Light emitting diodes: refining a tool for plant response analyses and improved plant performance

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Abstract

Light emitting diode (LED) technology has been increasingly used in the horticultural industry. In addition to emitting narrow-spectrum light that can target photosynthetic pigments, LEDs have high energy-conversion efficiencies that conventional lighting technologies, such as high pressure sodium (HPS) luminaires, cannot achieve. However, the adoption rate of LED technology is still low. HPS luminaires are still the preferable choice in the industry, and questions surrounding LED performance for plant cultivation (photosynthetic and photomorphogenic responses), reliability in operation, and eye safety have not yet been determined. This dissertation addresses these questions from the perspective of an LED user and the plant, with an emphasis on the spectral quality of photosynthesis, which is the basis of wavelength selection for plant growth in horticultural lighting applications. The first study was an assessment of 12 eye protection devices subjected to LED technology across the visible wavelength spectrum (380–720 nm), and up to 1,000 $W \cdot m^{-2}$ (equivalent to 5,000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Results showed that welding glasses or polarized glasses could limit possible ocular damage from monochromatic light. Sunglasses and safety goggles, however, appear inadequate for protecting one's eyes from infrared LEDs or LEDs emitting wavelengths above 700 nm. Alternation on transmitted spectrum occurred for certain eye protection devices that could lead to potential light hazards and LED users should acquire transmitted spectrum data of their respective eye protection device(s) prior to use. Following this investigation, an apparatus using welding glasses as filtering lenses was designed and developed to examine plant optical and spectral responses up to an irradiance level of 500 $W \cdot m^{-2}$ (equivalent to 2,500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) for the second study. Under these high monochromatic light conditions, lettuce leaves (shade plants) were able to tolerate higher irradiance levels of blue (470 nm) and green (530 nm) LED light than tomato leaves (sun plants). These results differed from previous knowledge of sun and shade plant tolerances under high light conditions. We postulate that this behavior was not previously reported because of multiple wavelength interaction effects that are present in broad-spectrum light sources. As such, we hypothesize that shade plants are not true shade plants; rather, they may be considered as "blue-light-spot plants" and can withstand strong blue light better than sun plants by regulating photosynthetic machinery through blue light. In the third study, an emission 530-nm peak was

observed as using the same apparatus developed in the second study, but under 410-nm light for tomato and lettuce leaves. To our knowledge, this observation has not been reported elsewhere, and it would appear to be a novel second photon re-emission process in plants after fluorescent emission. Data further indicate that green leaf coloration results not only from green light reflection, but also from green light emission from the leaves. Further, we observed that the 530-nm spectrum was not influenced by irradiated time; rather it is impacted by leaf water content. The fourth study is the main focus of this dissertation. It revisits the McCree curve, the standard for spectral quality of photosynthesis and the foundation of plant LED lighting research. Spectral quality data was collected with 1-nm resolution and a narrow light spectrum (10 nm full-width at half maximum) at a light intensity of 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ using tomato and lettuce plants. Both plants had spectral photosynthetic curves with two distinct peaks at 430 nm and 650 nm, and shoulders at 480 nm and 595 nm. Results indicate that there is a reverse correlation between the spectral quality of photosynthesis and the extracted pigment absorbance spectrum. This implies that the current understanding of photosynthetic activity in plants, previously based on extracted pigment data, is not completely accurate. In the fifth study, lettuce growth and its photosynthetic capacity were investigated under a manipulated LED spectrum. Four light spectra were outfitted from existing LEDs using optical filters: a double peak spectrum (595 nm and 655 nm; hereafter referred to as 595-nm light treatment) that excluded 630-nm, 602-nm, 613-nm, and 633-nm light emitted at an irradiance level of 50 $W \cdot m^{-2}$ (243-267 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Shifting and narrowing LED wavelengths from 602 nm to 613 nm and from 633 nm to 613 nm resulted in a biomass yield decrease of ~50 % and ~80 %, respectively. When compared to 595-nm and 602-nm light treatments, the exclusion of 630-nm light resulted in larger leaf areas, expanded plant structures, and the absence of purple coloration. Results suggested that not all the wavelengths in the visible spectrum have positive or neutral impact on plant productivity. Removing certain wavelengths could promote plant growth and beneficially alter plant architecture.

Résumé

La technologie des diodes électroluminescentes (DEL) est de plus en plus utilisée dans l'industrie horticole. En plus d'émettre de la lumière à spectre étroit pouvant cibler les pigments photosynthétiques, les DELs ont des rendements de conversion d'énergie élevés que les technologies d'éclairage classiques, tels que les luminaires au sodium à haute pression (HPS), ne peuvent atteindre. Cependant, le taux d'adoption de la technologie DEL reste faible. Les luminaires HPS restent le choix favoris dans l'industrie et les questions concernant les performances des DELs pour la culture des plantes (réponses photosynthétiques et photomorphogènes), la fiabilité de fonctionnement et la sécurité oculaire n'ont pas encore été déterminées. Cette thèse aborde ces questions du point de vue d'un utilisateur de diodes électroluminescentes et de la plante, en mettant l'accent sur la qualité spectrale de la photosynthèse, qui est la base de la sélection de la longueur d'onde pour la croissance des plantes dans les applications d'éclairage horticole. La première étude consistait à évaluer 12 dispositifs de protection des yeux lors de l'utilisation de la technologie DEL sur le spectre de longueurs d'onde visible (380–720 nm) et jusqu'à 1000 $W \cdot m^{-2}$ (équivalent à 5000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Les résultats ont montrés que des lunettes de soudage ou des lunettes à verres polarisés pouvaient limiter les dommages oculaires possibles dus à la lumière monochromatique. Cependant, les lunettes de soleil et les lunettes de protection sont inadéquates pour protéger les yeux des DELs infrarouges ou émettant des longueurs d'ondes supérieures à 700 nm. Une altération sur le spectre transmis s'est produite pour certains dispositifs de protection oculaire qui pourrait représenter un danger pour les yeux, et les utilisateurs de DEL devraient acquérir les données du spectre transmis de leurs dispositifs de protection oculaire respectifs avant utilisation. À la suite de cette enquête, un appareil utilisant des lunettes de soudage comme lentilles filtrantes a été conçu et développé pour examiner les réponses optiques et spectrales des plantes jusqu'à un niveau d'irradiance de 500 $W \cdot m^{-2}$ (équivalent à 2500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Dans ces conditions d'intense lumière monochromatique, les feuilles de laitue (plantes d'ombre) étaient capables de tolérer des niveaux plus élevés d'irradiance de la lumière DEL bleue (470 nm) et verte (530 nm) que les feuilles de tomate (plantes de soleil). Ces résultats différaient des connaissances existantes sur les tolérances des plantes d'ombre et de soleil sous des conditions de forte luminosité. Nous postulons que ce comportement n'avait pas été signalé auparavant en raison des effets d'interaction multiple des longueurs d'onde présentes dans les sources lumineuses à large spectre. De ce fait, nous émettons l'hypothèse que les plantes d'ombre ne sont pas de vraies plantes d'ombre; plus

précisément, elles pourraient être considérées comme des «plantes détectrices de lumière bleue» qui résistent mieux à la forte lumière bleue que les plantes de soleil en régulant le processus photosynthétique grâce à la lumière bleue. Dans la troisième étude, un pic réfléchi de 530 nm a été observé utilisant le même appareil que celui développé dans la deuxième étude, mais sous lumière inférieure à 410 nm pour les feuilles de tomate et de laitue. À notre connaissance, cette observation n'a pas été rapportée ailleurs et il semblerait qu'il s'agisse d'un deuxième procédé de ré-émission des photons chez les plantes après la ré-émission fluorescente. Les données indiquent en outre que la coloration des feuilles vertes résulte non seulement de la réflexion de la lumière verte, mais également de la lumière verte ré-émise par les feuilles. De plus, nous avons observé que le spectre à 530 nm n'était pas influencé par le temps d'exposition à la lumière; il est plutôt influencé par le contenu en eau des feuilles. La quatrième étude est le sujet principal de cette thèse. Elle ré-examine la courbe de McCree, le standard pour la qualité spectrale de la photosynthèse et le fondement de la recherche sur l'effet de l'éclairage DEL sur les plantes. Les données de qualité spectrale ont été recueillies avec une résolution de 1 nm et un spectre lumineux étroit (10 nm de largeur à mi-hauteur) à une intensité lumineuse de 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ en utilisant des plants de tomate et de laitue. Les deux plantes avaient des courbes photosynthétiques spectrales avec deux pics distincts à 430 nm et 650 nm et des pics secondaires à 480 nm et 595 nm. Les résultats indiquent qu'il existe une corrélation inverse entre la qualité spectrale de la photosynthèse et le spectre d'absorption du pigment extrait. Cela implique que la compréhension actuelle de l'activité photosynthétique chez les plantes, basée précédemment sur les données de pigment extrait, n'est pas complètement exacte. Dans la cinquième étude, la croissance de la laitue et sa capacité photosynthétique ont été étudiées sous un spectre de DEL manipulé. Quatre spectres de lumière ont été créés à partir de DELs en utilisant des filtres optiques: un spectre à double pic (595 nm et 655 nm; dorénavant appelé exposition à la lumière à 595 nm) excluant les spectres à 630 nm, 602 nm, 613 nm et 633 nm lumière émise à un niveau d'irradiance de 50 $W \cdot m^{-2}$ (243-267 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). La variation et le rétrécissement des longueurs d'onde des DELs de 602 nm à 613 nm et de 633 nm à 613 nm ont entraîné une diminution de la biomasse d'environ ~ 50 % et ~ 80 %, respectivement. Par rapport aux expositions à la lumière à 595 nm et à 602 nm, l'exclusion de la lumière à 630 nm a entraîné une plus grande surface foliaire, un élargissement de la structure de la plante et l'absence de coloration pourpre. Les résultats suggèrent que toutes les longueurs d'onde du spectre visible n'ont pas un impact positif ou neutre sur le développement des plantes. L'élimination de certaines

longueurs d'onde pourrait favoriser la croissance des plantes et modifier avantageusement leur architecture.

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Contribution to original knowledge

In this work, earlier plant and LED lighting studies were reviewed and compared while applying current knowledge with respect to plant physiology and LED lighting technology. From a user's perspective, it was important to design a test that evaluated eye safety. From a plant's perspective, plant responses (spectral quality of photosynthesis, optical and spectral properties, as well as photomorphogenic development) were assessed using a wide range of wavelengths and intensities using the same LED assembly systems. Together, this work has contributed new knowledge to this scientific field and to the horticultural industry. Specifically, our understanding of the effect of wavelength on the spectral quality of photosynthesis, the foundation of plant cultivation, and plant-LED research has been expanded in the following manner:

1. This work offers comprehensive and detailed information on the spectral quality of photosynthesis. This will help when improving the design of lighting systems and wavelength selection. It has the potential to enhance plant productivity for plant cultivation facilities using either sole or supplemental lighting.
2. This work provides new insight into photosynthesis in plants with respect to the role of chlorophyll and the oxygen evolving complex (OEC).
3. This work demonstrates that individual wavelengths of light can have positive, negative or neutral effects on plant growth.
4. This work has shown that light directionality potentially influences photosynthetic machinery.
5. This work provides evidence that a shade plant can withstand higher light intensity for particular wavelengths than a sun plant.

Contribution of authors

In accordance with the McGill Guidelines for a Manuscript-Based Thesis, the contributions made by the candidate and the co-authors to the completion of this work are described here.

Bo-Sen Wu is the principal author of this work, supervised by Dr. Mark Lefsrud from the Department of Bioresource Engineering, McGill University, Quebec, Canada.

Dr. Mark Lefsrud, the supervisor and director of the thesis, co-authored all manuscripts, and provided scientific guidance in the planning and execution of the work, as well as co-editing and reviewing manuscripts.

Dr. Valérie Orsat, the co-supervisor of this thesis, co-authored two chapters (2nd and 4th) and made valuable comments to improve the manuscripts.

Journal Papers

1. **Wu, B.-S.** and M. Lefsrud. 2018. Photobiology eye safety for horticultural LED lighting: Transmittance performance of eyewear protection using high-irradiant monochromatic LEDs. *Journal of Occupational and Environmental Hygiene*: 15(2):133-142.
2. Eichhorn Bilodeau, S., **B.-S. Wu**, A.-S. Rufyikiri, S. MacPherson, and M. Lefsrud. 2019. An update on plant photobiology and implications for cannabis production. *Frontiers in Plant Science* 10: 296.
3. **Wu, B.-S.,** V. Orsat, and M. Lefsrud. Re-interpreting the photosynthesis action radiation curve in plants (submitted to *Plant Science*, under review).
4. **Wu, B.-S**., V. Orsat, and M. Lefsrud. Electrical lighting technology in plant photobiology (submitted to *Environmental and Experimental Botany*, under review).
5. **Wu, B.-S**. and M. Lefsrud. Short-term high irradiance exposure to sun and shade plant leaves using narrow-spectrum LEDs (will be submitted to *Journal of Plant Physiology*).

6. **Wu, B.-S.** and M. Lefsrud. Revisiting the questions of why leaves are green and leaves absorb green light: UV-light-induced spectrum peaking at 530 nm. (Pending, due to patent application).
7. Lefsrud, M. and **B.-S. Wu**. The photosynthesis action radiation (PAR) curve of tomato and lettuce plants: 1-nm resolution at 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. (Pending, due to patent application).
8. **Wu, B.-S.** and M. Lefsrud. Manipulating light-emitting diode spectra with optical filters to investigate lettuce growth (Will be submitted to *Photochemical and Photobiological Sciences*).

CHAPTER 1

General introduction

This chapter provides background information and the rationale that lead to the development of this research. The hypothesis and the objectives of this research are stated, and thesis organization may be found at the end of this chapter.

1.1 Background

The basis of the spectral quality of photosynthesis, or the photosynthetically active radiation (PAR) curve, is derived from earlier studies that used different lighting technologies and varied methodologies (Hogewoning et al., 2012b; Hoover, 1937; Inada, 1976; McCree, 1972a). These studies reported that blue and red wavelengths (400–500 nm and 600–700 nm, respectively) were the primary wavelengths within the visible wavelength spectrum (380–720 nm) that drive photosynthesis. However, shifting within red and blue peaks (10–40 nm), in addition to different PAR curve shapes, was observed. Moreover, narrow light spectrum properties have been reported differently, including light measurement units, full width at half maximums (FWHMs) of narrow light spectra, and light intensity levels, leading to inconsistencies within and amongst these studies. These differences have further contributed to discrepancies in spectral quality of photosynthesis data, while making the unification of plotted curves a nearly impossible task. To add to this, the McCree curve that is considered the standard for spectral quality of photosynthesis was constructed under varying and low light intensities with a broad FWHM (25 nm) (McCree, 1972a; van Iersel, 2017). This factor is often neglected and can lead to misinterpretation of results. Detailed information on the photosynthetic rates and light intensities used for each wavelength in McCree's work remains unclear, making the re-interpretation of this pioneering work difficult.

Using the McCree curve, researchers started by examining the effect of monochromatic light on whole plant growth with developments using the latest light technology: light-emitting diode (LED) (Bula et al., 1991; Kim et al., 2005a; Yanagi et al., 1996). Pilot experiments with LEDs established the basal components for the plant-growing light spectrum (between 460 nm and 650 ± 10 nm LED light), resulting in countless studies aimed at determining the optimal plant-growing light spectrum (Brown et al., 1995; Chen et al., 2017; Deram et al., 2014; Fan et al., 2013; Son and Oh, 2013). Supplementing other wavelengths with LEDs, such as 520 nm and 735 nm, increased plant productivity (Kim et al., 2005a), or enhanced photosynthetic machinery (Terashima et al., 2009; Zhen and van Iersel, 2017). With these positive results, the era of plant LED light research began. Despite large efforts made toward determining the optimal plant-growing light spectrum, the exact spectral effects on whole plant growth remain elusive (Mitchell, 2015; Olle and Viršile, 2013; Ouzounis et al., 2015). This uncertainty prohibits the adoption of LED units in the horticultural industry and further development of horticultural LEDs by leading manufacturers. High pressure sodium (HPS) luminaires and metal halides are still the preferable light source for sole or supplemental lighting (Stober et al., 2017).

Regardless of the challenges described above, LEDs represent a promising lighting technology compared to conventional lighting sources for controlled environment plant production (Massa et al., 2008; Morrow, 2008). Advantages include: a controllable light beam, low heat emission, cool emitting surfaces, and multiple wavelength options, which have been reviewed extensively elsewhere (Massa et al., 2015; Morrow, 2008; Yeh and Chung, 2009). Taken together, LEDs are a superior tool for plant spectral response investigations. Nevertheless, an LED is a semiconductor device requiring a higher degree of LED hardware controls to maintain its junction temperature, accompanied by stable spectral properties (Chang et al., 2012; Van Driel and Fan, 2013). Without confirmation and regular measurements of LED spectral properties over the plant growth period, incorrect data interpretation could occur. Furthermore, high heat load and humidity in greenhouses decrease lifespan for LEDs and its drivers (Gu and Narendran, 2004; Schanda et al., 2014; Xi et al., 2005a; Yang and Cai, 2013). Manufacturers also limit

LED wavelength selection, and users are not able to manipulate their spectral properties under normal operation. Other lighting technologies such as optical filters can be used for wavelength manipulation, but they emit low intensity light with limited emitting areas. This prohibits the exploration of specific wavelengths and leaves a large sum of wavelengths in the photosynthetically active radiation (PAR) spectrum untouched.

LED technology has the potential to benefit horticulture in numerous ways, yet uncertainties in reliability and performance for plant productivity must be addressed before mass adoption across the industry. As LEDs are more readily adopted in general lighting applications, it is important to note that LED photobiological eye safety has been reviewed and evaluated for its high intensity. However, these data and guidelines cannot be directly applied to horticulture because of the specific light units used when human vision is considered (International Electrotechnical Commission, 2006; Lau, 2013).

1.2 Statement of research objectives

The main objective of this thesis was to revisit the McCree curve and have a comprehensive understanding of the spectral quality of photosynthesis. Specific objectives are listed as follows:

1. Safety glasses transmittance performance: measure the transmittance performance of different types of eye protection devices under high-irradiant LED light (20 to 1500 $W \cdot m^{-2}$, equals ~100 to 7000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) intended for operators in the horticultural industry.
2. Plant responses under high intensity light: investigate the absorptance, reflection, and transmittance of leaves under high intensity (20 to 1000 $W \cdot m^{-2}$, approx. ~100 to 5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$).
3. Revisit and re-interpret the McCree curve: determine the spectral quality of photosynthesis by determining photosynthetic rates of tomato and lettuce plants using a narrow spectrum with a FWHM of 10 nm and a 1-nm resolution.

4. Optical filters: manipulate LED spectrum compositions (600–640 nm) using optical filters to investigate the effect on lettuce growth, based on the peak or valley wavelength findings in the PAR spectrum from Objective 3.

1.3 Organization of thesis

Chapter 2 encompasses the literature review and brief discussion of topics involved and relating to this research. Chapters 3 to 7 describe the research and experiments that fulfilled each research objective. Between each chapter, connecting texts provide the transition and rationale between each study. Chapter 8 provides a summary of each study, describes the significant contributions to knowledge, and suggests further studies for the research topic. References follow. International System of Units (SI) are used throughout but imperial units were preferred for certain descriptions and measurements, in which case SI units follow in parentheses.

Connecting text

In the literature review, sections have appeared in publications as follows:

Chapter 2.1, "Re-interpreting the photosynthesis action radiation curve in plants", is authored by **Wu, B.-S.,** V. Orsat, and M. Lefsrud and submitted as a manuscript for peer-reviewed publication (*Plant Science*, under review).

Chapter 2.2 was published as part of the following peer-reviewed article:
Eichhorn Bilodeau, S., **B.-S. Wu**, A.-S. Rufyikiri, S. MacPherson, and M. Lefsrud. 2019. An update on plant photobiology and implications for cannabis production. *Frontiers in Plant Science* 10: 296.

Chapter 2.3, "Electrical lighting technology in plant photobiology", is authored by **Wu, B.-S.,** V. Orsat, and M. Lefsrud and submitted as a manuscript for peer-reviewed publication (*Environmental and Experimental Botany*, under review).

CHAPTER 2

Literature review

2.1 Re-interpreting the photosynthesis action radiation curve in plants

The spectral quality of photosynthesis (or photosynthetically active radiation [PAR] curves) has been determined for many crop species under various narrow light spectra (Bulley et al., 1969; Govindjee and Rabinowitch, 1968; Hoover, 1937; Inada, 1976; McCree, 1972a). Data analyzed in these pioneering studies indicated that blue and red wavelengths (400–500 nm and 600–700 nm) are the most efficient for photosynthetic machinery in the visible spectrum (380–720 nm), and our current knowledge of the spectral quality of photosynthesis in higher plants is predominantly based on the McCree curve (McCree, 1972a). Other researchers have replicated McCree's work (Balegh and Biddulph, 1970; Hogewoning et al., 2012b; Inada, 1976); however, shifts of the red and blue peaks and varying PAR curve shapes have since been reported (Balegh and Biddulph, 1970; Bulley et al., 1969; McCree, 1972a; Sager and Giger Jr, 1980).

During this particular period in plant research, early PAR curve measurements were taken under narrow spectrum light with limited lighting technology. These spectra were obtained using either optical filters (Bulley et al., 1969; Hogewoning et al., 2012b; Hoover, 1937) or a monochromator (Balegh and Biddulph, 1970; McCree, 1972a). In both cases, the filtered light had inconsistent full width at half maximum (FWHM) and low intensity levels of narrow light spectrum across the test wavelength range. The FWHM of the narrow spectra obtained using optical filters, for example, ranged from 10–40 nm (Hogewoning et al., 2012b; Hoover, 1937; Inada, 1976). The controllability of peak wavelengths of narrow light spectra was limited by the availability of optical filters, resulting in different measured wavelengths amongst these studies (i.e. different wavelength increments). The use of a monochromator allowed for precise and accurate control of spectral properties that the filtering technology could not achieve. This approach, however, led to significant

intensity loss and/or a limited photon flux area. At this time, light intensity obtained from a monochromator was dependent on the FWHM of the narrow light spectrum: higher light intensities with a broad FWHM (McCree, 1972a) or low light intensity with a narrow FWHM (Balegh and Biddulph, 1970). The light intensity that was used was, in general, less than 150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. These approaches allow for narrow light spectrum while using conventional lighting technology, but there is limited exploration of the spectral quality of photosynthesis with higher light intensities or a consistent wavelength increment.

Besides the differences in light spectrum properties, various experimental designs were developed for these studies, resulting in dissimilarities in methods used to acquire spectral quality data. Typical approaches were completed at a constant light intensity (Bulley et al., 1969; Hoover, 1937; Inada, 1976). This approach provides information on spectral quality of photosynthesis under the same baseline, but it was determined under different units of light measurements, including foot-candles (Hoover, 1937), irradiance (Bulley et al., 1969; Inada, 1976), and photon flux density (Balegh and Biddulph, 1970). As such, different light intensity baselines likely contributed to discrepancies in spectral quality of photosynthesis data. McCree (1972a) employed another approach for spectral quality measurements; a constant photosynthetic rate was obtained by adjusting the irradiance level at different wavelengths. This approach was conducted based on the observation that irradiance did not affect photosynthetic activity, and it was unknown if the relationship between irradiance and photosynthesis activity remained the same at a higher irradiance level since the experiment was conducted under low intensity ($< 30\ W \cdot m^{-2}$). More importantly, irradiance levels used at each wavelength for the McCree curve were not disclosed.

The large diversity of narrow light spectra used in these studies creates a challenge when trying to resolve and unify a plant's true response concerning its spectral quality of photosynthesis. Often, it leads to a misinterpretation of spectral quality data. A comprehensive understanding of spectral quality with high wavelength resolution and narrow FWHM is still a virtually impossible task, since the difficulties in obtaining narrower spectra across the test wavelength range still

remain beyond current technological capacity. The effect of spectral quality on photosynthesis has been reviewed extensively (Bugbee, 2016; van Iersel, 2017). Therefore, the goal of this work was to consider and re-interpret early spectral photosynthetic curve study data with the spectral properties used at the time, and to highlight some neglected aspects in these early studies in an attempt to better understand the plant's true physiological response to different light properties.

Narrow spectrum properties

The spectral properties of narrow light spectra used in early spectral quality determination studies, along with the observed peak wavelengths are listed in Table 1. FWHMs and light intensities used in the earlier studies range between 11–34 nm and are less than 150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, respectively. To correlate a plant's photosynthetic activity under natural light conditions, the light intensity used ideally needs to be greater than ~100–150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ depended on the emitting wavelengths when using narrow spectrum light (Figure 1). For instance, the intensities have to be approximately 100 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and greater than 150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ when using blue and amber LED light, respectively, in order to mimic natural light conditions in these wavelength regimes. However, most of the earlier experiments were conducted at unrealistically low light intensity, as low as 20 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (Balegh and Biddulph, 1970), or very broad spectra with a full bandwidth (up to 100 nm) and potential wavelength interaction effects (Hoover, 1937; Inada, 1976; McCree, 1972a).

Table 1. Full width at half maximum, light intensity, experimental design, and peaks observed in early spectral photosynthetic curve determination studies. Light intensities ($\mu mol \cdot m^{-2} \cdot sec^{-1}$) were converted from reported original units. CL: Constant Light Intensity; CP: Constant Photosynthetic Rate Measurements; FWHM: Full Width at Half Maximum.

Study	FWHM (nm)	Light intensity ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	Approach	Peaks (nm)
Hoover (1937)	20-30	40-60	CL	440 and 650
Hogewoning et al. (2012b)	10-25	100	-	409 and 618
Bulley et al. (1969)	11 or 17	140-200	CL	435 and 665
McCree (1972a)	25	16-150	CP	450 and 670
Inada (1976)	17-34	100-150	CL	430 and 680
Balegh and Biddulph (1970)	10	19.8	CL	437 and 670

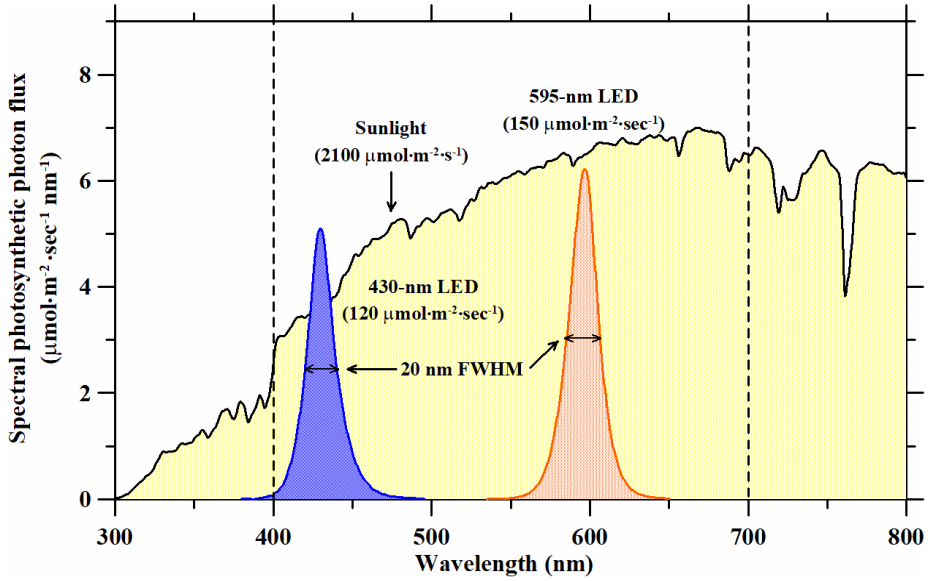

Figure 1. A comparison of sunlight, 430 nm, and 595 nm light emitting diode (LED) light spectra measured using a spectroradiometer (PS-300, Apogee, Logan, UT). The sunlight spectrum was measured in Montreal, QC, Canada.

Action spectrum

An action spectrum is the rate of photosynthetic activity plotted against wavelengths of light. It can depict the response of a photosynthetic organism's biological system to electromagnetic radiation. An action spectrum is constructed

from measurements of oxygen production (Evans and Anderson, 1987), carbon dioxide use (Bulley et al., 1969; Hoover, 1937), or net photosynthetic rate (Inada, 1976; McCree, 1972a) at different wavelengths, as shown by the following equation:

$$\textbf{Action spectrum} = \frac{\textbf{photoactivity in light} - \textbf{photoactivity in dark}}{\textbf{Irradiance}} \qquad \text{Equation 1}$$

Action spectrum data from early reports were compared and re-plotted in Figure 2. Overall, all curves exhibited different shapes and had different peaks within the blue and red wavelength regions. Some studies reported much more pronounced blue peaks (Balegh and Biddulph, 1970; Hogewoning et al., 2012b; Hoover, 1937). The first action spectrum curve using narrow light spectra was determined under a photometric unit (foot-candle) using young wheat plants by Hoover (1937). The Hoover curve shows two distinct peaks at 440 nm and 650 nm. Because of limited lighting and optical technologies employed at this time, the light intensity used in this study was less than 300 foot-candles (~ 40–60 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), with inconsistent broad FWHMs (20–30 nm). The observed peaks were similar to those reported in other studies; however, the curve was somewhat different in shape from other curves, particularly the blue peak (Figure 2). More specifically, the height of the blue peak was approximately 80 % of the red peak (Hoover, 1937), whereas other curves showed a blue peak that was approximately 50–60 % of the red peak. Further studies indicated that the Hoover curve was likely determined with pale coloured leaves, as the aforementioned blue peaks could only be replicated under these conditions (Burns, 1942; Gabrielsen, 1940). McCree (1972a) reached the same conclusion as a similarly shaped curve was observed with a pale-green lettuce plant.

That said, a high blue peak similar to that exhibited by the Hoover curve was observed in a study published later, yet no pale leaves were reported (Balegh and Biddulph, 1970). A distinct blue peak at approximately 435 nm, yet with lower blue light efficiency was reported for radish (*Raphanus sativus* L. var. Early Scarlet Globe) and other typical greenhouse plants (33 species) (Bulley et al., 1969; Inada, 1976). Differences in the spectral quality of photosynthesis within the blue light range have been discussed (Inada, 1976; McCree, 1972a; Yabuki and Ko, 1973).

McCree (1972a) pointed out that neither irradiance nor absorbed photon flux is a perfect light measurement unit for “photosynthetically active radiation”. Both units overestimate blue light efficiency relative to the red, but irradiance overestimates more than the absorbed photon flux. Moreover, different relative blue light efficiencies were observed for arborous and herbaceous plants when constructing PAR curves with a radiant unit (Figure 2) (Inada, 1976). It is possible that the difference in the spectral quality of photosynthesis observed with blue light may be caused by the degree of leaf greenness, an increase in inactive absorbance by carotenoids or light-screening compounds such as anthocyanins, an increase in leaf absorptance within the leaf tissue, or an increase in reflected blue light caused by the presence of epicuticular wax deposits (Inada, 1976; Yabuki and Ko, 1973). High intensity blue light triggers an avoidance response in chloroplasts; their movement away from the strong light, causing a reduction in photosynthetic efficiency (Wada, 2013). When using the narrow blue light spectrum with an intensity that is excessive compared to the blue light intensity of sunlight, it might cause a reduction in blue light spectral efficiency. Under this circumstance, the blue light spectral efficiency of photosynthesis reported in earlier studies may possibly contain a chloroplast avoidance response, which lowers the spectral efficiency in the blue range (Figure 1) (Bulley et al., 1969; Inada, 1976; McCree, 1972a).

