kaczperski et al. (1994) found that pansy (*Viola* ×*wittrockiana* 'Majestic Giant Yellow') seedlings displayed accelerated growth at a CO_2 concentration of 1000 µmol·mol^{-1} compared to 500 µmol·mol^{-1}.

However, plants grown under elevated CO_2 concentrations have been found to display acclimation responses, limiting the potential benefits from this enrichment (Arp, 1991; Makino and Mae, 1999). One such response involves a reduction in Rubisco content under elevated CO_2, which is typically accompanied by a decrease in leaf N content (Ainsworth and Rogers, 2007; Evans, 1989). One reason for this reduced Rubisco content is the plant's inability to utilize additional carbohydrate provided by the increased photosynthesis under elevated CO_2, ultimately leading to a decrease in source activity as a means of regulation (Drake et al., 1997). Additionally, decreased Rubisco may be due to a lower requirement for this protein at increased CO_2 concentrations (Drake et al., 1997). A trend of reduced Rubisco content was observed in the

present study, with decreases in φ for seedlings produced at the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$ (Table 18). However, this decrease in Rubisco did not affect A_{OP}, suggesting that plants may have reallocated resources from Rubisco as result of the elevated CO_2 concentration. Additionally, it has been estimated that 35% of Rubisco could be lost under elevated CO_2 concentrations prior to any differences in A being noticed (Drake et al., 1997). Since ETR was rate limiting well below operating condition at the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$, φ likely decreased as an acclimation response.

A trend of decreased g_s and g_m was also observed for petunia seedlings grown under the elevated CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$ (Table 18). Decreases in g_s under elevated CO_2 may be caused by changes in stomatal density, index, or aperture (Ainsworth and Rogers, 2007; Assmann, 1999; Drake et al., 1997). Likewise, g_m has been found to decrease under increased CO_2 concentrations (Düring, 2003; Flexas et al., 2007). Düring (2003) hypothesized that both g_s and g_m adapt alongside elevated CO_2 concentrations to match CO_2 supply with the demand in chloroplasts. In alignment with the present study, Herrick et al. (2004) found that the increased photosynthetic activity resulting from CO_2 enrichment more than compensated for the diffusional limitation imposed by stomatal closure. Additionally, reduced g_s may help to improve water-use efficiency by reducing water loss through transpiration (Drake et al., 1997). Thus, the reduction in g_s and g_m under the CO_2 concentration of 900 $\mu mol \cdot mol^{-1}$ appears to have had no detrimental effect on the overall photosynthetic capacity of petunia seedlings in the present study, and was likely an acclimation response to CO_2 demand.

4.6 Conclusion

While previous studies have evaluated the feasibility of seedling production in indoor environments using LED SSL, few have reported physiological responses to light and CO_2 inputs

under these conditions. With increased understanding of these responses, production in controlled environments can be more effectively optimized. Based on our results, petunia seedlings showed significantly higher A per unit LA under increased intensities of blue radiation. However, the increase in LA observed under an increased percentage of red wavelengths ultimately led to greater light interception and dry mass accumulation under high light intensities. Therefore, though there is potential for blue radiation to stimulate "sun-type" leaf and chloroplast responses, future research is needed to elucidate how these responses can be fully utilized in controlled environments to optimize seedling production. For example, increased light intensities under elevated CO_2 concentrations may alleviate RuBP limitations and allow for increased A. However, a concurrent increase in LA must be present to fully utilize this incident radiation. Additionally, acclimation to elevated CO_2 concentrations may limit potential gains from this input. Therefore, the present study provides a foundation for future research to elucidate how light intensity and quality can be utilized alongside elevated CO_2 to optimize seedling growth and decrease production time for the efficient use of energy and indoor space.

4.7 Acknowledgements

I gratefully acknowledge Dr. Roberto Lopez, Dr. Krishna Nemali, Jillian Malecki, and Zachary Oravec for intellectual, growth chamber, and laboratory assistance; Ball Horticultural Co. for seed; Sun Gro Horticulture for growing media; Everris NA Inc. for fertilizer; and Philips Lighting and Hort Americas for LED lighting assistance and donations. The use of trade names in this publication does not imply endorsement by Purdue University of products named nor criticism of similar ones not mentioned.

4.8 Literature Cited

Ainsworth, E.A. and A. Rogers. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. Plant, Cell and Environ. 30:258–270.

Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant, Cell and Environ. 14:869–875.

Assmann, S.M. and K. Shimazaki. 1999. The multisensory guard cell. Stomatal responses to blue light and abscisic acid. Plant Physiol. 119:809–815.

Bainbridge G., P. Madgwick, S. Parmar, R. Mitchell, M. Paul, J. Pitts, A.J. Keys, and A.J. Parry. 1995. Engineering rubisco to change its catalytic properties. J. Exp. Bot. 46:1269–1276.

Banerjee, C. and L. Adenaeuer. 2014. Up, up and away! The economics of vertical farming. J. Agr. Studies 2:40–60.

Barnes, C., T. Tibbitts, J. Sager, G. Deitzer, D. Bubenheim, G. Koerner, and B. Bugbee. 1993. Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux. HortScience 28:1197–1200.

Bunce, J.A. 2016. Light dependence of carboxylation capacity for C_3 photosynthesis models. Photosynthetica 54:484–490.

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

Callan, E.J. and C.W. Kennedy. 1995. Intercropping stokes aster: Effect of shade on photosynthesis and plant morphology. Crop Sci. 35:1110–1115.

Cope, K.R., M.C. Snowden, and B. Bugbee. 2014. Photobiological interactions of blue light and photosynthetic photon flux: Effects of monochromatic and broad-spectrum light sources. Photochemistry and Photobiology 90:574–584.

Cosgrove, D.J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67:584−590.

Croce, R., M.G. Müller, R. Bassi, and A.R. Holzwarth. 2001. Carotenoid-to-chlorophyll energy transfer in recombinant major light-harvesting complex (LHCII) of higher plants. I. Femtosecond transient absorption measurements. Biophysical J. 80:901–915.

Currey, C.J., A.P. Torres, R.G. Lopez, and D.F. Jacobs. 2013. The quality index: A new tool for integrating quantitative measurements to assess quality of young floriculture plants. Acta Hort 1000:385–391.

Drake, B.G., M.A. González-Meler, and S.P. Long. 1997. More efficient plants: A consequence of rising atmospheric CO_2? Annu. Rev. Plant. Physiol. Plant Mol. Biol. 48:609–639.

Düring, H. 2003. Stomatal and mesophyll conductances control CO_2 transfer to chloroplasts in leaves of grapevine (*Vitis vinifera* L.). Vitis 42:65–68.

Evans, JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9–19.

Farquhar, G.D., S. von Caemmerer, and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90.

Faust, J.E., V. Holcombe, N.C. Rajapakse, and D.R. Layne. 2005. The effect of daily light integral on bedding plant growth and flowering. HortScience 40:645−649.

Flexas, J., M.F. Ortuño, M. Ribas-Carbó, A. Diaz-Espejo, I.D. Flórez-Sarasa, and H. Medrano. 2007. Mesophyll conductance to CO_2 in *Arabidopsis thaliana*. New Phytol. 175:501–511.

Flexas, J., M. Ribas-Carbó, A. Diaz-Espejo. J. Galmés, and H. Medrano. 2008. Mesophyll conductance to CO_2: Current knowledge and future prospects. Plant, Cell and Environ. 31:602–621.

Frantz, J.M. and P. Ling. 2011. Growth, partitioning, and nutrient and carbohydrate concentration of *Petunia ×hybrida* Vilm. are influenced by altering light, CO_2, and fertility. HortScience 46:228−235.

Graper, D.F. and W. Healy. 1991. High pressure sodium irradiation and infrared radiation accelerate *Petunia* seedling growth. J. Amer. Soc. Hort. Sci. 116:435–438.

Graper, D.F. and W. Healy. 1992. Modification of petunia seedling carbohydrate partitioning by irradiance. J. Amer. Soc. Hort. Sci. 117:477–480.

Hernández, R. and C. Kubota. 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ. Expt. Bot. 121:66–74.

Herrick, J.D., H. Maherali, and R.B. Thomas. 2004. Reduced stomatal conductance in sweetgum (*Liquidambar styraciflua*) sustained over long-term CO_2 enrichment. New Phytol. 162:387–396.

Hoenecke, M.E., R.J. Bula, and T.W. Tibbits. 1992. Importance of 'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427−430.

Hogewoning, S.W., E. Wientjes, P. Douwstra, G. Trouwborst, W. van Ieperen, R. Croce, and J. Harbinson. 2012a. Photosynthetic quantum yield dynamics: From photosystems to leaves. Plant Cell 24:1921–1935.

Hogewoning, S.W., G. Trouwborst, E. Meinen, and W. van Ieperen. 2012b. Finding the optimal growth-light spectrum for greenhouse crops. Acta Hort. 956:357–363.

Hogewoning, S.W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Expt. Bot. 61:3107–3117.

Huché-Thélier, L., L. Crespel, J.L. Gourrierec, P. Morel, S. Sakr, and N. Leduc. 2016. Light signaling and plant responses to blue and UV radiations – perspectives for applications in horticulture. Environ. Expt. Bot. 121:22–38.

Kaczperski, M.P., A.M. Armitage, and P.M. Lewis. 1994. Accelerating growth of plug-grown pansies with carbon dioxide and light. HortScience 29:442 (abstr.).

Kigel, J. and D.J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049−1056.

Kinoshita, T, M. Doi, N. Suetsugu, T. Kagawa, M. Wada, and K. Shimazaki. 2001. Phot1 and phot2 mediate blue light regulation of stomatal opening. Nature 414:656−660.

Kopsell, D. A., C.E. Sams, T.C. Barickman, and R.C. Morrow. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. J. Amer. Soc. Hort. Sci. 139:469–477.

Lindhout, P. and G. Pet. 1990. Effects of CO_2 enrichment on young plant growth of 96 genotypes of tomato (*Lycopersicum esculentum*). Euphytica 51:191–196.

Long, S.P. and C.J. Bernacchi. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Expt. Bot. 54:2393–2401.

Long, S.P., E.A. Ainsworth, C.J. Bernacchi, P.A. Davey, G.J. Hymus, A.D.B. Leakey, P.B.Morgan, and C.P. Osborne. 2006. Long-term responses of photosynthesis and stomata to elevated [CO_2] in managed systems. Ecol. Studies 187:253–270.

Makino, A. and T. Mae. 1999. Photosynthesis and plant growth at elevated levels of CO_2. Plant Cell Physiol. 40:999–1006.

Massa, G.D., H. Kim, R.M. Wheeler, and C.A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43:1951–1956.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, and K. Kurata. 2007. Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleracea* L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. Soil Sci. Plant Nutr. 53:459–465.

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

McCree, K.J. 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9:191–216.

Muneer, S., E.J. Kim, J.S. Park, and J.H. Lee. 2014. Influence of green, red and blue light-emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). Int. J. Mol. Sci. 15:4657–4670.

Murchie, E.H. and P. Horton. 1998. Contrasting patterns of photosynthetic acclimation to the light environment are dependent on the differential expression of the responses to altered irradiance and spectral quality. Plant Cell Environ. 21:139–148.

Nemali, K.S. and M.W. van Iersel. 2004. Light effects on wax begonia: Photosynthesis, growth respiration, maintenance respiration, and carbon use efficiency. J. Amer. Soc. Hort. Sci. 129:416–424.

Nemali, K.S. and M.W. van Iersel. 2008. Physiological responses to different substrate water contents: Screening for high water-use efficiency in bedding plants. J. Amer. Soc. Hort. Sci. 133:333–340.

Oh, W., E.S. Runkle, and R.M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience 45:1332–1337.

Ohashi-Kaneko, K., M. Takase, N. Kon, K. Fujiwara, and K. Kurata. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and Komatsuna. Environ. Control Biol. 45:189–198.

Park, Y. and E.S. Runkle. 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Expt. Bot. 136:41–49.

Piel, C., E. Frak, X.L. Roux, and B. Genty. 2002. Effect of local irradiance on CO_2 transfer conductance of mesophyll in walnut. J. Expt. Bot. 53:2423–2430.

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

Pramuk, L.A. and E.S. Runkle. 2005. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40:1336–1339.

Prior, S.A., G.B. Runion, S.C. Marble, H.H. Rogers, C.H. Gilliam, and H.A. Torbert. 2011. A review of elevated atmospheric CO_2 effects on plant growth and water relations: Implications for horticulture. HortScience 46:158–162.

Randall, W.C. and R.G. Lopez. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. HortScience 49:589–595.

Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience 50:705–713.

Ruiz-Espinoza, F.H., B. Murillo-Amador, J.L. García-Hernández, L. Fenech-Larios, E.O. Rueda-Puente, E. Troyo-Diéguez, C. Kaya, and A. Beltrán-Morales. 2010. Field evaluation of the relationship between chlorophyll content in basil leaves and a portable chlorophyll meter (SPAD-502) readings. J. Plant Nutr. 33:423–438.

Runkle, E.S. and R.D. Heins. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Amer. Soc. Hort. Sci. 126:275–282.

Shaahan, M.M., A.A. El-Sayed, and A.A.A. Abou El-Nour. 1999. Predicting nitrogen, magnesium, and iron nutritional status in some perennial crops using a portable chlorophyll meter. Scientia Hort. 82:339–348.

Sharkey, T.D., C.J. Bernacchi, G.D. Farquhar, and E.L. Singsaas. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant, Cell and Environ. 30:1035–1040.

Sukenik, A., J. Bennett, and P. Falkowski. 1987. Light-saturated photosynthesis – limitation by electron transport or carbon fixation? Biochim. Biophys. Acta 891:205–215.

Thomas, J.F., C.D. Raper, Jr., C.E. Anderson, and R.J. Downs. 1975. Growth of young tobacco plants as affected by carbon dioxide and nutrient variables. Agron. J. 67:685–689.

Tremblay, N. and A. Gosselin. 1998. Effect of carbon dioxide enrichment and light. HortTechnology 8:524–528.

van Ieperen, W. 2012. Plant morphological and developmental responses to light quality in a horticultural context. Acta Hort. 956:131–139.

Wang, Y., B.L. Dunn, D.B. Arnall, and P. Mao. 2012. Use of an active canopy sensor and SPAD chlorophyll meter to quantify geranium nitrogen status. HortScience 47:45–50.

Weston, E., K. Thorogood, G. Vinti, and E. López-Juez. 2000. Light quantity control leaf-cell and chloroplast development in *Arabidopsis thaliana* wild type and blue-light-perception mutants. Planta 211:807–815.

Wollaeger, H.M. and E.S. Runkle. 2014. Producing commercial-quality ornamental seedlings under sole-source LED lighting. Acta Hort. 1037:269–276.

Yorio, N.C., G.D. Goins, H.R. Kagie, R.M. Wheeler, and J.C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience 36:380–383.

