PROMOTING FLOWERING OF CHRYSANTHEMUM AND PETUNIA WITH NOVEL NIGHT INTERRUPTION APPROACHES

by

ERIC JOSEPH STALLKNECHT

(Under the Direction of Marc W. van Iersel)

ABSTRACT

Many horticultural crops require photoperiodic manipulation to initiate flowering and obtain a salable product when grown out of season. The current studies aim to reevaluate two common photoperiod manipulation practices by using light emitting diodes' (LEDs) innate production of narrow bandwidths of light and their instantaneous output regulation. Night interruptions (NI) with varying amounts of narrow bandwidth far-red (FR) (peak at 730 nm) were unable to promote flowering of the short-day plant *Chrysanthemum morifolium*. Far-red light caused shade avoidance syndrome (SAS) late in skotoperiods when circadian-controlled internal factors are most abundant. Cyclical NI with simulated irrigation booms slightly hastens flowering of the long-day plant *Petunia* × *hybrida* while causing no reductions in number of inflorescence. An industry standard of 4-hrs of continuous NI resulted in faster flowering and more inflorescences than any cyclical NI frequency tested. Both novel night interruption approaches need refinement for more realistic use.

INDEX WORDS: Light emitting diode, LED, controlled environments, cyclical, far-red, phytochrome, phytochrome-photoequilibrium, PPE, shade avoidance syndrome

PROMOTING FLOWERING OF CHRYSANTHEMUM AND PETUNIA WITH NOVEL NIGHT INTERRUPTION APPROACHES

by

ERIC JOSEPH STALLKNECHT

BS, University of Georgia, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

PROMOTING FLOWERING OF CHRYSANTHEMUM AND PETUNIA WITH NOVEL NIGHT INTERRUPTION APPROACHES

by

ERIC JOSEPH STALLKNECHT

Major Professor:	Marc van Iersel
Committee:	Paul Thomas
	Erico Mattos
	Anish Malladi

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2018

DEDICATION

I would like to personally dedicate this thesis to the love of my life, family, friends, and mentors who have supported me throughout this journey. While individual names are too numerous to list, know, if you are reading this dedication than you are very much the key to my success and I could never thank you enough.

ACKNOWLEDGEMENTS

Firstly, I would like to give a very special acknowledgement to the Horticulture department and its faculty. You welcome students with open arms and nurture their growth and development with enthusiasm and encouragement. Dr. Paul Thomas and Dr. Tim Smalley who provided me opportunities during my undergraduate studies that fueled my desire to further my education.

I could never begin to repay Dr. Marc van Iersel for all the guidance and support he has given me the past two years. Working in your lab has been a privilege and one of the best experiences of my life. Above all else, I will always have the pleasure of calling you a friend.

I would very much like to thank our lab technicians, Sue Dove and Michael Martin, who have answered my endless questions and helped fix everything I broke along the way. None of this would have been possible without you.

Last, but certainly not least, I would like to acknowledge my parents and the love of my life for their unwavering inspiration. Seeing the work ethic needed to be successful was the greatest gift my parents could have ever bestowed upon me and is undoubtedly the most valuable lesson I have ever learned. The love and care given to me by Annika Kohler has been eternal. You were with me before this journey began and I cannot wait to see you succeed through yours.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER

1 Introduction and Literature Review 1

Introduction 1

Literature Review 3

Literature Cited 14

2 Morphological and Developmental Responses of *Chrysanthemum morifolium* to Night Interruption with Different Red:Far-red Ratios 19

Abstract 20

Introduction 20

Materials and Methods 23

Results 26

Discussion 28

Literature Cited 31

Tables and Figures 36

3 Night Interruption with Light Emitting Diodes Applied Using Simulated Moving Greenhouse Booms Promotes Flowering of *Petunia* × *hybrida* 41

Abstract 42

Introduction 42

Materials and Methods 45

Results 49

Discussion 50

Literature Cited 55

Tables and Figures 57

4 Conclusions 64

LIST OF TABLES

Page

Table 2.1: Multiple regression analysis of phytochrome-photoequilibrium, night interruption application times, and their interaction on *Chrysanthemum morifolium* morphology and flowering 36

Table 3.1: Growth chamber environmental conditions during cyclical night interruption of *Petunia* × *hybrida* 57

Table 3.2: *Petunia* × *hybrida* cyclical night interruption properties 58

LIST OF FIGURES

Page

Figure 2.1: Spectroradiometer readings of main lighting source in growth chamber 37

Figure 2.2: Average phytochrome-photoequilibrium distribution over experimental units.....38

Figure 2.3: Phytochrome-photoequilibrium and night interruption time effects on the morphology of *Chrysanthemum morifolium*.....39

Figure 2.4: Night interruption timing and phytochrome-photoequilibrium effects on the number of days to first open flower of C*hrysanthemum morifolium*.....40

Figure 3.1: Spectroradiometer readings of sole-source lighting in growth chamber and germination room for *Petunia* × *hybrida*.....60

Figure 3.2: Photosynthetic photon flux density over time of two randomly selected cyclical night interruption treatments for *Petunia* × *hybrida*.....61

Figure 3.3: Light contamination measured in a control treatment for *Petunia* × *hybrida* 62

Figure 3.4: The effects of cyclical night interruption on *Petunia* × *hybrida* growth and development.....63

CHAPTER 1

Introduction and Literature Review

Introduction

Controlled environments use electrical lighting to expand growing seasons and alter plant development to produce high quality products (Morrow and Robert, 2008). High pressure sodium lamps and incandescent bulbs have traditionally provided this electrical lighting. Light emitting diodes (LEDs) can advantageously replace these older lighting sources because of their reduced operating costs, increased intensities and electrical efficiencies, and controllable light spectrum (Morrow and Robert, 2008; Tan et al., 2012). These characteristics make them practical tools for growers to control crop growth and development while helping researchers broaden their understanding of photomorphology. For our purposes, LEDs' production of narrow bandwidths of light make them unrivaled tools to study how different wavelengths of light effect plant growth and development.

Because LEDs are still expensive options compared to older light sources, it is important, and the primary goal of this research, to expand on their current range of uses and capabilities. Furthering knowledge into LEDs' best practical applications, coupled with their advancing sophistication, will help lead to their eventual incorporation into controlled environments.

Flowering of some out-of-season ornamental crops requires photoperiod manipulation to achieve proper floral development. For instance, flower initiation of *Chrysanthemum morifolium* (mums), a photoperiodic short-day (SD) plant, in long summer months is currently achieved by

physically darkening crops with opaque cloth; creating non-natural short days. Performing this task each day requires excessive labor or expensive automated systems. Using night interruption with specific wavelengths of light to promote flowering of mums could reduce costs for growers by removing the above-mentioned labor and materials. It is hypothesized that reducing the phytochrome-photoequilibrium (PPE) of the growing environment with narrow bandwidth far-red LEDs can induce flowering during noninductive long days. This is an understudied area of horticulture and could expand the usefulness and practicality of LEDs.

Reducing the number of LEDs required to provide night interruption in large greenhouses could contribute to their use around the country. Because installing LED fixtures is still costly, a simple way to decrease large initial investments is to simply have fewer LEDs performing an identical task in a unique way. Typically, night interruption of long-day (LD) plants uses stationary overhead lighting systems. Instead of fixed overhead lights, I am hoping to move the lights themselves around greenhouses to accomplish the same goal. Many greenhouses have movable irrigation booms, capable of supporting LEDs fixtures attached to the bottom. These booms could then hypothetically systematically apply night interruption throughout a greenhouse section to promote flowering. This new technique requires quantification of light requirements to promote flowering of specific long-day crops and adapting that information for use on greenhouse booms.

Literature review

Light Emitting Diodes

Light emitting diodes' (LEDs) advantages over traditional lighting sources make them an emerging technology in the horticultural industry. LEDs' inherent ability to manipulate light intensity and spectrum make them extremely adaptable to changing crop needs (Schubert et al., 2005; Schubert et al., 2006) and desirable growing tools. Yet, the economic feasibility of switching to an all-LED lighting system was out of the question until recently. Thanks to their use in electronic devices, primarily displays, they have proliferated in the marketplace. Economics of scale have driven down production prices per unit. LED light output and electrical efficiency have increased dramatically as they became more advanced (Tan et al., 2012). Some LEDs now have better electrical efficiency than high pressure sodium lamps typical used in greenhouses (Bugbee, Both, unpublished). Trends of decreasing price and increasing power are projected to continue over time (Tan et al., 2012). This supports the possibility of LEDs becoming the sole source of supplemental lighting in controlled environments.

Currently, the economic feasibility of switching to LEDs is debated. One perspective argues that despite high initial investment costs, LEDs will eventually pay for themselves from energy savings and reduced replacement costs over their extremely long lifespans (Ouzounis et al., 2015), about 100,000 hours (Folta et al., 2005) compared to high pressure sodium lamps. However, high pressure sodium lamps and replacement bulbs are much cheaper than LEDs and are often more or equally energetically efficient. These differing perspectives put LED economics in limbo because they both hold merit. As LEDs continue to develop we will gain a more definitive answers of whether LEDs high investment costs are practical. In the interim,

LED controllability, and number of applications, can hopefully be improved to help offset these high initial capital costs.

LEDs' controllability is derived from two manipulative properties of these lights; light quantity and light quality. Firstly, light quantity measures how much light reaches an area, measured in $\mu mol \cdot m^{-2} \cdot s^{-1}$. There are three approaches of controlling LED light quantity: distance, current regulation, and pulse width modulation. As LED fixtures move toward a crop canopy light intensity increases. Light emitting diodes radiate less heat than high pressure sodium lamps, which can often damage plants, allowing for closer placement to crops without physical damage. Rather than radiating heat, LED dissipate excess heat from heat sinks. LEDs, like other lights, can still damage plant tissue if in extreme proximity. LEDs can easily be dimmed by adjusting the electrical current, operating under maximum capacity to reduce heat generation or excess light that may damage plant tissue. Photons given off relate proportionally to the electrical current moving through the LED. Less electricity = fewer photons, more electricity = more photons. Lastly, LEDs can turn on and off instantly because they are solid state devices (Marrow, 2008; Schubert et al., 2005; Schubert et al., 2006). No warm up, or cool down period is required like older lighting sources. This can be used to manipulate the amount of light given off by LEDs, using pulse width modulation (PWM). The fraction of time within each on/off cycle at which lights are pulsed for is called duty cycle. Lights are turned on and off very rapidly (milli- to micro seconds) allowing for precise amounts of light to reach crops. The number of ways light quantity can be regulated make LEDs unique, lending themselves to greater applications in light control systems.

Light emitting diodes are better at manipulating light quality than traditional lights. They can produce narrow bandwidths of light in a plethora of colors (wavelengths). What wavelength

LEDs produce is easily controlled in two ways; by altering diode gap distance or changing the materials that constitute the semiconductor itself (Haitz et al., 1995). Coatings may also be applied to LEDs to alter one narrow bandwidth into a wider spectrum of visible light. For instance, white LEDs are typically blue LEDs with phosphorous coatings. LEDs of various wavelengths can also be aggregated together to further expand the spectra reaching plants when working in unison or be controlled individually to give a desired spectrum. Specific spectra of light induce unique physiological responses in plants; whether that be blue light keeping propagated cuttings shorter, red and far-red preventing flowering in short day plants (Wollaeger and Runkle, 2014), or ultra-violet increasing anthocyanin concentrations in lettuce (Li and Kubota, 2009). LEDs precise light quality control makes them ideal tools to study photomorphology.

The positive qualities of LEDs are clear. Learning to take advantage of these positive qualities will take time. Light emitting diodes are functional replacements to traditional lighting sources. Used at their maximum capacity they can influence plant physiological and morphological responses like flowering, nutrient accumulation, pigmentation, or elongation. They have long life spans and compact size, making them ideal for controlled environments where space is often severely limited. Older, HPS lights use heavy metals which often get deposited into landfills and become pollutants while LEDs do not. Meaning, this source of heavy metal pollutants can be reduced (Lim et al., 2010). The additive advantages of LEDs can offset their high price, hopefully tipping the scale in their favor over traditional lighting sources.

Photoreceptors and Phytochromes

Plants use photoreceptors to perceive different wavelengths of light in the visible spectrum, and slightly outside of it (Casal, 2000). Plants detect certain wavelengths of light in

their environment with different types of photoreceptors, including light-oxygen-voltage, xanthopsins, rhodopins, and blue-light sensors using flavin adenine dinucleotides (Möglich et al., 2010). Light perception through photoreceptors determines if environmental skotoperiods (dark periods) are conducive to physiological changes like tuberization, flowering, seed germination, or vegetative growth at proper times in a plant's life cycle (Casal, 2000; Demotes-Mainard et al., 2016). Often initiating physiological responses in plants requires more than one photoreceptor. For example, flower initiation of *Arabidopsis* requires the combined effects of cryptochrome and phytochrome (Song et al., 2015).