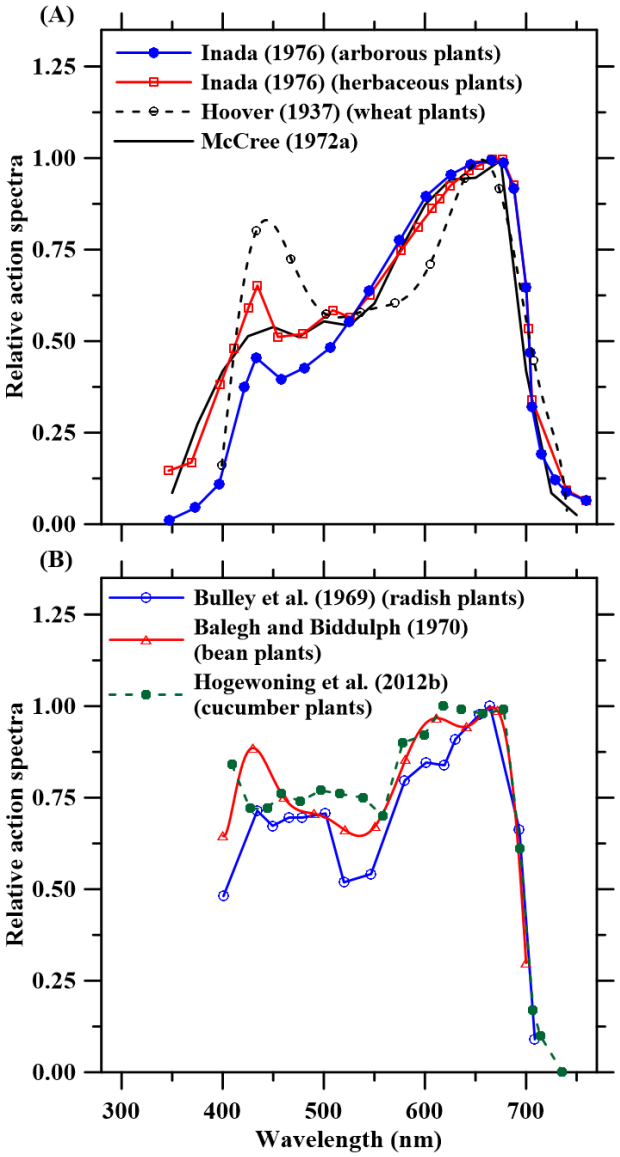

Figure 2. A comparison of action spectra reported in earlier studies (Balegh and Biddulph, 1970; Bulley et al., 1969; Hogewoning et al., 2012b; Hoover, 1937; Inada, 1976; McCree, 1972a). All curves are plotted using original data and normalized to a maximum of 1.

When comparing the narrow light properties used amongst all the earlier PAR curve determination studies, they all appear to have an effect on the spectral quality of photosynthesis. For the studies that did not observe distinct blue peaks, the FWHM was larger than 25 nm (Hogewoning et al., 2012b; McCree, 1972a). Contrarily, studies that used light with a FWHM lower than 20 nm observed a distinct blue peak at approximately 435–440 nm (Balegh and Biddulph, 1970; Bulley et al., 1969; Hoover, 1937). For the study published by Inada (1976), the blue

peak was observed but the FWHM used ranged between 17–34 nm, and it was not specified for the corresponding wavelengths. Thus, we could not conclude any such effect on the spectral quality of photosynthesis for this study. Hogewoning et al. (2012b) reported a blue peak located at 409 nm rather than at 435–440 nm, as described in other studies. The FWHM of the 409-nm light spectrum used in this study was 40 nm. It is possible that this pronounced 409 nm peak may be caused by an interaction effect between wavelengths within this broad 40 nm FWHM spectrum.

Differences in spectral quality data between studies were much greater than differences observed within these studies (Inada, 1976; McCree, 1972a). This observation implies that the true spectral quality of photosynthesis might be identical (or at least similar) amongst plant species but using a different investigative tool (narrow light spectrum with different FWHMs) results in other representations of the curves, based on spectral properties of the narrow spectrum and measured wavelength.

The use of narrow spectrum light could resolve the spectral quality of the photosynthesis quandary. However, since obtaining a narrow spectrum with a 1 nm full width is nearly impossible, photosynthetic data measured at each wavelength using a "narrow spectrum" (e.g. 70–100 nm full width) represents a convolution of the spectral bandpass function with an unknown quantity at each intended wavelength measurement. Potential interactive wavelength effects caused by a "narrow light spectrum" might influence measured photosynthetic rates. The extent of such effects depends on spectra. Further, for the standard curve determined by McCree (1972a), the FWHM used (25 nm) was wider than that of a light emitting diode (LED)'s FWHM (~20 nm). Although the difference in FWHM is only 5 nm, differences in spectral full bandwidths could be at least 30 nm, depending on spectral compositions. In this case, the photosynthetic machinery activated by the narrower LED light spectrum might not be as the McCree curve predicts at the same measured wavelength.

In the red wavelength region, peaks of action spectra are located between 660–680 nm (Inada, 1976; McCree, 1972a). Action spectrum curves in the red region are nearly identical in shape between earlier studies (Figure 2), with the exception of a few inconsistent valleys and low efficiency observed between 600–650 nm (Balegh and Biddulph, 1970; Bulley et al., 1969; Hoover, 1937). Reported valleys were at 620 nm and 640 nm, and these were not observed in other studies (Balegh and Biddulph, 1970; Bulley et al., 1969). For the Hoover curve, the spectral efficiency in the 600–650 nm range is approximately 10-20 % lower than reported in other studies. This might be due to an under-representation of the curve after correcting the remaining infrared wavelengths in the narrow light spectra without considering the observed Emerson effect (Emerson et al., 1957). In the 500–630 nm wavelength range, all plant extracted pigment absorbance is low, whereas the spectral efficiency of photosynthesis in this range was the opposite, or even higher than some blue wavelengths. This disproportional relationship might be explained by the scattering effect, as opposed to leaf absorptance and extracted pigment absorbance (Brodersen and Vogelmann, 2010; Terashima et al., 2009).

Quantum yield

The quantum yield spectra measured by McCree (1972a) and Inada (1976) are presented in Figure 3. We did not present the quantum yield spectra determined by Evans and Anderson (1987) and Hogewoning et al. (2012b), since (1) Evans and Anderson (1987) conducted the experiment using oxygen evolution units, which may only represent the spectral efficiency of Photosystem II (PSII) rather than the whole photosynthetic machinery, and (2) the Hogewoning curve is nearly identical to the curves presented in Figure 3, with the exception of the 409 nm peak addressed above. Quantum yield peaks occurred close to 600–620 nm, with a shoulder present at approximately 420 nm for both studies (Inada, 1976; McCree, 1972a) (Figure 3). In the blue range, the peak and valley were almost at the same wavelengths (approximately 420 nm and 490 nm, respectively) between the two curves, although the Inada curve oscillated more compared to the McCree curve (Inada, 1976; McCree, 1972a). This difference reinforces the statement that FWHMs impact the curve shape.

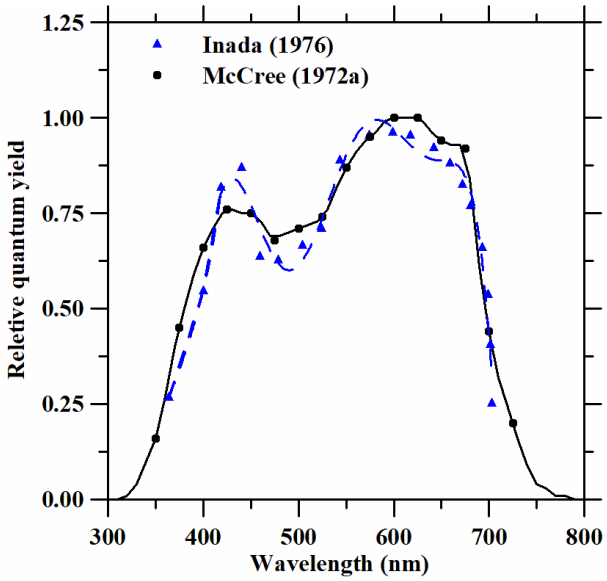

Figure 3. A comparison of relative quantum yield as reported by Inada (1976) and McCree (1972a). All curves are plotted using original data and normalized to a maximum of 1.

Unlike other studies conducted with a few plant species and limited growth conditions, McCree (1972a) first investigated the action spectra of 22 plant species with different growth and environmental factors. The photosynthetic spectral quantum yield or the CO_2 consumed by plant leaves per mole of photons absorbed was obtained by correlating the monochromatic irradiance level ($W \cdot m^{-2}$) required to obtain a certain rate of photosynthesis in cut leaves to their absorptance spectrum, measured in an integrating sphere with a spectrophotometer. The assay covered the wavelength range from 350 nm to 750 nm, in 25 nm waveband increments. McCree (1972b) then determined that quantifying PAR in quantum flux units based on moles of photons would yield data more closely correlated to the actual photosynthetic rate than radiant units, since photosynthesis is a quantum photochemical process, with one carbon fixed and one molecule of oxygen evolved per roughly 10 photons (quanta) of light absorbed. Though both units of measurement, irradiance level, ($W \cdot m^{-2}$) and photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$), overestimate the effect of blue vs. red light, this phenomenon is smaller when light energy is measured in quantum units (Inada, 1976; McCree, 1972b). To date, these data are still considered the standard for the spectral quality of photosynthesis and PAR. However, McCree

(1972a) did not provide specific information on the irradiance levels used for each wavelength, which may be relevant when interpreting his data (van Iersel, 2017).

PAR is described as photosynthetic photon flux density (PPFD, $\mu mol \cdot m^{-2} \cdot s^{-1}$), measured in quantum units of moles of photons per incident surface area per time elapsed in the wavelength range of 400–700 nm. Photon flux density is broadly considered to be the available estimate of potential photosynthetic flux, since the two are positively correlated. PAR is determined by integrating PPFD values within the limits of the action spectrum (McCree, 1971, 1972b). Based on McCree's findings with respect to the quantum yield curve, it is a generally accepted principle that all photons emitted within the 400–700 nm range are approximately used equally for photosynthesis by plants. The following equation shows a rectangular hyperbola between net photosynthetic rate and irradiance:

$$P = -bI / (1+aI) \text{ or } I/P = (aI/b) + (1/b) \qquad \text{Equation 2}$$

Where P equals the net photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), I represents irradiance ($W \cdot m^{-2}$), a is the slope at zero I, and 1/b is the value of P at infinite I. Based on this equation, as irradiance increases, photosynthetic rate would increase as well, along a hyperbolic curve of slope b, until the point of light saturation of the photosystems results in a leveling off of the increase in photosynthetic rate as it reaches maximum capacity, P_{max}, at the limit b/a (Inada, 1976). Using these data, it was concluded that the PAR action spectrum curve could be extrapolated to serve as a measure of potential photosynthetic quantum yield applicable to the upper limits of photosynthesis, under much higher irradiance, based on the same basic shape of spectral quantum yield response curve to irradiance at each wavelength obtained at unnaturally low irradiance (McCree, 1972a).

Analysis of some of the hyperbolas obtained for plotted P/I values in Inada (1976) did not show a clear linear relationship, such as at 459 nm and 546 nm. This result implies that perhaps some wavelengths of light are actually used more or less efficiently than others when under different light conditions for certain plants. Light below 400 nm and above 700 nm induces photosynthetic activity, which was not previously considered in PAR (Inada, 1976; McCree, 1972a). This led to the use of

yield photon flux (YPF). YPF weighs photosynthetic activity between 360–760 nm, based on the McCree quantum yield curve, under the assumption that the curve retains its form under different light conditions (Barnes et al., 1993; Sager et al., 1988). Whether the quantum yield curve really does keep its famous form, it remains to be determined under different light conditions or higher wavelength resolutions (Lefsrud et al., 2008). Bugbee (2016) pointed out that using these early action spectra data would not be as appropriate when predicting whole plant photosynthesis under higher light intensities.

There has always been a misinterpretation or misunderstanding of spectral quality data determined in early studies regarding radiant and quantum units. The McCree action spectrum that shows peaks at 450 nm and 670 nm was constructed with the radiant unit, irradiance. These peaks, reported by McCree (1972a), led to pilot experiments investigating photomorphogenic development (Bula et al., 1991; Yanagi et al., 1996), and later plant LED lighting experiments aimed at determining maximum plant productivity using 460 nm and 660 nm LED light. However, the baselines of light units used between wavelength selection and applications for whole plant cultivation are different. The result for the McCree action spectrum is based on irradiance, whereas the quantum unit PAR is used for whole plant cultivation, as was suggested by McCree (1972b). Thus, a variation in photomorphogenic development using the quantum unit is expected, as predictions made with McCree action spectrum data were conducted using irradiance. These two units are fundamentally different when photon energy is or isn't considered. Over the past four decades, studies have reported spectral quality data constructed with the quantum unit, which has peaks at ~600 nm, for both action spectrum and quantum yield (Inada, 1976; McCree, 1972a; Sager et al., 1982).

Although not considered when measuring, the spectral quality on photosynthesis synergistic effects of different wavelengths and photomorphogenesis are worth mentioning. All spectral quality curves are developed from quantifying photosynthetic activity under narrow light spectra. In this case, potential synergistic effects of different wavelengths may be detected (McCree, 1972a; van Iersel, 2017). Emerson et al. (1957) first observed that combined red and far-red light leads to

higher photosynthetic rates than the sum of the two wavelengths of light alone. In addition, different degrees of wavelength supplementation based on intensity have been reported (Terashima et al., 2009). Combined white and red light results in a higher photosynthetic rate than the addition of green light at lower light intensities, whereas at high light intensity, white light mixed with green light results in a higher photosynthetic rate than adding red light at higher intensities (Terashima et al., 2009). These findings imply that plants utilize light in a more complex manner according to wavelength and intensity.

2.2 Interactive effects between monochromatic light and plant development

Light wavelength and intensity are used to quantify light in plant lighting experiments, and it is now widely accepted that both influence photosynthesis and photomorphogenesis (Olle and Viršile, 2013; Singh et al., 2015). With the McCree curve and improvements in lighting technology, photomorphogenic responses with whole plant measurements have been investigated under various wavelengths and intensities of narrow spectrum light for greenhouse crops (Hoenecke et al., 1992; Kim et al., 2004a; Li and Kubota, 2009; Martineau et al., 2012; Stutte et al., 2009). In contrast to photosynthesis that is associated with growth from direct light energy, photomorphogenesis is defined as the effect of light on plant development. Several plant responses such as germination and flowering result from the mere presence of light and are not influenced greatly by its intensity (Hall et al., 2014; Kołodziejek and Patykowski, 2015). Therefore, the outcome of a plant's response under any light spectrum results from the interactive effects of photosynthesis and photomorphogenesis. These two responses are difficult to separate from each other for long-term whole plant growth.

Photomorphogenesis is the light-mediated development of plants regulated by five different photoreceptors (Figure 4) (Folta and Carvalho, 2015; Pocock, 2015). They mediate and modulate dozens of structural plant developments such as height, leaf size, and flowering. These changes to plant architecture affect long-term plant development and subsequent photosynthetic surfaces, which are not considered when interpreting spectral quality data.

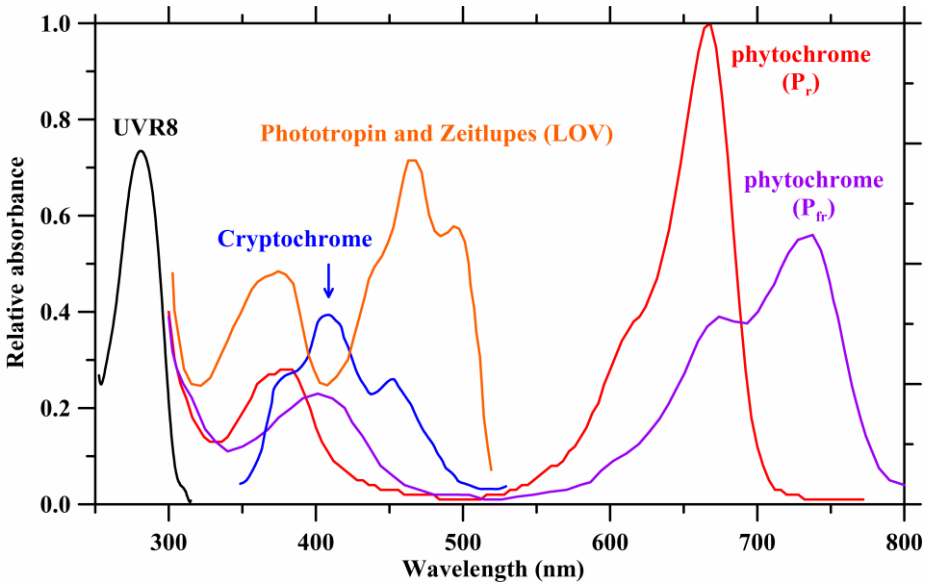

Figure 4. Absorbance spectra of photoreceptors. Spectrum data are derived from Taiz and Zeiger (2002), Galvão and Fankhauser (2015), and Sager et al. (1988).

Red (625–700 nm) and far-red (> 700 nm) light

Red light impacts photomorphogenesis, leaf nutrient content, and stem growth. It is essential for chlorophyll synthesis and for straightening the epicotyl or hypocotyl hook of dicot seedlings (Goins et al., 1997; Johkan et al., 2012; McNellis and Deng, 1995; Poudel et al., 2008). These processes are under the influence of phytochrome control. Phytochrome is sensitive to red (~650–670 nm) light and far-red (FR) light (~705–740 nm), and to a lesser extent, blue light (~400–500 nm). For any one phytochrome, there exists a photo-equilibrium of two interconvertible forms, red and FR absorbing forms (also known as Pr and Pfr, respectively). Pfr is the active form of phytochrome and it elicits physiological responses (Shinomura et al., 2000). Pr, the other form of phytochrome, is the inactive form that switches to Pfr upon absorbing ~650–670 nm light (Folta and Carvalho, 2015; Nagatani, 2010). In long day plants, various experiments suggest that flowering is promoted mostly when red light (or light creating a high Pfr/Pr ratio) is delivered during the early part of the photoperiod and when FR light (or light creating a lower Pfr/Pr ratio) is delivered toward the end of the photoperiod (Evans, 1976; Kadman-Zahavi and Ephrat, 1976; Lane et al., 1965; Thomas and Vince-Prue, 1996).

The effect of red light on plant physiology has been investigated (Poudel et al., 2008; Vu et al., 2014). Poudel et al. (2008) reported that red light induced an

increase in rooting percentage and root numbers in grape (*Vitis vinifera*) plants. Wu and Lin (2012) showed that king protea (*Protea cynaroides* L.) plantlets grown in red light produce a higher number of roots and new leaves. Vu et al. (2014) reported that "*Lapito*" tomato plants grown solely under red LED light produce a higher total root surface area, length, and number of root tips in comparison with other light treatments. Lower leaf nitrogen content was found in rice (*Oryza sativa* L.) and spinach (*Spinacia oleracea* L., cv. Megaton) grown under red light (Matsuda et al., 2004; Matsuda et al., 2007; Ohashi et al., 2005). In addition, reductions in photosynthetic rate observed for plants grown under red light are reportedly due to stomata being controlled more by blue light than by red light (Bukhov et al., 1996; Sharkey and Raschke, 1981; Zeiger, 1984).

Red light further regulates flowering quality, quantity, and flowering duration (Bula et al., 1991; Tennessen et al., 1994). According to Guo et al. (1998) and Thomas and Vince-Prue (1996), inhibition of flowering with red light is effected by red-light receptors including phytochromes (Kelly and Lagarias, 1985). The number of visible flower buds in marigold plants was approximately five times higher when grown with fluorescent light supplemented with red LEDs, as well as under fluorescent light, when compared to monochromatic blue or red light. No flower buds formed in salvia plants when grown under monochromic blue or red light or when fluorescent light supplemented with FR light was used for marigold (*Tagetes minuta*) plants.

Plants grown under canopy shade conditions or in the proximity of other plants show a range of responses to changes in R: FR ratios of ambient light. This response, known as shade avoidance or the near neighbor detection response, is characterized by an acceleration of flowering time (i.e. becoming visible within the expanded floral bud) and rapid elongation of stems and leaves (Halliday et al., 1994; Smith, 1994). Kasperbauer (1988) determined that FR light reflected from neighboring seedlings increased the R: FR ratio plants received, inducing a density-dependent increase in stem length, chloroplast content, chlorophyll *a/b* ratio, and CO_2 fixation rate, along with decreased leaf thickness. In recent years, the effect of FR light (or a low R: FR ratio) has been intensively investigated in different plant species and

development stages (Finlayson et al., 2010; Li and Kubota, 2009; Mickens et al., 2018; Park and Runkle, 2018). Supplemental FR treatments increased dry mass for many greenhouse crops during vegetative development (Hogewoning et al., 2012a; Lee et al., 2016; Mickens et al., 2018; Park and Runkle, 2018), but conflicting results on leaf area have been published. Hogewoning et al. (2012a) reported no significant difference in leaf area for tomato (*L. esculentum* 'Mecano') and cucumber (*Cucumis sativus* 'Venice'), whereas an increase in leaf area was observed for lettuce, petunia (*Petunia* × *hybrida),* geranium (*Pelargonium* × *hortorum*), and coleus (*Solenostemon scutellariodes*) (Lee et al., 2016; Mickens et al., 2018; Park and Runkle, 2018). Such differences in leaf area responses among species are still unknown and need to be addressed. For an extensive examination of FR light refer to the following recent review (Demotes-Mainard et al., 2016)

Blue (450–520 nm) and UV (< 400 nm) light

Blue and UV-A light trigger cryptochrome (320–500 nm) and phototropin (phot1 and pho2; 320−500 nm) function (Jones, 2018). These two photoreceptors regulate various physiological and developmental processes including chloroplast relocation, germination, elongation, and stomatal opening, which impacts water transpiration and CO_2 exchange (Cosgrove, 1981; Schwartz and Zeiger, 1984). Blue light mediates chlorophyll and chloroplast development, enzyme synthesis, plant density, and regulates responses to biotic environmental stresses (Goins et al., 1997; Schuerger et al., 1997). Walters and Horton (1995) reported that blue light deficiency can impact the light saturation rate of photosynthesis and can change the chlorophyll *a/b* ratio in *Arabidopsis thaliana*. Blue light causes thickness of the epidermis and palisade mesophyll cells in *Betula pendula* (Sæbø et al., 1995). Lee et al. (2014) concluded that shorter blue wavelengths (< 445 nm) promote stem growth, plant height, and anthocyanin synthesis in green perilla (*Perilla frutescens* var. *japonica* Hara *cv. Soim*) plants.

Blue light activates Zeitlupe (ZTL) family function, a group of proteins that plays a role in circadian clock regulation, wherein their light-dependent function allows modulation of internal timing signals (Kim et al., 2007). Wavelengths of light that are shorter than the PAR spectrum (e.g. violet light and UV [< 400 nm]

radiation) have limited photosynthesis; however, discrete photomorphogenic effects are observed when UV-B (290–320 nm) sensing systems are triggered (Folta and Carvalho, 2015; Frohnmeyer and Staiger, 2003). UV-B radiation is perceived via the UV-B photoreceptor *UV resistance locus 8* (*UVR8*). Although UV-B represents a threat to plant integrity in large quantities, smaller quantities of UV-B have important benefits such as promoting pest resistance, increasing flavonoid accumulation, improving photosynthetic efficiency, and serving as an indicator of direct sunlight and sunflecks (Ballaré et al., 2012; Moriconi et al., 2018; Wargent and Jordan, 2013; Zoratti et al., 2014). Further to this, some UV-B responses can also be modulated by a *UVR8*-independent signal and UV-A radiation, since plants' responses to UV-B light are regulated by both UVR8-dependent and -independent pathways (Jenkins, 2017; Li et al., 2015; Morales et al., 2013).

Green (520–560 nm) light

Green light is often considered unavailable for plant growth since plant photosynthetic pigments have limited absorbance for these wavelengths. However, there is evidence that green light is available for active plant growth, yet this phenomenon is light wavelength- and light intensity-dependent (Johkan et al., 2012; Kim et al., 2004a; Kim et al., 2005a). Green light influences plant morphology, including leaf growth, stomatal conductance, and early stem elongation (Folta, 2004; Kim et al., 2004a, b). Kim et al. (2004c) first examined the effect of green light on plant growth and photomorphogenesis, later concluding that it impacted plant growth at low light intensity (~150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) (Kim et al., 2005a). A low percentage (≤ 24 %) of green light enhanced plant growth, whereas plant growth was inhibited under a higher percentage of green light (Folta and Maruhnich, 2007; Kim et al., 2004a; Kim et al., 2005a; Lee et al., 2011; Liu et al., 2017). Lee et al. (2011) reported that lady slipper orchid grown under a combined LED lighting regime (8:1:1 ratio; 660 nm, 525 nm, and 450 nm) had at least 60 % greater shoot dry mass when compared to blue or red LED emissions alone, or to a combination of red and blue lights at the same light intensity. Furthermore, green light exhibits better leaf tissue penetration ability (Brodersen and Vogelmann, 2010), resulting in better plant canopy penetration than either red or blue light (Klein, 1992). The issue with green light is that it exerts an antagonistic effect on other blue light-induced

responses, including stomatal closure (Frechilla et al., 2000) or anthocyanin accumulation (Zhang and Folta, 2012).

2.3 Electrical lighting technology in plant photobiology

Electrical lighting systems that are currently in use for plant photobiology are fluorescent bulbs, high-intensity discharge (HPS) luminaires, and solid-state light emitting diodes (LEDs) (van Ieperen and Trouwborst, 2007). Conventional lighting systems (i.e. fluorescent bulbs and HPS luminaires) serve different purposes, such as supplementing lighting, plant propagation, and germination (Langhans and Tibbitts, 1997). Although being slowly replaced by LEDs because of their low efficiency (< 20 %), they indeed deliver sufficient intensity and high intensity uniformity (Brault et al., 1989; Mansour and Arafa, 2014; Nelson and Bugbee, 2014; Shur and Zukauskas, 2005). Currently, HPS luminaires are still the industry standard for plant cultivation in greenhouses as supplemental light (Stober et al., 2017).

LEDs represent a promising lighting technology when compared to conventional lighting for controlled environment plant research and production (Massa et al., 2008; Morrow, 2008). The advantages include: long lifespan (30,000–50,000 h), controllable light beam and intensity, narrow spectral emission, and multiple wavelength options (Massa et al., 2015; Morrow, 2008; Yeh and Chung, 2009). These advantages have significantly increased their application in academic studies and vertical farming (Bantis et al., 2018; Blanchard and Runkle, 2010; Martineau et al., 2012; Mickens et al., 2018; Stober et al., 2017). ~~Besides plant productivity,~~ LEDs have also been widely used to investigate plant responses to radiation and intensity, abiotic stress, chlorophyll fluorescence, and photosynthetic activity (Johkan et al., 2012; Meng et al., 2019; Miyashita et al., 2005; Parrine et al., 2018). Taken together, it is clear that the era of LED research on plant photobiology has begun.

That said, LEDs are not as robust as conventional lighting sources with respect to reliable spectral properties (Chang et al., 2012; Van Driel and Fan, 2013). Their spectral properties are greatly impacted by junction temperature. Inadequate thermal management can result in peak wavelength shift, intensity decline, or even a reduced

lifespan (Li et al., 2014; Schanda et al., 2014). LED luminaires require a higher degree of hardware control to ensure consistent spectral properties when compared to conventional lighting systems. In a greenhouse environment, environment factors such as ambient temperature and moisture are harsher and fluctuate more when compared to a fully enclosed indoor environment. Moreover, these factors vary according to plant species, greenhouse type, geographical locations, and time of the day (McCartney and Lefsrud, 2018). These varying conditions make the maintenance of LED spectral performance difficult for manufacturers and users for supplemental lighting purposes. It could lead to misinterpretations when correlating wavelength/intensity and plant response, if regular confirmation of LED spectral properties does not occur.

Several researchers have thoroughly reviewed and discussed photomorphogenic responses and the advantages of LED technology, when compared to conventional lighting technology in the horticultural industry (Massa et al., 2008; Morrow, 2008; Pocock, 2015; Singh et al., 2015). However, LED reliability issues, possible outcomes and interaction effects with external environmental factors have not been investigated, and recent developments in conventional lighting sources could compete with LEDs (Nelson and Bugbee, 2014). Herein, we present the important aspects of electrical lighting technology in plant photobiology, with an emphasis on LED technology for greenhouse applications.

Light emitting mechanisms

Both fluorescent bulbs and HPS luminaires emits light by sending an electric discharge through ionized gas, also known as gas discharge luminaires (Figure 5). Fluorescent bulbs emit visible light via fluorescence, as 253.7 nm light produced by the ionization of mercury vapor at low pressure excites a phosphor coating. Fluorescent bulbs are typically used for seed germination and seedling growth in growth chambers, due to their uniform light distribution over plant growing areas. They provide spectral emissions that result in healthy and productive plants (Langhans and Tibbitts, 1997). Typically, fluorescent bulbs last for more than 9,000 hours under standard test conditions; however, this depends on starting and stopping frequencies since the emissive coating (usually phosphor) on the electrodes is

rapidly eroded during start-up. Typical fluorescent bulbs used in growth chambers are cool-white fluorescent bulbs as they are more efficient compared to other fluorescent bulbs. HPS luminaires are the most efficient high-intensity gas discharge lighting units. They deliver sufficient intensity and increase greenhouse temperature through their high heat emission (> 200 ºC) (Brault et al., 1989; Nelson and Bugbee, 2014). HPS luminaires have a longer lifespan (24,000-32,000 h) than fluorescent bulbs (Mansour and Arafa, 2014; Sincero and Perin, 2007). Frequent starts reduce the lifespan of HPS luminaires, as does excessive voltage (power surges).

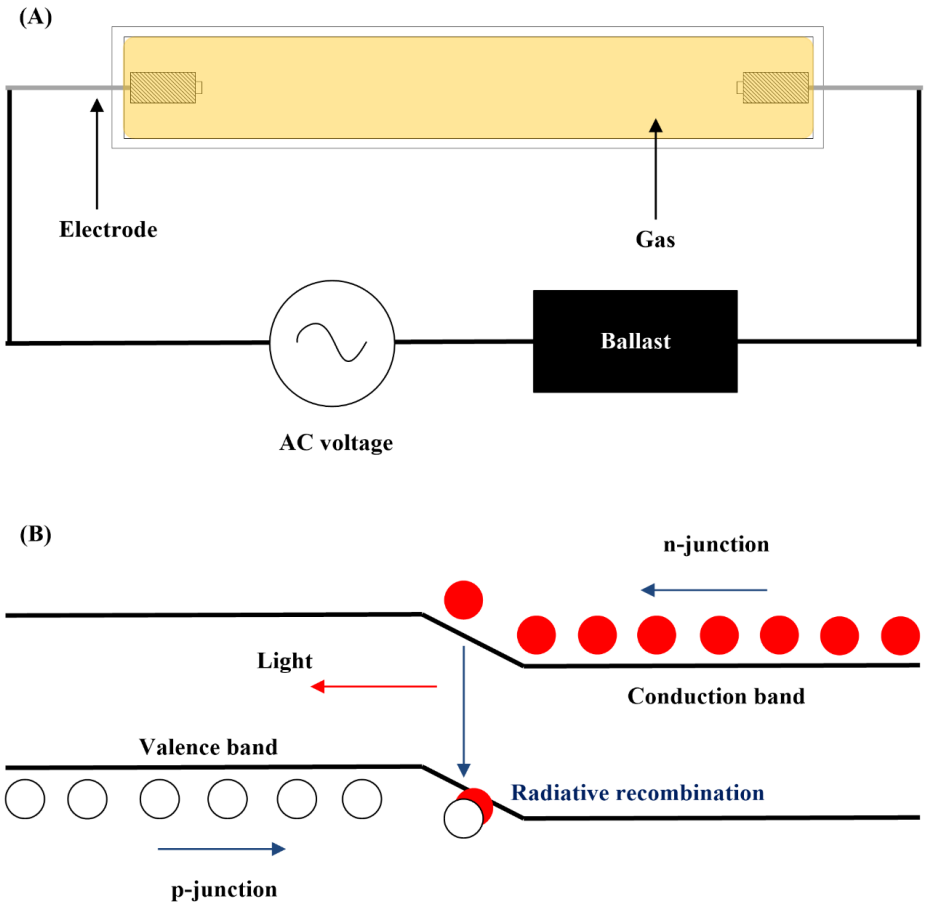

Figure 5. Light emitting mechanisms for (A) gas discharge luminaires and (B) light emitting diodes (LEDs) (adapted from Byszewski et al. (1996) and Singh et al. (2015).

