EVALUATION OF DIFFERENT LIGHTING STRATEGIES USED TO IMPROVE EFFICIENCIES IN PLANT GROWTH FOR CONTROLLED ENVIRONMENT AGRICULTURE

by

CLAUDIA ANN ELKINS

(Under the Direction of Marc W. van Iersel)

ABSTRACT

Crop growth in controlled environments can be improved with targeted strategies to deliver supplemental light. Three studies evaluated elements that can affect photosynthesis and growth. Study one used chlorophyll fluorescence to quantify the daily photochemical integral (DPI, electron transport rate integrated over 24 hours). Daily photochemical integral increased when the same daily light integral (DLI) was provided over longer photoperiods at a lower photosynthetic photon flux density (*PPFD*) for *Lactuca sativa* grown in a growth chamber. Study two applied these findings to greenhouse production using an adaptive lighting control system. Supplemental light-emitting diode (LED) arrays were provided to achieve a similar target DLI across set photoperiods. Supplemental light increased growth of *Rudbeckia fulgida* seedlings more efficiently at longer photoperiods and lower *PPFD*. Study three evaluated supplementation with far-red light on growth of *Digitalis purpurea* seedlings in a growth chamber. Plant dry weight increased with increasing far-red without detrimental morphological effects.

INDEX WORDS: Photochemistry, daily photochemical integral, far-red, light-emitting diodes, chlorophyll fluorescence, *Digitalis*, *Rudbeckia*, lettuce, quantum yield of photosystem II, photoperiod.

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DEDICATION

I dedicate this thesis to my late husband Gene. He always encouraged and happily supported my wild and crazy ideas. The wildest and craziest was to join the Navy Reserves in 1983 while raising a one-year old son and working full-time. Joining the Navy gave me the opportunity to go to graduate school on the Post-9/11 GI Bill. I also dedicate this thesis to my children, Jasper, Marty, and Kim, and daughter-in-law Jasmine. It was their love, support, encouragement, and holding down the fort while I was away, that sustained me when I did not think I could do the work. And to my grandchildren, I hope I have set a good example that you should be open to new adventures and that you are never too old to learn something new.

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Introduction and Literature Review

Introduction

Horticulture operations are important to the United States (U.S.) economy. In 2014, they sold $13.8 billion in floriculture, nursery, and other specialty crops, including $797 million for food crops grown under protection (USDA, 2015). Many operations consist of controlled environment agriculture (CEA) systems such as plant factories, vertical farms, and greenhouses, where crops are grown under tightly controlled environmental conditions. These systems allow growers to consistently produce high-quality crops year-round, with a predictable growing cycle, enabling them to get their crop to market on schedule and within quality specifications.

Lighting is an important component in the production of high-quality, high-value horticultural crops grown in controlled environments. Electric lighting is the sole source of light for plants grown in plant factories, and it is used as supplemental and photoperiodic light in greenhouses to augment sunlight (Morrow, 2008). Supplemental light is often needed from late fall through early spring for greenhouse crops when the total amount of accumulated light, or daily light integral (DLI), provided by the sun is insufficient (Albright et al., 2000; Clausen et al., 2015), especially at higher latitudes (Albright et al., 2000; Hemming, 2011).

The U.S. horticulture industry spends $600 million annually on supplemental lighting to grow plants in controlled environments (Stober et al., 2017). Current light types include high-pressure sodium (HPS, the current industry standard), metal halide (MH), fluorescent, and light-

emitting diode (LED) arrays. Although it costs more to install LED lights (Nelson and Bugbee, 2014), their use is increasing because they are more energy efficient (Bourget, 2008), easier to control (van Iersel, 2017), allow for tunable quality and quantity of the light spectrum, and can save growers money on energy costs in the long term. To reduce energy costs for the industry, we need to understand how efficiently plants use the light they receive. Only then can we develop strategies to provide supplemental light only when needed and without a reduction in growth.

Light (photons) is a necessary component of photosynthesis; however, light capture must be balanced with light use. Too much light may damage plants (Barber and Andersson, 1992) and too little light may inhibit plant growth (Fankhauser and Batschauer, 2016). To study how plants use the light they receive, we must understand the process of photosynthesis, especially the light reactions. Photons absorbed by the plant have one of three fates: 1) used to drive photosynthesis (photochemistry), 2) dissipated as heat, or 3) reemitted as light from chlorophyll *a* (chlorophyll fluorescence) (Baker, 2008). These three fates compete, so an increase in one process must be accompanied by a decrease in one or both of the other two processes. Measuring chlorophyll fluorescence is quick and nondestructive, and it can be used to determine the efficiencies of photosynthesis, such as quantum yield of photosystem II (Φ_{PSII}), electron transport rate (ETR), and daily photochemical integral (DPI), defined as ETR integrated over a 24-hour period (Weaver and van Iersel, 2019). Understanding how efficiently plants use the light they receive should allow growers to provide supplemental light only when plants need it and in the most efficient, cost-effective way.

Literature Review

Lighting in horticulture

Greenhouses and plant factories have different lighting needs. Greenhouses rely on the sun as the primary light source for plant growth, but supplemental lighting can boost crop yields, especially on cloudy days or during the winter when sunlight intensities are low (Albright et al., 2000; van Iersel, 2017). Plant factories rely on electric light as the sole source of energy for plant growth (van Iersel, 2017). According to a 2017 U.S. Department of Energy report, CEA operations use a mixture of lighting technologies that include conventional fixtures (e.g., HPS, MH, and fluorescent) and newer LED light fixtures. Greenhouses and non-stacked indoor operations use more HPS/MH fixtures (98% and 89%, respectively) and fewer LED fixtures (2% and 4%, respectively) compared to vertical farms that use 66% LED, 34% fluorescent, and less than 1% HPS/MH fixtures (Stober et al., 2017).

Light-emitting diode fixtures in CEA systems are becoming more popular because they have unique properties that conventional lights do not have (Gupta and Agarwal, 2017). They come in a wide range of colors that can be tailored to produce a desired response in plants. Li and Kubota (2009) found that providing far-red light expanded the leaf area in lettuce (*Lactuca sativa*), which increased light interception and biomass. Light-emitting diodes can also be dimmed to control light intensity, rather than the on/off functionality of HPS lamps, which can help growers reach precise target daily light integrals (van Iersel, 2017). These two unique properties of LEDs were used in the research studies.

Photosynthesis and light

Photosynthesis is the process by which plants synthesize carbohydrates from water, carbon dioxide (CO_2), and photons of light. It can be broken down into two sets of reactions.

First, the light reactions require photons in order to proceed. This is where light energy is converted into chemical energy by the two pigment-protein complexes photosystem II (PSII) and photosystem I (PSI). Second, the light-independent reactions, also referred to as the Calvin-Benson-Bassham (CBB) cycle, require products of the light reactions, ATP and NADPH, to fuel the CBB cycle (Ruban, 2015). The CBB cycle is where CO_2 is fixed and carbohydrates necessary for growth are synthesized. Research in subsequent chapters will provide light to plants and study how efficiently they use that light, focusing on the light reactions of photosynthesis as these reactions provide a direct way of measuring light use efficiency.

Chlorophyll fluorescence

Measuring chlorophyll fluorescence is a powerful, yet simple, tool commonly used to measure the efficiency of the light reactions of photosynthesis (Maxwell and Johnson, 2000). It can be used to develop optimal lighting strategies for plants grown in controlled environments. Chlorophyll fluorescence is one of the three possible competing fates for light energy absorbed by a plant. As such, measuring chlorophyll fluorescence can provide information about the other two: Φ_{PSII}, defined as the fraction of absorbed light energy used for photochemistry, and non-photochemical quenching (NPQ), an indication of the amount of absorbed light energy converted to heat (Baker and Rosenqvist, 2004; Genty et al., 1989). If there is an increase in the efficiency of one, there will be a decrease in the yield of one or both of the other two (Maxwell and Johnson, 2000).

Chlorophyll fluorescence is a non-destructive method that has long been used to track crop responses to light (Pocock, 2015) and used as an evaluation tool to improve production of greenhouse-grown crops (Baker and Rosenqvist, 2004). Pocock (2016) used chlorophyll fluorescence measurements to determine how quickly lettuce plants responded to changes in

light spectrum. van Iersel et al. (2016) used chlorophyll fluorescence measurements to develop a biofeedback system to control ETR, maintain a target Φ_{PSII}, and ultimately optimize photosynthesis. Weaver and van Iersel (2019) obtained chlorophyll fluorescence measurements over a 35-d production cycle to characterize photochemical responses of lettuce to photosynthetic photon flux density (*PPFD*) and daily light integral (DLI). Using simulations, they confirmed their hypothesis that for a given DLI, the DPI would increase given a low *PPFD* and a longer photoperiod, and the increase could be maximized with a uniform *PPFD* over the entire photoperiod (Weaver and van Iersel, 2019).

Quantum yield of photosystem II and electron transport rate

A unitless measure, Φ_{PSII} is the ratio between electrons transported through PSII and total absorbed photons. It is an indication of how efficiently leaves use absorbed light energy to drive photochemistry. A higher Φ_{PSII} indicates greater relative photochemistry.

To understand photosynthetic capacity, we can use Φ_{PSII} and *PPFD* values to calculate the linear ETR through PSII (Baker and Rosenqvist, 2004; Genty et al., 1989; Maxwell and Johnson, 2000). Electron transport rate is a calculated estimate of the rate of the light reactions of photosynthesis (Maxwell and Johnson, 2000), using the equation $ETR = \Phi_{PSII} \times PPFD_{absorbed} \times 0.5$, where $PPFD_{absorbed} = 0.84 \times PPFD$, with the assumption that leaves absorb 84% of the photons that reach them (Björkman and Demmig, 1987).

Adaptive supplemental lighting control

Adaptive supplemental lighting control is a system that offers precise control over different lighting variables that can affect growth such as DLI, photoperiod, and *PPFD* (van Iersel and Gianino, 2017). The system works especially well with LEDs because they are dimmable. The system consists of 1) dimmable LED light fixtures, 2) drivers that regulate power

to the LEDs, 3) quantum sensors that measure *PPFD*, and 4) a datalogger that collects *PPFD* measurements and adjusts the LED light intensity by sending a dimming signal to the drivers. To reach a target DLI within a set photoperiod, the datalogger uses the *PPFD* measurements to continually calculate how much light has been received during the photoperiod, calculate how much more light is needed to reach the target DLI, and determine the average *PPFD* required for the remainder of the photoperiod.

Photoperiod, daily light integral, and daily photochemical integral

Manipulating photoperiod and DLI have long been used to study plant growth and develop lighting strategies to improve crop production. Photoperiod is the continuous interval within a 24-h period in which plants are exposed to light. Daily light integral is the *PPFD* integrated over a 24-h period, expressed as moles of photons per square meter of ground area per day ($mol \cdot m^{-2} \cdot d^{-1}$). Koontz and Prince (1986) found a 30% to 50% increase in fresh and dry weights of four loose leaf lettuce cultivars grown under a 24-h photoperiod compared with plants grown under a 16-h photoperiod, both with a similar DLI. Across a range of DLIs, Craker et al. (1983) found that increasing the photoperiod from 8 to 16 h, but providing the same DLI, increased the fresh weight of radish (*Raphanus sativus*). Daily photochemical integral, the ETR through PSII integrated over a 24-h period, is expressed as moles of electrons per square meter of leaf per day ($mol \cdot m^{-2} \cdot d^{-1}$) (Weaver and van Iersel, 2019). Quantifying DPI can be used to determine the effectiveness of supplemental lighting control strategies (Weaver and van Iersel, 2019).

Far-red light and plant morphology and physiology

Plants exposed to far-red light (wavelengths from 700 to 800 nm) sense they are being shaded. In the shade underneath a canopy, the fraction of far-red light of the total amount of light

increases. Additionally, light availability can limit growth of shaded plants. Plants can respond to high fractions of far-red light by growing taller or increasing leaf size to capture more light. Chia and Kubota (2010) found that providing a near-saturation dose of far-red light at the end of the day resulted in 22% to 34% longer hypocotyls of two tomato rootstock cultivars 'Aloha' (*Solanum lycopersicum*) and 'Maxifort' (*S. lycopersicum* × *S. habrochaites*), which is important for grafting success. Li and Kubota (2009) also found greater leaf expansion, 28% more fresh weight, and 15% more dry weight of lettuce plants treated with supplemental far-red light compared to those without supplemental far-red light. Park and Runkle (2016) found that adding far-red light to a mix of red and blue light increased shoot dry weight and photosynthetic efficiency (calculated as shoot dry weight per unit leaf area) in snapdragon (*Antirrhinum majus*) under sole-source lighting, without excessive extension growth.

Phytochrome photoequilibrium

Phytochromes are important pigment-protein photoreceptors that detect light quantity and quality (Demotes-Mainard et al., 2016). They continually monitor surrounding light conditions and regulate processes within the plant. Phytochromes exist in two interconvertible forms: phytochrome red (Pr) and phytochrome far-red (Pfr). The Pr state absorbs red light with a maximum peak near 660 nm and is considered the biologically inactive form. Phytochrome is originally synthesized in the Pr form. When red light enters the plant cell during the day, Pr is quickly converted to Pfr. The Pfr form absorbs far-red light, with a maximum peak near 730 nm. It is considered the biologically active form that can directly regulate responses within cells and impact gene expression, and it regulates several processes within the plant including growth and the shade-avoidance response (Franklin and Whitelam, 2005). Plants can respond to shade by making larger leaves to capture more light and increase photosynthesis and growth.

Relevance to studies

The topics above are important for the three studies presented in this thesis. Manipulating photoperiod, DLI, and far-red light can be used to elicit a desired plant response. First, photons are needed for photosynthesis. Measuring chlorophyll fluorescence allows for the calculation of Φ_{PSII}. The Φ_{PSII} and a known *PPFD* can be used to calculate ETR through PSII and integrated over 24 h to yield the DPI. Quantifying DPI can be used to assess the effectiveness of supplemental lighting control strategies and help growers save money by providing supplemental light to plants only when needed.

The first study was a short-term physiological study that quantified DPI in lettuce across different photoperiods with the same DLI in a growth chamber. Findings from the first study were applied to a growth trial in a greenhouse using *Rudbeckia fulgida* seedlings and an adaptive lighting control system. The system controlled the LED light output in such a way that the combination of sunlight and LED light did not exceed a target DLI. The third study looked at the effect of far-red light on *Digitalis purpurea* seedling grown in a growth chamber, with the goal of determining an optimal level of supplemental far-red light.

