IMPROVING PHOTOSYNTHETIC LIGHTING EFFICIENCY IN CONTROLLED ENVIRONMENT AGRICULTURE: THE IMPORTANCE OF LIGHT ACCLIMATION AND FAR-RED LIGHT

by

SHUYANG ZHEN

(Under the Direction of Marc van Iersel)

ABSTRACT

Photosynthetic lighting is often needed to produce high quality crops in controlled environment agriculture but can substantially increase production costs. To improve lighting efficiency and reduce costs, a better understanding of how crops use light for photosynthesis is needed. We used chlorophyll fluorescence and gas exchange measurements to quantify the photosynthetic light responses of three horticultural crops as affected by acclimation to different light intensities, and the effect of far-red light on photosynthesis. Quantum yield of PSII (Φ_{PSII}), a common measure of photochemical efficiency, decreased with increasing light intensity, indicating that supplemental lighting is used less efficiently for photochemistry when provided at high ambient light intensity. Electron transport rate, which is often closely correlated with photosynthetic rate, increased asymptotically with increasing light. The high light-adapted species sweetpotato (*Ipomea batatas*) used high light more efficiently for electron transport than light-intermediate lettuce (*Lactuca sativa*) and shade-tolerant pothos (*Epipremnum aureum*). Plants acclimated to high light (full sun) also tended to have higher Φ_{PSII} than those acclimated to low light (44% or 75% shade). Far-red light increased Φ_{PSII} and net photosynthetic rate of lettuce

when added to red/blue or warm-white LED light, which over-excites photosystem II (PSII). The addition of far-red light helps to balance the excitation between the two photosystems, thus increasing photosynthetic efficiency, by preferentially exciting photosystem I (PSI). This indicates that different wavelengths interactively affect photosynthetic efficiency, likely through affecting the excitation balance between PSI and PSII. We also determined which wavelengths of far-red light increase photochemical efficiency of lettuce. Longer wavelengths within the 678-703 nm range were increasingly used more efficiently by PSI than by PSII, as indicated by the increasing Φ_{PSII} when light of longer wavelengths was added to red/blue light. The enhancement of Φ_{PSII} tended to be smaller as wavelengths increased from 721 to 731 nm, probably due to lower leaf light absorption at longer wavelengths. Photons at 752 nm no longer increase Φ_{PSII}, likely because they do not excite PSI. Additional measurements with narrow-band far-red wavelengths that are currently unavailable (especially 732-751 nm) will provide more information on the efficiency of different far-red wavelengths at enhancing photochemistry.

INDEX WORDS: quantum yield of photosystem II, electron transport rate, light acclimation, non-photochemical quenching, photosynthesis, shade, chlorophyll fluorescence, Emerson enhancement effect, excitation energy distribution, laser diodes, light emitting diodes, state transitions

IMPROVING PHOTOSYNTHETIC LIGHTING EFFICIENCY IN CONTROLLED ENVIRONMENT AGRICULTURE: THE IMPORTANCE OF LIGHT ACCLIMATION AND FAR-RED LIGHT

by

SHUYANG ZHEN

B.S., Huazhong Agricultural University, China, 2011

M.S., University of Maine, 2013

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

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2017

IMPROVING PHOTOSYNTHETIC LIGHTING EFFICIENCY IN CONTROLLED ENVIRONMENT AGRICULTURE: THE IMPORTANCE OF LIGHT ACCLIMATION AND FAR-RED LIGHT

by

SHUYANG ZHEN

Major Professor:	Marc van Iersel
Committee:	Anish Malladi
	Robert Teskey
	Paul Thomas
	Deepak Mishra

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2017

DEDICATION

I would like to dedicate this work to my parents and my sister, who have always supported and believed in me.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Marc van Iersel, for his guidance and support during this process and for always encouraging me to think critically. Marc has been a patient and supportive advisor and a good friend. His kindness and sense of humor are appreciated. Thank you for advising me on numerous occasions, and for providing me with opportunities that facilitate my academic career. I hope to become a great advisor to my future students like you have been to me.

I thank my committee, Dr. Malladi, Dr. Teskey, Dr. Thomas, and Dr. Mishra for their support, advice, and feedbacks on my research.

I thank our lab technician Sue Dove and all the former and current members of the horticultural physiology lab for their assistance and friendship. I also thank University of Georgia Horticulture Department faculty and staff for their support and assistance. I thank all the Horticulture graduate students for their friendship and encouragements.

I would like to thank my master's advisor, Stephanie Burnett for continuing to support me during my study at the University of Georgia.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF FIGURES ix

CHAPTER

1 INTRODUCTION 1

Research Objectives 3

Literature Cited 5

2 LITERATURE REVIEW 8

Benefits of LEDs for Photosynthetic Lighting 8

Photosynthetic Responses to Different Light Intensities 9

Wavelength Dependence of Photosynthesis 10

Synergistic Effect among Wavelengths on Photosynthesis 11

Species and Light Acclimation Effects on Photosynthetic Responses 13

Optimizing Photosynthetic Lighting Efficiency 14

Literature Cited 15

3 PHOTOCHEMICAL ACCLIMATION OF THREE CONTRASTING SPECIES TO DIFFERENT LIGHT LEVELS: IMPLICATIONS FOR OPTIMIZING SUPPLEMENTAL LIGHTING 22

Abstract 23

Introduction 24

Materials and Methods 27
Results and Discussion 32
Conclusions 41
Acknowledgements 41
Literature Cited 42
4 FAR-RED LIGHT IS NEEDED FOR EFFICIENT PHOTOCHEMISTRY AND PHOTOSYNTHESIS 53
Abstract 54
Introduction 55
Materials and Methods 59
Results and Discussion 64
Conclusions 72
Acknowledgements 73
Literature Cited 73
5 IDENTIFYING THE SPECTRAL RANGE OF FAR-RED LIGHT THAT ENHANCES PHOTOCHEMISTRY OF LETTUCE GROWN UNDER DIFFERENT SPECTRA 87
Abstract 88
Introduction 89
Materials and Methods 92
Results and Discussion 98
Conclusions 106
Acknowledgements 107

Literature Cited 107

6 CONCLUSIONS 118

LIST OF FIGURES

Page

Figure 3.1: Leaf chlorophyll content index of sweetpotato, lettuce, and pothos 47

Figure 3.2: Dark-adapted F_v/F_m of sweetpotato, lettuce and pothos at three shading levels..... 48

Figure 3.3: Light response curves of quantum yield of photosystem II (Φ_{PSII}) (A-C), linear electron transport rate (ETR) (D-F), and non-photochemical quenching (NPQ) (G-I) for sweetpotato (A, D, G), lettuce (B, E, H), and pothos (C, F, I) grown under full sun, 44% shade, and 75% shade..... 49

Figure 3.4: Initial slope of the Φ_{PSII} – *PPF* curve, an indicator of how fast Φ_{PSII} decreased when plant was transferred from dark to light, of sweetpotato, lettuce, and pothos (A) and by shading level (B) 50

Figure 3.5: Initial slope of the ETR – *PPF* curve, an estimate of the maximum rate of increase in ETR per unit increase in incident *PPF,* of sweetpotato, lettuce, and pothos (A) and by shading level (B) 51

Figure 3.6: Initial slope of the NPQ – *PPF* curve, which is indicative of the rate of increase in heat dissipation of the absorbed light upon transfer of plants from dark to light, of sweetpotato, lettuce, and pothos (A) and by shading level (B)..... 52

Figure 4.1: Normalized spectral distribution of red/blue, warm-white, and far-red light emitting diodes…. 80

Figure 4.2: A typical fluorescence trace of a lettuce leaf under changing light conditions illustrates changes in fluorescence yield upon transitioning of the leaves from dark to

different intensities of red/blue light, and when far-red light (110 μmol m^{-2} s^{-1} in the 700-770 nm wavelength range) was added to or removed from red/blue light..... 82

Figure 4.3: Quantum yield of photosystem II (Φ_{PSII}) (A, B) and non-photochemical quenching (NPQ) (C, D) of chlorophyll fluorescence as affected by adding far-red light to different intensities of red/blue (A, C) or warm-white light (B, D)..... 83

Figure 4.4: The effect of adding far-red light to different intensities of red/blue or warm-white light on net photosynthetic rate (Pn) (A, B) and Pn/*PPFD* (C, D) 84

Figure 4.5: Quantum yield of photosystem II (Φ_{PSII}) (A) and non-photochemical quenching (NPQ) (B) of chlorophyll fluorescence as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1}) 85

Figure 4.6: Net photosynthetic rate (P_n) as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1})..... 86

Figure 5.1: Normalized spectral distribution of sunlight, red/blue light-emitting diodes (LED) light, and halogen light..... 112

Figure 5.2: Pigment concentration of lettuce grown under red/blue LED light or sunlight 113

Figure 5.3: Absorption, transmission, and reflectance spectra of red/blue-grown lettuce leaves (a) and sunlight-grown lettuce leaves (b) 114

Figure 5.4: Quantum yield of photosystem II (Φ_{PSII}) (a, b) and non-photochemical quenching (NPQ) (c, d) of lettuce grown under red/blue light (a, c) or sunlight (b, d) in response to increasing photosynthetic photon flux density (*PPFD*) of red/blue light or halogen light..... 115

Figure 5.5: Quantum yield of photosystem II (Φ_{PSII}) upon adding different wavelengths of far-red light (FWHM 2-3 nm) to red/blue light for lettuce grown under red/blue light (a) or sunlight (b) 116

Figure 5.6: Quantum yield of photosystem II (Φ_{PSII}) as a function of the incident photon flux density (PFD) (a, b) and absorbed PFD (c, d) of far-red light with a peak wavelength of 700 or 723 nm, respectively 117

CHAPTER 1

INTRODUCTION

Horticultural crops such as vegetables, cut flowers, and ornamental bedding plants are often produced year-round inside controlled environment agriculture facilities to meet market needs. Insufficient sunlight, especially during winter, frequently limits plant growth and reduces plant quality. Photosynthetic lighting is often needed to increase photosynthesis, improve crop quality and yield, and shorten the production cycle (Nelson, 2012). Typical uses of photosynthetic lighting include off-season vegetable production and winter production of ornamental crops in greenhouses for spring/summer sales in northern climates (Gaudreau and Chartbonneau, 1994; Lopez and Runkle, 2008). Recently, there is increasing interest in growing food in indoor vertical farms, where the light needed to grow the plants is entirely provided by electric light. Crop production costs increase substantially because of the high electrical consumption of lighting. For example, van Iersel and Gianino (2017) estimated that the cost of supplemental lighting provided by high pressure sodium (HPS) lamps can account for about 30% of the farm gate value for vegetable greenhouses. In indoor production facilities, lighting accounts for 50-60% percent of the operating costs (van Iersel, 2017; Zeidler et al., 2013). The high cost of photosynthetic lighting in controlled environment agriculture has brought the need for more efficient use of light.

Photosynthetic lighting efficiency can be improved by implementing energy-efficient lighting sources, such as light emitting diodes (LEDs) (Nelson and Bugbee, 2014). In addition to the efficiency of the lights, the overall efficiency at which the electrical energy is converted into

plant biomass depends on how efficiently plants are able to use light for photosynthesis. Photosynthetic light use efficiency can be improved by providing plants with the light levels that are most efficient for photosynthesis. The efficiency with which plants use the absorbed light for photosynthesis, however, decreases with increasing light intensity (Baker, 2008; Demmig-Adams et al., 1996; van Iersel et al., 2016b). As a result, photosynthetic light provided at high intensities or in addition to a high ambient light intensity would result in a proportionally smaller increase in photosynthesis and presumably growth (van Iersel and Gianino, 2017). This response may also differ among species and is affected by light acclimation, as plants adapted/acclimated to different light levels often differ in their efficiency at using light for photosynthesis (Anderson and Osmond, 1987; Anderson et al., 1995; Björkman, 1981; Seemann et al., 1987; Valladares and Niinemets, 2008). Few studies have investigated the feasibility of optimizing photosynthetic lighting in controlled environments based on plant's physiological responses to light (van Iersel et al., 2016a, 2016b) and there is a need for information on how photosynthetic lighting can be optimized for crops adapted/acclimated to different light environments.

In addition to optimizing the light intensity for plant growth, photosynthetic light use efficiency can be improved by providing plants with the optimal spectrum for photosynthesis. The efficiency of photosynthesis is wavelength dependent: when measured under monochromatic light, the quantum yield for CO_2 fixation per absorbed photon is highest under red light (about 600-680 nm), followed by blue and green light (Evans 1987; Inada 1976; McCree 1972). Far-red light ($\lambda > 700$ nm) has long been considered to be photosynthetically ineffective, due to its low quantum yield of photosynthesis (McCree, 1972). As a result, red and blue light is more widely used in LED grow lights in controlled environment agriculture facilities while other colored lights are often not included, or only included at small amounts. However,

the effects of different wavelength lights on photosynthesis are not simply independent or additive: light of different wavelengths may have synergistic effects on photosynthesis (Emerson et al., 1957). The quantum yield spectrum of photosynthesis ignores the synergistic effects among wavelengths and thus may not accurately estimate the photosynthetic efficiency of different wavelengths. A better understanding of the synergistic effects on photosynthesis among wavelengths, which remain under-studied, can provide more accurate estimates of the photosynthetic efficiency of different wavelengths and improve our understanding of the optimal spectrum for photosynthesis.

The quantum yield of photosystem II (Φ_{PSII}), which is the fraction of light absorbed by the leaves that is used in the photochemical reactions to drive electron transport (Maxwell and Johnson, 2000), is a common measure of photosynthetic light use efficiency. Quantum yield of PSII can be easily measured using chlorophyll fluorescence (Genty et al., 1989). In addition, chlorophyll fluorescence can be used to estimate the electron transport rate (ETR), which is often closely correlated with overall photosynthetic rate (Beer et al., 1998; Flexas et al., 1999), and the degree of heat dissipation, determined by a parameter called non-photochemical quenching (NPQ), under various light conditions. The real-time, non-invasive nature of chlorophyll fluorescence measurements makes it an excellent tool to monitor photosynthetic efficiency.

Research Objectives

The aim of this research was to improve our understanding of the photosynthetic responses to different light intensities and spectra, and how the responses differ among species/plants acclimated to different light environments.

The first study quantified the photosynthetic light responses of three horticultural crops as affected by acclimation to different light intensities. The objectives of this study were 1) to determine the photochemical responses of different species to a wide range of light intensities; 2) to quantify how light acclimation affects crops' photosynthetic efficiency; and 3) to examine if chlorophyll fluorescence measurements can be used to develop improved supplemental lighting strategies for crops adapted/acclimated to different light environments.

The second study re-visited the Emerson enhancement effect on photosynthesis among different wavelengths and re-evaluated the effect of far-red light on photosynthesis. We specifically wanted to answer the following questions, which have not been addressed in past research: 1) how does far-red light affect Φ_{PSII} and NPQ (*i.e.* heat dissipation of the absorbed light energy) and does this depend on the intensity of the far-red light?; 2) how does far-red light affect Φ_{PSII} and NPQ when added to different light intensities?; and 3) does the effect of far-red light differ when added to different spectra (red/blue vs. white light)? Our goal is to provide a better understanding of the interactive effects of light with different wavelengths on Φ_{PSII} and photosynthesis.

Our third study compared the efficiency of different far-red wavelengths at enhancing photochemistry of lettuce grown under different spectra. We used laser diodes to obtain narrow-band far-red light to 1) identify the spectral range of far-red light that enhances photochemistry; 2) determine the relative efficiency of different wavelengths and intensities of far-red at enhancing photochemistry; and 3) determine if acclimation to different light spectra (red/blue vs. sunlight) affects the photosynthetic responses measured under different light spectra (red/blue vs. halogen light).

Literature Cited

Anderson, J.M. and C.B. Osmond.1987. Shade - sun responses: Compromises between acclimation and photoinhibition, p. 1-36. In: D. J. Kyle, C. B. Osmond and C.J. Arntzen (eds.). Photoinhibition. Elsevier Science Publishers, Amsterdam.

Anderson, J.M., W.S. Chow, and Y. Park. 1995. The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. Photosyn. Res. 46:129-139.

Baker, N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59:89-113.

Beer, S., B. Vilenkin, A. Weil, M. Veste, L. Susel, and A. Eshel. 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. Marine Ecol. Prog. Ser. 174:293-300.

Björkman O. 1981. Responses to different quantum flux densities, p. 57-107. In: O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.). Physiological plant ecology I. Encyclopedia of plant physiology, Vol. 12A, Springer-Verlag, Berlin, Germany.

Demmig-Adams, B., W.W. Adams, III, D.H. Barker, B.A. Logan, D.R. Bowling, and A.S. Verhoeven. 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant. 98:253-264.

Emerson, R., R. Chalmers, and C. Cederstrand. 1957. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci. USA 43:133-143.

Evans, J.R. 1987. The dependence of quantum yield on wavelength and growth irradiance. Aust. J. Plant Physiol. 14:69-79.

Flexas, J. J.M. Escalona, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell Environ. 22:39-48.

Gaudreau, L. and J. Chartbonneau. 1994. Photoperiod and photosynthetic photon flux influence growth and quality of greenhouse-grown lettuce. HortScience 29:1285-1289.

Genty, B., J. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990:87-92.

Inada, K. 1976. Action spectra for photosynthesis in higher plants. Plant Cell Physiol. 17:355-365.

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

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – A practical guide. J. Expt. Bot. 51:659-668.

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

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS ONE 9(6):e99010.

Nelson, P.V. 2012. Greenhouse operation and management. 7th ed. Prentice Hall, Upper Saddle River, NJ.

Seemann, J.R., T.D. Sharkey, J. Wang, and C.B. Osmond. 1987. Environmental effects on photosynthesis, nitrogen –use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiol. 84:796-802.

Valladares, F. and Ü. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annu. Rev. Ecol. Evol. Syst. 39:237-257.

van Iersel, M.W. 2017. Optimizing lighting in controlled environment agriculture. In S.D. Gupta (ed.) Light emitting diodes for agriculture - Smart lighting. Springer, Singapore. (in print).

van Iersel, M.W. and D. Gianino. 2017. An adaptive control approach for LED lights can reduce the energy costs of supplemental lighting in greenhouses. HortScience 52:72-77.

van Iersel, M.W., E. Mattos, G. Weaver, R.S. Ferrarezi, M.T. Martin, and M. Haidekker. 2016a. Using chlorophyll fluorescence to control lighting in controlled environment agriculture. Acta Hort. 1134:427-433.

van Iersel, M.W., G. Weaver, M.T. Martin, R.S. Ferrarezi, E. Mattos, and M. Haidekker. 2016b. A chlorophyll fluorescence-based biofeedback system to control photosynthetic lighting in controlled environment agriculture. J. Amer. Soc. Hort. Sci. 141:169-176.

Zeidler C., D. Schubert, and V. Vrakking. 2013. Feasibility study: Vertical farm EDEN. German aerospace center, Inst. Space Systems, Dept. System Analyse Space Segment (SARA), Bremen, Germany.

CHAPTER 2

LITERATURE REVIEW

Benefits of LEDs for Photosynthetic Lighting

Light emitting diodes (LEDs) are increasingly used for supplemental lighting in greenhouses or as sole-source lighting for indoor production of high value crops (Mitchell et al., 2012). In addition to their high efficiency, LEDs have narrow spectral output (*i.e.*, distinct colors) available in the wavelength range from UV to far-red light (Bourget, 2008; Morrow, 2008). This enables precise control of the light spectral composition for controlling plant growth and morphology, phytochemical composition, flowering, and potentially optimizing the spectrum for photosynthesis (Cope et al., 2014; Craver et al., 2017; Ouzounis et al., 2015; van Iersel, 2017; Wollaeger and Runkle, 2015).

Compared to conventional light sources, such as high-intensity discharge (HID) lamps, LEDs emit little radiant heat, which allows them to be installed close to plant surfaces, e.g., in indoor multi-layer production systems, without scorching the leaves (Morrow, 2008; Randall and Lopez, 2015). Decreasing the distance between plants and lights increases the energy efficiency of photosynthetic lighting because more light reaches the canopy without an increase in electrical costs. Lighting efficiency can be further improved by intra-canopy lighting. Placing LEDs inside the canopy increases the light level within the inner canopy where low light availability normally limits photosynthesis (Gómez et al, 2013).

Another feature of LEDs is the controllability of light output. The light intensity of LEDs can be easily and precisely controlled (van Iersel and Gianino, 2017). As the efficiency at which plants use light for photosynthesis is dependent on light intensity, LEDs have the potential to provide plants with the light levels that are optimal for photosynthesis. However, there has been little research on controlling photosynthetic lighting based on plant light use efficiency, and the controllability of LEDs remains underutilized (van Iersel, 2017).

Photosynthetic Responses to Different Light Intensities

Not all the light absorbed by plants is used in the photochemical reactions of photosynthesis for subsequent carbon assimilation. Some of the absorbed light can be dissipated as heat, and another small fraction is re-emitted as chlorophyll fluorescence (Maxwell and Johnson, 2000). The ability of plants to use the absorbed light for photosynthesis, rather than dissipating it as heat or through chlorophyll fluorescence, decreases with increasing light intensity (Baker, 2008; Demmig-Adams et al., 1996; van Iersel et al., 2016b). Heat dissipation of the absorbed light, on the other hand, increases with increasing light intensity (Demmig-Adams and Adams, 1992; Demmig-Adams et al., 1996). Chlorophyll fluorescence is dependent on the efficiency of the other two processes: chlorophyll fluorescence yield decreases in response to increases in the efficiency of photochemistry and/or thermal dissipation of the absorbed light energy (Baker et al., 2007; Maxwell and Johnson, 2000).

Chlorophyll fluorescence has long been used to monitor plant photosynthetic performance. Chlorophyll fluorescence provides fast, non-invasive measurements of the quantum yield of photosystem II (Φ_{PSII}), a common measure of photosynthetic light use efficiency (Genty et al., 1989). Chlorophyll fluorescence can also be used to estimate the

electron transport rate (ETR) and the degree of heat dissipation, determined by a parameter called non-photochemical quenching (NPQ), under various light conditions (Maxwell and Johnson, 2000).

Wavelength Dependence of Photosynthesis

Photosynthetic responses to light are wavelength-dependent: when measured under monochromatic light, the efficiency of photons to drive photosynthesis is higher under red light (about 600-680 nm) than that under blue and green light (Evans, 1987; Inada, 1976; McCree, 1972a). Photosynthetic efficiency also declines sharply at wavelengths above 685 nm (Emerson and Lewis, 1943; Hogewoning et al., 2012). Action or quantum yield spectra of photosynthesis (photosynthetic rate per unit incident or absorbed photon flux) are commonly used to describe the wavelength dependence of photosynthesis (McCree, 1972a).

Absorption by photosynthetic carotenoids (where excitation energy is transferred to chlorophylls with an efficiency significantly less than 100%) and non-photosynthetic flavonoids and carotenoids partly account for the lower quantum yield of blue and green light (Hogewoning et al., 2012; Hoover, 1937; Terashima et al., 2009). Besides that, a major cause for the wavelength dependency of photosynthetic efficiency is the imbalanced excitation of the two photosystems – photosystem I (PSI) and photosystem II (PSII), that carry out the photochemical reactions of photosynthesis (Evans, 1987; Hogewoning et al., 2012). As the two photosystems operate in series to drive linear electron transport from H_2O to the terminal electron acceptors (usually $NADP^+$), excitation of PSI and PSII should be approximately equal to achieve optimal efficiency of photochemistry (Allen, 2003; Butler, 1978). Under light that over-excites one

photosystem relative to the other, the rate of photochemical reactions is limited by the activity of the under-excited photosystem, resulting a decrease in photosynthetic efficiency.

Due to differences in pigment composition, antenna size, and density of PSI and PSII, excitation energy distribution between the two photosystems is often imbalanced, especially under narrow spectrum light (Hogewoning et al., 2012; Laisk et al., 2014). Most of the shorter wavelengths within the 400-680 nm range over-excite PSII, while longer wavelength far-red light ($\lambda > 680$ nm) tends to over-excite PSI (Evans, 1987; Hogewoning et al., 2012; Laisk et al., 2014).

Synergistic Effect among Wavelengths on Photosynthesis

Far-red light ($\lambda > 700$ nm) has long been considered to be photosynthetically ineffective according to the action/quantum spectra of photosynthesis (McCree, 1972a). Emerson and Lewis (1943) first described the 'red drop', a sharp decline in quantum yield of O_2 evolution at wavelengths above 685 nm. They observed that the quantum yield at 700 nm was less than half of that at 685 nm (Emerson and Lewis, 1943). Emerson and coworkers subsequently found that the photosynthetic rate under simultaneous illumination of long- ($\lambda > 685$ nm) and short-wavelength lights was greater than the sum of the rates from applying the two lights separately (Emerson et al., 1957, Emerson and Rabinowitch, 1960; Myers, 1971). This indicates that the effects of different wavelength lights on photosynthesis are not simply independent or additive: light of different wavelengths may have synergistic effects on photosynthesis.

With the assumption that the quantum yield of photosynthesis at shorter wavelengths is maximum and constant, Emerson and others ascribed this synergistic effect on photosynthesis to the enhancement of quantum yield of longer wavelength light by shorter wavelengths (Emerson

et al., 1957; Emerson and Rabinowitch, 1960; Myers and Graham, 1963). The reverse effect, the enhancement of quantum yield of shorter wavelength light by far-red light, has not received much attention and sometimes is thought not to be present (Govindjee et al., 1964; Myers and Graham, 1963). Later studies on the photosynthetic responses to light have paid little attention to the synergistic effects among wavelengths on photosynthesis, partly due to the influential conclusion by McCree (1972b) that that enhancement effects are insignificant in white light.

It is now known that the low photosynthetic efficiency of far-red light is caused by unbalanced excitation of PSI and PSII (Myers, 1971). Far-red light preferentially excites PSI, while shorter wavelengths generally excite PSII more than PSI (Evans, 1987; Hogewoning et al., 2012). Since PSI tends to be under-excited relative to PSII under shorter wavelength light, this limits the overall rate of photochemistry and the subsequent CO_2 assimilation. When shorter wavelength light is supplemented with far-red light that preferentially excites PSI, the excitation balance between the two photosystems can be restored. This can synergistically increase photochemistry and photosynthesis.

For an enhancement of photosynthesis to occur when combining two lights, 1) either of the lights alone should provide unequal excitation of the two photosystems, and 2) the two lights should complement each other, *i.e.* one light over-excites PSI and the other light over-excites PSII (Myers, 1971). It is thus expected that neither of the two lights would be optimal for photosynthesis when applied alone, and that the interaction between the two lights would be synergistic: photosynthetic efficiency of both lights would be improved by each other. Therefore, ignoring the synergistic effects among wavelengths or exclusion of far-red light can lead to inaccurate measures of the photosynthetic activity of light.

Species and Light Acclimation Effects on Photosynthetic Responses

Differences in plants' ability to use light for photosynthesis exist among species. Species adapted to high light usually possess adaptive characteristics (e.g., high rubisco content) that optimize photosynthesis under high light, whereas shade-adapted species optimize photosynthesis in low light by maximizing light harvesting (Björkman, 1981; Seemann et al., 1987). High light-adapted species generally have greater photosynthetic capacity, i.e., a higher maximum photosynthetic rate and higher light-saturation point, than shade-adapted species (Björkman, 1981). In contrast, shade-adapted species tend to reach maximum photosynthetic capacity at much lower light intensity and are more susceptible to photoinhibition under high light (Demmig-Adams and Adams, 1992).

Plants often have the ability to acclimate, i.e., exhibit phenotypic (morphological and physiological) plasticity, to their light environments within the life cycle of a plant (Anderson et al., 1995; Björkman, 1981; Valladares and Niinemets, 2008). Light acclimation has been shown to induce a wide range of phenotypic modifications that alter plants' photosynthetic capacities and their efficiency at using high/low light or different light spectra. For instance, compared with shade-acclimated plants, plants acclimated to high light often have increased electron transport capacities by increasing the content of electron transport components, such as cytochrome f (on per chlorophyll basis), plastoquinone pool, plastocyanin, ferredoxin, and ATP synthase (Anderson and Osmond, 1987; Anderson et al., 1995; Björkman, 1981; Chow et al., 1988; Walters, 2005). Such increases in the content of electron transport components, e.g., a bigger plastoquinone pool, allow a greater fraction of reaction centers to be "open" (i.e., oxidized and capable of using excitation energy from light for photochemistry) under a given light intensity, thus resulting in higher Φ_{PSII}. Changes in chlorophyll content (Givnish, 1988;

Niinemets, 2010), rubisco content and activity (Seemann, 1989), and xanthophyll cycle pigment pool size (involved in heat dissipation of the absorbed light) (Demmig-Adams and Adams, 1992; Logan et al., 1998) could also occur in response to acclimation to different light intensities, allowing plants to use the light levels that they are acclimated to more efficiently.