LED lighting technology and its history in the plant photobiology field has been reviewed and presented in numerous studies (Cho et al., 2017; Kasap, 2001; Lafont et al., 2012; Morrow, 2008; Singh et al., 2015; Yeh and Chung, 2009). LEDs

are optoelectronic apparatuses comprising two components (dye and packaging) that are doped with chemical impurities at a p–n junction leading to light generation (Lafont et al., 2012). As an electron (p junction) meets a hole (n junction) in the depletion region, it drops from the conduction band to the valance band, resulting in photon emission (Figure 5) (Singh et al., 2015). The conduction band refers to the energy of free electrons that originate from the n junction, while the valence band refers to the valence energy of the holes that originate from the p junction. Photons released from LEDs correspond to the energy difference between the conduction and valence bands, referred to as the bandgap (Kasap, 2001) (Figure 6). The electromagnetic energy of photons and their wavelength proportionally depend on the bandgap of the semiconductor used; they are determined by the materials forming the p-n junction in LEDs (Yeh and Chung, 2009). The following equation relates the emitted wavelength (λ) and the energy bandgap:

$$\boldsymbol{\lambda = \frac{hc}{E_g} = \frac{1.24}{E_g}\ \mu m} \qquad \text{Equation 3}$$

Where h is Planck's constant, c is the speed of the light, and E_g is the energy bandgap (eV).

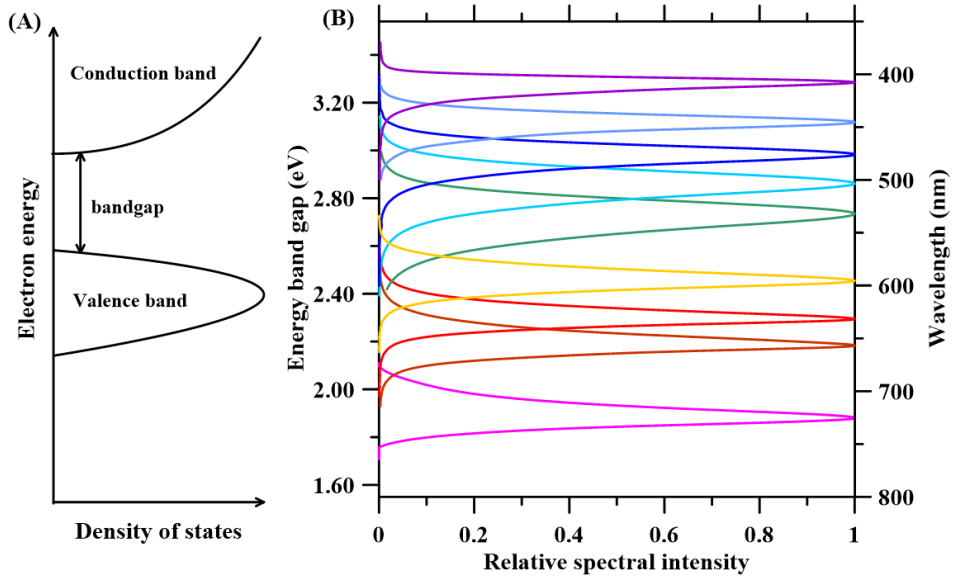

Figure 6. (A) Band structure and (B) representative LED spectra with corresponding energy bandgap.

The foundation of LED applications in greenhouse applications is based on early studies that used narrow spectrum light (using both LEDs and blue fluorescent bulbs) (Bula et al., 1991; Kim et al., 2005b; Yanagi et al., 1996). Results from these studies provided insight into the effect of wavelengths on photosynthesis and photomorphogenesis. Importantly, plant yield was greatly affected by wavelength selection, setting the stage for future LED plant experiments (Chen et al., 2017; Deram et al., 2014; Johkan et al., 2012; Naznin et al., 2016; Ouzounis et al., 2016; Son et al., 2017). Despite vast efforts placed in this field of study, doubt still remains with respect to the use of typical LED wavelengths (460 nm and 660 nm ± 10 nm) in industrial applications (Stober et al., 2017). This LED system, which emits a purplish light, induces higher plant productivity and exhibits lower energy consumption. Yet, it has not replaced conventional lighting units such as HPS luminaires and metal halide. According to a recent survey conducted by the US Department of Energy, HPS luminaires and metal halide are still the preferred choices for non-stacked indoor farms and enclosed greenhouses (> 90 %) (Stober et al., 2017). Reasons such as high capital costs and little knowledge of commercial greenhouse applications prohibit the replacement of HPS and metal halides by LEDs within the industry (Mitchell, 2015; Nelson and Bugbee, 2014).

Thermal stress and management for LEDs

LEDs have cooler light-emitting surfaces and a lower heat load compared to conventional lighting systems. However, substantial heat (at least a half of the input power) is still generated. Apart from the radiative recombination events, electrons and holes can also recombine non-radiatively, releasing excited energy as heat that results in self-heating. This non-radiative recombination event results in the reduction of photon conversion efficiency, a peak wavelength shift, and a temporary decline in light intensity (Gu and Narendran, 2004; Schanda et al., 2014; Xi et al., 2005a; Yang and Cai, 2013). If a constant high heat load occurs in LEDs, it will accelerate all LED failure mechanisms that could shorten longevity and cause permanent LED degradation (Li et al., 2014).

Junction temperature is a primary factor influencing LED performance and lifespan (Xi et al., 2005b). As junction temperature increases in LEDs, a shift in

peak wavelengths, toward longer wavelengths (10–15 nm) occurs, due to the decrease in bandgap energy (Gu and Narendran, 2004; Peng et al., 1999). Thermal stress leads to an increase in non-radiative recombination events in LEDs, where charge carriers combine without releasing photons, resulting in reduced intensity (10-25 %) (Chang et al., 2012; Liu et al., 2009). Schanda et al. (2014) reported that junction temperatures affected intensity and peak wavelength, but changes were greater for red LEDs than for blue LEDs. LED intensity significantly decayed when the junction temperature exceeded 100 ºC (Christensen and Graham, 2009; Su et al., 2012). Therefore, the thermal management of LEDs is of primary importance and is directly related to their reliability and efficiency (Christensen and Graham, 2009).

Passive heat sinks and cooling fans are typical methods used for LED thermal management in plant lighting applications. These methods might guarantee stable and effective heat dissipation for LEDs, but this depends on the external environment and operational methods. Passive heat sinks and fan-based cooling systems ensure a stable LED junction temperature, accompanied by stable LED spectral properties, which works best in an indoor environment (i.e. laboratory environment and growth chamber) with consistent temperatures and air flow. When using supplementing LED lighting in a greenhouse, the reliability and performance of these LEDs need to be monitored continuously, as different heat loads resulting from solar radiation may occur (McCartney and Lefsrud, 2018). When the ambient temperature in a greenhouse changes, the LED junction temperature changes accordingly, resulting in inconsistent LED spectral properties emitted to plants. To minimize these changes, the LED junction temperature should be monitored, controlled, and maintained with an active program-controlled cooling system. For plant lighting experimentations with different light intensities, using the same LED systems with the same cooling system requires consistent heat dissipation. While adjusting intensity by controlling its voltage/current outputs (depending on current or voltage control modes), different heat loads would consequently result in different LED peak wavelengths at different target intensities, since more of the input power is converted to heat resulting in higher temperatures.

The lifespan of LED luminaires is often advertised. Most manufacturers estimate that the lifetime of an LED is between 30,000–50,000 h, with a 70% initial lumen maintenance level, depending on the junction temperature of the diodes (Cree, 2017; Philips Lumileds Lighting, 2017). This estimation is based on the Arrhenius model that may be used to extrapolate test results at high temperatures that are expected when the LED unit is operational (Pecht et al., 2014). However, this estimate is inadequate when accurately predicting LED lifespan, as LED lighting configurations are exposed to different thermal environments. Furthermore, this model ignores others stresses, such as hygro-mechanical and electrical stresses, that could result in lifespan decline (Van Driel and Fan, 2013). Lifespan testing for LEDs is impractical since testing a certain condition would take at least 3–5 years operating 24/7 (30,000–50,000 h). A recent consumer's survey for general lighting applications revealed that an LED lifespan ranged from 1,460–27,375 h and that nearly 80 % of LED lighting products failed in the first year (Casamayor et al., 2015), indicating that the need for better design and maintenance.

The lifespan of LED sub-components is also problematic but often neglected. For instance, LED driver lifespans depend on operating temperature, ranging between 16 % and 20 % of the LED diode lifespan (5,000–10,000 h), depending on the capacitors and ambient temperatures. In this scenario, high costs would be incurred, as frequent driver replacement for greenhouse operations is certain, and whole luminaire replacement may occur if a driver is not replaceable. As such, an LED lifespan survey for plant photobiology field would be most useful for scientists in the plant photobiology field.

When comparing the power consumption and lifespan of LEDs and conventional light sources, LEDs offer substantial electricity savings that could recover their high capital cost over time (Singh et al., 2015). However, it has been reported that double-ended HPS luminaires with electronic ballasts have nearly two times higher efficacy, and better longevity than single-ended HPS luminaires (Johnson and Bugbee, 2017; Nelson and Bugbee, 2014). Further, limited emitting areas for each LED need to be considered. An HPS luminaire, for example, is able to cover a large growing area with sufficient intensity for plant growth, since it is an

area light source. Unlike HPS luminaires, the light covering area of each LED diode is relatively small. Therefore, each LED fixture is composed of tens to hundreds of LEDs. If one single LED experiences changes in wavelength/intensity, color inconsistency, or any failure type, it could result in dark spots/areas and the entire luminaire would need to be replaced (Pecht et al., 2014).

Light uniformity

Light uniformity refers to either uniform light intensity or the horizontal intensity gradient. In growth chambers equipped with fluorescent bulbs, intensity uniformity may vary, and it is dependent on chamber size and the wall coating reflectance. The highest light intensity occurs in the chamber center and slowly decreases toward each wall. The difference between the highest and lowest light intensities in a chamber sized 1.8 m × 0.8 m is approximately 10 % (5 to 10 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) (Langhans and Tibbitts, 1997). For both HPS and LED luminaires, secondary optics including reflectors and/or lenses, are critical components used to control intensity uniformity within a target area. HPS luminaires require reflectors to maximize their intensity performance and collimate the light over the top of the plant canopy, since HPS bulbs produce omnidirectional lights in 360 degrees (Cole and Driscoll, 2014). HPS intensity uniformity and HPS lighting designs for a large scale greenhouse have been characterized by design software and a decision model, as it is a difficult task to test and evaluate multiple HPS luminaires for greenhouse installations (Both et al., 1994; Both et al., 2000; Ciolkosz et al., 2002; Ciolkosz et al., 2001). Simulated results showed that lower intensities occur at the perimeter of a greenhouse and a higher luminaire density is required for uniform light intensity in the outer row (Both et al., 2000; Ciolkosz et al., 2001).

In the case of the LED luminaires, the types of the secondary optics used vary based on the user's requirements. Optic manufacturers provide different secondary optics with diverse beam angle options, such as reflectors and total internal reflection (TIR) optics. For small scale plant LED lighting experiments (i.e. growth chamber), LEDs with a low beam angle have been used (Li et al., 2016; Poulet et al., 2014). Low beam angle lenses direct light toward target plants and they improve over-all photon utilization efficiency by collimating light from different LEDs

(Poulet et al., 2014).

Light intensity can be improved by operating in close proximity to plants without increasing energy output; this is a definite advantage over traditional luminaries (Both et al., 2017; Gómez et al., 2013; Massa et al., 2008). However, such an approach does not take into consideration changes in intensity/wavelength uniformity and photosynthetic surface expansion (plant growth) over time. Placing LED luminaires close to the plants results in reduced light intensity uniformity. Additionally, if LEDs emitting multiple wavelengths are used, heterogeneity of the wavelength ratio or combination over the photosynthetic surfaces occurs that can result in misinterpretations of certain wavelength effects (Hogewoning et al., 2010a). Such wavelength ratios/combination heterogeneity often occurs using bar-type and strip LED luminaries emitting mixed wavelengths, because of their linear diode arrangements. For example, plants would receive a different red/blue ratio of light spectrum when placed at different locations under the bar-type LED luminaires with red and blue diode ratio of 3:1 (Figure 7). These heterogeneous intensity patterns can be avoided by maintaining a longer distance between luminaires and the top of the plant canopy, and/or reallocating plants between each spot over time (Gómez and Mitchell, 2015; Hogewoning et al., 2010b). Importantly, intensity and wavelength measurements need to be performed in a detailed manner. As an example, a grid size measurement rather than single-spot measurement could be made.

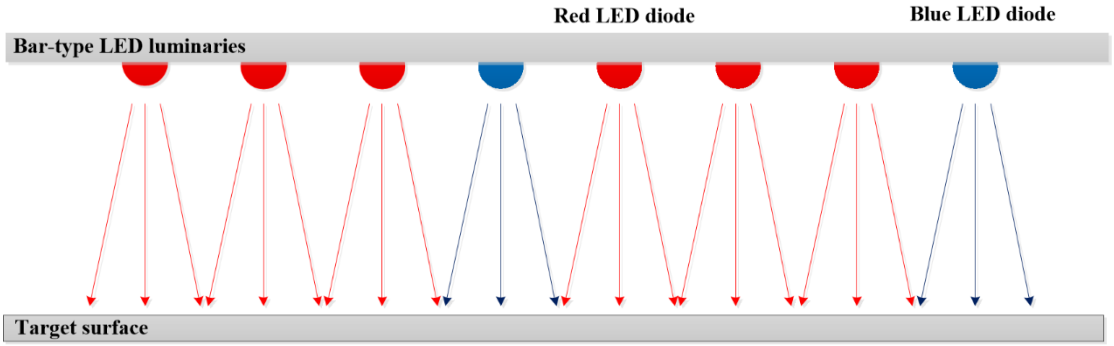

Figure 7. A simplified schematic diagram for a bar-type LED luminaire and its wavelength distribution.

When considering larger scale lighting systems for plant growth experiments, commercial high-wattage LED luminaires (500-1000 W) are usually chosen because of their higher intensity output (Magagnini et al., 2018; Nelson and Bugbee, 2014). These LED luminaires are usually high bay LED luminaires equipped with optics. The types of optics, such as reflectors and lenses, are manufacturer-, application-, and lighting requirement-dependent. For luminaires employing integrated LED chips (or chip-on-board LED), 60-120 º lenses are often used.

When using commercial high-wattage LED luminaires, heterogeneity of wavelength ratios or combinations rarely occurs, but severe heterogeneity in light intensity uniformity has been observed (Nelson and Bugbee, 2014; Wallace and Both, 2016). Both studies reported that light intensity declined dramatically (> 60 %) as the sensor moved one meter away from a position directly below the center of the luminaries (Nelson and Bugbee, 2014; Wallace and Both, 2016). This severe heterogeneity could lead to different plant growth rates and morphology for plants grown in greenhouses.

To our knowledge, the characterization of intensity uniformity and plant lighting system designs for multiple LED luminaries has not yet been reported (Nelson and Bugbee, 2014). A few approaches have been proposed according to target PPFDs or fixture numbers (Aldrich and Bartok, 1994; Kubota et al., 2016), but these approaches are originally designed for roadway lighting for human vision using conventional lighting sources. Further evaluations for greenhouse applications are required. In the general lighting field, different approaches such as secondary lens design and specific diode arrangements have been developed to obtain high intensity uniformity for low wattage LED luminaries, which might be used as a reference for greenhouse lighting designs (ASABE standad S640, 2017; ASABE standad S642, 2018; Chen et al., 2011; Whang et al., 2009).

Wavelength availability

Conventional light sources have fewer wavelength selection and no narrow spectrum options available. However, different spectral compositions exist and are observed in plant lighting studies (Bergstrand and Schüssler, 2013; Dąbrowski et al.,

2015; Johkan et al., 2012; Poel and Runkle, 2017). Blue fluorescent bulbs were used in early plant studies, as blue LEDs were not available at that time (Brown et al., 1995; Goins et al., 1997; Hoenecke et al., 1992). Currently, cool-white fluorescent bulbs are commonly seen in the market and used for plant germination (van Ieperen and Trouwborst, 2007). They served as controls when conducting plant LED experiments (Johkan et al., 2012; Johkan et al., 2010; Wollaeger and Runkle, 2014); however, spectral compositions differ amongst manufacturers and studies (Figure 8). Different light-emitting recipes (and phosphor coatings for the white LEDs) result in diverse spectral compositions that all appear "white" in colour, or have the same Kelvin colour temperature. Apart from the usual colours, fluorescent bulbs with different spectral compositions, including full spectrum and different blue and red wavelengths, have recently been unveiled. These products might not be able to serve as controls for LED specific wavelength investigations, however, they may prove interesting for some users' specific requirements.

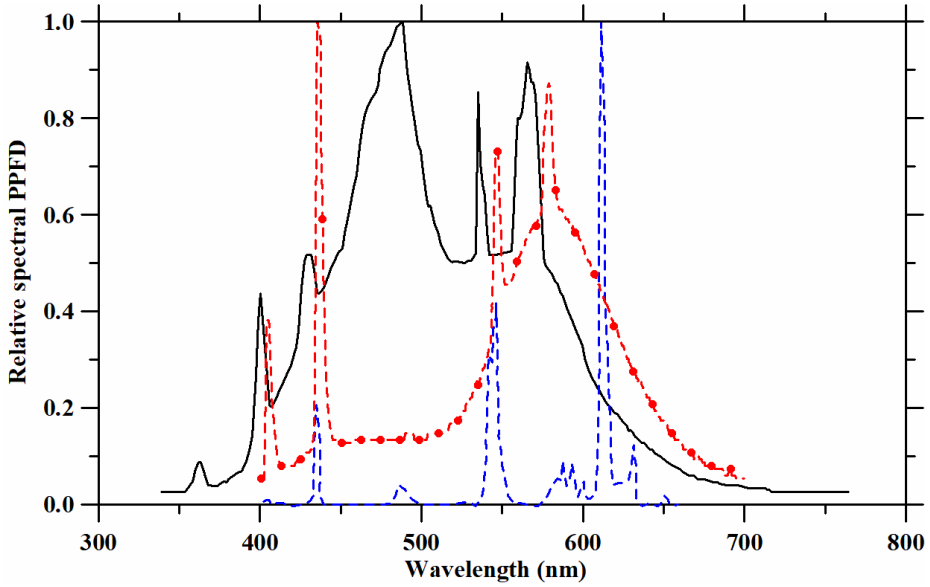

Figure 8. Relative fluorescent bulb spectra derived from Dąbrowski et al. (2015) (black sold line), Johkan et al. (2012) (blue dotted line), and Wollaeger and Runkle (2014) (red dotted line with circles).

HPS luminaires emit yellow (or amber) light but have different spectral compositions based on their designs (Bergstrand and Schüssler, 2013; Poel and Runkle, 2017) (Figure 9). Single-ended HPS luminaires exhibits a dominant peak at

595 nm (Gómez and Mitchell, 2015; Guo et al., 2016; Islam et al., 2012; Poel and Runkle, 2017). Double-ended HPS luminaires, the re-designed HPS luminaries with higher photon emission efficiency and better light uniformity, has peaks at 570 nm and 605 nm, with a valley at 590 nm (Bergstrand and Schüssler, 2013; Pepin et al., 2013; Randall and Lopez, 2015; Trouwborst et al., 2010). The difference in wavelength output from the single-ended and double-ended HPS requires further investigation from a plant perspective.

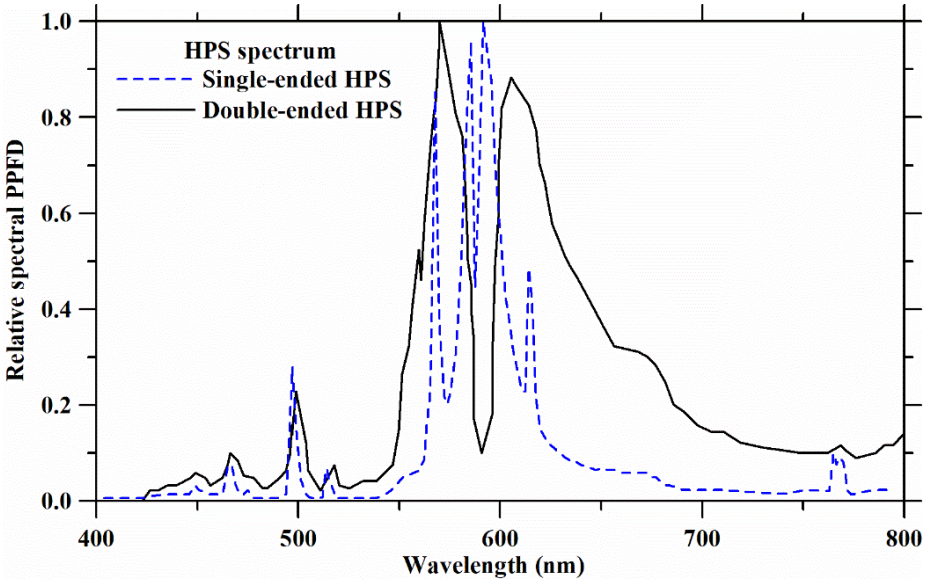

Figure 9. Single- and double-ended HPS luminaire spectra. The spectra for single- and double-ended HPS luminaires are derived from Poel and Runkle (2017) and Bergstrand and Schüssler (2013), respectively.

As the spectral compositions of conventional light sources are diverse, whether or not these conventional light sources are capable of serving as the "standard" light source (or control) when conducting plant LED light experimentations merits further discussion. In addition, whether or not these luminaires are manufactured for indoor or outdoor lighting, they are all designed to accommodate human vision, and this needs to be considered when using conventional lighting sources. Using colour metrics to describe luminaire properties might be inappropriate.

A wide selection of wavelengths and narrow spectra LEDs are available (Eichhorn Bilodeau et al., 2019; Stutte et al., 2009; Wu and Lefsrud, 2018). As a

result, LED technology is more suited to plant wavelength response investigations than conventional lighting systems. This advantage allows researchers to characterize and study the optimal irradiance and/or wavelength combination for maximum plant productivity. Nevertheless, the commercial availability of different wavelengths is greatly limited by LED manufacturers and luminaire suppliers (Cree, 2017; Philips Lumileds Lighting, 2017). Although there are nearly 100 different LED peak wavelengths available (Stutte et al., 2009), wavelengths used in plant experiments only represent approximately 10 different peak wavelengths in the visible spectrum. The nominal LED wavelengths that are predominantly used are 460 nm and 650 ± 10 nm. A large number of studies have applied these LEDs with different ratios to different crop types for plant lighting experimentation (Chen et al., 2014; Martineau et al., 2012; Naznin et al., 2016). Most commercial LED luminaires in the industry comprise these two wavelengths (460 and 650 nm). After these two common wavelengths, other monochromatic LEDs include 440 nm, 500 nm, 520 nm, 640 nm, 680 nm, and 720 nm with a variation of ± 10 nm (Johkan et al., 2012; Lefsrud et al., 2008; Mizuno et al., 2011; Zhen and van Iersel, 2017). White LEDs with a Kelvin color temperature between 3000–5000 K, use a 460-nm LED as its photon driver, with a phosphor layer producing a broad 595-nm light that is used for plant production (Han et al., 2017; Swan and Bugbee, 2017). A limited selection of LED wavelengths leaves a large sum of wavelengths in the PAR spectrum untouched, and their effects on plant growth unknown, which limits the exploration on wavelength investigations. In all, this technology allows for the possibility of more peak wavelengths of light, but at present, LED technology remains limited by manufacturing capability.

Challenges in manufacturing and usability

LEDs that are based on III-phosphide and III-nitride exhibit high efficiency (30–50 %) for the emission of red and blue light, respectively (Craford, 2007; Nelson and Bugbee, 2014). Weak carrier confinement for green wavelengths (500–600 nm) results in a lower efficiency (30%) for both semiconductors (Lafont et al., 2012). In horticultural applications, wavelengths located between 520 nm and 590 nm have not been well explored, and specific plant responses remain unexamined. To determine the effects of these wavelengths on plant growth, users will need to

find a solution to increase the low intensity outputs of these wavelengths. Another issue for LED devices is colour consistency; LED colour components vary between identical LEDs. After slicing a wafer into a LED chip, each chip must be individually tested and placed in a performance bin for process variability. Processed LED chips are grouped based on performance, including: colour, forward voltage, and flux for consistency. This results in the production of LEDs that deviate in performance from one another. Deviations in wavelength from each bin group are usually within ± 10 nm, which may contribute to the discrepancy seen between studies using the same LEDs, but without the confirmation of peak wavelength using a spectroradiometer.

In recent years, increasing irradiance intensity and narrow light bandwidth have resulted in photobiology eye safety concerns within the lighting industry for end users (Lau, 2013). International standard groups and diode manufacturers have published evaluations and assessments of monochromatic LEDs (Cree, 2016; International Electrotechnical Commission, 2006; Osram, 2012; Philips-Lumileds, 2010a). These reports provide a guideline for LED users specific to photobiology eye safety concerns and inform users of tests conducted under light units of radiance ($W \bullet m^{-2} \bullet sr^{-1}$). This information is important for general lighting applications, but they cannot be applied directly to and/or by users in the plant photobiology field because different light measurement units are applied (radiance [$W \bullet m^{-2} \bullet sr^{-1}$] and photosynthetic photon flux density [$\mu mol \cdot m^{-2} \cdot sec^{-1}$]). In enclosed environments with sole LED light, workability and occupational health are neglected during plant lighting design (Takao, 2016). Users are potentially at risk of being exposed to ocular light hazards from varying spectral compositions and irradiance levels. Plant lighting LED manufacturers should provide information on spectral weighting radiance and recommended exposure duration in accordance with these standards (Both et al., 2017; Wu and Lefsrud, 2018). Further, the sharp blue peaks (5-10 nm FWHM) in fluorescent bulbs (Figure 8) may require more attention when considering photobiology eye safety.

2.4 Summary

Early attempts to quantify the spectral quality of photosynthesis under either photometric or radiant units have proven inadequate. Several earlier studies correlated the effects of quanta on spectral quality, but under unrealistic light levels relative to sunlight. Various FWHMs, measured wavelengths and methodologies create a difference in curve shape, potentially leading to a misinterpretation of the collected spectral quality data. The McCree curve (McCree, 1972a) is still considered the standard for the spectral quality of photosynthesis, although certain relevant information is missing, making it hard to re-interpret these results. Furthermore, since the curve was constructed under a broad FWHM, data collected at each wavelength represents a convolution of the spectral-bandpass function, with an unknown quantity in a broad FWHM as the intended measurement. When using a narrow spectrum light with different FWHM other than those that comprise the McCree curve, different data regarding photosynthetic activity are expected. Since then, subsequent studies have suggested that it is inadequate to correlate plant responses to the McCree curve at higher light intensity levels (Bugbee, 2016).

Recent studies have attempted to replicate the McCree PAR curve with LED lighting technology and optical filters. However, challenges occur with wavelength selection and achieving higher light intensity levels. A comprehensive spectral quality of photosynthesis analysis of different plants with better controllability over wavelength, FWHM and higher light intensity is required. The influence of light properties, as well as synergistic or antagonistic effects on photomorphogenesis, should be considered going forward, although these properties cannot be detected with spectral quality measurements and monochromatic light. Importantly, these observations collectively raise the question of whether or not deploying these weighing factors, including certain wavelengths and light intensity, is required for quantifying PAR in future studies.

Researchers have structured plant spectral responses through long-term plant cultivation of various species grown under different light conditions with a narrow light spectrum (Chen et al., 2017; Deram et al., 2014; Johkan et al., 2012; Naznin et al., 2016; Ouzounis et al., 2016; Son et al., 2017). Wavelength selection is usually

based on the standard curve determined by McCree (1972a). It is an alternate way of determining spectral responses through whole plants, other than short-term and single-point spectral quality measurements on a single leaf. The outcome of whole plant cultivation and analysis represents the interaction effects between photosynthesis and photomorphogenesis. Photomorphogenic responses cannot be determined from spectral quality data collected with monochromatic light. However, these interaction effects are difficult to distinguish one from another, making plant spectral response analyses difficult. Further, using the McCree curve to select LED wavelengths and predict whole plant cultivation would be inappropriate, since different FWHMs are used. These observations raise the question of whether or not deploying weighing factors for certain wavelengths based on intensity ranges is required for quantifying "photosynthetically active radiation". As plant responses when grown under LED light remain elusive, clearly more work needs to be done in the area.

Each lighting technology has its advantages and disadvantages. Indeed, LEDs offer many advantages over conventional light sources, rendering them suitable as plant lighting sources. However, LEDs, as a sophisticated semiconductor device, require a higher degree of hardware and lighting design with controls when compared to other lighting sources. LED users need to be aware and cautious regarding maintenance of optical, mechanical and spectral properties when designing and using the systems for their operations. Changes in spectral properties would result in misinterpretations, which could cause discrepancies between studies using the same reported light properties. Proper and regular light measurements taken over the plant growth period is recommended, particularly when plants are grown in thermal stress environments. New wavelengths of narrow spectrum light can be feasibly created with optical filters but require better control and design for desired wavelengths and light intensities. Fluctuating data slow further advances in plant lighting LEDs, as several uncertainties remain and invention costs are high. Notably, LED photobiological eye safety concerns need to be addressed, whether in academic research or commercial operations, as LED light intensity increases with ongoing developments.

Connecting text

Chapter 3 has been published and is cited as the following:

Wu, B.-S., and M. Lefsrud. 2018. Photobiology eye safety for horticultural LED lighting: Transmittance performance of eyewear protection using high-irradiant monochromatic LEDs. *Journal of Occupational and Environmental Hygiene* 15(2):133-142.

In Chapter 2, the current state and challenges of horticultural LED applications were reviewed. In the last section, an increase in LED intensity and photobiological eye safety concerns was described, yet existing safety guidelines cannot be directly applied to users in academic research or in the horticultural industry. Therefore, in Chapter 3, the performance of protective eyewear was examined using high intensity monochromatic light, as an alternate way to ensure photobiological eye safety.

CHAPTER 3

Photobiology eye safety for horticultural LED lighting: transmittance performance of eyewear protection using high-irradiant monochromatic LEDs

Abstract

Light emitting diodes have slowly gained market share as horticultural lighting systems in greenhouses due to their rapid improvement in color performances and light outputs. These advancements have increased the availability of the full spectrum of visible wavelengths and the corresponding irradiance outputs available to plants. However, light emitting diodes owners have limited information on the proper options for personal eyewear protection as the irradiance levels have increased. The objective of this study was to measure the light transmittance performance of 12 eyewear protection including welding goggles, safety goggles, polarized glasses and sunglasses across the human visible spectrum (380–740 nm) up to an irradiance level of 1500 $W \bullet m^{-2}$ from high-irradiant light emitting diodes assemblies. Based on the spectral measurements, certain transmitted spectra exhibited spectrum shifts or an alteration in the bimodal distribution which were different than the light emitting diodes spectra, due to the uneven transmittance efficiencies of the glasses. As for the measured transmittance percentages in two experiments, each type of eyewear protection showed distinct transmittance performances, and the performance of the tested eyewear protection was not impacted by irradiance but was dependent on the wavelength. The mean light transmittance was 1.77 % for the welding glasses, 13.12 % for the polarized glasses, 15.27 % for the safety goggles, and 27.65 % for the sunglasses. According to these measured results and the spectral weighting exposure limits from the International Electrotechnical Commission 62471 and EU directive 2006/25, consumers and workers using horticultural lighting can select welding goggles or polarized glasses, to limit the possible ocular impact of the high irradiance of monochromatic light in electrical lighting environment. Sunglasses and safety goggles would not be advised as protection, especially if infrared radiation was used.