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CHAPTER 2

Longer Photoperiods with the Same Daily Light Integral Increase Daily Electron Transport Through Photosystem II in Lettuce[1]

[1] Claudia A. Elkins and Marc W. van Iersel. To be submitted to HortScience for publication.

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Abstract

Controlled environment crop production recommendations often use the daily light integral (DLI) as a guide to the light requirements of specific crops. Sole-source electric lighting, used in plant factories, and supplemental electric lighting, used in greenhouses, may be required to attain a specific DLI, but can be expensive to purchase and operate. Electric lighting can be wasteful if it is not provided in a way that promotes efficient photochemistry. Because the quantum yield of photosystem II (Φ_{PSII}), the fraction of absorbed light used for photochemistry, decreases with increasing photosynthetic photon flux density (*PPFD*), we hypothesized that the daily photochemical integral (DPI), the total electron transport through photosystem II (PSII) integrated over 24 hours, would increase if the same DLI was provided at a lower *PPFD* over a longer photoperiod. To test this, chlorophyll fluorescence was measured to determine the Φ_{PSII} and the electron transport rate (ETR) of lettuce (*Lactuca sativa* 'Green Towers') under controlled lighting conditions in a growth chamber. Daily light integrals of 15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$ were tested with photoperiods of 7, 10, 13, 16, 19, and 22 hours. The *PPFD*s for the DLI-photoperiod combinations ranged from 189 to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Combined over the DLI treatments, the Φ_{PSII} decreased from 0.67 at a *PPFD* of 189 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 0.28 at 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while ETR increased from 55 to 99 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as *PPFD* increased from 189 to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The DPI increased linearly as the photoperiod increased, but this response depended on DLI. With a 7-hour photoperiod, the DPI was ≈2.7 $mol \cdot m^{-2} \cdot d^{-1}$, regardless of DLI. However, with a 22-hour photoperiod, the DPI was 4.54 $mol \cdot m^{-2} \cdot d^{-1}$ (74% increase) with a DLI of 15 $mol \cdot m^{-2} \cdot d^{-1}$ and 5.78 $mol \cdot m^{-2} \cdot d^{-1}$ (109% increase) with a DLI of 20 $mol \cdot m^{-2} \cdot d^{-1}$. A greater DPI with longer photoperiods suggests that plants grown under these conditions will have

increased photosynthesis and as a result will grow faster. This was confirmed in subsequent growth chamber and greenhouse trials.

Introduction

The United States (U.S.) spends $600 million annually on horticultural lighting in controlled environments like indoor plant factories and greenhouses (Stober et al., 2017). To reduce energy costs, it is important to understand how efficiently plants use the light they receive. Only then can strategies be developed to provide lighting when needed, with the intensity needed, and without a reduction in plant growth.

Light (photons) is necessary for photosynthesis; however, light must be provided at appropriate intensities. Too much light may damage plants (Barber and Andersson, 1992) and too little light may inhibit growth (Fankhauser and Batschauer, 2016). To understand how efficiently plants use the light they receive, researchers must quantify the process of photosynthesis and the light reactions in particular. Photons absorbed by photosynthetic pigments in plants have one of three fates: 1) they can be used to drive the light reactions of photosynthesis (photochemistry), 2) they can be dissipated as heat, or 3) they can be re-emitted as light from chlorophyll *a* (chlorophyll fluorescence). These three fates compete, so an increase in one process must be accompanied by a decrease in one or both of the other processes (Baker, 2008; Maxwell and Johnson, 2000).

Measuring chlorophyll fluorescence is easy and widely used to study the light reactions of photosynthesis. It provides a noninvasive method to quantify the quantum yield of photosystem II (Φ_{PSII}), a unitless measure of the efficiency with which absorbed photons are used to drive photochemistry (Genty et al., 1989). As the photosynthetic photon flux density (*PPFD*)

increases, a larger portion of the photosystem II (PSII) reaction centers become closed (unable to accept additional excitation energy). This occurs because once the electron acceptor Q_A in PSII has accepted an electron, it cannot accept another until the first one is transferred to the electron carrier Q_B. The decrease in "open" electron acceptors results in a decrease in Φ_{PSII} (Baker, 2008). Previous studies have shown that Φ_{PSII} decreases with increasing *PPFD* (Weaver and van Iersel, 2019, 2020; Zhen and van Iersel, 2017; Zou et al., 2019).

The Φ_{PSII}, combined with a known *PPFD*, can be used to calculate the linear electron transport rate (ETR) through PSII and estimate the overall rate of the light reactions of photosynthesis (Baker and Rosenqvist, 2004; Genty et al., 1989; Maxwell and Johnson, 2000). Election transport rate is calculated as $\Phi_{PSII} \times PPFD_{absorbed} \times 0.5$ (two photons are needed to move one electron through the entire electron transport chain).Typically, $PPFD_{absorbed}$ is determined as = $0.84 \times PPFD$, assuming leaves absorb 84% of the photons that reach the leaf surface, which is a common leaf absorptance coefficient for C_3 plants (Björkman and Demmig, 1987).

Electron transport rate, in turn, can be used to determine the daily photochemical integral (DPI), defined as the ETR integrated over a 24-h period (Weaver and van Iersel, 2019). Our goal was to quantify the effect of *PPFD* and photoperiod on DPI, while maintaining a static DLI. Because Φ_{PSII} decreases with increasing *PPFD*, we hypothesized DPI would be greater when the same DLI was provided over longer photoperiods at lower *PPFD*s. Results should afford a better understanding of the relationship between *PPFD*, photoperiod, DLI, and DPI, leading to development of more efficient lighting strategies.

Materials and Methods

Plant material. Lettuce (*Lactuca sativa* 'Green Towers') seeds were sown in 15-cm diameter round pots filled with a soilless growing medium (Fafard 3B; SunGro Horticulture, Agawam, MA). The seeds were germinated indoors on an ebb-and-flow bench under white light-emitting diode (LED) arrays (Fat Jeff; Aurora, St. Petersburg, FL) with a *PPFD* of 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ plus 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (700–800 nm). They were fertigated, as needed, with 100 $mg \cdot L^{-1}$ N water-soluble fertilizer solution (15N–2.2P–12.5K, Peters Excel 15–5–15 Cal-Mag Special; ICL Fertilizers, Dublin, OH). After 10 d, the plants were thinned to one plant per pot, moved to a growth chamber (E15; Conviron, Winnipeg, Manitoba, Canada), and grown under cool-white fluorescent light with a *PPFD* of ≈250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 14-h photoperiod, ambient CO_2, ≈37% relative humidity, and constant air temperature of 22.7 ºC. Plants were watered using the same fertilizer solution. New seeds were sown every 5 d to maintain a steady supply of plants of similar age. Thirty-six different lettuce plants were used during the study period from 16 Jan. to 6 Feb. 2018.

Experimental setup. Two DLIs (15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$) were each applied across six photoperiod treatments (7, 10, 13, 16, 19, and 22 h) to evaluate the effects on Φ_{PSII}, ETR, and DPI. To measure these parameters, individual plants were moved daily into a second growth chamber (E15, Conviron) that was divided into two separate sections with identical measurement setups. Chlorophyll fluorescence measurements were collected using a modified version of the system described in van Iersel et al. (2016). Each section of the growth chamber was lit using custom-made LED arrays (PhytoSynthetix, Boulder, CO), with the spectral distribution shown in Fig. 2.1. The fixtures were powered by dimmable drivers. The drivers in each section were connected to separate dataloggers (CR1000; Campbell Scientific, Logan, UT). A quantum sensor (LI-190;

LI-COR Biosciences, Lincoln, NE) and a chlorophyll fluorometer (MiniPam; Heinz Walz, Effeltrich, Germany) with a leaf clip were placed in each section, and the analog outputs from each were recorded by the datalogger. The datalogger was also connected to the serial communication port of the fluorometer, allowing the datalogger to trigger fluorometer measurements. The *PPFD*s required to reach the target DLI ranged from 189 to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$, depending on the DLI and photoperiod. To control lights, we programmed the target DLI and photoperiods into the datalogger program before each test. From that information, the datalogger calculated the required *PPFD* (DLI divided by photoperiod in seconds). Then, based on readings from the quantum sensor, the datalogger sent a voltage signal to the dimmable driver, using an analog output module (SDM-AO4A; Campbell Scientific) to adjust the output from the LED fixtures to achieve the required *PPFD*. With this setup, we could achieve precise control over the *PPFD* and DLI; the standard deviation of the mean *PPFD* for all 36 tests averaged 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while the DLI in each run was within 0.001 $mol \cdot m^{-2} \cdot d^{-1}$ of the target DLI.

Two mature plants, approximately four weeks old, were selected each day data were collected. One plant was placed in each section and a fully expanded, uppermost leaf was chosen for chlorophyll fluorescence measurements. The leaf clip was attached to the leaf and the plant was kept in the dark for a minimum of 30 min to adapt to the dark, after which the dark-adapted Φ_{PSII} was measured. After the lights came on, Φ_{PSII} measurements were taken and ETR calculated every 15 min using the approach described by van Iersel et al. (2016) throughout the entire photoperiod. During the measurement period, air temperature was 22.8 ± 0.2 ºC (mean ± SD), vapor pressure deficit was 1.7 ± 0.2 kPa, and CO_2 level was ambient inside the growth chamber.

Experimental design and data analysis. The six photoperiods were randomized between days. Once the photoperiod for a specific day was determined, the DLIs of 15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$ were randomly assigned to the two sections of the growth chamber. Each treatment combination (DLI × photoperiod) was repeated three times. Linear and multiple regression analyses were performed using SigmaPlot (version 11.0; Systat Software, San Jose, CA), using *PPFD*, photoperiod, and DLI as continuous variables. Photoperiod by *PPFD* or DLI interactions were included in the models to determine whether in the models this interaction was significant.

Results and Discussion

Fig. 2.2 shows representative Φ_{PSII} and ETR data of lettuce for three different photoperiods tested at a DLI of 20 $mol \cdot m^{-2} \cdot d^{-1}$. The area under each ETR curve represents the DPI. It took approximately 1 to 2 h for plants to reach a stable Φ_{PSII} and ETR once lights were turned on, and plants exposed to the highest *PPFD* and shortest photoperiod took the longest to stabilize. Typically, plants reach steady-state photosynthesis after 5 to 10 min in the light (Kalaji et al., 2014). By comparison, our data suggest it takes longer for Φ_{PSII} and ETR to reach a steady state.

The relatively longer time required for photochemistry to stabilize is likely due to complex regulation of the light reactions, which involves multiple processes at different time scales. First, when dark-adapted plants with fully open reaction centers are exposed to light, there is a rapid (≈1 s) increase in chlorophyll fluorescence (the Kautsky effect). This increase in fluorescence, and decrease in Φ_{PSII}, occurs because of a reduction of the electron acceptor Q_A, resulting in temporary closure of some of the PSII reaction centers (Maxwell and Johnson, 2000). In response to continued light exposure, plants upregulate heat dissipation (Demmig-

Adams et al., 2012). This results in non-photochemical quenching of chlorophyll fluorescence, with plants typically reaching a steady-state within 15 to 20 min (Maxwell and Johnson, 2000). Our data indicate that achieving a true steady-state of photochemistry can take several hours.

Photochemistry produces adenosine triphosphate (ATP) and reduced compounds (ferredoxin, nicotinamide adenine dinucleotide phosphate—NADPH) that are used in multiple metabolic processes. Because levels of ATP and NADPH are relatively stable in light-exposed leaves, photochemistry needs to be in balance with the usage of ATP and NADPH (Geiger and Servaites, 1994). Photochemistry not only supports carbon fixation in the Calvin-Benson-Bassham (CBB) cycle, but also processes like photorespiration (Krall and Edwards, 1992), nitrate (Tischner, 2000) and sulfate reduction (Takahashi et al., 2011), and the Mehler reaction (Polle, 1996). Steady-state electron transport cannot be reached until all these processes have reached steady state. For example, regulation of nitrate reduction depends on both CBB cycle activity and photochemistry. Carbohydrate accumulation, resulting from CBB cycle activity, induces upregulation of nitrate reductase mRNA transcript levels (Lillo, 1994), presumably followed by increased levels of nitrate reductase. In turn, nitrate reductase activity is regulated by NADPH (Lillo, 1994). The interplay among these different processes may explain the relatively slow stabilization of photochemistry.

Independent of DLI, the Φ_{PSII} decreased linearly ($r^2 = 0.77$, $P < 0.001$) from 0.67 to 0.29 as *PPFD* increased from 189 to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2.3A). The trend was the same for plants exposed to either DLI, 15 or 20 $mol \cdot m^{-2} \cdot d^{-1}$. This is consistent with previous studies that found Φ_{PSII} decreased as *PPFD* increased. Weaver and van Iersel (2019) reported that the Φ_{PSII} of 'Green Towers' lettuce grown in a greenhouse under natural light decreased exponentially as *PPFD* increased from 0 to ≈1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These plants were exposed to a wider range of

*PPFD*s and that may explain the differences in trends, linear versus exponential, between the two studies. When comparing data obtained by Weaver and van Iersel (2019) across the range of *PPFD*s used in this study (189 to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$), the decline in Φ_{PSII} was roughly linear, and further declines in Φ_{PSII} at *PPFD*s above 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were small. Over the range of *PPFD*s the two studies have in common, ≈200 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the results are consistent. Zhen and van Iersel (2017) also found that the Φ_{PSII} of sweet potato (*Ipomea batatas*), lettuce, and pothos (*Epipremnum aureum*), grown in a greenhouse, decreased with increasing *PPFD,* and the rate of decrease was species-dependent.

Two processes can explain decreasing Φ_{PSII} with increasing *PPFD*. First, in response to increased light, a larger fraction of PSII reaction centers will be in a closed state and unable to accept additional excitation energy (Baker, 2008; Maxwell and Johnson, 2000). Conversely, at low light levels, PSII reaction centers will be open most of the time, allowing for more efficient photochemistry. Second, in response to increased light, plants upregulate photoprotective processes, such as the xanthophyll cycle, to reduce photoinhibition (Demmig-Adams et al., 2012; Horton, 2012; Murchie and Lawson, 2013; Ruban, 2017). Photoinhibition refers to damage to PSII reaction centers and occurs when they receive more excitation energy than can be processed. Upregulation of heat dissipation reduces the amount of excitation energy reaching PSII and thus reduces the risk of photoinhibition, but also reduces Φ_{PSII}.