Zanin, G. and P. Sambo. 2006. Using SPAD-meter in nitrogen fertilization of *Rosa chinensis* Jacq. var. *mutabilis*. HortScience 41:969–970.

Zeiger, E., L.D. Talbott, S. Frechilla, A. Srivastava, and J. Zhu. 2002. The guard cell chloroplast: A perspective for the twenty-first century. New Phytol. 153:415−424.

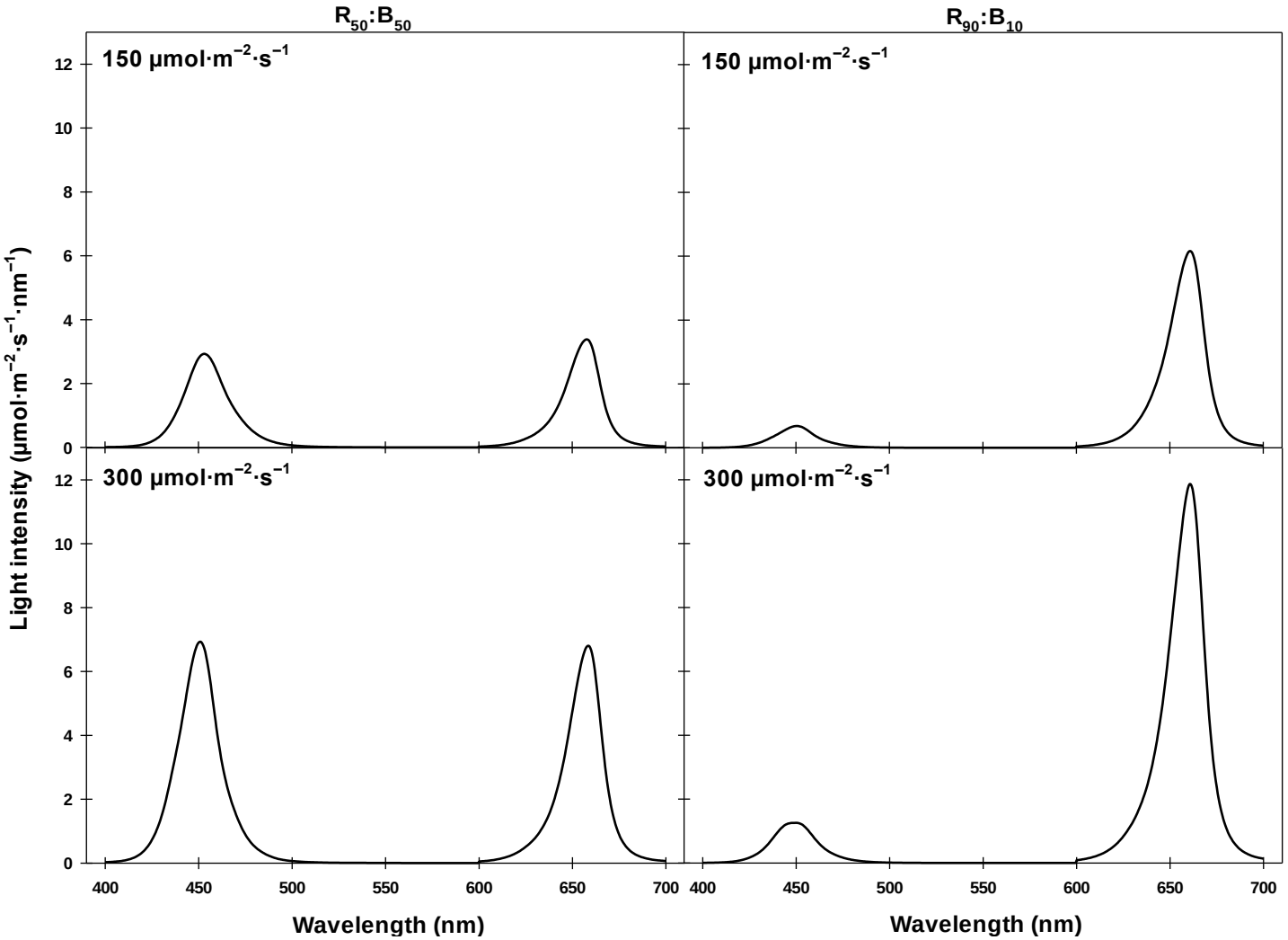


Figure 5. Spectral quality delivered from sole-source light-emitting diode (LED) arrays with red:blue light quality ratios (%) of 50:50 (R_{50}:B_{50}) and 90:10 (R_{90}:B_{10}) at target light intensities of 150 and 300 µmol·m^{-2}·s^{-1}.

Figure 6. Relationship between leaf photosynthetic rate (A) and photosynthetic photon flux density (*PPFD*) for petunia (*Petunia* ×*hybrida* 'Dreams Midnight') seedlings grown in walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality ratios (%) of 50:50 (R_{50}:B_{50}) and 90:10 (R_{90}:B_{10}) at target light intensities of 150 and 300 µmol·m^{-2}·s^{-1}. The measurements were taken 22 d after germination using a portable photosynthesis meter (LI-6400XT; LI-COR Inc.). An exponential rise to maximum equation was fitted for all treatment combinations to describe the response. The fitted equations at the CO_2 concentration of 450 µmol·mol^{-1} for the LED SSL treatments (light intensity_light quality) of 150_ R_{50}:B_{50}, 300_ R_{50}:B_{50}, 150_ R_{90}:B_{10}, and 300_ R_{90}:B_{10} were $A = -2.02 + 22.52 (1 - e^{-0.0024 \times PPFD})$, $R^2 = 0.99$; $A = -1.99 + 21.48 (1 - e^{-0.0024 \times PPFD})$, $R^2 = 0.99$; $A = -1.70 + 18.06 (1 - e^{-0.0039 \times PPFD})$, $R^2 = 0.99$; and $A = -2.09 + 17.89 (1 - e^{-0.0038 \times PPFD})$, $R^2 = 0.99$, respectively. The fitted equations at the CO_2 concentration of 900 µmol·mol^{-1} for the LED SSL treatments of 150_ R_{50}:B_{50}, 300_ R_{50}:B_{50}, 150_ R_{90}:B_{10}, and 300_ R_{90}:B_{10} were $A = -2.07 + 26.74 (1 - e^{-0.0024 \times PPFD})$, $R^2 = 0.99$; $A = -2.60 + 29.78 (1 - e^{-0.0020 \times PPFD})$, $R^2 = 0.99$; $A = -1.96 + 21.59 (1 - e^{-0.0038 \times PPFD})$, $R^2 = 0.99$; and $A = -2.67 + 22.19 (1 - e^{-0.0035 \times PPFD})$, $R^2 = 0.99$, respectively. Fitted curves represent the mean responses of four samples across three experimental replications of the study over time (n = 12).

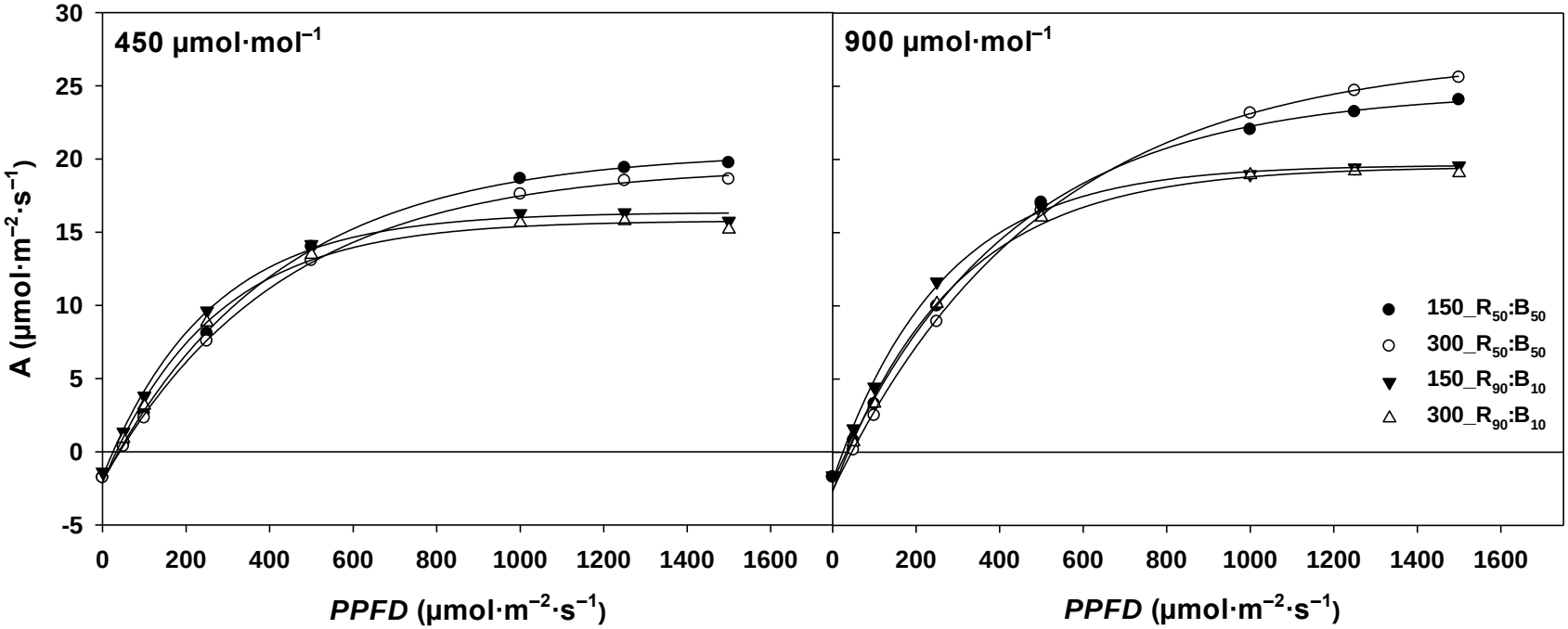

450 µmol·mol⁻¹
900 µmol·mol⁻¹
A (µmol·m⁻²·s⁻¹)
PPFD (µmol·m⁻²·s⁻¹)
150_R50:B50
300_R50:B50
150_R90:B10
300_R90:B10

Figure 7. Relationship between leaf photosynthetic rate (A) and leaf internal carbon dioxide concentration (C_i) for petunia (*Petunia* ×*hybrida* 'Dreams Midnight') seedlings grown in walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality ratios (%) of 50:50 (R_{50}:B_{50}) and 90:10 (R_{90}:B_{10}) at target light intensities of 150 and 300 µmol·m^{-2}·s^{-1}. The measurements were taken 29 d after germination using a portable photosynthesis meter (LI-6400XT; LI-COR Inc.). A rectangular hyperbola was fitted for all treatment combinations to describe the response. The fitted equations at the CO_2 concentration of 450 µmol·mol^{-1} for the LED SSL treatments (light intensity_light quality) of 150_ R_{50}:B_{50}, 300_ R_{50}:B_{50}, 150_ R_{90}:B_{10}, and 300_ R_{90}:B_{10} were $A = -6.87 + [(23.90 \times C_i) / (153.81 + C_i)]$, $R^2 = 0.99$; $A = -13.02 + [(42.37 \times C_i) / (142.58 + C_i)]$, $R^2 = 0.99$; $A = -7.56 + [(24.39 \times C_i) / (145.77 + C_i)]$, $R^2 = 0.99$; and $A = -12.15 + [(37.71 \times C_i) / (134.99 + C_i)]$, $R^2 = 0.99$, respectively. The fitted equations at the CO_2 concentration of 900 µmol·mol^{-1} for the LED SSL treatments of 150_ R_{50}:B_{50}, 300_ R_{50}:B_{50}, 150_ R_{90}:B_{10}, and 300_ R_{90}:B_{10} were $A = -8.39 + [(23.56 \times C_i) / (137.48 + C_i)]$, $R^2 = 0.99$; $A = -9.98 + [(36.15 \times C_i) / (214.77 + C_i)]$, $R^2 = 0.99$; $A = -9.87 + [(23.89 \times C_i) / (139.56 + C_i)]$, $R^2 = 0.99$; and $A = -10.69 + [(31.73 \times C_i) / (212.61 + C_i)]$, $R^2 = 0.99$, respectively. Fitted curves represent the mean responses of four samples across three experimental replications of the study over time (n = 12).

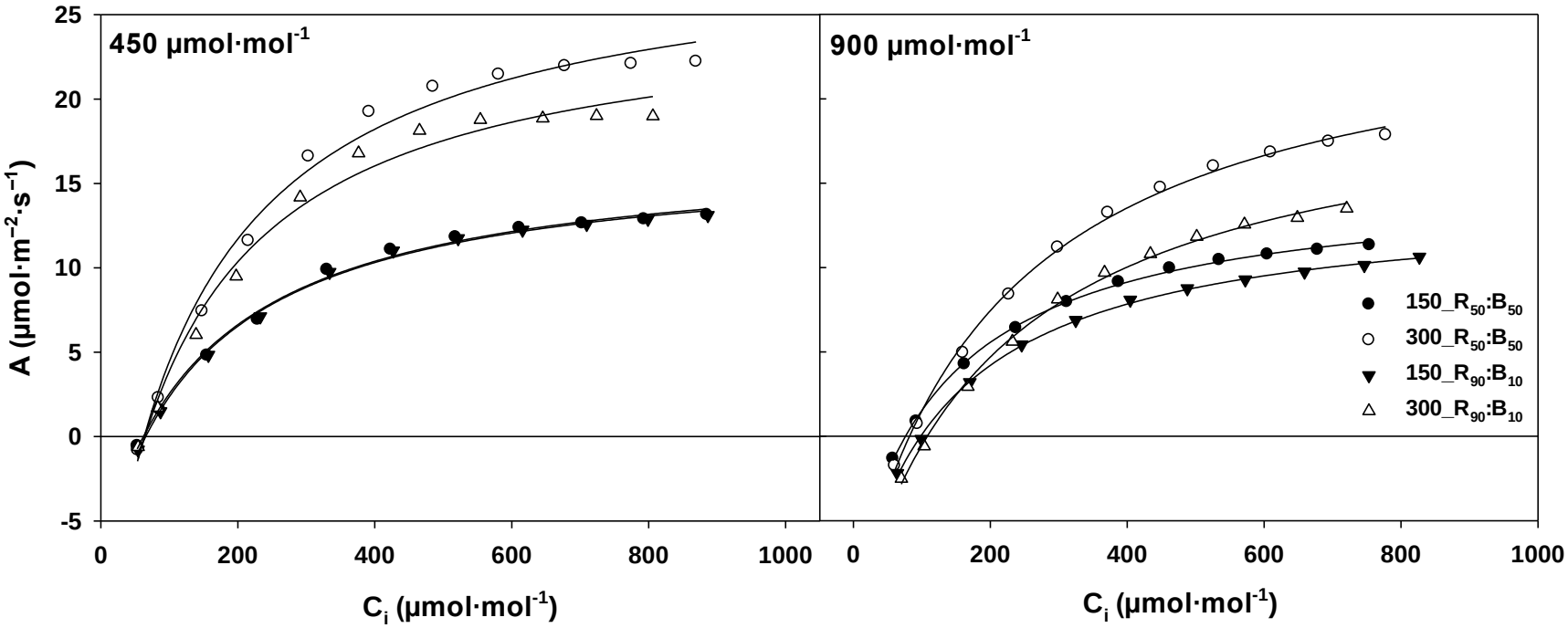

450 µmol·mol-1
900 µmol·mol-1
A (µmol·m-2·s-1)
Ci (µmol·mol-1)
Ci (µmol·mol-1)
25
20
15
10
5
0
-5
0
200
400
600
800
1000
150_R50:B50
300_R50:B50
150_R90:B10
300_R90:B10

Table 12. Average carbon dioxide (CO_2) concentration, air temperature (Temp; day/night), and relative humidity (RH; day/night) ± SD logged every 15 min by a data logger (DL1 Datalogger; Environmental Growth Chambers) for separate walk-in growth chamber environments with CO_2 concentration set points of 450 and 900 µmol·mol^{-1}. Mean values reported were averaged across three experimental replications.