3.1 Introduction

Light emitting diodes (LEDs) have begun to slowly replace conventional lighting technologies (e.g. high-intensity discharge luminaires and compact fluorescent bulbs) in horticultural industry due to their rapid and ongoing improvements in color performances, packaging technology, and light outputs (Murphy Jr, 2012; Singh et al., 2015; U.S. Department of Energy, 2014, 2015; Xie and Hirosaki, 2007). However, as plants use LED light to photosynthesize, an electrical lighting environment is created where LED users are potentially at risk of being exposed to ocular light hazards from varying spectral compositions and irradiance levels. Safety concerns regarding the narrow spectral compositions and high irradiance outputs of LEDs have arisen amongst LED users as the technology slowly penetrates into horticultural lighting and other lighting applications (Lau, 2013; U.S. Department of Energy, 2015). In this scenario, international standard groups and diode manufacturers have begun to assess and evaluate LED photobiological safety issues (Cree, 2016; International Electrotechnical Commission, 2006; Osram, 2012; Philips-Lumileds, 2010a). Guidance identifying radiation hazards for all luminaires and luminaire systems emitting wavelengths from 200 nm to 3000 nm is provided to evaluate photobiological safety in the International Electrotechnical Commission (IEC) 62471 (International Electrotechnical Commission, 2006). It is the most relevant standard with respect to LED photobiological safety, and defines the risk groups and radiation hazards of luminaire systems based on spectral weighting light intensity (irradiance ($W \bullet m^{-2}$) or radiance ($W \bullet m^{-2} \bullet sr^{-1}$)), as well as their corresponding exposure periods and their wavelengths. Diode manufacturers also have conducted LED photobiological testing and issued risk group assessment reports for their standalone emitters, in accordance with the standard (Cree, 2014, 2016; Osram, 2012; Philips-Lumileds, 2010a, b). According to these reports, attention to LED photobiological safety should be given for lower wavelength lights (< 480 nm) and infrared radiation (700-1000 nm) due to the induction of different ocular damages and burning sensations in the skin surrounding the eyes, respectively (Cree, 2016; Osram, 2012; Philips-Lumileds, 2010a, b).

Unlike with other lighting applications, the use of spectrum and irradiant outputs from monochromatic LEDs varies in the horticultural industry. LEDs emitting blue (400-500 nm) and infrared wavelengths are widely being used since they have a variety of important photomorphogenic and physiological roles in plants. In conjunction with red LEDs (600-700 nm), the effects of blue LED light have been the major subject of countless studies for plant productivity due to its important role in CO_2 exchange and the high photon-conversion efficiency of blue LEDs (~50 %) (Craford, 2007; Deram et al., 2014; McCree, 1972a; Naznin et al., 2016; Son and Oh, 2013; Young, 1991). The infrared radiation is mainly used in far red light (710–850 nm) applications in greenhouse production due to the absorbance sensitivity of phytochrome, disease development, and nitrate accumulation (Kim et al., 2005b; Sager et al., 1982). Supplementing beneficial LED wavelengths with far red light also allows plant stem elongation (Brown et al., 1995; Chia and Kubota, 2010), and better flowering for long-day photoperiod plants (Deitzer et al., 1979; Kohyama et al., 2014). Besides these wavelengths, LEDs that have different wavelengths, such as UV A&B (280-400 nm) and green (500–600 nm), are also applied to study influences on plant physiological responses (Folta, 2004; Kim et al., 2004a, b). However, wavelengths between 500–700 nm at a high irradiance level may also cause retinal photothermal injuries (Boulton et al., 2001; Sliney, 1983). Increased production of reactive oxygen species and DNA damage occur as human retinal pigment epithelial cells are exposed to monochromatic LED light (Chamorro et al., 2013). In addition to the effect of wavelength on ocular damage, irradiance output is also a factor that can cause ocular damages (Ide et al., 2015; Laube et al., 2004). A typical commercial horticultural blue/red LED luminaire (e.g. 500 W output and 90^o light bean angle), for instance, can radiate at least 100 $W•m^{-2}$ (or approximately 54 $W•m^{-2}•sr^{-1}$), which is ~ 80 times brighter than the minimum recommended indoor light level for indoor working environments (500 lux) (DiLaura et al., 2011). The number or configuration of the LED luminaires depends on plant growing areas or targeted irradiance levels over the plant canopy. Moreover, the brightness of LEDs has increased by 20x every decade and at a higher rate for certain LEDs (Haitz and Tsao, 2011). Under these circumstances, LED photobiological tests from diode manufacturers are difficult to apply for LED owners in the industry. Information on photobiological testing for these commercial horticultural LED luminaires from

manufacturers is lacking as well. Therefore, owners need to assess the risk of the lighting environment or select proper personal eyewear protection against light hazards from high light horticultural LED lights, as there are only exposure guidelines for electrical lighting systems but no regulations for the occupational photobiological safety for horticultural working environment specifically (European Union, 2006; Takao, 2016).

Sunglasses are the typical personal eyewear protection recommended to shield eyes from bright sunlight, lower wavelength lights, and harmful radiation. Current market available sunglasses are all able to reduce a significant amount of UV wavelength, and transmit sufficient amounts of visible light (particularly green and red wavelengths for traffic signals) as stated in standards (European Standards Organisation (CEN), 2005; Tuchinda et al., 2006). However, most sunglasses are sold without any information about their detailed transmittance performances in the visible spectrum (Bruzell et al., 2007; Gursoy et al., 2015). This makes the selection of proper eyewear protection difficult for users' specific requirements. Moreover, wearing a set of glasses that inefficiently filters certain spectral regions may cause chronic diseases with damage to the lens and retina (Fishman, 1986). This can be even more harmful to the eyes when wearing glasses with improper transmittance characteristics in certain wavelength regions (Fishman, 1986)**.** Due to the lack of this knowledge, transmittance performances of the available sunglasses or filtering lenses have been characterized based on purposes and lighting applications, including UV protection, dental curing luminaires and surgical LED luminaires (Abdulrahim et al., 2015; Anderson and Gebel, 1977; Bruzell et al., 2007; Ide et al., 2015; Moseley, 1985). For each use, studies were conducted to evaluate eyewear protection and provide detailed information on their transmittance performances, but these studies focused either on specific wavebands or special luminaires with specific types of eyewear protection. Knowledge on an eyewear protection's transmittance performance with respect to monochromatic LED luminaires and different irradiance levels is still unknown for LED users in the horticultural industry. The performances amongst different types of eyewear protections available in the market also need to be examined for user's selection.

Hereby, the objective of this study was to provide information on the transmittance performances of different types of eyewear protection under monochromatic LED light for the LED users in the horticultural industry. To achieve this objective two experiments were conducted: (1) examining the transmittance performances of each set of eyewear protection using high-irradiant output LEDs across the visible spectrum (380–730 nm) at a constant irradiance level; (2) testing the dependence of the transmittance performances on different irradiance levels using blue, green, and red LEDs. The results of this study will provide more specific information for the LED users in the horticultural industry to select proper eyewear that will provide optimal protection against ocular tissue damage.

3.2 Methods

LED assemblies and eyeglasses

A total of 12 different eyewear protections (glasses) (Table 2 and Figure 10) and a transparent lab safety goggle (A700, Honeywell, Morris Plains, NJ) were examined under 10 different monochromatic high-irradiant LED assemblies. The glasses were purchased in local markets in the city or through online vendors. They were divided into four groups based on their types: welding goggles, safety glasses, polarized glasses, and sunglasses. The LED assemblies consisted of the following nominal wavelengths in order: 405 (UV, EFEV-1AE1; Edison Opto, Hsinchu, Taiwan), 447 (Royal-Blue, LXML-PR01, Philips-Lumileds, San José, CA), 470 (Blue, LXML-PB01, Philips-Lumileds, San José, CA), 505 (Cyan, LXML-PE01, Philips-Lumileds, San José, CA), 530 (Green, LXML-PM01, Philips-Lumileds, San José, CA), 560 (Lime, LXML-PX02, Philips-Lumileds, San José, CA), 595 (PC-Amber, LXM2-PL01, Philips-Lumileds, San José, CA), 627 (Orange-Red, LXM2-PD01, Philips-Lumileds, San José, CA), 655 (Deep-Red, LXM3-PD01, Philips-Lumileds, San José, CA), and 735 nm (Far red, ELSH-Q91LX, Everlight, Hsinshu, Taiwan) (Figure 11). Each assembly had seven diodes emitting the same wavelength onto a thermal pad and was attached with a concentrated lens (Focal length=25 mm, No. 263, Polymer Optics, Wokingham, Berkshire, UK). The LED assemblies' light outputs were adjusted by controlling the current outputs of a programming DC power supply (DP832, Rigol Tech., Beaverton, OR).

Table 2. Brand and classification of 12 eyewear protection devices used in this study.

Number	Brand	Type	Color
1	McMaster-Carr (Elmhurst, IL)	welding goggles	green
2	McMaster-Carr (Elmhurst, IL)	welding goggles	green
3	McMaster-Carr (Elmhurst, IL)	welding goggles	green
4	Radnor*	safety goggles	green
5	Stanley*	safety goggles	gray
6	Ray-Ban (Rochester, NY)	polarized glasses	green
7	Fisherman (Berkeley, CA)	polarized glasses	brown
8	zeroUV (Huntington Beach, CA)	polarized glasses	black
9	Burberry (London, UK)	sunglasses	brown
10	DxTreme*	sunglasses	black
11	Chanel (Paris, France)	sunglasses	black
12	Dereon*	sunglasses	brown

*company information could not be found online.

Figure 10. The images of the 12 glasses used in this study. Refer to Table 2 for the brand and type.

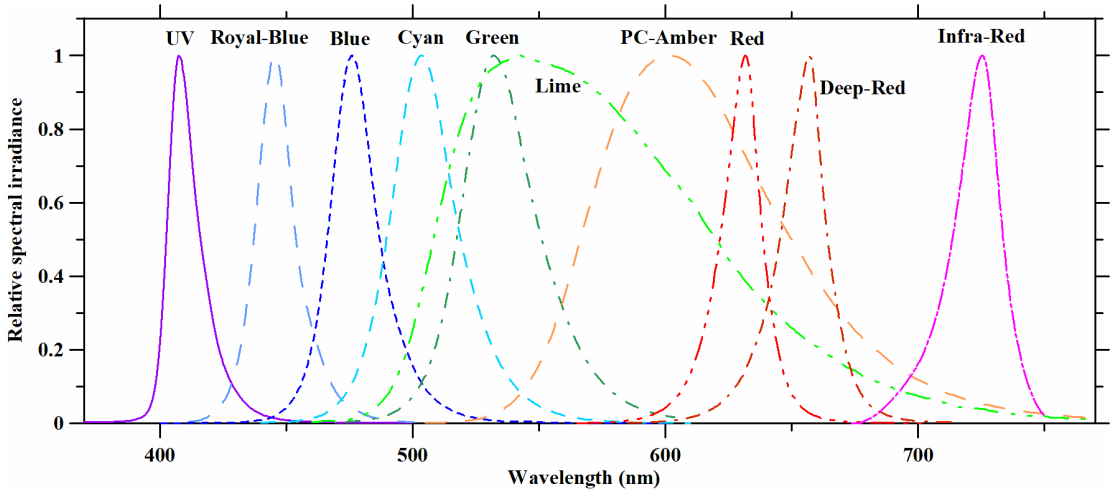

Figure 11. The normalized spectral compositions of the 10 LED assemblies including UV, royal-blue, blue, cyan, green, lime, pc-amber, red, deep-red, and infra-red LEDs (in the order of the nominal wavelengths).

Apparatus and measurements procedure

In experiment 1, 10 LED assemblies examined the transmittance performances of the glasses across the human visible spectrum (380–750 nm) at a set irradiance level of 1,000 W•m^{-2}. In experiment 2, the response of the glasses to changing irradiance levels was tested with 3 LED wavelengths (460 nm, 530 nm, and 655 nm). LED assemblies were selected based on typical LEDs utilized in horticultural production. The tested irradiance levels were 200 W•m^{-2}, 750 W•m^{-2}, 1000 W•m^{-2}, and 1500 W•m^{-2}. Both experiments were conducted with the same procedure described below. Light transmittance measurements were acquired with and without a set of glasses (blanks and samples) as the beams from the LEDs were aimed at the centers of the glasses where the light sensor was located. The glasses were positioned upon the light sensor head with a clamp holding the glasses and secured in place. To focus the light from the diodes on to the sensor head, the LEDs were placed 25 mm (focal length of the lens) above the sensor. The orientation from the top is the LED assembly, goggle, and then the light sensor. As for the light sensor, after recommendations from the sensor manufacturer, a combination of a spectroradiometer (300–1000 nm, PS-300; Apogee, Logan, UT) and a silicon cell pyranometer (MP-200; Apogee, Logan, UT) with a calculation provided by the manufacturer for the spectral errors of the pyranometer was required, as using either one as a standalone sensor prevented us from accurately measuring such high light

intensities. The spectroradiometer was used to make measurements on spectral composition data for the LED assemblies, and the pyranometer measured irradiance levels. The theoretical spectral errors were then calculated based on the following equations described by Ross and Sulev (2000) with the use of the LED assemblies' normalized spectral compositions and sunlight spectrum measured by the spectroradiometer as a reference light sensor, to obtain the corrected irradiance levels of the pyranometer

$$\beta_{PYR} = \frac{\int_{\lambda_{\min}}^{\lambda_{\max}} \varepsilon_{PYR}(\lambda) \cdot G^{E}(\lambda) d(\lambda) \cdot \int_{\lambda_{\min}}^{\lambda_{\max}} \varepsilon_{REF}(\lambda) \cdot I^{E}(\lambda) d(\lambda)}{\int_{\lambda_{\min}}^{\lambda_{\max}} \varepsilon_{REF}(\lambda) \cdot G^{E}(\lambda) d(\lambda) \cdot \int_{\lambda_{\min}}^{\lambda_{\max}} \varepsilon_{PYR}(\lambda) \cdot I^{E}(\lambda) d(\lambda)} \qquad \text{Equation 4}$$

where β_{PYR} is spectral error (%); $\varepsilon_{PYR}(\lambda)$ is the relative spectral sensitivity of the pyranometer which obtained from the manufacturer; $G^{E}(\lambda)$ is global spectral irradiance ($W \bullet m^{-2} \bullet nm^{-1}$) which was LED's spectral irradiance in this study, $\varepsilon_{REF}(\lambda)$ is the relative spectral sensitivity of the reference light sensor, which was the spectroradiometer in this study; $I^{E}(\lambda)$ is direct solar, diffuse or reflected spectral irradiance. Once the spectral errors were obtained, the apparent irradiance levels acquired from the pyranometer were corrected by the spectral errors, to obtain the actual irradiance level. During measurements, the measured order of glasses was randomized as illuminating under the same LED assembly. All measurements were performed per set of glasses for each wavelength in three replicates, and were made in an otherwise darkened room.

Data analysis

Measurements for each set of the glasses were repeated three times and averaged. The transmittance was reported as a percentage, which represents the ratio of LED light that has penetrated the sample to that without the sample.

3.3 Result

Transmitted spectra

The light characteristics of the LEDs (blanks) and the ones that were passed

through the tested eyewear protections (samples) had been measured using the spectroradiometer in experiment 1 and 2. Using the spectroradiometer to examine the LEDs' spectral compositions of the blanks and the samples showed no difference on spectral trace reading or FWHM under each LED light treatment, except for a few exceptions (illustrated in Figure 12). While examining the spectral trace readings that passed through some sets of glasses under the 447-nm and 470-nm LED assemblies, the transmitted spectral trace readings were found to be different since there was either a centroid wavelength shift or an alteration in the bimodal distributions at the tails of the LED spectra. For the 447-nm LED assembly, the transmitted spectra filtered through both of the sunglasses (sample 10 and 12 from Table 2) were altered to the bimodal distributions. The main centroid wavelengths of the transmitted spectra of these two glasses were still same as the unimodal 447-nm LED spectrum (blank), but secondary peaks were found at 461 nm and 463 nm for sample 10 and 12, respectively. As for the 470-nm LED assembly, the centroid wavelengths of the welding goggles (sample 1, 2, and 3 from Table 2) appeared to be shifted by approximately 10-25 nm toward larger wavelengths.

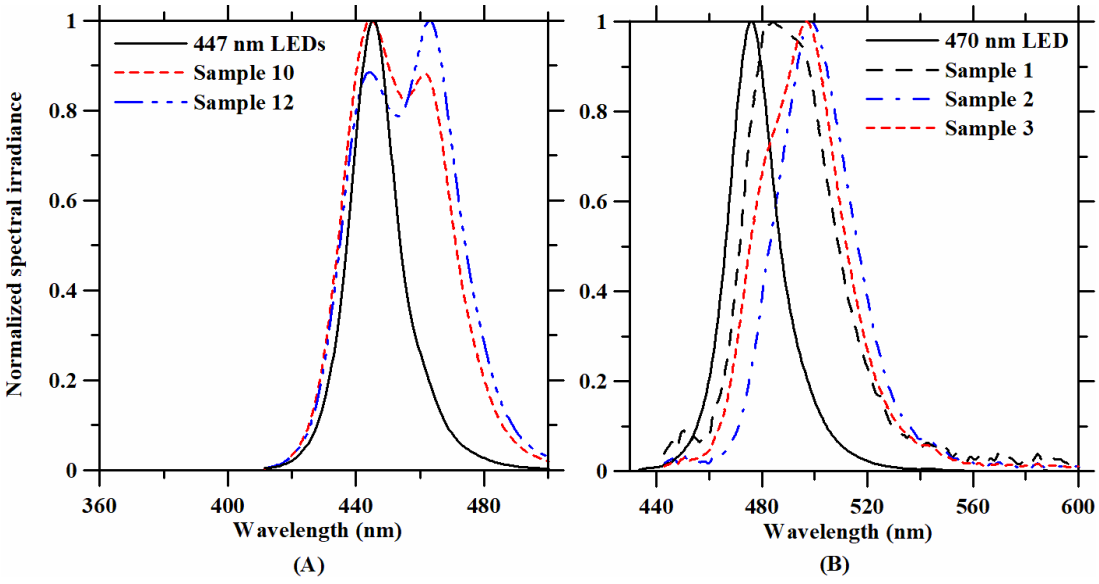

Figure 12. The normalized spectral irradiances of (A) the 447-nm LED assembly and light passing through the sunglasses (samples 10 and 12 from Table 2), and (B) the 470-nm assembly and light penetrating the welding glasses (sample 1, 2, and 3 from Table 2).

Calculation of the theoretical spectral errors for each light configuration (with and without samples) showed the lowest and highest errors for the 405 and 735-nm LED assemblies, which were -38.4 % and 42.5 %, respectively. The spectral errors for LED assemblies over the visible spectrum were nearly the linear response as shown in Figure 13. As for the LED light passing through the filtered glasses, the theoretical spectral errors remained the same since they consisted of the same LED spectral compositions, except the shifted spectra mention. The changes in spectral errors under 447-nm LED assembly were low, which decreased from -21.7 % to -19.28 % and -18.5 % for the sample 10 and 12. The spectral errors for the 470-nm LED assembly were -5.95 % for the sample 1, -3.61 % for the sample 2, and -4.97 % for the sample 3. Within these two wavelengths, the errors increased as the peak wavelengths increased. Irradiance levels were underestimated between 2.4-38.4 % and overestimated between 4.4-42.5 % for the peak wavelengths of LEDs that were between 405-505 and 530-735 nm, respectively. Therefore, the spectral errors were not considered as calculating the transmittance percentages for unchanged spectra after filtering, but were only considered for the results of the shifted spectra.

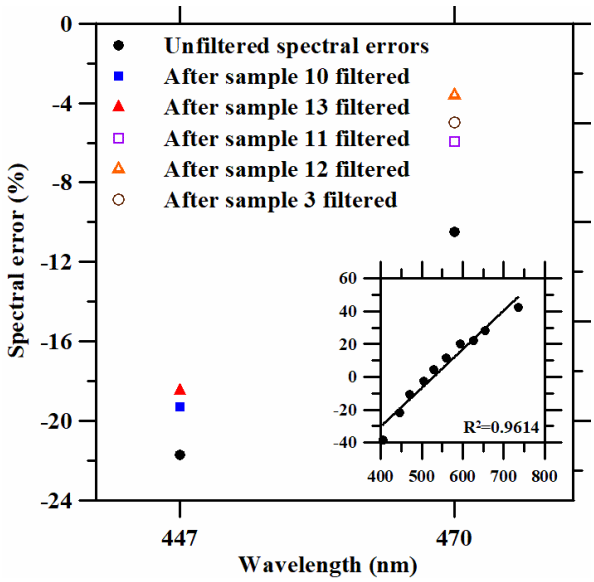

Figure 13. The spectral errors for the LED assemblies used in this study and for filtered spectra under certain eyewear protection samples

Transmittance performances

The transmittance performances of the tested eyewear protections at an irradiance level of 1000 $W \cdot m^{-2}$ using monochromatic LED assemblies (experiment 1) are shown in Table 3 and Figure 14. Overall, each type of tested eyewear protection exhibited distinct transmittance performances. The mean transmittance percentage was 1.77 % for the welding glasses, 13.12 % for the polarized glasses, 15.27 % for the safety goggles, and 27.65 % for the sunglasses. The welding goggles had the lowest transmittance percentages among all the tested glasses in this study, which were approximately between 0.1-9.5 % (Table 3). The percent light-reductions of the welding goggles were at least 95 %, except the welding goggles (sample 1 from Table 2) under the 735-nm LED assembly, which was around 90 %. Note that the transmittance data examined using the 405 and 447.5-nm LED assemblies are not shown in Table 3 since the irradiance levels penetrating the glasses were detected as zero by the pyranometer. As for the sunglasses and polarized glasses, they both had low UV light transmittance (< 10 %) but the polarized glasses had better light-reduction performance than the sunglasses for the rest of the tested wavelengths (447-735 nm), especially under the 735-nm LED light. With the 405-nm LED light, the percentage transmittance for both types of glasses was between 0.1-10 %. The mean transmittance percentages were 15 % and 30 % for the sunglasses and polarized glasses, respectively, between 447 and 655 nm. For the 735-nm LED light, the transmittance percentages were ~35 % for the polarized glasses and ~90 % for the sunglasses.

Table 3. Transmittance percentages (average and standard deviation) for welding and safety glasses examined with 8 LED assemblies at an irradiance level of 1000 $W \bullet m^{-2}$.

Nominal wavelength of LEDs (nm)	McMaster-Carr (1)	McMaster-Carr (2)	McMaster-Carr (3)	Radnor
	Transmittance percentage (Average ± St. Dev., %)			
470	0.07 ± 0.06	0.27 ± 0.06	0.53 ± 0.06	0.10 ± 0.00
505	0.50 ± 0.00	1.96 ± 0.23	2.99 ± 0.26	0.96 ± 0.21
530	0.90 ± 0.10	4.28 ± 0.06	5.58 ± 0.21	2.20 ± 0.10
560	0.70 ± 0.10	2.23 ± 0.06	5.46 ± 0.06	2.10 ± 0.20
595	0.40 ± 0.00	0.30 ± 0.00	5.85 ± 0.10	1.82 ± 0.06
627	0.07 ± 0.06	0.33 ± 0.06	0.62 ± 0.06	0.13 ± 0.06
655	0.10 ± 0.00	0.00 ± 0.00	9.54 ± 0.12	1.16 ± 0.15
735	0.13 ± 0.06	0.17 ± 0.06	9.92 ± 0.12	2.86 ± 0.12

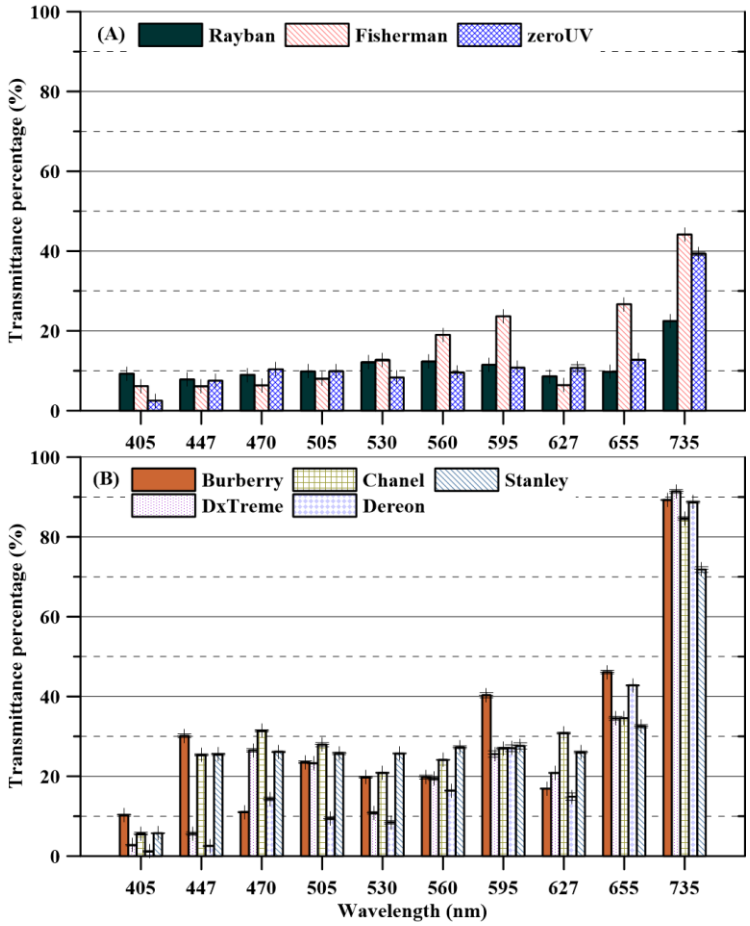

Figure 14. The transmittance percentages of (A) polarized glasses and (B) sunglasses.

When testing the two sets of safety goggles (samples 4 and 5 in Table 2), they had opposite transmittance performances compared to each other (Table 3 and Figure 14). The safety goggles (sample 4 in Table 2) had low transmittance performance (0.1-3 % for all tested wavelengths), which was similar to the welding goggles. However, the transmittance percentages for the safety goggles (sample 5 in Table 2) was approximately ~30 % for wavelengths between 405-660 nm and ~70 % for the 735-nm LED assembly. For the transparent lab safety goggles, the transmittance percentages were between 95-99 % under each LED assembly.

Results of the transmittance percentages of the tested eyewear protections at different irradiance levels (experiment 2) are summarized in Table 4. The results were only presented for each type of glass that had the highest transmittance measured in experiment 1, since their performance was not impacted by irradiance due to the varied irradiance levels. For each set of the tested glasses, the differences on transmittance percentages under different irradiance levels were all less than 2%.

Table 4. The transmittances percentages of each type of eyewear protection under three LEDs at different irradiance levels. N.D. - not detected.

Nominal wavelength of LEDs (nm)	Irradiance level ($W \cdot m^{-2}$)	Brand and type of eyewear protection			
		McMaster-Carr (1) (welding goggles)	Stanley (safety glasses)	Rayban (polarized glasses)	Chanel (Sunglasses)
		Transmittance percentages (Average ± St. Dev., %)			
470	200	N. D.	26.06 ±0.78	8.29 ±0.29	30.63 ±0.29
	750	0.49 ±0.08	26.25 ±0.20	9.05 ±0.20	31.11 ±0.15
	1000	0.53 ±0.06	26.14 ±0.20	8.98 ±0.06	31.41 ±0.26
	1500	0.53 ±0.06	26.14 ±0.20	8.98 ±0.06	31.41 ±0.26
530	200	5.25 ±0.29	24.20 ±0.29	12.52 ±0.29	19.80 ±0.51
	750	5.60 ±0.23	25.33 ±0.13	12.22 ±0.15	20.76 ±0.28
	1000	5.58 ±0.21	25.68 ±0.15	12.12 ±0.17	20.84 ±0.17
	1500	5.42 ±0.07	23.22 ±0.14	12.19 ±0.07	21.69 ±0.14
655	200	9.25 ±0.28	32.70 ±0.28	9.25 ±0.28	33.81 ±0.28
	750	9.04 ±0.20	31.35 ±0.23	9.92 ±0.01	34.39 ±0.13
	1000	9.54 ±0.12	32.57 ±0.40	9.74 ±0.06	34.56 ±0.06
	1500	9.51 ±0.10	31.86 ±0.17	9.93 ±0.07	34.59 ±0.04

3.4 Discussion

Transmitted spectra and the used light sensor

In the current study, the transmitted spectra and corrected irradiance levels for the blanks and samples were acquired using the spectroradiometer and the pyranometer, respectively. Comparing the data measurement sensitivity of the two sensors shows that the pyranometer's spectral response is inadequate to measure narrow spectrum light due to its low spectral sensitivity and its design to measure broad spectrum light (i.e. sunlight). According to the calculation of spectral errors acquired from Eq.1, the spectral error of the pyranometer was nearly a linear response as the LED wavelength increased, which was similar to the spectral response of the pyranometer. Moreover, we considered spectral errors under the light configuration with shifted spectra (different light spectra with/without glasses) and modified the apparent irradiance levels of the pyranometer through the scaled modification since the effect of low spectral response from the pyranometer was eliminated as the spectra remained the same after filtering. Therefore, the data presented in this study was rectified.

With respect to the changes on spectral compositions after passing the 447-nm and 470-nm LED light through the glasses, some spectra appeared to shift towards the green wavelength range and were altered to a larger bandwidth of light. This might be due to uneven transmittance efficiencies in these wavelength ranges. Research by Bruzell et al. (2007) found a similar unexpected response and concluded that it was caused by auto fluorescence (Bruzell et al., 2007). The auto fluorescence occurred when measuring transmittance percentages for eyewear protections, however, the fluorescent spectra presented was mainly between 525-700 nm and occurred high transmittance (> 1,000 %), which was not consistent with our result with lower transmittance percentages. Therefore, we concluded that the shift might due to uneven transmittance efficiencies. Further investigation would help validate these results and determine if autofluorescence is occurred, as it was not measured in our study. As using these eyewear protections (welding goggles and sunglasses), it would reduce the effect of blue light hazard and allow users to stay under an intense blue light environment for a longer period of time. However, it is unknown if all other glasses would have a similar effect on changing spectral

composition to lower energy of wavelength, as seen with the tested glasses in this study. This observation would therefore suggest that users would need to examine transmitted spectrum of eyewear protection using a spectroradiometer, in case that transmitted spectra appear to shift to higher energy of wavelength or a narrower spectrum is presented.

Transmittance performance

In the experiment 1, we found that the transmittance performances of all the tested eyewear protections were not influenced by the materials and tinted colors but were dependent on the types of glasses. These observations are similar to the results found by Adrian et al. (1976) and Borgwardt et al. (1981). Both studies concluded that no correlation regarding cost, lens color, or composition was found to predict the performances of the tinted lenses in the visible spectrum (Adrian et al., 1976; Borgwardt et al., 1981). However, it is contrary to the conclusion made by a recent study (Abdulrahim et al., 2015). Abdulrahim et al. (2015) examined 20 commercial eyeglasses from local markets, and concluded that the materials and tinted colors of glasses had an impact on transmittance performance. Some previous studies have shown that certain lens colors enhances contrast, color vision, and visual acuity (Kelly, 1990; Wolffsohn et al., 2000), but not much data is available on light transmittance performance with respect to lens colors. In the current study, no correlation was found between lens colors and transmittance for the tested sunglasses across the test wavelength range, however, the colors of the lenses only included black and brown. As such, we cannot conclude the interaction between lens color and transmittance performance (Adrian et al., 1976; Borgwardt et al., 1981).

Amongst all types of the tested eyewear protections examined in this study, the welding goggles had the highest light reduction performance as expected, since this type of goggle was designed for protecting a welders eyes from intense radiation (such as an electric arc, sparks, debris, or even heat in a welding work places (Finch, 2007; Tenkate, 1998)). The measured data indicates that welding goggles can minimize ocular light hazard but might cause low visibility for LED owners in an electrical lighting environment due to their high light-reduction performance. Wearing welding goggles enhances owners' feasibility and ensures safety against

any intense light hazard, especially for UV light and infrared radiation, but it might decrease the LED owners' color perception in an electrical lighting environment. Furthermore, low labor productivity and occupational health hazards may occur for workers equipped with this eyewear protection due to low visibility and color discrimination. As for the polarized glasses, the eyewear protection type that had the second highest light-reduction performance in this study, they are recommended for outdoor activities, since they are designed to block intense reflected light from surfaces such as smooth water as they reduce glare through the polarization coating on the lenses. As our measured data showed, all the tested polarized glasses had proper light transmittance performances across the visible wavelength according to the selection guideline of sunglasses recommended by Fishman (1986). Although a few sets of polarized sunglasses had higher transmittance percentages in far red light, overall, they still reduced a significant amount of visible and far red light and were better than the tested sunglasses. In greenhouses where electrical luminaires are used, glare might be caused from luminaires or smooth surfaces (Takao, 2016). These direct and reflected glares can disable labors' visibility and cause stress in such an environment. Equipping workers with polarized glasses could avoid these visual discomforts in closed LED lighting environments and provide better color discrimination due to their glare-free design and proper transmittance, unlike high light-reduction welding goggles. When evaluating the performances of the tested sunglasses, we found that they transmitted far red light nearly freely, even though they can greatly decrease the level of exposure to UV radiation and moderately block other wavelengths in the visible spectrum. Similar transmittance patterns for sunglasses were found in other studies (Abdulrahim et al., 2015; Gursoy et al., 2015). Although sunglasses normally were worn to reduce brightness during daily activities, they cannot protect against infrared radiation from the sun. Any person wearing it as protection from infrared radiation, either from natural sources or others, increases the risk of an eye injury on themselves. For the safety goggle, we found that their capacities to reduce light are not unified. Safety glasses, therefore, would be suggested for LED user for ocular LED safety if their transmittances are examined.