Phytochrome was the first photoreceptor discovered and is the best understood (Briggs and Olney, 2000). Phytochrome absorbs a wide range of wavelengths. The greatest difference in absorption between the two forms are in the red and far-red part of the spectrum. As dimeric chromoproteins, phytochrome starts in a phytochrome red (Pr) shape that absorbs red wavelengths and changes its conformation into the phytochrome far-red (Pfr) shape. Oppositely, Pfr absorbs far-red light and changes conformation back to the initial Pr form. Phytochrome conformational changes occur rapidly in the presence of light. Pfr has a natural half-life of about 1-2 hours, slowly reverting to the Pr form under dark conditions. This slow reversion of phytochrome in the absence of light is how plants measure photoperiod length. Cellular function between phytochrome conformation forms naturally differs (Casal, 2000; Devlin et al., 1999, Sharrock and Clack, 2002). Pfr is considered to be the biologically active form, acting as a transcription factor in the nucleus.

At least five forms of phytochrome, phyA - E, exist in plant systems. Each has unique biological functions, some even acting antagonistically (Casal, 2000, Devlin et al., 1999). By studying *Arabidopsis* mutants deficient in different forms of phytochrome their functions have

been deciphered. The two most studied forms, PhyA and PhyB, were found to mediate far-red and red responses, respectively (Devlin et al., 1999). PhyA is the most abundant form in the presence of light, and phyB in absence of light. PhyB and phyD mediate shade avoidance syndrome responses in plants (Devlin et al., 1999). Shade avoidance causes physiological changes in plant growth, ranging from increased plant height, greater leaf area, to decreased time to flower (Sharrock and Clack, 2002). Low proportions of red to far-red causes shade avoidance. Height increases from shade avoidance could be detrimental to crop quality and should be taken into consideration when evaluating any beneficial effects of night interruption lighting high in far-red light.

Photoperiod

Changing light conditions over seasons or days affects developmental processes (Searle and Coupland, 2004). Photoperiod refers to the duration of day length and influences developmental processes like flowering and tuberization. Early observations of photoperiodic requirements for flowering categorically determined lists of short-day, long-day, and day-neutral species (Garner and Allard, 1920). Photoperiodic flowering was later discovered to be controlled by skotoperiod duration (dark interval) and not photoperiod (light interval). Referring to photoperiod as the controlling factor of flowering remains an artifact of those original studies. Further development of molecular biology would later reveal mechanisms behind photoperiodism. Light perception is not isolated to leaves. Varying organs of plants perceive light with differing effects. Light absorbed by stems causes different physiological effects then light absorbed by meristems or leaves; e.g. floral induction begins in leaves, and nowhere else (Giakountis and Coupland, 2008; Kim et al., 2008; Shim and Imaizumi, 2014). Photoperiodism's effect on flowering helped horticulturists grow crops out-of-season by extending day lengths,

interrupting night intervals, or creating artificial dark periods (Thomas and Vince-Prue, 1996). Photoperiod is directly linked with light quality and circadian rhythms to initiate flowering, and trigger other developmental changes (Song et al., 2010).

Circadian rhythms

Time is a fundamental factor controlling physiological changes. Plants base developmental and physiological changes off fluctuating environmental conditions and corresponding circadian rhythms (Song et al., 2010). A rhythmic fluctuation of gene expression occurs in plants independently of environmental stimuli over a 24-hour period. Plants exposed to continuous light periods continue oscillating specific gene expression patterns (Alabadí et al., 2001). These "clock" genes are locked in complex multiple feedback loops, where one gene influences multiple feedback loops (Alabadí et al., 2001; Hernando et al., 2016). Circadian clock genes are fundamentally conserved in biological systems; ranging from simple cyanobacteria and terrestrial plants to animals (Alabadí et al., 2001; Harmer et al., 2000). My primary interest in understanding circadian rhythms is how they correlate with flower initiation. Several genes involved in flower initiation have oscillating peak expressions throughout the day, making them circadian. These peaks must correspond with appropriate environmental ques to trigger biochemical cascades beginning flower initiation (Shim and Imaizumi, 2014; Song et al., 2015). Circadian genes, or genes regulated by circadian genes, of interest include: *GIGANTEA* (*GI*), *FLAVIN-BINDING KELCH REPEAT F-BOX 1* (*FKF1*), *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), and *PSEUFO-RESPONSE REGULATOR 5* (*PRR5*) (Craig and Runkle, 2016; Kim et al., 2008). These genes interact upstream of the floral initiator *FLOWERING LOCUS T* (*FT*). *FT*'s main promoter, *CONSTANS* (CO), interacts heavily with circadian genes of interest listed above (Pin and Nilsson, 2012; Shim and Imaizumi, 2014). Theoretically, applying light

treatments at known peak gene expression times could strengthen flower initiation responses. Differences in peak gene expression occur between long and short-day plants (Song et al., 2015).

Flower initiation

The external coincidence model describes how external stimuli like temperature and photoperiod interact with internal biological factors to trigger physiological changes ranging from flower initiation to tuberization (Song et al., 2015, Valverde et al., 2004). Internal biological factors, *e.g.* proteins and mRNA, have peak abundances over the duration of a day, often governed by the circadian clock. As photoperiod changes with season, peak abundance levels of transcripts can fluctuate in response. For example, if a protein promoting flowering reaches maximum abundance at the end of long summer days, short winter days may not allow enough time for its translation and accumulation, limiting its ability to promote flowering. Peaks in internal expression of genes controlling flowering must coincide with external stimuli, environmentally activated factors, to induce specific physiological responses. External factors are environmental conditions acting upon a plant. These may range from but are not limited to: temperature, day length, light quality, and light quantity. Matching external stimuli with peak expression of internal factors can determine if plants flower or not, making consideration of both factors pivotal for flowering. This means lighting treatments applied to plants, external stimuli, must be done with consideration of the natural fluctuation of internal factors. Molecular machinery driving flower initiation is conserved in plants (Searle and Coupland, 2004). Inferences into the molecular biology of flower initiation requirements is based on work with *Arabidopsis* and *Oryza*, a model long- and short-day crop, respectively. However, trying to equate molecular processes across all plants based off findings in *Arabidopsis* and *Oryza* should be met with appropriate skepticism.

Among others, two distinct factors often interact to control flowering of photoperiodic plants; circadian rhythm-controlled genes and environmental light quality. Circadian genes and internal factors have been discussed previously, so this section will focus on the addition of external stimuli to regulate flower response. *FT* mRNA creation requires physical coincidence of internal factors, like high CO protein pools, with external factors like suitable temperature, photoperiod, light quantity, and light quality. CO, the direct promoter of FT, protein biosynthesis occurs when circadian genes *GI* and *CCA1* peak in expression levels and interact with light-dependent factor FKF1 in *Arabidopsis* (Valverde et al., 2004). Light-dependent factors regulate CO protein levels by post-translationally stabilizing or destabilizing them, or by regulating CO mRNA creation through transcription factors (Pin and Nilsson, 2012; Song et al., 2015). Light absorbed at several key wavelengths; blue, red, and far-red regulate FT mRNA synthesis, and therefore flowering in *Arabidopsis*. Blue wavelengths act through FKF1 photoreceptors to promote CO expression and flower initiation in *Arabidopsis* (King et al., 2008, Song et al., 2015). Phytochrome A&B and cryptochrome control portions of the light dependent process. Phytochrome A and B have antagonistic effects on FT mRNA synthesis. PHYA absorbs far-red light and stabilizes CO proteins, promoting flowering in LD plants. PHYB absorbs red light and has the opposite effect of destabilizing CO proteins, and inhibiting flowering of LD plants. Specific mechanisms in which PHYB destabilizes CO are currently unclear (Song et al., 2015). Cryptochrome absorbs blue wavelengths and stabilizes CO proteins to ultimately promote synthesis of FT mRNA as well.

FT proteins are synthesized in companion cells, loaded into the phloem, and transported to the shoot apical meristem where they cause a signal cascade of organ differentiation into flower primordia (Giakountis and Coupland, 2008, Pin and Nilsson, 2012). Flower initiation

often, but not always, requires the combined effects of multiple pathways. The photoperiodic pathway, the vernalization pathway, ambient temperature pathway, and autonomous pathways all can influence flower development (Valentim et al., 2015). Past lighting studies have predominantly looked at the light-dependent pathway, namely how light quality effects regulation of FT, but not interactions between multiple pathways. While being the simplest approach, attributing flowering to a single pathway may oversimplify flowering responses.

Long-day Flower Initiation

Long-day (LD) plants flower during summer when days are long, and nights are short. LD plants require a certain critical night length to promote flowering; this is often species and even cultivar dependent. Factors like plant maturation and vernalization may be required for flowering to occur (Eckardt, 2007). The relative abundance of Pr to Pfr during night periods determines if conditions are appropriate for flowering. Low proportions of Pr to Pfr cause flowering in LD plants. LD plants convert less Pfr back to Pr over short night intervals. This creates low proportions of Pr to Pfr and initiates flowering. LD plants grown under short day conditions require night interruption or day extensions to promote flowering (Runkle and Heins, 2001). These techniques artificially decrease the relative pools of Pr to Pfr. LEDs with a PPE of ~0.7 are as effective as high-pressure sodium lamps and incandescent lamps in promoting flowering of LD plants with night interruption and day extensions (Craig and Runkle, 2016, Meng and Runkle, 2017; Runkle et al., 1998).

Night Interruption Lighting with Greenhouse Irrigation Booms

Night interruption applied in many shorter periods (cyclical night interruption) rather than one long continuous period can give sufficient stimuli to initiate flowering of LD plants grown under unfavorable winter conditions (Runkle et al., 1998; Blanchard and Runkle, 2010).

Rotating high pressure sodium lamps sufficiently promote flowering of LD plants grown under short day conditions (Blanchard and Runkle, 2010). Many greenhouses have movable irrigation boom systems to automate irrigation and reduce labor costs. It is possible to apply periodic night interruption by mounting LEDs under these booms. LEDs mounted to booms would mimic this periodic night interval interruption. Quantifying light intensities and durations required for periodic interruptive lighting using LEDs needs investigation. Attaching LEDs to a boom, instead of installing them throughout the entire greenhouse, would greatly reduce the capital costs for growers.

Short-Day Flower Initiation

Short-day (SD) plants flower when nights are long. The required night duration to promote flowering is species specific. Factors like temperature, maturity, and light quality influence flowering (Eckardt, 2007). Red and far-red spectra work antagonistically through phytochrome pigments, with red light activating the pigments biological function, and far-red deactivating its biological function. The biological function of Pfr in SD plants inhibits flower initiation. Long nights allow a large amount of Pfr to slowly convert back to Pr, reducing flower inhibition and promoting flowering. High proportions of Pr to Pfr promote flowering in SD plants. Lower Pfr pools reduce the inhibitory signal for flowering. Applying high red to far-red light proportions during night intervals causes lower proportions of Pr to Pfr, weakening flowering response. Applications of far-red during skotoperiods should diminish the pool size of Pfr and would be expected to promote flowering of SD plants.

There is little information on flower promotion of SD plants grown under long day conditions. Craig and Runkle (2013) demonstrated that moderate to high proportions of red to far-red light (>0.66) delay flowering of SD crops when interrupting long night periods. Higher

red to far-red ratios caused greater amounts of flower inhibition. These night interruptions reduced flowering percentages but did not prevent flowering outright. An opposite physiological effect may occur when low ratios of red to far-red (<0.28) are applied during short night periods. It is thought that far-red alone does not control flowering, needing red wavelengths for plants to perceive a periodic effect (Craig and Runkle, 2013). Because of this, specific proportions of red and far-red are likely needed to promote flowering. These exact proportions of red to far-red are unknown. Blue light, another regulatory wavelength, was not needed to prevent flowering of SD crops (Casal, 2000).

Morphological changes resulting from far-red

Different proportions of red to far-red wavelengths cause unique morphological responses (Demotes-Mainard et al., 2016). While primarily focusing on flowering response, other important factors of crop quality must be addressed. Low proportions of red to far-red causes stretching and promotion of flowering of LD plants (Runkle and Heins, 2001). Stretching is a shade response plants use to counteract low light levels in an environment. Stem elongation and decreased branching allows plants to reach light higher in canopies. These shade avoidance responses, particularly stretching, could reduce the economic value of crops where far-red was used to promote flowering. Ideally, lighting treatments will promote flowering, and generate minimal height increases. Using different proportions of red to far-red will help establish the minimum amount of far-red light needed to induce flowering while minimizing stem elongation.

Literature cited

Alabadí, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Más, P. and Kay, S.A., 2001. Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science*, *293*(5531), pp.880-883.

Blanchard, M.G. and Runkle, E.S., 2010. Intermittent light from a rotating high-pressure sodium lamp promotes flowering of long-day plants. *HortScience*, *45*(2), pp.236-241.

Briggs, W.R. and Olney, M.A., 2001. Photoreceptors in plant photomorphogenesis to date. Five phytochromes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiology*, *125*(1), pp.85-88.

Casal, J.J., 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochemistry and Photobiology*, *71*(1), pp.1-11.

Craig, D.S. and Runkle, E.S., 2013. A moderate to high red to far-red light ratio from light-emitting diodes controls flowering of short-day plants. *Journal of the American Society for Horticultural Science*, *138*(3), pp.167-172.

Craig, D.S. and Runkle, E.S., 2016. An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants. *Environmental and experimental botany*, *121*, pp.132-138.

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., Crespel, L., Morel, P., Huché-Thélier, L., Boumaza, R. and Vian, A., 2016. Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany*, *121*, pp.4-21.