Plants can also dynamically adjust their photosynthetic apparatus when grown under different light spectra to optimize photosynthetic efficiency. Short-term responses (taking place within minutes) to imbalanced excitation of the two photosystems include the re-allocation of a mobile pool of light harvesting complex II (LHCII) to the under-excited photosystem, a process termed state transition that helps to direct more energy to the under-excited photosystem (Allen 1992, 2003; Haldrup et al. 2001). Adjustments in photosystem stoichiometry, for example an increase in the PSI/PSII ratio of plants grown under light that over-excites PSII, can take place over days to correct unbalanced excitation of the two photosystems and increase the photosynthetic efficiency (Chow et al., 1990; Fujita, 1997; Hogewoning et al., 2012). A decrease in the amount of LHCII per PSII core has also been reported in plants grown under blue light (which over-excites PSII), decreasing the amount of excitation energy partitioned to PSII (Hogewoning et al., 2012). Those changes in photosystem composition as a result of acclimation to the light environment may affect how efficiently plants use light of different spectra for photosynthesis, as well as the interactive effects among different wavelengths.

Optimizing Photosynthetic Lighting Efficiency

One straightforward way to improve the efficiency of photosynthetic lighting is to implement energy-efficient lighting sources, such as LEDs (Nelson and Bugbee, 2014). In addition, photosynthetic lighting efficiency depends on how efficiently plants are able to use

light for photosynthesis. Supplemental lighting in greenhouses is often simply controlled by a timer or based on ambient light levels. The ability of a crop to use the provided light for photosynthesis is seldom considered when developing supplemental lighting strategies. Few studies have investigated the feasibility of optimizing photosynthetic lighting in controlled environments based on plant's physiological responses to light (van Iersel et al., 2016a, 2016b) and there is a need for information on how photosynthetic lighting can be optimized for crops adapted/acclimated to different light environments.

Currently, the optimization of the photosynthetic light spectrum is primarily based on the action or quantum yield spectra of photosynthesis developed by McCree (1972a), assuming that different wavelengths affect photosynthesis independently and additively and that far-red has little photosynthetic efficiency. Red and blue light is more widely used in LED grow lights in controlled environment agriculture facilities while other color lights are often not included, or only included at small amounts. However, the action or quantum yield spectrum of photosynthesis ignores the synergistic effects among wavelengths and thus may not accurately estimate the photosynthetic efficiency of combining different wavelengths. A better understanding of the synergistic effects on photosynthesis among wavelengths, which remain under-studied, can provide more accurate estimates of the photosynthetic efficiency of different wavelengths and improve our understanding of the optimal spectrum for photosynthesis.

Literature Cited

Allen, J.F. 1992. Protein phosphorylation in regulation of photosynthesis. Biochim. Biophys. Acta 1098:275-335.

Allen, J.F. 2003. State transitions – a question of balance. Science 299:1530-1532.

Anderson, J.M. and C.B. Osmond.1987. Shade - sun responses: Compromises between acclimation and photoinhibition, p. 1-36. In: D. J. Kyle, C. B. Osmond and C.J. Arntzen (eds.). Photoinhibition. Elsevier Science Publishers, Amsterdam.

Anderson, J.M., W.S. Chow, and Y. Park. 1995. The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. Photosyn. Res. 46:129-139.

Baker, N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59:89-113.

Baker, N.R., J. Harbinson, and D.M. Kramer. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 30:1107-1125.

Björkman, O. 1981. Responses to different quantum flux densities, p. 57-107. In: O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.). Physiological plant ecology I. Encyclopedia of plant physiology, Vol. 12A, Springer-Verlag, Berlin, Germany.

Bourget, C.M. 2008. An introduction to light-emitting diodes. HortScience 43: 1944-1946.

Butler, W.L. 1962. Effects of red and far-red light on the fluorescence yield of chlorophyll *in vivo*. Biochim. Biophys. Acta 64:309-317.

Chow, W.S., A. Melis, and J.M. Anderson. 1990. Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc. Natl. Acad. Sci. 87:7502-7506.

Chow, W.S., L. Qian, D.J. Goodchild, and J.M. Anderson. 1988. Photosynthetic acclimation of *Alocasia macrorrhiza* (L.) G. Don to growth irradiance: Structure, function and composition of chloroplasts. Austral. J. Plant Physiol. 15:107-122.

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

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

Demmig-Adams, B. and W.W. Adams, III. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:599-626.

Demmig-Adams, B., W.W. Adams, III, D.H. Barker, B.A. Logan, D.R. Bowling, and A.S. Verhoeven. 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant. 98:253-264.

Emerson R. and C.M. Lewis. 1943. The dependence of the quantum yield of chlorella photosynthesis on wave length of light. Amer. J. Bot. 30:165-178.

Emerson R. and E. Rabinowitch. 1960. Red drop and role of auxiliary pigments in photosynthesis. Plant Physio. 35: 477-485.

Emerson, R., R. Chalmers, and C. Cederstrand. 1957. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci. USA 43:133-143.

Evans, J.R. 1987. The dependence of quantum yield on wavelength and growth irradiance. Aust. J. Plant Physiol. 14:69-79.

Fujita, Y. 1997. A study on the dynamic features of photosystem stoichiometry: Accomplishments and problems for future studies. Photosyn. Res. 53:83-93.

Genty, B., J. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990:87-92.

Givnish, T.J. 1988. Adaptation to sun and shade: a whole-plant perspective. Austral. J. Plant Physiol. 15:63-92.

Gómez, C., R.C. Morrow, C.M. Bourget, G.D. Massa, and C. Mitchell. 2013. Comparison of intracanopy light-emitting diode towers and overhead high-pressure sodium lamps for supplemental lighting of greenhouse-grown tomatoes. HortTechnology 23:93-98.

Govindjee, R., Govindjee, and G. Hoch. 1964. Emerson enhancement effect in chloroplast reactions. Plant Physiol. 39:10-14.

Haldrup, A., P.E. Jensen, C. Lunde, and H.V. Scheller. 2001. Balance of power: a view of the mechanism of photosynthesis state transitions. Trends Plant Sci. 6:301-305.

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

Hoover, W.H. 1937. The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. Smithsonian Institution Misc. Collections 95:1-13.

Inada, K. 1976. Action spectra for photosynthesis in higher plants. Plant Cell Physiol. 17:355-365.

Laisk, A., V. Oja, H. Eichelmann, and L. Dall'Osto. 2014. Action spectra of photosystems II and I and quantum yield of photosynthesis in leaves in State 1. Biochim. Biophys. Acta 1837:315-325.

Logan, B.A., B. Demmig-Adams, W.W. Adams, III, and S.C. Grace. 1998. Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. J. Exp. Bot. 49:1869-1879.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – A practical guide. J. Expt. Bot. 51:659-668.

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

McCree, K.J. 1972b. Significance of enhancement for calculation based on the action spectrum for photosynthesis. Plant Physiol. 49:704-706.

Mitchell, C, A.-J. Both, M. Bourget, J. Burr, C. Kubota, R. Lopez, R. Morrow, and E. Runkle. 2012. LEDs: The future of green- house lighting! Chron. Hort. 52:6–11.

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

Myers J. and J.R. Graham. 1963. Enhancement in Chlorella. Plant Physiol. 38:105-116.

Myers, J. 1971. Enhancement studies in photosynthesis. Annu. Rev. Plant Physiol. 22:289-312.

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS ONE 9(6):e99010.

Niinemets, Ü. 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecol. Res. 25:693-714.

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

Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-

source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience 50:705-713.

Seemann, J.R., T.D. Sharkey, J. Wang, and C.B. Osmond. 1987. Environmental effects on photosynthesis, nitrogen –use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiol. 84:796-802.

Terashima I., T. Fujita, T. Inoue, W.S. Chow, and R. Oguchi. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50:684-697.

Valladares, F. and Ü. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annu. Rev. Ecol. Evol. Syst. 39:237-257.

van Iersel, M.W. 2017. Optimizing lighting in controlled environment agriculture. In S.D. Gupta (ed.) Light emitting diodes for agriculture - Smart lighting. Springer, Singapore. (in print).

van Iersel, M.W. and D. Gianino. 2017. An adaptive control approach for LED lights can reduce the energy costs of supplemental lighting in greenhouses. HortScience 52:72-77.

van Iersel, M.W., E. Mattos, G. Weaver, R.S. Ferrarezi, M.T. Martin, and M. Haidekker. 2016a. Using chlorophyll fluorescence to control lighting in controlled environment agriculture. Acta Hort. 1134:427-433.

van Iersel, M.W., G. Weaver, M.T. Martin, R.S. Ferrarezi, E. Mattos, and M. Haidekker. 2016b. A chlorophyll fluorescence-based biofeedback system to control photosynthetic lighting in controlled environment agriculture. J. Amer. Soc. Hort. Sci. 141:169-176.

Walters, R.G. 2005. Towards an understanding of photosynthetic acclimation. J. Expt. Bot. 56:435-447.

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

CHAPTER 3

PHOTOCHEMICAL ACCLIMATION OF THREE CONTRASTING SPECIES TO DIFFERENT LIGHT LEVELS: IMPLICATIONS FOR OPTIMIZING SUPPLEMENTAL LIGHTING[1]

[1]Zhen, S. and M.W. van Iersel. Submitted to Journal of American Society for Horticultural Science.

Abstract

Photosynthetic responses to light are dependent on light intensity, vary among species, and can be affected by the light environment (e.g., light intensity, spectrum, and photoperiod) that plants are acclimated to. Understanding how these factors affect photochemistry is important for improving supplemental lighting efficiency in controlled environment agriculture. We used chlorophyll fluorescence to determine the photosynthetic responses of three species with contrasting light requirements [sweetpotato (*Ipomea batatas*), lettuce (*Lactuca sativa*) and pothos (*Epipremnum aureum*)] to a wide range of photosynthetic photon flux (*PPF*). We also quantified how these responses were affected by light acclimation to three shading treatments – full sun, 44% shade, and 75% shade. The quantum yield of photosystem II (Φ_{PSII}), the fraction of absorbed light that is used in photochemistry, decreased exponentially with increasing *PPF* in all three species. In contrast, linear electron transport rate (ETR), which is often closely correlated with photosynthetic rate, increased asymptotically with increasing *PPF*. Within each shading level, sweetpotato tended to have the highest Φ_{PSII} and ETR, followed by lettuce and pothos, especially when *PPF* was relatively high (> 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). This indicates that the high light-adapted species sweetpotato can use high light more efficiently for electron transport than light-intermediate lettuce and shade-tolerant pothos. Within a species, plants acclimated to more shade tended to have a more rapid decrease in Φ_{PSII} as *PPF* increased, and had lower ETR at the same *PPF* than plants grown under full sun. Non-photochemical quenching (NPQ; an indicator of the amount of absorbed light energy that is dissipated as heat) was up-regulated with increasing *PPF*; faster up-regulation was observed in pothos as well as plants grown under 75% shade. Overall, our results show that high *PPF* results in high ETR, low Φ_{PSII}, and high NPQ, but the magnitude of these responses depends on species and acclimation to ambient light levels. The

results indicate that supplemental lighting is used less efficiently for electron transport at high ambient *PPF*, and the differences in photosynthetic responses among species/plants acclimated to different light intensity should be taken into consideration when developing supplemental lighting strategies.

Additional index words: electron transport rate, light acclimation, non-photochemical quenching, photosynthesis, quantum yield of photosystem II, shade

Introduction

Supplemental lighting is commonly used for producing horticultural crops under low natural light conditions to increase photosynthesis, improve crop quality and yield, and shorten the production cycle (Nelson, 2012). Typical uses of supplemental lighting include off-season vegetable production and winter production of ornamental crops in greenhouses for spring/summer sales in northern climates (Gaudreau and Chartbonneau, 1994; Lopez and Runkle, 2008). Crop production costs increase substantially because of the high electrical consumption of lighting. For example, van Iersel and Gianino (2017) estimated that the cost of supplemental lighting provided by high pressure sodium (HPS) lamps can account for about 30% of the farm gate value for vegetable greenhouses. More efficient methods for supplemental lighting would increase the profitability of greenhouses.

One straightforward way to improve the efficiency of supplemental lighting is to implement energy-efficient lighting sources (Nelson and Bugbee, 2014). In addition to the efficiency of the lights, the overall efficiency at which the electrical energy is converted into plant biomass depends on how efficiently plants are able to use light for photosynthesis. Not all

the light absorbed by plants is used in the photochemical reactions of photosynthesis for subsequent carbon assimilation. Some of the absorbed light can be dissipated as heat, and another small fraction is re-emitted as chlorophyll fluorescence (Maxwell and Johnson, 2000). The ability of plants to use the absorbed light for photosynthesis, rather than dissipating it as heat or through chlorophyll fluorescence, decreases with increasing light intensity (Baker, 2008; Demmig-Adams et al., 1996; van Iersel et al., 2016b). Meanwhile, heat dissipation of the absorbed light is up-regulated with increasing light intensity (Demmig-Adams and Adams, 1992; Demmig-Adams et al., 1996). As a result, supplemental light provided at high intensities or in addition to a high ambient light intensity would result in a proportionally smaller increase in photosynthesis (van Iersel and Gianino, 2017) and presumably growth. Clearly, it is not beneficial to provide supplemental light when plants cannot use that light efficiently for photosynthesis. To optimize supplemental lighting, we need to gain quantitative information on how efficiently plants use light over a wide range of light intensities and provide the optimal amount of light when crops can use that light most efficiently to produce biomass (van Iersel, 2017).

Differences in plants' ability to use light for photosynthesis exist among species. Species adapted to high light usually possess adaptive characteristics (e.g., high rubisco content) that optimize photosynthesis under high light, whereas shade-adapted species optimize photosynthesis in low light by maximizing light harvesting (Björkman, 1981; Seemann et al., 1987). High light-adapted species generally have greater photosynthetic capacity, i.e., a higher maximum photosynthetic rate and higher light-saturation point, than shade-adapted species (Björkman, 1981). In contrast, shade-adapted species tend to reach maximum photosynthetic capacity at much lower light intensity and are more susceptible to photoinhibition under high

light (Demmig-Adams and Adams, 1992). Consequently, the photosynthetic responses to supplemental light are expected to differ among species adapted to different light intensities.

While adaptation of photosynthesis to light environment involves changes in the genetic makeup of a species that take place over generations, plants often have the ability to acclimate, i.e., exhibit phenotypic (morphological and physiological) plasticity, to their light environment over a much shorter timescale, typically ranging from minutes to months or years within the life cycle of a plant (Anderson et al., 1995; Björkman, 1981; Valladares and Niinemets, 2008). We use the term light acclimation specifically to refer to changes in the photosynthetic characteristics in response to different levels of shading for weeks. Light acclimation has been shown to induce a wide range of phenotypic modifications that alter plants' photosynthetic light use efficiency, including changes in leaf anatomical structures (Evans and Poorter, 2001; McMillen and McClendon, 1983), chlorophyll content and chlorophyll a/b ratio (Givnish, 1988; Niinemets, 2010), changes in electron transport capacity per unit chlorophyll (Anderson and Osmond, 1987), rubisco content and activity (Björkman, 1981; Seemann, 1989), xanthophyll cycle pigment pool size (involved in heat dissipation of the absorbed light) (Demmig-Adams and Adams, 1992; Logan et al., 1998), and maximum photosynthetic capacity (Oguchi et al., 2005). Therefore, light acclimation should be taken into consideration when developing supplemental light strategies, but this topic has not been addressed in past research.

The Φ_{PSII}, which is the fraction of light absorbed by the leaves that is used in the photochemical reactions to drive electron transport (Maxwell and Johnson, 2000), is a common measure of photosynthetic light use efficiency. Quantum yield of PSII can be easily measured using chlorophyll fluorescence (Genty et al., 1989). In addition, chlorophyll fluorescence can be used to estimate the ETR, which is often closely correlated with overall photosynthetic rate (Beer

et al., 1998; Flexas et al., 1999), and the degree of heat dissipation under various light conditions. The real-time, non-invasive nature of chlorophyll fluorescence measurements makes it an excellent tool to monitor photosynthetic efficiency.

Currently, supplemental lighting in greenhouses is often simply controlled by a timer or based on ambient light levels. The ability of a crop to use the supplemental light for photosynthesis is seldom considered when developing supplemental lighting strategies. Few studies have investigated the feasibility of optimizing supplemental lighting in controlled environments based on plant's physiological responses to light (van Iersel et al., 2016a, 2016b) and there is a need for information on how supplemental lighting can be optimized for crops adapted/acclimated to different light environments. Chlorophyll fluorescence measurements are especially well-suited for this purpose, since they can be used to determine how efficiently plants use the provided light. Therefore, our objectives were: 1) to determine the photochemical responses of different species to a wide range of light intensities; 2) to quantify how light acclimation affects crops' photosynthetic efficiency; and 3) to examine if chlorophyll fluorescence measurements can be used to develop improved supplemental lighting strategies for crops adapted/acclimated to different light environments.

Materials and Methods

Plant material and growing conditions

Sweetpotato 'Desana Lime' stem cuttings were rooted in 1.7-L round, plastic containers filled with a soilless substrate (Fafard 1P, 80% peat: 20% perlite (v/v); Sun Gro Horticulture, Agawam, MA). Lettuce 'Green Ice' was grown from seeds in the same containers and substrate. Young pothos plants were pruned to a few newly formed shoots and then transplanted using the same containers and substrate. Sweetpotato was rooted on a shaded mist bench, and lettuce and

pothos were hand-watered in a glass-covered greenhouse for 2 weeks to get them established. After that, all the plants were placed on ebb-and-flow benches inside a glass-covered greenhouse and sub-irrigated daily with a nutrient solution containing 100 $mg \cdot L^{-1}$ N, made with a water-soluble fertilizer (15N-2.2P-12.45K; 15-5-15 Cal-Mag, Everris, Marysville, OH). During the growing period (20 Mar. – 5 May 2014), average greenhouse temperature and vapor pressure deficit were 21.2 ± 1.5 °C and 1.3 ± 0.3 kPa (average ± SD).

Shading treatments

The ebb-and-flow benches were not covered (full sun), covered with a single layer of commercial 30% shadecloth, or with a single layer of commercial 70% shadecloth on all sides of polyvinyl chloride (PVC) structures installed on top of the benches. Six plants per species were randomly placed on each bench and grown inside the shading structures or in full sun. Quantum sensors (SQ-110; Apogee Instruments, Logan, UT) connected to a data logger (EM50; Decagon Devices, Pullman, WA) were placed on top of each bench at plant height and monitored *PPF* every minute. Daily maximum and average *PPF* and daily light integral (DLI) of all three shading treatments were obtained from the light measurements. Compared with the full sun treatment, the average *PPF* in the treatments that received commercial 30% and 70% shadecloth was reduced by 44% and 75%, respectively. The shading treatments are thus referred to as full sun, 44% shade, and 75% shade. The maximum *PPF* plants experienced over the entire growing period was 1942, 1508, and 665 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the full sun, 44% shade, and 75% shade treatment, respectively. Average DLI was 28.7 ± 10.7, 16 ± 5.9, and 7.0 ± 2.6 $mol \cdot m^{-2} \cdot d^{-1}$ (average ± SD) in the three treatments.

Leaf chlorophyll content index was measured using a chlorophyll meter (CCM-200 plus; Apogee Instruments) on upper-most fully expanded leaves after plants had acclimated to their different light environments for 4 weeks. The measured leaves developed under the different shading treatments.

Light response of chlorophyll fluorescence

LED light. After 4 weeks of light acclimation, light response curves of chlorophyll fluorescence were taken under LED light inside a growth chamber (E15; Conviron, Winnipeg, MB, Canada). A custom-built, dimmable 400-W light emitting diodes (LED) unit, consisting of four 100-W, warm white LED modules (3000 K; EpiLEDs, Tainan, Taiwan), capable of providing a *PPF* of 0 to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, was mounted on aluminum heat sinks with cooling fans installed on top of the heat sinks. The spectral distribution of the LEDs was measured using a spectrometer (UniSpec; PP Systems, Amesbury, MA). The LED light had a primary peak at 578 nm and a secondary peak at 444 nm. About 98.5% of its total photons were within the 400 – 700 nm wavelength range, and the other 1.5% of photons were > 700 nm.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured on upper-most fully expanded leaves that developed under the different shading treatments. Data were collected using a pulse-amplitude modulated fluorometer (Mini-PAM; Heinz Walz, Effeltrich, Germany). One plant per species from each shading level (three species × three shading levels; nine plants in total) were randomly placed inside the growth chamber the night prior to data collection to dark-adapt the plants. Minimum and maximum fluorescence yield of dark-adapted leaves (F_0 and F_m, respectively) were determined to calculate the ratio of variable to maximum fluorescence (F_v/F_m), the maximum quantum yield of PSII for photochemistry when

all reaction centers are "open" (i.e., oxidized), where $F_v = F_m - F_0$. Then, LEDs were switched on at a low light intensity (< 20 μmol·m^{-2}·s^{-1}). Plants were given 15 to 20 min for photosynthesis to stabilize under the light level before steady-state and maximum fluorescence in the light (F_t and F_m', respectively) were determined. Quantum yield of PSII of light-adapted leaves was calculated as $\Phi_{PSII} = (F_m' - F_t)/F_m'$ (Genty et al., 1989). During the photochemical reactions, the excitation energy from some of the absorbed photons is used to excite electrons in the reaction center of PSII. The resulting electron transport ultimately leads to the production of ATP and ferredoxin, the energy and reducing power that are subsequently used for carbon fixation. The ETR was calculated using the following equation: $ETR = \Phi_{PSII} \times PPF \times 0.84 \times 0.5$ (Baker et al., 2007), where *PPF* is the incident light intensity at the site of leaf fluorescence measurements and was measured using a quantum sensor embedded in the leaf-clip (2030-B; Heinz Walz) of the fluorometer. This quantum sensor was calibrated under the LED light against a second quantum sensor (LI-190; LI-COR, Lincoln, NE). This estimation of ETR was based on the common assumptions that 84% of the incident *PPF* was absorbed by the leaves (Björkman and Demmig, 1987) and that the absorbed *PPF* was equally partitioned between PSI and PSII (Baker, 2008; Maxwell and Johnson, 2000). Non-photochemical quenching, which provides an index of the amount of absorbed light that is dissipated as heat, was calculated as $NPQ = (F_m - F_m')/ F_m'$ (Maxwell and Johnson, 2000).

The *PPF* inside the growth chamber, monitored by a quantum sensor (LI-190; Li-Cor BioSciences, Lincoln, NE) connected to a datalogger (CR1000; Campbell Scientific, Logan, UT) placed in the middle of the growth chamber at plant height, was increased stepwise to a *PPF* of ~1800 μmol·m^{-2}·s^{-1} over a period of about 12 h. Light intensity was increased by ~10 – 150 μmol·m^{-2}·s^{-1} during each increase, with small increments when *PPF* was low and then gradually

bigger adjustments as *PPF* increased. Plants were given 15 to 20 min under each *PPF* level, and $\Phi_{PSII,}$ ETR, and NPQ at each *PPF* were determined in a similar manner as described above until the highest *PPF* of ± 1800 μmol·m^{-2}·s^{-1} was reached. This entire procedure was replicated six times using six sets of plants on 6 d (each day was treated as a block).

The *PPF* inside the growth chamber was not uniform, with higher light intensity in the middle than toward the sides of the growth chamber. The *PPF* at the site of leaf fluorescence measurements thus varied substantially from plant to plant. As plants were randomly placed inside the growth chamber each day, several treatments (i.e., species and shading level combinations) only had one or a few data points at high light intensities (*PPF* > 1000 μmol·m^{-2}·s^{-1}) across all six replications. In such cases, those data points at high light levels were highly influential in subsequent curve-fitting and could potentially introduce bias in the analyses and were thus excluded from the data analysis.

Experimental design and statistical analysis

The experimental design was a factorial (3 species × 3 shading levels) carried out in a randomized complete block design with six blocks. Data were analyzed using regression (linear, exponential rise to maximum, and exponential decay to minimum) and two-way analysis of variance (ANOVA) in SAS (version 9.2; SAS Institute, Cary, NC). For $\Phi_{PSII,}$ ETR, and NPQ, the light response curve obtained from each plant (i.e., one replicate of a species × shading level combination) was separately fitted using regression. Initial slopes of the Φ_{PSII} – *PPF*, ETR – *PPF*, and NPQ – *PPF* curves, which estimate the rates of change in Φ_{PSII}, ETR, and NPQ when a plant is transferred from dark to low light, were derived from the corresponding light response curves fitted through data obtained from each plant. Predicted $\Phi_{PSII,}$ ETR, and NPQ at a *PPF* of

500 μmol·m^{-2}·s^{-1} were derived from the fitted regression functions. The initial slopes of the curves and predicted $\Phi_{PSII,}$ ETR, and NPQ at *PPF* of 500 μmol·m^{-2}·s^{-1} were then analyzed using ANOVA with $P < 0.05$ considered to be statistically significant.

Results and Discussion

Chlorophyll content and plant morphology

The leaf chlorophyll content index of the shade-adapted species pothos was three times higher than that of lettuce, which needs intermediate light levels for best growth, and seven times higher than that of sweetpotato, a high light plant (Fig. 1). Shade-adapted species typically have high leaf chlorophyll content compared to high light plants, a trait that maximizes light capture (Valladares and Niinemets, 2008). Plants not only inherit adaptive traits that developed over generations to optimize fitness under a certain light condition, but also show morphological and physiological acclimation in response to their light environment (Anderson et al., 1995; Björkman, 1981; Valladares and Niinemets, 2008), which vary both over time and within the plant canopy. Many plants have increased leaf chlorophyll content to increase light capture when grown under shade (Evans and Poorter, 2001; Givnish, 1988; Nemali and van Iersel, 2004; Niinemets, 2010), although this response is species-dependent (Logan et al., 1998; Murchie and Horton, 1997). The chlorophyll content index of the three species was not significantly affected by acclimation to different shade levels ($P = 0.62$, data not shown).

Morphological modifications in response to light were not the focus of this study and were thus not quantified. However, we did observe that lettuce grown under shade (especially 75% shade) had pronounced stem elongation, and the leaves of shade-grown pothos tended to be less variegated.

Dark-adapted F_v/F_m

Maximum quantum yield of PSII for photochemistry (F_v/F_m) of plants that had been dark-adapted was significantly affected by the interaction between species and light acclimation under different shading levels (Fig. 2). Specifically, F_v/F_m of sweetpotato did not differ among the three shading levels; F_v/F_m of lettuce, however, was lower in the two shaded treatments compared to that in the full sun treatment (Fig. 2). Pothos showed the opposite trend from lettuce: F_v/F_m was highest in plants grown under 75% shade and lowest in plants grown under full sun (Fig. 2), likely due to photoinhibition (i.e., damage to PSII) by excess light under full sun condition. The PSII reaction center D_1 protein degrades during photoinhibition, and the repair of damaged D_1 protein is slow (takes hours), causing a fraction of the PSII reaction centers to be non-functional, which decreases F_v/F_m (Ruban, 2015). Low-light grown plants have been reported to have a slower rate of D_1 protein turnover (i.e., slower repair cycle of photodamaged PSII reaction centers) (Aro et al., 1993) and a lower capacity for xanthophyll cycle-mediated thermal dissipation of excess absorbed light (Demmig-Adams and Adams, 1992), and thus are often more susceptible to photoinhibition. Alternatively, a reduction in F_v/F_m could be the result of the sustention of high level of xanthophyll cycle pigment zeaxanthin, and consequently sustained thermal dissipation of the absorbed light (which corresponds to lower Φ_{PSII}), in response to chronic stresses such as excess light and cold (Demmig-Adams and Adams, 2006; Demmig-Adams et al., 2012).