CO_2 Set Point (µmol·mol^{-1})	**CO_2 (µmol·mol^{-1})**	**Day Temp (ºC)**	**Night Temp (ºC)**	**Day RH (%)**	**Night RH (%)**
450	446 ± 19	22.0 ± 0.2	18.0 ± 0.2	55.0 ± 1.1	60.0 ± 1.0
900	926 ± 55	22.2 ± 0.9	18.5 ± 0.5	55.9 ± 2.1	60.9 ± 2.9

Table 13. Average blue (400 to 500 nm), red (600 to 700 nm), and photosynthetic (400 to 700 nm) photon flux density (*PPFD*) ± SD delivered from sole-source light-emitting diodes (LEDs) with red:blue light quality ratios (%) of 50:50 and 90:10 with target light intensities of 150 and 300 µmol·m^{-2}·s^{-1}. Light treatments were represented in two separate growth chambers with carbon dioxide (CO_2) concentrations of 450 and 900 µmol·mol^{-1}. Mean values reported are the average of nine spectral scans across three experimental replications.

CO_2 (µmol·mol^{-1})	**Intensity (µmol·m^{-2}·s^{-1})**	**Quality [red:blue (%)]**	**Blue (400-500 nm)**	**Red (600-700 nm)**	***PPFD* (400-700 nm)**
450	**150**	**50:50**	75.6 ± 13.2	74.5 ± 10.8	150.1 ± 18.9
		90:10	14.9 ± 3.1	135.4 ± 19.8	150.3 ± 22.8
	300	**50:50**	149.8 ± 34.7	150.1 ± 23.0	299.9 ± 49.0
		90:10	30.4 ± 6.0	272.7 ± 39.0	303.1 ± 44.8
900	**150**	**50:50**	75.3 ± 14.5	75.2 ± 10.0	150.5 ± 24.1
		90:10	14.8 ± 3.2	135.9 ± 20.1	150.7 ± 22.6
	300	**50:50**	149.6 ± 30.2	150.9 ± 19.2	300.5 ± 41.3
		90:10	30.8 ± 6.3	270.2 ± 38.3	301.0 ± 44.3

Table 14. Average air temperature (day/night) and leaf temperature (day/night) ± SD logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc.). Data was collected in two separate walk-in growth chamber environments with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality ratios (%) of 50:50 and 90:10 at target light intensities of 150 and 300 µmol·m^{-2}·s^{-1}. Mean values reported were averaged across three experimental replications.

CO_2 (µmol·mol^{-1})	**Intensity (µmol·m^{-2}·s^{-1})**	**Quality [red:blue (%)]**	**Day Air (ºC)**	**Night Air (ºC)**	**Day Leaf (ºC)**	**Night Leaf (ºC)**
450	**150**	**50:50**	22.8 ± 0.8	18.5 ± 0.2	21.0 ± 1.1	18.0 ± 1.1
		90:10	22.8 ± 0.6	18.4 ± 0.2	21.4 ± 1.1	18.0 ± 1.2
	300	**50:50**	23.1 ± 0.6	18.3 ± 0.3	21.6 ± 1.1	18.1 ± 1.2
		90:10	22.9 ± 0.7	18.7 ± 0.2	21.8 ± 1.0	18.5 ± 1.2
900	**150**	**50:50**	22.6 ± 0.7	18.4 ± 0.6	21.6 ± 1.2	18.3 ± 1.3
		90:10	22.6 ± 0.9	18.5 ± 0.6	21.5 ± 1.2	18.0 ± 1.3
	300	**50:50**	22.8 ± 0.6	18.4 ± 0.6	22.1 ± 1.3	18.5 ± 1.4
		90:10	22.7 ± 0.8	18.4 ± 0.6	21.8 ± 1.2	18.1 ± 1.4

Table 15. Morphological data including stem length, stem caliper, leaf area (LA), stem (SDM), leaf (LDM), root (RDM), and total dry mass (TDM), leaf mass area (LMA), quality index (QI), and relative chlorophyll content (RCC) harvested 14, 21, and 28 d after germination for petunia (*Petunia ×hybrida* 'Dreams Midnight') seedlings. Seedlings were grown in 128-cell trays using walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality (LQ) ratios (%) of 50:50 and 90:10 at light intensities (LIs) of 150 and 300 µmol·m^{-2}·s^{-1}.

CO_2 (µmol·mol^{-1})	Intensity (µmol·m^{-2}·s^{-1})	Quality [red:blue (%)]	Length (mm)	Caliper (mm)	LA (cm^2)	SDM (mg)	LDM (mg)	RDM (mg)	TDM (mg)	LMA (mg·cm^{-2})	QI	RCC
						Day 14						
450	**150**	**50:50**	2.6[z]b[y]	0.8c	1.4c	0.44c	2.5c	0.8	3.7c	1.8	2.4	36.2b
		90:10	3.3a	1.0b	2.6b	0.88a	4.9b	1.0	6.8b	2.0	3.2	36.8b
	300	**50:50**	3.2a	1.1a	3.3a	0.74ab	9.0a	1.7	11.4a	2.7	5.9	44.4a
		90:10	3.3a	1.1a	3.7a	0.67b	9.8a	1.8	12.2a	2.6	6.2	42.9a
LI			***[x]	***	***	NS	***	***	***	***	***	***
LQ			***	***	***	***	***	NS	***	NS	NS	NS
LI×LQ			***	*	**	***	**	NS	*	NS	NS	*
900	**150**	**50:50**	2.9c	0.9	2.1	0.38	3.9	0.6	5.0	1.9	2.4	39.1
		90:10	3.3a	1.0	2.8	0.43	5.3	1.1	6.8	1.9	3.5	37.5
	300	**50:50**	3.0bc	1.1	2.9	0.54	9.6	1.4	11.5	3.2	5.9	45.2
		90:10	3.1ab	1.1	3.4	0.66	11.1	1.8	13.5	3.2	7.1	41.5
LI			NS	***	***	***	***	***	***	***	***	***
LQ			***	***	***	*	***	***	***	NS	***	***
LI×LQ			*	NS	NS	NS	NS	NS	NS	NS	NS	NS
						Day 21						
450	**150**	**50:50**	3.6c	1.2c	6.9c	1.2d	13.8	1.8	16.8	2.0	7.4	45.6c
		90:10	4.5a	1.4b	12.7a	2.7c	25.0	4.3	32.0	2.0	15.0	44.7c
	300	**50:50**	4.2b	1.5a	10.5b	3.4b	38.2	7.9	49.5	3.6	27.9	54.5a
		90:10	4.7a	1.6a	13.5a	4.1a	48.3	9.3	61.7	3.6	32.6	51.0b

Table 15 continued

LI			***	***	***	***	***	***	***	***	***	***
LQ			***	***	***	***	***	***	***	NS	***	***
LI×LQ			*	**	***	**	NS	NS	NS	NS	NS	*
900	**150**	**50:50**	4.3	1.2	8.5 c	1.9	19.9	3.4	25.3	2.3	11.4	48.2 c
		90:10	4.8	1.4	12.0 ab	2.9	27.9	5.2	36.0	2.4	16.5	47.6 c
	300	**50:50**	4.6	1.6	11.5 b	4.3	49.1	8.6	61.9	4.3	31.8	57.4 a
		90:10	4.9	1.7	12.8 a	4.7	57.7	9.3	71.7	4.5	36.5	53.6 b
LI			**	***	***	***	***	***	***	***	***	***
LQ			***	***	***	***	***	***	***	NS	***	***
LI×LQ			NS	NS	***	NS	NS	NS	NS	NS	NS	**
							Day 28					
450	**150**	**50:50**	5.0	1.4 c	18.9	4.6	44.7	6.6	55.8	2.4 b	22.6 c	50.6 c
		90:10	5.8	1.6 b	24.7	6.9	63.2	11.6	81.7	2.6 b	36.5 b	51.3 c
	300	**50:50**	5.7	1.8 a	19.8	10.1	95.5	17.0	122.5	4.7 a	59.7 a	61.1 a
		90:10	6.6	1.9 a	24.9	12.0	115.0	20.2	147.2	4.8 a	65.6 a	56.7 b
LI			***	***	NS	***	***	***	***	***	***	***
LQ			***	***	***	***	***	***	***	NS	***	***
LI×LQ			NS	***	NS	NS	NS	NS	NS	*	*	***
900	**150**	**50:50**	5.4	1.6	20.8	6.6	58.8	10.0 c	75.5	2.8	34.1	54.2 c
		90:10	6.3	1.8	25.3	8.4	78.1	14.1 b	100.6	3.2	45.3	54.5 c
	300	**50:50**	6.2	2.0	22.4	12.2	116.8	20.7 a	149.7	5.3	72.9	63.4 a
		90:10	6.9	2.1	25.2	13.9	136.6	22.0 a	172.5	5.5	77.6	60.2 b
LI			***	***	NS	***	***	***	***	***	***	***
LQ			***	***	***	***	***	***	***	NS	***	**
LI×LQ			NS	NS	NS	NS	NS	*	NS	NS	NS	***

[z]Mean values are based on 10 samples from each treatment across three experimental repetitions (n = 30).

[y]Means sharing a letter within a harvest date and CO_2 concentration are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

[x]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 16. Morphological data including stem length, stem caliper, leaf area (LA), stem (SDM), leaf (LDM), root (RDM), and total dry mass (TDM), leaf mass area (LMA), quality index (QI), and relative chlorophyll content (RCC) harvested 14, 21, and 28 d after germination for petunia (*Petunia ×hybrida* 'Dreams Midnight') seedlings. Seedlings were grown in 288-cell trays using walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality (LQ) ratios (%) of 50:50 and 90:10 at light intensities (LIs) of 150 and 300 µmol·m^{-2}·s^{-1}.

CO_2 (µmol·mol^{-1})	Intensity (µmol·m^{-2}·s^{-1})	Quality [red:blue (%)]	Length (mm)	Caliper (mm)	LA (cm^2)	SDM (mg)	LDM (mg)	RDM (mg)	TDM (mg)	LMA (mg·cm^{-2})	QI	RCC
						Day 14						
450	**150**	**50:50**	2.6[z]c[y]	0.8c	1.5c	0.24c	2.9	0.7c	3.9d	2.0	2.2c	39.3
		90:10	3.1ab	1.0b	2.5b	0.41b	4.7	1.1b	6.2c	1.9	3.3b	38.6
	300	**50:50**	2.9b	1.0ab	2.4b	0.64a	7.4	1.9a	9.9b	3.1	5.8a	45.3
		90:10	3.1a	1.1a	2.9a	0.66a	9.0	1.7a	11.3a	3.2	5.8a	43.2
LI			***x	***	***	NS	***	***	***	***	***	***
LQ			***	***	***	***	***	NS	***	NS	NS	**
LI×LQ			***	*	**	***	**	NS	*	NS	NS	NS
900	**150**	**50:50**	2.6b	0.8c	1.8b	0.42c	3.9c	0.9c	5.2c	2.1	2.8c	40.5c
		90:10	2.9a	0.9b	2.3a	0.70a	5.5b	1.3b	7.5b	2.3	4.2b	39.8c
	300	**50:50**	2.8ab	1.0ab	2.2a	0.54bc	8.8a	1.7a	11.1a	3.9	6.1a	46.5a
		90:10	2.9a	1.0a	2.3a	0.57ab	8.8a	1.8a	11.1a	3.8	6.1a	43.4b
LI			NS	***	**	NS	***	***	***	***	***	***
LQ			**	**	***	***	**	***	***	NS	**	***
LI×LQ			NS	**	***	**	**	**	**	NS	**	**
						Day 21						
450	**150**	**50:50**	3.3c	1.1	5.7	1.1	11.6	2.1	14.7	2.0	7.2	47.4
		90:10	4.0a	1.2	8.2	1.5	16.8	3.8	22.1	2.1	11.3	46.0
	300	**50:50**	3.6b	1.2	6.5	1.8	25.4	5.7	32.9	4.0	18.3	54.8
		90:10	4.0a	1.3	8.3	2.3	32.2	7.0	41.5	4.0	22.2	52.0

Table 16 continued

LI			NS	***	NS	***	***	***	***	***	***	***
LQ			***	***	***	***	***	***	***	NS	***	***
LI×LQ			*	NS	NS	NS	NS	NS	NS	NS	NS	NS
900	**150**	**50:50**	3.7	1.1 c	6.3	1.5	16.3	3.5	21.3	2.6 c	10.6 c	49.0 c
		90:10	4.2	1.2 b	7.8	2.2	21.3	4.7	28.2	2.8 c	14.2 b	49.0 c
	300	**50:50**	3.8	1.3 a	6.4	2.7	33.6	6.9	43.2	5.2 a	23.4 a	58.2 a
		90:10	4.1	1.3 a	7.7	2.8	35.8	7.3	45.9	4.7 b	23.7 a	53.6 b
LI			NS	***	NS	***	***	***	***	***	***	***
LQ			***	***	***	*	***	***	***	NS	**	***
LI×LQ			NS	**	NS	NS	NS	NS	NS	NS	*	***
							Day 28					
450	**150**	**50:50**	4.6	1.2	12.8	2.5	30.2	5.1	37.9	2.4 b	16.2	51.2 c
		90:10	5.0	1.4	14.9	3.8	40.2	10.1	54.1	2.7 b	30.1	51.6 c
	300	**50:50**	4.5	1.4	11.1	5.1	56.8	11.1	73.0	5.1 a	36.9	59.1 a
		90:10	4.9	1.5	14.1	6.1	70.6	13.2	89.9	5.0 a	43.9	55.9 b
LI			NS	***	**	***	***	***	***	***	***	***
LQ			***	***	***	***	***	**	***	NS	***	*
LI×LQ			NS	NS	NS	NS	NS	NS	NS	*	NS	**
900	**150**	**50:50**	4.5	1.3	13.1	3.7	37.4	8.2	49.4	2.9	24.8	54.8
		90:10	5.0	1.4	14.5	4.8	43.5	8.6	56.9	3.2	26.4	54.1
	300	**50:50**	4.8	1.6	13.2	6.4	72.5	12.9	91.9	5.6	45.6	60.5
		90:10	5.2	1.6	14.2	6.7	82.7	14.3	103.6	5.9	49.2	58.0
LI			***	***	NS	***	***	***	***	***	***	***
LQ			***	NS	*	**	***	*	***	NS	NS	**
LI×LQ			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Mean values are based on 10 samples from each treatment across three experimental repetitions (n = 30).