Devlin, P.F., Robson, P.R., Patel, S.R., Goosey, L., Sharrock, R.A. and Whitelam, G.C., 1999. Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. *Plant Physiology*, *119*(3), pp.909-916.

Eckardt, N.A., 2007. Measuring daylength: Pharbitis takes a different approach.

Folta, K.M., Koss, L.L., McMorrow, R., Kim, H.H., Kenitz, J.D., Wheeler, R. and Sager, J.C., 2005. Design and fabrication of adjustable red-green-blue LED light arrays for plant research. *BMC Plant Biology*, *5*(1), p.17.

Giakountis, A. and Coupland, G., 2008. Phloem transport of flowering signals. *Current opinion in plant biology*, *11*(6), pp.687-694.

Haitz, R.H., Craford, M.G. and Weissman, R.H., 1995. Light emitting diodes. *Handbook of optics*, *2*, pp.121-129.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.S., Han, B., Zhu, T., Wang, X., Kreps, J.A. and Kay, S.A., 2000. Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science*, *290*(5499), pp.2110-2113.

Hernando, C.E., Romanowski, A. and Yanovsky, M.J., 2017. Transcriptional and post-transcriptional control of the plant circadian gene regulatory network. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1860*(1), pp.84-94.

Kim, S.Y., Yu, X. and Michaels, S.D., 2008. Regulation of CONSTANS and FLOWERING LOCUS T expression in response to changing light quality. *Plant physiology*, *148*(1), pp.269-279.

King, R.W., Hisamatsu, T., Goldschmidt, E.E. and Blundell, C., 2008. The nature of floral signals in Arabidopsis. I. Photosynthesis and a far-red photoresponse independently

regulate flowering by increasing expression of FLOWERING LOCUS T (FT). *Journal of Experimental Botany*, *59*(14), pp.3811-3820.

Li, Q. and Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*, *67*(1), pp.59-64.

Lim, S.R., Kang, D., Ogunseitan, O.A. and Schoenung, J.M., 2010. Potential environmental impacts of light-emitting diodes (LEDs): metallic resources, toxicity, and hazardous waste classification. *Environmental science & technology*, *45*(1), pp.320-327.

Meng, Q. and Runkle, E.S., 2017. Moderate-intensity blue radiation can regulate flowering, but not extension growth, of several photoperiodic ornamental crops. *Environmental and Experimental Botany*, *134*, pp.12-20.

Möglich, A., Yang, X., Ayers, R.A. and Moffat, K., 2010. Structure and function of plant photoreceptors. *Annual review of plant biology*, *61*.

Morrow, R.C., 2008. LED lighting in horticulture. *HortScience*, *43*(7), pp.1947-1950.

Ouzounis, T., Rosenqvist, E. and Ottosen, C.O., 2015. Spectral effects of artificial light on plant physiology and secondary metabolism: a review. *HortScience*, *50*(8), pp.1128-1135.

Pin, P.A. and Nilsson, O., 2012. The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant, cell & environment*, *35*(10), pp.1742-1755.

Runkle, E.S., Heins, R.D., Cameron, A.C. and Carlson, W.H., 1998. Flowering of herbaceous perennials under various night interruption and cyclic lighting treatments. *HortScience*, *33*(4), pp.672-677.

Runkle, E.S. and Heins, R.D., 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. *Journal of the American society for horticultural science*, *126*(3), pp.275-282.

Schubert, E.F., 2018. *Light-emitting diodes*. E. Fred Schubert.

Schubert, E.F., Kim, J.K., Luo, H. and Xi, J.Q., 2006. Solid-state lighting—a benevolent technology. *Reports on Progress in Physics*, *69*(12), p.3069.

Searle, I. and Coupland, G., 2004. Induction of flowering by seasonal changes in photoperiod. *The EMBO Journal*, *23*(6), pp.1217-1222.

Sharrock, R.A. and Clack, T., 2002. Patterns of expression and normalized levels of the five Arabidopsis phytochromes. *Plant Physiology*, *130*(1), pp.442-456.

Shim, J.S. and Imaizumi, T., 2014. Circadian clock and photoperiodic response in Arabidopsis: from seasonal flowering to redox homeostasis. *Biochemistry*, *54*(2), pp.157-170.

Song, Y.H., Ito, S. and Imaizumi, T., 2010. Similarities in the circadian clock and photoperiodism in plants. *Current opinion in plant biology*, *13*(5), pp.594-603..

Song, Y.H., Shim, J.S., Kinmonth-Schultz, H.A. and Imaizumi, T., 2015. Photoperiodic flowering: time measurement mechanisms in leaves. *Annual review of plant biology*, *66*, pp.441-464.

Tan, S.T., Sun, X.W., Demir, H.V. and DenBaars, S.P., 2012. Advances in the LED materials and architectures for energy-saving solid-state lighting toward “lighting revolution”. *IEEE Photonics Journal*, *4*(2), pp.613-619.

Thomas, B. and Vince-Prue, D., 1996. *Photoperiodism in plants*. Academic Press.

Valentim, F.L., van Mourik, S., Posé, D., Kim, M.C., Schmid, M., van Ham, R.C., Busscher, M., Sanchez-Perez, G.F., Molenaar, J., Angenent, G.C. and Immink, R.G., 2015. A quantitative and dynamic model of the Arabidopsis flowering time gene regulatory network. *Plos One*, *10*(2), p.e0116973.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. and Coupland, G., 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science*, *303*(5660), pp.1003-1006.

Wollaeger, H.M. and Runkle, E.S., 2014. Growth of impatiens, petunia, salvia, and tomato seedlings under blue, green, and red light-emitting diodes. *HortScience*, *49*(6), pp.734-740.

Garner, W.W. and Allard, H.A., 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *Monthly Weather Review*, *48*(7), pp.415-415.

CHAPTER 2

Morphological and Developmental Responses of *Chrysanthemum morifolium* to Night Interruption with Different Red:Far-Red Ratios [1]

[1] Eric J. Stallknecht and Marc W. van Iersel. To be submitted to HortScience for publication.

Abstract.

Chrysanthemum morifolium (chrysanthemum) is a popular short-day plant grown in summer months for fall markets. Although often grown in summer, chrysanthemum requires long skotoperiods for flowering. Using narrow bandwidth far-red (730 nm) light emitting diodes (LEDs) to induce flowering mediated through phytochrome photoreceptors, instead of physical darkening with opaque black cloth, could reduce production costs and increase profits for growers. We hypothesized that night interruption (NI) high in far-red light applied early in skotoperiods would cause increased photo-conversion of far-red absorbing phytochrome (Pfr) to red-absorbing phytochrome (Pr) to promote flowering. We also expected NI high in far-red to induce shade avoidance syndrome (SAS). To test this, we applied 200 min NIs at three different times (beginning, middle, or end) during skotoperiods with a range of phytochrome-photoequilibriums (PPE) (0.3-0.8). Decreasing PPE and applying NI at the end of skotoperiods increased SAS severity. Plants receiving NI in the middle of skotoperiods flowered faster, had more inflorescences, and higher flowering percentages compared to plants receiving NI at the beginning or end of the skotoperiods.

Introduction

Controlled environment agriculture uses electrical lighting to improve the quality of its horticultural products (Morrow and Robert, 2008). Electrical lighting can improve crop quality in two ways in controlled environments. First, greenhouses use supplemental photosynthetic lighting to compensate for low light levels during short winter days and overcast weather. These conditions impair growing high-quality crops in greenhouses due to limited solar radiation, and as a result reduce photosynthesis. Supplemental lighting can increase crop photosynthesis and

growth. Second, electrical lighting can beneficially alter crop development. Night interruption (NI) lighting can alter skotoperiods to promote or inhibit flowering of long-and short-day plants (Craig and Runkle, 2013, 2016; Meng and Runkle, 2017). Light intensities as low as 1-2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ can induce flowering in certain long-day ornamental crops when applied as photoperiod extensions (Whitman et al., 1998).

Plants detect photoperiods with photoreceptors to help them adapt to environmental conditions to ensure survival and reproduction (Doi et al., 2004; Song et al. 2015). Photoperiod is important for horticultural crops because it directly affects flowering (Searle and Coupland, 2004). Phytochrome photoreceptors help plants quantify time by influencing internal circadian rhythms (Casal, 2000; Doi et al., 2004; Pittendrigh et al., 1964). Phytochromes absorb a wide range of light, with the greatest difference in absorption between its two forms in the red (R) (~630nm*)* and far-red (FR) (~730nm) range (Briggs and Olney, 2001; Casal, 2000; Devlin et al., 1999). Phytochrome, a dimeric chromoprotein, is synthesized in the phytochrome red (Pr) form, which absorbs R wavelengths, photo-converting it into phytochrome far-red (Pfr). Oppositely, Pfr photo-converts back to Pr after absorbing FR light. Pfr has a natural half-life of one to two hrs, and slowly reverts into the synthesized Pr form during dark periods *(*Vierstra, 1994*).* Pfr is the biologically active form (Quail and Peter, 2000). It enters nuclei and acts as a transcription factor, controlling flowering *(*Devlin et al., 1999; Quail and Peter, 2000; Sharrock and Clack, 2002*).* Phytochrome far-red is thought to inhibit flowering in SDP (Borthwick and Cathey, 1962). Under long night conditions, when SDP flower naturally, Pfr pools that have accumulated during the day revert to Pr through continuous dark reversion. This diminishes Pfr's inhibitory regulation and allows flowering of SDP. Because Pfr helps establish photoperiods and influences the floral induction pathway directly, it is important to quantify its relative abundance to control

photoperiodic plants transition into the reproductive stage (Craig and Runkle, 2013; Song et al., 2010).

The phytochrome-photoequilibrium (PPE) estimates relative amounts of phytochrome Pfr and Pr within a plant based on the light spectrum (Sager et al., 1988). Knowing relative abundance of phytochrome's two forms helps characterize morphological changes based on environment light spectra. Because conformational changes between phytochrome's two forms occurs spontaneously in the presence of light, estimates of PPE are calculated based on proportions of R and FR wavelengths in the environment. Phytochrome-photoequilibrium is functionally derived from the equation Pfr/(Pr+Pfr), *i.e.* the fraction of total phytochrome in the Pfr form. Environments high in FR wavelengths have low PPEs and have been shown to promote flowering in the species *Arabidopsis thaliana, Petunia × hybrida, Zinnia elegans,* and *Hordeum vulgare* (Demotes-Mainard et al., 2016). Phytochrome-photoequilibrium gives a metric to quickly gauge relative phytochrome abundance without difficult and time-consuming protein extractions, where the protein under evaluation is both light and time sensitive. Night interruption light with a high R:FR ratio, resulting in a high to moderate PPE, can delay flowering of some short-day plants (SDP) (Craig and Runkle, 2013). However, little research has been conducted on the alternative scenario, promoting flowering of SDP with low PPEs, induced by light with a low R:FR ratio.

Chrysanthemum morifolium is a photoperiodic SDP with a strong photoperiodic requirement for flower initiation, making it an ideal species to study flower promotion and development (Higuchi et al., 2013; Meng and Runkle, 2017). Chrysanthemums are often grown in summer for sale as fall and winter bedding plants. This presents a unique problem, because summer months have long days (LD) that inhibit flowering of SDP. Current production methods overcome this

inhibition by covering entire crops with opaque black cloth to prevent sunlight from reaching the plants for a portion of the day. This effectively creates short days (SD) or, more importantly, long nights and induces flowering out of season. While ultimately working, the labor and materials to cover and uncover crops can be expensive. We hypothesize that night interruption with a low red:far-red ratio, resulting in low PPE, applied earlier in the skotoperiod will promote flowering of chrysanthemum growing under otherwise noninductive long days by photo-converting the most Pfr into Pr. Since environments with low PPE are often indicative of shading (Demotes-Mainard et al., 2016; Devlin et al., 1999; Franklin, 2008), we also hypothesize that night interruption with a low red:far-red ratio will alter plant morphology, indicative of shade-avoidance syndrome (SAS), primarily height increases. Using overhead LED lights to promote flowering, instead of using black cloth, if successful, could reduce production input costs and make flower promotion of chrysanthemum simpler for growers.

Materials and Methods

Controlled environment. A 54 m^3 walk-in cooler, retrofitted into a walk-in growth chamber, was used for this study. Plants were grow on a metal shelving rack with three, 2.4 m × 0.6 m shelves each. Ebb and flow benches sub-irrigated each shelf with a water-soluble fertilizer solution (100 $mg \cdot L^{-1}$ N; Peters Excel 15-5-15 Cal-Mag special; ICL Fertilizers, Dublin, OH). Each rack shelf was divided into two equal sections containing 0.74 m^2 of growing space lit by two LED bars providing white light (SpydrX Plus with PhysioSpec indoor spectrum; Fluence Bioengineering, Austin, TX). The photosynthetic photon flux density from these LEDs was measured with a portable quantum sensor (SQ-110; Apogee Instruments, Logan, UT) and averaged 399 ± 56 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± sd) at canopy height at transplant. Plants were grown under 14-hr

photoperiods and received a daily light integral of 20.1 ± 2.8 $mol \cdot m^{-2} \cdot d^{-1}$. The main lighting fixtures spectrum is depicted in Fig. 2.1. A datalogger (CR1000; Campbell Scientific, Logan, UT) was used to monitor environmental conditions using various sensors and to control CO_2 augmentation. To maintain the CO_2 level, the datalogger opened a solenoid valve controlling flow from a compressed CO_2 cylinder for 1 s intervals whenever the CO_2 level dropped below 800 $\mu mol \cdot mol^{-1}$. CO_2 level was measured with a CO_2 transmitter (GMD20, Vaisala, Helsinki, Finland) and averaged 800 ± 87 $\mu mol \cdot mol^{-1}$. Air temperature was maintained with thermostat at 20.8 ± 1.1 °C throughout the study period with no diurnal fluctuations. Average relative humidity was 60 ± 8% and controlled with a dehumidifier (FAD704DWD13, Electrolux Home Products, Charlotte, NC). Air temperature and relative humidity measured with a combined probe (HMP50, Vaisala, Helsinki, Finland).