Electron transport rate increased in an asymptotic manner from 55 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a *PPFD* of 189 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to an asymptote of 99 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($r^2 = 0.58$, $P < 0.001$) as *PPFD* increased to 794 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2.3B). The trend was the same for both DLIs. Weaver and van Iersel (2019) likewise found that ETR of 'Green Towers' lettuce responded to *PPFD* with an exponential rise to a maximum but reported a higher asymptote (121 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Zhen and

van Iersel (2017) found that the asymptote of the ETR curve depended on the light intensity plants received prior to the measurements, i.e., photochemistry acclimates to the growing conditions. Different growing conditions and the ability of plants to acclimate to different light conditions may explain the difference in asymptotes between the current study and that reported by Weaver and van Iersel (2019). Plants in this study were grown in a growth chamber with constant *PPFD* and a DLI of 12.6 $mol \cdot m^{-2} \cdot d^{-1}$, while Weaver and van Iersel (2019) grew plants in a greenhouse with an average DLI of 13.9 $mol \cdot m^{-2} \cdot d^{-1}$ and much higher peak *PPFD*s. It is plausible that greenhouse-grown plants had a greater photochemical capacity. It is important to note that all plants in this study were grown under the same lighting conditions, then transferred, for one day, to another set of lighting conditions for measurement. Therefore, plants in this study were not able to acclimate to the measurement lighting conditions.

Daily photochemical integral increased linearly as the photoperiod increased from 7 to 22 h, from 2.61 to 4.54 $mol \cdot m^{-2} \cdot d^{-1}$ (74% increase) and from 2.77 to 5.78 $mol \cdot m^{-2} \cdot d^{-1}$ (109% increase) for DLIs of 15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$, respectively ($R^2$ = 079, P <0.001; Fig. 2.4). The effect of photoperiod on DPI was greater for a DLI of 20 $mol \cdot m^{-2} \cdot d^{-1}$ because of the wider range of measurement *PPFD*s (253–794 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and thus Φ_{PSII} values (0.28–0.64), compared to the *PPFD*s (189–595 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and Φ_{PSII} values (0.39–0.68) for a DLI of 15 $mol \cdot m^{-2} \cdot d^{-1}$.

van Iersel (2017) made the case for using longer photoperiods with lower *PPFD* as a strategy to increase photosynthetic efficiency and crop growth in controlled environments. This study confirms that a target DLI delivered over longer photoperiods with lower *PPFD*s indeed results in greater DPIs in lettuce. Other studies have also shown that longer photoperiods with lower *PPFD*s do translate into more growth, but the increases in growth were much lower than the increases in DPI found in this study. In a growth trial conducted in a growth chamber, Palmer

(2018) observed an 18% increase in lettuce shoot dry weight as photoperiod increased from 10 to 20 h with a DLI of 16 $mol \cdot m^{-2} \cdot d^{-1}$. Weaver and van Iersel (2020) found that for greenhouse-grown lettuce, dry weight increased by 28% when the photoperiod increased from 12 to 21 h, even though all plants received the same DLI (13.9 $mol \cdot m^{-2} \cdot d^{-1}$). In a greenhouse trial with *Rudbeckia fulgida* var. *sullivantii* 'Goldsturm', Elkins (2020, Ch. 3) determined that shoot and root dry weight increased linearly from 0.23 to 0.30 g/plant (30%) and 0.071 to 0.088 g/plant (24%), respectively, as photoperiods increased from 12 to 21 h, while maintaining a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$.

Much larger increases in DPI (74% to 109%) were observed in this study in response to longer photoperiods, as compared to growth responses (18% to 30%) in other studies. Plants in this study were grown under identical lighting conditions with relatively low *PPFD*, then transferred for one day to another set of lighting conditions for measurement. Therefore, our plants were not necessarily acclimated to the measurement *PPFD*. If, for example, our plants that were measured at a high *PPFD* and short photoperiod had been grown at high *PPFD*, acclimation to high *PPFD* might have given the plants a greater capacity for photochemistry, as demonstrated by Zhen and van Iersel (2017), and those plants might have had higher ETRs and DPIs, thus making the treatment differences smaller. Nonetheless, a greater DPI and more growth can be achieved in lettuce grown with longer photoperiods and lower *PPFD*.

Conclusions

To our knowledge, this is the first study to quantify DPI over different photoperiods with the same DLI. Lettuce DPI greatly increased with longer photoperiods and lower *PPFD*s because plants exhibited decreased Φ_{PSII} with increasing *PPFD*. The short-term

physiological responses found in this study are consistent with results from longer-term growth chamber and greenhouse studies where growth was measured. This has practical implications for controlled environment agriculture. Longer photoperiods with lower *PPFD* can increase growth and reduce capital expenses, because fewer lights would need to be installed to provide the appropriate *PPFD* and DLI. Because our study was designed so that plants did not have a chance to acclimate to the measurement lighting conditions, potential future work should study how acclimation affects DPI.

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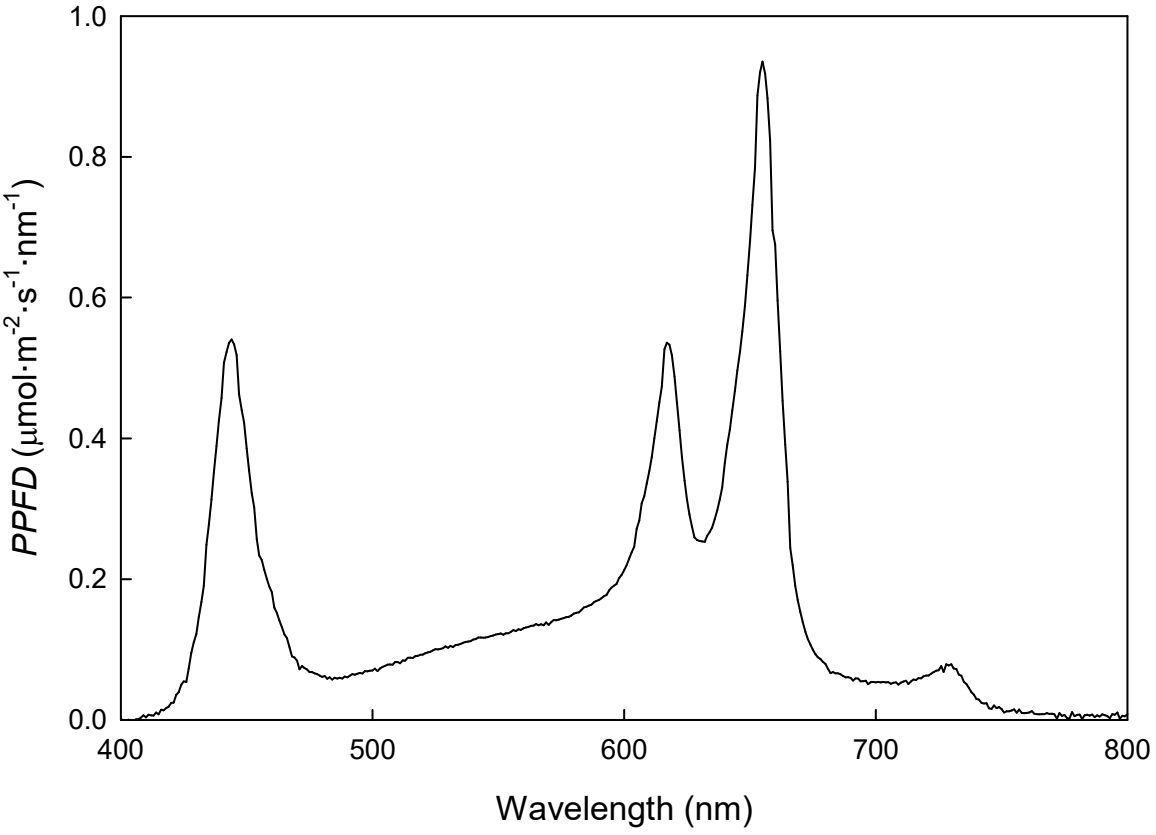

Fig. 2.1. Spectral distribution of the custom-made light-emitting diode (LED) array used for photochemical measurements.

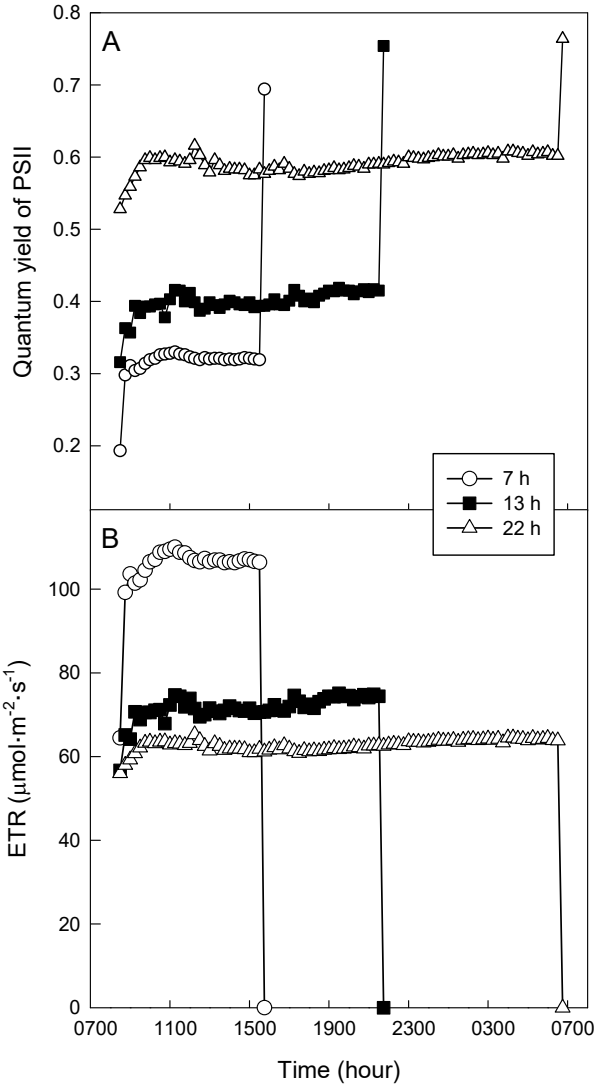

Fig. 2.2. The quantum yield of photosystem II (Φ_{PSII}; A) and electron transport rate (ETR; B) of lettuce (*Lactuca sativa* 'Green Towers') for three different photoperiods (7, 13 and 22 h) with a daily light integral (DLI) of 20 $mol \cdot m^{-2} \cdot d^{-1}$ over the course of the photoperiod. The area under the ETR curve represents the daily photochemical integral (DPI). The first data point, in all three data sets, is the first measurement with the lights on and the last data point is the first measurement with the lights off. Data are from one representative plant from each of the three treatments.

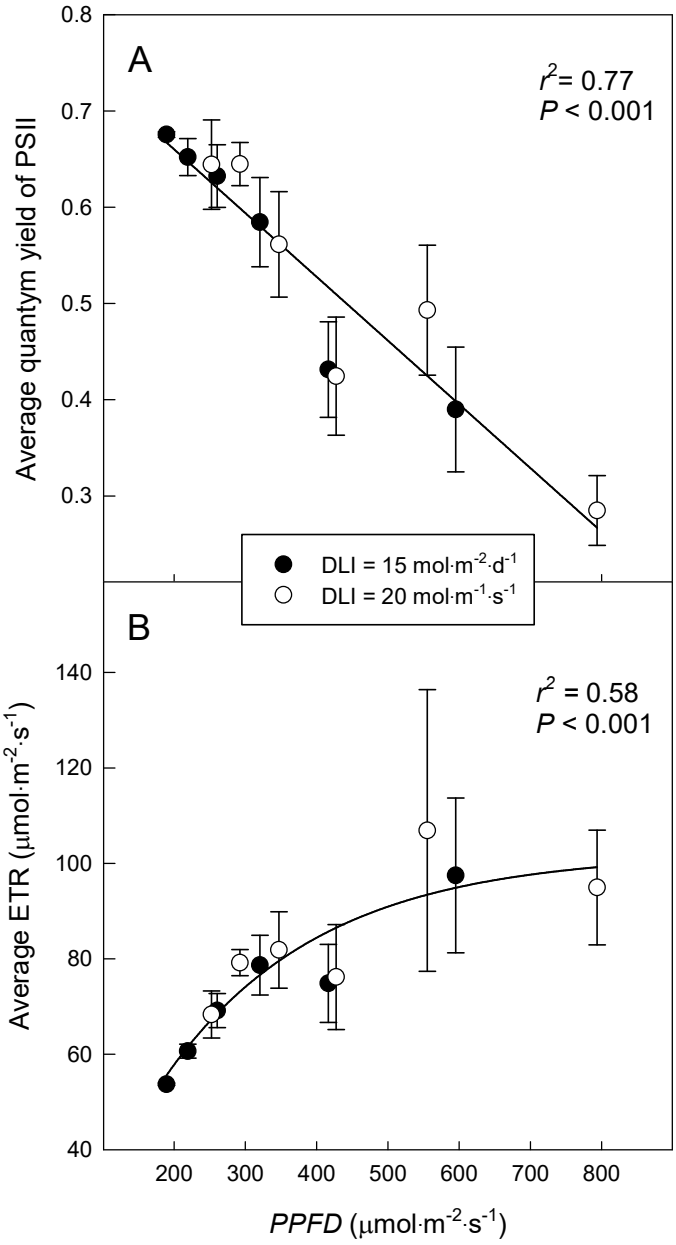

Fig. 2.3. The quantum yield of photosystem II ($\Phi_{PSII} = 0.792 - 0.000663 \times PPFD$); A) and electron transport rate [$ETR = -10.1 + 112.1 \times (1-e^{-0.00463 \times PPFD})$]; B) of lettuce (*Lactuca sativa* 'Green Towers'), averaged over the entire photoperiod, as a function of photosynthetic photon flux density (*PPFD*) (n=3). Plants were measured under two daily light integrals (DLIs), 15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$, but DLI had no effect on these parameters.