The reduction in F_v/F_m of lettuce grown under shade, however, was unexpected because it is less likely for photoinhibition or sustained thermal dissipation to occur under lower light conditions. The maximum Φ_{PSII} estimated from the $\Phi_{PSII} - PPF$ curves (i.e., the Φ_{PSII} at $PPF = 0$

μmol·m^{-2} ·s^{-1}; see section "quantum yield of photosystem II (Φ_{PSII})" below for more details), however, did not differ among the three light acclimation treatments for lettuce, even though it is essentially the same as F_v/F_m. For pothos, F_v/F_m and Φ_{PSII} at *PPF* = 0 μmol·m^{-2} ·s^{-1} provided consistent results (data not shown). Therefore, we suspect that the observed reduction in F_v/F_m of lettuce grown under shade might be a measurement artifact.

At all shading levels, sweetpotato consistently had higher F_v/F_m than pothos (Fig. 2), indicating that sweetpotato had a greater capacity for using the absorbed light for photochemistry, when all the PSII reaction centers are "open", than pothos. A high capacity for photochemistry, and a high light-saturated maximum photosynthetic rate that is often observed in high light plants (Björkman, 1981), allows high light-adapted plants like sweetpotato to use high light more efficiently for photosynthesis and reduces the risk of photoinhibition.

Quantum yield of photosystem II (Φ_{PSII})

Quantum yield of PSII of all three species was greatest in the dark and decreased exponentially as *PPF* increased (Fig. 3A-C). This decrease in Φ_{PSII} with increasing *PPF* level is commonly observed as a greater fraction of PSII reaction centers become "closed" (photo-reduced) under higher light, and are thus unable to use the absorbed light for photochemistry (Baker, 2008; Maxwell and Johnson, 2000).

The rate at which Φ_{PSII} decreased with increasing *PPF* differed among species and among shading levels: within a species, plants grown under heavier shade tended to have more rapid decrease in Φ_{PSII} (i.e., photochemistry became less efficient more quickly as *PPF* increased); and within a shading level, Φ_{PSII} tended to decrease fastest in pothos and slowest in sweetpotato as *PPF* increased (Fig. 3A-C). Specifically, the initial slope of the Φ_{PSII} – *PPF* curve, which is an

indicator of how fast Φ_{PSII} decreased when a plant is transferred from dark to low *PPF*, was steeper (i.e., decreased faster) for pothos (Fig. 4A) as well as for plants grown under 75% shade (Fig. 4B).

To further illustrate how the efficiency at which a plant used the absorbed light for photochemistry varied among species and was affected by acclimation to different shade levels, Φ_{PSII} at a *PPF* of 500 μmol·m^{-2}·s^{-1}, obtained from the fitted Φ_{PSII} – *PPF* curves, was compared among species and among shading levels (note that there was no interactive effect). The Φ_{PSII} of sweetpotato at *PPF* of 500 μmol·m^{-2}·s^{-1} was 11% and 81% higher than that of lettuce and pothos, respectively, indicating that sweetpotato was able to use high light most efficiently, while pothos was least efficient (Fig. 4C). In addition, Φ_{PSII} at a *PPF* of 500 μmol·m^{-2} ·s^{-1} was highest for plants grown under full sun and lowest for plants grown under 75% shade (Fig. 4D), indicating that plants acclimated to a shade environment were not able to use high light as efficiently as plants that had acclimated to a full sun environment.

Electron transport rate (ETR)

In contrast to the decrease in Φ_{PSII} in response to increasing *PPF*, ETR through PSII increased asymptotically as *PPF* increased (Fig. 3D-F). During the light reactions of photosynthesis, the transport of electrons through the electron transport chain results in the production of ATP and ferredoxin that are subsequently used in carbon assimilation (Blankenship, 2014) and other processes requiring energy (ATP) or reducing power (ferredoxin). A higher ETR, which requires a high *PPF* to achieve, has been shown to closely correspond to a higher rate of carbon assimilation/oxygen evolution, especially in the absent of severe stresses, such as drought, that increase the strength of alternative electron sinks, e.g., photorespiration

(Beer et al., 1998; Flexas et al., 1999). However, Φ_{PSII} is lower under high *PPF* (see Fig. 3A-C; Baker, 2008; Demmig-Adams et al., 1996; van Iersel et al., 2016b), meaning that a high ETR, and correspondingly a high rate of carbon assimilation and plant growth, is achieved at the expense of photochemical efficiency (also see van Iersel et al., 2016b).

Consistent with the higher Φ_{PSII} observed in sweetpotato and in plants grown under full sun, ETR at a given *PPF* tended to be greater for sweetpotato than that for lettuce and pothos; higher ETR was also seen in plants acclimated to full sun compared to plants grown under shade, especially under high *PPF* (Fig. 3D-F). Compared with shade-adapted or -acclimated plants, plants adapted or acclimated to high light often have increased electron transport capacities by increasing the content of electron transport components such as cytochrome f (on per chlorophyll basis), plastoquinone pool, plastocyanin, ferredoxin, and ATP synthase (Anderson and Osmond, 1987; Anderson et al., 1995; Björkman, 1981; Chow et al., 1988; Walters, 2005). Such increases in content of electron transport components, e.g., a bigger plastoquinone pool, allows a greater fraction of reaction centers to be "open" (i.e., oxidized and capable of using excitation energy from light for photochemistry) under a given *PPF*, thus resulting in higher Φ_{PSII}. In addition, a greater capacity for using the absorbed light for electron transport decreases the need for xanthophyll cycle-mediated thermal dissipation, which is discussed in more detail in the section on non-photochemical quenching below.

The initial slope of the ETR – *PPF* curve, which gives an estimate of the maximum rate of increase in ETR per unit increase in incident *PPF*, was 15% higher for sweetpotato than for pothos (Fig. 5A). This is in line with the higher dark-adapted F_v/F_m of sweetpotato compared with that of pothos. Light acclimation to different shading levels, on the other hand, had no effect on the initial slope of the ETR – *PPF* curve (Fig. 5B).

The ETR of pothos at a *PPF* of 500 μmol·m^{-2} ·s^{-1} was 40% and 35% lower than that of sweetpotato and lettuce, respectively (Fig. 5C), indicating that pothos could not use high light as efficiently for electron transport and, presumably, for subsequent carbon assimilation compared with high light-adapted plants such as sweetpotato. The ETR at a *PPF* of 500 μmol·m^{-2} ·s^{-1} also decreased with increasing shading level (Fig. 5D), consistent with the reduction in Φ_{PSII} in response to shade acclimation (see Fig. 4C and D).

Non-photochemical quenching

Non-photochemical quenching of chlorophyll fluorescence, indicative of the degree of change in heat dissipation of the absorbed light relative to that in the dark-adapted state (Maxwell and Johnson, 2000), was up-regulated as *PPF* increased (Fig. 3G-I). As NPQ competes with photochemistry for the same excitation energy, a decrease in Φ_{PSII} under increasing *PPF* is often companied by an increase in NPQ (see Fig. 3A-C; Demmig-Adams et al., 1996; Matos et al., 2009; van Iersel et al., 2016b). This increase in NPQ under high *PPF* was likely attributable to the up-regulation of the xanthophyll cycle-mediated heat dissipation of the excess absorbed energy, a process that is activated by accumulation of H^+ in the thylakoid lumen (Demmig-Adams and Adams, 1996; Eskling et al., 1997). Accumulation of H^+ in the lumen in turn results from increasing rates of electron transport as *PPF* increases (Baker et al., 2007; Rochaix, 2014). The xanthophyll cycle-mediated heat dissipation contributes to a major part of NPQ and is thought to protect the photosynthetic apparatus from damage (i.e., photoinhibition through damage to PSII, which is another component of NPQ) by safely dissipating the excess light energy as heat (Demmig-Adams and Adams, 1992, 2006).

The need for this photoprotective mechanism varies depends on 1) the plant's capability of utilizing light for photochemistry, and 2) factors that alter the plant's photosynthetic capacity, including light acclimation and environmental stresses, e.g., excess light, cold, nutrient deficiency, and drought (Demmig-Adams and Adams, 1992; Demmig-Adams et al., 2012; Logan et al., 1998; Verhoeven et al., 1997). It is expected that plants with a low capacity for using light for photosynthesis would have greater need to dissipate excitation energy through alternate pathways, e.g., the xanthophyll cycle. Adams and Demmig-Adams (1992) compared the changes in xanthophyll cycle activity in response to diurnal changes in light intensity between slow-growing species with low photosynthetic capacity and fast growing crops with high photosynthetic capacity. They found that although the slow-growing species had similar or a smaller xanthophyll pool size on a per chlorophyll basis, under high light conditions they converted a much higher fraction of their total xanthophyll pigment pool to zeaxanthin and antheraxathin, the two de-epoxidized forms of xanthophylls that lead to dissipation of excess absorbed light as heat. Maintaining a high fraction of de-epoxidized xanthophylls (i.e., zeaxanthin and antheraxathin) results in faster up-regulation of NPQ in response to excess light (Demmig-Adams and Adams, 2006; Demmig-Adams et al., 2012, Logan et al., 1998).

The species differences in the regulation of NPQ are evident in our data: NPQ of pothos increased more quickly upon transitioning of plants from dark to light than that of sweetpotato and lettuce, as indicated by the steeper initial slope of the NPQ – *PPF* curve of pothos (Fig. 6A). In addition, the initial slope of NPQ – *PPF* was higher in plants grown under 75% shade than plants grown in full sun and 44% shade (Fig. 6B), suggesting that acclimation to lower light level also resulted in faster up-regulation of heat dissipation under increasing light. The NPQ of pothos at a *PPF* of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was twice that of lettuce and sweetpotato (Fig. 6C),

indicating greater upregulation of heat dissipation, resulting in lower Φ_{PSII} in pothos (see Fig. 4C). Non-photochemical quenching at a *PPF* of 500 μmol·m^{-2} ·s^{-1} was also higher for plants grown under 75% shade (Fig. 6C and D), which had lower Φ_{PSII} at that light intensity (see Fig. 4D).

Implications for optimizing supplemental lighting

Sweetpotato, lettuce, and pothos showed vast differences in how they used light for photochemistry. In addition, their photosynthetic performance was affected by acclimation to different shade levels. As a result, crop-specific strategies should be used when using supplemental light for different crops; variations in the crop light use caused by light acclimation should also be taken into account. Although Φ_{PSII} decreased with increasing *PPF* for all three species (see Fig. 3A-C), sweetpotato and lettuce maintained higher Φ_{PSII} under high light, i.e., used the provided light more efficiently to drive electron transport. Pothos, on the other hand, had a lower Φ_{PSII} at the same *PPF* and increased NPQ more rapidly with increasing *PPF*. Consequently, for each unit of supplemental light provided, greater increases in ETR, and presumably in carbon assimilation and growth, can be achieved in sweetpotato and lettuce than in pothos, especially when ambient *PPF* is high.

Consistent with the decreases in Φ_{PSII} with increasing *PPF*, the increase in ETR per unit *PPF* supplemented diminishes with increasing light intensity for all species. However, this diminishing return at higher light levels is most pronounced in pothos: ETR of pothos increased more slowly with increasing *PPF* and tended to reach a maximum at much lower *PPF* compared to that of sweetpotato and lettuce (Fig. 3D-F). Light supplemented to a high ambient *PPF* (e.g., 500 μmol·m^{-2} ·s^{-1}) would be expected to result in little, if any, increase in ETR in pothos. It is

thus most beneficial to provide supplemental light to pothos when ambient *PPF* is low. In contrast, supplemental light provided at high ambient *PPF* can be used with relatively high efficiency to further increase ETR of sweetpotato and lettuce, especially when it is desired to speed up crop growth and shorten production cycles while slightly or moderately compromising light use efficiency. van Iersel and Gianino (2017) similarly reported that plants with different photosynthetic capacities responded differently to supplemental light provided at different ambient light intensities. They simulated the responses of net photosynthesis (A_n) of two species to supplemental light provided at different ambient *PPF* and found that the same amount of supplemental light resulted in less increase in A_n when provided at higher ambient *PPF*. However, the high light-adapted species *Campanula portenschlagiana* (with higher photosynthetic capacity) still showed pronounced increases in A_n when supplemental light was provided at an ambient *PPF* of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, whereas little increase in A_n was observed in the low light-adapted plant *Heuchera americana* when supplemental light was provided at the same ambient *PPF* (van Iersel and Gianino, 2017).

The simulation by van Iersel and Gianino (2017) did not account for the effects of light acclimation on a crop's photosynthetic performance. As shown by our data, light acclimation significantly affected the light responses of Φ_{PSII}, ETR, and NPQ, and thus must be taken into consideration when developing lighting strategy for a specific crop. Compared with shade-acclimated plants, high light-grown plants of all three species were able to maintain higher Φ_{PSII} at high *PPF* and had greater ETR and slower up-regulation of NPQ (i.e., heat dissipation), thereby could use supplemental light more efficiently for photosynthesis when provided at high ambient *PPF*. To get the maximum benefit from supplemental light, it is important that the plants are acclimated to relatively high light. Providing supplemental light to a crop that was

previously grown under low light conditions is likely to be relatively ineffective, because the shade-acclimated crop will not be able to use that light efficiently.

The light response curves of Φ_{PSII} and ETR obtained using chlorophyll fluorescence measurements provide important quantitative information on how efficiently various crops use the provided light for photosynthesis, and how their responses are affected by light acclimation. Given the relative simplicity of the measurements, chlorophyll fluorescence can be used as an effective tool to optimize crop-specific lighting strategies (van Iersel et al., 2016a).

Conclusions

High *PPF* is needed to achieve high ETR and presumably a high rate of photosynthesis and growth. However, Φ_{PSII} and the rate of increase in ETR per unit increase in *PPF* decrease with increasing *PPF*, suggesting that supplemental lighting is used less efficiently for photochemistry when provided at high ambient *PPF*. The photosynthetic responses differ among species and are affected by light acclimation, with high light adapted species and plants acclimated to high light environment being more efficient in using light for electron transport than low light adapted/acclimated plants. Supplemental lighting strategies thus should be species-specific and take account of the effect of light acclimation.

Acknowledgements

We thank the American Floral Endowment and the Georgia Research Alliance for financial support for this research. This work also was supported by the USDA National Institute of Food and Agriculture, Hatch project 1011550. We thank Drs. Robert Teskey and Paul Thomas for providing feedback on this manuscript. We thank Sue Dove for technical support.

Literature Cited

Adams, III, W.W. and B. Demmig-Adams. 1992. Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. Planta 186:390-398.

Anderson, J.M. and C.B. Osmond.1987. Shade - sun responses: Compromises between acclimation and photoinhibition, p. 1-36. In: D. J. Kyle, C. B. Osmond and C.J. Arntzen (eds.). Photoinhibition. Elsevier Science Publishers, Amsterdam.

Anderson, J.M., W.S. Chow, and Y. Park. 1995. The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. Photosyn. Res. 46:129-139.

Aro, E., S. McCaffery, and J.M. Anderson. 1993. Photoinhibition and D1 Protein degradation in peas acclimated to different growth irradiances. Plant Physiol. 103:835-843.

Baker, N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59:89-113.

Baker, N.R., J. Harbinson, and D.M. Kramer. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 30:1107-1125.

Beer, S., B. Vilenkin, A. Weil, M. Veste, L. Susel, and A. Eshel. 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. Marine Ecol. Prog. Ser. 174:293-300.

Björkman O. 1981. Responses to different quantum flux densities, p. 57-107. In: O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.). Physiological plant ecology I. Encyclopedia of plant physiology, Vol. 12A, Springer-Verlag, Berlin, Germany.

Björkman, O. and B. Demmig. 1987. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77k among vascular plants of diverse origins. Planta 170:489–504.

Blankenship, R.E. 2014. Molecular mechanisms of photosynthesis. 2nd ed. Wiley, Oxford, UK.

Chow, W.S., L. Qian, D.J. Goodchild, and J.M. Anderson. 1988. Photosynthetic acclimation of *Alocasia macrorrhiza* (L.) G. Don to growth irradiance: Structure, function and composition of chloroplasts. Austral. J. Plant Physiol. 15:107-122.

Demmig-Adams, B. and W.W. Adams, III. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:599-626.

Demmig-Adams, B. and W.W. Adams, III. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci.1:21-26.

Demmig-Adams, B. and W.W. Adams, III. 2006. Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. New Phytol. 172:11–21.

Demmig-Adams, B., W.W. Adams, III, D.H. Barker, B.A. Logan, D.R. Bowling, and A.S. Verhoeven. 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant. 98:253-264.

Demmig-Adams, B., C.M. Cohu, O. Muller, and W.W. Adams, III. 2012. Modulation of photosynthetic energy conversion in nature: from seconds to seasons. Photosyn. Res. 113:75-88.

Eskling, M., P. Arvidsson, and H. Åkerlund. 1997. The xanthophyll cycle, its regulation and components. Physiol. Plant. 100:806-816.

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

Flexas, J. J.M. Escalona, and H. Medrano. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell Environ. 22:39-48.

Gaudreau, L. and J. Chartbonneau. 1994. Photoperiod and photosynthetic photon flux influence growth and quality of greenhouse-grown lettuce. HortScience 29:1285-1289.

Genty, B., J. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990:87-92.

Givnish, T.J. 1988. Adaptation to sun and shade: A whole-plant perspective. Austral. J. Plant Physiol. 15:63-92.

Logan, B.A., B. Demmig-Adams, W.W. Adams, III, and S.C. Grace. 1998. Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth *PPFD*s in the field. J. Expt. Bot. 49:1869-1879.

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

Matos, F.S., R. Wolfgramm, F.V. Gonçalves, P.C. Cavatte, M.C. Ventrella, and F.M. DaMatta. 2009. Phenotypic plasticity in response to light in the coffee tree. Environ. Expt. Bot. 67:421-427.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – A practical guide. J. Expt. Bot. 51:659-668.

McMillen, G.G. and J.H. McClendon. 1983. Dependence of photosynthetic rates on leaf density thickness in deciduous woody plants grown in sun and shade. Plant Physiol. 72:674-678.

Murchie, E.H. and P. Horton. 1997. Acclimation of photosynthesis to irradiance and spectral quality in British plant species: Chlorophyll content, photosynthetic capacity and habitat preference. Plant Cell Environ. 20:438-448.

Nelson, J.A. and B. Bugbee. 2014. Economic analysis of greenhouse lighting: Light emitting diodes vs. high intensity discharge fixtures. PLoS ONE 9(6):e99010.

Nelson, P.V. 2012. Greenhouse operation and management. 7th ed. Prentice Hall, Upper Saddle River, NJ.

Nemali, K.S. and M.W. van Iersel. 2004. Acclimation of wax begonia to light intensity: Changes in photosynthesis, respiration, and chlorophyll concentration. J. Amer. Soc. Hort. Sci. 129:745-751.

Niinemets, Ü. 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecol. Res. 25:693-714.

Oguchi, R., K. Hikosaka, and T. Hirose. 2005. Leaf anatomy as a constraint for photosynthetic acclimation: Differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. Plant Cell Environ. 28:916-927.

Rochaix, J. 2014. Regulation and dynamics of the light-harvesting system. Annu. Rev. Plant Biol. 65:287–309.

Ruban, A.V. 2015. Evolution under the sun: Optimizing light harvesting in photosynthesis. J. Expt. Bot. 66:7-23.

Seemann, J.R., T.D. Sharkey, J. Wang, and C.B. Osmond. 1987. Environmental effects on photosynthesis, nitrogen –use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiol. 84:796-802.

Seemann, J.R. 1989. Light adaptation/acclimation of photosynthesis and the regulation of rubulose-1,5-bisphosphate carboxylase activity in sun and shade plants. Plant Physiol. 379-386.

Valladares, F. and Ü. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annu. Rev. Ecol. Evol. Syst. 39:237-257.

van Iersel, M.W. 2017. Optimizing lighting in controlled environment agriculture. In S.D. Gupta (ed.) Light emitting diodes for agriculture - Smart lighting. Springer, Singapore. (in print).

van Iersel, M.W. and D. Gianino. 2017. An adaptive control approach for LED lights can reduce the energy costs of supplemental lighting in greenhouses. HortScience 52:72-77.

van Iersel, M.W., E. Mattos, G. Weaver, R.S. Ferrarezi, M.T. Martin, and M. Haidekker. 2016a. Using chlorophyll fluorescence to control lighting in controlled environment agriculture. Acta Hort. 1134:427-433.

van Iersel, M.W., G. Weaver, M.T. Martin, R.S. Ferrarezi, E. Mattos, and M. Haidekker. 2016b. A chlorophyll fluorescence-based biofeedback system to control photosynthetic lighting in controlled environment agriculture. J. Amer. Soc. Hort. Sci. 141:169-176.

Verhoeven, A.S., B. Demmig-Adams, and W.W. Adams,III. 1997. Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach expose to high light and N stress. Plant Physiol. 113:817-824.

Walters, R.G. 2005. Towards an understanding of photosynthetic acclimation. J. Expt. Bot. 56:435-447.

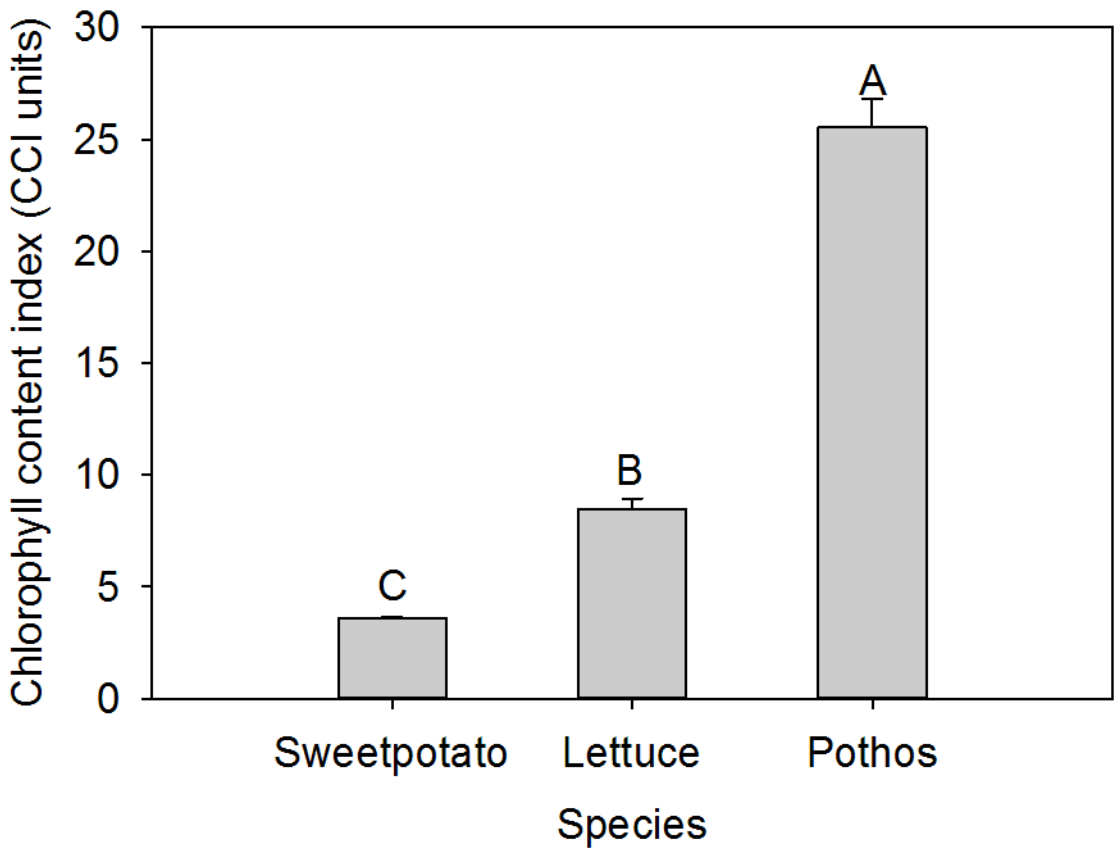


Figure 3.1. Leaf chlorophyll content index of sweetpotato, lettuce, and pothos. Error bars represent standard error (n = 18; 3 shading levels × 6 replications). Different letters indicate significance at $P < 0.05$. The chlorophyll content index of these species was not affected by shading.

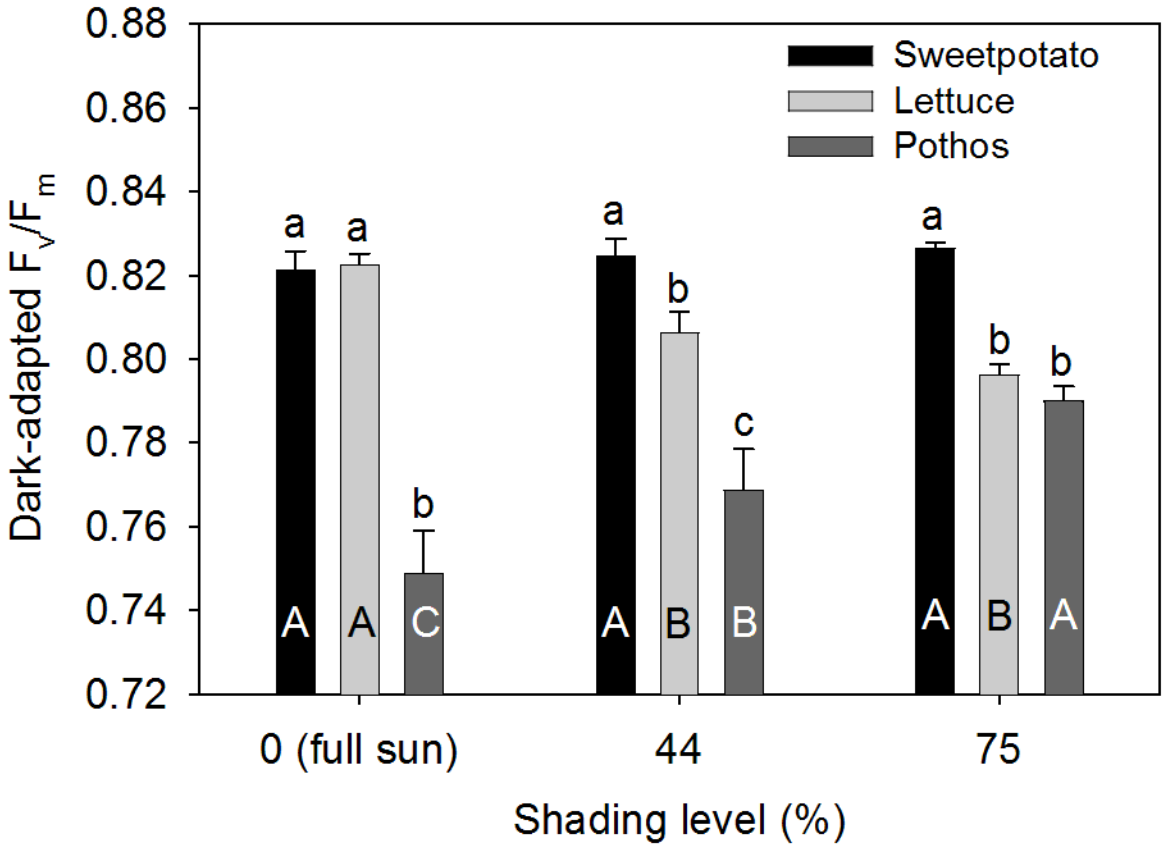


Figure 3.2. Dark-adapted F_v/F_m of sweetpotato, lettuce and pothos at three shading levels. Within each species, different uppercase letters indicate significance at $P < 0.05$ among the shading levels (n = 6). Within each shading level, different lowercase letters indicate significance at $P < 0.05$ among the species. Error bars represent standard error (n = 6).