Plant material. On June 8 2017, rooted chrysanthemum *'Paradise pink'* cuttings (Ball Seed Company, West Chicago, IL) arrived by mail and were immediately transplanted into 10-cm square black pots with soilless potting media (Fafard professional potting mix; Sun Gro Horticulture, Agawam, WA) and acclimated for 7 d under a noninductive 14-hr photoperiod prior to NI treatments beginning.

Treatments. Night interruptions with a range of PPEs were applied to chrysanthemum growing under noninductive 14-hr photoperiods to elucidate their effects on flowering and morphology. Treatments began on 15 June 2017. Night interruption was applied at either the beginning (NI BEG), middle (NI MID), or end (NI END) of the skotoperiod. Each NI lasted 200-minutes, or exactly 1/3 of the 10-hr skotoperiod. To achieve a gradient of PPEs, a custom-built FR LED bar (peak 730nm) was placed at the end of an experimental block (Fig. 2.2). This FR LED bar provided a maximum of 81 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to experimental units closest to it and 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to

units furthest away. The white LED bars were used at 10% of their full power (45 ± 4.5 μmol·m^{-2}·s^{-1}) to create a range of white:far-red ratios. The white LED bars at 10% power produce an integrated total of 0.54 ± 0.05 mol·m^{-2} over 200-min NI times. A spectroradiometer (SS-110; Apogee Instruments, Logan, UT) was used to quantify each experimental unit's spectral composition. The spectroradiometer's accompanying software (Apogee Spectrovision) was used to estimate PPE for each experimental unit.

By combining FR and white LED bars, we created an environment where one side of a plot has a high PPE and the other has low PPE, ranging from 0.8 to 0.3 (Fig. 2.2). Night interruption treatments occur nightly for 60 d. Treatment applications concluded after 60 d when plants became too tall for the study space, and began blocking the FR LEDs, resulting in uneven distributions over experimental plots. Plants were grown for another 50 d under noninductive photoperiods inside walk-in growth chamber.

Data collection. The number of days to first bud break (first petal emergence) was monitored for each plant. The total inflorescence number (all buds and open flowers), percent flowering plants (percent of plants with at least one fully opened flower), height (pot rim to shoot apical meristem), shoot dry weight, and plant compactness (dry shoot weight / final height) were measured at the end of the study.

Experimental design and statistical analysis. To test our hypotheses, we used a randomized complete block design for NI application times, with a non-randomized split-plot for PPE gradient. There were two blocks, each with three plots. Plots contain nine experimental units (sub-plots) with two chrysanthemums per unit (sub-plot). Statistical analysis was done in R (Version 3.3.3, The R Foundation) at α=0.05. Multiple regression analysis, with timing of NI as a

class variable and PPE as a continuous variable, was conducted on all data collected. Cook's distance was used to test for and remove highly influential outliers.

Results

Morphology. Phytochrome-photoequilibrium and NI application time had significant interacting effects on chrysanthemum height (Table 2.1). Phytochrome-photoequilibrium differences in the NI BEG treatment did not influence chrysanthemum height (Fig. 2.3A), with < 0.4 cm difference among all plants. For contrast, plants with a PPE of 0.3 in the NI MID and NI END treatment (dashed lines, Fig. 2.3A) were 5.9 cm 12.0 cm, respectively, taller than plants with a PPE of 0.8. Plants with a PPE of 0.8, regardless of NI time, showed minor height differences (< 1 cm). However, plants with a PPE < 0.7 experienced stretching with increasing magnitudes in response to decreasing PPE later in skotoperiods (NI MID and END treatments) (Fig. 2.3A). Plants with a PPE of 0.3 in the NI END treatment were 31.7 cm tall, 5.7 cm and 11.5 cm taller than plants with the same PPE in the NI MID and NI BEG treatments. Therefore, the same PPE (0.3) causes an 18% and 36% height increase in NI MID and NI END, respectively, when compared to NI BEG. Phytochrome-photoequilibrium significantly influenced shoot dry weight while NI application time and their interaction did not (Table 2.1). Shoot dry weight was lowest in plants with a PPE of 0.3, with little difference in shoot dry weights with PPEs between 0.8 and 0.5 (Fig. 2.3B). PPEs <0.5 appear to cross a threshold where shoot dry weight becomes more severely reduced. For instance, plants with a PPE of 0.3 average 6.8 g less shoot weight than plants with a PPE of 0.5. Yet, the difference in shoot dry weight between PPEs of 0.5 and 0.8 is only 1.3 g.
There was a significant interactive effect between PPE and NI application time on chrysanthemum compactness (Table 2.1). Compactness trends (Fig. 2.3C) are the inverse of

those of plant height (Fig. 2.3A). When applied at the beginning of the skotoperiod, there is no effect of PPE on compactness, while increasing PPE results in greater compactness when NI is applied in the middle and especially late in the skotoperiod.

Flowering. Phytochrome-photoequilibrium and NI application time both affect days to first open flower (Table 2.1). Lower NI PPE slightly reduces days to first open flower (Fig. 2.4). Plants closest to the FR LEDs (lowest PPE, 0.3), flowered 90 d after NI treatments began, 4 d earlier than plants with the highest PPE (0.8), a 4.4% reduction in days to flower. Chrysanthemums flowered significantly earlier when subject to NI during the middle of the skotoperiod (after 81 d), compared to the beginning (94 d) or end (99 d).

The total number of visible inflorescences differed among all three NI application times, but was not affected by PPE or the interaction between PPE and NI time. At study termination, plants in NI MID treatments averaged 12.8 visible inflorescences; 5.1 more inflorescences than NI BEG and 7.0 more than NI END plants.

NI application time also affected chrysanthemum flowering percentage (Table 2.1). Flowering percentage in the NI BEG and NI MID treatments was significantly higher than NI END. NI MID plants had the highest flowering percentage at 95%, NI BEG at 75%, and NI END has the lowest percent flowering plants at 44%. Overall, NI END resulted in the poorest flowering response, with a combination of most days to flower, lowest flowering percentage, and fewest inflorescences. Oppositely, plants in the NI MID treatment had the highest flowering percentage, fewest days to first open flower, and most inflorescences.

Discussion

Morphology. Certain species of plants react to shaded environments, with low PPE and low R:FR ratio, by elongating their internodes to reach higher in canopies for increased solar radiation exposure (Demotes-Mainard et al., 2016; Devlin et al., 1999; Keuskamp, et al., 2010). As hypothesized, reducing NI PPE (reducing R:FR ratio) increased height (Fig. 2.3A), but only when NI was applied at the middle or end of the skotoperiod. Height increases (internode extensions) likely result from shade-avoidance syndrome (SAS), as increases in height occurred with little change in shoot dry weight except at extremely low PPEs (< 0.5, Fig. 2.3B), which decreased shoot dry weight. Morphological changes caused by SAS in the current study perhaps are best exemplified by plant compactness, the ratio of shoot dry weight to plant height (Fig. 2.3C). Lowering PPE later in the skotoperiod creates less compact plants in a far-red dosage dependent manner, with decreasing compactness equating to more severe SAS. Shade-avoidance syndrome was moderate in the NI MID treatment, but this NI timing has the best flowering response: the most inflorescences, highest flowering percentage, and quickest to flower. Because of this, further work aiming to promote flowering of chrysanthemum with low NI PPE should focus on the middle of skotoperiods.

What mediated interactions between PPE and NI application time to cause SAS in our study is unknown, but appears to be circadian controlled. Many genes regulating SAS are circadian controlled, rhythmically changing their transcript (mRNA) abundance over a 24-hr cycle (Casal, 2000; Doi et al., 2004, Nozue and Maloof, 2006). This is observed in many species, including *Arabidopsis* and snapdragon (*Antirrhinum majus*) (Neily et al., 1997; Soy, et al., 2012). It is possible that circadian-controlled genes regulating SAS in chrysanthemum reach near maximum or minimum abundance levels late in skotoperiods/early morning, allowing for the greatest

response to low PPEs. PHYTOCHROME-INTERACTING FACTORs (PIF) are well-studied candidates for genes controlling SAS, with some gene transcripts known to have diurnal abundance fluctuations. These genes may influence both flowering and stem elongation (Kim et al., 2008; Quail and Peter, 2000; Nozue and Maloof, 2006). A study by Soy et al. (2012) demonstrated circadian fluctuations of PIF4-5 transcripts, having peak abundances late in skotoperiods in arabidopsis. They detected greatest hypocotyl elongation late in skotoperiods/early mornings. Bayer et al. (2016), while not quantifying gene transcript abundance, also observed the same increase in elongation pattern at the end of skotoperiods in *Hibiscus acetosella*. The findings from these two studies are congruent with our results, showing FR has greatest effects on SAS later in skotoperiods when certain PIF transcripts are most abundant. Understanding diurnal transcript abundance fluctuations, and therefore the possible sensitivity of plants to FR, will hopefully lead to more efficient NI treatments application times for the manipulation of plant morphology.

Flowering. We hypothesized that lower PPEs applied earlier in the skotoperiod would generate greater phytochrome photo-conversion of Pfr into Pr to promote flowering, which might manifest as reduced days to flower, more inflorescences, or increased flowering percentage of chrysanthemum. However, no interaction between PPE and NI timing was seen. We observed that low PPE slightly reduced days to flower, regardless of NI application time. A previous study by Craig and Runkle (2013) into floral inhibition of chrysanthemum showed that NI in the middle of long nights with PPEs < 0.63 reduced the number of days to first open flower. We similarly found that lowering NI PPE, regardless of NI time, reduced the number of days to flower. FR is known to induce changes that post-translationally stabilize CONSTANS (CO) proteins through phytochrome-A (PhyA), which promotes expression of Flowering Locus-t (FT)

to induce flowering in Arabidopsis (Casal, 2007; Song et al., 2015). Through this pathway, FR could accelerate flowering independent of NI timing, comparable to our results. Another plausible explanation for the reduced days to flower in response to low PPE is FR negating R inhibition caused by our main lighting fixtures, which are high in R (Fig. 2.1). It is possible that FR did not promote flowering per se, but instead counteracted the inhibitory effect of R on flowering. Far-red can counteract inhibitory R signals to promote flowering of morning-glory (*Ipomoea nil*) and chrysanthemum (Cathey and Borthwick, 1957; Takimoto et al., 1965). Although the effect was relatively small, our high intensity FR accelerated flowering in chrysanthemum exposed to LDs. Whether high FR truly promoted flowering or provided the least inhibitory flowering environment is unclear. Removing R wavelengths from further NI studies may help to differentiate between the two.

Unexpectedly, NI application time had greater effects on chrysanthemum flowering than PPE. Night interruption in the middle of the skotoperiod resulted in the fewest days to flower, highest flowering percentage, and most inflorescences. This result runs contrary to our hypothesis that the greatest reversion of Pfr to Pr could be accomplished by applying FR earlier in the skotoperiod to produce the strongest flowering response. What explicitly caused this is unclear. It is possible that other internal factors, besides phytochrome, regulate flowering of chrysanthemum during the middle of skotoperiods. Blue light, acting through cryptochrome photoreceptors, regulates multiple plant responses, including plant morphology and flowering in arabidopsis (Pin and Nilsson, 2012). The white LED lighting fixtures, which we used to produce a gradient of PPE, produced 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of blue between 450–490 nm with a peak intensity of 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 450 nm. Therefore, it is conceivable that blue light influenced flowering acting through cryptochrome rather than FR acting through PhyA. Cryptochrome promotes

flowering similarly to PhyA in arabidopsis by stabilizing CO proteins that promote Flowering Locus-T protein synthesis (Pin and Nilsson, 2012; Song et al., 2015). The precise nature of chrysanthemum flower regulation, or SD plants in general, is not well understood. Omitting blue or applying blue with varying intensities and NI timings could help determine whether blue lights affects chrysanthemum flowering.

Conclusion. Night interruption of short nights with PPEs < 0.6 cause SAS in chrysanthemum when applied during the middle or end of the skotoperiod. The greatest extension growth resulted from NI at the end of the 10-hr skotoperiod with a PPE of 0.3, while almost none occurred from NI at the beginning with the same PPE. This reveals that SAS in chrysanthemum is regulated by the interaction of internal plant PPE and internal circadian-controlled factors. Night interruption at the beginning and end of the skotoperiod created 17.3-hr long days and resulted in reduced flowering responses compared to a 10-hr skotoperiod interrupted for 3.3 hr. What caused these differences in flowering response are currently unknown. Night interruption in the middle of skotoperiods is often used to keep short-day plants vegetative, but resulted in the best flowering response in this study. Based on our results, NI at the end of 10-hr skotoperiods is better at keeping chrysanthemum vegetative than any other NI time.