[y]Means sharing a letter within a harvest date and CO_2 concentration are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

[x]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 17. Physiological parameters from leaf photosynthesis photosynthetic photon flux density (*PPFD*) response curves [A-*PPFD* analysis (see Figure 6 and "Materials and Methods" for more details)] including maximum gross photosynthetic rate ($A_{g,max}$), maximum net photosynthetic rate ($A_{n,max}$), light compensation point (LCP), light saturation point (LSP), and quantum yield (α) for petunia (*Petunia ×hybrida* 'Dreams Midnight') seedlings grown in walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality (LQ) ratios (%) of 50:50 and 90:10 at target light intensities (LIs) of 150 and 300 µmol·m^{-2}·s^{-1}.

CO_2 (µmol·mol^{-1})	Intensity (µmol·m^{-2}·s^{-1})	Quality [red:blue (%)]	$A_{g,max}$	$A_{n,max}$	LCP	LSP	α
			(µmol·m^{-2}·s^{-1})				(mol·mol^{-1})
450	**150**	**50:50**	22.6[z]	20.6	38.3	1260	0.055
		90:10	18.1	16.4	24.8	766	0.071
	300	**50:50**	21.8	19.8	40.5	1329	0.051
		90:10	17.9	15.8	32.9	792	0.068
LI			NS[y]	NS	*	NS	NS
LQ			***	***	***	***	***
LI×LQ			NS	NS	NS	NS	NS
900	**150**	**50:50**	26.9	24.8	33.9	1233b[x]	0.065
		90:10	21.6	19.6	24.6	777c	0.083
	300	**50:50**	29.9	27.3	45.4	1506a	0.060
		90:10	22.2	19.5	37.6	868c	0.077
LI			NS	NS	***	***	**
LQ			***	***	***	***	***
LI×LQ			NS	NS	NS	*	NS

[z]Mean values are based on four samples from each treatment across three experimental repetitions (n = 12).
[y]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[x]Means sharing a letter within a CO_2 concentration are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

Table 18. Physiological parameters from leaf photosynthesis internal carbon dioxide response curves [A-C_i analysis (see Figure 7 and "Materials and Methods" for more details)] including CO_2 compensation point (Γ), internal CO_2 concentration at the operating point (C_{iOP}), assimilation rate at the operating point (A_{OP}), Rubisco efficiency (φ), stomatal conductance to CO_2 (g_s), mesophyll conductance to CO_2 (g_m), and electron transport rate (ETR) for petunia (*Petunia ×hybrida* 'Dreams Midnight') seedlings grown in walk-in growth chambers with carbon dioxide (CO_2) concentration set points of 450 and 900 µmol·mol^{-1} and sole-source light-emitting diode (LED) treatments with red:blue light quality (LQ) ratios (%) of 90:10 and 50:50 at target light intensities (LIs) of 150 and 300 µmol·m^{-2}·s^{-1}.

CO_2 (µmol·mol^{-1})	Intensity (µmol·m^{-2}·s^{-1})	Quality [red:blue (%)]	Γ (µmol·mol^{-1})	C_{iOP} (µmol·m^{-2}·s^{-1})	A_{OP}	φ	g_s (mmol·m^{-2}·s^{-1})	g_m	ETR (µmol e$^-$·m^{-2}·s^{-1})
450	**150**	**50:50**	62.5[z]	388.2ab[x]	10.3c	152c	171.5b	46.4	47.9c
		90:10	65.4	392.4a	10.2c	141c	182.3b	43.3	47.3c
	300	**50:50**	63.5	373.5b	17.7a	468a	234.5a	140.6	89.1a
		90:10	64.2	353.2c	15.1b	363b	165.7b	129.1	82.0b
LI			NS[y]	***	***	***	*	***	***
LQ			NS	NS	***	**	**	NS	***
LI×LQ			NS	**	***	*	***	NS	***
900	**150**	**50:50**	76.2	683.7ab	11.2bc	98b	56.4b	16.8	48.8c
		90:10	98.3	743.2a	10.2c	62b	79.9ab	15.4	47.3c
	300	**50:50**	82.7	693.1ab	17.6a	207a	95.3a	80.9	86.1a
		90:10	108.6	649.5b	13.2b	97b	61.5ab	68.3	77.8b
LI			*	*	***	***	NS	***	***
LQ			***	NS	***	***	NS	NS	***
LI×LQ			NS	*	**	**	**	NS	**

[z]Mean values are based on four samples from each treatment across three experimental repetitions (n = 12).
[y]NS, *, **, *** Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[x]Means sharing a letter within a CO_2 concentration are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant interaction between LI and LQ.

CHAPTER 5. FINAL CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Introduction

The overarching objective of this research was to evaluate the use of light-emitting diodes (LEDs) for both indoor and greenhouse production of annual bedding plant seedlings (plugs). From an applied standpoint, the conclusions from this research provide information regarding the use and selection of commercially available LED arrays for seedlings production in multiple environments. Fundamentally, this research also contributes significantly to the understanding of plant responses to light and carbon dioxide (CO_2) in controlled environments. With the basic knowledge obtained from these studies, light and CO_2 inputs can be optimized leading to decreased production time, desired photomorphogenic responses, and an efficient use of energy and space.

5.2 Use of Light-emitting diodes (LEDs) as an Alternative Supplemental Lighting Source to High-pressure Sodium (HPS) Lamps (Chapter 2)

The results of Chapter 2 indicate that low-profile LEDs may be used as an equivalent supplemental lighting (SL) source to high-pressure sodium (HPS) lamps for the production of annual bedding plant seedlings in commercial greenhouses. These findings coincide with those of Hernández and Kubota (2014) and Poel and Runkle (2017), who found that SL source had little effect on the morphology, physiology, or quality of seedlings produced in a greenhouse environment. One of the novel aspects with the use of LEDs is the ability to target specific wavelengths of light to elicit desired responses. However, based on the results from this study, wavelength specificity has limited greenhouse application. Specifically, when the relative

contribution of radiation provided by SL to DLI is low, the photomorphogenic responses expected from the inclusion of specific wavelengths are not readily observed.

While applications focused solely on increasing greenhouse DLI have shown limited benefits from wavelength specificity, creative uses for LED SL may see benefits from this technology. For example, Owen and Lopez (2015) found that 5 to 7 d of end-of-production SL from LEDs with red:blue light ratios (%) of 0:100, 50:50, or 100:0 enhanced the development of dark red foliage of lettuce (*Lactuca sativa* 'Cherokee'), 'Magenta', 'Ruby Sky', and 'Vulcan' compared to HPS lamps at a light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, intracanopy lighting, where radiation is supplied within the foliar canopy, has become a possible means of providing SL to high-wire crops due to the relative coolness of LEDs (Gómez and Mitchell, 2016). Thus, future avenues of research regarding greenhouse LED SL should likely focus on value-added benefits for specialty crops and strategies that capitalize on benefits of LEDs other than wavelength specificity.

5.3 Morphological and Developmental Responses of Annual Bedding Plant Seedlings to Light Intensity and Quality Under Sole-source Lighting (Chapter 3)

The results of Chapter 3 demonstrate the capability of producing coreopsis (*Coreopsis grandiflora* 'Sunfire'), pansy (*Viola* ×*wittrockiana* 'Matrix™ Yellow'), and petunia (*Petunia* ×*hybrida* 'Purple Wave') seedlings in a LED sole-source lighting (SSL) environment. The highest quality seedlings for all species were consistently produced under high light intensities, with little to no effect from light quality. Thus, for most production applications, priority should be placed on a high photosynthetic photon flux density (*PPFD*) output from a light source rather than minute changes to spectral composition. The LED arrays used for this study were commercially available fixtures with spectral ratios predetermined by the manufacturer. While

the addition of green and far-red wavelengths from the fixtures selected provided valuable insight, the slight difference in red:blue light ratios among the treatments complicated the evaluation of light quality effects.

While light quality had little effect on seedling morphology, the addition of far-red radiation during propagation reduced the time to flower for pansy 'Matrix^{TM} Yellow'. This response appears to be species-specific, with coreopsis 'Sunfire' and petunia 'Purple Wave' displaying no differences in flowering from the inclusion of far-red wavelengths. However, all three species displayed similar photomorphogenic responses to far-red radiation including increased stem length and leaf area. Similar results were found by Park and Runkle (2017) who concluded that flowering and photomorphogenic responses were likely regulated separately within the plant. Thus, for plants possessing a long-day photoperiodic response where earlier flowering is desired, extensive research is needed to determine cultivar- and species-specific far-red radiation requirements during propagation under SSL conditions.

5.4 Physiological Responses and Acclimation of *Petunia ×hybrida* 'Dreams Midnight' Seedlings to Light and Carbon Dioxide Under Sole-source Lighting (Chapter 4)

The results of Chapter 4 display that petunia 'Dreams Midnight' seedlings possessed the highest photosynthetic capacity under increased percentages of blue light. Specifically, under the red:blue light ratio of 50:50 and a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, petunia seedlings displayed significantly higher maximum net photosynthetic rate ($A_{n,max}$), Rubisco efficiency (φ), photosynthesis at operating C_i concentration (A_{OP}), and electron transport rate (ETR) compared to those under the light ratio of 90:10. However, petunia seedlings produced under the light ratio of 90:10 and a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ possessed the greatest leaf area, which ultimately allowed for increased light interception and greater dry mass accumulation. Thus,

even though photosynthesis (A) per unit of leaf area was greatest under the increased percentage of blue radiation, the restriction of leaf area expansion commonly observed under blue wavelengths likely limited benefits from this increase. Future research is required to determine whether an increase in leaf area under a high percentage of blue radiation would ultimately yield greater dry mass accumulation alongside increases in A. Methods to achieve this increased leaf area under high percentages of blue radiation may involve the inclusion of green or far-red wavelengths. For example, green radiation absorbed by cryptochrome has been found to reverse blue-light inhibition responses in a manner similar to shade avoidance (Zhang and Folta, 2012). Thus, the inclusion of green or far-red wavelengths may lead to increased leaf area expansion regardless of the typical inhibition responses observed under blue radiation.

While petunia seedlings displayed increased dry mass accumulation under the carbon dioxide (CO_2) concentration of 900 $\mu mol \cdot mol^{-1}$, acclimation responses to this enriched atmosphere were also observed, such as decreased φ. However, this decrease in φ did not have any apparent effect on A, as A_{OP} was similar between both CO_2 concentrations. Rather, we conclude that A was ultimately limited by Ribulose-1,5-bisphosphate (RuBP) regeneration. Therefore, we propose that seedling production under elevated CO_2 concentrations should prioritize increased light intensities in an attempt to increase ETR and prevent a RuBP-limited state. Additionally, if elevated CO_2 concentrations are utilized, they must coincide with increased light intensity to take full advantage of the input. While a trend of greater dry mass accumulation under the CO_2-enriched environment was observed, the increases were mostly due to the suppression of photorespiration. Thus, elevated CO_2 concentrations for indoor production would not be recommended at the light intensities tested for this study.

While the fundamental knowledge gained from this research is beneficial in understanding seedling physiological responses to light and CO_2, whether or not production in a SSL environment provides a profitable alternative to greenhouse production has yet to be determined. Thus, future research is needed not only to evaluate whether A and growth can be maximized through the manipulation of light quality and concurrent increases in light intensity and CO_2, but also to determine the timing and necessity of these inputs and whether the decreased production time and increased seedling quality are profitable pursuits.

5.5 Literature Cited

Gómez, C. and C.A. Mitchell. 2016. Physiological and productivity responses of high-wire tomato as affected by supplemental light source and distribution within the canopy. J. Amer. Soc. Hort. Sci. 141:196–208.

Hernández, R. and C. Kubota. 2014. Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. Scientia Hort. 173:92–99.

Owen, W.G. and R.G. Lopez. 2015. End-of-production supplemental lighting with red and blue light-emitting diodes (LEDs) influences red pigmentation of four lettuce varieties. HortScience 50:676–684.

Park, Y. and E.S. Runkle. 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Expt. Bot. 136:41−49.

Poel, B.R. and E.S. Runkle. 2017. Spectral effects of supplemental greenhouse radiation on growth and flowering of annual bedding plants and vegetable transplants. HortScience 52:1221–1228.

Zhang, T. and K. Folta. 2012. Green light signaling and adaptive response. Plant Signal. Behav. 7:1–4.

APPENDIX

NON-DESTRUCTIVE IMAGING TO ESTIMATE LEAF AREA, BIOMASS, AND GROWTH RATE OF ANNUAL BEDDING PLANT SEEDLINGS

Abstract

Monitoring the growth and development of bedding plant seedlings (plugs) is essential to ensure production timing and quality. While methods for the estimation of leaf area (LA), biomass, and growth rate through digital imaging have been described for many phenotyping applications, little information exists for the monitoring of these attributes during greenhouse plug production. We tested the use of non-invasive fluorescence-based imaging methods to estimate LA and track the growth rate of entire plug trays. Our hypothesis was that the fluorescence from seedlings could be used to separate plant material from the surrounding area during image processing, thereby increasing the accuracy of measurements. Seeds of pansy (*Viola* ×*wittrockiana*'Matrix™ Yellow'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), tomato (*Lycopersicum esculentum* 'Early Girl'), and zinnia (*Zinnia elegans* 'Zahara Fire') were sown in 128-cell trays and grown in a greenhouse environment under an average daily light integral (DLI) of 16.1 ± 3.5 $mol \cdot m^{-2} \cdot d^{-1}$ and an average daily temperature of 20.1 ± 0.67 °C. Data collection occurred every two days starting from the third day after germination, with one tray from each species randomly selected for imaging and destructive measurements. Leaf area estimation was made by exposing seedlings to a flash of blue light (470 nm) to create a fluorescent image using a top-view image station. This fluorescent image provided an alpha channel mask used to separate the plant material in a digital red:green:blue (RGB) image from the background area. The image processing was rapid and automatic after collection using

OpenCV software to estimate the pixel area. Destructive data collection of each tray for LA and dry mass immediately followed imaging. Regression analyses indicated a strong linear relationship (r^2 = 0.95 to 0.99) between destructively measured LA and non-invasive pixel area from fluorescence imaging for all four species. Additionally, a strong linear relationship (r^2 = 0.88 to 0.96) was observed between imaged LA and total dry mass for plug trays of all four species. The imaging method also allowed for the calculation of relative leaf growth rate (RLGR) based on LA data. A linear relationship between RLGR and relative growth rate (RGR) was observed for all four species, displaying the potential to track growth rate nondestructively. Therefore, the proposed imaging technique could be utilized by the commercial greenhouse industry to quickly and efficiently estimate production timing and quality of entire plug trays by non-destructively monitoring LA, biomass, and growth rate.