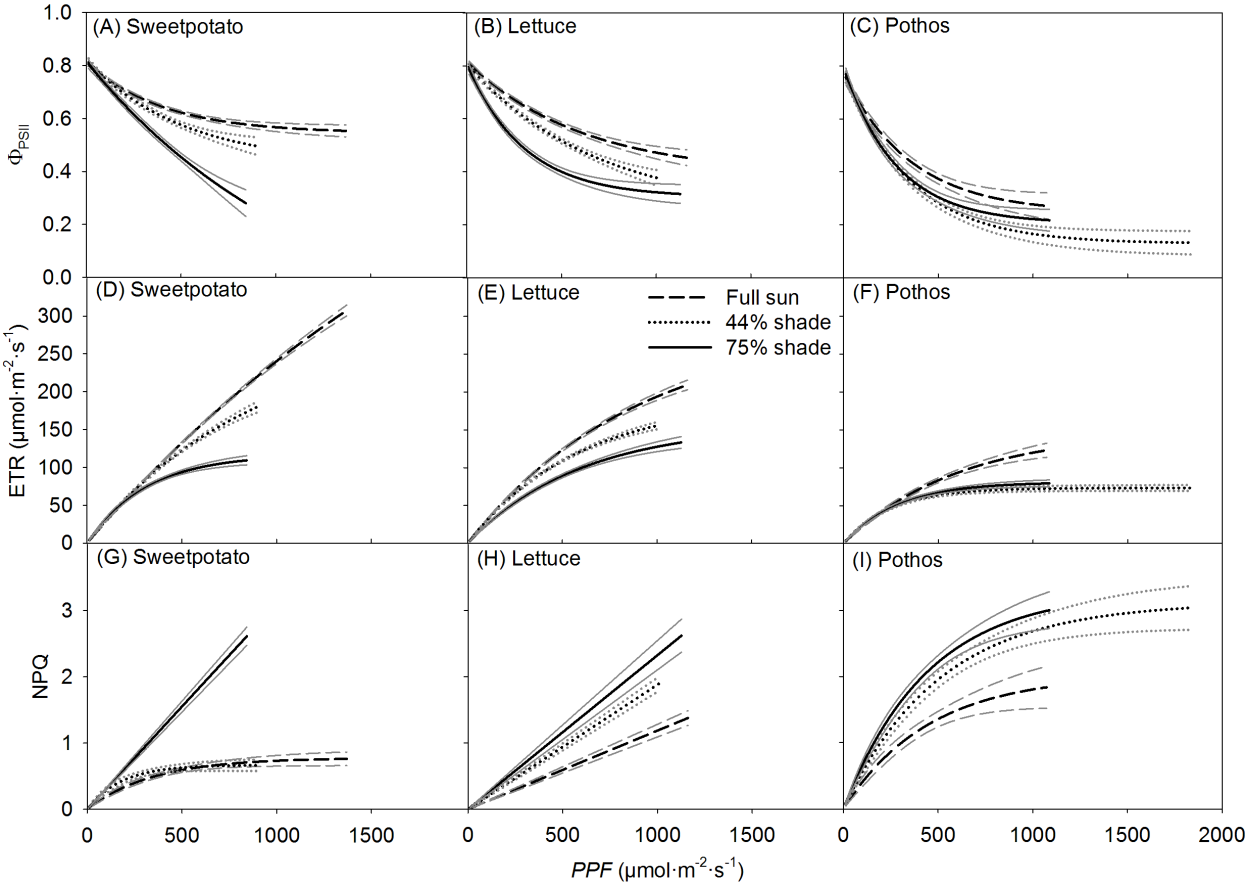


Figure 3.3. Light response curves of quantum yield of photosystem II (Φ_{PSII}) (A-C), linear electron transport rate (ETR) (D-F), and non-photochemical quenching (NPQ) (G-I) for sweetpotato (A, D, G), lettuce (B, E, H), and pothos (C, F, I) grown under full sun, 44% shade, and 75% shade. *PPF* stands for photosynthetic photon flux. Each regression curve was fitted using data pooled from six replications with gray lines representing the 95% confidence intervals.

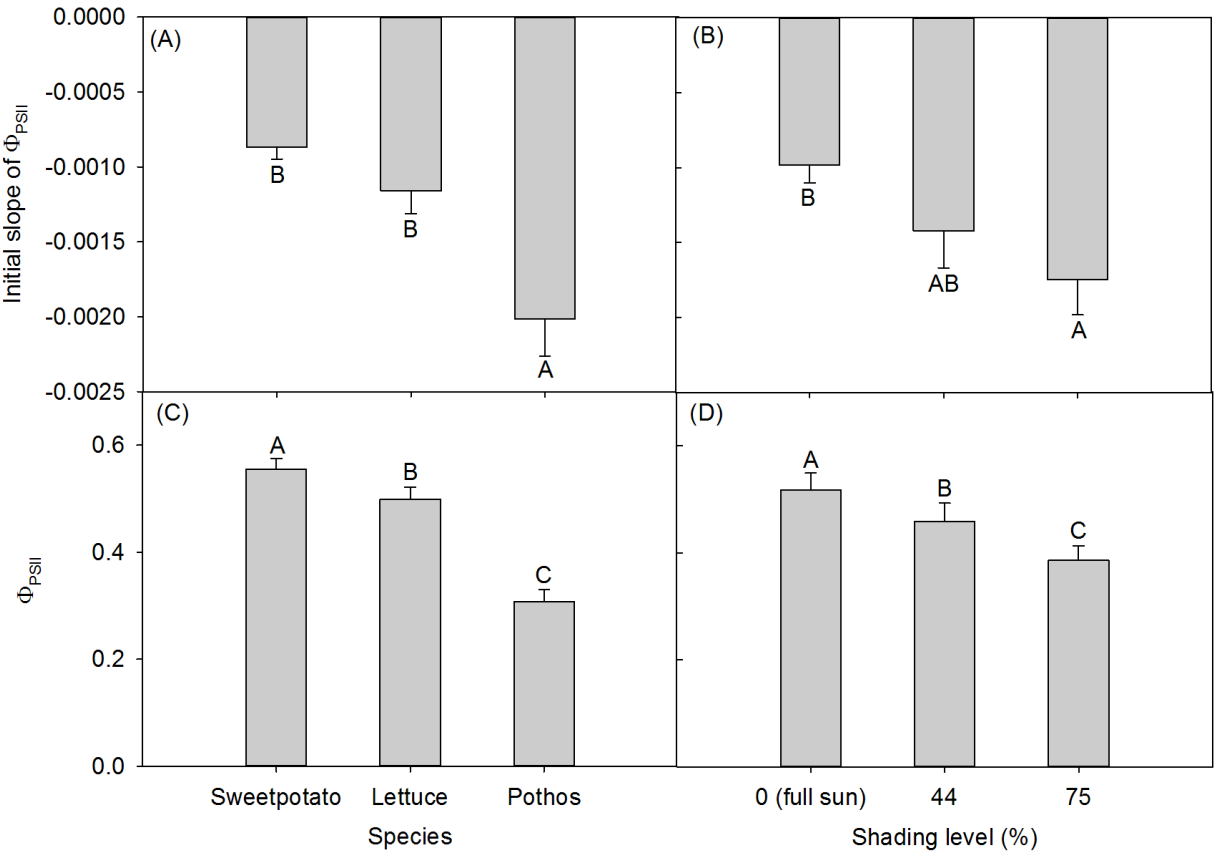


Figure 3.4. Initial slope of the Φ_{PSII} – *PPF* curve, an indicator of how fast Φ_{PSII} decreased when plant was transferred from dark to light, of sweetpotato, lettuce, and pothos (A) and by shading level (B). Φ_{PSII} stands for quantum yield of photosystem II. *PPF* stands for photosynthetic photon flux. Predicted Φ_{PSII} at *PPF* of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of sweetpotato, lettuce, and pothos (C) and by shading level (D). There was no significant species × shading level interaction for both variables. Error bars represent standard error (n = 18; 3 species/shading levels × 6 replications). Different letters indicate significance at $P < 0.05$.

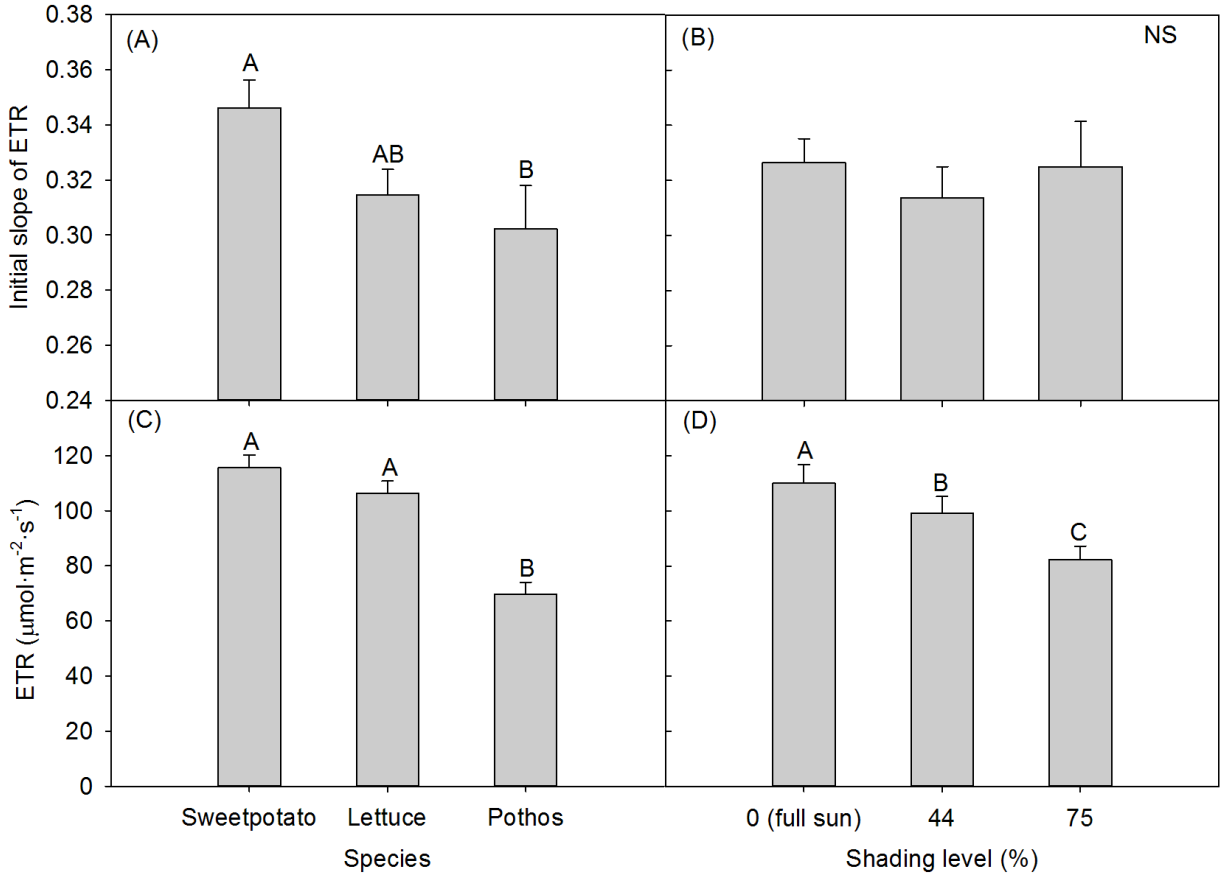


Figure 3.5. Initial slope of the ETR – *PPF* curve, an estimate of the maximum rate of increase in ETR per unit increase in incident *PPF,* of sweetpotato, lettuce, and pothos (A) and by shading level (B). ETR stands for linear electron transport rate through photosystem II. *PPF* stands for photosynthetic photon flux. Predicted ETR at *PPF* of 500 µmol·m^{-2} ·s^{-1} of sweetpotato, lettuce, and pothos (C) and by shading level (D). Error bars represent standard error (n = 18; 3 species/shading levels × 6 replications). Different letters indicate significance at $P < 0.05$. NS represents non-significance.

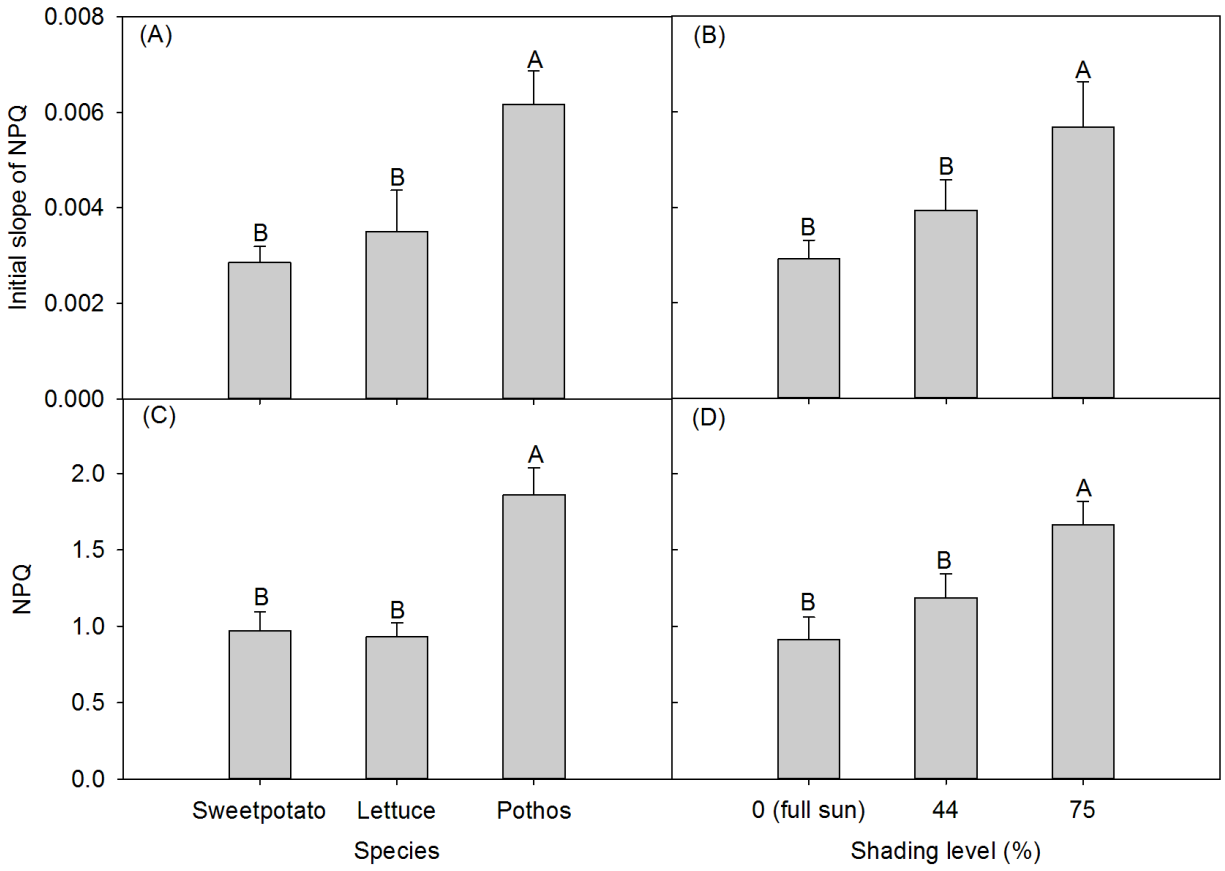


Figure 3.6. Initial slope of the NPQ – *PPF* curve, which is indicative of the rate of increase in heat dissipation of the absorbed light upon transfer of plants from dark to light, of sweetpotato, lettuce, and pothos (A) and by shading level (B). NPQ stands for non-photochemical quenching. *PPF* stands for photosynthetic photon flux. Predicted NPQ at *PPF* of 500 μmol·m^{-2} ·s^{-1} of sweetpotato, lettuce, and pothos (C) and by shading level (D). Error bars represent standard error (n = 18; 3 species/shading levels × 6 replications). Different letters indicate significance at $P <$ 0.05.

CHAPTER 4

FAR-RED LIGHT IS NEEDED FOR EFFICIENCT PHOTOCHEMISTRY AND PHOTOSYNTHESIS[2]

[2]Zhen, S. and M.W. van Iersel. 2017. Journal of Plant Physiology 209:115-122.

Reprinted here with permission of the publisher.

Abstract

The efficiency of monochromatic light to drive photosynthesis drops rapidly at wavelengths longer than 685 nm. The photosynthetic efficiency of these longer wavelengths can be improved by adding shorter wavelength light, a phenomenon known as the Emerson enhancement effect. The reverse effect, the enhancement of photosynthesis under shorter wavelength light by longer wavelengths, however, has not been well studied and is often thought to be insignificant. We quantified the effect of adding far-red light (peak at 735 nm) to red/blue or warm-white light on the photosynthetic efficiency of lettuce (*Lactuca sativa*). Adding far-red light immediately increased quantum yield of photosystem II (Φ_{PSII}) of lettuce by an average of 6.5 and 3.6% under red/blue and warm-white light, respectively. Similar or greater increases in Φ_{PSII} were observed after 20 min of exposure to far-red light. This longer-term effect of far-red light on Φ_{PSII} was accompanied by a reduction in non-photochemical quenching of fluorescence (NPQ), indicating that far-red light reduced the dissipation of absorbed light as heat. The increase in Φ_{PSII} and complementary decrease in NPQ is presumably due to preferential excitation of photosystem I (PSI) by far-red light, which leads to faster re-oxidization of the plastoquinone pool. This facilitates reopening of PSII reaction centers, enabling them to use absorbed photons more efficiently. The increase in Φ_{PSII} by far-red light was associated with an increase in net photosynthesis (P_n). The stimulatory effect of far-red light increased asymptotically with increasing amounts of far-red. Overall, our results show that far-red light can increase the photosynthetic efficiency of shorter wavelength light that over-excites PSII.

Keywords: chlorophyll fluorescence; excitation energy distribution; photosynthesis; quantum yield of PSII; state transitions

Abbreviations – Φ_{PSII}, quantum yield of PSII; LED, light emitting diode; LHCII, light harvesting complex II; NPQ, non-photochemical fluorescence quenching; *PPFD*, photosynthetic photon flux density; PSI, photosystem I; PSII, photosystem II; qE, high energy-dependent quenching; *YPF*, yield photon flux

Introduction

Photosynthesis is dependent on both the quantity and quality of light that reaches the chloroplasts. While the most common measure of photosynthetic radiation, photosynthetic photon flux density (*PPFD*), weighs photons within the 400 to 700 nm wavelength range equally, photosynthetic responses to light are wavelength-dependent (Emerson and Lewis, 1943, Evans, 1987, Hogewoning et al., 2012, Hoover, 1937, Inada, 1976 and McCree, 1972a). The efficiency of photons to drive photosynthesis, measured as the amount of O_2 evolved or CO_2 fixed per mole of absorbed photons, is highest for red photons (roughly 600 – 680 nm), followed by blue and green photons (Inada, 1976 and McCree, 1972a). Yield photon flux (*YPF*) is an alternate measure of photosynthetic radiation which takes into account the spectral dependence of photosynthesis, weighing photons in the 360 to 760 nm range according to their relative quantum efficiency obtained by McCree (1972a) (Barnes et al., 1993). Photons with different wavelengths are treated as independent and additive in the calculation of *YPF* and *PPFD*.

Far-red light ($\lambda > 700$ nm) has long been considered to make a minimal contribution to photosynthesis, due to its poor absorption by leaves and low quantum yield of photosynthesis (McCree, 1972a). Emerson and Lewis (1943) first described the 'red drop', a sharp decline in quantum yield of O_2 evolution at wavelengths above 685 nm. They observed that the quantum

yield at 700 nm was less than half of that at 685 nm (Emerson and Lewis, 1943). Emerson and coworkers subsequently found that the photosynthetic rate under simultaneous illumination of long- ($\lambda > 685$ nm) and short-wavelength lights was greater than the sum of the rates from applying the two lights separately (Emerson et al., 1957, Emerson and Rabinowitch, 1960 and Myers, 1971). With the assumption that the quantum yield of photosynthesis at shorter wavelengths is maximum and constant, Emerson and others ascribed this synergistic effect on photosynthesis to the enhancement of quantum yield of longer wavelength light by shorter wavelengths (Duysens and Amesz, 1962, Emerson et al., 1957, Emerson and Rabinowitch, 1960 and Myers and Graham, 1963). This interpretation has been widely accepted by other researchers (Govindjee et al., 1964 and Myers and Graham, 1963). The reverse effect, the enhancement of quantum yield of shorter wavelength light by far-red light, has not received much attention and sometimes is thought not to be present (McCree, 1972b).

It is now known that the low quantum yield of photosynthesis under far-red light is caused by unbalanced excitation of the two photosystems, PSI and PSII, which operate in series to carry out photochemical reactions (Duysens and Amesz, 1962, Hill and Bendall, 1960 and Myers, 1971). To achieve optimal efficiency of photochemistry (electron transport from PSII to PSI and ultimately to ferredoxin), the two photosystems should be equally excited by light and operate at matching rates (Duysens and Amesz, 1962 and Myers, 1971). Far-red light preferentially excites PSI, while shorter wavelengths (about 400 - 670 nm) generally excite PSII more than PSI (Evans, 1987 and Hogewoning et al., 2012). Since PSI tends to be under-excited relative to PSII under shorter wavelength light, this limits the overall rate of photochemistry and subsequently CO_2 assimilation. When shorter wavelength light is supplemented with far-red light

that preferentially excites PSI, the excitation balance between the two photosystems can be restored. This can synergistically increase photochemistry and photosynthesis.

Plants can dynamically adjust their photosynthetic apparatus in response to ambient light conditions to optimize photosynthetic efficiency. When the two photosystems are unequally excited, a mobile pool of light harvesting complex II (LHCII) moves to the under-excited and thus rate-limiting photosystem to rebalance the excitation energy between the two photosystems (Allen, 1992, 2003 and Chow et al., 1981). This process is termed state transitions and takes place within minutes (Haldrup et al., 2001). Longer-term adjustment of photosystem stoichiometry can also take place on a time scale of days to correct unbalanced excitation of the two photosystems (Chow et al., 1990, Fujita, 1997 and Haldrup et al., 2001).

For an enhancement of photosynthesis to occur when combining two lights, 1) either of the lights alone should provide unequal excitation of the two photosystems, and 2) the two lights should complement each other, *i.e.* one light over-excites PSI and the other light over-excites PSII (Myers, 1971). It is thus expected that neither of the two lights would be optimal for photosynthesis when applied alone, and that the interaction between the two lights would be synergistic: photosynthetic efficiency of both lights would be improved by each other. Therefore, ignoring the synergistic effects among wavelengths (*e.g.* in the calculation of *YPF* and *PPFD*) or exclusion of far-red light (*e.g.* in the integration of *PPFD*) can lead to inaccurate measures of the photosynthetic activity of light.

Chlorophyll fluorescence has long been used to study the light reactions of photosynthesis. Chlorophyll fluorescence decreases in response to increases in the efficiency of photochemistry and/or thermal dissipation of the absorbed light energy (estimated as non-photochemical quenching (NPQ) using chlorophyll fluorescence analyses) (Baker et al., 2007

and Maxwell and Johnson, 2000). Enhancement of photosynthesis when combining long- and short-wavelengths is evident from the corresponding, rapid changes in chlorophyll fluorescence: the fluorescence yield under the combination of far-red light and shorter wavelength light is less than additive of that produced by the two lights separately (Govindjee et al., 1960). Fluorescence yield under shorter wavelength light decreases rapidly when far-red light is added (Bonaventura and Myers, 1969, Butler, 1962 and Myers, 1971). The rapid decrease in fluorescence yield is thought to be caused by increases in the efficiency of photochemistry (Bonaventura and Myers, 1969 and Myers, 1971). Although early work has shown that far-red light affects chlorophyll fluorescence, researchers were unable to use these measurements to quantify the quantum yield of photosystem II (Φ_{PSII}). Genty et al. (1989) were the first to describe how fluorescence measurements can be used to determine Φ_{PSII}. The subsequent development of pulse amplitude modulation fluorometry has made measurements of Φ_{PSII} fast and easy.

Understanding the wavelength-dependence of photosynthesis and the interaction among wavelengths is of particular importance for optimizing photosynthetic lighting provided by electric light sources in controlled environment agriculture. Electric lights, such as light emitting diodes (LEDs), are increasingly used to supplement sunlight in greenhouses or as the sole lighting source for indoor production of high value crops. Production costs, however, can increase substantially due to the high electrical consumption of lighting. One way to improve the photosynthetic lighting efficiency is to provide light with a spectral distribution that most efficiently drives photosynthesis. Currently, the optimization of photosynthetic light spectra is primarily based on the action or quantum yield spectra of photosynthesis developed by McCree (1972a), assuming that different wavelengths affect photosynthesis independently and additively and that far-red has little photosynthetic efficiency. The synergistic effects of different

wavelengths are largely overlooked. In this study, we revisited the Emerson enhancement effect, with a focus on the enhancement of photosynthetic efficiency of shorter wavelength light by long-wavelength light. Instead of measuring photosynthetic action or quantum yield of photosynthesis under low light conditions, as was done by McCree (1972a), we focus on the first steps of the light reactions of photosynthesis: the quantum yield of PSII (Φ_{PSII}), which is the fraction of light absorbed by the leaves that is used for photochemical electron transport (Maxwell and Johnson, 2000). We specifically wanted to answer the following questions, which have not been addressed in past research: 1) how does far-red light affect Φ_{PSII} and NPQ (*i.e.* heat dissipation of the absorbed light energy) and does this depend on the intensity of the far-red light?, 2) how does far-red light affect Φ_{PSII} and NPQ when added to different intensities of *PPFD*?, and 3) does the effect of far-red light depend on the spectrum of the *PPFD* (red/blue vs. white light)? Our goal is to provide a better understanding of the interactive effects of light with different wavelengths on Φ_{PSII} and photosynthesis.

Materials and Methods

Plant material and growing conditions

Lettuce (*Lactuca sativa* 'Green Towers') plants were grown inside a greenhouse at the University of Georgia (Athens, GA, USA) in 1.7 L, round, plastic containers filled with a soilless substrate (Fafard 2P; 60% peat and 40% perlite; Sun Gro Horticulture, Agawam, MA, USA). Plants were placed on ebb-and-flow benches and sub-irrigated daily with a nutrient solution containing100 mg L^{-1} nitrogen, made with a water-soluble fertilizer (15N-2.2P-12.45K Cal-Mag; Everris, Marysville, OH, USA). During the growing period (31 October 2015 to 7 January 2016), the average daily temperature and vapor pressure deficit were 20.3 ± 0.9 °C and 1.0 ± 0.4 kPa.

Daily light integral ranged from 0.4 to 9.1 mol m^{-2} d^{-1} with an average of 4.6 mol m^{-2} d^{-1}. No Supplemental lighting was provided. Three groups of lettuce were seeded on the following dates: 30 October, 13 November, and 29 November 2015. Measurements were made on plants that were 31 to 39 days old.

LED lights

After plants reached maturity, plants were moved into an enclosed chamber where chlorophyll fluorescence and photosynthesis measurements were made under different LED lights. Three types of LEDs - red/blue (54 W; PopularGrow, Shenzhen Houyi Lighting, Shenzhen, China), warm-white (Bridgelux, Livermore, CA) and far-red (Epistar, Hsinchu, Taiwan) were used. The spectral distributions of the LEDs were measured using a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, USA) and normalized to their respective peaks (Fig. 1). Around 90% of the photons from red/blue LED light were within the 433-473 nm and 618-658 nm wavelength ranges, centered at 453 nm (blue) and 638 nm (red), respectively. The blue (400-500 nm):green (501-600 nm):red (601-700 nm):far-red (701 – 800 nm) ratio (B:G:R:FR ratio) of the red/blue LED light was 23.3:0.9:75.8:0 (each component expressed as % of total photons). The warm-white light had a primary peak at 599 nm and a secondary peak at 453 nm, and 4.4% of its total photons were > 700 nm. The B:G:R:FR ratio of the warm-white light was 12.1:42.9:40.6:4.2. The far-red LED light (peak wavelength at 735 nm) had 90% of its photons within the 701-769 nm wavelength range, and 8.2% of its total photons were between 601 and 700 nm. Yield photon flux was calculated by the spectroradiometer software.

The warm-white and far-red LEDs were mounted on aluminum heat sinks with cooling fans added on top of the heat sinks. Two DC power supplies (PPS2320A, Circuit Specialists, Tempe, AZ and E3631A, Agilent Technologies, Santa Clara, CA, USA) were used to power the LEDs. The intensity of the LEDs was controlled by adjusting the current output from DC power supplies.

Treatments and measurements

Dark measurements.

Plants were placed in the dark chamber for at least an hour prior to data collection to dark-adapt them. Chlorophyll fluorescence measurements were made on the upper-most fully expanded leaves using a pulse-amplitude modulated fluorometer (Mini-PAM; Heinz Walz, Effeltrich, Germany). Minimal fluorescence level (F_o) in the dark was recorded five times per second by the fluorometer. A saturating light pulse was applied to the dark-adapted leaves to transiently close all the PSII reaction centers, resulting in the maximal fluorescence (F_m) (Maxwell and Johnson, 2000). Net photosynthetic rate (P_n) was measured on the same leaves using a photosynthesis system (CIRAS-2; PP Systems, Amesbury, MA, USA). The CO_2 concentration within the leaf cuvette was maintained at 394 ± 12.5 μmol mol^{-1}. Cuvette temperature was set at 25 ºC. Vapor pressure deficit inside the cuvette was 1.7 ± 0.3 kPa.