Literature Cited

Borthwick, H.A. and H.M. Cathey. 1962. Role of phytochrome in control of flowering of chrysanthemum. *Bot. Gaz. 123*(3):155-162.

Bayer, A., J. Ruter, and M.W. van Iersel. 2016. Elongation of Hibiscus acetosella Under Well-watered and Drought-stressed Conditions. *HortScience 51*(11):1384-1388.

Briggs, W.R. and M.A. Olney. 2001. Photoreceptors in plant photomorphogenesis to date. Five phytochromes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiol. 125*(1):85-88.

Whitman, C.M., R.D. Heins, A.C. Cameron, and W.H. Carlson. 1998. Lamp type and irradiance level for daylength extensions influence flowering of Campanula carpaticaBlue Clips', Coreopsis grandifloraEarly Sunrise', and Coreopsis verticillataMoonbeam'. *J. of the Amer. Soc. for Hort. Sci. 123*(5): 802-807.

Casal, J.J. 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochemistry and Photobiology, 71*(1):1-11.

Cathey, H.M. and H.A. Borthwick. 1957. Photoreversibility of floral initiation in chrysanthemum. *Bot. Gaz. 119*(2):71-76.

Craig, D.S. and E.S. Runkle. 2013. A moderate to high red to far-red light ratio from light-emitting diodes controls flowering of short-day plants. *J. of the Amer. Soc. for Hort. Sci. 138*(3):167-172.

Craig, D.S. and E.S. Runkle. 2016. An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants. *Environ. and expt. bot. 121*:132-138.

Demotes-Mainard, S., T. Péron, A. Corot, J. Bertheloot, J. Le Gourrierec, Pelleschi- S. Travier, L. Crespel, P. Morel, L. Huché-Thélier, R. Boumaza and A. Vian. 2016. Plant responses to red and far-red lights, applications in horticulture. *Environ. and Expt. Bot. 121*:4-21.

Devlin, P.F., P.R. Robson, S.R. Patel, L. Goosey, R.A. Sharrock and G.C. Whitelam. 1999. Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. *Plant Physiol. 119*(3):909-916.

Doi, K., T. Izawa, T. Fuse, U. Yamanouchi, T. Kubo, Z. Shimatani, M. Yano and A. Yoshimura. 2004. Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes & Dev. 18*(8):926-936.

Franklin, K.A. 2008. Shade avoidance. *New Phytologist 179*(4):930-944.

Higuchi, Y., T. Narumi, A. Oda, Y. Nakano, K. Sumitomo, S. Fukai, and T. Hisamatsu. 2013. The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. *Proc. of the Natl. Acad. of Sci. 110*(42):17137-17142.

Keuskamp, D.H., R. Sasidharan and R. Pierik. 2010. Physiological regulation and functional significance of shade avoidance responses to neighbors. *Plant signaling & behavior 5*(6):655-662.

Kim, S.Y., X. Yu, and S.D. Michaels. 2008. Regulation of CONSTANS and FLOWERING LOCUS T expression in response to changing light quality. *Plant phys. 148*(1):269-279.

Meng, Q., and E.S. Runkle. 2017. Moderate-intensity blue radiation can regulate flowering, but not extension growth, of several photoperiodic ornamental crops. *Environ. and Expt Bot. 134*:12-20.

Morrow, R.C. 2008. LED lighting in horticulture. *HortScience 43*(7):1947-1950.

Neily, W.G., P.R. Hicklenton, and D.N. Kristie. 1997. Temperature and developmental stage influence diurnal rhythms of stem elongation in snapdragon and zinnia. *J. of the Ameri. Soc. for Hort. Sci. 122*(6):778-783.

Pin, P.A. and O. Nilsson. 2012. The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant, cell & environ. 35*(10):1742-1755.

Pittendrigh, C.S. and D.H. Minis. 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *The Ameri. Naturalist 98*(902):261-294.

Quail, P.H. 2000, December. Phytochrome-interacting factors. In *Seminars in cell & dev. biol.* 11(6):457-466. Academic Press.

Sager, J.C., W.O. Smith, J.L. Edwards, and K.L. Cyr. 1986. Use of spectral data to determine photosynthetic efficiency and phytochrome photoequilibria. *Ameri. Soc. of Agr. Eng. Microfiche collection (USA).*

Schubert, E.F., J.K. Kim, H. Luo and J.Q. Xi. 2006. Solid-state lighting—a benevolent technology. *Reports on Prog. in Phys. 69*(12):3069.

Searle, I. and G. Coupland. 2004. Induction of flowering by seasonal changes in photoperiod. *The EMBO J. 23*(6):1217-1222.

Sharrock, R.A. and T. Clack. 2002. Patterns of expression and normalized levels of the five Arabidopsis phytochromes. *Plant Physiol. 130*(1):442-456.

Song, Y.H., S. Ito and T. Imaizumi. 2010. Similarities in the circadian clock and photoperiodism in plants. *Current opinion in plant Biol. 13*(5):594-603.

Song, Y.H., J.S. Shim, H.A. Kinmonth-Schultz, and T. Imaizumi. 2015. Photoperiodic flowering: time measurement mechanisms in leaves. *Ann. Rev. of plant Biol. 66*:441-464.

Soy, J., P. Leivar, N. González-Schain, M. Sentandreu, S. Prat, P.H. Quail and E. Monte. 2012. Phytochrome-imposed oscillations in PIF3 protein abundance regulate hypocotyl growth under diurnal light/dark conditions in Arabidopsis. *The Plant J. 71*(3):390-401.

Takimoto, A. and K.C. Hamner. 1965. Effect of far-red light and its interaction with red light in the photoperiodic response of Pharbitis nil. *Plant physiol. 40*(5):859.

Thomas, B. and D. Vince-Prue. 1996. *Photoperiodism in plants*. Academic Press.

Valverde, F., A. Mouradov, W. Soppe, D. Ravenscroft, A. Samach and G. Coupland. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science 303*(5660):1003-1006.

Vierstra, R.D. 1994. Phytochrome degradation. In *Photomorphogenesis in Plants*. 141-162. Springer, Dordrecht.

Table 2.1. Multiple regression analysis of phytochrome-photoequilibrium (PPE), night interruption (NI) application times, and their interaction on chrysanthemum morphology and flowering.

Response variable	Predictor variables		
	PPE	NI timing	Interaction
Days to flower (d)	0.008[Y]	< 0.001	NS
Percent flowering (%)	NS	< 0.001	NS
Number of inflorescences (#)	NS	< 0.001	NS
Height (cm)	< 0.001	< 0.001	< 0.001
Shoot dry weight (g)	0.032	NS	NS
Compactness (g/cm) [Z]	< 0.001	NS	<0.001

[Y] Values indicate significance level; NS indicates $P > 0.05$.

[Z] Compactness calculated by dividing plant weight by height.

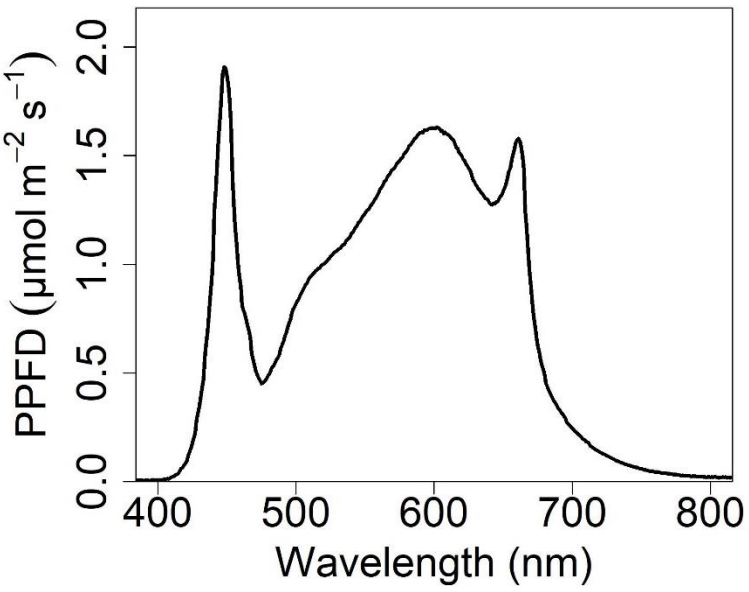

Figure 2.1. Light spectrum under the main lighting source (white LED lights) taken at transplant canopy height. Phytochrome photoequilibrium (PPE) = 0.86. The light spectrum (PhysioSpec Indoor spectrum; Fluence BioEngineering) is high in red and blue, but virtually devoid of far-red.

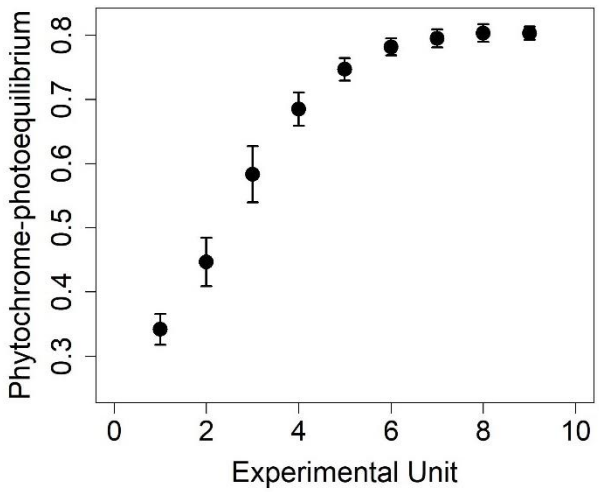

Figure 2.2. Average phytochrome photoequilibrium (PPE) distribution ± SD over all experimental units at transplant canopy height during night interruption. Far-red LEDs were placed over experimental unit one and provided a gradient of far-red light. All plants also received light from white LEDs placed above the crop, creating a range of PPEs.

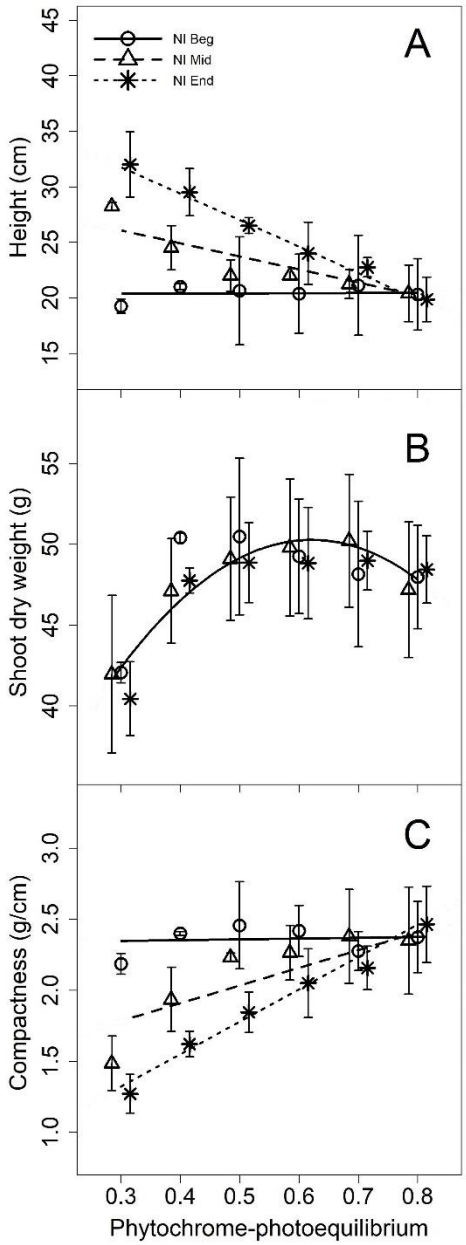

Figure 2.3. (A-C) The effect of phytochrome-photoequilibrium (PPE) and night interruption (NI) time on morphology of *Chrysanthemum morifolium*. Means ± SD. Shoot dry weight was not affected by NI treatment time. The quadratic regression shown is pooled data from all NI application times. Lines in Fig. A and C indicate the results of multiple regression (see table 2.1).

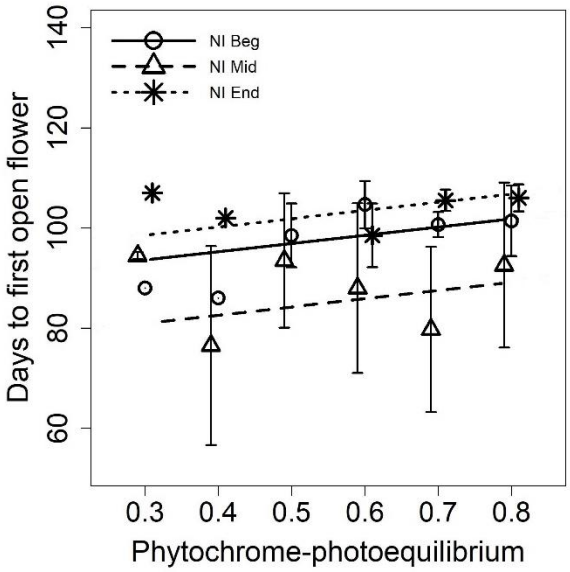

Figure 2.4. Night interruption timing and phytochrome-photoequilibrium effects on the number of days to first open flower. Means ± SD. Lines indicate the results of multiple regression (see table 2.1).