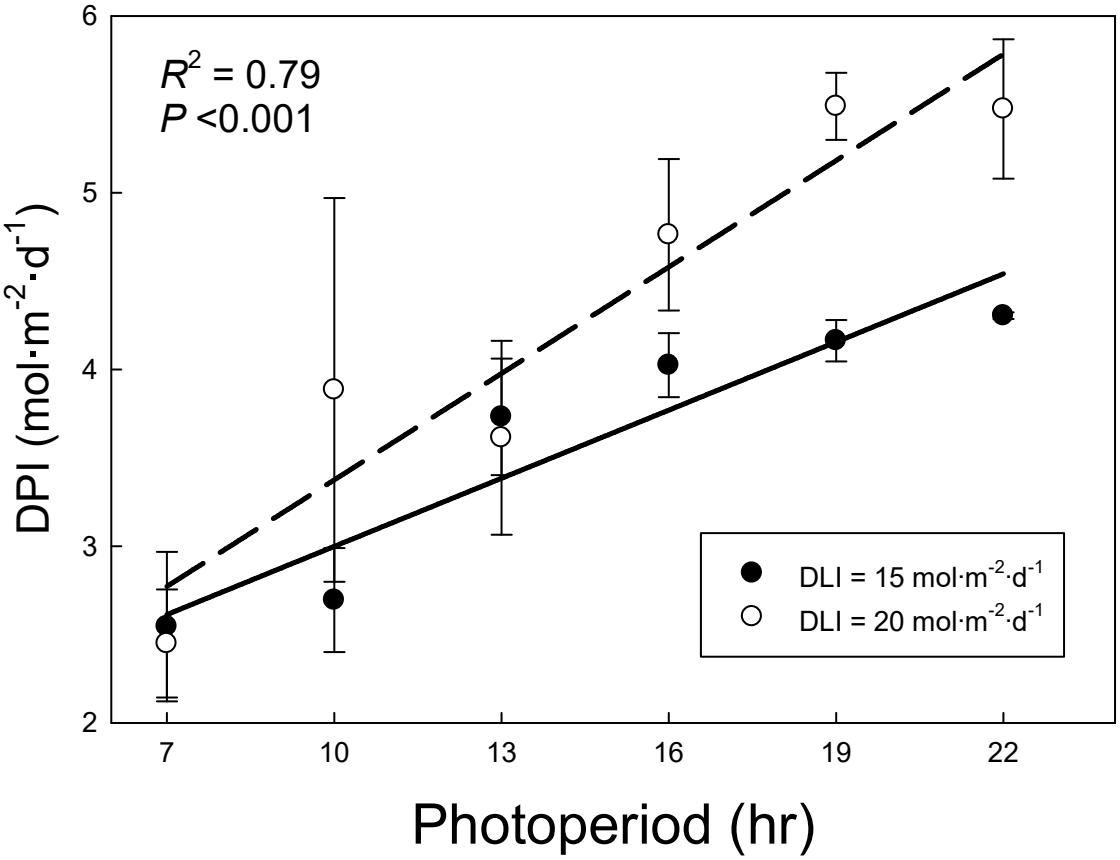

Fig. 2.4. Daily photochemical integral [DPI = 1.508 + (0.00953 × Photoperiod × DLI)] of lettuce (*Lactuca sativa* 'Green Towers') as a function of photoperiod. Plants measured under longer photoperiods, or under the same photoperiod but a lower target daily light integral (DLI) received a lower instantaneous photosynthetic photon flux density (*PPFD*). There was a significant interaction between DLI and photoperiod as indicated by the different slopes of the regression lines.

CHAPTER 3

Longer Photoperiods with the Same Daily Light Integral Improve Growth of Rudbeckia Seedlings Grown in a Greenhouse[1]

[1] Claudia A. Elkins and Marc W. van Iersel. To be submitted to HortScience for publication. This work was funded by USDA-NIFA-SCRI Award Number # 2018-51181-28365 Project 'Lighting Approaches to Maximize Profits.'

Abstract

Supplemental light can increase growth and accelerate production cycles of greenhouse-grown crops but can also be expensive if not provided in a way that promotes efficient photochemistry. The use of light-emitting diodes (LEDs) has the potential to reduce costs as the output can be precisely controlled to meet crop needs. Because light is used more efficiently to drive photosynthesis at lower photosynthetic photon flux densities (*PPFD*s), we hypothesized that providing *Rudbeckia fulgida* var. *sullivantii* 'Goldsturm' with the same daily light integral (DLI), spread out over a longer photoperiod and at lower *PPFD*s, should improve growth. A DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$was provided in the greenhouse over 12, 15, 18, or 21-hour photoperiods via sunlight in addition to adaptive lighting control of supplemental LED lights. Control plants without supplemental lighting had an ≈12-hour photoperiod and received an average DLI of 5 $mol \cdot m^{-2} \cdot d^{-1}$, ≈58% less light than the four treatments. As photoperiod increased from 12 to 21 hours, increased shoot dry weight (30%), root dry weight (24%), plant height (15%), compactness (17%), leaf area (16%), and chlorophyll content index (48%), and a decrease in specific leaf area (26%) were observed. There was no significant effect of photoperiod on root mass fraction. Control plants were smaller for all growth parameters measured compared to the 12-hour photoperiod, ranging from 26% to 90%. Treatment effects on canopy size, seen early in the study, were indicative of plant growth over the entire study. Longer photoperiods did not induce a shade-avoidance response, based on specific leaf area and compactness data. A 24% increase in root dry weight for the 21-hour photoperiod could shorten the cropping cycle by one to two weeks compared to the 12-hour photoperiod. This could result in more crop turns per year and increased profits. In addition, fewer lights would be needed for adequate growth, which could reduce capital costs.

Introduction

There are more than 2,000 farms in the United States (U.S.) engaged in seedling (plug) and cutting (liner) production, with 345 ha under protection and annual sales of $644 million (USDA, 2019). Many greenhouse operations use supplemental electric lighting to improve growth and yield of crops. At higher latitudes, where larger seasonal fluctuations in the daily light integral (DLI) occur, supplemental light is vital for year-round production (Albright et al., 2000). However, supplemental lighting costs can be high. Electricity alone can account for 30% of total production costs (van Iersel and Gianino, 2017; Watson et al., 2018). One way to offset supplemental lighting costs is to shorten cropping cycles and increase the number of crop turns per year. To achieve this, effective lighting strategies should be developed that result in more efficient photochemistry that lead to more efficient photosynthesis and enhanced growth.

Dimmable light-emitting diodes (LEDs) can be used to develop improved lighting strategies because their light output (photosynthetic photon flux density, *PPFD*) can be precisely controlled and programmed to respond to environmental parameters (e.g., sunlight) in real-time (van Iersel and Gianino, 2017; van Iersel et al., 2016; Weaver et al., 2019). An adaptive lighting control system measures the *PPFD* of the overall light environment (sunlight + supplemental light) at the canopy level and uses that data to reach a target DLI. The *PPFD* of supplemental light is continually adjusted so that sunlight combined with supplemental light reaches, but does not exceed, a target DLI. On overcast or seasonally low DLI days, more supplemental light will be provided to reach the target DLI. On sunny days or days with longer daylength, when sunlight alone exceeds the target DLI, the lights will not provide any supplemental lighting.

To develop effective lighting strategies, it helps to understand how plants absorb and

use light to provide energy for growth. This process starts with plants absorbing light, which is a function of canopy size. Because of their small canopy, seedlings often intercept little of the total light available to them. Growing seedlings at high densities (up to 4,000 plants/m^2) can make supplemental lighting more cost-effective (Graper et al., 1989; van Iersel, 2017).

Photons absorbed by photosynthetic pigments can be used for electron transport in the light reactions of photosynthesis (photochemistry), dissipated as heat, or reemitted as chlorophyll fluorescence. Chlorophyll fluorescence can be used to measure the quantum yield of photosystem II (Φ_{PSII}). This is a ratio between electrons transported through photosystem II (PSII) and absorbed photons, a measure of photochemical efficiency (Maxwell and Johnson, 2000). With Φ_{PSII} and known *PPFD*, the electron transport rate (ETR) through PSII can be estimated (Maxwell and Johnson, 2000). High *PPFD*s are needed for high ETRs, but that also means low Φ_{PSII} values due to excess light energy being dissipated as heat to prevent photoinhibition (Demmig-Adams et al., 2012; Ruban, 2015). Light drives photochemistry more efficiently at lower *PPFD*s (Koontz and Prince, 1986; Soffe et al., 1977). In addition, *PPFD* has a direct effect on plant photomorphogenesis. Plants grown under low light may develop shade acclimation characteristics, including increased leaf area.

These associations led to our hypothesis that the same DLI, provided over a longer photoperiod and hence a lower instantaneous *PPFD*, would increase growth via better light interception (increased canopy size) and more efficient photochemistry. Previously, Weaver and van Iersel (2020) observed that lettuce (*Lactuca sativa* 'Green Towers') growth increased when the same DLI was provided over a longer photoperiod. In this study, (*Rudbeckia fulgida* var. *sullivantii* 'Goldsturm') was selected. It is an obligate long day plant (Yuan et al., 1998) that prefers full sun but can grow in partial shade. Seedlings were grown

in a greenhouse with supplemental LED light to reach a target DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ using an adaptive lighting system.

Materials and Methods

Experimental setup, plant material, and growing conditions. An adaptive lighting system was used to study the effect of providing a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ over four different photoperiod treatments (12, 15, 18, and 21 h). The study was conducted in a glass-covered greenhouse at the University of Georgia in Athens, GA. The adaptive lighting system consisted of five ebb-and-flow trays with inside dimensions of 1.5 m × 0.9 m × 4 cm, covered with landscape fabric (Scotts Pro Weed Control; Greenscapes Inc., Calhoun, GA), and placed side-by-side on a single greenhouse bench. Each tray was a separate block and divided into five sections, for a total of 25 growing sections. Each section was 0.9 m long × 0.3 m wide and separated by aluminum sheet metal dividers, 0.9 m long × 0.3 m high, to prevent light contamination from neighboring sections. Black shade cloth, 3 m wide × 12 m long was draped over the top and sides of the adaptive lighting system to mimic winter DLI (not daylength) conditions. The shade cloth blocked ≈80% of the solar radiation.

One cool-white LED light bar (SPYDRx with PhysioSpec greenhouse spectrum; Fluence Bioengineering, Austin, TX) was mounted 38 cm above the tray bottom in the center of four of the five sections in each block. The LED light bars were connected to and powered by four separate dimmable drivers (SPYDRx; Fluence Bioengineering). Each driver controlled five light bars, with one light bar from each driver assigned to one section in each of the five trays. The dimming inputs of the drivers were connected to an analog output module (SDM-A04A; Campbell Scientific, Logan, UT) that was connected to a datalogger (CR1000; Campbell

Scientific). This resulted in four treatments with and one treatment without supplemental light. Quantum sensors (LI-190; LI-COR BioSciences, Lincoln, NE) were placed in the five growing sections of the center block and connected to the datalogger. The sensors were positioned approximately 10 cm from the southern edge of the tray, 15 cm above the bottom of the tray and centered between the two aluminum dividers. In the four lit sections, the quantum sensors were directly under the light bar. The datalogger recorded the quantum sensor measurements every 15 min and controlled the dimmable LED drivers by sending a 0-10,000 mV signal to the driver via the analog output module. This allowed for precise control of the supplemental *PPFD*, using the approach described by Weaver and van Iersel (2020). Briefly, the datalogger used the *PPFD* measurements to calculate how much light had been received at any time during a day, determined how much additional light was needed to reach a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$, and determined the average *PPFD* required during the remainder of the photoperiod. The datalogger recalculated the required *PPFD* every 2 s. The datalogger also recorded temperature and relative humidity as measured by a combined probe (HMP50; Vaisala, Helsinki, Finland) housed in a radiation shield placed on the bench adjacent to the middle block.

Supplemental lighting treatments consisted of four different photoperiods (12, 15, 18, and 21 h). The DLI in each section with a quantum sensor was 12.0 ± 0.0 $mol \cdot m^{-2} \cdot d^{-1}$ (mean ± standard deviation, SD) from a combination of sunlight plus supplemental light. The section without supplemental lighting (control) received an average DLI of 5.0 ± 1.7 mol m^{-2} d^{-1} from sunlight only. To reach the target DLI for the different photoperiods, supplemental lights turned on at 0000, 0300, 0600, or 0900 $_{HR}$ for photoperiods of 21, 18, 15, and 12 h, respectively, and all lights turned off at 2100 $_{HR}$. The natural photoperiod decreased from 12 h 55 min on 30 Aug. to 11 h 22 min on 14 Oct.

On 20 Aug. 2019, seeds of *R. fulgida* were sown in 72-cell trays (25 cm × 50 cm) filled with a soilless propagation medium (Fafard germinating mix with RSi; SunGro Horticulture, Agawam, MA). The trays were cut in half, creating an experimental unit of 36 plants per tray. Seed trays were covered with a clear plastic humidity dome and placed on a five-shelf stainless steel cart retrofitted with white LED lights (225 LED ultrathin grow light panel, Apluschoice, La Puente, CA) , small fans, and dataloggers (HOBO U12; Onset Computer Corp., Bourne, MA). Seeds germinated under these conditions for 10 d under a 10-h photoperiod, *PPFD* of 279 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and air temperature of 24.3 ± 2.2 ºC (mean ± SD). They were fertigated, as needed, by overhead misting with a 100 $mg \cdot L^{-1}$ N water-soluble fertilizer solution (15N–2.2P–12.5K, Peters Excel 15–5–15 Cal-Mag Special; ICL Fertilizers, Dublin, OH). On 30 Aug. 2019, 25 of the 36-cell trays were thinned to one plant per cell, resulting in a plant density of 600 plants/m^2, and moved to the 25 separate sections of the adaptive lighting system in the greenhouse. During the study period, 30 Aug. to 14 Oct. 2019, plants were subirrigated as needed (every 2 to 3 d) with a 100 $mg \cdot L^{-1}$ N water-soluble fertilizer solution (15N–2.2P–12.5K, Peters Excel 15–5–15 Cal-Mag Special, ICL Fertilizers), the air temperature was 27.0 ± 1.1 ºC (mean ± SD), and the vapor pressure deficit was 1.35 ± 0.13 kPa.

Experimental design, data collection, and analysis. A custom-made imaging system was used to non-destructively measure projected canopy size over the course of the study. The imaging system consisted of a grow tent, 0.6 m wide × 0.6 m long × 1.2 m high with a mylar reflective interior lining, with a LED light fixture (Pro 325e; LumiGrow, Emeryville, CA). A digital monochrome camera (CM3-U3-31S4M-CS; FLIR Systems, Wilsonville, OR) with a band-pass filter (680 to 740 nm; Omega Optical, Brattleboro, VT) was mounted inside

at the top of the grow tent. Chlorophyll fluorescence images of each 36-cell tray were taken weekly, beginning 30 Aug. and ending 11 Oct. 2019, for a total of seven weeks. To collect each image, the blue LEDs (peak λ 440 nm) in the light fixture were turned on and the camera with the band-pass filter captured leaf fluorescence from the chlorophyll in the plant tissue. The chlorophyll fluorescence images were analyzed using ImageJ (National Institute of Health, Bethesda, MD) software to determine projected canopy size.