Introduction

The production of bedding plant seedlings (plugs) is a labor-intensive process that has been expedited by advances in automation technologies. One such advancement is the development of automated transplanting equipment, which facilitate the replacement of unmarketable or missing seedlings in a plug tray (Ryu et al., 2001; Tai et al., 1994). The development of this technology has garnered substantial commercial interest, with benefits including reduced labor costs, improved production efficiency, and increased uniformity (Ryu et al., 2001; Tai et al., 1994). An essential component for the success of these transplanters is a vision system, which allows for the determination of empty cells or poor-quality seedlings in a plug tray (Tong et al., 2013). However, with recent advances in imaging technologies, vision

systems for the determination of plug quality may be expanded upon as novel methods of measuring plant growth responses are developed.

In recent years, high-throughput automated imaging has become an increasingly useful tool for the evaluation of individual plant morphological and physiological traits (Rahaman et al., 2015; Sozzani et al., 2014). These novel image-capture and processing techniques allow for the collection of data non-destructively, while traditional destructive measurements tend to be more complex, costly, and time-consuming (Rahaman et al., 2015). One metric commonly used to evaluate plant growth and productivity is leaf area (LA) (Cemek et al., 2011). Leaf area measurements typically require the manual removal of leaves and use of a hand or bench-top scanner (Cemek et al., 2011; Humplik et al., 2015; Misle et al., 2013). However, the accurate estimation of LA through non-destructive means has become viable with the development of imaging methods (Cemek et al., 2011; Misle et al., 2013; Walter et al., 2007). For example, Tong et al. (2013) developed an image-processing procedure by which LA and quality of vegetable seedlings could be evaluated by extracting the contour of leaves from red:green:blue (RGB) images collected using a charge-coupled device (CCD) camera. Seedling quality was determined using a LA threshold, with poor quality seedlings possessing a LA below a specified value (Tong et al., 2013).

In addition to LA, one of the most useful traits for evaluating crop production is biomass (Humplik et al., 2015). However the determination of biomass requires that plants be destructively harvested, limiting the utility and commercial relevance of this data (Golzarian et al., 2011; Humplik et al., 2015). Therefore, methods to determine biomass non-destructively are desired for measuring and monitoring growth. To address this need, plant phenotyping facilities are employing the use of digital cameras with subsequent software analysis to evaluate growth

non-destructively (Humplik et al., 2015; Tackenberg, 2007). For example, the prediction of biomass for multiple species of tree seedlings has been conducted using stereoscopic RGB images to measure LA and height (Montagnoli et al., 2016). Additionally, Lati et al. (2013) was able to estimate the biomass of sunflower (*Helianthus annuus*) and corn (*Zea mays*) by measuring the volume of 3D models developed from digital images taken at two viewpoints.

While promising technologies exist for monitoring growth through digital means, these measurements are not without limitations. Leaf area can be distorted by overlapping leaves and irregular growth, which is most prevalent when using top-down imaging (Humplik et al., 2015; Tessmer et al., 2013). One method to overcome this limitation is called High-throughput Plant Growth Analysis (HPGA), which identifies individual leaf tips and then uses the surrounding short curvature to estimate LA regardless of overlap (Tessmer et al., 2013). Zhang et al. (2012) found that with *Arabidopsis thaliana*, overlapping or irregular growth of leaves was not paramount to measuring leaf function. Specifically, these authors stated that the LA obtained through top-down imaging was directly related to the available area for photosynthesis (Zhang et al., 2012). Thus, appropriate imaging methods may vary based on species and application needs.

A high-quality bedding plant plug is one that has a high root and shoot dry mass, reduced LA, well-developed root system, and thick stem diameter (Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). Growers desire consistent, rapid, and uniform production of plug trays that possess these qualities as they are more easily processed, shipped, and mechanically transplanted (Pramuk and Runkle, 2005). Providing an automated and non-destructive means by which growers could monitor these quality attributes during plug production would facilitate timely adjustments to inputs and scheduling while also ensuring that quality standards are consistently met. While imaging methods for the estimation of LA,

biomass, and growth rate exist for many species, to our knowledge, there is little information regarding the monitoring of these attributes during bedding plant plug production. Therefore, we tested the use of automated and non-invasive imaging using fluorescence to estimate LA and track the growth rate of entire plug trays. Our hypothesis was that the fluorescence from seedlings could be used to separate plant material from the surrounding area during image processing, thereby increasing the accuracy of measurements.

Materials and Methods

Seeds of pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), tomato (*Solanum lycopersicum* 'Early Girl'), and zinnia (*Zinnia elegans* 'Zahara Fire') were sown in 128-cell trays (14-mL individual cell volume), cut into 40-cell sections, and placed in a glass-glazed greenhouse at Purdue University, West Lafayette, IN (lat. 40 °N). Trays were filled with a commercial soilless medium comprised of (by vol.) 65% peat, 20% perlite, and 15% vermiculite (Fafard Super Fine Germinating Mix; Sun Gro Horticulture, Agawam, MA). Growing conditions were managed by exhaust fan and evaporative-pad cooling, radiant hot water heating, and retractable shade curtains via an environmental control system (Maximizer Precision 10; Priva Computers Inc., Vineland Station, Ontario, Canada). Trays were placed under 86% shade cloth (8635-O-FB; Ludvig Svensson, Inc., Charlotte, NC) and manually misted to facilitate germination. Air temperature and solar photosynthetic photon flux density (*PPFD*; 400-700 nm) were measured every 15 s using precision thermistors (ST-100; Apogee Instruments, Inc., Logan, UT) and quantum sensors (SQ-110; Apogee Instruments, Inc.), respectively, and the average was logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc., Logan, UT). The germination environment average daily temperature

(ADT) and daily light integral (DLI) from 18 Oct. to 25 Oct. 2016 were 20.5 ± 1.5 °C and 2.0 ± 0.7 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

The shade cloth was removed upon hypocotyl emergence for zinnia, tomato, petunia, and pansy on 22 Oct., 23 Oct., 24 Oct., and 25 Oct., respectively. Plugs were irrigated with water-soluble fertilizer (Jack's LX 16N–0.94P_2O_5–12.3K_2O Plug Formula for High Alkalinity Water; J.R. Peters, Inc., Allentown, PA) providing (in $mg \cdot L^{-1}$): 100 nitrogen (N), 10 phosphorus (P), 78 potassium (K), 18 calcium (Ca), 9.4 magnesium (Mg), 0.10 boron (B), 0.05 copper (Cu), 0.50 iron (Fe), 0.25 manganese (Mn), 0.05 molybdenum (Mo), and 0.25 zinc (Zn). Supplemental lighting was provided by 1000-W high-pressure sodium (HPS) lamps and all species were placed under a 16-h photoperiod. Canopy air temperature and solar *PPFD* were measured every 15 s using precision thermistors [fan-aspirated solar radiation shields (ST-110; Apogee Instruments, Inc.)] and quantum sensors (SQ-110; Apogee Instruments, Inc.), respectively, and the average was logged every 15 min by a data logger (Model CR1000; Campbell Scientific). The propagation environment ADT and DLI from 22 Oct. to 20 Nov. 2016 were 20.1 ± 0.67 °C and 16.1 ± 3.5 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

Data collection occurred every two days, beginning three days post germination, and continued until canopy closure. Based on these criteria, the duration of data collection for pansy, petunia, tomato, and zinnia was 27, 25, 15, and 19 d, respectively. For each data collection day, one tray from each species was randomly selected for both imaging and destructive measurements. A top-view image station (Fig. 8; Aris B.V., The Netherlands) was used to estimate LA non-invasively (imaged LA). This was achieved by first collecting a RGB color image of the tray using a digital camera. The tray was then exposed to a flash of blue light (470 nm) to create a fluorescent image. This fluorescent image provided an alpha channel mask,

which was used to separate the plant material in the original RGB image from the background area (Fig. 9). Pixel area of the resulting image was estimated using OpenCV software, with final values converted to cm^2. Immediately following the collection of imaged LA, trays underwent destructive data collection. For each tray, LA was measured using a LA meter (LI-3100; LI-COR Inc., Lincoln, NE) by removing the seedling leaves at the axil. Roots, stems, and leaves from each tray were separated and dried in an oven at 70 °C for at least 4 d and the dry mass of each was recorded. The experiment was conducted using a completely randomized design, with each species evaluated independently using linear regression analyses with SAS (SAS version 9.4; SAS Institute, Cary, NC) regression procedure (PROC REG).

Classical growth analyses were performed using a combination of both imaged and destructive growth data (Hunt and Cornelissen, 1997; Poorter, 1989; Thorne, 1960). Relative growth rate (RGR) is defined as the increase in total dry mass (TDM) per unit of TDM present per unit of time (t):

$$RGR = \frac{lnTDM_2 - lnTDM_1}{t_2 - t_1}$$

Relative growth rate can also be broken down into the components of net assimilation rate (NAR) and leaf area ratio (LAR) as follows:

$$RGR = NAR \times LAR$$

Net assimilation rate is defined as the increase in plant mass per unit of LA per unit of time. Thus, NAR provides an estimate for the photosynthetic efficiency of a leaf and was calculated as:

$$NAR = \frac{TDM_2 - TDM_1}{LA_2 - LA_1} \times \frac{lnLA_2 - lnLA_1}{t_2 - t_1}$$

Leaf area ratio is the product of specific leaf area (SLA) and leaf mass ratio (LMR) and provides a measure of LA per unit of TDM:

$$LAR = SLA \times LMR$$

Specific leaf area is defined as the LA per unit of leaf dry mass (LDM). This metric provides a means of estimating leaf thickness:

$$SLA = \frac{LA}{LDM}$$

Leaf mass ratio (LMR) provides the fraction of dry matter partitioned to the leaves:

$$LMR = \frac{LDM}{TDM}$$

Relative leaf growth rate (RLGR) is defined as the increase in LA per unit of LA present per unit of time, with the following equation used to calculate RLGR based on imaged LA:

$$RLGR = \frac{lnLA_2 - lnLA_1}{t_2 - t_1}$$

Results and Discussion

Relative growth rate is a valuable tool in evaluating growth potential, as it is independent of plant size (Hunt and Cornelissen, 1997). Therefore, all four species were included in the same regression analysis for RGR in this study. The analysis indicated a linear decrease in RGR over time (r^2 = 0.44; Fig. 10A). A decrease in RGR is commonly observed as plants age (van Iersel, 1997). Seedlings typically undergo an exponential growth phase shortly after germination, followed by a steady decline in RGR as the plants near maturity (Hunt and Cornelissen, 1997). This decrease over time can be further explained by evaluating the physiological (NAR) and morphological (LAR) components that factor into the calculation of RGR (Hunt and Cornelissen, 1997).

Net assimilation rate decreased linearly in all four species over time (r^2 = 0.23; Fig. 10B). As discussed previously, NAR provides a means of estimating the photosynthetic efficiency of a leaf. This efficiency often decreases with plant age due to increased intracanopy shading (Monteith, 1977; Thorne, 1960). As LA increases over time, the plant's ability to efficiently intercept and utilize radiation is diminished due to leaf overlap. Plug production would likely accentuate this decrease in photosynthetic efficiency due to the high plant density in 128-cell plug trays and high amount of intercanopy shading that is commonplace.

Leaf area ratio can be further divided into the components SLA and LMR, with SLA generally being more influential (Hunt and Cornelissen, 1997; Poorter and Lambers, 1991; Poorter and Remkes, 1990). Similar to NAR, SLA decreased linearly in all four species over time (r^2 = 0.38; Fig. 10C). Hunt and Cornelissen (1997) found that in herbaceous species, a high RGR was dependent on a high allocation of assimilates to produce thin leaves with limited performance. In our study, we found that SLA decreased over time, indicating that seedlings were allocating more resources toward producing thicker leaves with a higher performance. Thus, a positive linear relationship exists between SLA and RGR (r^2 = 0.56), whereby the formation of thicker leaves in all four species led to decreased growth rates (Fig. 11). Increased leaf thickness is indicative of optimal irradiance. For example, Allard et al. (1991) found that shaded tall fescue (*Festuca arundinacea*) allocated more dry mass to LA than plants grown under a high irradiance. Similarly, Evans and Poorter (2001) found that acclimation to low irradiance resulted in a doubling of SLA in ten dicotyledonous species. Leaf mass ratio showed little change over time (Fig. 10D), providing further evidence that SLA is the primary parameter dictating changes in LAR and RGR in our study. Additionally, the present data support previous findings that RGR has a greater dependence on LAR than NAR (Hunt and Cornelissen, 1997;

Poorter and Remkes, 1990). Therefore, tracking changes in LA through imaging provides a promising means of monitoring RGR for entire plug trays.

Regression analyses indicated a strong linear relationship (r^2 = 0.95 to 0.99) between imaged LA and destructive LA for all four species (Fig. 12A). Based on these results, imaging provided an accurate and non-destructive estimation of LA. Similarly, Cemek et al. (2011) found that LA of green pepper (*Capsicum annuum* var. cayenne) could be predicted from leaf length and width measurements using linear models. Lati et al. (2013) also found that LA of corn and sunflower could be accurately estimated, with ~4-5% error, using 3D stereovision modeling. However, while accurate estimations of seedling LA were possible in the present study, the imaging method appears to be most accurate early during production due to leaf overlap. Leaf area underestimation (>10%) began to occur at 19, 15, 9, and 13 d after germination for pansy, petunia, tomato, and zinnia, respectively (data not shown). As stated previously, imaging methods to alleviate this underestimation are possible using HPGA systems (Tessmer et al., 2013). However, the underestimation of LA due to overlap had little effect on the accuracy of estimation for plug biomass accumulation. Regression analysis indicated a linear relationship between imaged LA and TDM for pansy (r^2 = 0.95), petunia (r^2 = 0.88), tomato (r^2 = 0.93), and zinnia (r^2 = 0.96) plug trays (Fig. 12B). While underestimation did occur later in production, imaging of plugs was sufficient for measuring functional LA, which directly affects biomass accumulation and growth. Thus, estimated LA provides a viable means of predicting biomass accumulation nondestructively. Walter et al. (2007) found similar results in that LA estimations obtained through the phenotyping procedure GROWSCREEN were indicative of fresh and dry weight gains for tobacco (*Nicotiana tabacum* ‘Samsun’) seedlings. While a strong correlation was present between LA and fresh weight, these authors found that greater dry mass per leaf

occurred under high light intensities leading to a weaker correlation between the two variables (Walter et al., 2007). While a strong linear relationship between LA and TDM was present in the current study, changes to the production environment may alter these findings. Therefore, research applications investigating environmental extremes may find estimations using these relationships to be less accurate.