CHAPTER 3

Night Interruption with Light Emitting Diodes Applied Using Simulated Moving Greenhouse Booms Promotes Flowering of *Petunia* × *hybrida* [1]

[1] Eric J. Stallknecht and Marc W. van Iersel. To be submitted to HortScience for publication.

Abstract

Long-day plants often require night interruption (NI) to promote flowering when grown out-of-season. Cyclical NI, NI delivered in many short periods rather than one long continuous period, is less studied than other methods but can efficiently administer NI. Many greenhouses have movable electric booms capable of supporting lighting fixtures to successfully administer cyclical NI. We hypothesized that cyclical NI from Light Emitting Diode (LED) fixtures can promote flowering of *Petunia* × *hybrida* as well as 4 hr of continuous NI. A growth chamber with programmable LEDs accurately mimicked moving irrigation booms, simulating movement over a crop. Cyclical NI reduced time to flowering by as much as 3 d compared to short-day controls. Cyclical NI frequencies ≤ 0.1 passes/minute were ineffective at promoting flowering. Increasing frequencies past 0.2 passes/minute did not further reduce time to flower. The traditional NI of 4-hr of continuous light promoted flowering better than any cyclical NI frequency studied. Cyclical NI had no effect on the number of inflorescences produced compared to short-day controls.

Introduction

Greenhouses use supplemental lighting to enhance crop growth and regulate photoperiodic responses. Winter-time production in greenhouses can require high intensity supplemental lighting to overcome limited solar radiation caused by short photoperiods and prolonged overcast weather. Daylength-sensitive crops may also require photoperiodic manipulation to promote or inhibit flowering. Typically, to promote flowering of long-day plants noninductive skotoperiods are interrupted with overhead lighting. While night interruption (NI) techniques can vary in

duration, intensity, and spectra, 4-hr of continuous light in the middle of skotoperiods is often used.

Irrigation booms are common in greenhouses because they efficiently apply irrigation, plant growth regulators, fertilizers, and pesticides while reducing labor costs (Schulz, 2008). Many irrigation booms can support the addition of small lighting fixtures that can be used to manipulate photoperiodic crops (Schulz, 2008; Kuack, 2013). Incandescent bulbs have traditionally filled this role. Light emitting diodes (LEDs) are gradually replacing traditional lighting sources like high pressure sodium lights and incandescent bulbs thanks to their high light output, spectral diversity, and electrical efficiency (Morrow, 2008). Their compact size and controllability make them perfect for boom lighting.

Cyclical or intermittent lighting applies night interruption in multiple short sequences, rather than a long continuous NI period (Runkle, et al., 1998). Research into cyclical/intermittent NI started in the 20th century and demonstrated the ability of cyclical NI to influence flowering of photoperiodic plants. For instance, cyclical NI administered with incandescent and fluorescent lamps could prevent flowering of short-day *Chrysanthemum morifolium* growing in inductive photoperiods (Borthwick and Cathey, 1962). Despite research into cyclical NI beginning almost 60 years ago, little published information exists on its best uses to promote flowering. One primary benefit of cyclical lighting is reduced electrical demand, lowering energy consumption by as much as 60% (Bickford and Dunn, 1972).

Research into cyclical lighting increased with high pressure sodium lamps' (HPS) proliferation in the greenhouse industry. Research at Michigan State University by Blanchard and Runkle (2009 and 2010) and Runkle and Blanchard (2016) demonstrates that cyclical NI with HPS can efficiently manipulate photoperiodic responses, inhibiting flowering in short-day plants and

promoting flowering in long-day plants. They also demonstrate that rotating shields around HPS lamps can reduce the total number of lighting fixtures needed for NI. Cyclical NI applied with both HPS and incandescent lamps can promote flowering of the long-day plants Petunia, *Campanula*, *Rudbeckia*, and *Coreopsis* as well as 4 hr of continuous NI (Blanchard and Runkle, 2010; Runkle et al., 1998; Runkle and Blanchard, 2016).

Petunia is a quantitative long-day plant that flowers most rapidly under long days/short nights and slower under less inductive short days/long nights. Petunias grown during winter for spring sale benefit from night interruption (NI) of long skotoperiods to accelerate flowering. Four hours of low intensity NI, 1-2 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in the middle of the skotoperiod is an industry standard and enough to induce flowering of many long-day plants (Whitman et al., 1998; Runkle et al., 1998). The efficacy of cyclical NI has been successfully demonstrated in research and industry applications. Despite gradual incorporation of LEDs into greenhouses, little research has tested the efficacy of LEDs administering cyclical NI. The goal of this study was to test the efficacy of LED fixtures mounted on greenhouse booms to administer NI. This could greatly reduce the initial investment cost of buying LEDs, while expanding on current capabilities of greenhouses with irrigation booms. We hypothesize that cyclical NI administered using LED fixtures can promote flowering of *Petunia* × *hybrida* as well as 4 hr of continuous NI in the middle of the skotoperiod with stationary fixtures. Since the actual use of moving irrigation booms was not practical from an experimental perspective, we also wanted to test whether movement of a boom can be simulated by programming a dimmable LED to mimic movement of an LED fixture. Light emitting diodes are exceptionally controllable because they are solid-state devices capable of turning on and off near instantaneously, easily and accurately regulating their operating times and intensities.

Materials and Methods

Controlled environment. Two studies were conducted in a 54 m^3 walk-in refrigeration cooler, retrofitted into a walk-in growth chamber. The growth chamber contained three metal shelving racks, each with three 2.4 m × 0.6 m (1.44 m^2) shelves. This provides 13 m^2 (1.44 m^2 * 9 shelves) of total growing space. Irrigation was provided to crops with sub-irrigation ebb and flow trays on each shelf. Shelves were divided with a Styrofoam divider into two equal experimental units with 0.72 m^2 of growing space, creating 18 total experimental units. The main light source for each unit was a set of two LED bars (SpydrX Plus with PhysioSpec indoor spectrum; Fluence Bioengineering, Austin, TX). Their spectral composition was evaluated with spectroradiometer (SS-110; Apogee Instruments, Logan, UT, Fig. 3.1). The photosynthetic photon flux density was measured with a quantum sensor (SQ-110; Apogee Instruments, Logan, UT) and averaged 400 ± 57 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± sd). All plants were grown under daily 10-hr photoperiods, with a DLI of 14.4 ± 0.2 $mol \cdot m^{-2} \cdot d^{-1}$. A datalogger (CR6; Campbell Scientific, Logan, UT) recorded environmental conditions every 5 min and controlled CO_2 enrichment. To maintain an elevated CO_2 level, the datalogger opened a solenoid valve regulating flow from a compressed CO_2 cylinder for a 1 s interval whenever the CO_2 concentration dropped below 800 $\mu mol \cdot mol^{-1}$. CO_2 was measured with CO_2 transmitter (GMD20, Vaisala, Helsinki, Finland). Volumetric water content (VWC) sensors (EC-5, Decagon Devices, Pullman, WA) measured substrate water content in one pot in each experimental unit. Fertility was provided at each irrigation through the sub-irrigation system (100 $mg \cdot L^{-1}$ N; Peters Excel 15-5-15 Cal-Mag special; ICL Fertilizers, Dublin, OH). Air temperature and relative humidity were measured with a combined probe (HMP50, Vaisala, Helsinki, Finland). Humidity was regulated with dehumidifier

(FAD704DWD13, Electrolux Home Products, Charlotte, NC). Air temperature was regulated using a thermostat. Environmental conditions are summarized in Table 3.1.

Plant material for study 1. Petunia × *hybrida* 'Daddy blue' (Park Seed Wholesale, Greenwood, SC) were sown on Oct. 9 2017 into 72-cell plug trays. Seeds were germinated under white light from LEDs (Fat Jeff; Aurora, St. Petersburg, FL; spectrum in Fig. 3.1B) producing 230 $\mu mol \cdot m^{-2} \cdot s$, with 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red (700-800nm) and noninductive 8-hr photoperiods. This produced a DLI of 6.6 $mol \cdot m^{-2} \cdot day^{-1}$. Petunia seedlings were transplanted on Nov. 20 2017, 42 d after seeding, into15-cm round pots containing soilless potting media (Fafard's professional potting mix; SunGro Horticulture, Agawam, MA) and placed in the growth chamber. The seedlings were older than typical at transplanting (6 weeks old), because of the time required to form transplantable plugs in 72-cell trays. Transplants acclimated for 7 d under 10-hr photoperiods inside the growth chamber before NI treatments began.

Treatments in study 1. Treatments started on Nov. 27 2017 on seven-week-old petunia seedlings. Petunia received a range of cyclical NI treatments to observe their effects on growth and development. Night interruption treatment times, and their characteristics for study 1 are shown in Table 3.2. Cyclical NI treatments occurred from 2000h to 0800h (12 hr) with 1-hr dark periods before and after the 10-h photoperiod. The datalogger provided boom simulations by sending a voltage signal that first gradually increased and then decreased to dimming controllers in the LED drivers, effectively controlling LED light output. These boom simulations caused the light intensity to gradually increase to a maximum, simulating an approaching boom, and then decreased the light intensity to zero, simulating the boom moving away (Fig. 3.2). One simulated boom pass provided ~2300 $\mu mol \cdot m^{-2}$ over 10 s period. Each NI treatment simulated a boom traveling at ~9.1 m/min with a LED beam spread of ~1.5 m, this spread mimics an LED ~0.6 m

above the crop with a ~100° beam angle. This relatively slow boom speed was selected to accommodate most boom systems. Light intensity and boom speed were identical among all treatments. Cyclical NI frequencies were 0.0, 0.25, 0.5, 1, and 2 boom passes per minute. Plants receiving cyclical NI treatments with higher frequencies will, as a byproduct, accumulate higher integrated light totals and illumination times (Table 3.2). Integrated light intensity for each treatment was measured with a quantum sensor at transplant height. Total NI treatment illumination times are calculated according to

$$\text{Illumination time} = (\text{light spread} / \text{boom speed}) \times (\text{frequency} \times 12\ \text{h}) \quad \text{(Eq. 1)}$$

where: illumination time = total hours plants receive illumination, light spread = meters of thrown light, boom speed = meters/second, and frequency = irrigation boom passes/second. Study one contained two control treatments because of a datalogger software programming error. We planned to compare our cyclical treatment to an industry standard of 4 hr of continuous NI in the middle of the skotoperiod, but we lost this treatment.

Plant material for study 2. On Mar. 12 2018 *Petunia* × *hybrida* 'Daddy blue' seeds (Park Seed Wholesale) were sown into 288-cell plug trays to expediate transplant production compared to study 1. Seedlings were germinated under the same light conditions as in study 1 (Fig. 3.1B) and a noninductive 8-hr photoperiod. Petunia seedlings were transplanted on Apr. 13 2018 (4 weeks old) into 15-cm round pots filled with soilless potting media (Fafard's professional potting mix; SunGro Horticulture) and moved into the growth chamber.

Treatments in study 2. Treatments began on Apr. 13 2018 on four-week-old petunia seedlings. Cyclical NI treatments in study 2 matched the intensity and simulated boom speeds of study 1.

However, study 2 uses two, lower frequencies (0.2 and 0.1 passes/min) to gain a better understanding of minimal inputs needed to stimulate flowering. No acclimation period was used for study two to maximize petunia seedling exposure to night interruption. Night interruption treatment times and their integrated NI light totals are shown in Table 3.2.

Light contamination. Light intensities as low as 1-2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ can cause flowering responses in photoperiodic plants. Therefore, it was important to quantify any light contamination among treatments because they all occur inside the same growth chamber. Light contamination was measured with multiple quantum sensors recording *PPFD* during cyclical NI applications in the experimental unit receiving NI, as well as in the surrounding experimental units (Fig. 3.3B). Some light bleed-over was evident from measurements in a control treatment, which should receive no light during its skotoperiod (Fig. 3.3A). Yet, small rhythmic *PPFD* fluctuations were visible, ranging from 0 to 0.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This quantity of bleed over was considered negligible, providing 0.005 – 0.002 $mol \cdot m^{-2} \cdot night^{-1}$.

Data collection. Height at first fully-open bloom, shoot dry weight, days to first fully open flower since treatment commencement, nodes under first fully-opened flower, total number of visible inflorescence (all flowers and visible flower buds), and compactness (g shoot dry weight / cm plant height) were recorded. Parameters were included in consideration of previous research on cyclical NI (Runkle et al., 1998; Blanchard and Runkle 2009 and 2010; Runkle and Blanchard 2016).

Experimental design and statistics. A completely randomized block with three blocks design was used to test the ability of cyclic NI lighting with LEDs to promote flowering of petunia. Each experimental unit contained 10 plants for a total of 180 plants in each study. Statistical analysis

was done in R (Version 3.3.3, The R Foundation) with significance set at α=0.05 for all tests. Pairwise comparisons between treatments done with Tukey's honestly significant difference test.