On 14 Oct. 2019, 55 d after sowing, plants in most treatments had reached a marketable transplant size and were harvested. Some plants died over the course of the study. The number of viable plants (≥ four true leaves) per 36-cell tray were counted. Chlorophyll content index (CCI) was measured on five fully-expanded leaves per experimental unit using a handheld meter (CCM-200 plus; Apogee Instruments Inc., Logan, UT). Five representative plants were selected from each tray and height was measured (from substrate surface to shoot tip). Additionally, shoots were cut at the substrate level, leaves were counted, and total leaf area was measured (LI-3100, LI-COR Biosciences). Finally, foliage was dried in an oven at 80 ºC for 7 d and then weighed. Roots of these plants were washed, dried in an oven at 80 ºC for 7 d, then weighed. Shoots from the remaining plants were cut at substrate level, leaf area measured, dried in an oven at 80 ºC for 7 d, then weighed. Leaf area was calculated as the sum of leaf area of the five representative plants and the leaf area of the remaining plants divided by the number of plants harvested. Specific leaf area (SLA) was calculated as leaf area divided by shoot dry weight. Compactness was calculated as shoot dry weight divided by height. Root mass fraction was calculated as root dry weight / (shoot + root dry weight) based on data from the five representative plants.

This study was designed as a randomized complete block with five blocks (replications) and five treatments per block. JMP Pro (version 14.1.0; SAS Institute, Cary, NC) was used to analyze the data, using analysis of variance (ANOVA) to test for differences among the control and other treatments using Dunnett's test ($\alpha = 0.05$). Effects of the four photoperiods were tested using mixed model regression analyses (linear and quadratic) with photoperiod as a continuous variable and block as a random variable. SigmaPlot (version 11.0; Systat Software, Inc., San Jose, CA) was used to analyze the relationship between projected canopy size and final dry weight.

Results and Discussion

The calculated threshold *PPFD*s at the start of the photoperiod were 158.7, 185.2, 222.2, and 275.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the 21, 18, 15, and 12 h photoperiods, respectively. These thresholds were recalculated constantly throughout the day so that when the *PPFD* from the sun exceeded these thresholds, the threshold *PPFD* decreased to ensure the target DLI was not exceeded by the end of the photoperiod. Thus, early in the day, *PPFD* remained at the initial calculated threshold. As the sunlight became more intense, the supplemental lights were dimmed or completely turned off and the threshold *PPFD* decreased. This decrease in threshold *PPFD* was more pronounced on sunny compared to overcast days (Fig. 3.1). This resulted in precise control and all supplemental lighting treatments received a DLI of 12.0 ± 0.0 $mol \cdot m^{-2} \cdot d^{-1}$ every day.

The number of viable plants (≥ four true leaves) per 36-cell tray ranged from 26 to 35, with an average of 29.8 ± 2.3 plants, and this was not affected by treatment. Shoot and root dry weights of control plants averaged 0.04 and 0.007 g/plant, 83% and 90% lower,

respectively, than the 12-h photoperiod treatment. The difference in dry weight between the control and 12-h photoperiod treatment was much greater than the difference in DLI (≈58%). Randall and Lopez (2015) also reported disproportionate increases in shoot and root dry mass for seedlings in response to supplemental light (control DLI 6.6 $mol \cdot m^{-2} \cdot d^{-1}$, DLI with supplemental light 10.6 $mol \cdot m^{-2} \cdot d^{-1}$). This 38% increase in DLI increased shoot dry mass of vinca (*Catharanthus roseus*), impatiens (*Impatiens walleriana*), and geranium (*Pelargonium* × *hortorum*) seedlings by 50% to 74% and root dry mass by 81% to 104%. In the current study, shoot and root dry weight increased linearly, from 0.23 to 0.30 g/plant (30% increase) and from 0.071 to 0.088 g/plant (24% increase), respectively, as photoperiod increased from 12 to 21 h, while maintaining a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 3.2A and B). Other studies have reported a similar increase in plant dry weight in response to extending the photoperiod while maintaining the total amount of light delivered daily. Soffe et al. (1977) determined that by extending the photoperiod in growth chambers from 12 to 16 h while maintaining a constant 5 $MJ \cdot m^{-2} \cdot d^{-1}$, plant dry weight increased by 20% for radish (*Raphanus sativus*), cabbage (*Brassica olearacea capitata*) and oilseed rape (*Brassica napus*); 40% for spinach beet (*Beta vulgaris*), beetroot (*Beta vulgaris*), and celery (*Apium graveolens*); and 100% for lettuce. Koontz and Prince (1986) reported a 30% to 50% increase in dry weight of four loose-leaf lettuce cultivars by extending the photoperiod from 16 to 24 h, while providing approximately the same DLI (22.4 and 23.9 $mol \cdot m^{-2} \cdot d^{-1}$, respectively). Recently, Weaver and van Iersel (2020) used a similar approach to the one used in this study to extend photoperiods without changing the DLI (17 $mol \cdot m^{-2} \cdot d^{-1}$) and found a 28% increase in dry weight of lettuce grown under a 21 h versus a 12 h photoperiod.

Data from this study show no evidence that photoperiod treatments influenced root mass fraction (Fig. 3.2C). Average root mass fraction of the four photoperiods was 0.23, 26% higher than that of the control plants (0.17).

Plant height increased linearly from 12.8 to 14.6 cm plant (15%) as photoperiods increased from 12 to 21 h (Fig 3.3A). This suggests plants elongated as a shade response to lower *PPFD* associated with longer photoperiod. However, compactness increased linearly from 8.8 to 10.3 $g \cdot m^{-1}$ (17%) as photoperiod increased from 12 to 21 h (Fig. 3.3B), suggesting that increased height was not caused by more elongation, but rather increased growth. Control plants had an average height of 1.17 cm and a compactness of 3.0 $g \cdot m^{-1}$, 54% shorter and a 66% decrease in compactness, respectively, when compared to plants grown under the 12 h photoperiod.

Several factors may contribute to increased plant dry weight in response to an extended photoperiod delivered using methods such as day extension, night break, or providing the same DLI over a longer photoperiod. These include leaf expansion, chlorophyll content, and photosynthesis (Adams and Langton, 2005). Previous studies reported increased leaf expansion with extended photoperiods and a constant DLI (Langton et al., 2003; Milford and Lenton, 1976; Soffe et al., 1977). Increased leaf expansion is often accompanied by an increase in SLA, an indication of larger, thinner leaves (Adams and Langton, 2005). In this study, leaf area increased in a quadratic manner from 56 to 65 cm^2/plant (16% increase) as photoperiod increased from 12 h to 15 or 18 h, and then decreased back to 56 cm^2/plant with a 21-h photoperiod (Fig. 3.3C). Leaf area for the control plants was 15 cm^2/plant. However, SLA decreased linearly from 266 to 197 $cm^2 \cdot g^{-1}$ (26% decrease) as photoperiod increased from 12 to 21 h (Fig. 3.3D). This contradicts the initial hypothesis that lower *PPFD*s

associated with the longer photoperiods would induce a shade acclimation response resulting in larger, thinner leaves and increased SLA. Control plants had a much higher SLA (428 $cm^2 \cdot g^{-1}$) than any of the other treatments with higher DLIs. That is consistent with a shade acclimation response (Evans and Poorter, 2001; Gommers et al., 2013; Gong et al., 2015).

Despite a decrease in SLA with increasing photoperiod, projected canopy size increased linearly from 42.6 to 69.1 cm^2 (week three) and from 240.4 to 358.1 cm^2 (week five) as photoperiod increased from 12 to 21 h , and in a quadratic manner from 768.8 to 910.6 cm^2 for week seven (Fig. 3.4). Variation in projected canopy size, which determines how much light is captured by plants and thus important for growth, helps explain differences in plant dry weight among treatments. Increased projected canopy size with increasing photoperiod was evident early in the study and remained until end of the study. Control plants consistently had a small projected canopy area compared to all photoperiods; compared to the 12-h treatment, this difference widened from 28% after three weeks to 65% after seven weeks.

The importance of canopy size for growth is evident from the positive correlation between weekly projected canopy size and final shoot dry weight (Fig. 3.5). Plants with greater projected canopy size as early as week 2 had a larger final shoot dry weight. Thus, early differences in projected canopy size were indicative of plant growth over the entire study. Other studies had similar conclusions, in that differences in projected canopy size early during the growing cycle were predictive of differences in final biomass of leafy greens (Palmer, Kim, pers. comm.). Among all studies, correlation between projected canopy size and shoot dry weight became stronger over time. The strong correlation between final projected canopy size and shoot dry weight was therefore expected in this study.

Chlorophyll content index increased from 15.5 to 23.0 (48% increase) as photoperiod increased from 12 to 21 h (Fig. 3.6). Control plants had an average CCI of 9, 53% lower than plants grown under a 12-h photoperiod. Increased chlorophyll content, a common shade acclimation response, can increase light absorption (Evans and Poorter, 2001; Givnish, 1988; Nemali and van Iersel, 2004), which may result in increased photosynthesis and growth. Hurd (1973) observed a 34% increase in chlorophyll in tomato *(Lycopersicon esculentum*) grown with a 16-h day compared to an 8-h day, with the same DLI. Based on research by Gabrielsen (1948) on chlorophyll concentration and photosynthetic efficiency, Hurd (1973) estimated the 34% increase in chlorophyll would yield a 6% increase in photosynthesis under low light treatment(s).

A combination of reasons may explain increased growth with longer photoperiods and lower *PPFD*s. First, under low *PPFD*, plants have a higher Φ_{PSII} and presumably more photosynthesis over the course of a day. Previous studies have shown increased Φ_{PSII} in lettuce at lower *PPFD*s (Weaver and van Iersel, 2019, 2020; Zhen and van Iersel, 2017; Zou et al., 2019). At low *PPFD*s, the reaction centers of PSII are predominantly open. Therefore, a longer photoperiod, during which reaction centers are open a greater proportion of the time, will result in greater electron transport through PSII and more efficient photochemistry. In a recent study, Elkins (2020, Ch. 2) reported a 74% and 109% increase in lettuce electron transport integrated over 24 h as the photoperiod increased from 7 to 22 h, with DLIs of 15 and 20 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. Second, increased leaf area, which may be the result of increased photosynthesis and growth, can result in more light capture and greater photosynthesis on a whole-plant basis, thus perpetuating a cyclic positive feedback mechanism. Third, increased chlorophyll can result in increased light absorption and more

photochemistry. These factors likely combined to result in increased growth at longer photoperiods.

Conclusions

Supplemental lighting can increase growth of *R. fulgida* seedlings, especially when supplied over longer photoperiods at a lower *PPFD*. In plug production, root system size is an important indicator of plug quality and primary determinant of transplant readiness. Based on the observed 24% increase in root dry weight of *R. fulgida*, we estimate supplemental light with a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ and 21-h photoperiod can shorten cropping cycles by one to two weeks (55 days in the study period × 0.24 = 13.2 days) compared to a 12-h photoperiod. Shortening the cropping cycle can allow for more crops (turns) per year and increased profits. In addition, growers could install fewer lights to achieve adequate growth responses, reducing initial capital expense.

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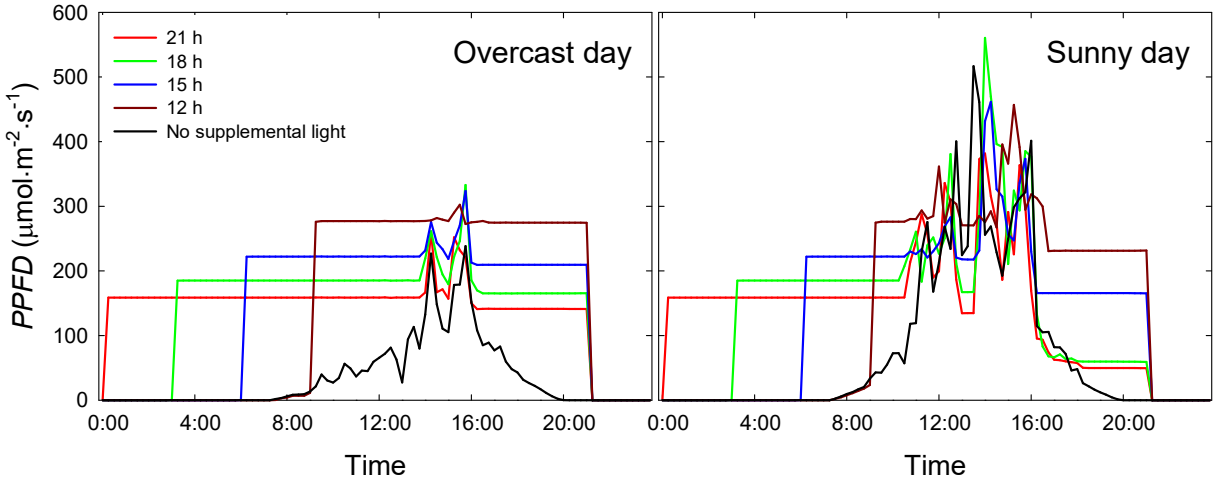

Fig. 3.1. Photosynthetic photon flux density (*PPFD*) over the course of a 24-h period on an overcast day (left) compared to a sunny day (right) as measured by a quantum sensor. The four photoperiod (12, 15, 18, and 21 h) treatments all had a daily light integral (DLI) of 12 $mol \cdot m^{-2} \cdot d^{-1}$.