Part 1: Far-red light effects on photochemistry and photosynthesis under different intensities of red/blue or warm white light.

Seven intensities of red/blue (or warm-white) light intensity, ranging from a *PPFD* of 50 to 750 μmol m^{-2} s^{-1}, were used to simulate the wide range of light levels that plants were exposed to inside the greenhouse. After the measurements in dark-adapted leaves were taken, red/blue (or

warm-white) LEDs were switched on to a *PPFD* of 50 μmol m^{-2} s^{-1}. Similar to the chlorophyll fluorescence measurements made on the dark-adapted leaves, steady-state fluorescence yield (F_t) in the light was recorded five times per second throughout the rest of the data collection period. After plants were given 15-20 min to acclimate to the light level and F_t was steady, a saturating pulse was applied to determine the maximal fluorescence in the light (Fm′). Quantum yield of PSII (Φ_{PSII}) of light-adapted leaves and non-photochemical quenching of chlorophyll fluorescence (NPQ) were calculated as $\Phi_{PSII} = (Fm' - F_t)/Fm'$ and $NPQ = (F_m - Fm')/Fm'$, respectively (Genty et al., 1989 and Maxwell and Johnson, 2000). P_n was also measured after plants had acclimated to the given light level. After that, far-red light (110 μmol m^{-2} s^{-1} in the 700-770 nm wavelength range) was added to the red/blue (or warm-white) light, and Φ_{PSII} and NPQ were determined immediately (within 1 min). Plants were then allowed to acclimate for about 20 min under the red/blue (or warm-white) plus far-red light before the stabilized Φ_{PSII}, NPQ, and P_n were determined. Far-red light was then switched off, and the intensity of red/blue (or warm-white) light was increased to the next level. Φ_{PSII}, NPQ, and P_n were again taken under the increased intensity of red/blue (or warm-white) light after plants were allowed to acclimate, and then with the addition of far-red light in a similar manner as described above (Φ_{PSII} and NPQ were determined both immediately and 20 min after the addition of far-red light). Measurements were taken in this fashion until the highest red/blue (or warm-white) light level of 750 μmol·m^{-2}·s^{-1} was reached. P_n/*PPFD* was calculated as the ratio of steady-state P_n to incident *PPFD* at each of the seven levels of *PPFD* used in this study.

This entire procedure was replicated five times using five different plants under red/blue light, and three times using three different plants under warm-white light. Note that leaf

temperature measured inside the gas exchange cuvette increased by 4.3 and 3 ºC from dark condition to when 750 μmol m^{-2} s^{-1} of red/blue and warm-white light, respectively, was given.

Part 2: Do different intensities of far-red light affect photochemistry and photosynthesis under constant red/blue light?

Six levels of far-red light intensity, ranging from 0 to 90 μmol m^{-2} s^{-1} within the 700-770 nm wavelength range, were added to red/blue light with a *PPFD* of 200 μmol m^{-2} s^{-1}. Only red/blue light was used for these studies, because the results from part 1 had indicated that far-red light enhanced photosynthetic efficiency of red/blue light more than that of white light. Data were collected in a similar manner as described above. F_o, F_m, and P_n were first determined in the dark. Red/blue light was then switched on, and F_t was recorded five times per second throughout the data collection. Φ_{PSII}, NPQ, and P_n were determined under red/blue light only. After that, Φ_{PSII} and NPQ were measured immediately after each increase in the intensity of far-red light. Stabilized Φ_{PSII}, NPQ and P_n were determined after plants were given about 20 min to acclimate after each increment of far-red light until the highest intensity of far-red light (*i.e.* 90 μmol m^{-2} s^{-1}) was added. This entire procedure was replicated four times using four different plants. Leaf temperature increased by 1.4 ºC when increasing far-red intensity from 0 to 90 μmol m^{-2} s^{-1}.

Statistical analysis

Data were analyzed using regression (polynomial, multiple linear, and exponential rise to maximum) and ANOVA in Statistical Analysis Systems (SAS Institute, Cary, NC, USA). Mean separation was performed using Fisher's protected least significant difference (LSD, $P = 0.05$).

Results and Discussion

Part 1: Far-red light effects on photochemistry and photosynthesis under different intensities of red/blue or warm white light.

Changes in chlorophyll fluorescence

Effects of actinic light intensity on chlorophyll fluorescence yield.

Chlorophyll fluorescence yield was minimal in the dark (F_o) and rose to a maximum (F_m) when a brief saturating pulse was applied, transiently closing all the PSII reaction centers (Fig. 2A). Upon transitioning from darkness to actinic light (*i.e.* red/blue or warm-white light) with a *PPFD* of 50 μmol m^{-2} s^{-1}, fluorescence yield exhibited a typical Kautsky effect (Butler, 1962 and Maxwell and Johnson, 2000), sharply increasing within seconds, and then slowly decaying over a time-scale of minutes back to a steady-state level (F_t), which was higher than F_o (Fig. 2A). The increase in the fluorescence yield under light-adapted steady-state (F_t) compared to that in the dark (F_o) is indicative of less efficient photochemistry. This likely resulted from a partial closure of the PSII reaction centers, not photoinhibitory damage to the reaction centers, under the relatively low levels of light used in this study (Maxwell and Johnson, 2000). A similar rapid increase in fluorescence yield followed by relaxation to a steady-state level that was higher than F_o was observed when increasing the intensity of actinic light to a *PPFD* of 100 μmol m^{-2} s^{-1} (Fig. 2A) or higher (data not shown). Maximal fluorescence under actinic light (Fm′), *e.g.* at *PPFD* of 50 μmol m^{-2} s^{-1}, was consistently lower than that in the dark (F_m), most likely resulting from the light-induced up-regulation of non-photochemical fluorescence quenching (NPQ) in the form of heat dissipation mediated by the xanthophyll cycle (Baker et al., 2007, Demmig-Adams et al., 1996 and Maxwell and Johnson, 2000).

Far-red light effects on chlorophyll fluorescence.

Fluorescence yield decreased immediately when far-red light was added to red/blue (or warm-white) light and reached a minimum within 10-15s (Fig. 2A and B). A decrease in fluorescence yield can be caused by an increase in the efficiency of photochemistry (*i.e.*, Φ_{PSII}) or NPQ: a greater proportion of the excitation energy/absorbed light is being used for photochemistry or quenched through non-photochemical processes, including xanthophyll cycle-dependent heat dissipation, photoinhibitory damage to PSII reaction centers (not likely to have occurred as discussed above), and state transitions (Baker et al., 2007, Krause and Weis, 1991, Maxwell and Johnson, 2000, Roach and Krieger-Liszkay, 2014 and Ruban, 2015). The rapid decrease in fluorescence yield upon adding far-red was most likely attributable to increased Φ_{PSII} rather than an increase in NPQ, as regulation of NPQ, either through the xanthophyll cycle or state transition, is a relatively slow process involving enzymatic reactions and typically requires at least minutes to occur (Roach and Krieger-Liszkay, 2014 and Ruban, 2015). Efficiency of photochemistry, on the other hand, can change on a millisecond time scale, driven by changes in the proportion of open reaction centers (Ruban, 2015). Under light conditions that over-excite PSII, the plastoquinone (PQ) pool, which is the intermediate electron transporter between PSII and PSI, gradually becomes reduced as electrons from PSII are being moved faster into the PQ pool than they can leave it (Allen, 2003). Reduction of the PQ pool, especially Q_A – the primary electron acceptor of PSII, prevents transfer of electrons away from PSII. Thus the PSII reaction centers become 'closed', *i.e.* incapable of using light for photochemistry (Maxwell and Johnson, 2000). Far-red light can increase the proportion of open PSII reaction centers through preferential excitation of PSI (Evans, 1987 and Hogewoning et al., 2012), which leads to faster re-oxidation of plastoquinones (Bonaventura and Myers, 1969 and Ruban, 2016). Re-oxidized

plastoquinones can accept electrons from excited PSII reaction center chlorophylls and thus help to re-open PSII reaction centers more quickly (Bonaventura and Myers, 1969, Baker, 2008 and Maxwell and Johnson, 2000), resulting in increased Φ_{PSII} (see Fig. 3A and B) and a consequent drop in fluorescence yield. The observed decrease in fluorescence yield and increase in Φ_{PSII} when adding far-red light to red/blue (Fig. 3A) or warm-white (Fig. 3B) LED light suggests that excitation energy is unequally distributed between the two photosystems under red/blue or warm-white light, with PSI being under-excited and thus limiting the overall rate and efficiency of photosynthesis.

After the rapid drop in fluorescence yield upon adding far-red light, fluorescence yield gradually increased over a time period of 6-8 min, until a steady state value was reached (Fig. 2A). Bonaventura and Myers (1969) similarly observed a gradual increase in fluorescence yield following an initial rapid drop when adding far-red light (710 nm) to shorter wavelength light (645 nm). They ascribed the slow increase in fluorescence yield to state transitions. We postulate that under red/blue or warm-white LED light, a mobile pool of LHCII moved from PSII to PSI to redistribute more light energy to PSI. The detachment of LHCII from PSII decreases the antenna absorption cross section of PSII and excitation energy transfer to PSII (Allen and Mullineaux, 2004), therefore possibly contributing to the slow decay of PSII fluorescence observed after switching on actinic light (Fig. 2A). As far-red light preferentially excites PSI, the addition of far-red can restore the balance of excitation between the two photosystems, or may even cause PSI to be overexcited relative to PSII when a large amount of far-red is added. It is likely that LHCII gradually migrated back to PSII from PSI after far-red light was added to red/blue or warm-white light, thus increasing the PSII antenna absorption cross section and the amount of light received by PSII, which in turn increased PSII chlorophyll fluorescence over a 6 to 8 min

period following the addition of far-red light (Fig. 2A). F_t in the presence of far-red light was greater than F_o, but lower than F_t under red/blue (or warm-white) light only (Fig. 2A). The decrease in steady-state fluorescence yield following the addition of far-red light indicates less efficient chlorophyll fluorescence, thus more energy was partitioned to photochemistry or NPQ processes (Maxwell and Johnson, 2000). However, the stabilized Fm′ was higher (thus NPQ was reduced) after the addition of far-red light (see Fig. 3C and D).

In contrast to the changes in fluorescence yield induced by adding far-red light, removal of far-red light from the actinic light caused a transient increase in fluorescence yield, which reached a maximum after 5-6 seconds and then slowly relaxed over a timescale of minutes (Fig. 2A and C). This transient increase in fluorescence yield after removal of far-red indicates that the efficiency of photochemistry was decreased, presumably due to the reduction of the PQ pool as a consequence of PSI operating slower than PSII under red/blue or warm-white light. The reduction of the PQ pool in turn leads to closure of PSII reaction centers and less efficient photochemistry (Maxwell and Johnson, 2000). The magnitude of this transient fluorescence rise upon removal of far-red light was much greater than that of the transient drop in fluorescence when adding far-red light (Fig. 1A). This differential change in fluorescence following the addition and removal of far-red light is thought to be indicative of the occurrence of state transitions (Haldrup et al., 2001 and Lunde et al., 2000). The gradual relaxation of fluorescence after the initial rapid rise upon removal of far-red (Fig. 2A) is likely caused by the movement of LHCII back to PSI, redirecting more excitation energy to PSI.

Quantum yield of photosystem II (Φ_{PSII})

The quantum yield of photosystem II (Φ_{PSII}) decreased with increasing red/blue (Fig. 3A) or warm-white (Fig. 3B) light intensity. As more light reaches the leaves, an increasing proportion of PSII reaction centers become closed (Baker, 2008), which in turn leads to decreased Φ_{PSII}. Φ_{PSII} increased within 10 - 15 s after adding far-red light, with a similar increase (6.5% on average) at all intensities of red/blue light (Fig. 3A). Under warm-white light, however, this immediate increase in Φ_{PSII} was 1% at the lowest PPFD and up to 8% at the highest *PPFD*, with an average increase of 3.6% (Fig. 3B). Similar or slightly greater increases in Φ_{PSII} were observed 20 min after adding far-red light (Fig. 3A and B). Two important differences in Φ_{PSII} under red/blue vs warm-white light were evident: 1) at the same *PPFD*, Φ_{PSII} under warm-white light was consistently higher than that under red/blue light and 2) the percent increase in Φ_{PSII} as induced by far-red light tended to be smaller under warm-white light compared to that under red/blue light, especially at low *PPFD* (Fig. 3A and B). One possible explanation for these observations is that while the red/blue light contains no far-red light, warm-white LEDs contain a small proportion of far-red light (Fig. 1), perhaps allowing for more efficient excitation of PSI. However, even the warm-white light does not contain sufficient amount of far-red to ensure that both photosystems operate at matching rates, as is clear for the increase in Φ_{PSII} when far-red light is added.

In addition, plants grown under red/blue LED light have been shown to use red/blue light more efficiently (*i.e.* have higher Φ_{PSII}) than plants grown under sunlight (unpublished data). This is likely due to longer-term adjustments of photosystem stoichiometry in response to ambient light conditions to optimize photosynthetic efficiency (Chow et al., 1990 and Fujita, 1997).

Non-photochemical quenching (NPQ)

A corresponding up-regulation of NPQ was observed alongside the decreasing Φ_{PSII} in response to increasing *PPFD* (Fig. 3C and D). An increase in NPQ is typically observed with increasing *PPFD* (Demmig-Adams et al., 1996 and Logan et al., 1998). Under high *PPFD*, accumulation of H^+ in the thylakoid lumen often occurs due to high rates of electron transport (Baker et al., 2007). A low lumen pH triggers the high energy-dependent quenching (qE) of the excess absorbed light as heat, which is a major component of NPQ, through activation of the xanthophyll cycle and protonation of the PsbS protein (Demmig-Adams and Adams, 2006 and Ruban 2015, 2016). This process is thought to serve a protective role against photodamage to the photosynthetic apparatus (Demmig-Adams and Adams, 1992, 2006 and Ruban, 2015).

Non-photochemical quenching measured immediately after adding far-red light showed a statistically significant increase at several *PPFD* levels (Fig. 3C and D), resulting from a negligibly small, but consistent, decrease in the Fm′ measured right after adding far-red light (Fig. 2A). However, this change was too small to be biologically meaningful. The curves describing the relationship between *PPFD* and NPQ were essentially identical before and immediately after adding far-red light.

Stabilized NPQ, measured after ~20 min of exposure to far-red light, on the other hand, was significantly lower at all *PPFD* levels (Fig. 3C and D). This relatively slow decrease in NPQ in response to the addition of far-red light may be caused by state transitions: the mobile pool of LHCII moved back to PSII on the order of minutes after adding far-red light, resulting in higher Fm′ (consequently lower NPQ; Fig. 2A) as a bigger PSII antenna captured more light energy, which in turn can increase fluorescence.

The increase in NPQ with increasing *PPFD* was slower with white light than with red/blue light. This is consistent with the higher Φ_{PSII} under warm-white light described above: a more efficient use of photons in photochemistry results in less need to dissipate excess excitation energy through NPQ.

Net photosynthetic rate (P_n)

Net photosynthetic rate (P_n) gradually increased with increasing red/blue or warm-white light, as more light was available to drive the light reactions of photosynthesis (Fig. 4A and B). At equal *PPFD* levels, white light resulted in higher P_n than red/blue light, consistent with our findings for Φ_{PSII}. However, P_n/*PPFD* at different incident light levels, indicative of the efficiency at which plant uses the incident light for photosynthesis, decreased with increasing *PPFD* (Fig. 4C and D). This response was expected as Φ_{PSII} decreased in response to increasing *PPFD* (Fig. 3), meaning that a smaller fraction of light absorbed by the leaves was used for photochemistry at higher *PPFD*. A greater fraction of the absorbed light energy was lost through NPQ as *PPFD* increased (Fig. 3C and D). Consistent with the increase in Φ_{PSII} by far-red, adding far-red light to the red/blue or warm-white light also increased P_n of lettuce (Fig. 4A and B). The increase in P_n by far-red light was greater than expected based on the increase in *PPFD* from the addition of the far-red light: for each 1% increase in *PPFD* provided by the far-red light, P_n increased by an average of 4% and 3% under the red/blue and warm-white light, respectively. The initial slope of the P_n – *PPFD* curve, which was derived from the fitted exponential rise to maximum function, shows the maximum rate of increase in P_n per unit increase in incident *PPFD*. For both red/blue and warm-white light, the initial slope of the P_n - *PPFD* curve was increased significantly by far-red light, from 0.060 to 0.066 mol CO_2 / mol *PPFD* under red/blue

light and from 0.071 to 0.078 mol CO_2 / mol *PPFD* under warm-white light – a 10% increase for both types of light. The initial slope of P_n was higher under warm-white light than under red/blue light, which is consistent with the higher P_n and Φ_{PSII} under warm-white light observed at the same *PPFD* levels as under red/blue light. This enhancement effect of far-red light on photosynthesis was also reflected in P_n/*PPFD*: far-red light increased P_n/*PPFD* by 41% for both red/blue and warm-white light at the lowest *PPFD* level (50 μmol m^{-2} s^{-1}), and the difference in P_n/*PPFD* with and without far-red light gradually decreased with increasing *PPFD* (Fig. 4C and D).

Part 2: Do different intensities of far-red light affect photochemistry and photosynthesis under constant red/blue light?

Φ_{PSII}, NPQ and P_n

The amount of far-red light added affected the enhancement of photosynthesis. Φ_{PSII} responded immediately to the addition of far-red light. Φ_{PSII}, both measured within 1 min (immediate) and 20 min (stabilized) after each increase in far-red light, increased asymptotically with increasing far-red light, with a 7.5% increase with the highest amount of far-red light added (Fig. 5A). This response suggests that at a given level of red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1} in this case), only a certain amount of far-red light (~50 μmol m^{-2} s^{-1} in this case) is needed to restore the balance between the excitation of the two photosystems. Far-red light increases Φ_{PSII} when the rate of photochemical reactions is limited by the re-oxidation of the PQ pool due to under-excitation of PSI. Once equal excitation of the two photosystems is reached, however, increasing far-red light induces no further increase in Φ_{PSII}.

Unlike the rapid response of Φ_{PSII} to far-red light, NPQ did not respond immediately to each increase in far-red light: NPQ measured immediately following an increase in far-red light was similar to the stabilized NPQ under the previous far-red light level (Fig. 5B). Instead, NPQ decreased slowly (as shown by the stabilized NPQ) over time with increasing far-red light (Fig. 5B). This decrease of NPQ by far-red light could be due to the occurrence of state transitions as well as down-regulation of xanthophyll cycle in response to more efficient photochemistry as discussed earlier. The time course of this response is in line with that of state transitions and xanthophyll cycle regulations, which take minutes to occur (Haldrup et al., 2001 and Ruban, 2015).

Consistent with the response of Φ_{PSII} to far-red light, P_n similarly increased asymptotically with increasing far-red light (Fig. 6). P_n increased by 18% with a 3% increase in *PPFD* (and 9% increase in *YPF*) at the highest amount of far-red added, suggesting that the increase of P_n by far-red light is not merely a direct effect from an increase in *PPFD* or *YPF*, but rather largely attributable to the increase in Φ_{PSII} (*i.e.* a true enhancement effect, where a greater proportion of absorbed light is used for photochemistry).

Conclusions

Our results show that different wavelengths of light can have synergistic effects on photochemistry and photosynthesis. Far-red light is needed for efficient photochemistry, especially under light with wavelengths that over-excite PSII. Adding far-red light to red/blue or warm-white LED light increases $\Phi_{PSII,}$ decreases NPQ, and enhances net photosynthetic rate. Chlorophyll fluorescence measurements provide a quick way to identify the interactive effect of different light sources on photochemistry. Both fast (< 1 min) and slow (~20 min) changes in

fluorescence yield upon altering the light conditions (*i.e.* intensity and quality) have been used to detect and distinguish between changes in photochemical activities and non-photochemical processes (*e.g.* heat dissipation and state transitions). The interactive effects of light of different wavelengths and the photosynthetic enhancement effect of far-red light should be taken into consideration to optimize photochemical efficiency and photosynthesis.

Acknowledgments

This work was supported by the Georgia Research Alliance and USDA National Institute of Food and Agriculture, Hatch project 1011550. We thank Drs. Robert Teskey, Anish Malladi, Paul Thomas, Deepak Mishra and an anonymous reviewer for providing feedback on this manuscript. We thank Sue Dove for technical support.

Literature Cited

Allen and Mullineaux, 2004 J.F. Allen, C.W. Mullineaux

Probing the mechanism of state transitions in oxygenic photosynthesis by chlorophyll fluorescence spectroscopy, kinetics and imaging. In Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: signature of photosynthesis

Springer, Dordrecht (2004), pp 447-461

Allen, 1992 J.F. Allen

Protein phosphorylation in regulation of photosynthesis

Biochim Biophys Acta, 1098(1992), pp. 275-335

Allen, 2003 J.F. Allen

State transitions – a question of balance

Science, 299(2003), pp.1530-1532

Baker et al., 2007 N.R. Baker, J. Harbinson, D.M. Kramer

Determining the limitations and regulation of photosynthetic energy transduction in leaves

Plant Cell Environ, 30(2007), pp.1107-1125

Baker, 2008 N.R. Baker

Chlorophyll fluorescence: probe of photosynthesis in vivo

Annu Rev Plant Biol, 59 (2008), pp.89-113

Barnes et al., 1993 C. Barnes, T. Tibbitts, J. Sager, G. Deitzer, D. Bubenheim, G. Koerner, B. Bugbee

Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux

HortScience, 28(1993), pp.1197-1200

Bonaventura and Myers, 1969 C. Bonaventura, J. Myers

Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*

Biochim Biophys Acta, 189(1969), pp.366-383

Butler, 1962 W.L. Butler

Effects of red and far-red light on the fluorescence yield of chlorophyll *in vivo*

Biochim Biophys Acta, 64(1962), pp.309-317

Chow et al., 1990 W.S. Chow, A. Melis, J.M. Anderson

Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis

Proc Natl Acad Sci, 87(1990), pp.7502-7506

Chow et al., 1981 W.S. Chow, A. Telfer, D.J. Chapman, J. Barber

State 1–state 2 transition in leaves and its association with ATP-induced chlorophyll fluorescence quenching

Biochim Biophys Acta, 638(1981), pp.60-68

Demmig-Adams et al., 1996 B. Demmig-Adams, W.W. Adams III, D.H. Barker, B.A. Logan, D.R. Bowling, A.S. Verhoeven

Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation

Physiol Plant, 98(1996), pp.253-264

Demmig-Adams and Adams, 1992 B. Demmig-Adams, W.W. Adams III Photoprotection and other responses of plants to high light stress

Annu Rev Plant Physiol Plant Mol Biol, 43(1992), pp.599-626

Demmig-Adams and Adams, 2006 B. Demmig-Adams, W.W. Adams III

Photoprotection in an ecological context, pp.the remarkable complexity of thermal energy dissipation

New Phytol, 172(2006), pp.11-21

Duysens and Amesz, 1962 L.N.M. Duysens, J. Amesz

Function and identification of two photochemical systems in photosynthesis

Biochim Biophys Acta, 64(1962), pp.243-260

Emerson et al., 1957 R. Emerson, R. Chalmers, C. Cederstrand

Some factors influencing the long-wave limit of photosynthesis

Proc Natl Acad Sci USA, 43(1957), pp.133-143

Emerson and Lewis, 1943 R. Emerson, C.M. Lewis

The dependence of the quantum yield of chlorella photosynthesis on wave length of light

Am J Bot, 30(1943), pp.165-178

Emerson and Rabinowitch, 1960 R. Emerson, E. Rabinowitch

Red drop and role of auxiliary pigments in photosynthesis

Plant Physio, 35(1960), pp.477-485

Evans, 1987 J.R. Evans

The dependence of quantum yield on wavelength and growth irradiance

Aust J Plant Physiol, 14(1987), pp.69-79

Fujita, 1997 Y. Fujita

A study on the dynamic features of photosystem stoichiometry: Accomplishments and problems for future studies

Photosyn Res 53(1997), pp.83-93

Genty et al., 1989 B. Genty, J.M. Briantais, N.R. Baker

The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence

Biochim Biophys Acta, 990(1989), pp.87-92

Govindjee et al., 1960 Govindjee, S. Ichimura, C. Cederstrand, E. Rabinowitch

Effect of combining far-red light with shorter wave light on the excitation of fluorescence in Chlorella

Arch Biochem Biophys, 89(1960), pp.322-323

Govindjee et al., 1964 R. Govindjee, Govindjee, G. Hoch

Emerson enhancement effect in chloroplast reactions

Plant Physiol, 39(1964), pp.10-14

Haldrup et al., 2001 A. Haldrup, P.E. Jensen, C. Lunde, H.V. Scheller

Balance of power: a view of the mechanism of photosynthesis state transitions

Trends Plant Sci, 6(2001), pp.301-305

Hill and Bendall, 1960 R. Hill, F. Bendall

Function of the two cytochrome components in chloroplasts: A working hypothesis

Nature, 186(1960), pp.136 - 137

Hogewoning et al., 2012 S.W. Hogewoning, E. Wientjes, P. Douwstra, G. Trouwborst, W. van Ieperen, R. Croce, J. Harbinson

Photosynthetic quantum yield dynamics: From photosystems to leaves

Plant Cell 24(2012), pp.1921-1935

Hoover, 1937 W.H. Hoover

The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation

Smithsonian Institution Misc Collections, 95(1937), pp.1-13

Inada, 1976 K. Inada

Action spectra for photosynthesis in higher plants

Plant Cell Physiol, 17(1976), pp.355-365

Krause and Weis, 1991 G.H. Krause, E. Weis

Chlorophyll fluorescence and photosynthesis: the basics

Annu Rev Plant Physiol Plant Mol Biol, 42(1991), pp.313-349

Logan et al., 1998 B.A. Logan, B. Demmig-Adams, W.W. Adams III, S.C. Grace

Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field

J Expt Bot, 49(1998), pp.1869-1879

Lunde et al., 2000 C. Lunde, P.E. Jensen, A. Haldup, J. Knoetzel, H.V. Scheller

The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis

Nature, 408(2000), pp.613-615

Maxwell and Johnson, 2000 K. Maxwell, G.N. Johnson

Chlorophyll fluorescence – a practical guide

J Expt Bot, 51(2000), pp.659-668

Myers, 1971 J. Myers

Enhancement studies in photosynthesis

Annu Rev Plant Physiol, 22(1971), pp.289-312

Myers and Graham, 1963 J. Myers, J.R. Graham

Enhancement in Chlorella

Plant Physiol, 38(1963), pp.105-116

McCree, 1972a K.J. McCree

The action spectrum, absorptance and quantum yield of photosynthesis in crop plants

Agric Meteorol, 9(1972a), pp.191-216

McCree, 1972b K.J. McCree

Significance of enhancement for calculation based on the action spectrum for photosynthesis