As discussed previously, using RGR to evaluate the growth of plug trays allows multiple species to be compared and analyzed through a single correlation. Due to the existing linear relationship between SLA and RGR (Fig. 11), we propose that RLGR might provide an accurate means of estimating RGR. Using LA estimates obtained through imaging, RLGR was calculated nondestructively. Regression analysis indicated a positive linear relationship between RLGR and RGR (r^2 = 0.70; Fig. 13). While the estimation of biomass is a valuable tool for research and phenotyping applications, the calculation of RLGR and RGR provides growers with useful information regarding changes in the growth of plug trays. Through the utilization of these methods, growers can nondestructively track growth over time and adjust inputs and scheduling based on this feedback.

Conclusion

Monitoring the growth of bedding plant plugs is essential to ensure uniform, rapid, and consistent timing and quality. However, visual growth assessments are often erroneous, while manual measurements are time consuming and impractical for use in the industry. In this study, we have proposed a fluorescence-based imaging method that allows for the accurate and immediate evaluation of growth for entire plug trays. The basis for this growth analysis is dependent upon the accurate estimation of LA through imaging software. A strong correlation

between LA and the accumulation of biomass exists for many plant species. Generally, as LA increases the amount of light intercepted by the plant also increases. By capitalizing on this existing correlation, we found that RGR of plug trays could be estimated based on RLGR calculations made through imaging. These measurements have both industry and research application by facilitating the evaluation of growth for high-density plug trays while also enabling the use of experimental designs with repeated measures.

Acknowledgements

I gratefully acknowledge Dr. Roberto Lopez, Dr. Krishna Nemali, Jillian Malecki, and Zachary Oravec for intellectual, greenhouse, and laboratory assistance; Ball Horticultural Co. for seed; Sun Gro Horticulture for growing media, and Everris NA Inc. for fertilizer. The use of trade names in this publication does not imply endorsement by Purdue University of products named nor criticism of similar ones not mentioned.

Literature Cited

Allard, G., C.J. Nelson, and S.G. Pallardy. 1991. Shade effects on growth of tall fescue: I. leaf anatomy and dry matter partitioning. Crop Sci. 31:163−167.

Cemek, B., A. Unlukara, and A. Kurunc. 2011. Nondestructive leaf-area estimation and validation for green pepper (*Capsicum annuum* L.) grown under different stress conditions. Photosynthetica 49:98−106.

Evans, J.R. and H. Poorter. 2001. Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant, Cell and Environ. 24:755−767.

Golzarian, M.R., R.A. Frick, K. Rajendran, B. Berger, S. Roy, M. Tester, and D.S. Lun. 2011. Accurate inference of shoot biomass from high-throughput images of cereal plants. Plant Methods 7:2. doi:10.1186/1746-4811-7-2.

Humplík, J.F., D. Lazár, A. Husičková, and L. Spíchal. 2015. Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. Plant Methods 11:29. doi:10.1186/s13007-015-0072-8.

Hunt, R. and J.H.C. Cornelissen. 1997. Components of relative growth rate and interrelations in 59 temperate plant species. New Phytol. 135:395−417.

Lati, R.N., S. Filin, and H. Eizenberg. 2013. Estimating plant growth parameters using an energy minimization-based stereovision model. Computers and Electronics in Agr. 98:260−271.

Misle, E., B. Kahlaoui, M. Hachicha, and P. Alvarado. 2013. Leaf area estimation in muskmelon by allometry. Photosynthetica 51:613−620.

Montagnoli, A., M. Terzaghi, N. Fulgaro, B. Stoew, J. Wipenmyr, D. Ilver, C. Rusu, G.S. Scippa, and D. Chiatante. 2016. Non-destructive phenotypic analysis of early stage tree seedling growth using an automated stereovision imaging method. Front. in Plant Sci. 7:1644. doi:10.3389/fpls.2016.01644.

Monteith, J.L. 1977. Climate and the efficiency of crop production in Britain. Philosophical Transaction Royal Soc. London B. 281: 277−294.

Oh, W., E.S. Runkle, and R.M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience 45:1332−1337.

Poorter, H. 1989. Interspecific variation in relative growth rate: On ecological causes and physiological consequences, p. 45−68. In: H. Labers, M.L. Cambridge, H. Konings, T.L. Pons (eds.). Causes and consequences of variation in growth rate and productivity in plants. SPB Academic Publishing, The Hague, The Netherlands.

Poorter, H. and C. Remkes. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83:553−559.

Poorter, H. and H. Lambers. 1991. Is interspecific variation in relative growth rate positively correlated with biomass allocation to the leaves? Amer. Naturalist 138:1264−1268.

Pramuk, L.A. and E.S. Runkle. 2005. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40:1336−1339.

Rahaman, M., D. Chen, Z. Gillani, C. Klukas, and M. Chen. 2015. Advanced phenotyping and phenotype data analysis for the study of plant growth and development. Front. Plant Sci. 6:619. doi:10.3389/fpls.2015.00619.

Randall, W.C. and R.G. Lopez. 2014. Comparison of supplemental lighting from high-pressure sodium lamps and light-emitting diodes during bedding plant seedling production. HortScience 49:589−595.

Ryu, K.H., G. Kim, and J.S. Han. 2001. Development of robotic transplanter for bedding plants. J. Agr. Eng. Res. 78:141−146.

Sozzani, R., W. Busch, E. Spalding, and P.N. Benfey. 2014. Advanced imaging techniques for the study of plant growth and development. Trends Plant Sci. 19:304−310.

Tackenberg, O. 2007. A new method for non-destructive measurement of biomass, growth rates, vertical biomass distribution and dry matter content based on digital image analysis. Ann. Bot. 99:777−783.

Tai, Y.W., P.P. Ling, and K.C. Ting. 1994. Machine vision assisted robotic seedling transplanting. Amer. Soc. Agr. Eng. 37:661−667.

Tessmer, O.L, Y. Jiao, J.A. Cruz, D.M. Kramer, and J. Chen. 2013. Functional approach to high-throughput plant growth analysis. BMC Systems Biol. 7:S17.

Thorne, G.N. 1960. Variations with age in net assimilation rate and other growth attributes of sugar-beet, potato, and barley in a controlled environment. Ann. Bot. 24:356−371.

Tong, J.H., J.B. Li, and H.Y. Jiang. 2013. Machine vision techniques for the evaluation of seedling quality based on leaf area. Biosystems Eng. 115:369−379.

van Iersel, M. 1997. Root restriction effects on growth and development of salvia (*Salvia splendens*). HortScience 32:1186−1190.

Walter, A., H. Scharr, F. Gilmer, R. Zierer, K.A. Nagel, M. Ernst, A. Wiese, O. Virnich, M.M. Christ, B. Uhlig, S. Jünger, and U. Schurr. 2007. Dynamics of seedling growth acclimation towards altered light conditions can be quantified via GROWSCREEN: A setup and procedure designed for rapid optical phenotyping of different plant species. New Phytol. 174:447−455.

Zhang, X., R.J. Hause, Jr., and J.O. Borevitz. 2012. Natural genetic variation for growth and development revealed by high-throughput phenotyping in *Arabidopsis thaliana*. G3: Genes|Genomes|Genetics 2:29−34.

Figure 8. Top-view image station (Aris B.V., The Netherlands) where leaf area of plug trays was estimated non-destructively.

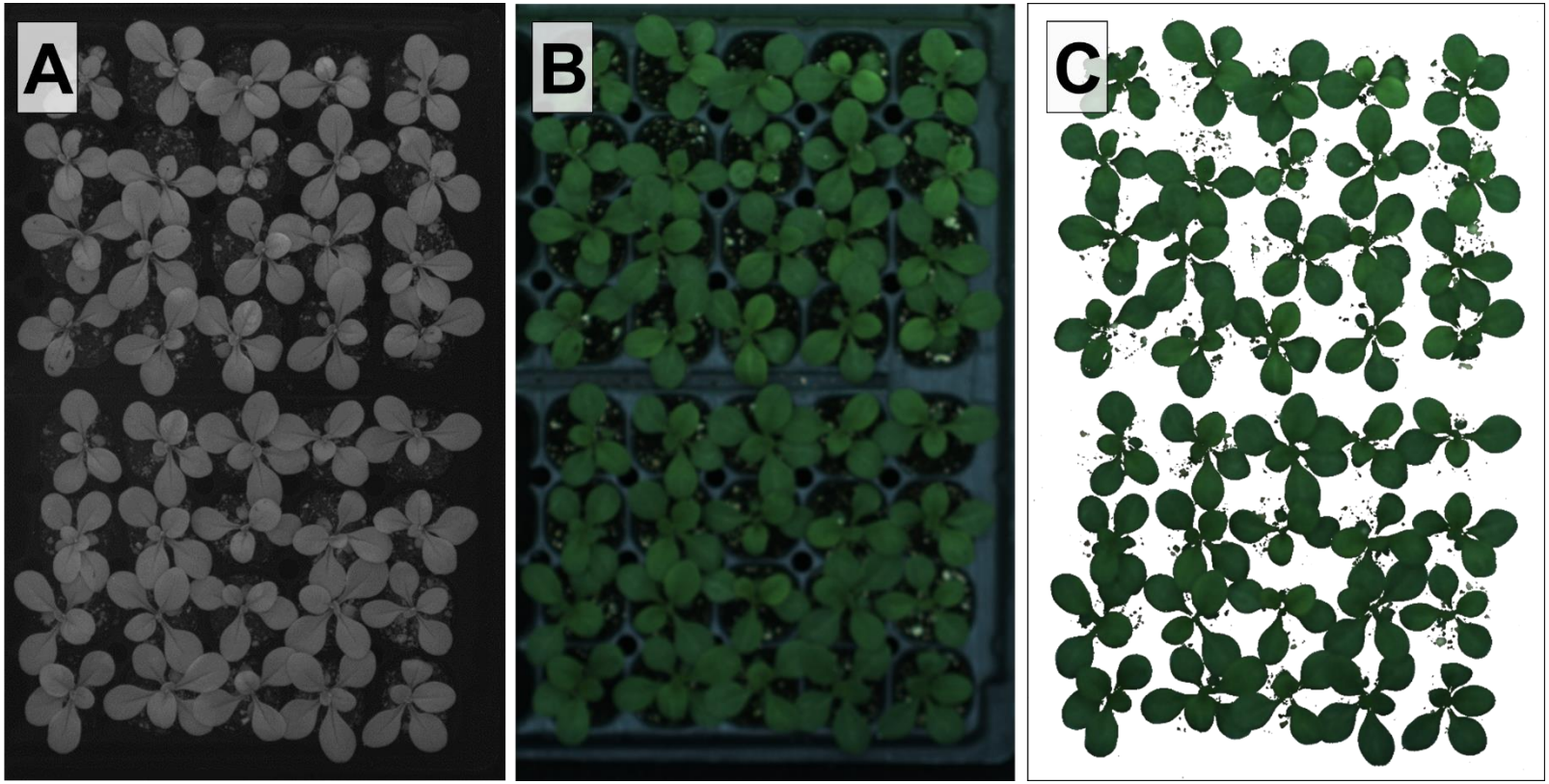
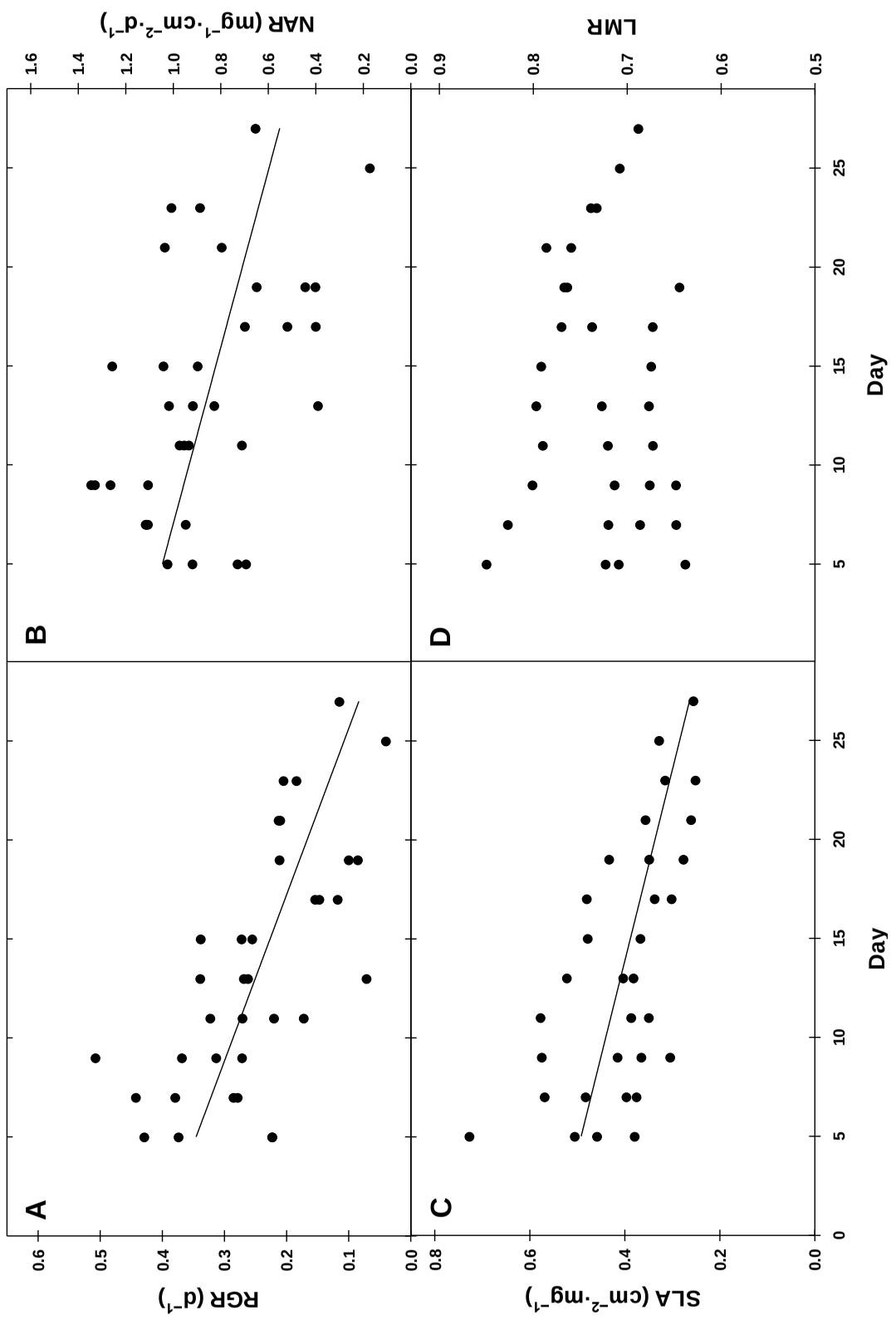


Figure 9. Non-invasive top-view imaging of a petunia (*Petunia ×hybrida* 'Dreams Midnight') plug tray 15 d after germination. Trays were exposed to a flash of blue light (470 nm) to create a fluorescent image used as an alpha channel to create a mask (A). The mask was overlaid on a red:green:blue (RGB) image (B) to separate plant material from the surrounding area during image processing (C).