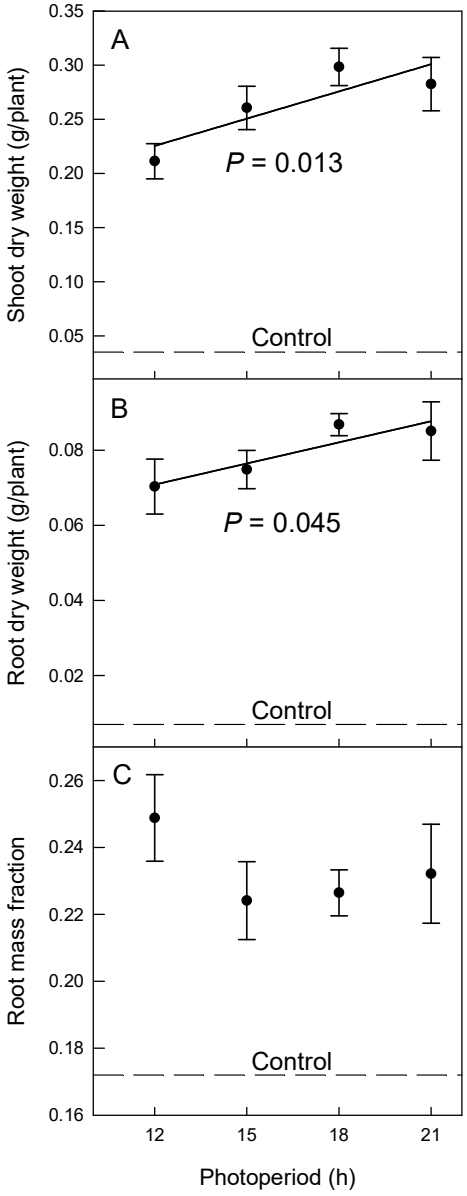

Fig. 3.2. Shoot dry weight (A), root dry weight (B), and root mass fraction (root dry weight / total plant dry weight) (C) of *Rudbeckia fulgida* var. *sullivantii* as a function of photoperiod. All plants received a daily light integral (DLI) of 12 $mol \cdot m^{-2} \cdot d^{-1}$, except for control plants that received no supplemental light (dashed lines, mean DLI of 5.0 $mol \cdot m^{-2} \cdot d^{-1}$). Control plants differed from the four other treatments for all three parameters ($P \leq 0.02$). Regression lines indicate significant effects of photoperiod.

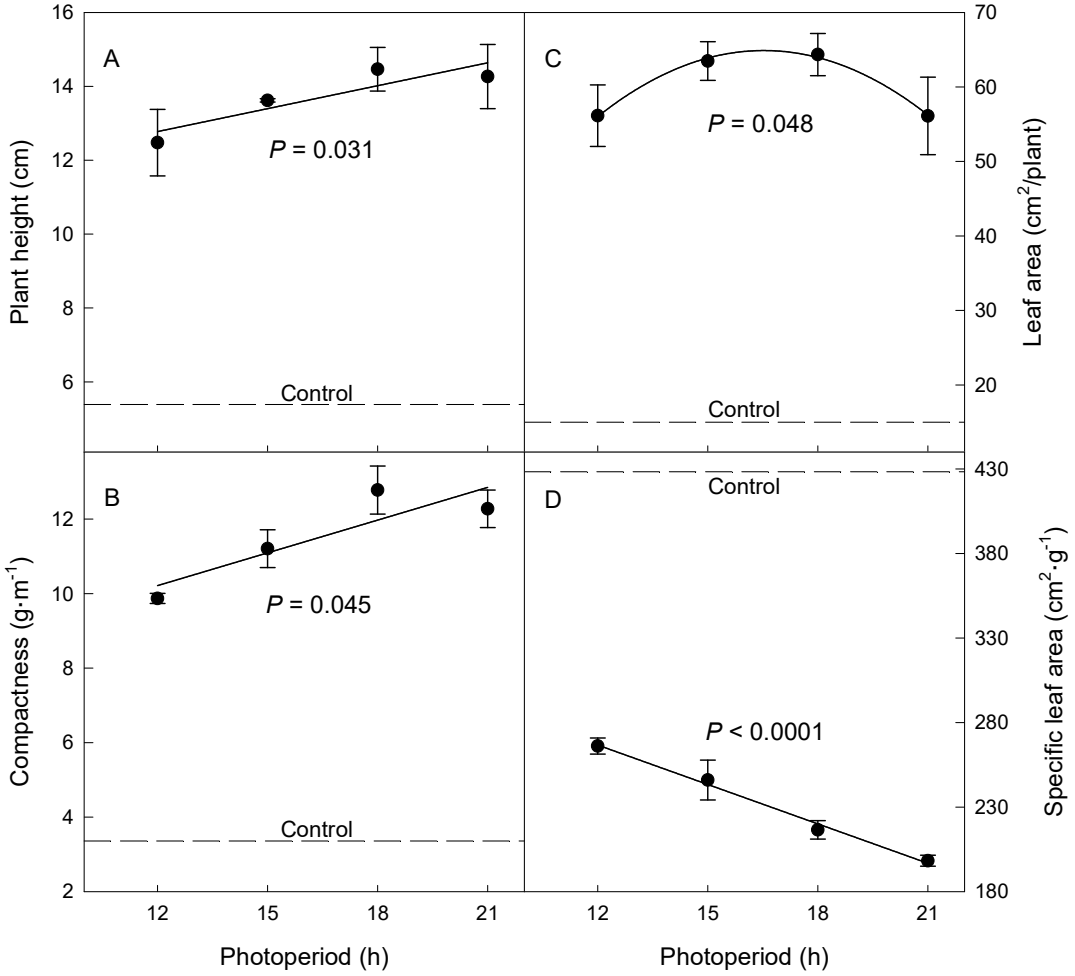

Fig. 3.3. Plant height (A), compactness (B), leaf area (C), and specific leaf area (D) of *Rudbeckia fulgida* var. *sullivantii* plants as a function of photoperiod. All plants received a daily light integral (DLI) of 12 mol·m^{-2}·d^{-1}, except for control plants that received no supplemental light (dashed line, mean DLI of 5.0 mol·m^{-2}·d^{-1}). Control plants differed from the four other treatments for all three parameters ($P < 0.0001$). Regression lines indicate significant effects of photoperiod.

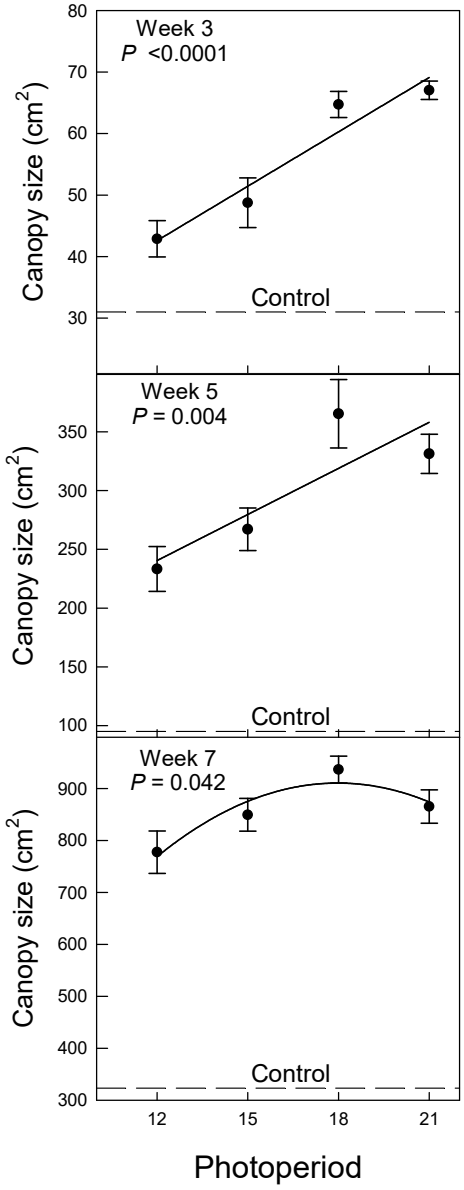

Fig. 3.4. Projected canopy size of *Rudbeckia fulgida* var. *sullivantii* plants as a function of photoperiod for weeks 3, 5, and 7 of the study period. All plants received a daily light integral (DLI) of 12 $mol \cdot m^{-2} \cdot d^{-1}$, except for the control plants that received no supplemental light (dashed line, average DLI of 5.0 $mol \cdot m^{-2} \cdot d^{-1}$). Control plants had significant smaller projected canopy size than plants in any of the other treatments at any of these time points ($P \leq 0.006$).

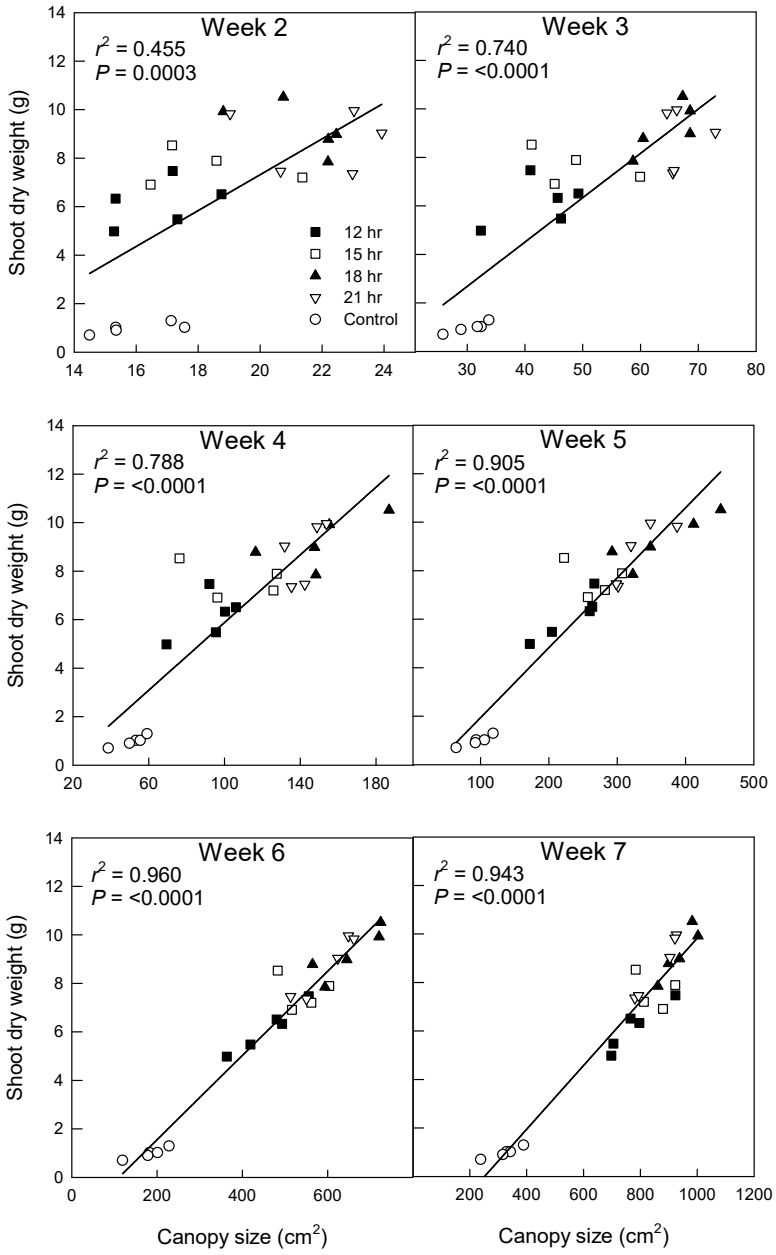

Fig. 3.5. Total final shoot dry weight of *Rudbeckia fulgida* var. *sullivantii* plants as a function of projected canopy size at different times during the growing cycle. The treatments included photoperiods of 12, 15, 18, and 21 h, all with a daily light integral (DLI) of 12 mol·m^{-2}·d^{-1} and a control treatment without supplemental light that received an average DLI of 5 mol·m^{-2}·d^{-1}.

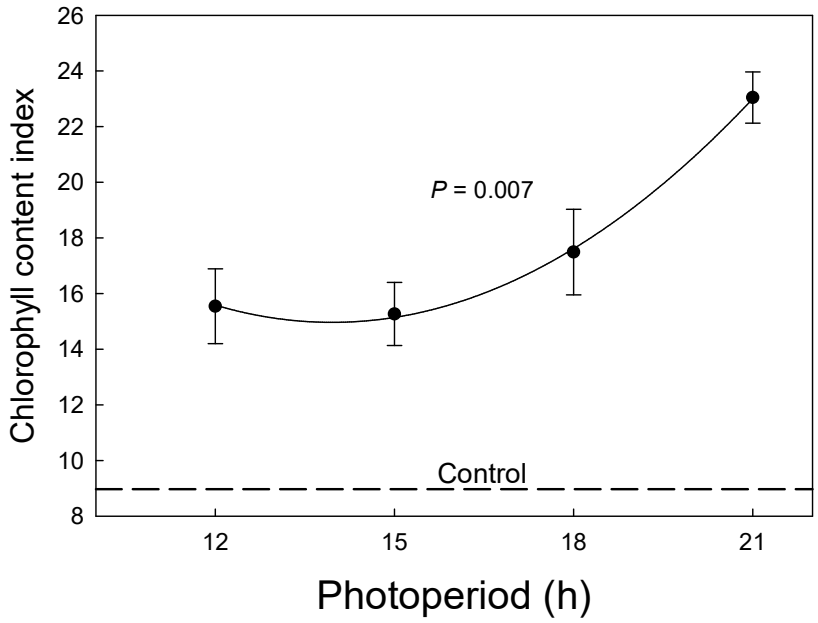

Fig. 3.6. Chlorophyll content index of *Rudbeckia fulgida* var. *sullivantii* plants as a function of photoperiod. All plants received a daily light integral (DLI) of 12 $mol \cdot m^{-2} \cdot d^{-1}$, except for control plants that received no supplemental light (dashed line, average DLI of 5.0 $mol \cdot m^{-2} \cdot d^{-1}$).Control plants had a lower chlorophyll content index than plants in any of the other treatments (P = 0.004).

CHAPTER 4

Supplemental Far-red LED Light Increases Growth of *Digitalis purpurea* Seedlings Under Sole-Source Lighting[1]

[1] Claudia A. Elkins and Marc W. van Iersel. To be submitted to HortTechnology for publication. This work was funded by USDA-NIFA-SCRI Award Number # 2018-51181-28365 Project 'Lighting Approaches to Maximize Profits.'

Abstract

Seedlings may be grown indoors where environmental conditions can be precisely controlled to assure consistent and reliable production. The optimal spectrum for production under sole-source lighting is currently unknown. Far-red light (λ = 700 to 800 nm) is photosynthetically active and can enhance leaf elongation, which may result in larger leaves and increased light interception. We hypothesized that adding far-red to sole-source lighting systems would increase the growth of foxglove (*Digitalis purpurea* 'Dalmatian Peach') seedlings grown under white light-emitting diode (LED) arrays, potentially shortening production times. Our objective was to determine how much far-red light is optimal. Foxglove seedlings were grown in a growth chamber with a photosynthetic photon flux density (*PPFD*) of 186 ± 6.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and supplemental far-red intensities ranging from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$. As far-red increased, shoot dry weight, root dry weight, and plant height increased by 38% ($P = 0.004$), 20% ($P = 0.029$), and 38% ($P = 0.025$) respectively, while root mass fraction decreased 16% ($P = 0.034$). Although we expected supplemental far-red to induce leaf expansion, specific leaf area and compactness, two measures of morphology, were unaffected. Because a 37% increase in total photon flux density (*PPFD* plus far-red) resulted in a 34.5% increase in total plant dry weight, the increased growth likely was due to increased photosynthesis rather than a shade-acclimation response. The growth response was linear across the 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ range of far-fed tested, so we were unable to determine a saturating photon flux density.