Plant Physiol, 49(1972b), pp.704-706

Roach and Krieger-Liszkay, 2014 T. Roach, A. Krieger-Liszkay

Regulation of photosynthetic electron transport and photoinhibition

Current Protein Peptide Sci, 15(2014), pp.351-362

Ruban, 2015 A.V. Ruban

Evolution under the sun: optimizing light harvesting in photosynthesis

J Expt Bot, 66(2015), pp.7-23

Ruban, 2016 A.V. Ruban

Nonphotochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage

Plant Physiol, 170(2016), pp.1903-1916

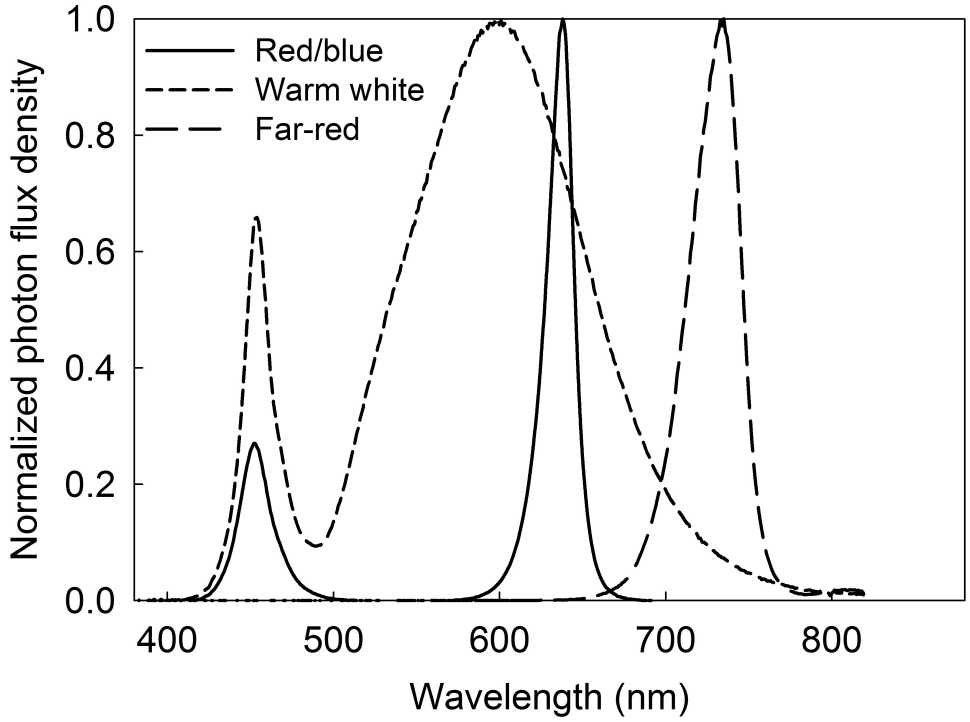
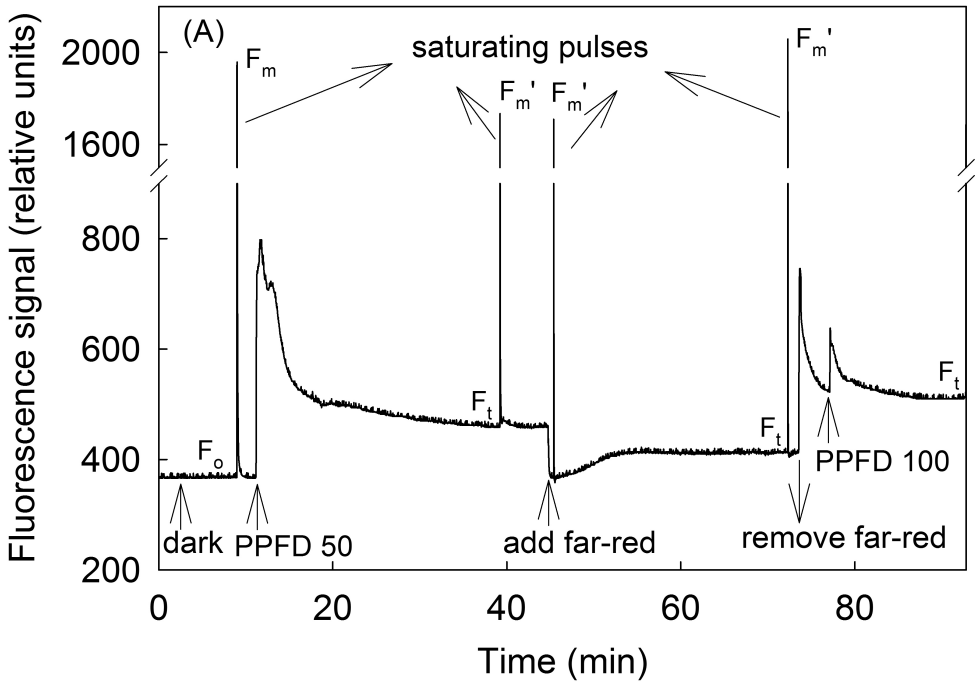
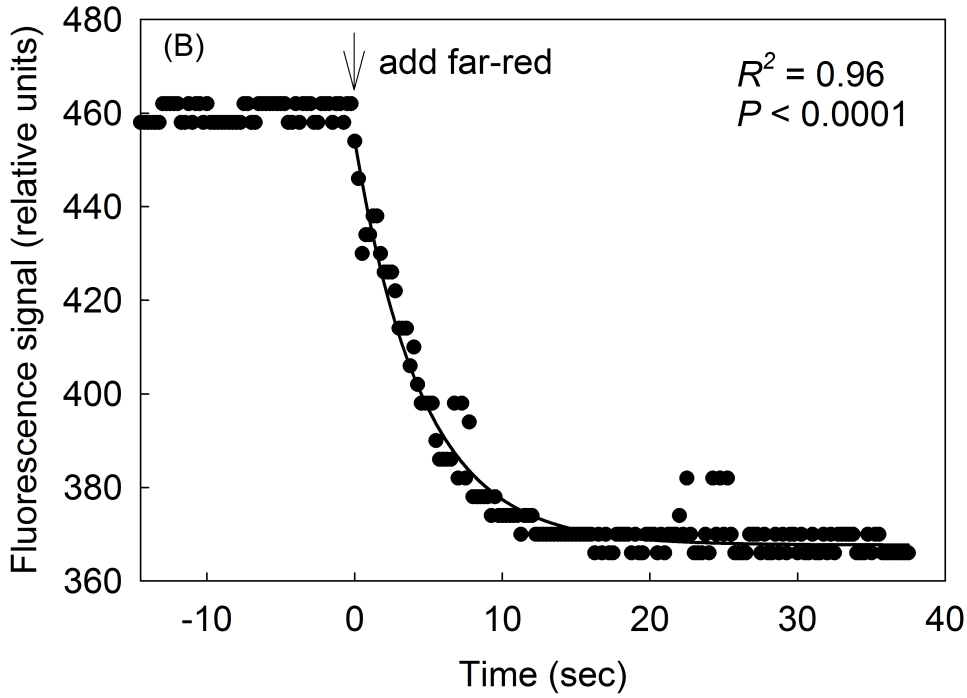


Figure 4.1. Normalized spectral distribution of red/blue, warm-white, and far-red light emitting diodes.


(A)
Fm
saturating pulses
Fm'
Fm'
Fm'
Ft
Ft
Ft
Fo
dark
PPFD 50
add far-red
remove far-red
PPFD 100
Fluorescence signal (relative units)
Time (min)
2000
1600
800
600
400
200
0
20
40
60
80


(B)
add far-red
R2 = 0.96
P < 0.0001
Fluorescence signal (relative units)
Time (sec)
480
460
440
420
400
380
360
-10
0
10
20
30
40

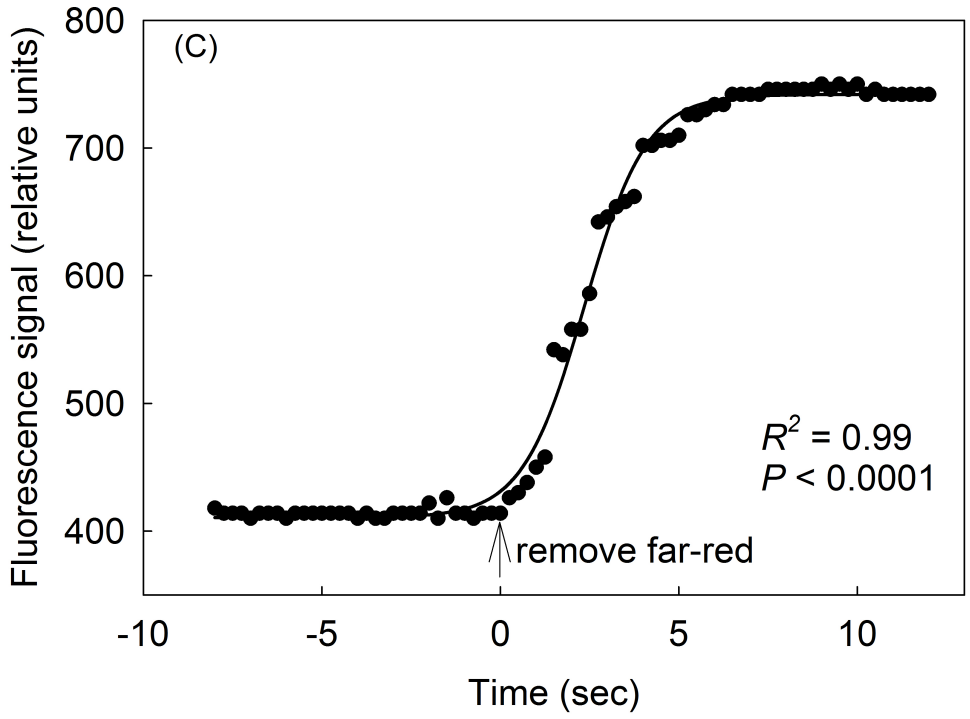


Figure 4.2. A typical fluorescence trace of a lettuce leaf under changing light conditions illustrates changes in fluorescence yield upon transitioning of the leaves from dark to different intensities of red/blue light, and when far-red light (110 μmol m^{-2} s^{-1} in the 700-770 nm wavelength range) was added to or removed from red/blue light (A). Saturating light pulses were applied to determine the quantum yield of photosystem II (Φ_{PSII}) and non-photochemical quenching (NPQ). PPFD 50 and PPFD 100 represent switching on or increasing red/blue light intensity to a photosynthetic photon flux density (*PPFD*) of 50 or 100 μmol m^{-2} s^{-1}. F_m and Fm′ are the maximal fluorescence yields in the dark and light, respectively. F_0 is the minimal fluorescence yield in the dark, and F_t is the steady-state fluorescence in the light. The changes in fluorescence yield as affected by the addition (B) or removal (C) of far-red light from the same dataset are shown on a much a shorter time scale.

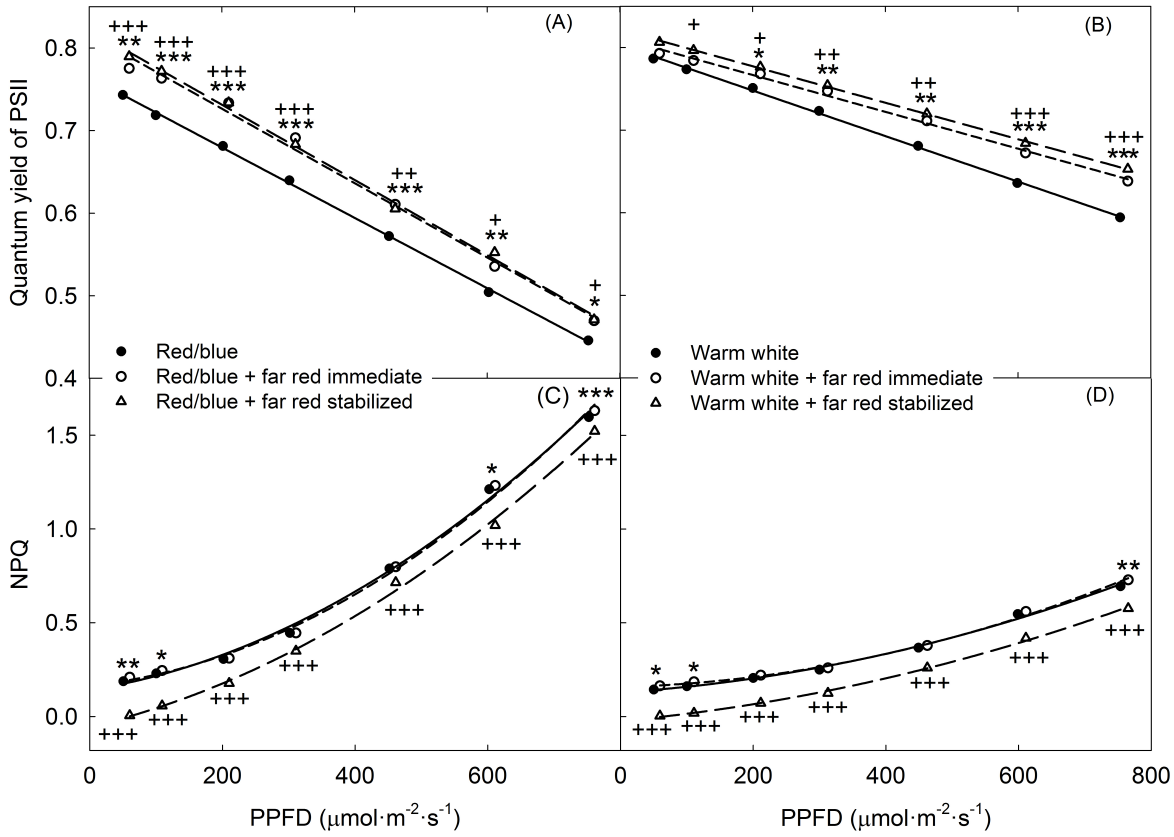


Figure 4.3. Quantum yield of photosystem II (Φ_{PSII}) (A, B) and non-photochemical quenching (NPQ) (C, D) of chlorophyll fluorescence as affected by adding far-red light to different intensities of red/blue (A, C) or warm-white light (B, D). Data points in A and C (red/blue) represent means from 5 replicates; data points in B and D (warm-white) represent means from 3 replicates. Within each *PPFD* level, ***, **, and * (or $^{+++}$, $^{++}$, and $^{+}$) indicate significant differences between light without added far-red and the immediate (or stabilized) response to the addition of far-red light at $P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively.

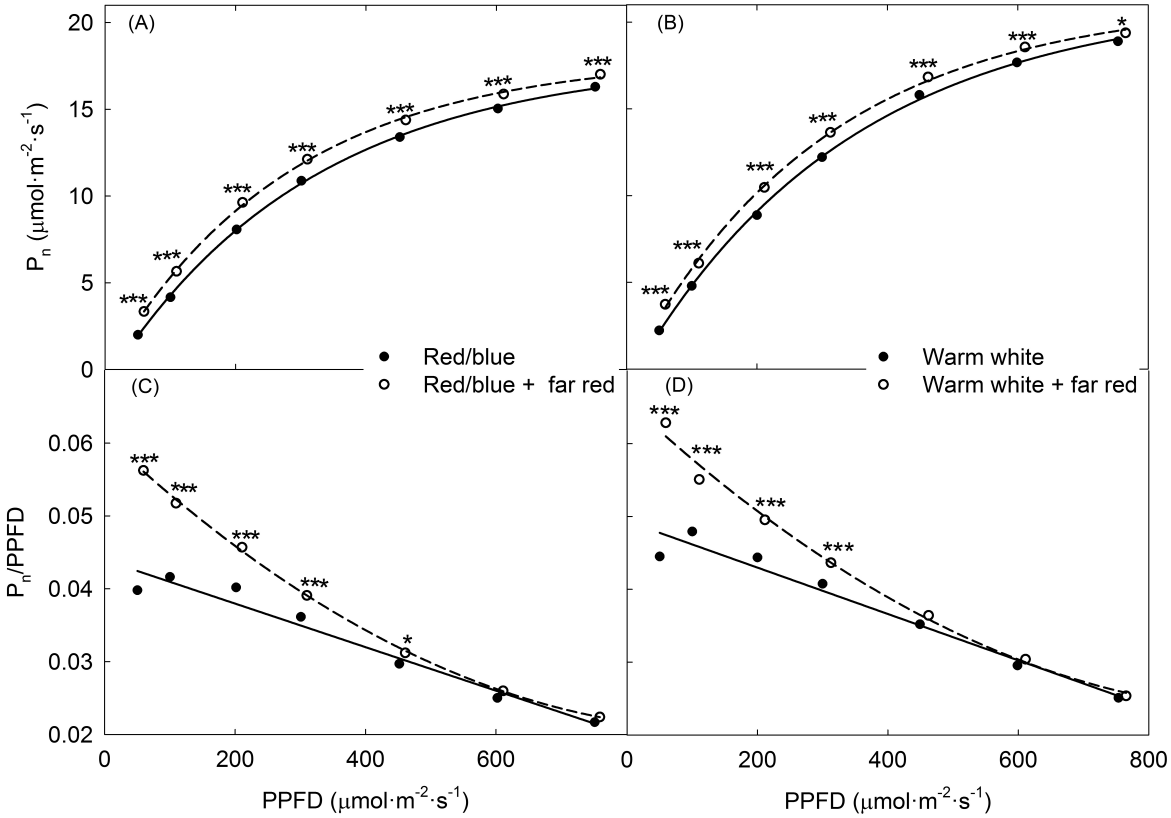


Figure 4.4. The effect of adding far-red light to different intensities of red/blue or warm-white light on net photosynthetic rate (Pn) (A, B) and Pn/*PPFD* (C, D). Data points in A and C (red/blue) represent means from 5 replicates; data points in B and D (warm-white) represent means from 3 replicates. Within each *PPFD* level, *** and * indicate significance at $P < 0.001$ and $P < 0.05$.

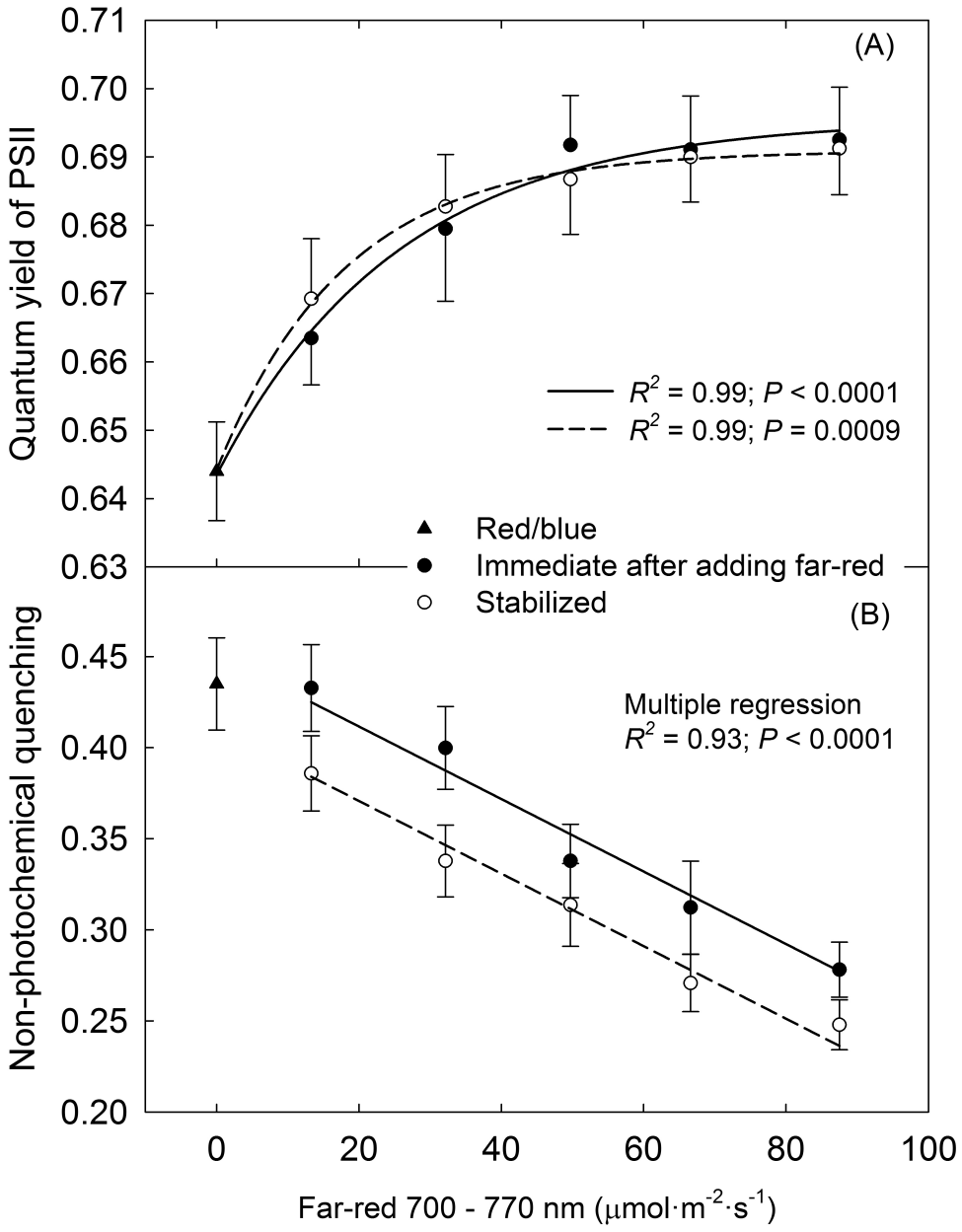


Figure 4.5. Quantum yield of photosystem II (Φ_{PSII}) (A) and non-photochemical quenching (NPQ) (B) of chlorophyll fluorescence as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1}). Data points represent means from 4 replicates with error bars representing standard error.

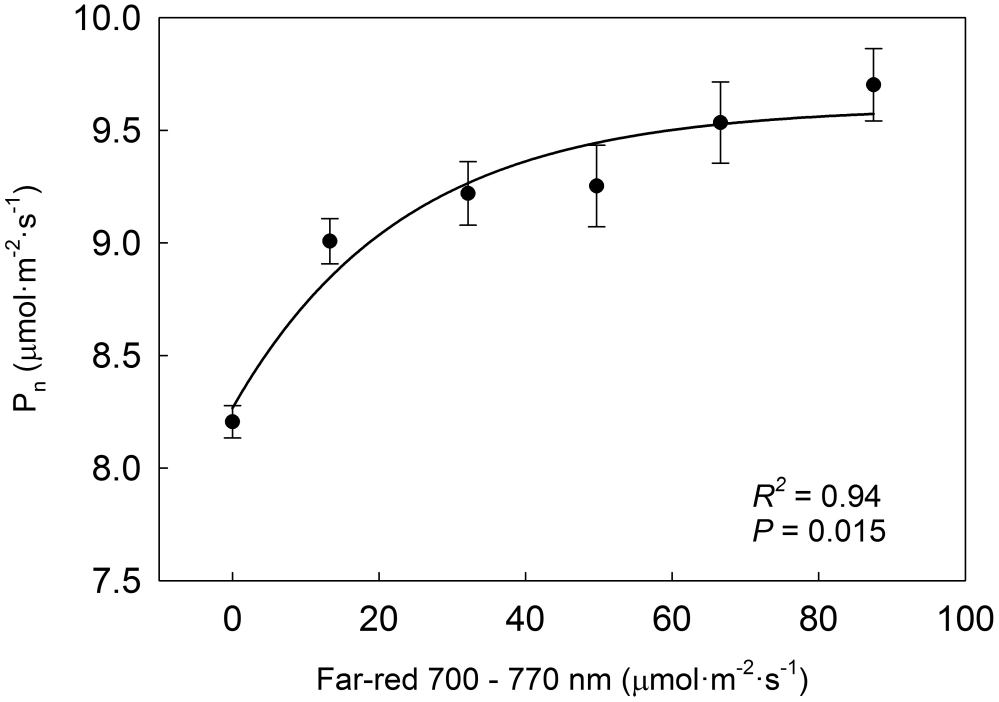


Figure 4.6. Net photosynthetic rate (P_n) as a function of the intensities of far-red light added to red/blue light (*PPFD* of 200 μmol m^{-2} s^{-1}). Data points represent means from 4 replicates with error bars representing standard error.

CHAPTER 5

IDENTIFYING THE SPECTRAL RANGE OF FAR-RED LIGHT THAT ENHANCES PHOTOCHEMISTRY OF LETTUCE GROWN UNDER DIFFERENT SPECTRA[3]

[3]Zhen, S., M. Haidekker, and M.W. van Iersel. Submitted to Photosynthesis Research.

Abstract

Far-red light preferentially excites photosystem I (PSI) and can enhance the efficiency of photosynthesis under light that over-excites photosystem II (PSII). In this work, we determined which wavelengths of far-red light increase photochemical efficiency of lettuce. We also quantified the interactive effect of acclimation to different light spectra (red/blue light or sunlight) and the spectrum of measuring light (red/blue or halogen light) on photochemistry. Lettuce grown under red/blue light had higher quantum yield of PSII (Φ_{PSII}) than those grown under sunlight; plants also had higher Φ_{PSII} under halogen light than under red/blue light (which over-excites PSII), likely because halogen light contains a large amount of far-red. The efficiency of different far-red wavelengths at exciting PSI was quantified by measuring Φ_{PSII} of lettuce under red/blue light with narrowband far-red light added (from 678 to 752 nm, with gaps between 704-720 and 732-751nm). Φ_{PSII} of lettuce increased linearly with increasing wavelengths (from 678 to 692 nm) of added light, and then had slower increase as wavelength further increased to 703 nm, suggesting that these longer wavelengths were increasingly used more efficiently by PSI than by PSII. Adding light at 721 nm resulted in similar Φ_{PSII} as that with 703 nm light added, but Φ_{PSII} tended to decrease as wavelength increased from 721 to 731 nm, probably due to lower leaf light absorption at longer wavelengths. Adding 752 nm light did not affect Φ_{PSII}, suggesting that photons at this wavelength no longer excite PSI.

Keywords: Emerson enhancement effect · Excitation energy distribution · Laser diodes · Photosystems · Quantum yield of PSII

Introduction

The efficiency of photosynthesis is wavelength dependent: when measured under monochromatic light, the quantum yield for CO_2 fixation per absorbed photon is higher under red light (about 600-680 nm) than that under blue and green light (Evans 1987; Inada 1976; McCree 1972a). Photosynthetic efficiency also declines sharply at wavelengths above 685 nm, first described as the 'red drop' by Emerson and Lewis (1943). Absorption by photosynthetic carotenoids (where excitation energy is transferred to chlorophylls with an efficiency significantly less than 100%) and non-photosynthetic flavonoids and carotenoids partly account for the lower quantum yield of blue and green light (Hogewoning et al. 2012; Hoover 1937; Terashima et al. 2009). Besides that, a major cause for the wavelength dependency of photosynthetic efficiency is the imbalanced excitation of the two photosystems – photosystem I (PSI) and photosystem II (PSII), that carry out the photochemical reactions of photosynthesis (Evans 1987; Hogewoning et al. 2012). Due to differences in pigment composition, antenna size, and density of PSI and PSII, excitation energy distribution between the two photosystems is often imbalanced, especially under narrow spectrum light (Hogewoning et al. 2012; Laisk et al. 2014).

As the two photosystems operate in series to drive linear electron transport from H_2O to the terminal electron acceptors (usually $NADP^+$), excitation of PSI and PSII should be approximately equal to achieve optimal efficiency of photochemistry (Allen 2003; Butler 1978). The efficiency of photochemistry is often measured as the quantum yield of PSII (Φ_{PSII}) – the fraction of the absorbed light that is used for photochemistry to drive electron transport (Genty et al. 1989; Maxwell and Johnson 2000). Under light that over-excites one photosystem relative to the other, the rate of photochemical reactions is limited by the activity of the under-excited

photosystem. Photosynthetic responses (e.g., photochemical efficiency and photosynthetic rate) are thus expected to differ under measuring light spectra that excite the photosystems differently (Murakami et al. 2017; Walters 2005). For instance, both Φ_{PSII} and net photosynthetic rate (A_n) of lettuce measured under red/blue light emitting diode (LED) light, which under-excites PSI (Evans 1987; Hogewoning et al. 2012), increases when far-red light is added to the red/blue measuring light (Zhen and van Iersel 2017). This increase in Φ_{PSII} after adding far-red light differs from the typical decrease in Φ_{PSII} with increasing light intensity (Baker 2008) and is thought to be caused by preferential excitation of PSI by far-red light (Evans 1987; Hogewoning et al. 2012). This helps to rebalance the excitation between the two photosystems.

The excitation balance between the two photosystems is also affected by acclimation to different light spectra. Plants possess several mechanisms that allow them to dynamically adjust their photosystem composition to optimize photosynthetic efficiency under different light environments. Short-term responses (taking place within minutes) to imbalanced excitation of the two photosystems include the re-allocation of a mobile pool of light harvesting complex II (LHCII) to the under-excited photosystem, a process termed state transition that helps to direct more energy to the under-excited photosystem (Allen 1992, 2003; Haldrup et al. 2001). Adjustments in photosystem stoichiometry, for example an increase in the PSI/PSII ratio of plants grown under light that over-excites PSII, can take place over days to correct unbalanced excitation of the two photosystems and increase the photosynthetic efficiency (Chow et al. 1990; Fujita 1997; Hogewoning et al. 2012). A decrease in the amount of LHCII per PSII core has also been reported in plants grown under blue light (which over-excites PSII), decreasing the amount of excitation energy partitioned to PSII (Hogewoning et al. 2012). Those changes in photosystem composition as a result of acclimation to the light environment may affect how efficiently plants

use light of different spectra for photosynthesis, as well as the interactive effects among different wavelengths.