In experiment 2, the transmittance performances of the eyewear protections were examined using three different nominal wavelength LEDs (Table 4), and found

to be independent of irradiance levels. This indicates that LED owners can use any irradiance level to examine transmittance performances of eyewear protections with their own LED system, and then select better protection that corresponds to the actual light levels used in their operation.

3.5 Suggestion and recommendation

Consumers and workers can select polarized glasses for the light environment with the wavelength of 400-700 nm based on our measured results. Sunglasses would not be advised as personal eyewear protection if LEDs emitting infrared wavelengths are used. Regarding the welding goggles, we suggest their use as a protective equipment when intense irradiances are used due to their high light-reduction nature. Simplified exposure limit values for the wavelength from 180-3000 nm in IEC 26471 and the EU Directive 2006/25/EC were listed in the Table 5 (European Union, 2006; International Electrotechnical Commission, 2006). The limits listed in the table are only the recommended values for the longest exposure durations for each hazard (or wavlength ranges). Noted that as users assess the light environment with the guidelines from the standards, spectral weighting functions (e.g. blue light and infrared readiation) should be considered as the irradiances are acquaired from any types of light sensors. The detailed spectral weighting functions and spectral weighting exposure limits can be found in the EU directive 2006/25 and Leccese et al. (2014).

Although there are no current selection guidelines for eyewear protection in an LED lighting environment, several recommendations such as total transmittance for sunglasses selection were made for general use (Adrian et al., 1976; Fishman, 1986). LED owners can obtain detailed knowledge from these studies and consider these recommendations as a selection criteria, based on their specific lighting environment.

Table 5. The simplified exposure limit values from 180-3000 nm (adapted from the EU directive 2006/25/EC and IEC 26471) (European Union, 2006; International Electrotechnical Commission, 2006). The full table can be found in the standards.

Wavelength (nm)	Hazard name	Exposure limit value		
		EU directive 2006/25/EC	IEC 26471	
180-400	Actinic UV	30 $J \cdot m^{-2}$ (8 h daily)	$30 \cdot t^{-1}$ $W \cdot m^{-2}$ ($t < 3{,}000$ sec)	
315-400	Eye UVA	10^4 $J \cdot m^{-2}$ (8 h daily)	$10000 \cdot t^{-1}$ $W \cdot m^{-2}$ ($t \leq 1{,}000$ sec) 10 $W \cdot m^{-2}$ ($t > 1{,}000$ sec)	
300-700	Blue light	0.01 $W \cdot m^{-2}$ ($t > 10{,}000$ sec)	*Skin and cornea*[1] $100 \cdot t^{-1}$ $W \cdot m^{-2}$ ($t \leq 100$ sec) 1.0 $W \cdot m^{-2}$ ($t > 100$ sec)	*Retina* $10^6 \cdot t^{-1}$ $W \cdot m^{-2} \cdot sr^{-1}$ ($t \leq 10{,}000$ sec) 100 $W \cdot m^{-2} \cdot sr^{-1}$ ($t > 10{,}000$ sec)
380-1400	Retinal thermal	$2.8 \cdot 10^7 \cdot C_a^{-1}$ $W \cdot m^{-2} \cdot sr^{-1}$ (for $t > 10$ sec)[2]	$6000/a$ $W \cdot m^{-2} \cdot sr^{-1}$	
780-3000	Eye Infrared	100 $W \cdot m^{-2}$ ($t > 1{,}000$ sec)	$18000 \cdot t^{-0.75}$ $W \cdot m^{-2}$ ($t \leq 1{,}000$ sec) 100 $W \cdot m^{-2}$ ($t > 1{,}000$ sec)	
380-3000	Skin thermal	$20000 \cdot t^{0.25}$ $J \cdot m^{-2}$ ($t < 10$ sec)	$20000 \cdot t^{-0.75}$ $W \cdot m^{-2}$ ($t < 10$ sec)	

[1]for small light source defined as one with $\alpha < 0.011$ rad.

[2]$C_a = 11$ for $\alpha \leq 11$ mrad, $C_a = a$ for $11 \leq \alpha \leq 100$ mrad, and $C_a = 100$ for $a > 100$ mrad.

To date, there are no occupational health or ocular safety regulations that can be directly applied for horticultural LED luminaires or lighting systems. Many LED owners have been working under intensive horticultural LED luminaires. In this study we compared the transmittance performances of 12 different eyewear protection equipments in 10 different high-irradiant colored LED assemblies which had nominal wavelengths between 405-735 nm (across 380 to 750 nm). Our results

indicate that 58 % (7/12) of the tested eyewear protection products could block 80 % of high irradiance light and were adequate as protective personal equipment against light hazards in wavelengths between 400-700 nm. Consumers and workers using LEDs in this wavelength range can select welding goggles and polarized glasses to avoid ocular light hazards. Sunglasses and safety goggles, however, are potentially inadequate to protect one's eyes from infrared LEDs or LEDs emitting wavelengths above 700 nm. We suggest that LED users should nonetheless acquire transmitted spectrum data of their respective eyewear protection before using it under potential light hazards.

Connecting text

Chapter 4, “Short-term exposure of sun and shade plant leaves to high irradiance using LEDs”, is authored by Bo-Sen Wu and Mark Lefsrud and will be submitted as a manuscript for peer review (*Journal of Plant Physiology*).

Plant responses under extreme high irradiance have been quantified under sunlight or a broad-spectrum of light, but not under narrow spectrum LED light. In Chapter 4, we quantified how plants respond to intense LED light through spectral and optical measurements using the filtering lenses described and used in Chapter 3.

CHAPTER 4

Short-term high irradiance exposure to sun and shade plant leaves using narrow-spectrum LEDs

Abstract

A plant's response to high light conditions using sunlight and conventional lighting systems has been well documented. Using light emitting diode (LED) assemblies that eliminate mixed wavelength effects, we investigated the optical and spectral properties of representative "sun plant" (tomato; *Solanum lycopersicum* cv. Beefsteak) and "shade plant" (lettuce; *Lactuca sativa* cv. Breen) leaves, when irradiated with up to 500 $W \cdot m^{-2}$ of light (equivalent to ~2500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Contrary to the established body of literature on sun and shade plants' responses to high light, the presence or absence of photobleached leaf pigments with the experimental light conditions suggests that lettuce leaves (shade plant) are able to tolerate higher irradiance levels of blue (470 nm) and green (530 nm) LED light than tomato leaves (sun plant). We hypothesize that shade plants are not true shade plants; rather, they may be considered as "blue-light-spot plants" that have a superior ability to tolerate high blue light and prepare for the next light beam using blue light as a regulator. Data further indicate that plants may have different photoprotection and photoinhibition mechanisms in photosystems that are wavelength-dependent. Most importantly, observed photobleaching when different leaf transmittance and reflectance measurement apparatuses are used, suggests that light directionality plays an important role in photosynthetic capacity. Taken together, observations made in this study suggest that the effect of monochromatic light on a higher plant's physiological response to high light conditions merits further investigation.

4.1 Introduction

Damage induced by high light conditions to the photosynthetic apparatus and leaves of higher plants has been well documented (Powles, 1984; Takahashi and Badger, 2011). As high levels of light penetrate the various tissue layers of an individual leaf, several photoprotection mechanisms are triggered, resulting in changes to the optical property of leaves, inhibition of leaf photosynthetic capacity (photoinhibition), and irreversible pigment photobleaching (photooxidation). Plant mechanisms involved in interacting with the light environment at the leaf level include leaf orientation and folding, enhanced reflectance through salt deposition and the formation of epicuticular wax layers (Smillie and Hetherington, 1999; Steyn et al., 2002). Within the leaf, internal measures taken to cope with excessive light energy include chloroplast movement, heat and fluorescent emissions, and the accumulation of screening compounds (e.g. anthocyanins and betalains) (Akoyunoglou and Anni, 1984; Boardman, 1977; Cosgrove, 1981; Sæbø et al., 1995; Schuerger et al., 1997; Senger, 1982; Tucker and Garratt, 1977). Knowledge of how leaves respond to different light conditions can be partially acquired and understood by determining the optical and spectral properties of leaf tissue.

Plants may be classified as sun or shade plants based on their photosynthetic capacities under low and high light conditions (Boardman, 1977). In general, sun and shade plants have contrasting photosystem characteristics, including total chlorophyll concentration per reaction center, photosystems I to II and chlorophyll b: chlorophyll a ratios, as well as photosynthetic electron transport capacity (Boardman, 1977; Sarijeva et al., 2007; Taiz and Zeiger, 2002). Sun and shade plants also exhibit differences in leaf anatomy and chloroplast structures that influence their ability to adapt to high light conditions. For instance, larger and irregularly arranged chloroplasts are observed in the leaves of shade plants when compared to chloroplasts of sun plants, which increase light absorptance efficiency under dim diffuse light (Boardman, 1977). Sun plants exhibit lower sensitivity to photoinhibition than shade plants, due to different photosystem II (PSII) repair cycle rates and redox state regulation (Öquist et al., 1992). In addition, different abilities when recovering from photoinhibition allow sun plants to grow better than shade plants when exposed to high light. This knowledge of how sun and shade plants

behave under various light conditions is based on studies of plants grown under broad spectrum light, such as sunlight or electrical lighting systems designed to simulate sunlight (Powles, 1984). As such, it is possible that such investigations of plants' physiological responses to high intensity light under broad light spectra may actually include plant responses to interactive effects of different wavelengths that are not yet fully understood.

With ongoing developments in light emitting diode (LED) technology, the use of LEDs in horticultural applications and plant studies has increased because of the availability of a wide spectral range, controllability of light intensity, and their small and relatively cool light emitting surface temperature, all of which cannot be achieved with conventional lighting (Morrow, 2008; Olle and Viršile, 2013). The low heat emission of LEDs allows them to be placed in close proximity to plants, creating high light conditions without heat stress. Previous studies have tested the effects of light spectral compositions and irradiance outputs on plant productivity and photomorphogenesis with LED assemblies emitting light up to 800 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (Bula et al., 1991; Deram et al., 2014; Johkan et al., 2010; Massa et al., 2005; Massa et al., 2008). Furthermore, studies on leaf optical properties of different greenhouse crops used broad and narrow spectrum light (Bergstrand et al., 2016; Critten, 1991; Massa et al., 2015). These studies either only conducted transmittance measurements or did not report irradiance levels. How plants response to high irradiance levels (greater than 800 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) using narrow spectrum light remain unknown. The objective of this study was to investigate the spectral and optical properties of representative sun and shade plants when grown under high irradiance levels, up to 500 $W \cdot m^{-2}$ (equivalent to ~2500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), via optical (reflectance, transmittance, and absorptance) and spectral property measurements using narrow spectrum LEDs. The data reported herein provide important information on the impact of monochromatic and high-irradiant light on higher plants.

4.2 Materials and methods

Plant germination and growth chamber condition

Tomato (*Solanum lycopersicum* cv. Beefsteak; lot A1, OSC, Ontario, Canada)

(sun plant) and lettuce (*Lactuca sativa* cv. Breen; pelleted MT0 OG, Johnny's Selected Seeds, Winslow, ME, US) (shade plant) were used as model plants for the experimental light conditions. Tomato and lettuce seeds were sown into rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a growth chamber (TC30, Conviron, Winnipeg, Canada) under cool-white fluorescent bulbs (4200 K, F72T8CW, Osram Sylvania, Wilmington, MA, US) at an average irradiance level of 55 $W \cdot m^{-2}$ (equivalent to ~250 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) with a 16-h photoperiod. The plants were exposed to a day/night temperature of 23/21 ± 1 ° C, and they were provided with full strength Hoagland nutrient solution every other day as described by Hoagland and Arnon (1950). Three plants were selected for experimentation 30 to 40 days after germination. The plants selected were consistent in size and age, and outliers in appearance were not included in the experiments.

Light sources

The light sources used in this study included a xenon bulb and three high-irradiance LED assemblies of nominal wavelengths: 470 nm, 530 nm and 655 nm (Philips-Lumileds, San José, CA). The relative spectral irradiance composition of these light sources is shown in Figure 15. The xenon bulb was used to establish a baseline for plant optical and spectral properties across the 400–750 nm spectrum at an irradiance level of 20 $W \cdot m^{-2}$ (equivalent to 70 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), prior to high irradiance lighting tests. Selection of the 470 nm and 655 nm LED assemblies was based on typical wavelengths used for plant growth in the blue and red wavelength ranges. LED assemblies were mounted on a water jacket (ST-011, Guangzhou Rantion Trading Co., Guangdong, China) and attached with cluster concentrator optics (25 mm focal length, No. 263, Polymer Optics, Wokingham, Berkshire, UK). Coolant set at 0 °C was circulated through the water jacket by using an Isotemp bath circulator (4100R20, Fisher Scientific, Hampton, NH, US) to dissipate heat generated by the LED assembly. The coolant prevented heat radiation interference when conducting experimentation and maintained the LEDs' optical characteristics (e.g. peak wavelengths and spectral irradiance compositions). The optic cluster concentrator concentrated all rays from the diodes on the LED assembly onto a small focal spot (12 mm diameter). Each LED assembly was powered by a DC power supply (DP832, Rigol Tech., Beaverton, OR, US) in a current-control mode

(20-3000 mA depending on the irradiance level). Measurements were made under the following LED irradiance levels: 20 $W \cdot m^{-2}$, 100 $W \cdot m^{-2}$, and 500 $W \cdot m^{-2}$. The irradiance levels and photosynthetic photon flux densities of each LED treatment are presented in Table 6.

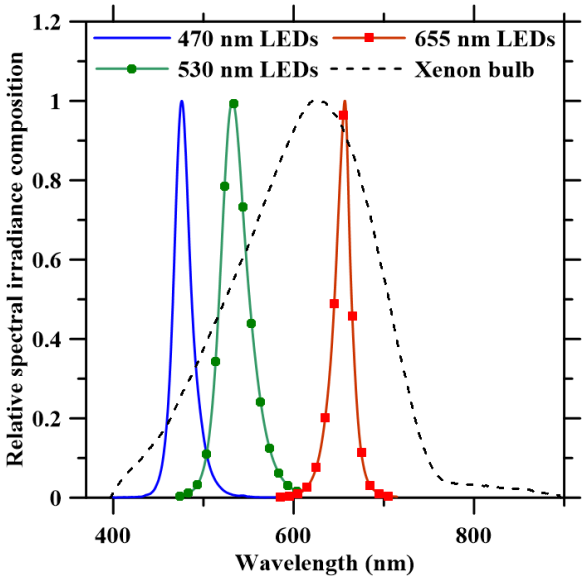

Figure 15. Relative spectral irradiance composition of xenon bulb and light-emitting diode (LED) assemblies. Spectral irradiance distributions were measured with a spectroradiometer (PS-300, Apogee).

Table 6. Irradiance levels and photosynthetic photon fluxes for the 470-nm, 530-nm, and 655-nm LED assemblies.

Nominal wavelength (nm)	20 $W \cdot m^{-2}$	100 $W \cdot m^{-2}$	500 $W \cdot m^{-2}$
	Photosynthetic photon flux ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)		
470	85.7	428.3	2141.3
530	89.8	449.2	2245.8
655	99.3	496.3	2481.3

Measurement apparatus and procedures

Optical and spectral properties of the plant leaves (transmittance, reflectance, and spectral composition) were obtained with and without (blank) a leaf using a spectroradiometer (PS-300, Apogee) and a 45° reflectance probe (AS-003, Apogee), from 231 nm to 1100 nm at 0.5-nm intervals. Four apparatus setups were required,

based on the different light sources and measurements recorded (Figure 16). When the xenon bulb was used as the light source (baseline testing), the leaf was placed between the bulb and the spectroradiometer to measure leaf transmittance (Figure 2A), and between the reflectance probe and a perfect absorptance (black) plate to measure leaf reflectance (Figure 16B). To measure transmittance when using an LED assembly as the light source, the LED assembly was positioned 25 mm above the spectroradiometer, and the leaf was placed onto the sensor (Figure 16C). The irradiated spherical area from the concentrated LED source was approximately 110 mm^2. To measure leaf reflectance using the LED assemblies, the leaf was placed between the probe and the clamp to secure the leaf in place (Figure 16D). The incident irradiance level could not be measured directly; therefore, a white halon reference standard (97 % reflectance from 300-1700 nm, AS-004, Apogee) was used to acquire incident irradiance levels.

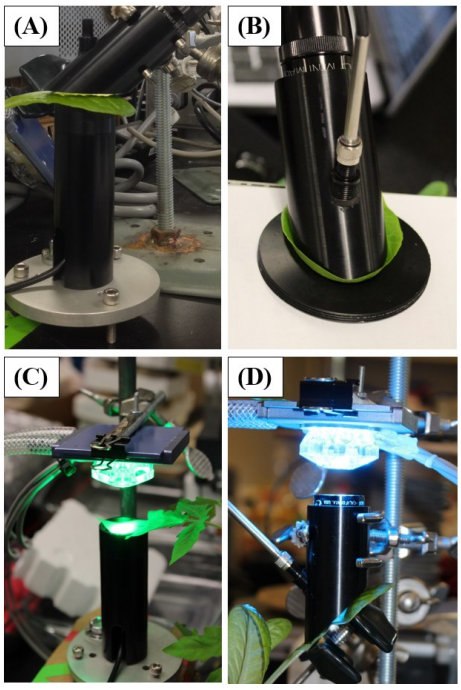

Figure 16. Apparatus setup for leaf transmittance and reflectance measurements. For the xenon bulb, leaf transmittance was measured using (A) and leaf reflectance was measured using (B). For the LED assemblies, transmittance was measured using (C) and leaf reflectance was measured using (D). LED assembly. When measuring leaf reflectance with the LED assemblies, the leaf was secured by the holder and rays that transmitted through the leaf went through the holder, avoiding any transmitted ray interference.

Each apparatus that was used to measure leaf transmittance or reflectance exhibited different ray profiles (directionality) due to the concentrated lens and the reflectance probe (Figure 17). Rays emitted from the transmittance and reflectance apparatuses to the leaves were composed of a mixture of perpendicular and intersecting rays and uni-directional rays, respectively. By using concentrated optics, rays striking the leaf surface hit a single spot; these included direct rays from the center LED diode (the perpendicular rays) and the intersecting rays from the LED diodes that were soldered in a circular arrangement on the LED base. When using the reflectance measurement apparatus, the leaf surface was struck by rays at a 45° angle due to the design of the reflectance probe.

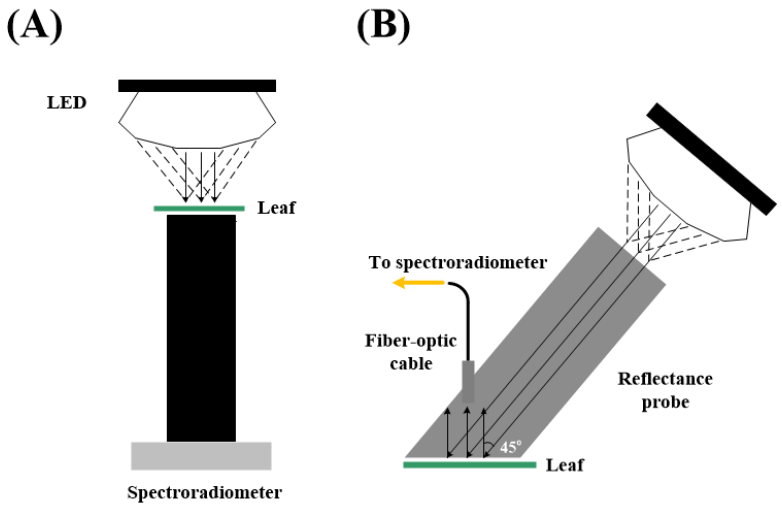

Figure 17. Diagram of A) leaf transmittance and (B) leaf reflectance measurement apparatuses when using an LED assembly. Arrows and dashed lines from the LED assemblies represented perpendicular and intersecting rays of the LED assembly, respectively. Under the reflectance apparatus, the intersecting rays from the LED assembly were absorbed in the reflectance probe. With this method, the only rays striking the leaf surface were at a 45° angle.

A filtering lens was placed on the spectroradiometer to measure incident irradiance levels (I_0) beyond the measuring limits of the spectroradiometer. To attenuate the light, a filtering lens was also placed between the light source and the probe for leaf transmittance and reflectance measurements. The incident irradiance levels were then calculated using the following equation:

$$I_0 = \frac{I_0'}{T}$$ Equation 5

Where I'_0 is the measured irradiance level that has passed through the filtering lens and T is the transmittance percentage of the filtering lens. Selection of the filtering lens and transmitted percentages were based on methods reported previously by Wu and Lefsrud (2018). To ensure constant corrected test irradiance levels, two sets of filtering lenses were used for each light configuration in this study: welding goggles (5444T, McMaster-Carr, Elmhurst, IL, US) and sunglasses (09612 90405, Chanel, Paris, France). When the filtering lens was needed to attenuate the light, one filtering lens was used to set the test irradiance levels, and the other was used to confirm the calculated incident irradiance. The difference in calculated incident irradiance levels made by the two sets of filtering lenses was less than 2 % for each light configuration.

Measurements for each light source were made in a dark room, using at least three mature, similarly sized plants with fully expanded leaves (three biological replicates). Tomato and lettuce leaves were randomly selected and remained attached to the plant. When using the xenon bulb, irradiance levels and spectral trace readings were recorded five minutes after the irradiance readings from the spectroradiometer. When using the LED assemblies, the reading from the spectroradiometer included spectral irradiance compositions and total transmitted/reflected irradiance levels; these were recorded upon initiation of the light treatment and every 10 minutes for an hour. Spectral irradiance compositions were recorded with and without LED light, to eliminate noise signals/background noise from the spectroradiometer. When the LED light was off (< 10 sec), leaf appearance was scrutinized for the presence of photo-bleaching or leaf coloration caused by the LED light treatments. After 1 h of LED light exposure, plants without any apparent photo-bleaching or leaf discoloration were removed from the apparatus and returned to the growth chamber for 14 days to observe any further changes caused by the light exposure. If photobleaching or leaf discoloration became apparent, images of the leaves were taken with a digital camera. Black image

backgrounds were positioned using ImageJ software (Bethesda, MD, US) to provide contrast. If photo-bleaching or leaf discoloration was detected visually but could not be seen clearly in the original images, the images were then presented in 8-bit black and white format (grayscale) to demonstrate contrast between the discolored and apparently normal leaf areas.

Data analysis

Total transmitted and reflected irradiance levels were averaged and compared to incident light levels without a leaf in the beam path. If the optical readings did not stabilize until the end of the measurement period, or if readings did not stabilize because of photobleaching or leaf discoloration, the last three data points were taken and averaged. Absorptance (A) of each leaf was calculated by subtracting the summed transmittance (T) and reflectance (R) from the total measured irradiance of the light source [A= 1- (T + R)]. The optical properties of the leaf were averaged and presented as a percentage.

Statistical analysis

Statistical analysis was done using JMP 10 (SAS, Cary, NC, US). Tukey-Kramer's HSD was used for multiple comparisons among irradiance treatment means from significant one-way analysis of variance (ANOVA) tests ($p < 0.05$). No between-crop and wavelength comparisons were conducted.

LED photobiological safety during the measurement

Although all the light beams from the LED light sources that provided high irradiance were focused with cluster concentrator optics and faced down toward the test leaves, a proper set of safety glasses (Wu and Lefsrud, 2018) or a box covering the measurement apparatus was applied to prevent any possible ocular light hazards during experimental trials.

4.3 Results

Optical properties under xenon bulb lighting

The spectra of optical properties (leaf transmittance, leaf reflectance, and calculated absorptance) for tomato and lettuce leaves exposed to irradiance levels of 20 $W \cdot m^{-2}$ using a xenon bulb are listed in Figure 18. Overall, tomato leaves had lower transmittance and reflectance percentages than lettuce leaves across the measured wavelength range (400–750 nm), but both plant species exhibited similar transmittance and reflectance spectra. For wavelengths between 480 nm and 700 nm, both plant species had combined leaf transmittance and reflectance maximums at 546 nm, and minimums at 485 nm and 675 nm. In the blue wavelength range (400–480 nm), the leaf reflectance percentages were nearly zero, but transmittance percentages were between 10 % and 20 % for both tomato and lettuce leaves. The average transmittance and reflectance percentages for tomato leaves were 11.0 ± 0.6 % and 5.1 ± 0.7 %, respectively, and 19.9 ± 1.5 % and 9.0 ± 1.5 % for the lettuce leaves, respectively. The absorptance spectrum characteristics of both species had maximum peaks at 480 nm and 685 nm (~90 % absorptance), and a valley at 550 nm (50–70 % absorptance). Average absorptance percentages were 69.5 ± 1.0 % and 83.0 ± 0.9 % for tomato and lettuce leaves, respectively. The maximum difference in the calculated absorptance percentages between the two plant species was ~20 %, between 530 nm and 570 nm.

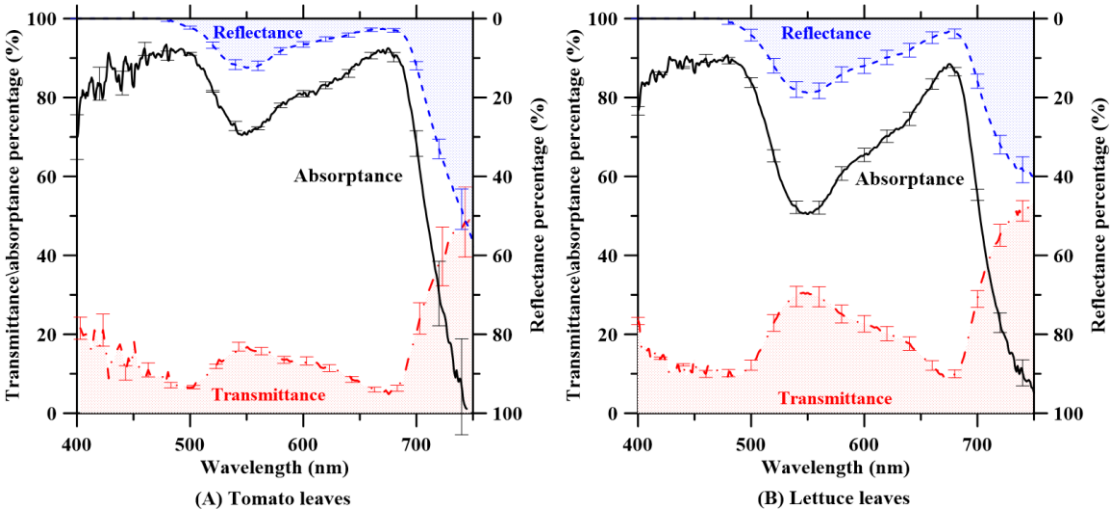

Figure 18. Optical properties spectra (leaf transmittance, leaf reflectance and calculated absorptance) of (A) tomato and (B) lettuce leaves using a xenon bulb with an irradiance level of 20 $W \cdot m^{-2}$.

Optical properties of tomato and lettuce leaves during the measurement period

Optical property response curves for tomato and lettuce leaves under 470-nm and 655-nm LED assemblies for 1 h are depicted in Figure 19. Data were normalized to measurements taken upon initiation of light exposure. With the exception of data recorded at 655 nm, optical properties of the leaves increased as exposure time increased, but different trends were observed when plant species, different optical properties, irradiance levels, and LED wavelengths were considered. Response curves with the 530-nm LED assembly are not depicted, as no change was observed. This same observation was made with the 655-nm light treatment at low irradiance levels.

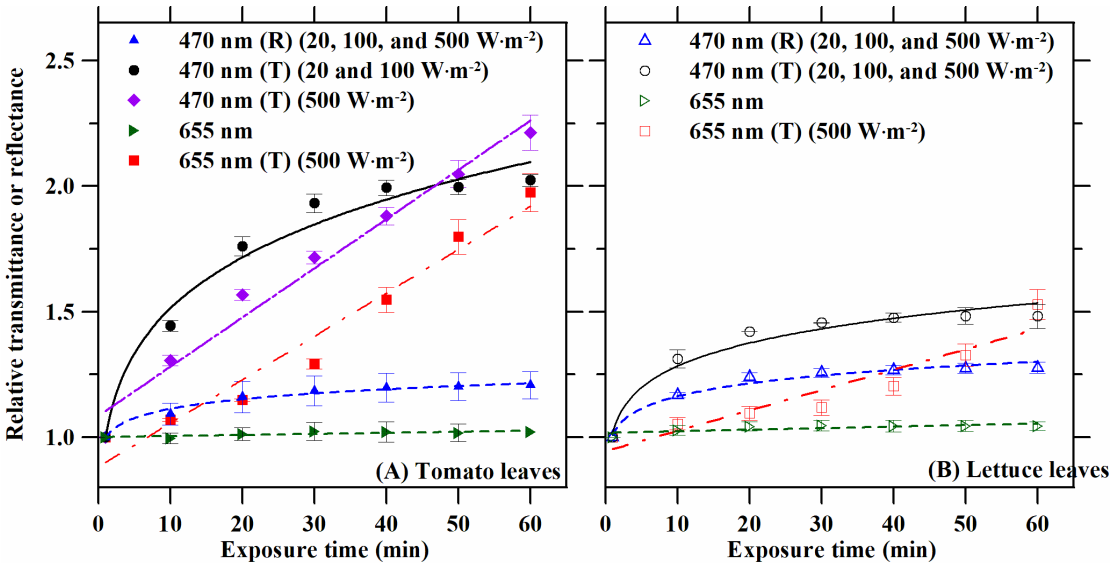

Figure 19. Relative leaf transmittance and reflectance percentages measured using 470-nm and 655-nm LED assemblies for (A) tomato leaves and (B) lettuce leaves exposed to different light treatments over a 1-h period. R and T represent reflectance and transmittance, respectively. The 655 nm data (green triangles) represent transmittance at 20 $W \cdot m^{-2}$ and 100 $W \cdot m^{-2}$, and reflectance at 20 $W \cdot m^{-2}$, 100 $W \cdot m^{-2}$, and 500 $W \cdot m^{-2}$.

Three main observations can be made from these data. First, greater changes in plant optical properties over the exposure period were observed for tomato leaves when compared to lettuce leaves for all experimental light conditions, with the exception of leaf reflectance under the 470-nm LED light assembly. Second, when

comparing the optical properties measured, changes in leaf transmittance were higher than leaf reflectance measurements taken under the same wavelength and irradiance levels for both species. After 1 h exposure to 470-nm LED light with an irradiance level of 20 $W \cdot m^{-2}$, leaf transmittance and reflectance percentages increased by ~100 %, and 20 % for tomato leaves, respectively, and by ~50 % and 30 % for lettuce leaves, respectively.

Finally, when considering the effect of irradiance levels on the optical properties of sun plant and shade plant leaves, different curve patterns for transmittance were observed for tomato leaves, but not for lettuce leaves exposed to 470-nm LED light. Response curves for tomato leaves were hyperbolic at lower irradiance levels ($\leq$ 100 $W \cdot m^{-2}$), but these curves became linear when the irradiance level increased to 500 $W \cdot m^{-2}$. In contrast, response curves for lettuce leaves were hyperbolic at all tested irradiance levels. A similar linear response curve was observed for lettuce leaves between the 470-nm and 655-nm light treatments at 500 $W \cdot m^{-2}$. Transmittance percentages of tomato and lettuce leaves both increased linearly as the exposure time increased for the 655-nm light treatment at 500 $W \cdot m^{-2}$. For data collected under 655-nm light that are not presented in Figure 19, no changes in transmittance and reflectance were observed. For all tested irradiance levels, optical properties measured for both plant species under the 530-nm light treatments remained stable (< 3 % differences).