Introduction

Young seedlings intercept little of the light available to them in a production space because their leaves are small relative to the size of the production space. For example, black-eyed Susan (*Rudbeckia fulgida* var. *sullivantii* 'Goldsturm') seedlings sown in a 72-cell plug tray (51 cm × 25 cm) had a total canopy size of ≈15 cm^2 after 10 d or ≈1% of the surface area of the tray (Elkins, 2020, Ch. 3). Plants this small may benefit from larger leaves to enhance light interception and presumably canopy photosynthesis and growth. This has the potential to shorten cropping cycles of ornamental plants grown in controlled environments. One approach to achieving enhanced growth is to add far-red light to the spectrum because 1) far-red light can trigger a shade avoidance and/or acclimation response (Franklin, 2008; Keuskamp et al., 2010; Possart et al., 2014) and 2) it is photosynthetically active (Zhen and Bugbee, 2020; Zhen and van Iersel, 2017).

Shade avoidance/acclimation responses are mediated by phytochrome, a pigment-protein photoreceptor that detects light and light quality. Phytochrome has two photo-interconvertible forms, the inactive red (Pr) and the active far-red (Pfr) forms. These allow plants to detect the red to far-red ratio (R:FR) of incident light that mediates a response in plants (Franklin and Whitelam, 2005; Possart et al., 2014; Ruberti et al., 2012). Red light is strongly absorbed, whereas far-red light is poorly absorbed and thus transmitted through leaves. Under-canopy plants are thus exposed to a low R:FR ratio, triggering shade responses (Casal, 2013; Franklin and Whitelam, 2005; Gommers et al., 2013). In response to shade, plants may respond morphologically, such as via elongated stems and petioles (shade avoidance) (Franklin, 2008; Franklin and Whitelam, 2005) or acclimate with traits such as increased specific leaf area (SLA) (shade tolerance) (Evans and Poorter, 2001; Gommers et

al., 2013; Gong et al., 2015).

For decades, far-red light has been considered outside of the range of photosynthetically active radiation (*PAR*, 400 to 700 nm) as established by Hoover (1937), McCree (1971) and Inada (1976), even though they demonstrated that far-red had at least some photosynthetic activity. Recently Zhen and van Iersel (2017) found a synergistic interaction between far-red and *PAR*, specifically an increase in net photosynthesis of lettuce (*Lactuca sativa*) in response to added far-red. Zhen and Bugbee (2020) found that adding far-red light (up to 40% of *PAR*) increased canopy photosynthesis of 14 diverse crop species while providing the same amount of *PAR*.

Previous studies examining the effect of far-red light on plant growth (Hurt et al., 2019; Li and Kubota, 2009; Meng et al., 2019; Meng and Runkle, 2019; Park and Runkle, 2016; 2017; Zou et al., 2019) and photosynthesis (Zhen and Bugbee, 2020; Zhen and van Iersel, 2017) have evaluated several intensities of far-red light ranging from 0 to 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Saturation levels have been determined for leaf and whole-plant photosynthesis, but not for growth response. Our objective was to evaluate18 intensities of supplemental far-red light, ranging from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, on the growth and morphology of foxglove (*Digitalis purpurea* 'Dalmatian Peach'), a species that performs well under a wide range of light conditions. We hypothesized that providing supplemental far-red light would result in leaf expansion, increased photosynthesis, and therefore more growth, but above a certain intensity, plant morphology would no longer respond to further increases in far-red light.

Materials and Methods

Controlled environment set up and treatments. A 54 m^3 walk-in cooler previously converted into a controlled environment growth chamber was used for this study. It contained three metal shelving racks with three, 2.4 m × 0.6 m shelves per rack. Ebb and flow trays were placed on each shelf and connected to a subirrigation system. Foil-covered Styrofoam was used to divide each shelf into two equal 1.2 m × 0.6 m growing sections for a total of 18 sections. The ends of each growing section were closed off with Styrofoam and an opaque black curtain was hung between the racks to prevent light pollution between neighboring sections. In each section, two white light-emitting diode (LED) light bars (SPYDRx Plus with PhysioSpec indoor spectrum; Fluence Bioengineering, Austin, TX) were installed 37.5 cm above the tray bottom. The photosynthetic photon flux density (*PPFD*) in each section was measured with a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT) and averaged 186 ± 6.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± SD) plus 4.0 to 4.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (700–800 nm). Five of the six sections in each rack were equipped with a custom-made dimmable far-red LED light bar (Fluence Bioengineering Inc.) installed 37.5 cm above the tray bottom to provide additional far-red light. The 15 far-red LED light bars were connected to five separate dimmable drivers (PLD100-ITCF14A-2100; FSP Powerland Technology Inc., Nanjing, China). Three far-red light bars, one from each rack, were connected to each dimmable driver. Dimming inputs of the drivers were connected to a 4-channel analog output module (SDM-AO4A; Campbell Scientific, Logan, UT), that was connected to a datalogger (CR6; Campbell Scientific), which allowed us to control the amount of additional far-red light by providing 0 (light off) to 10,000 mV (light on full power) dimming signals to the drivers.

Because all the far-red light bars provided different amounts of far-red at full power, we were not able to achieve five distinct levels of far-red light. Instead, we aimed to get a wide range of far-red intensities, with those intensities as evenly distributed as possible. This resulted in far-red light intensities ranging from of 7.9 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ among the 15 sections with a far-red light bar. Fig. 4.1 shows the spectral distribution of the white LED light bars with added low or high amounts of far-red. To measure the light spectrum, 18 readings, evenly spaced to cover the 1.0 m × 0.25 m area directly below the light bars were taken in each of the 18 sections using a spectroradiometer (SS-110; Apogee Instruments Inc.) and SpectroVision software (version 1.02.005, Apogee Instruments Inc.) with the integration range set to 700 to 800 nm to quantify the total amount of far-red in the spectrum. Table 4.1 shows the total far-red light (700–800 nm) in each treatment and corresponding values of phytochrome photoequilibrium (PPE) and red to far-red ratio (R:FR) (R; 635–685 nm, FR; 710–760 nm), as reported by SpectroVision. The default wavebands used by SpectroVision to calculate R:FR coincide with those with the greatest effect on PPE. Table 4.1 also includes the calculated *PPFD* to far-red ratio (*PPFD*; 400–700 nm, FR; 700–800 nm) and percent far-red calculated as: far-red (700–800 nm) / [*PPFD* (400–700 nm) + far-red (700–800 nm)] × 100.

Sensors were connected to a datalogger (CR6; Campbell Scientific)to measure and record environmental conditions in the chamber. Air temperature was controlled with a thermostat (PENN A19BAC, Johnson Controls, Milwaukee, WI) and humidity was controlled with a dehumidifier (FAD704DWD13; Electrolux, Charlotte, NC). Air temperature and relative humidity were measured with a combined probe (HMP50; Vaisala, Helsinki, Finland). Carbon dioxide (CO_2) concentration was measured with a CO_2 transmitter (GMC20; Vaisala), and the datalogger maintained the desired CO_2 concentration by triggering a solenoid valve to open and

release CO_2 from a compressed gas cylinder for 1 s intervals whenever the CO_2 concentration dropped below 800 μmol·mol^{-1}.

Plant material and growing conditions. On 25 Sept. 2018, foxglove (*Digitalis purpurea* 'Dalmatian Peach') seeds were sown in 72-cell trays filled with a soilless growing medium (Fafard 3B; SunGro Horticulture, Agawam, MA). One seedling tray was placed in each growing section. Seeds were germinated under white LED light only. Plants were subirrigated as needed, every 2 to 3 d, with a 100 ppm N water-soluble fertilizer solution (15N–2.2P–12.5K, Peters Excel 15–5–15 Cal-Mag Special; ICL Fertilizers, Dublin, OH). On 16 Oct. 2018, 21 d after seeds were sown, trays were thinned to one plant per cell and the far-red light treatments were initiated. Over the duration of the study, plants were grown at an air temperature of 21.5 ± 0.2 ºC, a 16 h photoperiod, *PPFD* of 186 ± 6.4 μmol·m^{-2}·s^{-1}, daily light integral (DLI) of 10.7 ± 0.4 mol·m^{-2}·d^{-1}, vapor pressure deficit of 1.3 ± 0.1 kPa, and CO_2 concentration of 800 ± 15 μmol·mol^{-1} (mean ± SD).

Plant measurements and analysis. On 19 Nov. 2018, 55 d after the seeds were sown, plants were harvested. Chlorophyll content index (CCM-200 plus; Opti-Sciences, Hudson, NH) measurements were taken on five mature leaves per treatment. The number of plants per 72-cell tray were counted. Five representative plants from each treatment tray were removed. Height (from media surface to shoot tip) was measured, leaf number was counted, and leaf area (LI-3100; LI-COR Biosciences, Lincoln, NE) was measured. Shoots were cut from the roots and dried in an oven at 80 ºC for ≥ 5 d to obtain dry weight. Roots were washed and dried as well. Shoots from the remaining plants in each tray were removed and dried to obtain dry weight. Mean shoot dry weight was calculated by adding the shoot dry weight of the five representative plants to the shoot dry weight of the remaining harvested plants, divided by the number of plants

harvested. For the five representative plants of each treatment, SLA was calculated by dividing total leaf area by total shoot dry weight, compactness was calculated by dividing mean shoot dry weight by mean height, and root mass fraction was calculated by dividing total root dry weight by total dry weight. SigmaPlot (version 11.0; Systat Software, Inc., San Jose, CA) was used to analyze the data using linear regression.

Results and Discussion

The number of viable plants (≥ four true leaves) harvested per 72-cell tray ranged from 41 to 72 with an average of 60 plants, and this was not affected by treatment. Foxglove seedlings that received more far-red light were larger, i.e., they had greater shoot and root biomass and were taller. Shoot dry weight increased linearly from 0.32 to 0.44 g/plant (38%) as far-red light increased from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4.2A). Previous studies reported similar increases in shoot dry weight in lettuce when far-red light was added to a constant *PPFD* (Meng and Runkle, 2019; Stutte et al., 2009) or when replacing some of the *PPFD* with far-red (Li and Kubota, 2009). Park and Runkle (2017) also reported increased shoot dry weight in geranium (*Pelargonium ×hortorum*), petunia (*Petunia ×hybrida*), and snapdragon (*Antirrhinum majus*), but not shade-tolerant impatiens (*Impatiens walleriana*) in response to added far-red, highlighting species-specific responses. This study observed a less-pronounced increase in root dry weight. As far-red light increased from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, root dry weight increased linearly from 0.083 to 0.100 g/plant (20%) (Fig. 4.2B). Meng and Runkle (2019) found a similar increase in root dry weight of red oakleaf lettuce ‘Cherokee’ (25%) and basil (*Ocimum basilicum*) (18% to 26%) when 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far red light was added to a mixture of blue and red light.

Root mass fraction decreased linearly from 0.198 to 0.166 (16%) as far-red light increased from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4.2C). This finding was not unexpected, and we consider two possible explanations for our results: 1) far-red light can increase biomass allocation for above-ground plant tissue, a shade-acclimation response to increase light capture (Valladares et al., 2016), or 2) it may simply be the result of bigger plants having a lower root mass fraction (Elkins, 2020, Ch. 3). Additionally, the increased plant growth with increased far-red light may be due to 1) a shade response resulting in a larger canopy and 2) increased photosynthesis.

Shade response. Two morphological responses to shade are commonly observed in plants. First, stem elongation is a typical shade-avoidance response mediated by phytochrome, which detect changes in the R:FR ratio (Franklin, 2008; Morgan and Smith, 1981; Ruberti et al., 2012). Added far-red light can increase stem elongation (Li and Kubota, 2009; Park and Runkle, 2017; Yang et al., 2012). In this study, as far-red light increased from 4.0 to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, plant height increased linearly from 8.4 to 11.6 cm, or by 38% (Fig. 4.3C), suggesting a shade-avoidance response. However, plant compactness, an indicator of stem elongation resulting from a shade-avoidance response, was not affected by far-red (Fig. 4.3D). Second, increased leaf expansion is a typical shade-acclimation response, which may also result in thinner leaves and increased SLA (Franklin, 2008). Prior studies have reported increased leaf area with addition of far-red light (Li and Kubota, 2009; Park and Runkle, 2017; Yang et al., 2012). However, our data for foxglove show no significant effect of far-red light on leaf area or SLA (Fig. 4.3A and B).

Increased photosynthesis. Far-red light increases the efficiency with which shorter wavelengths of light can be used for photosynthesis (Zhen and van Iersel, 2017). Photons with wavelengths up to at least 732 nm are photosynthetically active (Zhen et al., 2018). Recently,

far-red photons were shown to be as photosynthetically active as those in the 400 to 700 nm range (Zhen and Bugbee, 2020). Far-red light may thus increase growth through increased photosynthesis. Our lighting treatments supplied an average *PPFD* of 186 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and we added up to 69 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light, a 37% increase in total photon flux density. This resulted in a 34% increase in plant dry weight. The lack of far-red effects on compactness, leaf area, and SLA, combined with the known photosynthetic activity of far-red light suggests that the addition of far-red increased overall growth by increasing photosynthesis, rather than through shade avoidance/acclimation responses. It cannot be ruled out that an increase in *PPFD* would have a similar effect on growth of foxglove as increased far-red light.

Conclusions

The size of the root system is an important indicator of plug quality because that determines when the plug is ready to transplant. Based on the observed 20% increase in root dry weight of *D. purpurea*, we estimate that added far-red can shorten the cropping cycle by at least one week. The increased plant growth in response to added far-red was likely due to increased photosynthesis, rather than a shade-acclimation response, because we found no significant effect of far-red light on leaf area, SLA, and compactness, typical measures of a shade response. Because the observed effects appear to be due to the photosynthetic rather than morphological effects of far-red light, it is possible that increased *PPFD*, rather than far-red, would result in similar increases in growth.