Figure 10. Relative growth rate (RGR; A), net assimilation rate (NAR; B), specific leaf area (SLA; C), and leaf mass ratio (LMR; D) as a function of day for pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), tomato (*Solanum lycopersicum* 'Early Girl'), and zinnia (*Zinnia elegans* 'Zahara Fire') plugs. RGR = 0.41 + -0.01 × Day (r^2 = 0.44), NAR = 1.16 + -0.02 × Day (r^2 = 0.23), and SLA = 0.54 + -0.01 × Day (r^2 = 0.38).


A
B
C
D
RGR (d⁻¹)
NAR (mg⁻¹.cm⁻².d⁻¹)
SLA (cm⁻².mg⁻¹)
LMR
Day
Day

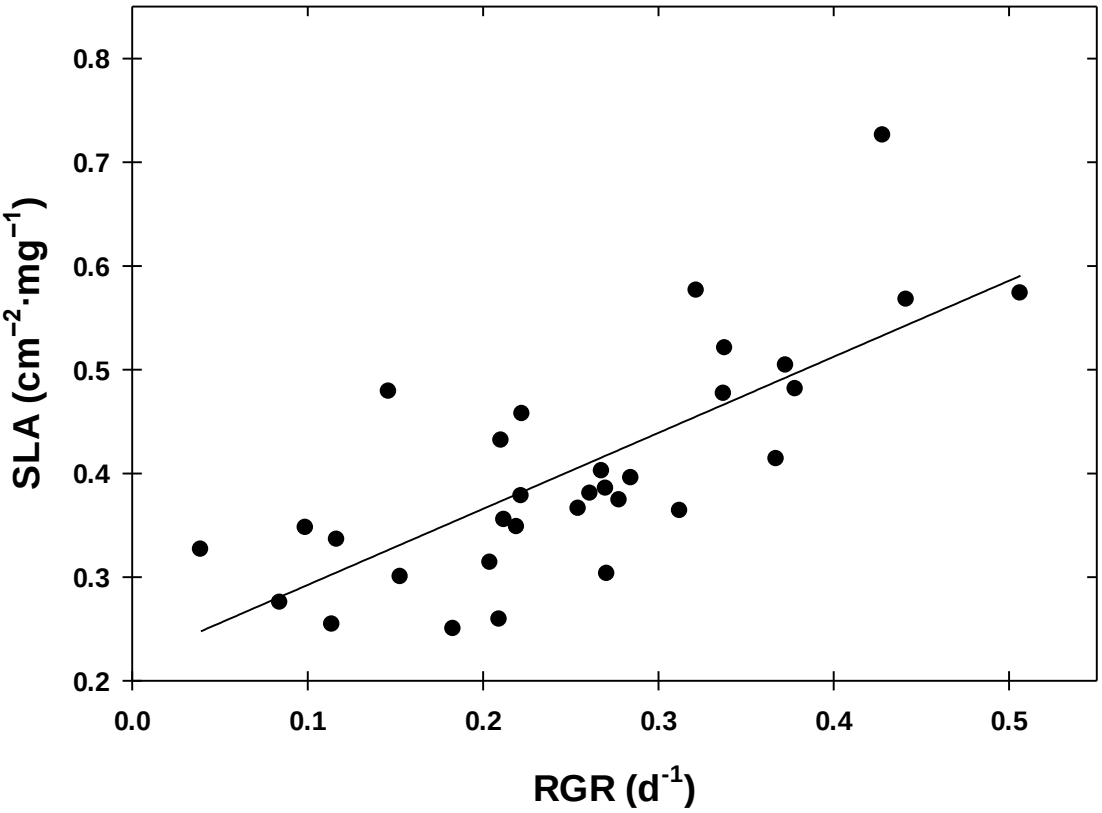


Figure 11. Specific leaf area (SLA) as a function of relative growth rate (RGR) for pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), tomato (*Solanum lycopersicum* 'Early Girl'), and zinnia (*Zinnia elegans* 'Zahara Fire') plugs. SLA = 0.21 + 0.75 × RGR (r^2 = 0.56).

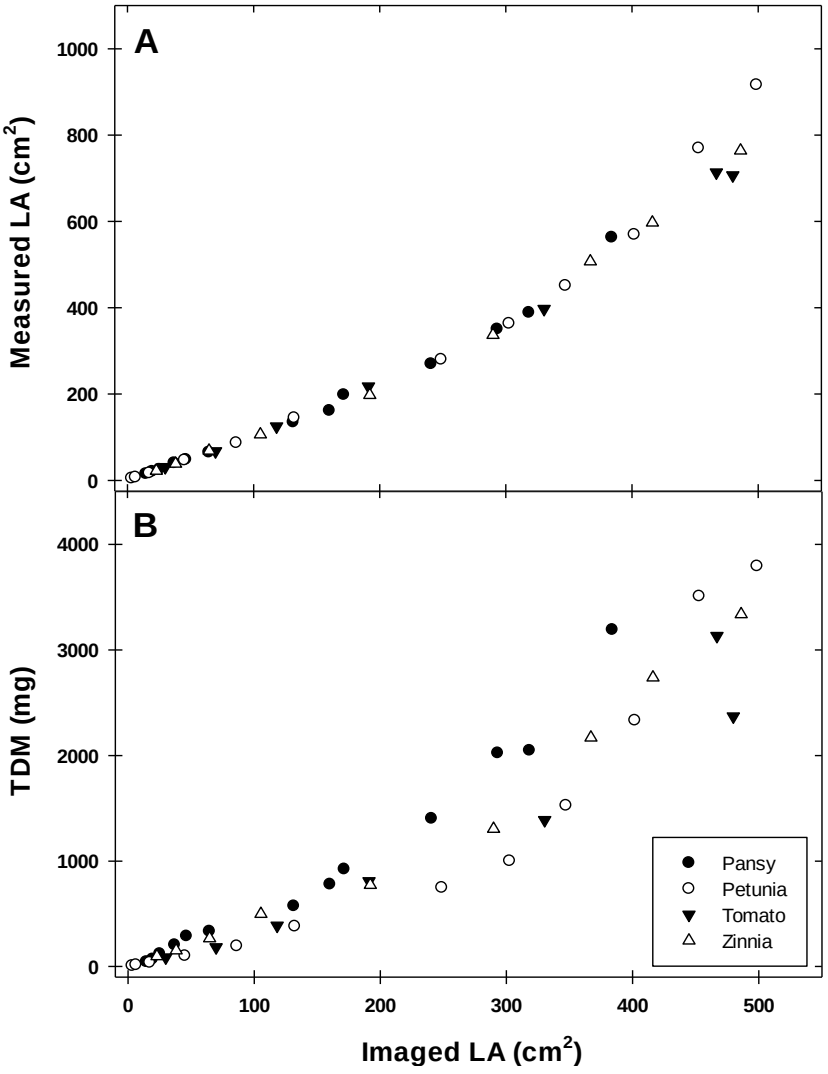


Figure 12. Linear relationship between destructively measured leaf area (measured LA) and non-invasive pixel area from top-view imaging (imaged LA) for pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow') (r^2 = 0.97), petunia (*Petunia* ×*hybrida* 'Dreams Midnight') (r^2 = 0.95), tomato (*Solanum lycopersicum* 'Early Girl') (r^2 = 0.98), and zinnia (*Zinnia elegans* 'Zahara Fire') (r^2 = 0.98) plug trays (A). Linear relationship between imaged LA and total (root + leaf + stem) dry mass (TDM) for pansy (r^2 = 0.95), petunia (r^2 = 0.88), tomato (r^2 = 0.93), and zinnia (r^2 = 0.96) plug trays (B). Measurements were conducted every two days starting from the third day after germination until canopy closure.

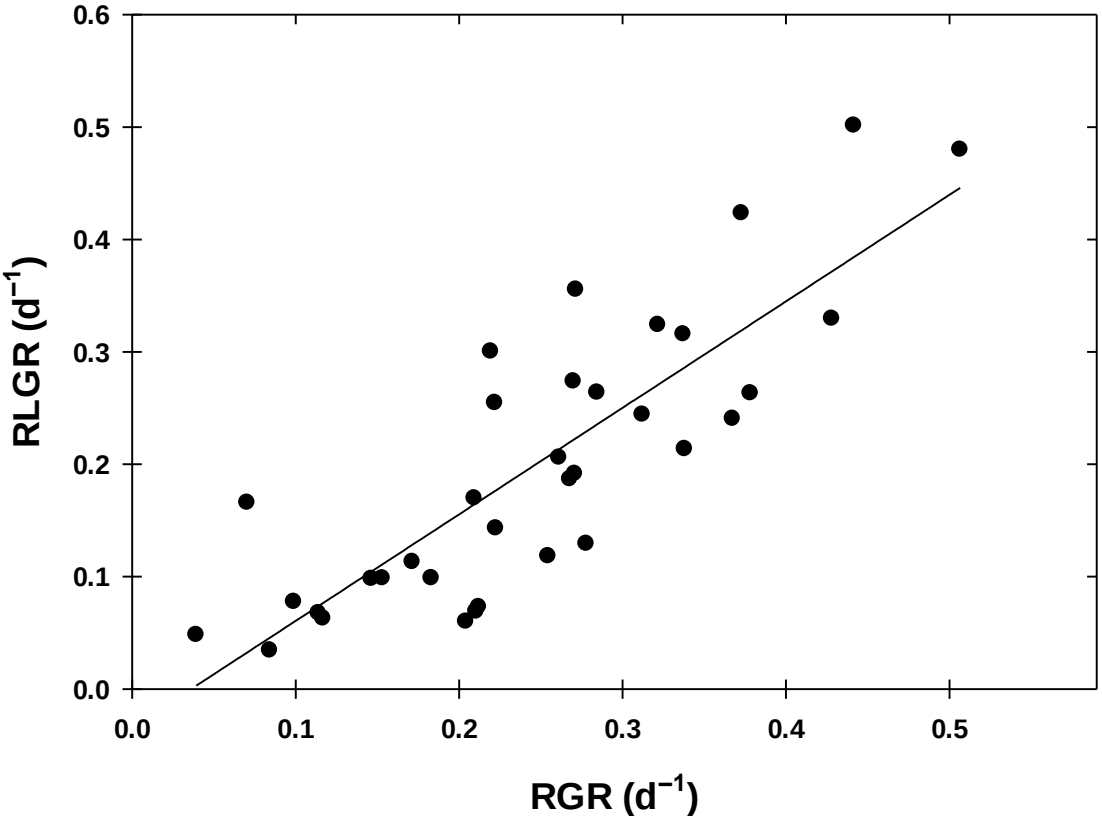


Figure 13. Relative leaf area growth rate (RLGR) as a function of relative growth rate (RGR) for pansy (*Viola* ×*wittrockiana* 'Matrix^{TM} Yellow'), petunia (*Petunia* ×*hybrida* 'Dreams Midnight'), tomato (*Solanum lycopersicum* 'Early Girl'), and zinnia (*Zinnia elegans* 'Zahara Fire') plugs. Relative leaf growth rate was calculated based on leaf area values obtained through top-view imaging. RLGR = -0.03 + 0.95 × RGR (r^2 = 0.70).

VITA

Joshua Craver was born on July 8th, 1989 to Ken and Pamela Craver. He was raised in Tyler, Texas along with two sisters, Robin and Emily, and his brother, Jacob. He graduated high school in 2008 and moved to Starkville, Mississippi the following fall to attend Mississippi State University. While he originally began his undergraduate education in landscape architecture, he quickly became fascinated with the plant sciences and switched his major to horticulture at the end of his first semester. He received his Bachelor of Science degree in Horticulture with a concentration in Floriculture and Ornamentals in 2012 under the advisement of Dr. Richard Harkess.

Upon graduation, Joshua began his graduate education at Kansas State University under the advisement of Dr. Kimberly Williams and Dr. Chad Miller. During this time, he became increasingly interested in teaching and served as a teaching assistant for the majority of his degree. Additionally, he was married to his wife, Shelby Steelhammer Craver, on June 22nd, 2013 who then joined him in Manhattan, Kansas for the remainder of his program. He received his Master of Science in Horticulture in 2014, and immediately after graduating moved to West Lafayette, Indiana to join Dr. Roberto Lopez in the Department of Horticulture and Landscape Architecture at Purdue University to pursue a Doctor of Philosophy in Horticulture. Upon graduation, Joshua plans to assume a teaching role focused on undergraduate education.

CURRICULUM VITAE – JOSHUA K. CRAVER

PROFESSIONAL HISTORY AND EXPERIENCE

Graduate Research and Teaching Assistant. *2014 - present*. Purdue University.
Teaching Assistant: HORT 301 *Plant Physiology*
HORT 420 *Ornamental Plant Production*
Primary Instructor: HORT 491 *Special Assignments – Greenhouse Crop Production*

Graduate Research and Teaching Assistant. *2012 - 2014*. Kansas State University.
Teaching Assistant: HORT 570 *Greenhouse Operations Management*
HORT 600 *Herbaceous Ornamental Landscape Plant Production*
HORT 625 *Floral Crops Production and Handling*

Intern at Metrolina Greenhouses. *2011.* Huntersville, NC.

EDUCATION

Ph.D. *2014 - present.* Purdue University
Major: Horticulture. GPA: 4.0
Advisors: Dr. Roberto Lopez and Dr. Cary Mitchell
Dissertation Research Title: "Manipulating light quality, light intensity, and carbon dioxide concentration to optimize indoor and greenhouse production of annual bedding plant seedlings."

M.S. *2014.* Kansas State University
Major: Horticulture. GPA: 4.0
Advisors: Dr. Kimberly Williams and Dr. Chad Miller
Thesis Research Title: "The effects of UVB radiation on intumescence development and the characterization of lesions from physiological disorders on ornamental sweet potato (*Ipomoea batatas*), tomato (*Solanum lycopersicum*), and interspecific geranium (*Pelargonium* spp.)."