Optical properties under the LED light (20–500 $W \cdot m^{-2}$)

At an irradiance level of 20 $W \cdot m^{-2}$, optical properties measured with the xenon bulb were slightly higher than those measured with the LED assemblies (Figure 18 and Figure 20). For tomato leaves, the difference between the two light sources was within 2 %. For lettuce leaves, the differences varied depending on the optical properties measured. Leaf transmittance and reflectance percentages of the lettuce leaves measured with the xenon bulb were approximately ~6 % higher and ~5 % lower when measured with the LED assemblies, respectively.

Under the 470-nm LED assembly, the average leaf transmittance and reflectance percentages were 5.3 ± 2.1 % and 3.7 ± 0.1 % for tomato leaves, and 7.0

± 1.9 % and 8.2 ± 0.7 % for lettuce leaves. Leaf reflectance percentages did not change as irradiance levels increased for both plant species; however, the transmittance percentages showed opposing responses between plant species to increased irradiance levels. For the tomato leaves, the transmittance percentage decreased by ~50 % and increased by ~50 % as the irradiance levels increased from 20 $W \cdot m^{-2}$ to 100 $W \cdot m^{-2}$ and 100 $W \cdot m^{-2}$ to 500 $W \cdot m^{-2}$, respectively. In contrast, transmittance percentages of the lettuce leaves under the 470-nm LED assembly increased by ~25 % and decreased by ~40 % as irradiance levels increased from 20 $W \cdot m^{-2}$ to 100 and 100 $W \cdot m^{-2}$ to 500 $W \cdot m^{-2}$, respectively. Under the 530-nm LED assembly, the average transmittance and leaf reflectance percentages were 12.9 ± 0.7 % and 11.9 ± 0.5 % for the tomato leaves, and 14.4 ± 3.5 % and 20.0 ± 4.2 % for the lettuce leaves, respectively. In addition, the irradiance levels did not impact optical properties of the tomato leaves, but they did for lettuce leaves. Specifically, a balancing effect between the leaf transmittance and reflectance percentages was observed as the irradiance levels increased, particularly from 100 $W \cdot m^{-2}$ to 500 $W \cdot m^{-2}$, and transmittance and reflectance percentages for the lettuce leaves decreased by ~40 % and increased by ~35 %, respectively. Average transmittance and reflectance percentages measured under the 655-nm light treatments were 8.0 ± 2.3 % and 4.7 ± 0.7 % for the tomato leaves, and 10.1 ± 1.1 % and 9.8 ± 0.5 % for the lettuce leaves, respectively. Similar trends in transmittance and irradiance levels were noted under the 655-nm and 470-nm light treatments for tomato leaves, in which transmittance percentages decreased and then increased with greater irradiance levels.

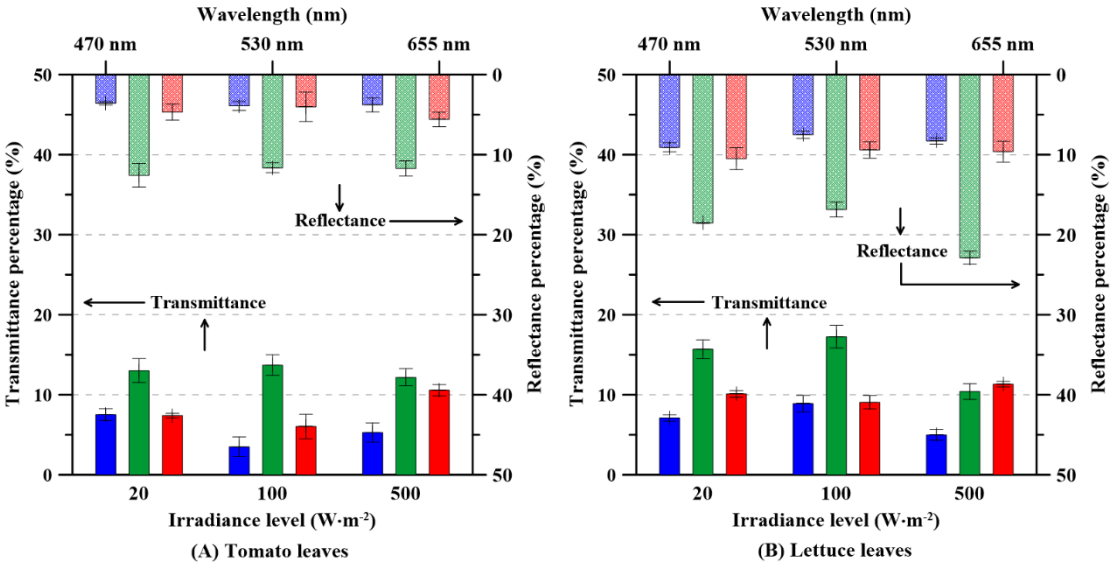

Figure 20. Leaf transmittance and reflectance percentages of (A) tomato and (B) lettuce leaves examined under the 470-nm, 530-nm, and 655-nm LED assemblies with irradiance levels of 20 W·m^{-2}, 100 W·m^{-2}, and 500 W·m^{-2}. The blue, green and red bars represent LED wavelengths of 470 nm, 530 nm, and 655 nm, respectively.

Table 7. Absorptance (%) of tomato leaves and lettuce leaves irradiated at 20 W·m^{-2}, 100 W·m^{-2} and 500 W·m^{-2} using three 470-nm, 530-nm and 655-nm LED assemblies.

Plant	Irradiance level (W·m^{-2})	470 nm	530 nm	655 nm
		Absorptance (%, mean. ± SD)		
Tomato	20	88.8 ± 0.8^{a}	74.4 ± 1.8^{c}	89.4 ± 2.8^{d}
	100	91.2 ± 2.5^{b}	74.6 ± 1.8^{c}	90.5 ± 2.8^{d}
	500	90.9 ± 0.3^{b}	75.0 ± 0.9^{c}	87.7 ± 2.5^{d}
Lettuce	20	83.8 ± 0.7^{a}	65.7 ± 1.2^{c}	79.3 ± 1.7^{d}
	100	83.6 ± 0.5^{a}	65.9 ± 1.2^{c}	81.5 ± 1.1^{d}
	500	86.8 ± 1.0^{b}	64.7 ± 3.4^{c}	79.0 ± 1.3^{d}

Absorptance (%) of the tomato and lettuce leaves at different irradiance levels was calculated from measured leaf transmittances and reflectance percentages and summarized in Table 7. Overall, absorptance was not impacted by increased irradiance, with the exception of lettuce leaves exposed to 500 W·m^{-2} under the 470-nm LED assembly. Absorptance of the tomato leaves was 6–10 % higher than the lettuce leaves.

Spectral properties of tomato and lettuce leaves

The spectral properties (spectral irradiance compositions) of the transmitted and reflected spectra for tomato leaves and lettuce leaves were measured and analyzed for all test LED light conditions. Apart from the LED spectra transmitted through or reflected by the leaves, fluorescent spectral readings (fluorescence emissions) were observed between 600 nm and 800 nm when measuring leaf reflectance under the 470-nm and 530-nm LED assemblies (Figure 21). When the 530-nm LED assembly was used, the relative fluorescent spectral readings presented only include the wavelengths above 650 nm, since the spectral composition below 650 nm has some residual spectral readings from the 530-nm LED assemblies. As for the 655-nm LED assembly, spectral readings between 600 nm and 800 nm only represent the transmitted and reflected spectra from the 655-nm light. No fluorescent spectral readings with the 655-nm light treatments were observed, as they were most likely buried by the 655-nm LED spectral readings. At all tested irradiance levels with the 470-nm and 530-nm LED assemblies, the peak wavelength of the reflected fluorescent spectral reading as determined by the spectroradiometer was 687 nm. When comparing relative fluorescent spectral readings between the two plant species under the same light conditions, spectral characteristics (peak wavelength and spectral readings) were almost identical, with the exception of wavelengths between 700 nm and 750 nm. In this waveband, tomato leaves had higher fluorescent emission (~10 %) than the lettuce leaves under the 470-nm LED assemblies. For the 530-nm LED assemblies, fluorescent spectral readings of the tomato leaves were slightly higher than the lettuce leaves, but this only occurred between 700 nm and 720 nm. Moreover, a secondary peak was observed at ~780 nm for both plant types under the 530-nm LED assembly; this was not observed for the 470-nm LED assembly. Different shapes for the fluorescent spectral readings were also observed when the leaves were irradiated with different wavelengths. When the tomato and lettuce leaves were irradiated under the 530-nm LED assembly, fluorescent spectral readings followed a saw tooth-shaped curve; however, readings under the 470-nm LED assembly appeared relatively smoother (Figure 21).

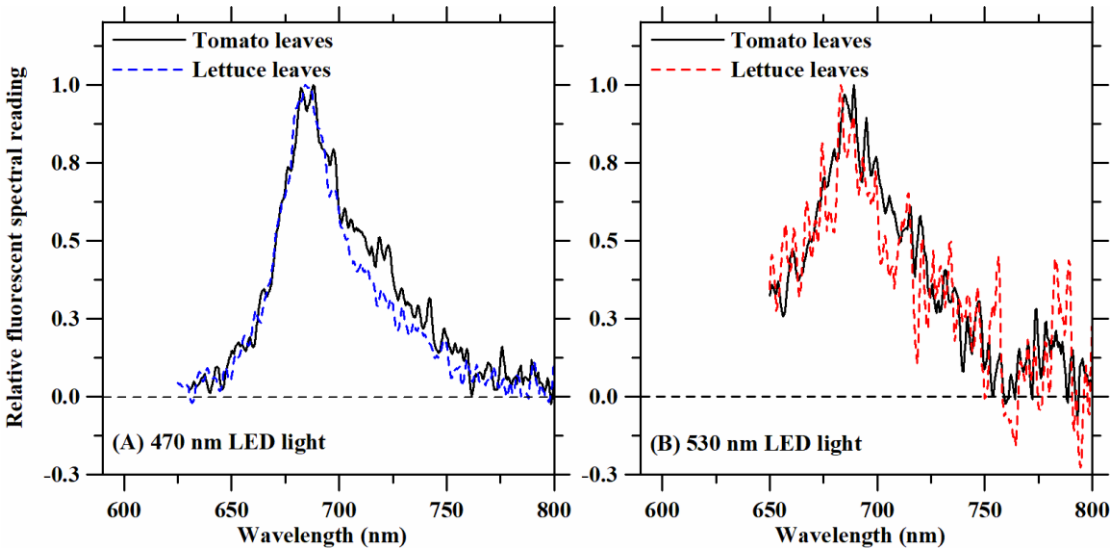

Figure 21. Relative fluorescent spectral readings of tomato and lettuce leaves under the leaf reflectance apparatus using the 470-nm and 530-nm LED assemblies. Data were normalized to the maximum reading of the fluorescent spectra (687 nm).

Fluorescent spectral readings were influenced by light exposure duration, and this response was plant species-dependent. Reflected fluorescent spectral readings of tomato leaves and lettuce leaves, upon initiation of exposure to 470-nm LED lighting and after 1 h at 20 $W \cdot m^{-2}$ are illustrated in Figure 22. Since fluorescent readings for different irradiance levels were the same, only data for irradiance at 20 $W \cdot m^{-2}$ are presented. The fluorescent spectral energy decreased as the irradiation time increased for both sun (tomato) and shade (lettuce) plant species, but the decrease observed for the tomato leaves was smaller than that of the lettuce leaves. After 1 h, the fluorescent spectral energy of the tomato and lettuce leaves decreased by ~15 % and 45 %, respectively. Further to this, the fluorescent spectral energy of the tomato leaves was nearly equal to that of the lettuce leaves after 1 h. When testing all irradiance levels with the 530-nm LED assembly, fluorescent spectral energy was not impacted by light exposure duration.

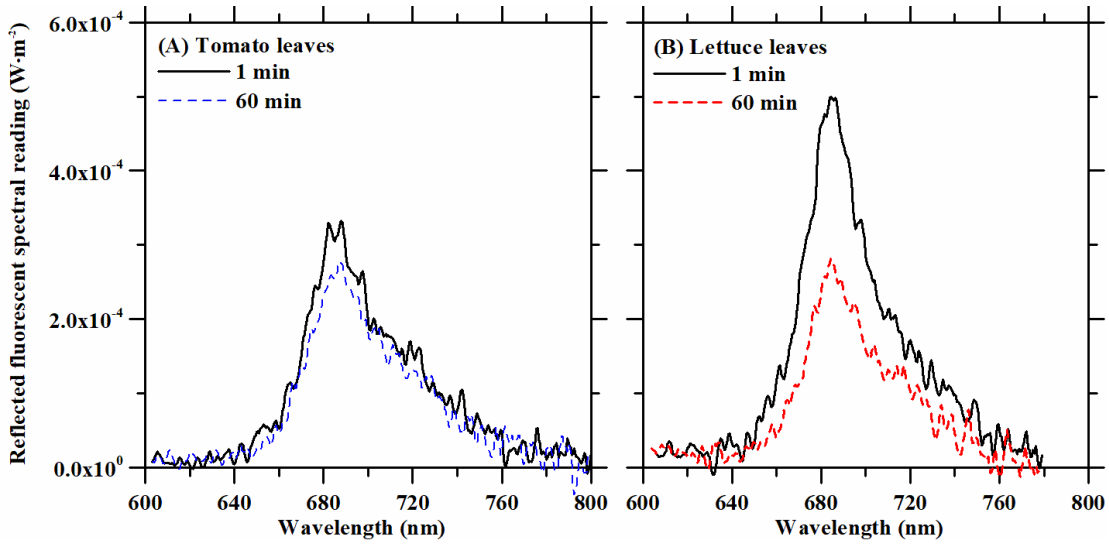

Figure 22. Comparison of the reflected fluorescent spectral readings over a period of one minute and 1 h for (A) tomato leaves and (B) lettuce leaves using the 470-nm LED assembly at an irradiance of 20 W·m^{-2}.

Pigment photo-bleaching and leaf coloration

Differences in leaf appearance were observed after measuring leaf transmittance with different wavelengths at 500 W·m^{-2} for both representative plant types (Figure 23 and Figure 24). Here, we assumed that pigment photo-bleaching occurred if the irradiated leaf area turned brown or white after exposure under the 470-nm and 655-nm LED assemblies. Leaf coloration remained mostly green when using the 530-nm LED assembly at 500 W·m^{-2}. With the 470-nm LED assembly, no photo-bleaching or changes in leaf coloration occurred in tomato and lettuce leaves during or immediately after the 1-h period at 500 W·m^{-2}. No further changes in leaf appearance were observed, even when this 1-h exposure to the 470-nm LED lighting was followed by exposure to fluorescent lights for 14 d in the growth chamber. In contrast, photo-bleaching was observed on all tomato leaves irradiated at 500 W·m^{-2} under the transmittance apparatus; this occurred 3 d after the 1-h exposure to LED lighting, when plants were placed back into the growth chamber with fluorescent lighting. With the 655-nm LED assembly, photo-bleaching appeared on both tomato and lettuce leaves after approximately 40 min exposure at 500 W·m^{-2} under the transmittance apparatus, yet slight differences in colour and appearance to the damage areas were observed between the two plant types. Damaged areas of the tomato leaves were larger and were browner when compared to lettuce leaves.

Specifically, photo-bleached areas were approximately ~110 mm^2 and ~50 mm^2 for the tomato and lettuce leaves, respectively. Most notably, when the reflectance apparatus was used at 500 $W \cdot m^{-2}$ with the 470-nm and 655-nm LED assemblies, no photo-bleaching or any apparent changes in leaf appearance were observed, either immediately after the 1-h exposure period or after being placed back into the growth chamber for 14 d.

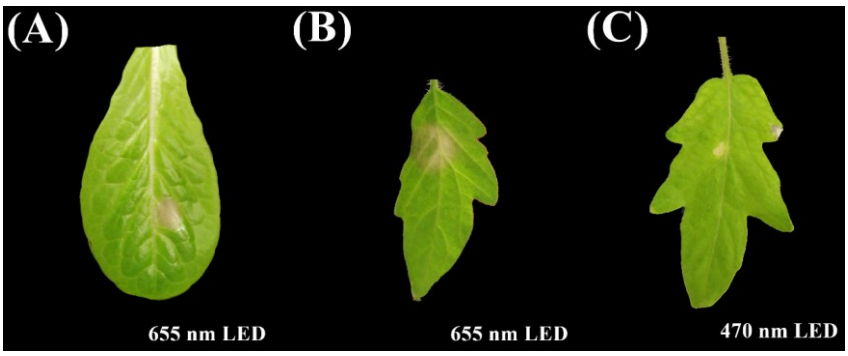

Figure 23. Representative images of tomato and lettuce leaves after exposure to 500 $W \cdot m^{-2}$ with different LED assemblies. (A) A lettuce leaf and a (B) tomato leaf were irradiated at 500 $W \cdot m^{-2}$ for 1-h with the 655-nm LED assembly; (C) a tomato leaf after irradiance at 500 $W \cdot m^{-2}$ for 1-h with the 470-nm LED assembly, followed by exposure to fluorescent light for 3 d in the growth chamber.

Figure 24 depicts representative tomato leaves placed under the transmittance and reflectance apparatuses with the 530-nm LED assembly at 500 $W \cdot m^{-2}$. Although the color of the irradiated leaf area changed after irradiance at 500 $W \cdot m^{-2}$ under the 530-nm LED assembly, the observed discoloration was different than the discoloration observed for the leaf areas irradiated with 500 $W \cdot m^{-2}$ with the 470-nm and 655-nm LED assemblies. When tomato leaves were irradiated under the transmittance apparatus at 500 $W \cdot m^{-2}$ with the 530-nm LED assembly, the irradiated leaf area changed to light green. Furthermore, a slight change in coloration (dozens of light green spots) was observed in the irradiated area with the reflectance apparatus (Figure 24B). No color changes for lettuce leaves were visually detected under the 530-nm LED assembly for all the tested irradiance levels. A summary of the observed photo-bleaching and discoloration responses when leaves were irradiated at 500 $W \cdot m^{-2}$ under different LED light assemblies and with different measurement apparatuses is provided in Table 8.

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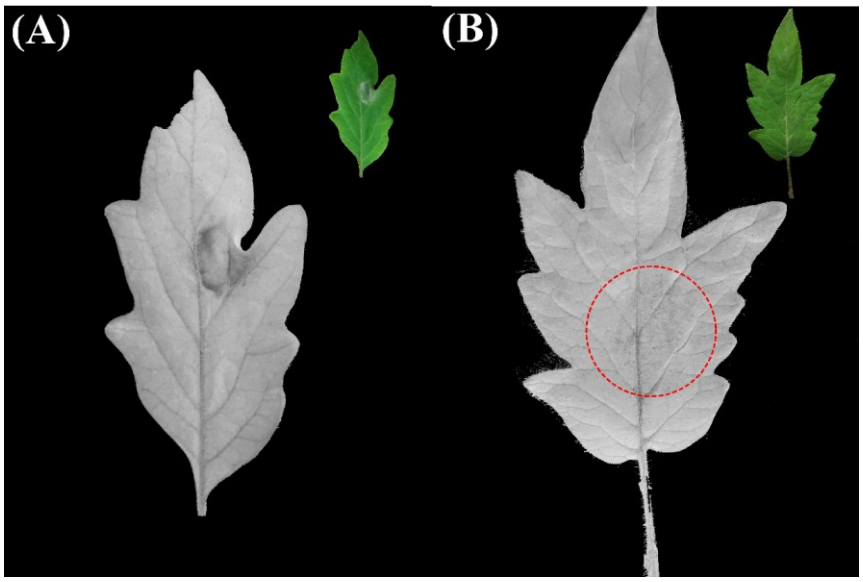

Figure 24. Changes in tomato leaf coloration after 1 h with the 530-nm LED assembly at 500 $W \cdot m^{-2}$ under (A) the transmittance and (B) reflectance apparatuses. The same leaf using grayscale is presented to demonstrate better contrast between the discolored and normal colored leaf areas.

Table 8. Summary of photo-bleaching (light-induced bleaching) of pigments and leaf discoloration in tomato and lettuce leaves irradiated at 500 $W \cdot m^{-2}$ under different LED light assemblies and measurement apparatuses. "O" and "Δ" represent observed photo-bleaching and discoloration of irradiated leaf areas, respectively. "X" indicated that no photo-bleaching or leaf coloration was observed.

Nominal wavelength (nm)	Tomato		Lettuce	
	Transmittance	Reflectance	Transmittance	Reflectance
470	O	X	X	X
530	Δ	Δ	X	X
655	O	X	O	X

4.4 Discussion

Low light plant responses (or baseline measurements)

Baseline leaf optical property spectra for tomato and lettuce leaves obtained using the xenon bulb were similar to those reported previously (Atrashevskii et al., 1999; Gates et al., 1965; Massa et al., 2015; McCree, 1972a). Calculated

absorptance (%) of the tomato and lettuce leaves were high under blue (400–500 nm) and red (600–700 nm) LED light, and low under the green (500–600 nm) LED light. In general, leaf optical properties in the visible wavelength spectrum (380–740 nm) are influenced by several factors, including photopigments in epidermal layers, intra-leaf structures and epicuticular waxes (Murakami and Matsuda, 2016; Vogelmann, 1993). With respect to wavelength, optical properties are mainly influenced by chlorophyll *a/b* for blue and red wavelengths, and by an elongated optical path that is caused by the scattering effect in the leaf interior for green wavelengths (Brodersen and Vogelmann, 2010; Terashima et al., 2009).

In this study, the optical properties of representative sun or shade plants were compared using the xenon bulb. Tomato leaves had higher absorptance percentages than the lettuce leaves across the tested wavelengths. This was expected, since sun plants often have higher chlorophyll content per unit leaf area than shade plants (Boardman, 1977; Lichtenthaler et al., 1981). Higher leaf absorptance percentages under the green wavelength range (500–600 nm) were observed in tomato leaves when compared to the lettuce leaves. Although the main pigment absorbance is low for green wavelengths, a considerable portion of green photons is still absorbed by leaves because of the elongated optical path length in spongy mesophyll, which is caused by the scattering or detour effect (Kok, 1948; Terashima et al., 2009). According to studies reporting on intra-leaf light absorptance profiles, green light is absorbed equally throughout the spongy mesophyll and it is able to penetrate deeper into the leaf, reaching chloroplasts in the lower cell better than other colors, which results in a substantial leaf absorptance of green wavelengths (Nishio, 2000; Sun et al., 1998). Therefore, for plants with thicker leaves, a typical characteristic of sun plants, leaf absorptance of green wavelengths is greater than absorptance in plants with thinner leaves, such as shade plants (Boardman, 1977; Mishra et al., 2012). Results obtained with the 530-nm LED assembly for both tomato and lettuce leaves support this hypothesis. Furthermore, data collected using the xenon bulb as a light source (baseline testing) for both tomato and lettuce plants corresponded to their classifications as either sun or shade plants.

High light plant responses

High light conditions (500 $W \cdot m^{-2}$) resulted in pigment photo-bleaching of the tomato leaves when the 470-nm and 655-nm LED assemblies were used. For lettuce, photo-bleaching was only observed with the 655-nm LED assembly. Different leaf discoloration was noted for tomato leaves exposed to the 530-nm LED assembly, this represents a wavelength range that is generally not considered useful for photosynthesis. Moreover, the use of different measurement apparatuses with the same irradiance under the 470-nm and 655-nm LED assemblies resulted in different photo-bleaching responses for both plant types. Together, these results suggest that plant behavior under high-irradiant narrow spectrum light conditions may be different than what we previously understood from the literature using broad spectrum light, and that an unknown light property, other than wavelength and irradiance, impacts photosynthetic mechanisms.

Photo-bleaching and its corresponding optical property response curves

In photo-protection mechanisms, blue light triggers chloroplast movement. This is one of the many mechanisms that plants have developed to photosynthesize efficiently under weak light and to avoid photo-inhibition from excessive light in nature (Banaś et al., 2012; Taiz and Zeiger, 2002; Wada, 2016). Under blue light, chloroplasts are re-distributed in palisade cells based on blue light levels (Banaś et al., 2012; Suetsugu and Wada, 2012). A common method used to detect or quantify chloroplast movement is to measure light transmittance (Wada, 2013). In this study, changes in leaf transmittance and reflectance over the course of the 1-h exposure to 470-nm LED light were observed when irradiance was lower than 100 $W \cdot m^{-2}$. This suggests that chloroplast movement occurred in the irradiated areas for both tomato and lettuce. Sun plants that have multiple layers of palisade cells (or thicker leaves) enable chloroplast translocation in the cells better than shade plants under blue light (Suetsugu and Wada, 2012; Wada, 2013, 2016), and our data support this observation. Under the 470-nm LED light, greater changes in transmittance at irradiance levels lower than 100 $W \cdot m^{-2}$ were observed for tomato leaves when compared to lettuce leaves, and this corresponds to the respective nature of sun and shade plants in terms of chloroplast movement in palisade cells (Davis and Hangarter, 2012; Wada, 2013). It is of interest to note, however, that these greater

changes in measured transmittance for tomato leaves suggests that the tomato leaves were additionally impacted by high irradiance under 470-nm LED lighting. Under these conditions, photo-bleaching was not observed on lettuce leaves but it was observed for tomato leaves under the transmittance apparatus. As irradiance was increased to 500 $W \cdot m^{-2}$, the optical property response curve became linear and the photo-bleaching of tomato leaves became apparent after time spent in the growth chamber.

Apart from a plant's adaptation to blue light intensity in its palisade cells, chloroplast movement redistributes photo-damage incurred by PSII throughout the leaf (Davis and Hangarter, 2012). Davis and Hangtarter (2012) observed that blue light alters the gradient of inhibition through a leaf's depth as chloroplasts are either in accumulated or avoidance positions. In the presence of strong blue light, photo-damage is distributed deeper into the leaf to prevent photo-inhibition. By combining these findings with our photo-bleaching results in tomato leaves caused by the 470-nm LED light treatment in this study, we suggest that the ability of photo-inhibition redistribution and blue-light acclimation in the lettuce leaves is better accomplished than in the tomato leaves. A similar phenomenon was observed when spectral properties were measured. Fluorescence emission data imply that lettuce leaves were able to adapt to 470-nm LED light better than tomato leaves. As exposure time increased, the fluorescent spectral reading did not change over time for the tomato leaves, but a change was observed for lettuce leaves. As PSII absorbs light, the light not only drives photochemistry, but can also be lost as heat dissipation or fluorescence emission (Duysens and Sweers, 1963; Taiz and Zeiger, 2002). Since PSII photochemistry and fluorescence emission are in direct competition, this is an alternative way of estimating PSII photochemistry from fluorescence emission (Baker, 2008; Taiz and Zeiger, 2002). In this study, fluorescence emissions differed between the two plant types, indicating that the lettuce leaves were able to adapt and utilize high-irradiant 470-nm LED light better and more efficiently than tomato leaves over time.

Photo-bleaching was observed for both plant types when the 655-nm LED lighting was used, yet damaged areas differed between tomato and lettuce leaves.

Pigment photo-bleaching caused by different wavelengths implies that red light may contribute to photo-protection and photo-inhibition using a pathway that is separate from blue light and PSII, as this photosystem is mainly associated with damage to photosynthetic machinery caused by excess light energy (Takahashi and Badger, 2011). It suggests that blue light may serve as a photo-protection signal to PSII, whereas red light is the main energy source, and that sun and shade plants respond differently than what has been previously reported regarding high light conditions. We believe that this behavior was not previously reported due to multiple wavelength interaction effects that are present in broad spectrum light sources. Our hypothesis is such that shade plants are not true shade plants but rather, "blue-light-spot plants" that can handle the bright blue light beams, regulating photosynthetic machinery by sensing blue light. It is possible that when the light beam disappears, plants prepare for the next beam. This deviates from what is currently presented in the literature. In a natural environment, plants can handle the bright sunlight that comes through the plant canopy and they can mediate leaf absorptance and photosynthetic activity in photosystems based on the proportions of blue light in sunlight. Plants have evolved under sunlight with a relatively consistent spectral composition, but irradiance fluctuates according to the external environmental conditions, such as weather and the time of day. Therefore, plants receive roughly the same proportion of the sunlight spectrum, but they may mediate the leaf absorptance spectrum (e.g. chloroplast movement and screening of light) and the photochemistry mechanisms within the photosystems to respond to specific wavelengths and irradiance levels. As the sunlight weakens or low blue light levels rise, they could gather chloroplasts to the leaf surface and prepare for the next beam. Thus, we propose that blue-light-spot plants can readily adapt to fluctuations in light levels, as opposed to sun plants that require more continuous high light.

Leaf coloration and light directionality

Tomato leaves did not undergo photo-bleaching when 530-nm LED lighting was used, but it did occur with the other two LED assemblies, exhibiting a lighter green color in the irradiated area relative to the rest of the leaf. Unlike blue and red light, green light can penetrate deeper into the leaf and its absorptance is caused mainly by the scattering effect in spongy mesophyll as described above. Therefore,

at high irradiance levels with 530-nm LED light, higher absorptance of green light should occur in tomato leaves when compared to lettuce leaves, and this is what was observed when measuring optical properties. Furthermore, due to the nature of the extracted pigment absorbance spectrum, the green light in the leaves was not absorbed by plant pigments (or antenna pigments in the photosystems) but by the leaf tissues, which might explain why no photo-bleaching occurred in tomato leaves when using 530-nm LED light.

Finally, the observation that photo-bleaching of pigments in leaf tissue only occurred with the transmittance apparatus, a setup in which light rays were direct, is important with respect to light directionality and photosynthesis capacity. Photo-bleaching did not occur with the reflectance apparatus, in which rays only hit leaves at 45º. As these apparatuses comprised different ray profiles (or light directionalities), these data support other studies in which internal leaf absorptance profiles are influenced when leaves were irradiated with rays from different directions (Brodersen and Vogelmann, 2010; Brodersen et al., 2008). Brodersen and Vogelmann (2010) reported that diffuse and low-angle light were better absorbed by the irradiated surface than the direct light. To our knowledge, the effect of a complex ray profile on the leaf's internal response using the mixture of rays described in this study has not yet been reported. The observed differences in photo-bleaching imply that ray profiles may strongly influence photosynthesis capacity, along with the two other important factors, wavelength and irradiance. The difference between photosynthesis and photo-inhibition (or even further photo-oxidation) is a spectrum of responses to light intensity associated with the amount of absorbed light energy by photosystems under the same wavelength. Low or moderate irradiance results in photosynthetic CO_2 utilization. As light energy exceeds the threshold of photosynthetic capacity and increases with time, photo-inhibition or irreversible photo-oxidation occurs. In this study, leaves that were irradiated by the same wavelengths and irradiance levels resulted in photo-bleaching when a different ray profile was used. As such, a complex ray profile may be more efficiently absorbed by photosystems and induce higher photosynthetic capacity than direct light with moderate irradiance. Furthermore, the smallest changes in the transmittance (%) under 470-nm LED lighting were approximately ~50 %. This is much higher than

what is reported with typical light conditions (~15 %), as reported by Gorton et al. (1999). This result reinforces the existence of a third unknown light property that affects photosynthesis. Brodersen et al. (2008) and Brodersen and Vogelmann (2010) similarly concluded that photosynthetic mechanisms at the internal leaf level react differently to the incident light directionality. However, further validation is required to better understand the effect of incident ray profiles and their mechanisms on leaf absorptance profiles.

Optical properties at high irradiance levels

The optical properties of tomato and lettuce leaves were not strongly influenced by irradiance, as photo-damage did not occur. Under all the light conditions examined, the leaf transmittance and reflectance percentages did not present an expressive change (< 2 %), with the exception of lettuce leaves exposed to high irradiance with the 530-nm LED assembly. When irradiance increased from 100 $W \cdot m^{-2}$ to 500 $W \cdot m^{-2}$, a balancing effect between transmittance and reflectance percentages occurred, as transmittance decreased and reflectance increased. This effect was not observed with any other light condition tested, including tomato leaves exposed to the same light conditions. We conjecture that this effect was caused by the interaction effect of anthocyanin accumulation and its reflected spectrum. Anthocyanin accumulation may have been triggered by 480–500 nm light in the 530-nm light spectrum, protecting leaf tissues from excessive irradiance levels as it acts as a light screening compound (Li and Kubota, 2009; Steyn et al., 2002). While the leaves were subjected to high irradiance with 530-nm LED light, the irradiance of blue light (480–500 nm) would be high enough to induce anthocyanin accumulation on leaf surfaces, and consequently reflect incident green light. Use of the 470-nm LED light would also trigger anthocyanin accumulation, but the balancing effect of optical properties was not as apparent. It is possible that the 470-nm LED light and the anthocyanin reflected spectra did not completely overlap.