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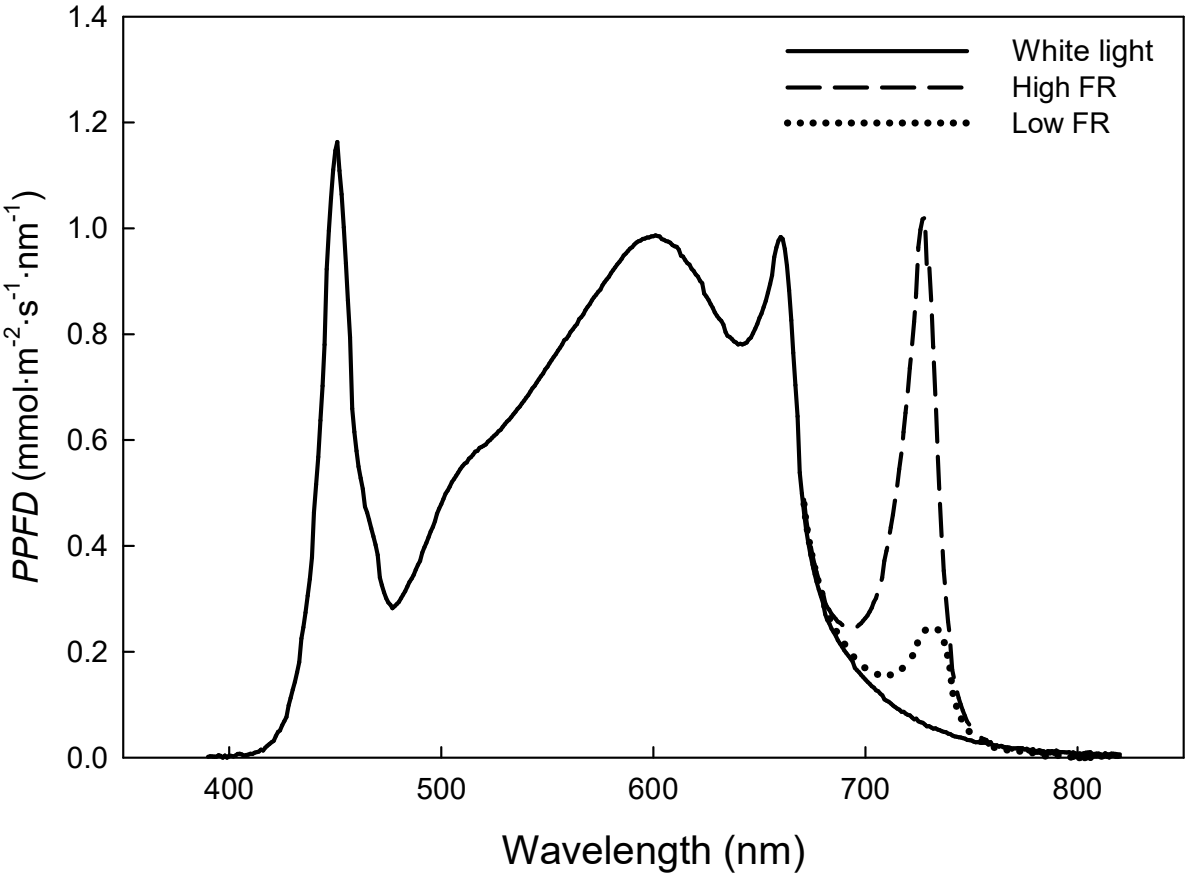

Fig. 4.1. Spectral distribution of white light bars with added low or high amounts of far-red light.

Table 4.1. Phytochrome photoequilibrium (PPE), red to far-red ratio (R:FR), *PPFD* to far-red ratio (*PPFD*:FR), and percent far-red corresponding to the total amount of far-red light provided in each treatment. The two white LED light bars in each section provided some far-red light. The top three rows represent treatments without a far-red LED light bar.

Far-red ($\mu mol \cdot m^{-2} \cdot s^{-1}$)[z]	PPE[y]	R:FR[x]	*PPFD*:FR[w]	Percent FR[v]
4.0	0.86	13.7	45.1	2.2
4.4	0.86	13.7	43.9	2.2
4.7	0.86	12.1	37.8	2.6
7.9	0.85	6.2	23.8	4.0
9.2	0.84	5.1	20.1	4.7
10.5	0.84	4.3	16.8	5.6
11.1	0.83	4.0	16.3	5.8
12.5	0.83	3.5	14.3	6.5
12.8	0.83	3.7	14.9	6.3
13.4	0.83	3.5	13.9	6.7
24.7	0.79	1.8	7.4	11.9
37.4	0.75	1.1	4.8	17.3
48.5	0.73	0.9	3.9	20.5
49.5	0.73	0.9	3.8	20.7
63.3	0.70	0.7	3.2	24.0
63.6	0.70	0.7	3.0	25.0
64.4	0.70	0.7	3.1	24.7

68.8	0.68	0.6	2.7	26.9

[z] Amount of far-red light: photon flux integral between 700 and 800 nm.

[y] PPE: Phytochrome photoequilibrium (Sager et al., 1988).

[x] R:FR: Ratio of photon flux integral of red (R; 635–685 nm) to far-red (FR; 710–760 nm).

[w] *PPFD*:FR: Ratio of photosynthetic photon flux density (*PPFD*; 400–700 nm) to far-red (FR; 700–800 nm).

[v] Percent FR: far-red (700–800 nm) / [*PPFD* (400–700 nm) + far-red (700–800 nm)] × 100.

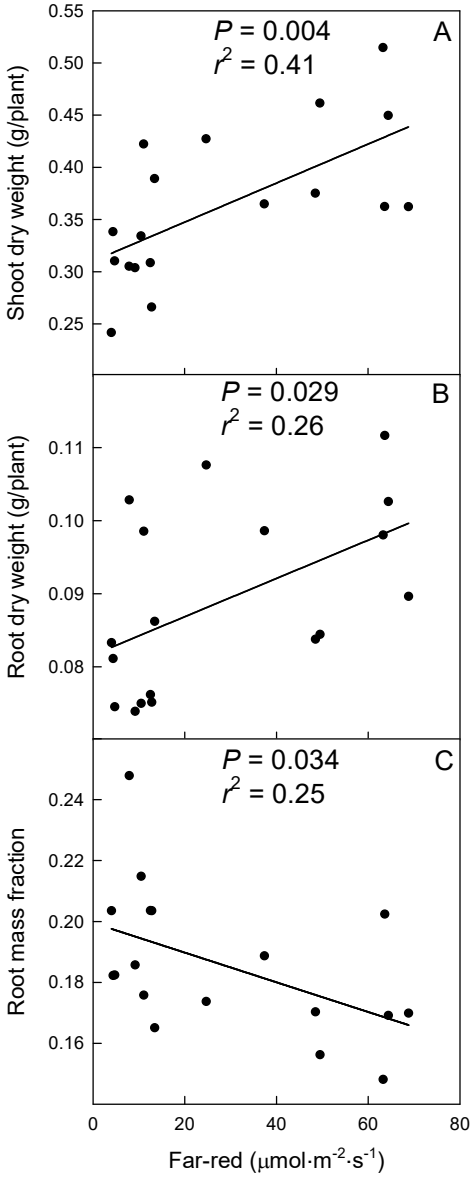

Fig. 4.2. Shoot dry weight (A), root dry weight (B), and root mass fraction (root dry weight / total plant dry weight) (C) of foxglove as a function of the amount of far-red light (λ 700–800 nm) provided. Plants also received an average photosynthetic photon flux density (*PPFD*) of 186.0 ± 6.4 μmol·m^{-2}·s^{-1}.

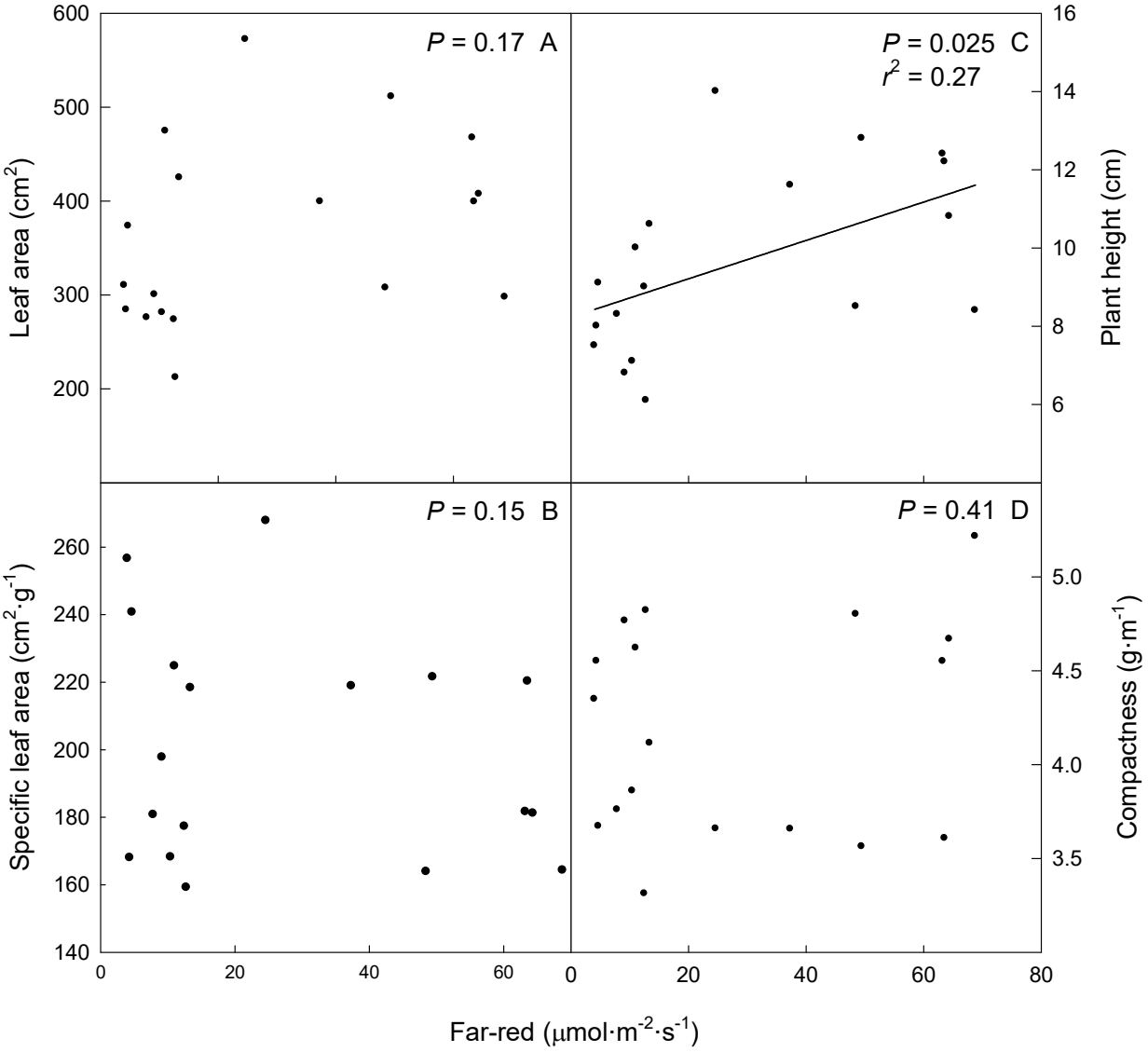

Fig. 4.3. Leaf area (A), specific leaf area (B), plant height (C), and compactness (D) of foxglove plants as a function of the amount of far-red (λ 700–800 nm) light provided.

CHAPTER 5

Conclusions

The main concepts that light is used more efficiently to drive photosynthesis at lower photosynthetic photon flux densities (*PPFD*) and that light spectrum can cause physiological and morphological responses in plants influenced this research. Our goal was to study how efficiently plants use the light they receive and develop lighting strategies to improve crop growth and quality. To accomplish this, tools to measure chlorophyll fluorescence and the unique qualities, dimmability and choice of spectrum, of light-emitting diodes (LEDs) were used.

Our first study, a short-term physiological study, confirmed the hypothesis that a similar daily light integral (DLI) provided over a longer photoperiod at a lower *PPFD*, would result in a greater daily photochemical integral (DPI). In lettuce, the effect of photoperiod on DPI was greater with the higher DLI, 20 versus 15 $mol \cdot m^{-2} \cdot d^{-1}$, because of the wider range of *PPFD*s, which in turn resulted in a wider range of values for quantum yield of photosystem II (Φ_{PSII}).

Findings from our first study were applied in a greenhouse setting, using an adaptive lighting control system, where a combination of sunlight and supplemental light was used to reach the same target DLI over different photoperiods. Results confirmed our hypothesis that providing *Rudbeckia fulgida* with the same DLI over a longer photoperiod at lower *PPFD*s would increase growth through better light interception and more efficient photochemistry. However, the large increases in DPI (74% to 109%) with longer photoperiods at lower *PPFDs* we saw in our first study translated into much smaller increases in growth (24% RDW and 30% SDW) in our greenhouse study. In addition, based on specific leaf area (SLA) and compactness,

longer photoperiods did not induce a shade response, as originally expected. Improved growth, especially root growth, is important in plug production because root system size is one of the most important indicators of plug quality and a primary determinant of transplant readiness. Growing plants under longer photoperiods with lower *PPFD* may shorten the production cycle.

The addition of far-red light can induce a shade-acclimation response, resulting in larger leaves that can capture more light, and is also photosynthetically active. In our third study, we hypothesized that adding far-red light to a broad-spectrum white light would result in larger leaves, more photosynthesis, and therefore more growth. Another aim was to determine the optimal intensity of far-red light. Increased growth was observed in *Digitalis purpurea* seedlings as far-red light (up to 68.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increased. However, increased growth in response to added far-red was likely due to increased photosynthesis, rather than a shade-acclimation response, because no significant effect of far-red light on leaf area, SLA, and compactness, typical measures of a shade response, was observed. Because the observed effects suggest a photosynthetic rather than morphological effect of far-red, it is possible that increased *PPFD*, rather than far-red, would result in similar increases in growth. Repeating this study using the same far-red light levels and adjusting the intensities of the white light, to hold the *PPFD* constant, could answer this question. In addition, because the growth response to increased far-red was linear across the range of far-red *PPFD*s tested, the photon flux density at which far-red responses saturate are unknown.

This research quantified the effect of photoperiod on both plant physiology and growth, but future research is needed to determine why large effects on DPI do not translate into similar large growth effects. Also, this research was unable to explain the mechanism for the observed growth responses under far-red light, although we specualte that it was due to the photosynthetic

activity of far-red light. This research, as a whole, has practical implications for the horticulture industry. Because providing supplemental light over longer photoperiods with lower *PPFD* or adding far-red light can result in more plant growth, cropping cycles could be shortened by one or two weeks, which means more crops per year and increased profits. In addition, growers could install fewer lights required for adequate growth, which would reduce capital expenses.