Results

Study 1. Flowering. Study 1 concluded on Dec. 23 2018, 26 d after daily NI treatments commenced. Petunia in study 1 began flowering shortly (18 d) after cyclical NI treatments began and may have florally initiated before NI treatments began. Still, cyclic NI applications during noninductive 14-hr skotoperiods reduced days to flower compared to both control groups, with plants receiving NI flowering ~2 d sooner (Fig. 3.4A). No differences in days to flower was seen among cyclical NI frequencies. The number of inflorescences was greatest with the highest cyclical NI frequency (2 passes/min, Fig. 3.4B). Plants in the 2 passes/min treatment produced ~8 additional inflorescences compared to the controls.

Morphology. Cyclical NI frequency had no significant effects on the number of nodes produced under the first open flower (Fig. 3.4C). Plants produced 12-13 nodes before first full open bloom. Petunias receiving NI were taller than control plants (data not shown). To best quantify whether observed morphological changes resulted from increased elongation and/or increased biomass accumulation, we calculated plant compactness, the ratio of mass to height. Petunias receiving 1 and 0.5 passes/min were both less compact than both control groups (Fig. 3.4D), while plants receiving 2 or 0.25 passes/min were only less compact from control-one, but not control-two. There were no differences in compactness among any of the NI treatments.

Study 2. Flowering. Study 2 concluded on 18 May 2018, 35 d after NI treatments initiated. Differences in time to flowering between NI treatments were more evident in study 2 relative to study 1. The number of days to flower was affected by both cyclical and continuous NI (Fig.

3.4E). Petunias receiving 4-hr continuous NI in the middle of the skotoperiod flowered faster than plants in all other treatments and 6.2 d earlier than control plants. Plants in the 1, 0.5 and 0.25 passes/min treatments flowered 2 to 3 d earlier than control plants, while plants in the 0.1 passes/min NI treatment did not flower earlier than control plants. Differing frequencies of NI had little effect on days to flower, with plants receiving 0.5 passes/min flowering slightly earlier than plants receiving 0.1 passes/min.

Notable differences were evident in the number of inflorescences present at the time of the first fully-open flower. Petunia receiving 4-hr continuous NI had more inflorescences than any other treatment, producing 12.1 more inflorescence than the control plants (Fig. 3.4F). The only cyclical NI frequency to result in significantly more inflorescences than control plants was the 1 pass/min treatment.

Morphology. The number of nodes petunias developed under their first fully open flower was unaffected by cyclical NI treatments relative to the control (Fig. 3.4G). However, plants in the 4-hr continuous NI treatment had ~1 fewer node under their first open flower than control plants. Petunia compactness was affected by both cyclical and continuous NI treatments (Fig. 3.4H). Plants in the 4-hr continuous treatment were less compact than control plants, but similar to the 1 passes/min and 0.5passes/min cyclical NI plants. The 0.5 passes/min treatment resulted in the least compact plants. Plants from the 0.1 passes/min treatment were not significantly less compact than the control.

Discussion

It was the goal of this research to understand if simulations of cyclical NI with LEDs attached to irrigation booms can promote flowering of petunia similarly to the commonly used method of NI

for 4-hr in the middle of skotoperiods. However, similar cyclical NI studies have never used LEDs or simulated irrigation booms. Blanchard and Runkle (2010) studied changing light intensity with constant cyclical frequency with high pressure sodium lamps or in the case of Runkle et al. (1998) and Runkle and Blanchard (2016) administering 6 and 2 minutes of NI, respectively, at different frequencies with incandescent lamps. The fundamental differences in how cyclical NI is applied and with what light source between all these studies makes exact comparisons difficult. At best, simple trends of more or less frequent cyclical NI can be compared.

Days to flower. Cyclical NI affected days to flower similarly in both studies. Study 1 shows all cyclical NI treatments with frequencies ≥ 0.25 passes/min reduced days to flower compared to the control (Fig. 3.4A). Yet, increasing cyclical NI frequency did not further reduce days to flower. Study 2 findings are comparable, with cyclical NI ≥ 0.2 passes/min reducing days to flower compared to the control, while maybe more importantly finding that cyclical NI of 0.1 passes/min failed to reduce days to flower (Fig. 3.4E). Previous research by Runkle and Blanchard (2016) on cyclical NI on *Petunia* × *hybrida* 'Dreams mix' flowering found similar results, with all cyclical NI frequencies used reducing days to flower compared to an uninterrupted 15-hr skotoperiod control, but no difference was seen among the different frequencies. They did not find a cyclical NI treatment that failed to promote flowering. Our results suggest a frequency threshold may exist for petunia 'Daddy blue' below 0.2 passes/min, below which cyclical NI no longer clearly accelerates flowering. Above this threshold, additional boom passes failed to further reduce days to flower. It is not possible to extrapolate what exact boom frequency thresholds may be for other petunia cultivars because of petunia's wide range of cultivar dependent photoperiodic requirements.

While cyclical NI did accelerate flowering compared to our short-day control in both studies, study 2 demonstrates that the industry standard 4-hr continuous NI most-effectively promoted flowering of this petunia cultivar (Fig. 3.4E). The integrated light quantity produced from 4-hr continuous and cyclical NI treatments varied widely in study 2, ranging from 3.4 to 0.2 $mol \cdot m^{-2}$/night (Table 3.2). The 4-hr continuous treatment provided twice as much light as the highest frequency cyclical NI treatment (1 passes/min). Therefore, it is possible that accelerated flowering resulted from increased light quantity (more photosynthesis) rather than 4 hr of continuous NI in the middle of the skotoperiod being the inherent correct time to administer NI. Based on our results, cyclical NI boom lighting is not currently an acceptable replacement for this existing NI standard for facilities already possessing overhead electrical lighting. That said, greenhouses not already possessing overhead fixtures could use cyclical NI boom lighting with frequencies $\geq$ 0.2 passes/min to promote flowering of petunia.

Pemberton and William (2006) found that NI could hasten petunia flowering anywhere from 4 to 30 d depending on cultivar. All petunia plants in studies 1 and 2 flowered, including all plants in 10-hr short-day controls. Likewise, Runkle and Blanchard (2016) saw that petunia grown under 9-hr photoperiods also reach complete flowering. Conversely, in an earlier cyclical NI study by Runkle et al. (2010), petunia grown under 9-hr photoperiods failed to reach complete flowering by study termination. These contrasting successes at keeping petunia vegetative leads us to believe our cultivar, 'Daddy blue', has a weak quantitative photoperiodic response. Weak photoperiodic requirements help to account for the limited success of cyclical NI in promoting flowering of petunia and the failure of short-day controls to remain vegetative. Since our petunia cultivar shows limited, but ultimately some, responsiveness to cyclical NI, it could be presumed that cyclical NI could work better on different cultivars of petunia where NI is more vital to

promoting flowering. Further study could benefit from using petunia cultivars with a stronger quantitative photoperiodic response and/or a qualitative long-day plant like *Campanula carpatica*.

Number of inflorescences. Petunias receiving cyclical NI frequencies ≥ 2 passes/min in study 1 and ≥ 1 passes/min in study 2 produced more visible inflorescence compared to short-day controls (Fig. 3.4B, F). Like days to flower, a frequency threshold may exist for inflorescence production, where > 1-2 passes/min increases petunia's production of inflorescence. Contradictory however, our results show that days to flower, and therefore the floral induction pathway, was influenced in both studies at lower (0.1-0.2 passes/min) cyclical NI frequencies than the number of inflorescences. Consequently, it is possible that the increase in the number of inflorescence under NI result from higher treatment light integrals (higher DLI), a byproduct of increasing frequency, rather than increasing frequency influencing the floral induction pathway. Blanchard and Runkle (2010) characterize a similar occurrence in *Petunia* × *hybrida* 'Easy wave coral reef', where greater cyclical NI light integrals (and thus higher DLI) increased the number of inflorescences. Results by Faust et al. (2005) and Warner (2010) also support this, finding the number of inflorescences that ***Petunia* × *hybrida*** and *Petunia axillaris* produce is highly dependent on DLI. Further exploration into cyclical NI could benefit from keeping either frequency or light integral constant, hopefully better separating the effects of frequency and amount of supplemental light provided on the number of petunia inflorescences.

Nodes. The number of nodes produced under the first full flower was unaffected by any cyclical NI frequency. However, the industry standard 4-hr continuous NI treatment did result in one less node, indicative of earlier flower initiation. Previous studies on petunia and other long-day plants examining cyclical NI found no significant effects on the number of nodes produced (Runkle, et

al. 1998; Blanchard and Runkle 2010; Runkle and Blanchard 2016). Based on multiple cyclical NI studies, including our own, it seems that few differences emerge in the number of nodes that petunia produces before flowering.

Compactness. Compactness was affected differently by cyclical NI frequencies in the two studies. Cyclical NI frequencies between 1 and 0.5 passes/min reduced petunia compactness in study 1, while frequencies ≥ 0.2 passes/min reduced compactness in study 2. Faust et al. (2005) suggests that plant compactness may be biased in response to delayed flowering and its effect on plant height or possibly when plant height is measured. We conducted linear regression analysis between plant height and days to flower to test this but saw no significant trend ($p = 0.43$). This suggest that compactness differences resulted from cyclical NI. It is not fully understood based on these two studies how exactly cyclical NI frequency affects compactness. This is made more difficult from study 1containing petunia seedlings possibly already florally initiated. Previous cyclic NI studies have often not reported plant compactness, suggesting that either the metric was no calculated or that no visible height differences emerged, and therefore researcher's saw no need to more fully characterize morphological differences. Because compactness is influenced by cyclical NI with moving booms in study 2, further work could benefit from including this metric.

Conclusion. It is possible to simulate an irrigation boom administering cyclical NI in a controlled environment using dimmable LED fixtures. Cyclical NI exceeding 0.2 boom passes/min accelerated flowering of petunia compared to a short-day control. The positive, yet somewhat small, promotion of flowering seen in our studies makes the technique's applicability questionable compared to traditional NI techniques for promoting flowering of *petunia* × *hybrida* 'Daddy blue', which has a weak quantitative photoperiodic response. This technique

could find more applicable use in promoting flowering of qualitative long-day plants or quantitative long-day plants with stronger photoperiodic requirements.

Literature Cited

Bickford, E.D. and Dunn, S., 1972. *Lighting for plant growth*.

Blanchard, M.G. and Runkle, E.S. 2009. Use of a cyclic high-pressure sodium lamp to inhibit flowering of chrysanthemum and velvet sage. *Scientia Horticulturae 122*(3):448-454.

Blanchard, M.G. and E.S. Runkle. 2010. Intermittent light from a rotating high-pressure sodium lamp promotes flowering of long-day plants. *HortScience 45*(2):236-241.

Borthwick, H.A. and H.M. Cathey. 1962. Role of phytochrome in control of flowering of chrysanthemum. *Bot. Gaz. 123*(3):55-162.

Faust, J.E., V. Holcombe, N.C. Rajapakse and D.R. Layne. 2005. The effect of daily light integral on bedding plant growth and flowering. *HortScience 40*(3):645-649.

Kuack, D. (2013). Propagation with LED lighting. *Greenhouse Grower 31*(*11*):8–9.

Morrow, R.C. 2008. LED lighting in horticulture. *HortScience 43*(7):1947-1950.

Olle, M. and A. Viršile. 2013. The effects of light-emitting diode lighting on greenhouse plant growth and quality. *Agri. Food Sci. 22*(2):223-234.

Pemberton, H. B., and R. R. William. 2006. Winter greenhouse performance and photoperiod responses of 51 cultivars of seed-grown trailing petunias. *HortScience* 41(4):1065-1065.

Runkle, E.S. and M.G. Blanchard. 2016, May. Investigating reciprocity of intensity and duration of photoperiodic lighting to regulate flowering of long-day plants. In *VIII Intl. Symp. on Light in Hort. 1134*:41-48.

Runkle, E.S., R.D. Heins, A.C. Cameron, and W.H. Carlson. 1998. Flowering of herbaceous perennials under various night interruption and cyclic lighting treatments. *HortScience 33*(4):672-677. *HortScience 33*(4):672-677.

Schulz, M. 2008. Boom systems: A solution for efficient irrigation. Greenhouse Product News *18* (*3*):26–31.

Tan, S.T., X.W. Sun, H.V. Demir, and S.P. DenBaars. 2012. Advances in the LED materials and architectures for energy-saving solid-state lighting toward “lighting revolution”. *IEEE Photonics J. 4*(2):613-619.

Thomas, B. and D. Vince-Prue. 1996. *Photoperiodism in plants*. Academic Press.

Valverde, F., A. Mouradov, W. Soppe, D. Ravenscroft, A. Samach and G. Coupland. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science 303*(5660):1003-1006.

Warner, R.M., 2010. Temperature and photoperiod influence flowering and morphology of four Petunia spp. *HortScience*, *45*(3), pp.365-368.

Whitman, C.M., R.D. Heins, A.C. Cameron and W.H. Carlson. 1998. Lamp type and irradiance level for daylength extensions influence flowering of *Campanula carpatica* 'Blue Clips', *Coreopsis grandiflora* 'Early Sunrise', and *Coreopsis verticillate* 'Moonbeam'. *J. Amer. Soc. Hort. Sci. 123*(5):802-807.