4.5 Conclusion

Our knowledge of how highly-irradiant, monochromatic light affects a plant's response is limited by conventional lighting technology. This study investigated the optical and spectral properties of representative sun and shade (tomato and lettuce) plant leaves when exposed to low and high irradiance ranging from 20 $W \cdot m^{-2}$ to 500 $W \cdot m^{-2}$ with LED light, using a broad-spectrum xenon bulb at 20 $W \cdot m^{-2}$ as a baseline. We determined that shade plants tolerate high-irradiant 470-nm light better than sun plants, and this finding differs considerably from previous studies in which sunlight or broad-spectrum light was used. In addition, different ray profiles affected pigment photo-bleaching. These data suggest that light directionality is as important as wavelength and irradiance, when considering light properties that strongly influence photosynthetic capacity.

Connecting text

Chapter 5, “Revisiting the questions of why leaves are green and why leaves absorb green light: the UV-light-induced spectrum peaking at 530 nm”, is authored by Bo-Sen Wu and Mark Lefsrud and will be submitted for peer review (Pending, due to patent application).

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In Chapter 4, a broad emission spectrum that peaked at ~530 nm and emitted 430-nm light on leaves was observed. However, this phenomenon occurred on the edge of the 430-nm light spectrum. Therefore, in Chapter 5, we investigated this peak using a shorter wavelength LED light.

CHAPTER 5

Revisiting the questions of why leaves are green and why leaves absorb green light: UV-light-induced spectrum peaking at 530 nm

Abstract

Leaves appear green in colour because they reflect green (500–600 nm) light that is not absorbed by plant pigments. However, a substantial remaining fraction of green light (~ 70 %) is absorbed, and this may be used to drive photosynthesis more efficiently than certain wavelengths of blue light. In this study, we observed that tomato and lettuce leaves emitted green light between 490–600 nm, peaked at 530 nm when using a 410-nm light emitting diode (LED). This 530-nm spectrum phenomenon was not reported in earlier studies, and it may represent a newly discovered second photon emission process in plants after fluorescent emission. This observation implies that green leaf coloration results not only from green light reflection but also from green light re-emitting from leaves. Although no known photoreceptor has been identified in the green wavelength range, the 530-nm peak provides indirect evidence that suggests otherwise. We hypothesized that the observed 530-nm spectrum is due to photons leaked from this green photoreceptor light. As the proposed green photoreceptor receives energy transferred from the photoreceptor that absorbs the 410-nm light, it emits energy as photons that cannot be used, resulting in the observed 530-nm spectrum. In addition, we observed that the 530-nm spectrum was not influenced by irradiated time; rather it was impacted by leaf water content. The 530-nm spectrum was highest in dried lettuce leaves (~0 % water content). We postulate that this putative green photoreceptor is linked to moisture in plant leaves and that this spectrum could be used to estimate leaf water content.

5.1 Introduction

Green (500–600 nm) light is often considered inefficient for photosynthetic activity in higher plants because of extracted pigment absorbance spectra (Singh et al., 2015; Taiz and Zeiger, 2002). As leaves do not absorb green light, it is reflected by leaf surfaces, resulting in the green leaf coloration. However, numerous studies have showed that a substantial amount of green light is absorbed by leaves and is available for photosynthesis (Johkan et al., 2012; Kim et al., 2005b; Nishio, 2000; Sun et al., 1998). Regarding photosynthetic machinery, the quantum yield of green wavelengths is greater than that of certain blue wavelengths (Inada, 1976; McCree, 1972a). More recently, it has been demonstrated that supplemental green light enhances plant growth and influences photomorphogenic responses (Folta and Maruhnich, 2007; Kim et al., 2005a).

It has been reported that the high leaf absorptance spectrum in the green wavelength range is mainly due to sieve and detour effects (Terashima et al., 2009; Vogelmann, 1993). These combined effects allow green light to be absorbed by unsaturated chloroplasts in the lower cell and be used more efficiently than other wavelengths in the photosynthetically active radiation (PAR) spectrum (400–700 nm) (Nishio, 2000; Sun et al., 1998). Light absorptance profiles of leaves irradiated with monochromatic light have been measured using a chlorophyll fluorescence technique. As light penetrates a leaf, blue and red light is absorbed in the upper leaf layer, whereas green light penetrates deeper into leaves (Brodersen and Vogelmann, 2010; Vogelmann and Han, 2000). Therefore, green light can be absorbed by chloroplasts that are not yet photosynthetically light saturated in the lower cell, resulting in increased absorptance in the green wavelength range (Terashima et al., 2009). However, the light absorptance profiles also show that at the leaf surface, the maximum fluorescence for both green and red light was between 30–40 % (Brodersen and Vogelmann, 2010; Sun et al., 1998). These light absorptance data do not agree with the considerable differences in extracted pigment absorbance between green light regimes (500-600 nm).

High absorptance in the green wavelength range may also be explained by refractive plant cells (Gausman et al., 1974; Vogelmann, 1993). The diffusive nature

of leaf tissues allows light to scatter, resulting in a longer light path length (detour effect). The intensification of absorbance is most pronounced at the wavelength that is weakly absorbed, up to 6-fold (Rühle and Wild, 1979). However, even applying this intensified factor, the new pigment absorbance (<10 % → 20–60 %) is still not comparable to the leaf absorptance percentage in the green wavelength range (60–80 %) (Gates et al., 1965; McCree, 1972a). Furthermore, both spectral photosynthetic activity and leaf absorptance should be similar according to the nature of the sieve and detour effects (Gates et al., 1965; Johkan et al., 2012; McCree, 1972a). However, as the wavelength increases from 500 nm to 530 nm, the leaf absorptance decreases rapidly as opposing to the increased photosynthetic activity (Gates et al., 1965; McCree, 1972a). Similar responses have been observed for photosynthetic and photomorphogenic responses (Johkan et al., 2012). These results suggest that there is another mechanism underlying green light utilization in plant leaves (Gates et al., 1965; Johkan et al., 2012; McCree, 1972a).

In our previous study (Chapter 4), we discovered an unusual leaf emission spectrum in the 510-550 nm wavelength range at the edge of a 430-nm light emitting diode (LED) light. We hypothesize that this unusual spectrum could be the missing evidence underlying substantial leaf absorptance in the green wavelength range. The aim of this study was to investigate this emission spectrum and confirm its presence when using a shorter wavelength LED light source.

5.2 Materials and methods

Light source and plant germination

The LED light source in this study is comprised of a 410-nm LED assembly (Edison Opto, Hsinchu, Taiwan) that is depicted in Figure 25 and described in Chapter 4. Briefly, the LED was mounted on a water jacket and equipped with concentrated optics. The LED assembly was powered by a DC power supply (DP832, Rigol Tech. Inc., Beaverton, OR, US). Its irradiance levels were controlled through a control-current mode. Tomato (*Solanum lycopersicum cv* 'Beefsteak', lot A1, OSC, ON, Canada) and lettuce (*Lactuca sativa cv*. Breen; pelleted MT0 OG, Johnny's Selected Seeds, Winslow, ME, US) plants were grown in a growth chamber (TC30, Conviron, Winnipeg, Canada) with an irradiance level of 55 $W \cdot m^{-2}$,

provided by cool-white fluorescent bulbs (4200 K, F72T8CW, Osram Sylvania, Wilmington, MA, US) with a 16-h photoperiod. The plants were exposed to an ambient CO_2 concentration, a day/night temperature of 23/21 ± 1 °C and 50 % relative humidity. The full strength Hoagland nutrient solution was replaced every other day as described by Hoagland and Arnon (1950).

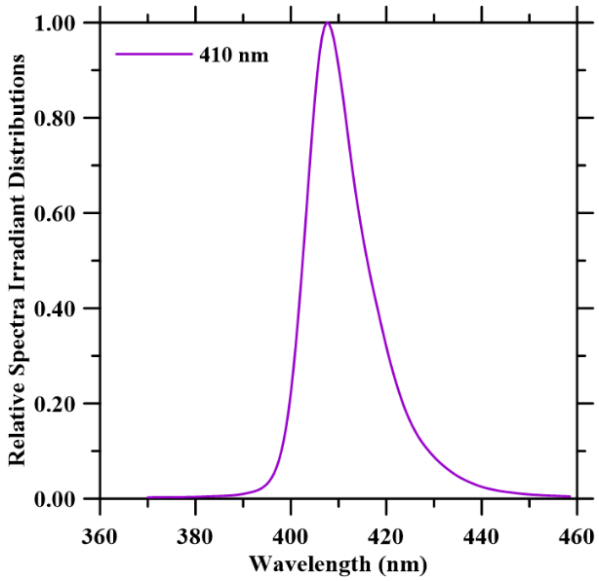

Figure 25. Relative reflected irradiance composition of the 410-nm LED assembly. Spectral irradiance was measured with a spectroradiometer.

Spectral irradiance measurements

Emission spectrum measurements were acquired using a spectroradiometer (PS-300, Apogee, Logan, UT) and a 45° reflectance probe (AS-003, Apogee, Logan, UT). The test leaves were secured in place between the probe and the clamp. The clamp was adjusted until the leaves in close proximation to the probe. Measurements were performed under an irradiance level of 20 $W \cdot m^{-2}$ and 50 $W \cdot m^{-2}$ (equivalent to ~70 and 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Since the incident irradiance level could not be measured directly, a white halon reference standard (97 % reflectance from 300-1700 nm, AS-004, Apogee, Logan, UT) was used to acquire the incident irradiance level. The emission reading was recorded upon initiating the 410-nm light and 5 min after the light treatment in a dark room. Background noise from the spectroradiometer was eliminated by recording the spectra with and without the LED light. Measurements were repeated three times on three biological replicates.

The emission spectrum was measured with other materials (fresh and dry leaves, black plastic bags, a black metal plate, and transparent plastic glasses) to establish spectrum baselines and to ensure no interference was created from the sensor or the measurement apparatus itself. The test was conducted under 20 $W \cdot m^{-2}$ only. The blank test data were consequently compared with the measured emission spectra from the leaves. To obtain leaves with different water content, leaves were left to air-dry in the laboratory after the first measurements were taken (fresh leaves). After the first emission spectrum of the fresh lettuce leaves was recorded, leaves were weighed as a baseline fresh mass and left in the laboratory to air-dry. The same procedure was followed for the rest of the measurements, using the same leaves but with different water content due to evaporation. After obtaining the last measurement, the leaves were dried in an oven at 65 ºC for 72 h. The dried leaf masses were then used to calculate water content of the same leaves compared to the mass measurements on different days.

5.3 Results

Baseline tests

The emission spectra of the baseline tests (black plastic bags, black metal plate, and transparent plastic glasses) using the 410-nm light are illustrated in Figure 26. Using different materials to examine the emission spectra in the PAR spectrum confirmed that no emission wavelength or photons were present. In the 500–800 nm wavelength range (excluding the tail of the reflected 410-nm LED light), spectral irradiance levels were all lower than 3.5×10^{-5} $W \cdot m^{-2}$.

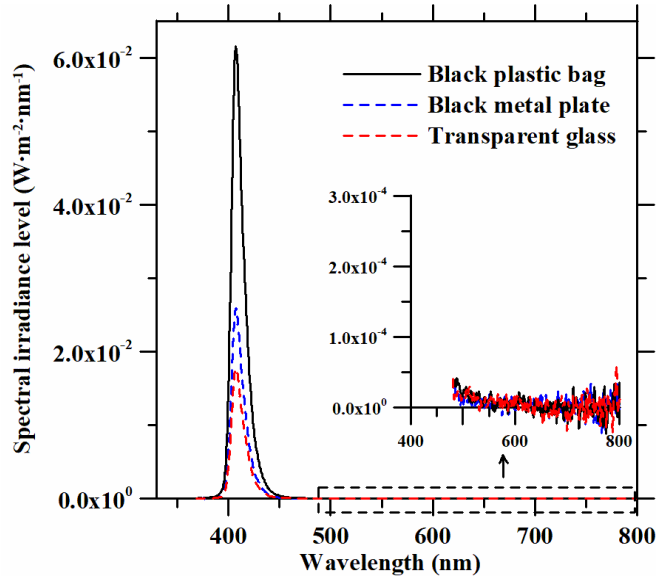

Figure 26. Reflected spectra of a black plastic bag, black metal plate, and transparent plastic glasses, using 410-nm LED light irradiated at 20 $W \cdot m^{-2}$. Spectral measurements were performed with a spectroradiometer and a 45° reflectance probe from 231 nm to 1100 nm at 0.5-nm intervals.

Emission spectra of fresh leaves

The emission spectra of fresh leaves irradiated at 20 $W \cdot m^{-2}$ and 50 $W \cdot m^{-2}$ from 480–800 nm are shown in Figure 27. Data from 400–500 nm are not shown since the spectrum of the 410-nm LED light emits at ~500 nm. The spectrum of the black metal plate collected using the reflectance probe was used as a blank reference. As expected, fluorescent emission spectra (~687 nm) were observed for both plant species. The fluorescent emission of the tomato leaves was higher than lettuce leaves under the 410-nm LED light at both tested light intensities. Secondary broad spectra were observed for leaves from both plant species. These observed spectra peaked at ~ 520–530 nm and in the 490-600 nm range. We named this spectrum after its main peak, the 530-nm spectrum, for the remainder of this study. No changes were observed for the 530-nm spectrum over the measurement periods (5 min). The 530-nm spectra for tomato and lettuce leaves were nearly identical at a low irradiance level (20 $W \cdot m^{-2}$). As the irradiance level increased to 50 $W \cdot m^{-2}$, however, both 530-nm spectra increased but exhibited differences between two species. Specifically, the lettuce leaf 530-nm spectrum was approximately 20 % higher than that of the tomato leaf.

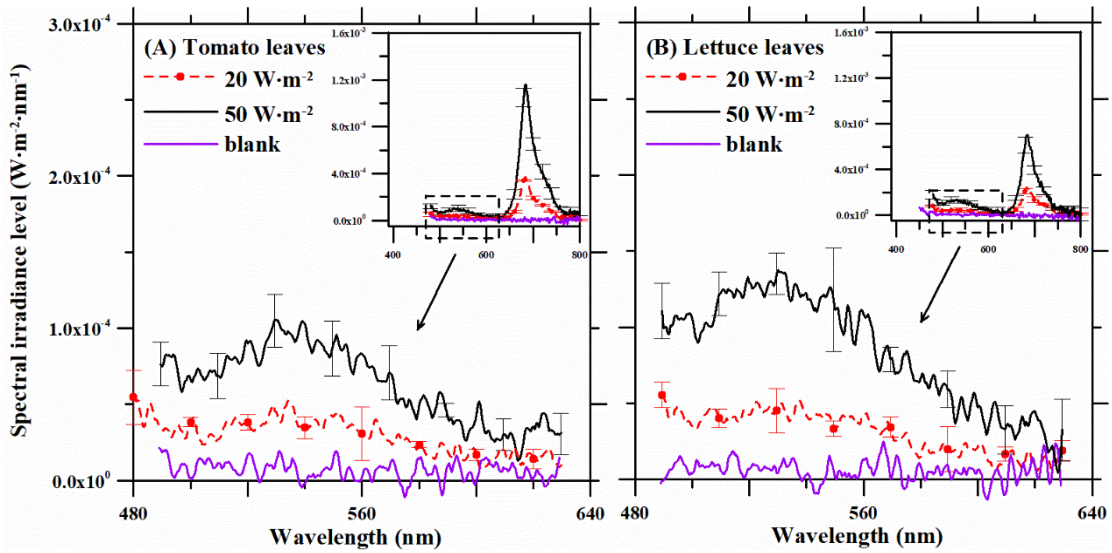

Figure 27. Emission spectra of fresh and dried leaves for (A) tomato and (B) lettuce leaves from 500–800 nm under a 410-nm LED light irradiated at 20 $W \cdot m^{-2}$ and 50 $W \cdot m^{-2}$.

Emission spectra of leaves with different water content

After drying, the tomato leaves were too fragile to be placed under the measurement apparatus for accurate emission measurements. Therefore, the 530-nm spectra data only represent that of the tomato leaves that were left to air-dry in the laboratory. Figure 28 shows the emission spectra of tomato and lettuce leaves with decreasing leaf water content. As the water content decreased, the fluorescent emission of the dried tomato leaves dramatically decreased (~30 %), whereas that of dried lettuce leaves was only slightly changed. Notably, the peak of the 530-nm spectrum increased nearly two-fold as leaf water content decreased from 88 % to nearly 0 %.

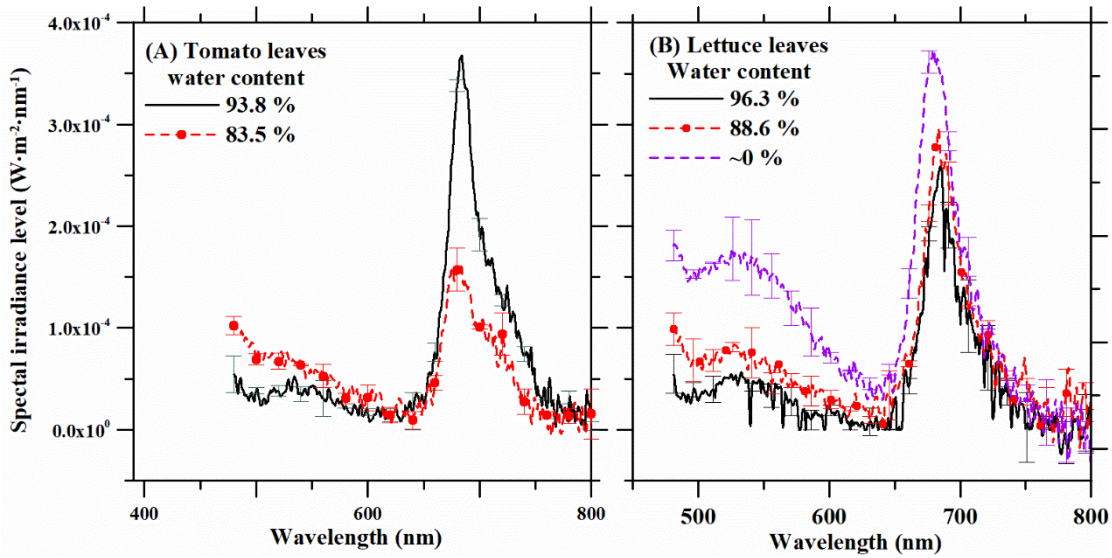

Figure 28. The emission spectra of (A) tomato and (B) lettuce leaves with different water content, irradiated at 20 $W \cdot m^{-2}$. Emission spectrum data for dried tomato leaves were not collected, as leaves were too fragile, prohibiting accurate optical measurements.

5.4 Discussion

The 530-nm spectrum was observed when leaves were irradiated with a 410-nm LED light. This 530-nm spectrum implies that green light reflection and green light re-emitting from leaves themselves both contribute to green leaf coloration. To our knowledge, this same effect has never been directly reported. A similar effect in green wavelength range was discovered and described in the 1960s (Deamer et al., 1967; Neumann and Jagendorf, 1964). These earlier studies observed a light-scattering effect that peaked near 535 nm in chloroplasts (Deamer et al., 1967; Neumann and Jagendorf, 1964). This differential in light scattering was later linked to ultrastructural changes in thylakoid membranes (Murakami and Packer, 1970), and the rapidly reversible non-photochemical quenching of chlorophyll fluorescence (qE) (Krause, 1973), confirming that the light scattering effect peaking at 535 nm was not linked to ΔpH; rather it was linked to qE. In this study, however, we report that the 530-nm spectrum was detected directly from the emission spectrum using 410-nm LED light, and no time-dependent changes were observed. Therefore, we believe that the 530-nm spectrum observed in this study is not the same 535-nm light scattering effect reportedly caused by qE and that was detected between changes in this time-dependent spectrum.

We hypothesize that the observed 530-nm spectrum might be caused by an unidentified green photoreceptor. With low pigment absorbance in the green wavelength range (5–10 %), applying the sieve or detour effects does not completely correlate with high leaf absorptance in the green wavelengths. Therefore, we postulate that there may be a photoreceptor that has an absorbance spectrum located in the green wavelength range, increasing the baseline of green light absorbance and LED light in this study, and consequently resulting in high leaf absorptance in the green, as the leaves absorbed the 410-nm LED light in the current study, it was emitted as a 530-nm spectrum, ranging from ~490–600 nm. Blank tests determined that this spectrum was not caused by the measurement apparatus and it was only emitted after the leaves absorbed the 410-nm light. One possible explanation for this effect is that as leaves absorb violet light via UV photoreceptors (cryptochromes or phototropins), energy is transferred to an unknown green photoreceptor. However, not all of the energy is absorbed, so that light is emitted or photons leak, peaking at 530 nm. In addition, this photon re-emitting process appears to be a subsequent emission, occurring after fluorescent emission from chlorophyll.

Although no photoreceptor in the green wavelength range has been identified, studies have demonstrated that green light impacts plant processes, and it exhibits wavelength- and light intensity-dependent effects (Johkan et al., 2012; Kim et al., 2005a; Terashima et al., 2009). At low light intensity (~150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), adding a small amount of green light (~24 %) to other wavelengths of light in the PAR spectrum is beneficial for shoot growth, yet higher percentage of green light reduces plant growth (Kim et al., 2005a). At high light intensity, supplementing green light into strong white light enhances photosynthetic activity (Terashima et al., 2009). While using green light alone (510 nm, 520 nm and 530 nm), the photosynthetic rate and dry mass of plants irradiated with 510-nm light was the highest, followed by 520-nm and 530-nm light (Johkan et al., 2012). Interaction effects between the wavelength effect on stomatal opening and the aforementioned hypothetical photoreceptor may explain these data. At low light intensity, a higher percentage of green light may be absorbed by the hypothetical photoreceptor, but photosynthetic capacity is limited since stomatal closure is caused by green light (Frechilla et al.,

2000; Kim et al., 2004b). At a higher light intensity, the hypothetical photoreceptor could use the green light energy that enhances the photosynthetic machinery by opening stomata (Terashima et al., 2009).

We postulate that the hypothetical photoreceptor could further explain the absorptance spectrum of anthocyanin. The main role of anthocyanin in plants is to protect against overexposure to UV light, yet it has absorptance peaks in the UV and green wavelengths. If green light is not used for photosynthesis and does not cause photo-inhibition or photo-damage, plants should not need to mediate light energy in the green spectrum. Changes to the 530-nm spectrum highlight an inverse correlation to leaf water content. As leaf water content decreased, the 530-nm spectrum increased. It is possible that the hypothetical photoreceptor might bond with water molecules. However, more experiments are needed to clarify the cause of the observed 530-nm peak in this study.

5.5 Conclusion

The role that green light plays in plant physiology has not been fully elucidated and green photoreceptors have not been identified. In this study, we discovered that tomato and lettuce leaves emitted 530-nm spectra when irradiated with a 410-nm LED light. The 530-nm spectrum may provide indirect evidence of an unknown green photoreceptor. We believe this is a second occurrence of photon emissions that are undetectable with monochromatic light, with the first being fluorescent emission from chlorophyll. Although the intensity of the 530-nm emission spectrum is low, it implies that green leaf coloration may result not only from reflecting green light, but re-emitting green light from leaves. Emission spectrum data collected using dried leaves highlight the relationship between a hypothetical photoreceptor and leaf water content.

Connecting text

Chapter 6, “The action spectrum of photosynthesis for tomato and lettuce leaves: 1-nm resolution at 30 μmol·m^{-2}·sec^{-1}”, is authored by Mark Lefsrud and Bo-Sen Wu and will be submitted as a manuscript for peer review (Pending, due to patent application).

Although McCree curve is the standard curve accepted for the spectral quality of photosynthesis, it was determined with a wide wavelength range (25 nm) and a broad light spectrum (25 nm). Therefore, in Chapter 6, we revisited McCree curve with a low wavelength range (or high wavelength resolution) and a narrow light spectrum (10 nm).

CHAPTER 6

The action spectrum of photosynthesis for tomato and lettuce leaves: 1-nm resolution at 30 µmol·m^{-2}·sec^{-1}

Abstract

The spectral quality for photosynthesis is well founded and has been investigated in higher plants for over 60 years. However, differences in action spectrum and quantum yield, with differently shaped curves and observed peaks have been reported. To date, the McCree curve is considered the standard photosynthetic response curve, but it was constructed with varying photosynthetic rates and a broad light spectrum (25 nm). Using this historical data as a baseline, the aim of this study was to collect repeated measurements of plant photosynthetic rates with a higher wavelength resolution. The action spectrum was measured with a light spectrum of 10 nm full width at half maximum (FWHM) and an intensity of 30 µmol·m^{-2}·sec^{-1}, with data points taken every 1 nm, using 3-4 week old tomato and lettuce plants. After data collection and analysis, both species had action spectrum curves with two distinct peaks at 430 nm and 650 nm, and shoulders at 480 nm and 595 nm. These 1-nm action spectrum data do not correlate with the extracted pigment absorbance spectrum. Furthermore, photo-damage efficiency, which strongly associates with oxygen evolving complex (OEC) absorptance, accords with spectral photosynthetic data in the green light region (500–600 nm). We hypothesize that chlorophylls and other photo-pigments are used as light energy dissipaters and that initiation of photosynthetic capacity for 595-nm light is associated with the oxygen-evolving complex only. Data presented in this study provide the most precise information on the spectral quality of photosynthesis to date, shedding new light on our understanding of how photosynthesis occurs in plants.

6.1 Introduction

The spectral quality of photosynthesis (action spectrum or/and quantum yield) in higher plants was initially determined using narrow light spectrum (Bulley et al., 1969; Govindjee and Rabinowitch, 1968; Hoover, 1937; Inada, 1976; McCree, 1972a). These studies concluded that blue (400–500 nm) and red (600–700 nm) wavelengths are the most efficient light in the visible wavelength range (380–720 nm). To date, the most comprehensive and well-accepted photosynthesis data set is the McCree action spectrum curve (McCree, 1972a), which has peak photosynthetic rates at 440 nm and 670 nm. Subsequent studies confirmed these data (Hogewoning et al., 2012b; Inada, 1976), resulting in the selection of 460 nm and 650 nm light spectrum as the principally applied photosynthetic light for research and plant productivity (Chen et al., 2017; Deram et al., 2014; Johkan et al., 2012; Naznin et al., 2016; Ouzounis et al., 2016; Son et al., 2017). However, high pressure sodium (HPS) luminaires that predominately emit ~595-nm light are still the industry standard (Stober et al., 2017). Blue/red light emitting diodes (LEDs), which emit "the most efficient light" for photosynthesis and plant growth, still cannot completely replace HPS luminaires as varied plant responses under the LED light have been reported (Olle and Viršile, 2013). This raises the question: Why is the McCree curve still the definitive reference for light selection?

Pioneering photosynthetic spectral quality curve studies determined that the red and blue wavelengths are the most efficient light in the visible spectrum (Hoover, 1937; Inada, 1976; McCree, 1972a). However, the observed peaks and curve shapes that were reported are quite diverse (Balegh and Biddulph, 1970; Bulley et al., 1969; Hoover, 1937; Inada, 1976; McCree, 1972a). Peaks in the blue and red wavelengths were between 420–450 nm and 660–680 nm, respectively. However, the red peak in the Hoover action spectrum had two sharp peaks at 440 nm and 620 nm for wheat, with a valley between both peaks (Hoover, 1937). Bulley et al. (1969) and Balegh and Biddulph (1970) obtained roughly the same results for radish, corn, and bean, yet they showed much lower rates in blue wavelength (~ 50–60 %) than in the red. Similar results were observed at low photosynthetic rates in the blue spectrum (Inada, 1976; McCree, 1972a), but a pronounced peak at ~ 440 nm is shown in the Inada action spectra (Inada, 1976). Inada (1976) and McCree (1972a) examined

more than 20 plant species, concluding that differences between species were small. The quantum yield spectra data were different between McCree (1972a) and Inada (1976), particularly in the blue spectrum. No distinct peak in the blue wavelengths for quantum yield spectra were observed in McCree (1972a), but Inada (1976) had a peak at 435 nm. Contrarily, the measured wavelength changed from 435 nm to ~470 nm, quantum yield decreased rapidly for Inada (1976), McCree (1972a) and Hoover (1937).

Despite differences in collected data, these photosynthetic studies have provided the basis of spectral efficiency for photosynthesis in higher plants. However, because lighting technology was limited at the time, these curves were measured by filtering a broad spectrum light (xenon bulbs) with large ranges of wavelengths (25 nm full width at half maximum [FWHM]; McCree (1972a)), varying FWHM (17–34 nm; (Inada, 1976), and limitation of filtering wavelength (infrared wavelengths: 700–1000 nm) that cause interference (Hoover, 1937). This variation in the measurement methods makes the re-interpretation and unification of these curves difficult. In recent years, control over spectral characteristics in lighting and optical technologies has improved. Unlike conventional lighting technology, LEDs can produce narrower spectra of light (~20 nm) and light intensity can be controlled. The maximum light intensity output of LEDs has increased 20-fold every decade (Haitz and Tsao, 2011) and new LEDs have reached energy conversion efficiency as high as 50 % (Nelson and Bugbee, 2014). Having this level of control over LED light output allows for an improved assessment of the spectral quality of photosynthesis in plants. However, obtaining sufficient intensity levels with narrow spectrum wavelengths (<10 nm) remains a challenge. Similar to earlier photosynthetic research, either a monochromator or optical filters have to be used to control wavelength, resulting in significant intensity loss (Hogewoning et al., 2012b).

The objective of this research was to measure the action spectrum curve at every nanometer with a narrow light spectrum (10 nm FWHM) and 1-nm resolution, using the latest, high-irradiant colored LEDs and a high wavelength precision monochromator. This experiment focused on the spectral responses of tomato and

lettuce plants across a wavelength range of 400–700 nm, at photosynthetic photon flux density of 30 μmol·m^{-2}·sec^{-1}. Collected data provide the most precise information to date on the impact of specific wavelengths of light on photosynthesis in higher plants.

6.2 Materials and experimental methods

Plant materials

Tomato (*Solanum lycopersicum* 'Beefsteak', lot A1, OSC, Ontario, Canada) and lettuce (*Lactuca sativa* cv. Breen; pelleted MT0 OG, Johnny's Selected Seeds, Winslow, ME) seeds were germinated in rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) within a growth chamber (TC30, Conviron, Winnipeg, Canada). Overhead cool-white fluorescent bulbs (4200 K, F72T8CW, Osram, Wilmington, MA) provided a photosynthetic photon flux density (PPFD) of 150 μmol•m^{-2}•sec^{-1} (400–700 nm). The plants were exposed to the following environmental conditions: day/night temperatures 23/21±1 °C, 16 h-photoperiod, and ambient CO_2 concentration. Full strength Hoagland nutrient solution was replaced every other day as described by Hoagland and Arnon (1950). Aluminum foil was placed on the rockwool to prevent algae growth. Plants selected for photosynthetic measurements 21–30 days after seeding and emergence of the second true leaf to allow for a relatively reproducible symmetrical leaf and plant distribution. Plants were selected for consistency in size and age, while outliers were excluded from any further experimentation.

Monochromatic lighting system and net photosynthetic measurements

The 1-nm-resolution action spectrum curve measurements were taken with two apparatuses; a monochromatic lighting system and a photosynthetic measurement apparatus depicted in Figure 29.