Although the enhancement of photosynthetic rate when combining longer wavelength far-red light ($\lambda > 680$ nm) and shorter wavelengths was first shown by Emerson and coworkers over half a century ago (Emerson et al. 1957; Emerson and Rabinowitch 1960), this synergistic effect of different wavelengths on photosynthesis has received little attention, partly due to the influential conclusion by McCree (1972b) that enhancement effects are insignificant in white light. The focus of later studies on the photosynthetic responses to light has been largely on quantifying the wavelength dependency of photosynthesis and obtaining more accurate quantum yield spectrum for CO_2 fixation or for electron transport at each photosystem, where the effects of different wavelengths are often perceived as independent and additive (Evans 1987; Hogewoning et al. 2012; Inada 1976; Laisk et al. 2014; McCree 1972a).

Recently, Hogewoning et al. (2012) observed that the quantum yield for CO_2 fixation of cucumber under broad spectral light (wavelength range of 400-725 nm) is higher than the weighted sum of the quantum yield determined at 19 wavelengths across the same spectral range, suggesting that photosynthesis is enhanced when combining different wavelengths, i.e., different wavelengths have interactive effects on photosynthetic efficiency. Our previous study revisiting the effect of far-red light on photosynthesis indicates that far-red LED light enhances both the Φ_{PSII} and A_n of lettuce when added to red/blue or warm-white LED light, which both over-excite PSII (Zhen and van Iersel 2017). Therefore, attention should be paid to the interactive effects among wavelengths, as different wavelengths do not act independently, and their effects on photosynthesis are not simply additive. The quantum yield spectrum of photosynthesis may underestimate the photosynthetic efficiency of wavelengths that over-excite one of the

photosystems, as synergistic effects on photosynthesis occur when combining these wavelengths with wavelengths that over-excite the other photosystem. Because most of the shorter wavelengths within the 400-680 nm range over-excite PSII, while longer wavelength far-red light ($\lambda > 680$ nm) tends to over-excite PSI (Evans 1987; Hogewoning et al. 2012; Laisk et al. 2014), the synergistic effect between far-red light and shorter wavelengths appears to be important for enhancement of photosynthesis. However, it is not clear which wavelengths of far-red light enhance photochemistry (Φ_{PSII}) and photosynthesis (A_n). The far-red LED light used for examining the enhancement effect in Zhen and van Iersel (2017) had a broad peak (peak at 735 nm; full width at half maximum (FWHM) of 35 nm). The relative excitation energy distribution between the two photosystems in the longer wavelength region ($\lambda > 680$ nm) so far has only been quantified at a few wavelengths with relatively poor spectral resolution (FWHM>10 nm) (Evans 1987; Hogewoning et al. 2012; Laisk et al. 2014). Therefore, we used laser diodes to obtain narrow-band far-red light (FWHM of 2-3 nm) to 1) identify the spectral range of far-red light that enhances photochemistry; 2) determine the relative efficiency of different wavelengths and intensities of far-red at enhancing photochemistry; and 3) determine if acclimation to different light spectra affects the photosynthetic responses measured under different light spectra.

Materials and Methods

Plant material and growth conditions

Lettuce (*Lactuca sativa* 'Green Towers') was seeded in 1.7 L containers filled with a soilless substrate (Fafard 2P; 60% peat and 40% perlite; Sun Gro Horticulture, Agawam, MA, USA) with controlled-release fertilizer (16N-2.6P-9.9K; Harrell's 16-6-12, Lakeland, FL, USA) incorporated at a rate of 4.7 g L^{-1}. Seedlings were grown in a growth chamber (E15; Conviron,

Winnipeg, MB, Canada) under red/blue LED light (12 h photoperiod at a photosynthetic photon flux density (*PPFD*) of 202 ± 36 μmol m^{-2} s^{-1}) or in a glass-covered greenhouse with no supplemental light. Plants in the growth chamber were rotated every 2 days throughout the growing period. Four groups of lettuce were seeded every 2 to 3 weeks between 16 December 2016 and 18 February 2017. Measurements of red/blue- and sunlight-grown lettuce were made on plants from the same age groups that were 34 to 43 days old.

The spectral distributions of the different light sources were measured using a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, USA) and normalized to their respective peaks (Fig. 1). The blue (400-500 nm):green (501-600 nm):red (601-700 nm):far-red (701-800 nm) ratio (B:G:R:FR ratio) of the red/blue LED light (peak wavelengths at 634 and 454 nm) was 30:1.8:68.2:0 (each component expressed as % of total *PPFD*). The B:G:R:FR ratio of sunlight inside the greenhouse (measured around solar noon) was 28.9:35.4:35.7:33. Note that sunlight has a large amount of far-red light.

Leaf absorption, reflectance, and transmission spectra

Leaf reflectance was measured using a spectrometer (UniSpec; PP Systems, Amesbury, MA, USA) equipped with a leaf clip, which measures the spectrum in ~3 nm intervals (3 wavelengths every 10 nm). Reflectance was calculated in 1 nm steps by interpolating the measured spectrum using reverse distance weighting. To determine leaf transmission, a spectroradiometer (SS-110; Apogee Instruments) was placed under a halogen light, and measurements were made with and without a leaf in the light path. Leaf absorption was calculated as 1 - leaf reflectance - transmission. Percent leaf light absorption under different light

sources was calculated by multiplying the leaf absorption spectrum by the spectral distribution of the light sources, and then integrated over a certain wavelength range, e.g., 400-700 nm.

Specific leaf area and pigment analysis

Specific leaf area (SLA, leaf area/dry mass) and pigment composition were determined using lettuce plants that received an average daily light integral (DLI, total *PPFD* integrated each day) of 8.7 and 9.9 mol m^{-2} d^{-1} under red/blue light and sunlight, respectively. Leaf area of three mature leaves per plant (six plants per growth light type, i.e., sunlight or red/blue LED light) was determined. Leaves were then oven-dried and weighed.

For pigment analysis, one leaf disk per leaf (3.14 cm^2) was randomly cut using a cork borer. Leaf margins and main veins were avoided. Leaf tissues were ground with pure methanol and centrifuged. The absorbance of the extract at 666, 653, and 470 nm was measured using a spectrophotometer (Spectronic 20 Genesys; Spectronic Instruments, Rochester, NY, USA). Chlorophyll (Chl) and total carotenoid concentrations were calculated using the equations from Wellburn (1994). Three leaf discs per plant (subsamples) were sampled, and pigment concentrations were averaged for each plant (three plants per growth light type).

Light acclimation and measuring light effects on light responses of Φ_{PSII} and NPQ

Light responses of Φ_{PSII} and non-photochemical quenching (NPQ) of lettuce grown under both sunlight and red/blue light were determined by subjecting plants to increasing *PPFD* (0 to 500 μmol m^{-2} s^{-1}) of red/blue light (same as described above) or halogen light (B:G:R:FR ratio of 7:27.6:65.4:89.2; Fig. 1). Halogen light was used as it contains a large amount of far-red light. The responses of Φ_{PSII} and NPQ to increasing halogen light were compared to those under the

red/blue measuring light, which contains no far-red light. Plants were dark-adapted overnight prior to data collection. Chlorophyll fluorescence measurements were made on recently matured leaves using a pulse-amplitude modulated fluorometer (Mini-PAM; Heinz Walz, Effeltrich, Germany). Minimum and maximum fluorescence yield of dark-adapted leaves (F_0 and F_m, respectively) were determined to calculate the maximum Φ_{PSII} with all the reaction centers being 'open' (Q_A, the primary electron acceptor of PSII, maximally oxidized) (Baker 2008). Then, steady-state and maximum fluorescence yield (F_t and F_m', respectively) at each *PPFD* level (25 to 500 μmol m^{-2} s^{-1} of red/blue or halogen light) were determined after plants were given 20 min to stabilize under that light level. Φ_{PSII} of light-adapted leaves was calculated as $\Phi_{PSII} = (F_m' - F_t)/F_m'$ (Genty et al. 1989), and NPQ was calculated as $NPQ = (F_m - F_m')/ F_m'$ (Maxwell and Johnson 2000). This entire procedure was replicated three times for both red/blue- and sunlight-grown lettuce. Lettuce used for these measurements received an average DLI of 8.7 mol m^{-2} d^{-1} under red/blue light and 8.1 mol m^{-2} d^{-1} under sunlight.

Narrow-spectrum far-red light

Laser diodes were used to provide narrow-spectrum far-red light with FWHM of 2-3 nm. Those narrow-spectrum far-red lights were used to determine the responses of Φ_{PSII} to different wavelengths and intensities of far-red light as described in the sections below. The laser diodes are temperature-tunable, i.e., the peak wavelength shifts toward longer wavelength with increasing temperature. A custom-built temperature controller with a Peltier thermoelectric cooler module was used to control the temperature of the laser within 10 to 60 °C, allowing for a ~10 nm wavelength tuning of each laser diode. The peak wavelengths at different temperatures were determined using a spectroradiometer (SS-110; Apogee Instruments). Four laser diodes

(HL6750MG, HL6738MG, HL7001MG, HL7302MG; Thorlabs, Newton, NJ, USA) were used to provide 18 narrow-bands within the 678-703 nm and 721-731 nm ranges. One additional laser diode (LT031MD; Meredith Instruments, Peoria, AZ, USA) was used to provide a single band centered at 752 nm. Note that the wavelength gaps (704-720 and 732-751 nm) were due to the unavailability of laser diodes emitting at these wavelengths. Wavelengths longer than 752 nm were not included, as our preliminary data indicated that they were ineffective in enhancing photochemistry. For simplicity, we refer to all wavelengths provided by the laser diodes as far-red light, including those < 700 nm.

Far-red light was projected from above onto the leaf through a diffuser, so that a circular leaf area with a diameter of 4 cm or greater was uniformly lighted. The incident far-red light intensity was measured using a silicon power sensor (S140A; Thorlabs) connected to a power meter (PM100; Thorlabs). The power output was converted to photon flux density (PFD) based on the amount of energy contained per mole of photons at different wavelengths and the area of the power sensor sensing area. The incident light intensity at different temperatures (i.e., different peak wavelengths) was adjusted using a potentiometer, which controlled the current to the laser diode, to obtain a PFD of 40 μmol m^{-2} s^{-1}. The position of the power sensor was noted, and subsequent Chl fluorescence measurements were made on leaves placed at the same spot as where the light intensity was measured.

Response of Φ_{PSII} to different wavelengths of far-red light

Response of Φ_{PSII} to different wavelengths of far-red light within 678-752 nm was quantified to identify the spectral range of far-red light that enhanced photochemistry. Plants were irradiated with a *PPFD* of 200 μmol m^{-2} s^{-1} red/blue light for about an hour before

stabilized Φ_{PSII} was determined. Then, far-red light of 40 μmol m^{-2} s^{-1} was added. Φ_{PSII} was measured shortly (12 s) after the addition, and far-red light was then turned off. After 10-15 min, far-red light of a different peak wavelength was added, and Φ_{PSII} was determined in the same fashion until all the wavelengths of far-red from each laser diode were added. After that, a different laser diode was calibrated, and Φ_{PSII} measurements were made in a similar manner as described above. Φ_{PSII} under 240 μmol m^{-2} s^{-1} red/blue light (i.e., red/blue light intensity increased from 200 μmol m^{-2} s^{-1} by 40 μmol m^{-2} s^{-1}) was also determined. This entire procedure was replicated four times for both red/blue- and sunlight-grown lettuce.

Note that a short time period of far-red exposure (12 s) was used because it allowed for the effects of far-red light on photochemistry to be quantified without altering non-photochemical processes, e.g., state transitions or xanthophyll cycle activity, that could be affected by far-red light on longer time scales (occur over minutes) (Allen 2003; Haldrup et al. 2001; Zhen and van Iersel 2017).

Sunlight-grown lettuce used for these far-red spectral response measurements received an average DLI of 9.9 mol m^{-2} d^{-1}, compared to that of 8.7 mol m^{-2} d^{-1} received by red/blue light-grown lettuce.

Response of Φ_{PSII} to increasing intensity of far-red light

Two wavelengths of far-red light, peaks at 700 or 723 nm (FWHM 2-3 nm), respectively, were used to investigate how Φ_{PSII} responded to increasing intensity of far-red light. Those two wavelengths were selected due to their contrasting absorption by the leaf (absorption at 723 nm was only about half of that at 700 nm). Ten intensities of far-red light (0-100 μmol m^{-2} s^{-1}) were added to red/blue light of 200 μmol m^{-2} s^{-1}. Prior to the measurements, plants were allowed to

acclimate to red/blue light for at least 40 min to reach a stabilized Φ_{PSII}. Then, far-red light of a given intensity was added for 12 s before Φ_{PSII} was determined, and far-red was then switched off. Each determination of Φ_{PSII} at a different far-red light intensity was about 40 min apart to allow for adjustment of far-red light intensity, and Φ_{PSII} under red/blue light was re-measured 10 min before each addition of far-red light to adjust for small variations in Φ_{PSII}, as Chl fluorescence were measured on slightly different parts of the same leaf. This entire procedure was replicated four times for both red/blue- and sunlight-grown lettuce, which received an average DLI of 8.7 and 17.4 mol m^{-2} d^{-1}, respectively. Leaf light absorption at the two wavelengths was measured on the same leaves after measurements of Φ_{PSII} with the addition of different intensities of far-red light were completed.

Results and Discussion

Morphology, pigment composition, and light absorption

Plants grown under red/blue light had substantially (54%) smaller leaves with lower SLA, i.e., thicker leaves, than plants grown under sunlight (SLA of 302 vs. 352 cm^2 g^{-1}; P = 0.0017). A lower SLA, or thickening of leaves, can result from a number of factors, including acclimation to high light (Björkman 1981, Evans and Poorter 2001, Givnish 1988). However, lettuce grown under red/blue light received less light than those grown under sunlight (average DLI of 8.7 vs. 9.9 mol m^{-2} d^{-1}). Acclimation to different spectra can also affect SLA, e.g., Hogewoning et al. (2010) observed a decrease in SLA in cucumber with increasing percentage of blue light in the range of 0-50%. Nonetheless, the proportion of blue light was approximately equal in red/blue light and sunlight (30.0 vs. 28.9% of total *PPFD*). A decrease in SLA has also been reported to occur in response to acclimation to high red:far-red light environment (McLaren

and Smith 1978, Shibuya et al. 2016). Sunlight contained a substantial amount of far-red light, while red/blue light had no far-red (Fig. 1). The lack of far-red light (thereby a high red/far-red ratio) may explain the lower SLA observed in lettuce grown under red/blue light.

Chlorophyll (Chl a + b) and total carotenoid concentrations per unit area were 73 and 38% higher, respectively, in red/blue-grown lettuce than in sunlight-grown plants (Fig. 2). This higher pigment concentration in red/blue-grown lettuce was partly attributable to a reduction in SLA (14% lower compared to that of sunlight-grown lettuce), i.e., higher dry mass per unit area. Plants acclimated to growth light that over-excites PSII have been reported to have a higher Chl a/b ratio, which often is associated with an increased PSI/PSII ratio in response to under-excitation of PSI (Chow et al. 1990; Hogewoning et al. 2012). However, the Chl a/b ratio was similar in red/blue- and sunlight-grown lettuce (average of 2.74 vs. 2.75; $P = 0.72$), and was similar to that reported for lettuce by Parry et al. (2014).

With thicker leaves (and thus a longer optical path) and higher pigment concentration, red/blue-grown lettuce absorbed light more efficiently than sunlight-grown plants at all wavelengths in the range of 400-820 nm, particularly in the green (~550 nm) and far-red (~720 nm) regions, where absorption is relatively low (Fig. 3) (see Björkman 1981; Terashima et al. 2009). The light absorption averaged within the 400-700 nm region was 0.93 and 0.90 for lettuce grown under red/blue light and sunlight, respectively. Lettuce grown under red/blue light absorbed 94.6% of red/blue light, while sunlight-grown lettuce absorbed 89.3% of sunlight and 92.2% of the red/blue measuring light (integrated over the 400-700 nm region). Note that the percent absorption of sunlight or red/blue light by lettuce was higher than the commonly assumed 84% absorption of incident light by green leaves, which was derived from the average absorption of 37 C_3 species under a filtered quartz-halogen light source that was rich in red light

(Björkman and Demmig 1987). Leaf light absorption under other light sources is dependent on the spectral distribution of the light. Other factors that determine light absorption, including leaf structure, pigment composition and concentration, often vary among species and are affected by growth conditions. For instance, the lettuce used in our study was fertilized, while the species used by Björkman and Demmig (1987) were mostly from native habitats and local gardens and likely not fertilized, which may result in low chlorophyll concentration. In addition, Björkman and Demmig (1987) determined the leaf absorption spectrum at 25 nm intervals, which could compromise the accuracy of the measurements. Therefore, the commonly assumed 84% absorption of incident light may not be an accurate estimate of light absorption by a specific leaf under a certain light source.

Within 678-752 nm (i.e., the wavelength range of far-red light provided by laser diodes), leaf absorption decreased from ~0.95 to ~0.2 for both red/blue- and sunlight-grown lettuce (Fig. 3). At 700 and 723 nm, the two wavelengths used to determine the response of Φ_{PSII} to increasing intensity of far-red light, absorption was 0.81 and 0.4, respectively, for red/blue-grown lettuce, and 0.73 and 0.32, respectively, for sunlight-grown lettuce.

Light acclimation and measuring light effects on light responses of Φ_{PSII} and NPQ

The light response of Φ_{PSII} was affected by the interaction among the light spectrum during growth (sun vs. red/blue light), the intensity-, and the spectrum of the measuring light (red/blue vs. halogen light) (Fig. 4a, b). Φ_{PSII} of both red/blue- and sunlight-grown lettuce decreased more slowly with increasing *PPFD* of halogen light than with that of red/blue light (Fig. 4a, b). One possible cause for this difference in the responses of Φ_{PSII} to the measuring light is that the excitation energy distribution between PSI and PSII differs under red/blue and halogen

light. Red and blue light tends to overexcites PSII relative to PSI (Evans1987; Hogewoning et al. 2012; Laisk et al. 2014). Halogen light, in contrast, contains a large amount of far-red light (Fig. 1) that preferentially excites PSI (Evans 1987; Hogewoning et al. 2012), likely preventing over-excitation of PSII/under-excitation of PSI. When PSII is over-excited relative to PSI, the plastoquinone (PQ) pool, which is the intermediate electron transporter between PSII and PSI, gradually becomes reduced due to faster influx of electrons from PSII into the PQ pool than they can leave it (Allen 2003). Consequently, a greater fraction of the PSII reaction centers become 'closed' as reduced plastoquinones cannot transfer electrons away from PSII, leading to a decrease in Φ_{PSII}. Far-red light preferentially excites PSI, resulting in faster re-oxidation of the PQ pool (Baker 2008; Bonaventura and Myers 1969). This in turn facilitates electron transport from PSII and helps to re-open the PSII reaction centers, increasing Φ_{PSII} (Zhen and van Iersel 2017).

Compared to sunlight-grown lettuce, lettuce grown under red/blue light had higher Φ_{PSII} under both measuring lights, and the difference was most pronounced when measured under red/blue light at high *PPFD* (Fig. 4a, b). We postulate that plants grown under red/blue light had adjusted their photosystem stoichiometry, i.e., had an increased PSI/PSII ratio compared to sunlight-grown lettuce, to correct for the under-excitation of PSI by red/blue light (see Chow et al. 1990; Fujita 1997; Murakami et al. 2017). This adjustment could allow red/blue-grown lettuce to have more balanced energy distribution between the two photosystems under red/blue light and thus use the light more efficiently than sunlight-grown lettuce.

Non-photochemical quenching (NPQ), an indicator of the amount of absorbed light that is dissipated as heat, of lettuce grown under either sunlight or red/blue LED light was lower under halogen light than that under red/blue measuring light (Fig. 4c, d). This is consistent with

the higher Φ_{PSII} under halogen light (Fig. 4a, b). The NPQ of sunlight-grown lettuce increased particularly rapidly in response to increasing intensity of red/blue light, which is consistent with the rapid decrease in Φ_{PSII} observed in sunlight-grown lettuce under increasing red/blue light (Fig. 4b). A lower Φ_{PSII} increases the need for dissipating excess energy as heat.

The observed differences in the light responses of Φ_{PSII} and NPQ as affected by the interaction of acclimation to light spectrum during growth and the spectrum of the measuring light likely translates into differences in the light response of A_n. Φ_{PSII} correlates with quantum yield of CO_2 assimilation in the absence of photorespiration (Baker 2008; Genty et al. 1989). At a given light intensity, a higher Φ_{PSII} is expected to correspond to a higher rate of photosynthesis (provided that allocation to alternative electron sinks is constant or negligible), as a greater fraction of light is being used in photochemical reactions to drive electron transport rather than being dissipated in non-photochemical pathways as heat or Chl fluorescence. Zhen and van Iersel (2017) showed that an increase in Φ_{PSII}, resulting from a more balanced excitation between the two photosystems upon adding far-red light to red/blue measuring light, corresponded to an increase in A_n of lettuce. The light response of A_n has been shown to depend on the interaction of growth light acclimation and the spectrum of the measuring light (Murakami et al. 2017), which is likely associated with differences in photochemistry efficiency as indicated by our Φ_{PSII} data. Therefore, evaluation of A_n among plants acclimated to different light spectra should account for the effect of the spectrum of the measuring light, as a mismatch between growth and measuring light could result in estimations of A_n that do not reflect the rate of photosynthesis under the light that plants were grown (Murakami et al. 2017; Walters 2005). This has important implications for leaf gas exchange systems, which commonly use a combination of red and blue LED light as measuring light. As red and blue light under-excites PSI (Hogewoning et al. 2012; Laisk et al.

2014), this likely leads to an underestimation of the photosynthetic rate under sunlight, which contains a substantial amount of far-red light. Zhen and van Iersel (2017) found that A_n of sunlight-grown lettuce measured under red/blue LED light increased with the addition of far-red light. We thus recommend that leaf gas exchange systems should include far-red light in the measuring light to improve the accuracy of the photosynthetic measurements.

Response of Φ_{PSII} to different wavelengths of far-red light

Quantum yield of PSII was 0.693 under 200 μmol m^{-2} s^{-1} of red/blue light (Fig. 5). The Φ_{PSII} of both red/blue- and sunlight-grown lettuce decreased upon adding 678 nm light to red/blue light, resulting in a similar Φ_{PSII} as increasing the intensity of red/blue light by the same PFD, i.e., 40 μmol m^{-2} s^{-1} (Fig. 5). This suggests that the excitation energy of light at 678 nm was distributed similarly between the two photosystems as that of red/blue light. Upon increasing light intensity, a decrease in Φ_{PSII} is common as a greater proportion of PSII reaction centers become 'closed' under higher light and thus are unable to use the absorbed light for photochemistry (Baker, 2008; Maxwell and Johnson, 2000). Φ_{PSII} of lettuce grown under both red/blue and sunlight increased linearly as far-red light with longer wavelengths up to 692 nm was added, and then tended to have slower increase as wavelength further increased to 703 nm (Fig. 5), indicating that the relative excitation energy distribution gradually shifted toward PSI as wavelengths increased from 678 nm to 703 nm. This shift in excitation energy distribution towards PSI can alleviate over-excitation of PSII relative to PSI, thus resulting in smaller decrease in Φ_{PSII} compared to that when adding light that more strongly over-excites PSII, e.g., 678 nm. An increase in Φ_{PSII} (thus enhancement of photochemistry) occurs when the added light preferentially excites PSI (Zhen and van Iersel 2017). Such an increase in Φ_{PSII}, compared to that

under a red and blue *PPFD* of 200 μmol m^{-2} s^{-1} without supplemental far-red light, occurred at wavelengths > 686 nm for red/blue-grown lettuce, or > 688 nm for sunlight-grown lettuce (Fig. 5), indicating that these wavelengths over-excited PSI, thus accelerating re-oxidation of the PQ pool, re-opening PSII reaction centers, and increasing Φ_{PSII} as a result (Baker 2008; Bonaventura and Myers 1969; Zhen and van Iersel 2017). The tendency for greater increases in Φ_{PSII} at longer wavelengths (up to 703 nm) suggests that longer wavelengths increasingly over-excited PSI more than PSII, perhaps due to the increasingly lower absorption by PSII than by PSI at longer wavelengths (Hogewoning et al. 2012). It is important to note that leaf light absorption decreased with increasing wavelength, from 0.94 at 686 nm to 0.79 at 703 nm for red/blue-grown lettuce and from 0.92 at 688 nm to 0.68 at 703 nm for sunlight-grown lettuce (Fig. 3a), meaning that on absorbed light basis, longer wavelengths resulted in greater enhancement of photochemistry. This is consistent with our hypothesis that longer wavelengths increasingly over-excited PSI compared to PSII.

Adding light at 721 nm resulted in similar Φ_{PSII} as that with 703 nm light added, and also an increase in Φ_{PSII} compared to that under only 200 μmol m^{-2} s^{-1} of red/blue light (Fig. 5), indicating that wavelength at 721 nm still preferentially excited PSI. Hogewoning et al. (2012) showed that the absorbance of purified PSII was close to zero at wavelengths > 700 nm, whereas purified PSI still had some absorbance at wavelengths > 721 nm. There was a tendency for Φ_{PSII} to decrease as wavelengths increased to 731 nm (Fig. 5), which is likely at least partly due to the drop in absorption in this region (absorption decreased from 0.46 to 0.31 for red/blue-grown lettuce as the wavelength increased from 721 to 731 nm, and from 0.37 to 0.26 for sunlight-grown lettuce; Fig. 3a, b).

The magnitude of increase in Φ_{PSII} in response to the addition of wavelengths that enhanced photochemical efficiency was 32% greater (averaged over the 688-731 nm region) in red/blue-grown lettuce than that in sunlight-grown lettuce (Fig. 5), which could be partly attributable to the higher (by 15%) light absorption in red/blue-grown lettuce within this wavelength region (Fig. 3C).

Due to the unavailability of laser diodes, there were no measurements made within 732-751 nm. The addition of light at 752 nm, on the other hand, did not affect Φ_{PSII} (Fig. 5). Leaf light absorption was low at this wavelength, with absorption of 0.20 and 0.19 for red/blue- and sunlight-grown lettuce, respectively (Fig. 3a, b). It is possible that photons with wavelength of 752 nm do not contain enough energy to drive the photochemical reactions in PSI. Our data also suggest that the upper wavelength limit of light that preferentially drives PSI (and thus enhances photochemistry) falls in the 731-752 nm range.

Response of Φ_{PSII} to increasing intensity of far-red light

The quantum yield of PSII increased asymptotically with increasing intensity of far-red light (700 or 723 nm; Fig. 6), indicating that the excitation balance between the two photosystems was gradually restored when more far-red light was added to red/blue light. Zhen and van Iersel (2017) similarly reported an asymptotic increase of Φ_{PSII} to increasing far-red LED light added to red/blue light. It is thought that far-red light increases Φ_{PSII} when the rate of photochemical reactions is limited by the activity of PSI, but increasing far-red light resulted in no further increases in Φ_{PSII} once PSI is no longer under-excited compared to PSII (Zhen and van Iersel 2017). On an incident light basis and at relatively low far-red PFD (< 50 μmol m^{-2} s^{-1}), 700 nm far-red light was more effective in increasing Φ_{PSII} than 723 nm light (Fig. 6a, b). This is due

to the greater light absorption at 700 nm than that at 723 nm (absorption of 0.81 at 700 nm and 0.4 at 723 nm for red/blue-grown lettuce, and of 0.74 at 700 nm and 0.32 at 723 nm for sunlight-grown lettuce). On an absorbed light basis, both 700 nm and 723 nm light resulted in similar increases in Φ_{PSII} for lettuce grown under each growth light (Fig. 6c, d).

Of the 200 μmol m^{-2} s^{-1} incident red/blue light, a comparable amount of light was absorbed by red/blue- and sunlight-grown lettuce (189 vs. 184 μmol m^{-2} s^{-1} absorbed). However, red/blue-grown lettuce required a notably larger amount of absorbed far-red light before Φ_{PSII} reached a maximum and showed little/no increase with further increases in far-red light intensity; the maximum increase in Φ_{PSII} was also larger in red/blue-grown lettuce compared to that in sunlight-grown lettuce (Fig. 6c, d). One possible explanation for this is that lettuce grown under red/blue light may have more PSI to correct for under-excitation of PSI (Chow et al. 1990; Fujita 1997; Hogewoning et al. 2012), thus requiring a greater amount of far-red light to saturate PSI. In addition, lettuce grown under red/blue light also had thicker leaves with higher pigment concentration, and were likely able to absorb and use more far-red light on a per unit leaf area basis.