Table 3.1. Growth chamber environmental conditions

Environmental condition	Study one	Study two
Air temperature (°C)	23.9 ± 0.6 [z]	24.5 ± 0.3
Relative humidity (%)	61 ± 5	58 ± 8
CO_2 ($\mu mol \cdot mol^{-1}$)	846 ± 117	849 ± 75
Volumetric water content (%)	34 ± 4	36 ± 7

[z] Average ± standard deviation

Table 3.2. Cyclical night interruption treatment properties. Increasing boom frequency causes increases in total time illuminated and light received.

Treatment		Frequency (boom passes/min)	Cumulative night interruption (hr) [z]	Integrated *PPFD* (mol/m^2/night)
Study one	1*	0	0	0.006 ± 0.001 [y]
	2*	0	0	0.005 ± 0.000
	3	2	4	3.36 ± 0.05
	4	1	2	1.81 ± 0.04
	5	0.5	1	0.84 ± 0.04
	6	0.25	0.5	0.46 ± 0.02
Study two	1*	0	0	0.002 ± 0.000
	2***	na	4	3.41 ± 0.02
	3	1	2	1.69 ± 0.03
	4	0.5	1	0.85 ± 0.03
	5	0.2	0.4	0.34 ± 0.01
	6	0.1	0.2	0.17 ± 0.00

* Control

*** 4-hr continuous night interruption.

[z] Total time plants receive illumination from any night interruption treatments.

[y] Average ± standard deviation. N=2

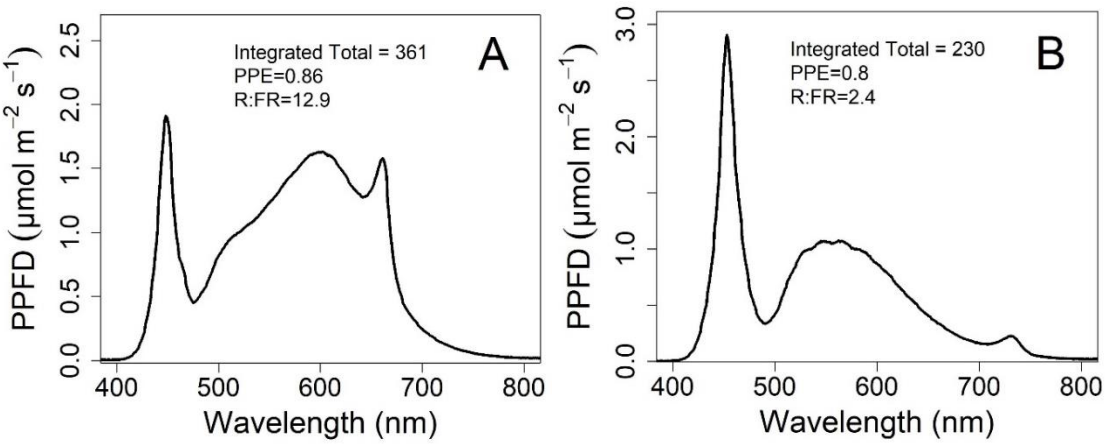

Figure 3.1. (A) Spectroradiometer readings of sole-source lighting in the growth chamber and germination room. (A) Growth chamber spectrum with little far-red (700-800 nm). (B) Germination spectrum with more blue light and a small amount of far-red light.

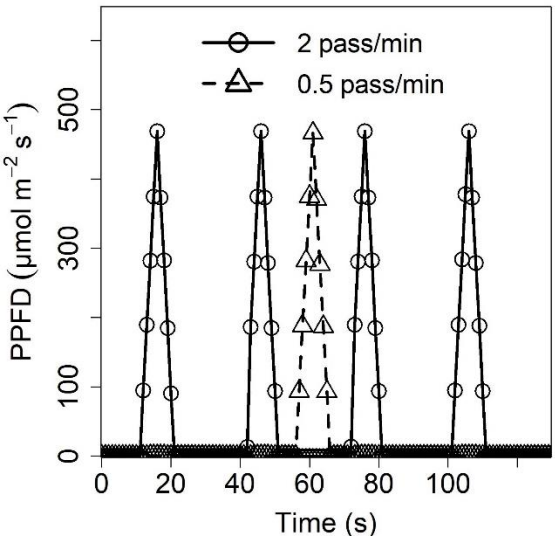

Figure 3.2. Photosynthetic photon flux density (*PPFD*) of two randomly selected treatments in study 1, demonstrating frequency differences between the 2 passes/min (solid line/circles) and 0.25 passes/min (dashed lined/triangles) cyclical night interruption treatments. Each simulated boom pass is identical and provides ~2300 µmol·m^{-2} over 10 s.

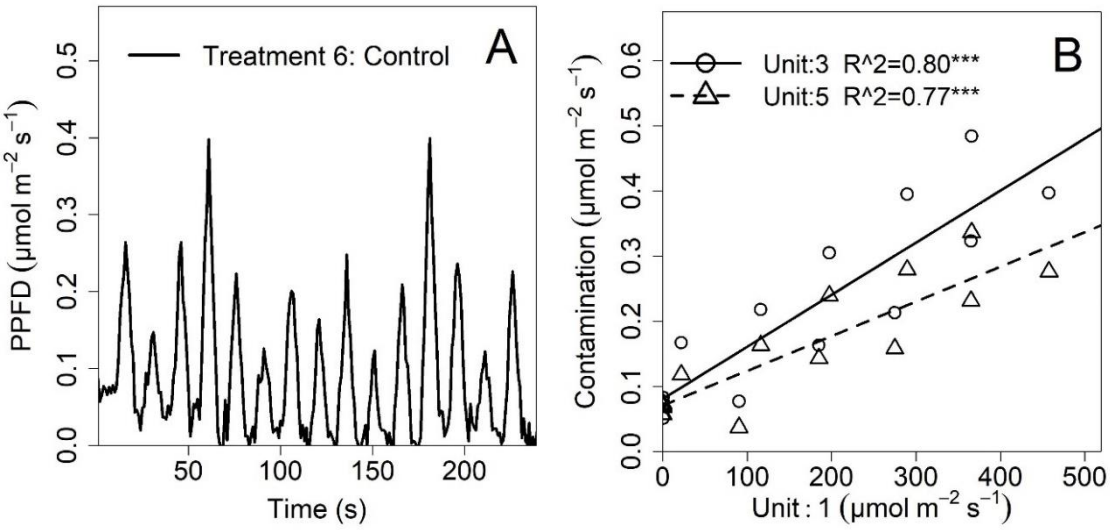

Figure 3.3. (A) Light contamination measured in a control treatment without night interruption, never exceeding 0.45 µmol·m^{-2}·s^{-1}. The rhythmic fluctuations mimic cyclical night interruption applications administered in other units in growth chamber. (B) Light contamination in two experimental units, coming from a 3rd experimental unit above the other two units. Light contamination in experimental units 3 and 5 are positively correlated with light in unit 1, but negligible.

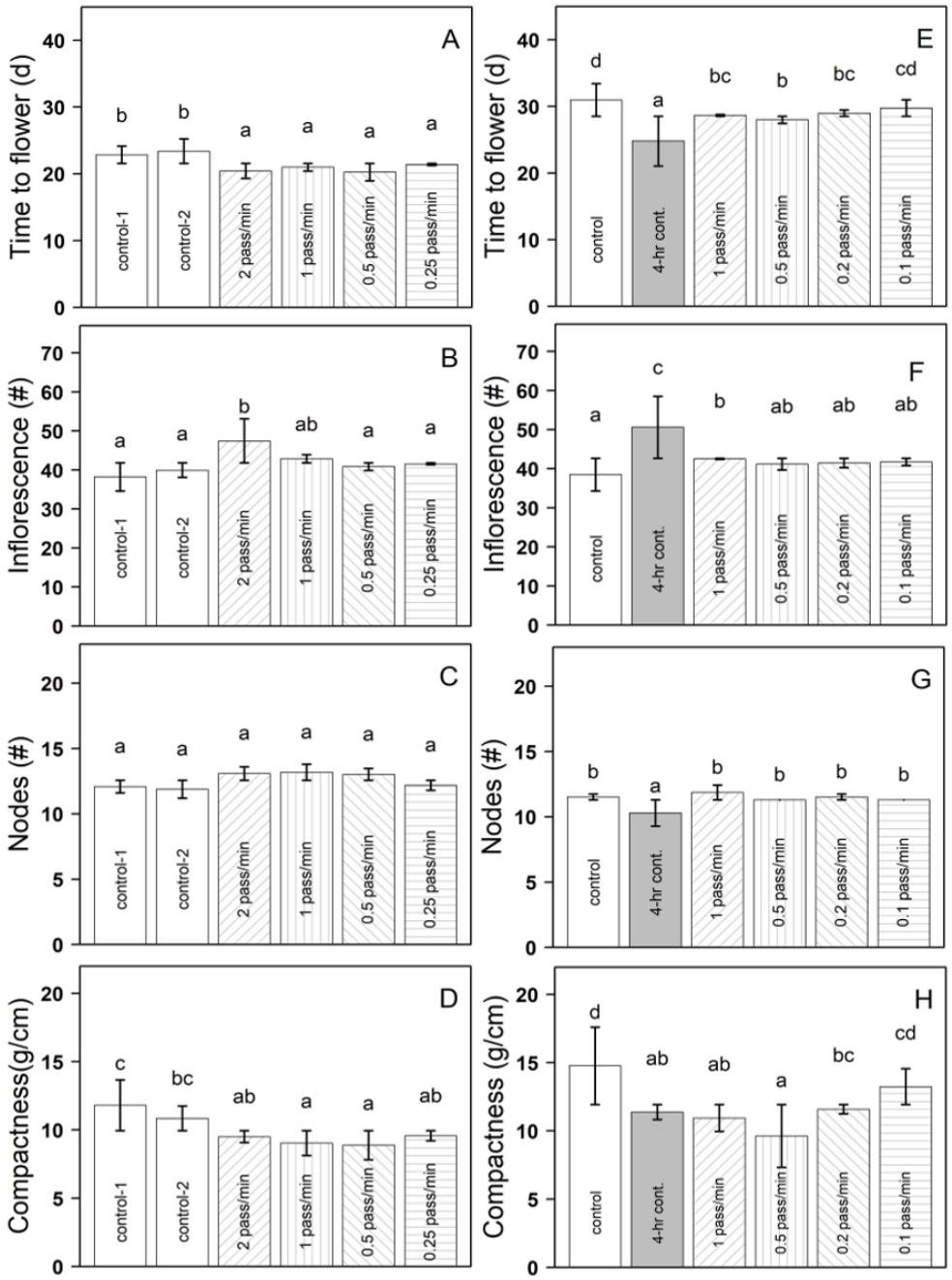

Figure 3.4. Effects of cyclical night interruption on petunia growth and development. (A-D) Results from study 1. Treatments began on 7-week old petunia seedlings. (E-H) Results of study 2. Treatments began on 4-week old seedlings. Means with the same letter are not significantly different ($P < 0.05$).

CHAPTER 4

Conclusions

Environments high in far-red (FR) light, characteristic of shading, cause shade avoidance syndrome (SAS) in many plant species. *Chrysanthemum morifolium* experiences SAS when night interruptions (NI) high in FR are applied at the end of short skotoperiods. The severity of SAS induced is codependently governed by an external and internal factor. Far-red light acts as this external factor and has a dosage dependent effect on SAS, where greater FR increases the magnitude of SAS. The internal factor, while undetermined form this study, has circadian fluctuations that likely begin accumulating in the middle of skotoperiods and peak at dawn. Knowing when FR influences SAS most could beneficially allow for, depending on intent, a way to avoid side effects caused by FR while trying to induce flowering or the best time to influence chrysanthemum height.

Contrary to our initial hypothesis, NI high in FR was ineffective at promoting flowering of chrysanthemum. We observed that FR only slightly hastened flowering while not influencing flowering percentage or number of inflorescence produced. Unexpectedly, we did find that NI applied during the middle of the skotoperiod, regardless of FR intensity, flowered quickest, had the most inflorescences, and the highest flowering percentage. Whether flowering was promoted or simply inhibited the least is currently unknown. Night interruption during the end of the skotoperiod was most effective at keeping chrysanthemum vegetative. This result contradicts traditional methods of NI in the middle of short skotoperiods to keep short-day plant vegetative.

Cyclical NI from simulated irrigation booms slightly accelerated flowering of *Petunia* × *hybrida* compared to 10-hr short-day controls. However, no additional benefits were seen from increasing boom frequency past a threshold between 0.1 and 0.2 passes/minute. This result has two broader implications. An infrequent threshold means large greenhouses with long bays, where frequency could be a limiting factor, can use this technique to accelerate flowering. Oppositely, in small greenhouses, where high frequencies could be achieved, no additional benefits would likely be gained from using higher frequencies.

Light emitting diodes are more controllable than traditional electrical light sources and allowed for successful computer simulations of irrigation booms administering NI. This new methodology empowers more accurate research into cyclical NI than ever before. Still, further study is needed in three major areas. 1) Using different petunia cultivars and crops with stronger photoperiodic requirements would help establish what frequency best promotes flowering and broaden its applications. 2) Keeping boom frequency or integrated light totals constant would help distinguish between their independent effects on flowering. 3) Finding frequency recommendations to inhibit flowering and keep short-day plants vegetative.