B.S. *2012*. Mississippi State University
Major: Horticulture (Floriculture and Ornamentals). GPA: 3.99
Undergraduate Research Title: "Determining rhizome maturity in reblooming iris."

AWARDS AND HONORS

North Central Extension and Research Activity-101 Travel Scholarship. *2018*
Second Place American Society for Horticultural Science Controlled Environments Working Group Student Oral Competition. *2017*
American Society for Horticultural Science Travel Scholarship. *2017*

North Central Extension and Research Activity-101 Travel Scholarship. *2016*
Allen Hammer Scholarship, Indiana Flower Growers Association. *2015*
First Place American Society for Horticultural Science Controlled Environments Working Group Student Oral Competition. *2015*
First Place American Society for Horticultural Science Graduate Student Poster Competition. *2015*
American Society for Horticultural Science Travel Scholarship. *2015*
American Society for Horticultural Science Outstanding Education Publication Award. *2014*
Fredrick N. Andrews Fellowship, Purdue University. *2014, 2015*
Third Place American Society for Horticultural Science Controlled Environments Working Group Student Oral Competition. *2014*
Richard Elmore Brown Outstanding College of Agriculture Graduate Teaching Award. *2014*
American Society for Horticultural Science Travel Scholarship. *2014*
First Place Agricultural Sciences Oral Presentation, Kansas Research Forum. *2014*
Paul Ecke Jr. Floriculture Scholarship, American Floral Endowment. *2014*
OFA Scholars Program. *2013*
Donoghue Scholar, Kansas State University. *2012, 2013*
College of Agriculture and Life Sciences and MAFES Undergraduate Student Research Award, Mississippi State University. *2012*
First Place J. B. Edmond Undergraduate Student Paper Award, Southern Region of the American Society for Horticultural Science. *2012*
Outstanding Undergraduate Student in Horticulture, American Society for Horticultural Science. *2012*
ASHS Collegiate Scholars Award. *2010, 2012*
Joseph B. Edmonds Scholarship, Mississippi State University. *2010-2012*
Overcash Scholarship, Mississippi State University. *2010-2011*
Second Place Overall Judging Competition, Annual Conference of the American Society for Horticultural Science. *2010*
Mississippi State University President's Scholar. *2008-2012*

SERVICE/INVOLVEMENT

American Floral Endowment Young Professionals Council. *2015-2017*
Purdue University Department of Horticulture and Landscape Architecture Graduate Student Organization Vice President. *2015-2018*
Mississippi State University Horticulture Club President. *2011-2012*
Mississippi State University Horticulture Club Vice President. *2010-2011*
National American Society for Horticultural Science –Associate Collegiate Branch Communications Officer. *2010-2011*
Mississippi State University Shackouls Honors College. *2008*
Mississippi State University Day One Leadership Community. *2008*

PROFESSIONAL ACADEMIC AFFILIATIONS AND HONOR SOCIETIES

Pi Alpha Xi, initiated *2013*
American Society for Horticultural Science, *2013-present*

GRANTS AWARDED

Fred C. Gloeckner Foundation, Inc. K.A. Williams, C.T. Miller and **J.K. Craver**. *2013*. Effects of UVB Radiation on Intumescence Development on *Ipomoea batatas*. FUNDED: $10,000 (1 year)

CoA Innovations in Teaching, Learning and Assessment Program. K.A. Williams and **J.K. Craver**. *2013*. Assessing Student Learning from a Hydroponics Production Module in HORT 570 Greenhouse
Operations Management. FUNDED: $2,400 (1 year)

PUBLICATIONS

Refereed

Craver, J.K., J.R. Gerovac, D.A. Kopsell, and R.G. Lopez. 2017. Light intensity and light quality from sole-source light-emitting diodes impact phytochemical concentrations within *Brassica* microgreens. J. Amer. Soc. Hort. Sci. 142(1):3-12.

Gerovac, J.R., **J.K. Craver**, J.K. Boldt, and R.G. Lopez. 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of *Brassica* microgreens. HortSci. 51(5):497-503.

Craver, J.K., C.T. Miller, K.A. Williams and N. Bello. 2014. UVB radiation affects intumescence development in ornamental sweetpotato (*Ipomoea batatas*). HortSci. 49:1277-1283.

Craver, J.K., C.T. Miller, K.A. Williams and D.L. Boyle. 2014. Characterization and comparison of lesions on ornamental sweetpotato 'Blackie', tomato 'Maxifort', interspecific geranium 'Caliente Coral', and bat-faced cuphea 'Tiny Mice'. J. Amer. Soc. Hort. Sci. 139(5):603-615.

Craver, J.K. and K.A. Williams. 2014. Assessing student learning from an experiential module in a greenhouse management course using hydroponics and recirculating solution culture. HortTechnology 24(5):610-617.

Proceedings of Scientific Symposia

Williams, K.A., **J.K. Craver**, C.T. Miller, N. Rud and M.B. Kirkham. 2015. Differences between the physiological disorders of intumescences and edemata. Acta Horticulturae. Acta Horticulturae 1104, 401-406.

Book Chapters

LED Lighting for Urban Agriculture

Craver, J.K., and R.G. Lopez. 2016. Control of morphology by manipulating light quality and daily light integral using LEDs, p. 203-217. *In:* LED Lighting for Urban Agriculture. Springer Science+Business Media, Singapore.

Williams, K.A., C.T. Miller, and **J.K. Craver**. 2016. Light quality effects on intumescence (oedema) on plants leaves, p. 275-286. *In:* LED Lighting for Urban Agriculture. Springer Science+Business Media, Singapore.

Light Management in Controlled Environments.

Currey, C.J., D.A. Kopsell, N.S. Mattson, **J.K. Craver**, R.G. Lopez, J.E. Erwin, and C. Kubota. 2017. Supplemental and sole-source lighting of leafy greens, herbs, and microgreens, p. 170-180 *In:* Light Management in Controlled Environments. Meister Media Worldwide.

Extension and Trade Articles

Craver, J.K. and R.G. Lopez. 2017. Producing high-quality plugs: Moving indoors Part IV. GrowerTalks 81(7):82−85.

Q. Meng and **J.K. Craver**. 2016. Lighting Highlights. GrowerTalks 80(3):90.

Craver, J.K., and R.G. Lopez. 2016. "Comparison of young and finish plant production under LED toplighting and HPS lamps. Greenhouse Grower 34(11):30–34.

Lopez, R.G. and **J.K. Craver**. 2016. How much do hanging baskets influence the light quality and quantity for crops grown below? e-GRO Alert 5(21):1–5.

Craver, J.K., and R.G. Lopez. 2015. "Sole-source lighting in horticulture: Bedding plant production." Greenhouse Grower 33(11):40–46.

Craver, J.K., and R.G. Lopez. 2015. "Sole-source lighting in horticulture: Microgreens production." Greenhouse Grower 33(10):56–60.

Craver, J.K., C.T. Miller, M.G. Cruz, and K.A. Williams. 2014. "Intumescences: Further investigations into an elusive disorder." Greenhouse Product News. 24(9): 32, 34, 36.

Craver, J.K., C.T. Miller and K.A. Williams. 2013. "Intumescences: A physiological disorder of greenhouse grown crops." Greenhouse Product News. 23(12):12-19.

Scientific Presentations and Abstracts

Craver, J.K.*, K. Nemali and R.G. Lopez. 2017. Physiological acclimation of petunia seedlings to varying light quality, light intensity, and carbon dioxide concentration for indoor production. 2017 American Society for Horticultural Science Annual Conference, Waikoloa, Hawaii. September 19-22, 2017.

Craver, J.K.*, K. Nemali and R.G. Lopez. 2017. Non-invasive imaging using fluorescence to measure growth rate of annual bedding plant seedlings. 2017 American Society for Horticultural Science Annual Conference, Waikoloa, Hawaii. September 19-22, 2017.

Craver, J.K.* and R.G. Lopez. 2016. Comparison of LED and HPS supplemental lighting for seedling production. 5th International Controlled Environment Conference, Canberra, Australia. September, 18-23, 2016.

Craver, J.K.* and R.G. Lopez. 2016. Daily light integral and light quality from sole-source LEDs impacts nutrient uptake and anthocyanin content of *Brassica* microgreens. 8th International Symposium on Light in Horticulture, East Lansing, Michigan. May 22-26, 2016.

Craver, J.K.* and R.G. Lopez. 2015. Daily light integral and light quality from sole-source light-emitting diodes affect seedling quality and subsequent flowering of long-day bedding plant species. 2015 American Society for Horticultural Science Annual Conference Abstract, p. 220.

Craver, J.K.*, J. Gerovac, R.G. Lopez and D.A. Kopsell. 2015. Daily light integral and light quality from sole-source light-emitting diodes impact phytochemical content of *Brassica* microgreens. 2015 American Society for Horticultural Science Annual Conference Abstract (196), p. 350.

Miller, C.T.*, **J.K. Craver**, M.G. Cruz and K.A. Williams. 2015. Screening of ornamental sweetpotato (*Ipomoea batatas*) cultivars for intumescence development. 2015 American Society for Horticultural Science Annual Conference Abstract (357), p. 253.

Craver, J.K.*, J. Gerovac and R.G. Lopez. 2015. Daily light integral and light quality from sole-source light-emitting diodes impact growth, morphology, and relative chlorophyll content of *Brassica* microgreens. 2015 Association of Education and Research Greenhouse Curators/North Central Extension and Research Activity-101 Annual Meeting, Columbus, Ohio. July 8-11, 2015.

Craver, J.K.*, C.T. Miller, K.A. Williams, and N. Bello. 2014. UVB radiation affects intumescence development in ornamental sweet potato (Ipomoea batatas). 2014 American Society for Horticultural Science Annual Conference Abstract, p. 97.

Craver, J.K.*, C.T. Miller, K.A. Williams, and D.L. Boyle. 2014. Characterization and comparison of lesions from physiological disorders on ornamental sweet potato (*Ipomoea batatas*), tomato (*Solanum lycopersicum* var. *hirsutum* 'Maxifort'), and interspecific geranium (*Pelargonium* x'Caliente Coral'). 2014 American Society for Horticultural Science Annual Conference Abstract (112), p. 109.

Craver, J.K.* and K.A. Williams. 2014. Assessing student learning from an experiential hydroponics production module in a greenhouse management course. 2014 American Society for Horticultural Science Annual Conference Abstract (079), p. 73.

Williams, K.A.*, **J.K. Craver**, C.T. Miller, N.A. Rud, and M.B. Kirkahm. 2014. Differences between the physiological disorders of intumescences and edemata. Poster presentation in Section 15: Ornamental horticulture in the global greenhouse. International Horticultural Congress, Brisbane, Australia. August 17-22, 2014.

Craver, J.K. and K.A. Williams*****. 2014. Assessing student learning from an experiential hydroponics production module in a greenhouse management course. Big XII Teaching and Learning Conference, Stillwater, Oklahoma. August 4-5, 2014.

Craver, J.K.*, C.T. Miller, and K.A. Williams. 2014. Effects of UVB radiation on intumescence development in *Ipomoea batatas*. K-State Research Forum; March 26, 2014.

Owen, J.S., K.A. Williams*, H.M. Stoven, **J.K. Craver**, and J. Brindley. 2013. Bluing of hydrangea 'Endless Summer' sepals is influenced by timing of aluminum sulfate drenches or aluminum chelate foliar sprays in three different locations and production systems. 2013 American Society for Horticultural Science Annual Conference Abstract (020), p. 93.

Craver, J.K.* and R.L. Harkess. 2012. Determining rhizome maturity in reblooming iris. 2012 Southern Regional Conference of the American Society for Horticultural Science. Birmingham, AL. February 3-6, 2012.

EXTENSION PRESENTATIONS

"Greenhouse Supplemental Lighting: Fundamental, Benefits, and Strategies." Great Plains Growers Conference. St. Joseph, MO. January 2018.

"Microgreens: Production and Opportunities." Great Plains Growers Conference. St. Joseph, MO. January 2018.

"Non-invasive Imaging Technique to Measure Growth of Annual Bedding Plant Seedlings." Indiana Flower Growers Association Purdue Annual Meeting. West Lafayette, IN. October 2017.

"Greenhouse Supplemental Lighting Strategies, Costs, and Plant Responses." Spring 2016 e-GRO Webinar Series. February 2016.

"LED Toplighting as an Alternative to HPS Lamps for Supplemental Lighting of Annual Bedding Plant Seedlings." Indiana Flower Growers Association Purdue Annual Meeting. West Lafayette, IN. October 2015.

"Sustainable Solutions for Producing Ornamental Plants and Vegetables: Advances in Greenhouse, High-tunnel, and Indoor Production". Westminster Village. West Lafayette, IN. June 2015.

"Comparison of Light Qualities and DLI for Sole-source Lighting of Microgreens and Bedding Plant Plugs." LED Symposium. Tucson, AZ. February 2015.

"Intumescences: A Physiological Disorder of Greenhouse-grown Crops." Indiana Flower Growers Association Purdue Annual Meeting. West Lafayette, IN. October 2014.

"Poinsettias Galore." Tuesday Talks in the Gardens. Manhattan, KS. November 2012.

"Poinsettias Galore." Manhattan Men's Garden Club. Manhattan, KS. December 2012.

PROFESSIONAL MEETINGS ATTENDED

2017 American Society for Horticultural Science Annual Conference, September 19-22, 2017. Waikoloa, Hawaii.

2016 National Floriculture Forum, January 24-29, 2016. The Netherlands and Essen, Germany.
8th International Symposium on Light in Horticulture. May 22-26, 2016. East Lansing, Michigan.
5th International Controlled Environment Conference. September 18-23, 2016. Canberra, Australia.

2015 LED Symposium, February 20, 2015. Tucson, Arizona.
Association of Education and Research Greenhouse Curators/North Central Extension and Research Activity-101 Annual Meeting, July 8-11, 2015. Columbus, Ohio.
AmericanHort Cultivate'15. July 11-14, 2015. Columbus, Ohio.
American Society for Horticultural Science Annual Conference, August 4-7, 2015. New Orleans, Louisiana.

2014 American Society for Horticultural Science Annual Conference, July 28-31, 2014. Miami, FL.

2013 OFA Short Course, July 13-16, 2013. Columbus, Ohio.
National Floriculture Forum, March 22-23, 2013. Portsmouth, New Hampshire.

2012 Kansas Greenhouse Growers Association 2012 Educational Conference, October 25, 2012. Manhattan, Kansas.
American Society for Horticultural Science Southern Region Conference, February 4-7, 2012. Birmingham, Alabama.

2011 American Society for Horticultural Science Annual Conference, September 25-28, 2011. Waikoloa, Hawaii.

2010 American Society for Horticultural Science Annual Conference, August 2-5, 2010. Palm Desert, California.