Conclusions

Acclimation to different growth light spectra (red/blue light or sunlight) and the spectrum of the measuring light (red/blue or halogen light) interactively affect the light response of Φ_{PSII}, likely via affecting the excitation balance between PSI and PSII. Far-red light is important in such interaction due to its role in exciting PSI, and should be included in the measuring light of leaf gas exchange systems to improve the accuracy of photosynthetic measurements. Far-red wavelengths within the 678-752 nm range differ in their efficiency at exciting PSI and enhancing

photochemistry under light that over-excites PSII. Longer wavelengths within 678-703 nm are increasingly used more efficiently by PSI than by PSII, as indicated by the increasing Φ_{PSII} when light of longer wavelengths was added. Wavelengths within 686 (or 688) to 731 nm enhanced Φ_{PSII} of lettuce grown under red/blue light (or sunlight) when added to red/blue measuring light, but the efficiency of these wavelengths at enhancing Φ_{PSII} tended to decrease as wavelength increased from 721 to 731 nm, probably due to the lower leaf light absorption at longer wavelengths. Wavelength at 752 nm did not affect Φ_{PSII}. Our data indicate that the upper wavelength limit of light that enhances photochemistry falls in between the 731-752 nm range.

Acknowledgments

This study was supported by the Georgia Research Alliance and USDA National Institute of Food and Agriculture, Hatch project 1011550. We thank Drs. Robert Teskey, Anish Malladi, Paul Thomas, and Deepak Mishra for providing feedback on this manuscript. We thank Sue Dove and Michael Martin for technical support.

Literature Cited

Allen JF (1992) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098:275-335

Allen JF (2003) State transitions – a question of balance. Science 299:1530-1532

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89-113

Björkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology I. Encyclopedia of plant physiology, vol 12A. Springer-Verlag, Berlin, pp 57-107

Björkman O, Demmig B (1987) Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77k among vascular plants of diverse origins. Planta 170:489–504

Bonaventura C, Myers J (1969) Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. Biochim Biophys Acta 189:366-383

Butler WL (1978) Energy distribution in the photochemical apparatus of photosynthesis. Ann Rev Plant Physiol 29:345-378

Chow WS, Melis A, Anderson JM (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc Natl Acad Sci 87:7502-7506

Emerson R, Chalmers R, Cederstrand C (1957) Some factors influencing the long-wave limit of photosynthesis. Proc Natl Acad Sci USA 43:133-143

Emerson R, Lewis CM (1943) The dependence of the quantum yield of chlorella photosynthesis on wave length of light. Am J Bot 30:165-178

Emerson R, Rabinowitch E (1960) Red drop and role of auxiliary pigments in photosynthesis. Plant Physio, 35:477-485

Evans JR (1987) The dependence of quantum yield on wavelength and growth irradiance. Aust J Plant Physiol 14:69-79

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767

Fujita Y (1997) A study on the dynamic features of photosystem stoichiometry: Accomplishments and problems for future studies. Photosyn Res 53:83-93

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87-92

Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. Austral J Plant Physiol 15:63-92

Haldrup A, Jensen PE, Lunde C, Scheller HV (2001) Balance of power: a view of the mechanism of photosynthesis state transitions. Trends Plant Sci 6:301-305

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

Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Ieperen W, Croce R, Harbinson J (2012) Photosynthetic quantum yield dynamics: From photosystems to leaves. Plant Cell 24:1921-1935

Hoover WH (1937) The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. Smithsonian Institution Misc Collections 95:1-13

Inada K (1976) Action spectra for photosynthesis in higher plants. Plant Cell Physiol, 17:355-365

Laisk, A, Oja V, Eichelmann H, Dall'Osto L (2014) Action spectra of photosystems II and I and quantum yield of photosynthesis in leaves in State 1. Biochim Biophys Acta 1837:315-325

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51:659-668

McCree KJ (1972a) The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric Meteorol 9:191-216

McCree KJ (1972b) Significance of enhancement for calculation based on the action spectrum for photosynthesis. Plant Physiol 49:704-706

Mclaren JS, Smith H (1978) Phytochrome control of the growth and development of *Rumex obtusifolius* under simulated canopy light environments. Plant Cell Environ 1:61-67

Murakami K, Matsuda R, Fujiwara K (2017) A basis for selecting light spectral distribution for evaluating leaf photosynthetic rates of plants grown under different light spectral distributions. Environ Control Biol 55:1-6

Parry C, Blonquist JM, Bugbee B (2014) *In situ* measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. Plant Cell Environ 37:2508-2520

Shibuya T, Endo R, Kitaya Y, Hayashi S (2016) Growth analysis and photosynthesis measurements of cucumber seedlings grown under light with different red to far-red ratios. HortScience 51:843-846

Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R (2009) Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol 50:684-697

Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56:435-447

Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307-313

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

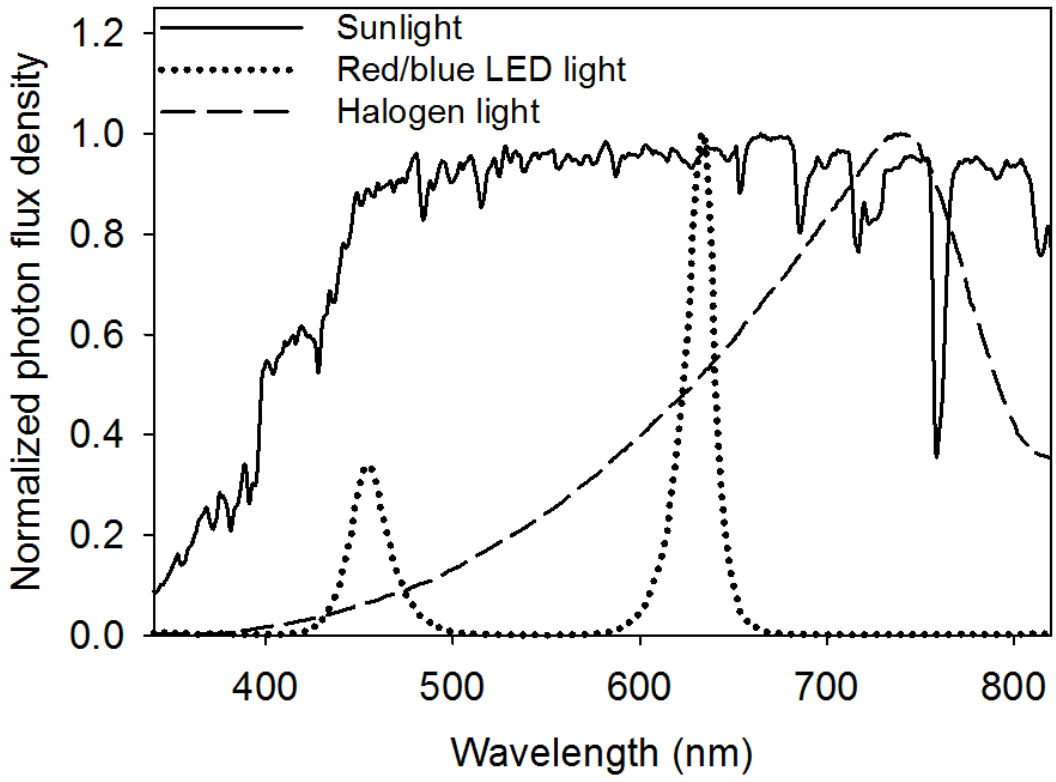


Figure 5.1. Normalized spectral distribution of sunlight, red/blue light-emitting diodes (LED) light, and halogen light

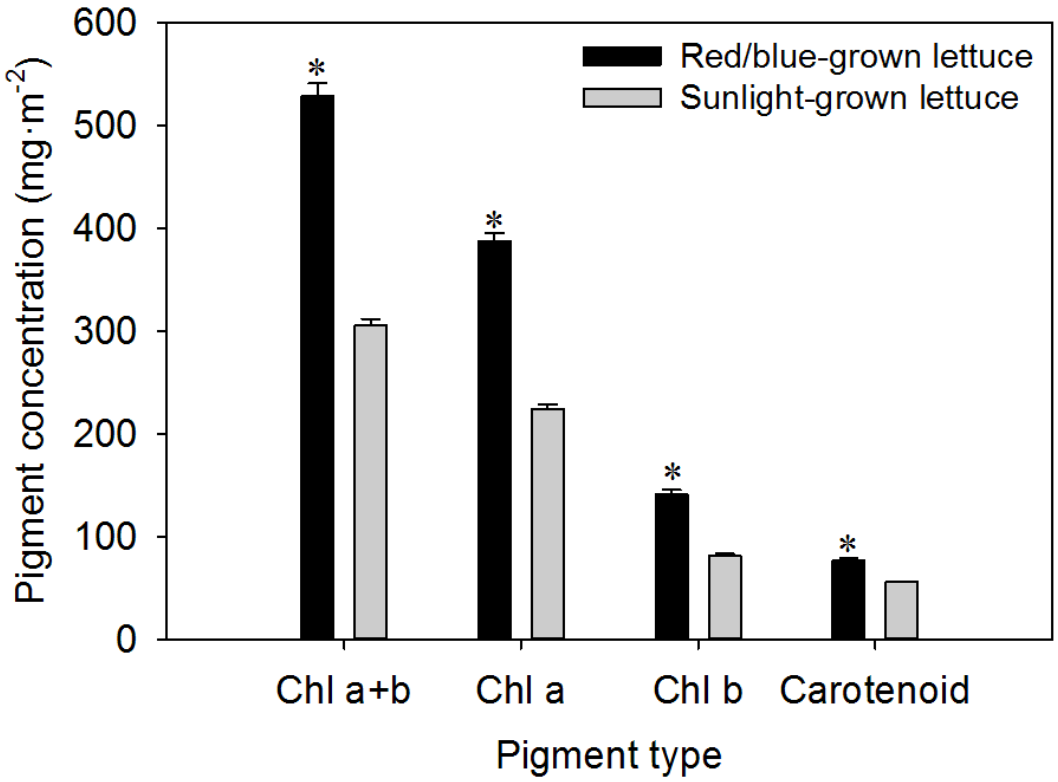


Figure 5.2. Pigment concentration of lettuce grown under red/blue LED light or sunlight. Error bar indicates one standard error (n=3). Within each pigment type, * indicates significance at $P < 0.05$.

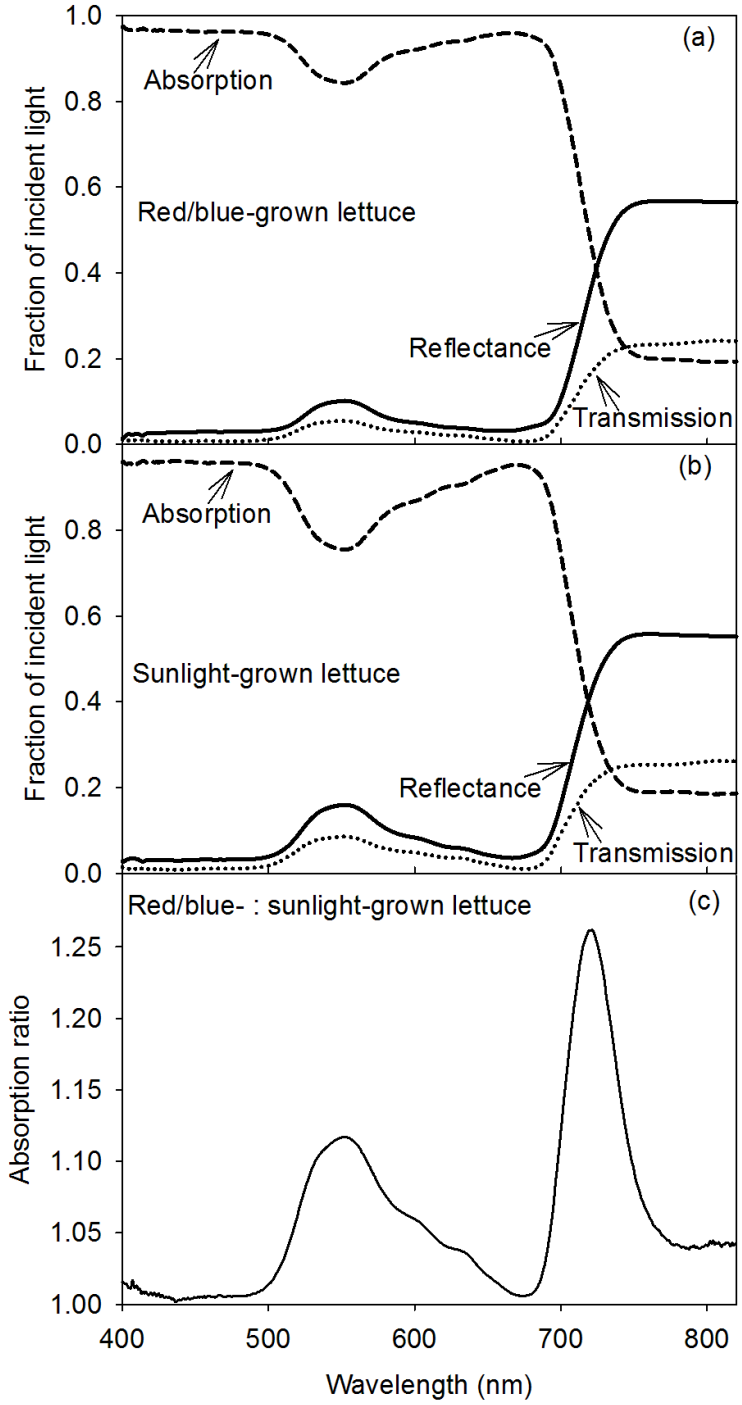


Figure 5.3. Absorption, transmission, and reflectance spectra of red/blue-grown lettuce leaves (a) and sunlight-grown lettuce leaves (b). Absorption ratio is calculated as fraction absorption of red/blue-grown lettuce to that of sunlight-grown lettuce (c)

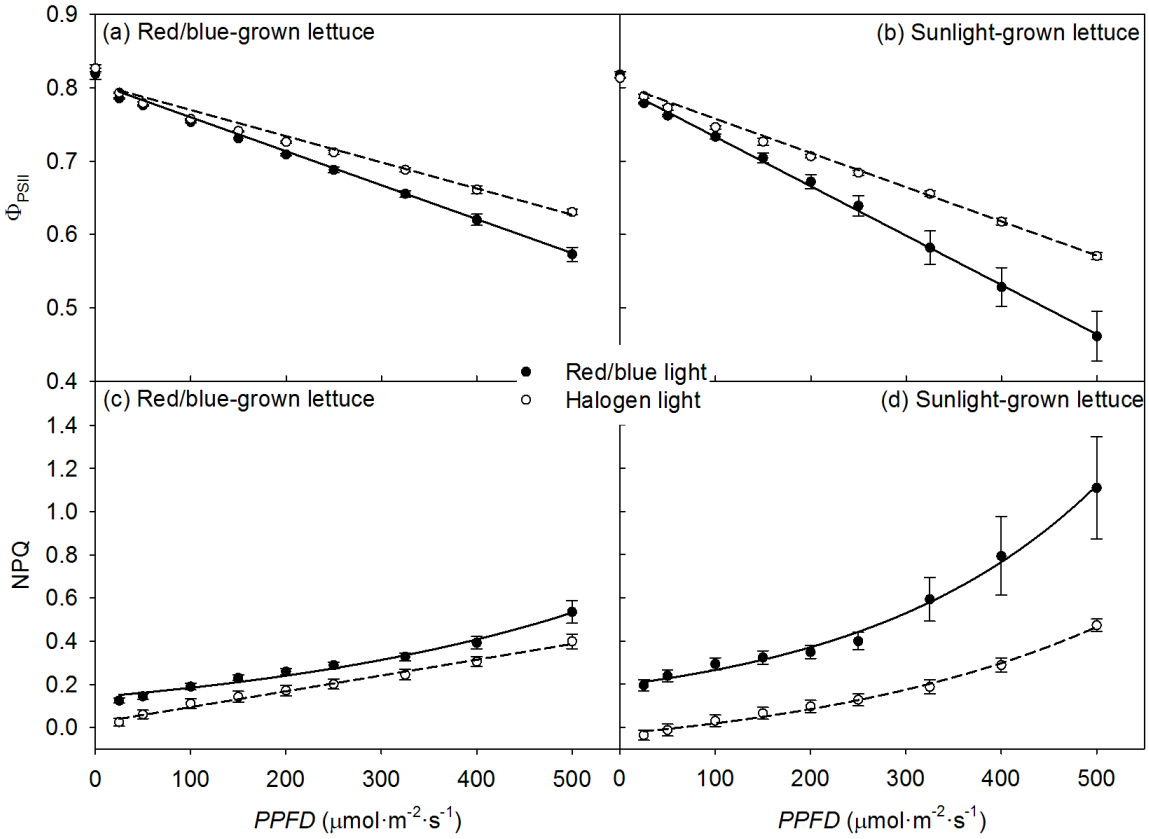


Figure 5.4. Quantum yield of photosystem II (Φ_{PSII}) (a, b) and non-photochemical quenching (NPQ) (c, d) of lettuce grown under red/blue light (a, c) or sunlight (b, d) in response to increasing photosynthetic photon flux density (*PPFD*) of red/blue light or halogen light. Error bars indicate ±SE (n=3)

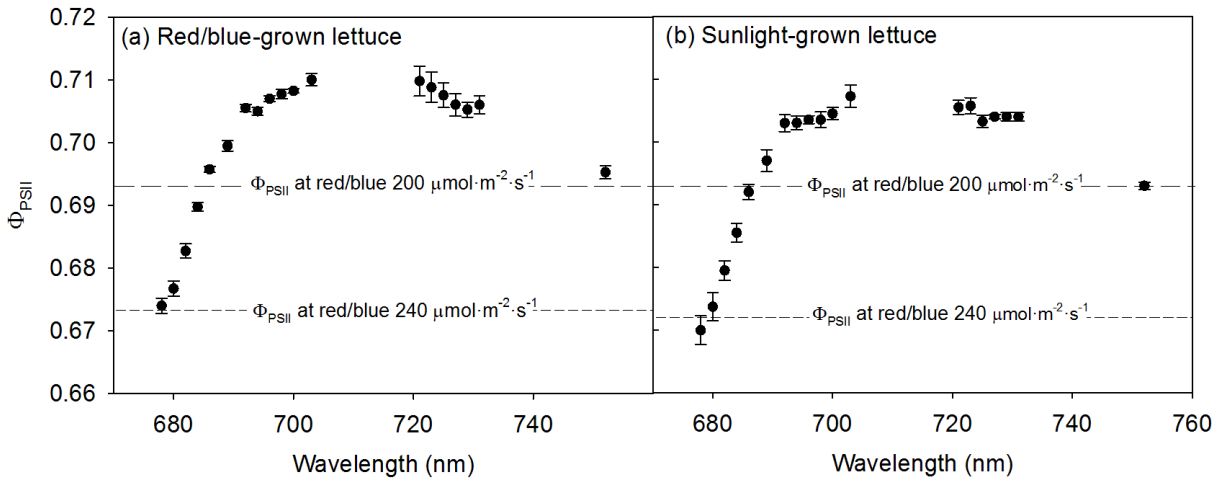


Figure 5.5. Quantum yield of photosystem II (Φ_{PSII}) upon adding different wavelengths of far-red light (FWHM 2-3 nm) to red/blue light for lettuce grown under red/blue light (a) or sunlight (b). Each far-red wavelength was added at a photon flux density of 40 μmol m^{-2} s^{-1}. Long-dashed line (top) indicates the initial Φ_{PSII} under red/blue light of 200 μmol m^{-2} s^{-1}, and the short-dashed line (bottom) indicates Φ_{PSII} under red/blue light of 240 μmol m^{-2} s^{-1}. Error bars indicate ±SE (n=4)

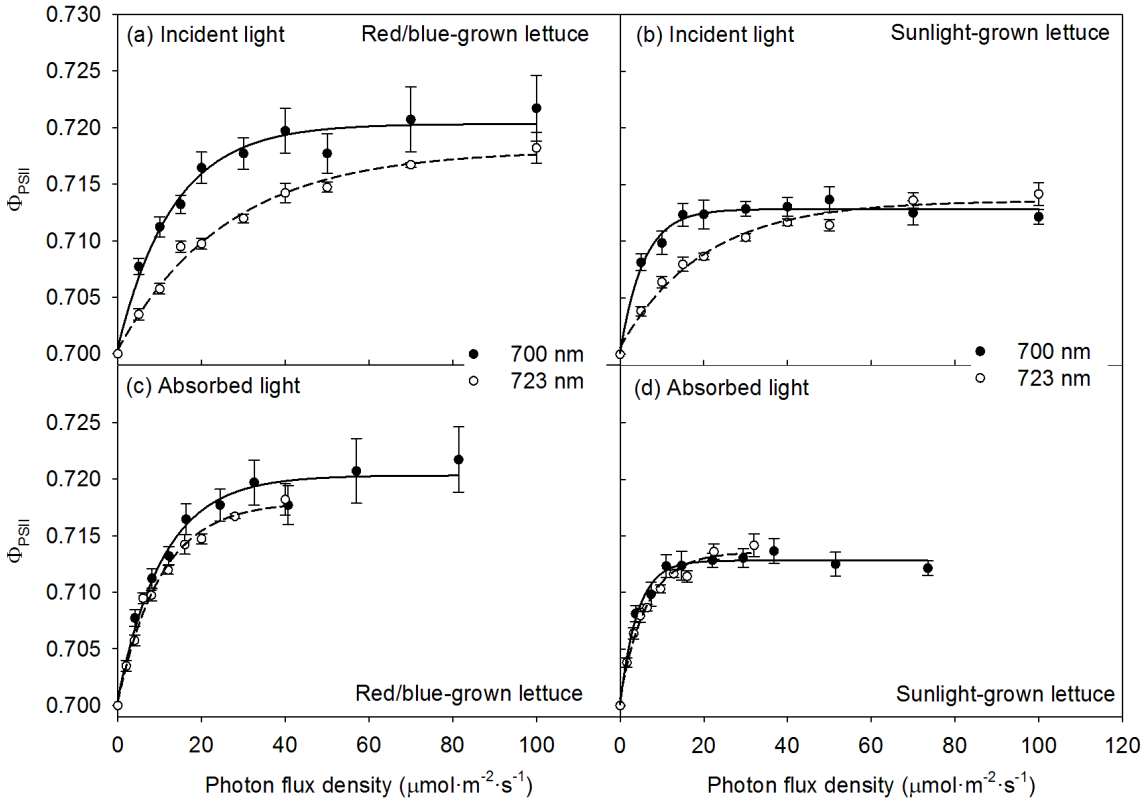


Figure 5.6. Quantum yield of photosystem II (Φ_{PSII}) as a function of the incident photon flux density (PFD) (a, b) and absorbed PFD (c, d) of far-red light with a peak wavelength of 700 or 723 nm, respectively. Lettuce grown under red/blue light (a, c) or sunlight (b, d) and measured under 200 μmol m^{-2} s^{-1} of red/blue light with the addition of different intensities of far-red light. Error bars indicate ±SE (n=4)

CHAPTER 6

CONCLUSIONS

The high cost of photosynthetic lighting in controlled environment agriculture necessitates the need for more efficient use of light. Photosynthetic light use efficiency can be improved by providing plants with the light levels and spectra that are most efficient for photosynthesis. The aim of this research was to improve our understanding of the photosynthetic responses to different light intensities and spectra, and how the responses differ among species/plants acclimated to different light environments.

Our first study quantified the photosynthetic light responses of three horticultural crops as affected by acclimation to different light intensities. Although there was a common trend for photochemical efficiency, determined as the quantum yield of PSII (Φ_{PSII}), to decrease with increasing light intensity, the three species used differed in their efficiency at using light for photochemistry. High light-adapted sweetpotato used light more efficiently for photochemistry to drive electron transport, which often corresponds to higher rate of CO_2 assimilation and growth, than light intermediate lettuce and shade-tolerant pothos, especially at high light levels. Plants acclimated to full sun also tended to have higher Φ_{PSII} than plants acclimated to shade. This indicates that supplemental light is used less efficiently for photochemistry when provided at high ambient PPFD, especially for plants adapted/acclimated to low light. The differences in light use efficiency among species and plants acclimated to different light levels should thus be considered when developing supplemental lighting strategies.

We acclimated plants to different light levels for 4 weeks. The ability of plants to use light was affected by the end of this 4-week period of light acclimation. It remains unclear how long it takes for different plants to acclimate to a light level, and how quickly plants can re-acclimate to a new light level. We also measured the photosynthetic responses on mature leaves that developed during the light acclimation period. It is not clear whether the ability to acclimate differs within a plant canopy, e.g., young vs. old leaves that have previously acclimated to a certain light level, and if the responses differ among species. More thorough examinations of how the photosynthetic responses are progressively affected during light acclimation, and how the responses differ within a plant canopy /among species can provide additional information for improving management strategies for photosynthetic lighting.

Few studies have investigated the feasibility of optimizing photosynthetic lighting in controlled environments based on how efficiently plants use light for photosynthesis. Such lighting control approach requires accurate and easy assessments of plant photosynthetic performance. Chlorophyll fluorescence provides fast, non-invasive measurements of plant light use efficiency, and can be used as a powerful tool in such plant-based lighting control systems. Nonetheless, chlorophyll fluorescence measurements are typically restricted to the single leaf level, and thus may not accurately reflect how efficiently light is being used at the whole plant or canopy level. Emerging techniques, such as remote sensing of chlorophyll fluorescence, may allow for accurate estimation of whole canopy light use efficiency in the near future.

To better understand the optimal spectrum for photosynthesis, the second study re-visited the Emerson enhancement effect on photosynthesis among different wavelengths and re-evaluated the effect of far-red light on photosynthesis. Contrary to the common perception that far-red is ineffective in driving photosynthesis, we found that far-red light increased the

photochemical efficiency and net photosynthetic rate of lettuce when added to red/blue or warm white LED light, which over-excites PSII. The addition of far-red helps to balance the excitation of the two photosystems due to preferential excitation of PSI by far-red light. Our results indicate that different wavelengths interactively affect photosynthetic efficiency, *i.e.*, wavelengths do not act independently, and their effects on photosynthesis are not simply additive.

Currently, the optimization of the photosynthetic light spectrum is primarily based on the action or quantum yield spectra of photosynthesis, which were developed by measuring photosynthesis under narrow wavelengths. Red and blue photons are thought to be more photosynthetically efficient than other photons, such as green, yellow, and far-red, according to the action/quantum yield spectra of photosynthesis. As a result, red and blue light is more widely used in LED grow lights in controlled environment agriculture facilities while other colored lights are often not included, or only included at small amounts. However, the action or quantum yield spectrum of photosynthesis ignores the synergistic effects among wavelengths and thus may not accurately estimate the photosynthetic efficiency of different wavelengths. The synergistic effects on photosynthesis among wavelengths remain under-studied. Additional studies are needed to examine if combining other wavelengths, e.g., green and far-red, interactively affect photosynthetic efficiency, and elucidate the mechanism if a synergistic effect is present. This information can provide more accurate estimates of the photosynthetic efficiency of different wavelengths and improve our understanding of the optimal spectrum for photosynthesis.

In this study, the effect of far-red light on photosynthesis was determined on leaf level during short time period (within hours). Although the physiological mechanism by which far-red enhances photosynthesis should be consistent and repeatable, it is hard to accurately predict the

whole plant responses based on leaf level measurements. Whole-plant gas exchange chambers can be used to measure the photosynthetic responses to far-red light at the plant or canopy level.

Far-red light is also known to affect plant morphology and reproductive physiology, such as promoting stem elongation and leaf expansion and regulating flowering responses. These effects can alter plant light interception, and thus may interact with the far-red photosynthetic responses to affect plant growth. Future research is needed to evaluate the longer-term effects of far-red light on whole plant growth, morphology, and physiology.

The third study quantified the efficiency of different far-red wavelengths at enhancing photochemistry under light that over-excites PSII. Longer wavelengths within the 678-703 nm range are increasingly used more efficiently by PSI than by PSII, indicated by the increase in Φ_{PSII} with increasing wavelengths of added light. Light at 721 nm appeared to be similarly efficient at enhancing Φ_{PSII} as 703 nm light, but longer wavelengths within 721 to 731 nm tended to be less efficient, probably due to the lower leaf light absorption at longer wavelengths. Adding 752 nm light did not affect Φ_{PSII}. Wavelength gaps exist within 704 to 720 nm and 732 to 751 nm due to the unavailability of laser diodes emitting at these wavelengths. Additional measurements with far-red wavelengths that are currently unavailable will provide more information on the efficiency of different far-red wavelengths at enhancing photochemistry and the upper wavelength limit of light that enhances photochemistry.