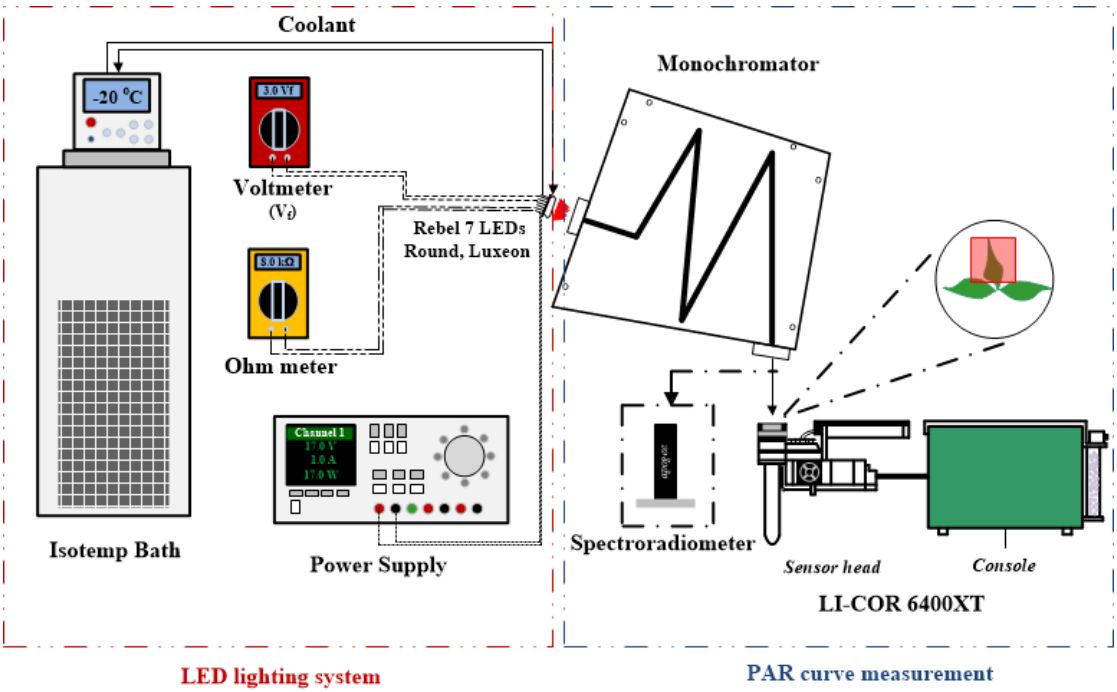

Figure 29. A simplified schematic diagram of the experimental setup for 1-nm-resolution action spectrum curve measurements.

Monochromatic light with 10 nm FWHM, along with the test wavelength range were obtained from a filtered, colored LED light sources provided by a high precision monochromator (Model 74125, Newport, Irvine, CA, US). Each colored LED assembly had a distinct color and peak wavelength. They were as follows: a 410 nm assembly (EFEV-1AE1, Edison Opto, Taiwan); a 447.5 nm LED assembly (LXML-PR01, Lumileds, Amsterdam, Netherlands); a 470 nm LED assembly (LXML-PB01, Lumileds); a 505 nm LED assembly (LXML-PE01, Lumileds), a 530 nm assembly (LXML-PM01, Lumileds), a 560 nm assembly (LXML-PX02, Lumileds), a 590 nm assembly (LXM2-PL01, Lumileds,), a 617 nm assembly (LXM2-PH01, Lumileds), a 627 nm assembly (LXM2-PD01, Lumileds), a 655 nm (LXM3-PD01, Lumileds), a 720 nm assembly (LXML-PF01, Lumileds); and a 735 nm assembly (ELSH-Q91LX, Everlight, Taiwan). Table 9 summarizes the optical characteristics and product attributes of the LED assemblies used in this study.

Table 9. LED assemblies used in this study.

Wavelength (nm)	Lumen (lm) or radiant power (mW)	FWHM (nm)	Maximum forward voltage (V)	Maximum temperature (°C)
410	-		-	-
448	9380	20	23.31	
470	672	20	23.73	
505	532	30	24.57	150
530	1358	30	24.57	
560	3220	100	19.25	
595	602	80	24.57	
617	1126	20	18.2	
627	826	20	18.2	135
655	4788 mW	20	19.6	
720	3360 mW	20	16.8	
735	-		-	-

Each assembly had seven diodes on a thermal pad, which were attached to a concentrated lens (No. 263, Polymer Optics, Wokingham, Berkshire, UK). The configuration of the lighting system is illustrated in Figure 29. Briefly, the LED assembly was placed on the entrance slit of the monochromator; and the monochromator was placed in a self-made frame, which allowed light outputs from the monochromator light to exit perpendicular to the leaf surface. The centroid wavelength and PPFD of the monochromatic light were adjusted with software provided by the monochromator manufacturer (Mono-Utility 5.0.4, Newport) and a DC power supply (DP832, Rigol Tech., Beaverton, OR, US), respectively. The light characteristic including centroid wavelength, PPFD, and FWHM of the monochromatic light was measured with a spectroradiometer (PS-300, Apogee, Logan, UT, US). Figure 30 compares the light distribution of a narrow bandwidth light (10 nm FWHM, used in this study) and a typical LED light (25 nm FWHM). The spectroradiometer was placed ~20 cm below the monochromator light exit and the irradiated area from the monochromator was approximately 1.5 cm × 1.5 cm (Figure 31).

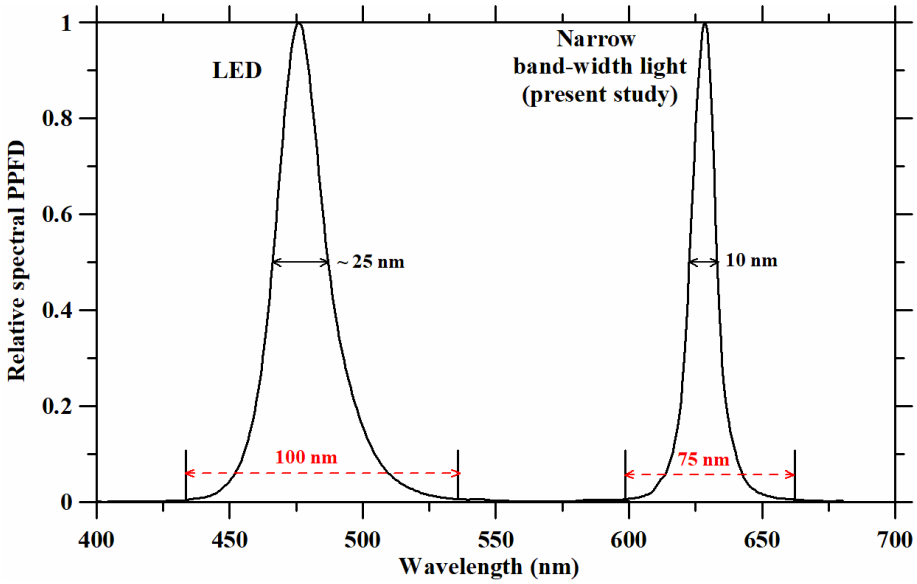

Figure 30. The relative spectral light distributions of a narrow band-width light (10 nm FWHM) and a 480-nm LED light (25 nm FWHM) filtered by the monochromator used in this study.

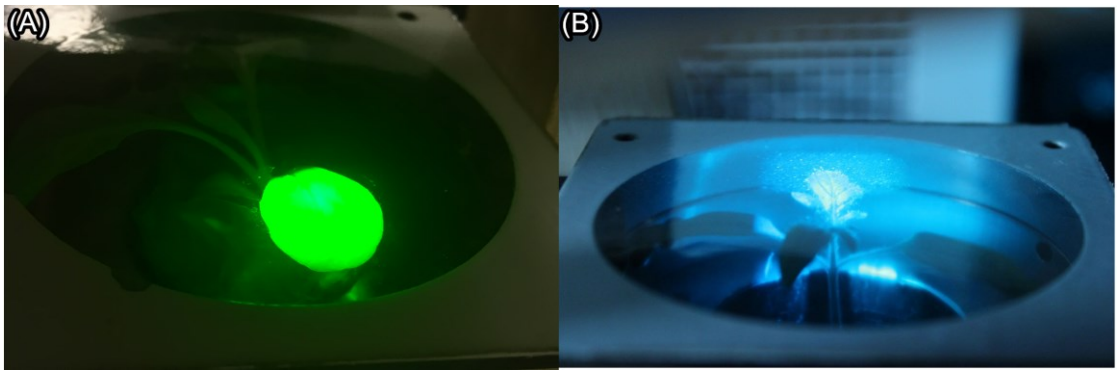

Figure 31. (A) Lettuce leaf under 530-nm light and (B) tomato leaf under 505-nm light.

A preliminary test showed that nearly ~80 % PPFD was lost through the filtering process in the monochromator. Therefore, to achieve the test PPFD level (30 μmol•m^{-2}•sec^{-1}) in the photosynthetically action radiation (PAR) spectrum (400–700 nm), an approach differing from that of typical LED operation methods was used. In addition, the LED assemblies were operated at three-fold higher than the maximum current outputs recommended by manufacturers (700–1000 mA). While overdriving the LED assemblies, coolant was circulated at -20 ºC in a water jacket

(ST-011, Guangzhou Rantion Trading Co., Guangdong, China) behind the mounted LED using an Isotemp bath circulator (4100R20, Fisher Scientific, Hampton, NH), to stabilize LED light characteristics and to prevent the LEDs from burning out while in overdrive. A low junction temperature allowed the LED assemblies to have a higher light output (~20–60 %) than if using a passive heat sink or fan-based cooling system. The overdriving process was limited to keep the LED junction temperature (T_j) below 90 % of the maximum operating junction temperature (< 130 ^oC). The LED junction temperature was monitored while measurements were taken and calculated with the following equation:

$$T_j = T_s + \Psi_{j-s} \cdot PD \qquad \text{Equation 6}$$

Where T_s is the temperature measured on the back surface of the LED board with a 10-K thermistor (Vishay, Malvern, PA), Ψ_{j-s} is the total thermal resistance of the diode (12 $^oC/W$) and thermal pad (4 $^oC/W$), and PD is the total power dissipation (in watts) of the center LED on the assembly, acquired from its thermal resistance and forward voltage. The thermal resistance and forward voltage were monitored using a digital voltmeter (F106, Fluke, Everret, WA, US) and an Ohm meter (XL-830L, Fluke), respectively.

Photosynthetic data for tomato and lettuce leaves were collected using the LI-6400 XT photosynthesis system (LI-COR, Lincoln, NE, US) equipped with a 6400-17 Whole Plant Arabidopsis Chamber (LI-COR). The LI-6400 system was calibrated with fresh soda lime (6–12 mesh) and a desiccant before conducting measurements. After calibration, CO_2 concentration and water vapor variations were kept to less than 0.05 $\mu mol \cdot sec^{-1}$ and 0.05 $mmol \cdot sec^{-1}$, respectively; the difference between these reference points and sample concentrations were expected to be within 0.01 $\mu mol \cdot sec^{-1}$ when measurements were taken. If not, the LI-6400 was re-calibrated again until criteria was met. A whole plant rooted in wet rockwool was placed in the Whole Plant Arabidopsis Chamber, and the test plant leaf (~1.5 by 1.5 cm) was placed against the top of the chamber cover, avoiding a heterogeneous light intensity distribution over the test leaf due to leaf tilt. If algae were observed on rockwool cube surface, there were removed using a razor blade to avoid interference. Parafilm

was placed on top of the rockwool cube to ensure moisture isolation from the test chamber. The LI-6400 was stabilized for 5 min, and the first reading normally took 20 minutes; all subsequent readings took approximately 2 min. The LI-6400 controlled flow rate (400 μL min^{-1}), CO_2 concentration (400 μL·L^{-1}), relative humidity (~50 %), and block temperature (23 °C) in the chamber.

The monochromator was placed ~20 cm above the LI-6400 sensor head and its monochromatic light exit faced downward and parallel with the test leaf (Figure 29). The spectroradiometer was placed on an adjustable jack and adjusted to the same distance as the test leaf, keeping PPFD constant. A plastic board (6 cm × 6 cm) with a hole (1.5 by 1.5 cm) in the center was used as a light distribution guide, placed on the spectroradiometer. The LED assembly's angle was adjusted until the highest light intensity of the monochromatic light was aimed at the center of the irradiated area using the plastic board guide. The plastic board guide was placed on the LI-COR sensor head and positioned above the center of the test leaf. This maintained uniform irradiance levels for the test leaves using the same light distribution between wavelength treatments; it was also maintained for other leaves of the plant that were not irradiated.

Photosynthetic measurement procedure

The monochromatic light with an assigned treatment wavelength and PPFD level was projected onto the test leaf. Each wavelength treatment lasted 5 min in duration, comprising 40 sec in the dark and 4 min 20 sec in the light, averaging 4 sec per signal (75 data points in total; Figure 32). If the CO_2 concentration in the chamber suddenly increased or decreased by more than 0.1 μmol·sec^{-1} while measurements were being taken, measurements periods were extended or rejected. Between each wavelength treatment, plants were placed in the dark for 2–5 min to allow for dark respiration and to eliminate carryover effects from the previous wavelengths. Three biological replicates for each plant species and each wavelength were measured. For each replicates, the order of the wavelengths tested were in 1-nm increments and 1-nm wavelength reductions. These were partially randomized (half increments and half reduction in wavelength) to minimize potential interaction effects between wavelengths. For each replicate, 15–30 wavelength blocks were

measured, depending on the coloured LEDs used. Between each measured wavelength block, wavelength measurement was overlapped for at least two wavelengths to allow for combined data blocks. After each measurement, the irradiated leaf and others leaves from the test plant were separated and placed on a white paper. Leaf areas were measured using software ImageJ software (NIH, Bethesda, MD, US) and this was used to determine the photosynthetic rate on a per unit leaf area basis.

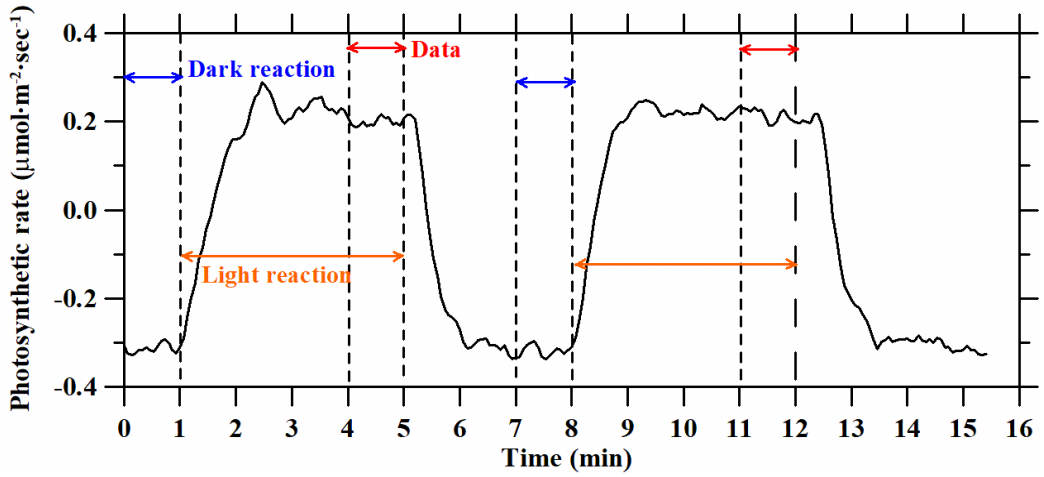

Figure 32. Photosynthetic response curve for the dark reaction (respiration) and light reaction (photosynthesis) for lettuce and tomato leaves. Photosynthetic rate was measured as μmol·m^{-2} ·sec^{-1} CO_2 used by any given plant.

Data analysis

Net photosynthetic rates (P_{net}) of the test leaf were calculated with the following equation:

$$P_{net} = \frac{P_{LI-COR,light} \cdot LA_{total} - P_{LI-COR,dark} \cdot LA_{dark}}{LA_{light}} - P_{Li-COR,dark} \qquad \text{Equation 7}$$

Where $P_{LI\text{-}COR,\ light}$ and $P_{LI\text{-}COR,\ dark}$ are photosynthetic rates measured in light and dark (μmol•m^{-2}•s^{-1}), respectively, and LA_{dark} and LA_{Total} are leaf areas (cm^2) that were in the dark and the total leaf area, respectively. $P_{LI\text{-}COR,\ light}$ was the average photosynthetic rate of last 20 data points for each measurement. After obtaining the net photosynthetic rate for each treatment wavelength across the block of

wavelengths, the response rate from different wavelength blocks were overlapped, based on the photosynthetic rates of the duplicate wavelengths. For example, curves between 450–470 nm and 468–490 nm were overlapped according to data at 469 nm and 470 nm, and the photosynthetic rates of these two wavelengths were normally similar. However, some discrepancies did occur because of small differences in sample CO_2 concentrations, as provided by the 6400 XT in between data point measurements. If differences were observed, the response rates were shifted with a correction factor to allow overlapping with the same wavelengths (468–470 nm in this case). After overlapping the net photosynthetic response rates of each measured wavelength block, the action spectrum responses from three replicates were averaged.

6.3 Results

Spectral properties

The average PPFD of the tested wavelengths was 30.09 ± 0.27 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, except for 570–590 nm, which ranged from 29.5–29.8 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. This slight decrease in irradiance was due to limited irradiance levels of the III-phosphide and III-nitride LEDs, as noted previously (Lafont et al., 2012). The FWHMs of the narrow light spectra ranged from 9 nm to 11 nm.

A 1-nm resolution action spectrum curve

The wavelength impact on net photosynthetic rates at 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ when using 1-nm resolution was determined for tomato and lettuce plants grown under a fluorescent-light spectrum (Figure 33). The 1-nm-resolution action spectra exhibited several distinct features. For both species, the action spectrum curves comprised four pronounced peaks (maximum photosynthetic rates), centered at 430 nm, 480/500 nm, 595 nm, and 650 nm. Major valleys (minimum photosynthetic rates) were located at 450 nm and 525/550 nm. Two major peaks were present in the 400–500 nm blue region, where the highest net photosynthetic rate was observed for both species. However, maximum peaks were opposed between species. In the blue region, the highest photosynthetic rates for the tomato and lettuce leaves were at 495 nm and 430 nm, respectively. The two blue peaks were separated by a distinct valley at 450 nm for both species. As the wavelength increased, the net photosynthetic rates

decreased until reaching the lowest photosynthetic rates observed at 525 nm for lettuce, and at 550 nm for tomato. Both species showed a decreasing trend in the response curve in the green wavelength region (500–600 nm). An increase in photosynthesis was located at ~595 nm. In the red wavelength region (600–700 nm), the curve of tomato had a valley and peak at ~620 nm and 650 nm, respectively, for the lettuce leaves. Unlike lettuce, the action spectrum curve for tomato leaves oscillated more sharply than that for the lettuce leaves.

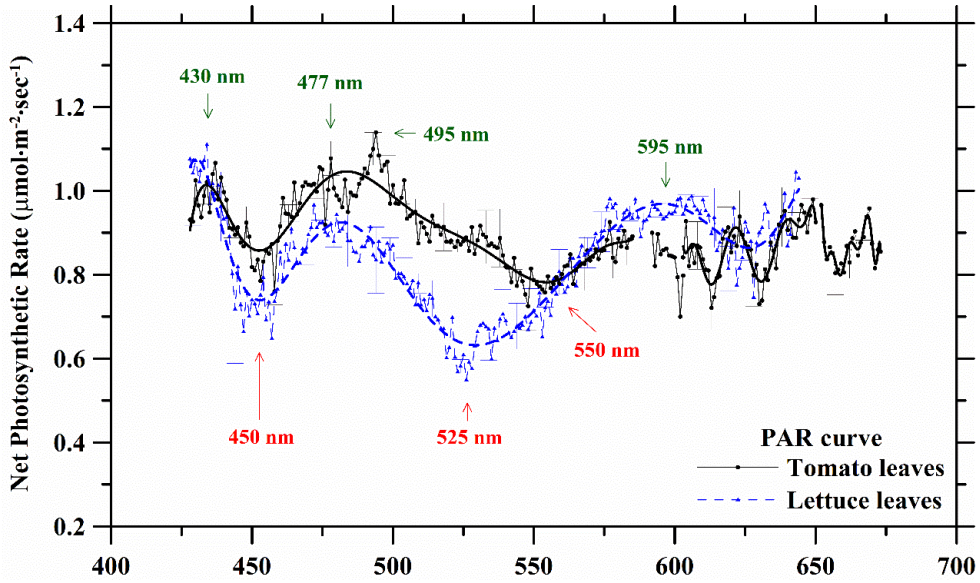

Figure 33. The 1-nm-resolution action spectrum curves of tomato and lettuce leaves from 428 nm to 650 nm.

6.4 Discussion

Comparison with early action spectra studies

The relative action spectra of the tomato and lettuce leaves obtained in this study are compared to those of earlier studies in Figure 34 (Balegh and Biddulph, 1970; McCree, 1972a). The McCree curve is included as it is considered the standard, and the photosynthetic response curve from Balegh and Biddulph is also compared because the PPFD was fixed along with measurement wavelength ranges. Others published data are presented as photometric units (Hoover, 1937) or radiant units (Inada, 1976); these have a different baseline when compared to the current data. It has been previously noted that using radiant units could underestimate the effectiveness of blue light (McCree, 1972a). For the McCree curve, we converted units to spectral photon flux density to coordinate with our own light measurement

units, following an approach established previously by Sager et al. (1982).

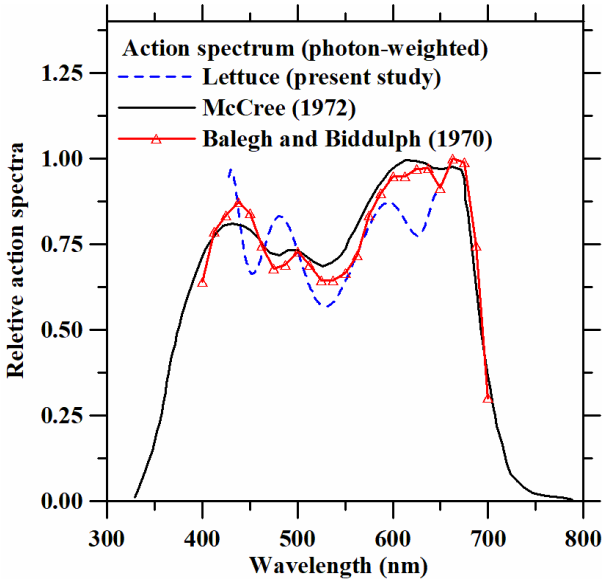

Figure 34. Comparison of relative action spectra. Curves from early studies are re-drawn from original data and normalized to a maximum of 1. McCree and Balegh curves represent the average of 22 higher plants and radish plants, respectively (Balegh and Biddulph, 1970; McCree, 1972a)

Overall, the action spectra obtained in the current study followed a similar trend to those presented in earlier studies, with the exception of some shifted photosynthetic maximums and minimums (Figure 34). Unlike the relatively smooth curve in the blue wavelengths and lower blue light efficiency relative to the red in the McCree curve, our data and those of Balegh and Biddulph (1970) had sharper peaks. Our data also had a higher blue light photosynthetic efficiency rate (~10–20 %) than these other two studies. Later research has since reported that variable responses in the blue photosynthetic rate could be caused by growing light conditions or leaf greenness (Inada, 1976; McCree, 1972a); however, it may be caused by other environmental factors. McCree (1972a) observed that plants grown in the field had lower responses in the blue wavelengths than in the growth chamber, but only for measurements taken at wavelengths less than ~410 nm. However, Hogewoning et al. (2012b) presented opposing data wherein different growing light conditions had no effect on quantum yield curve shape. Inada (1976) reported that the degree of leaf greenness affected blue light efficiency, but we did not observe such varied responses in blue light efficiency in our investigation. The leaf colour of

the tomato and lettuce leaves were dark green and light green, respectively, but they both had nearly identical responses in the blue wavelengths.

Differences in spectral quality data between studies are much greater than differences amongst species within a study (Bugbee, 2016). Importantly, the aforementioned spectral quality determination studies were conducted using different plant species, environmental conditions in growth chambers, and experimental designs that may have contributed to these dissimilarities (Balegh and Biddulph, 1970; Hogewoning et al., 2012b; McCree, 1972a). When comparing previously published data to those collected in this work, we observed that the FWHM of the light spectrum could be the main factor that contributes to the varied responses in blue light efficiency relative to red light. Photosynthesis is a wavelength-dependence process (Inada, 1976; McCree, 1972a). When using "boarder" narrow light spectrum [i.e. 25 nm used in McCree (1972a)], the wavelength dependence of photosynthesis could be under-represented because of the interactive wavelength effect. Using narrower light spectrum (10 nm in the current study) may allow for independent determination of the individual wavelengths of light on the spectral efficiency of photosynthesis. Net photosynthetic rates at each measured wavelength for the McCree curve were measured using the light spectrum with 25 nm FWHM. McCree's data at each measured wavelength actually represents the convolution of the spectral-bandpass function with an unknown quantity across nearly 100 nm (Figure 30). The narrower light spectrum (10 nm FWHM) used in the present study and the set of experiments performed by Balegh and Biddulph (1970) (10 nm FWHM) both showed pronounced peaks in the blue wavelengths. Distinct peaks were observed in other studies using the narrower light spectrum (< 25 nm FWHM) (Bulley et al., 1969; Inada, 1976). When considering the main peak in the blue wavelengths, it ranged between 430–440 nm in earlier studies (Balegh and Biddulph, 1970; Bulley et al., 1969; Hoover, 1937; Inada, 1976). This is within range of our measured peak wavelength at 430 nm. Therefore, we believe the blue peak is at 430 nm, with the 10-nm-FWHM and 1-nm wavelength resolution conducted in the current study.

Comparison of extracted pigment absorbance spectra

Environmental factors such as light irradiance level can affect plant growth and pigment accumulation, including lutein and β-carotene (Lefsrud et al., 2006; Lefsrud et al., 2005). However, the correlation between photosynthetic activity and extracted pigment absorbance peaks has not been determined. Two opposing opinions regarding their correlation exist. Some studies have pointed out that identifying the spectral quality of photosynthesis with particular pigments is difficult with leaves, as light-screening compounds are present (e.g. anthocyanins and betalains) (McCree, 1972a; Rabinowitch, 1945; Smillie and Hetherington, 1999; Steyn et al., 2002). Furthermore, the plant pigment absorbance spectrum varies (10-20 nm) according to the extraction solvents used; this is due to differences in polarity and the loss of pigment protein-interactions (Porra, 2002). Notably, these solvents do not exist in leaf tissues or in plant photosystems. It is possible that this contributes to differences observed between the extracted and true pigment absorbance spectra.

The opposing hypothesis is that major pigments play an important role as they harvest light energy in photosynthesis (Massa et al., 2008; Singh et al., 2015), and this has led to targeted pigment absorbance peaks for maximum photosynthesis and plant productivity using 460-nm and 650-nm LEDs (Chen et al., 2017; Hernández and Kubota, 2016; Naznin et al., 2016; Ouzounis et al., 2016; Piovene et al., 2015; Swan and Bugbee, 2017; Wang et al., 2016). In theory, these purplish lighting systems can induce high photosynthetic capacity and replace any other lighting system. Nowadays, however, amber-based HPS luminaires are still the preferable choice for greenhouse growers (Stober et al., 2017). Opposing evidence to the pigment theory, yet in accordance with the McCree curve has been reported (Han et al., 2017; Mizuno et al., 2011; Zhen and van Iersel, 2017). Han et al. (2017) showed that higher dry mass and leaf growth rates (2-3 times) are obtained for lettuce plants grown under combined blue, amber, and red light when compared to combined red and blue light at 150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. At a higher intensity range (50-750 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), using warm white LED light (low 460-nm and high broad 595-nm light) consistently induced higher photosynthetic rates for lettuce (*Lactuca sativa cv,* 'Green Towers') when compared to 453+638 nm light (Zhen and van Iersel, 2017).

To further clarify the correlation between a plant's action and major pigment absorbance spectra, they were overlapped with other possible determining factors for photosynthetic capacity in Figure 35. For the major pigment absorbance spectrum, neither the chlorophyll *a* nor *b* peak is in agreement with the action spectrum data. Surprisingly, the relationship between the 1-nm action spectrum and the extracted pigment absorbance spectrum happens to be in reverse. Specifically, peaks in the 1-nm action spectrum match the valleys of the chlorophyll *a* absorbance spectrum or it is between the chlorophyll *a* and *b* intersection. Similarly, valleys in the 1-nm action spectrum are in accordance with the chlorophyll absorbance peaks. The 430-nm peak and the 450-nm valley line up with the chlorophyll *a*/*b* intersection and the chlorophyll *b* peak, respectively. This relationship implies that major pigments might not be just used to funnel energy. We hypothesize that this coincidence could indicate that chlorophyll is used to dissipate light energy at 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, which is considered high energy by photosynthetic reaction centers in plants.

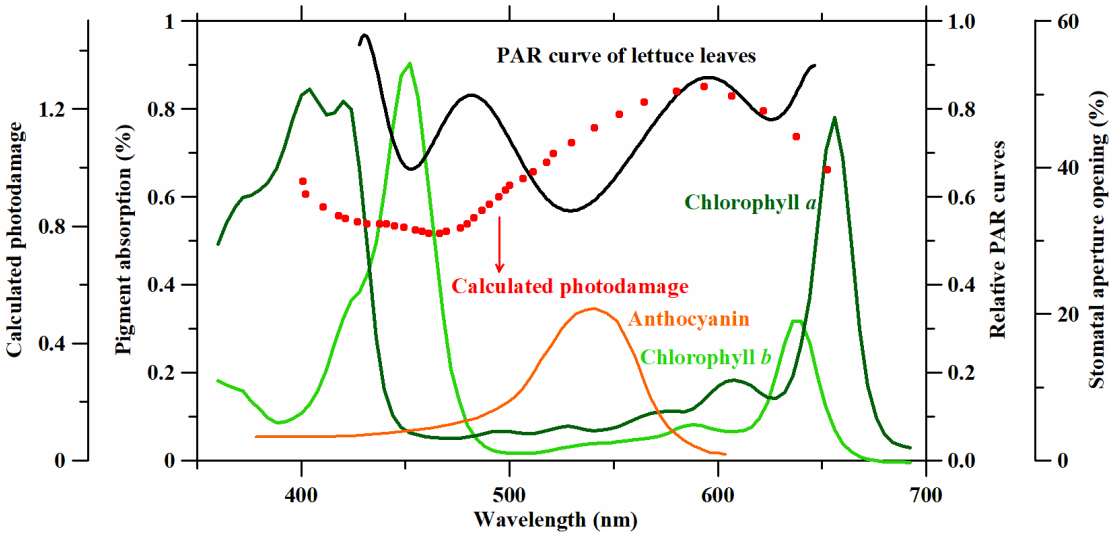

Figure 35. Comparison of the 1-nm-resolution action spectrum, the shifting pigment absorbance spectrum (unpublished data), the anthocyanin absorbance spectrum, and calculated photodamage efficiency. Pigment absorbance data (chlorophyll *a* and *b*) is derived from Taiz and Zeiger (2002). The photodamage efficiency is redrawn from data provided by Takahashi et al. (2010).

595-nm light

The 595-nm light effect may be mediated by the oxygen-evolving complex (OEC), a $Mn_4CaO_5^-$ cluster involved in photosynthesis (Umena et al., 2011). The OEC is located in photosystem II (PSII) and is responsible for photo-oxidation of water molecules. In addition, it has been suggested that manganese (Mn) contributes to photosynthesis (Bishop, 1928; Habermann, 1960; McHargue, 1922). Studies have attempted to determine the OEC structure using X-ray spectroscopy (DeRose et al., 1994; Iuzzolino et al., 1998; Sauer et al., 2008), but this remains challenging as X-rays can damage the OEC and the Mn-cluster is complex (Grabolle et al., 2006; Umena et al., 2011; Yano et al., 2005). Its absorptance characteristics also remain unknown.

The photo-damage efficiency of PSII provides indirect evidence of a link between 595-nm light and OEC involvement in photosynthesis (Figure 35) (Hakala et al., 2005; Takahashi and Badger, 2011; Takahashi et al., 2010). Studies have demonstrated that primary photo-damage to PSII is associated with light absorptance by the Mn-cluster in OEC (Hakala et al., 2005; Tyystjärvi, 2008), and that photo-damage to PSII is extensive upon exposure to UV and amber light (Takahashi et al., 2010). Therefore, these studies indirectly imply that the photo-damage efficiency of OEC may be represented by its absorptance spectrum and that peak absorptance of the OEC occurs in the amber wavelengths. Although the water-splitting process within the OEC has not been clarified, we observe a high correlation between the photo-damage efficiency of OEC and the spectral quality of photosynthesis between 500–600 nm in the current study. As such, we hypothesize that the water-splitting process initiates light energy absorptance by the OEC without light energy transfer from antenna pigments. In this way, amber light allows the water-splitting event in the OEC to occur, resulting in the electron transport chain and subsequent use of photosynthetic machinery, at a moderate light intensity. This hypothetical event agrees with the measured spectral quality of photosynthesis, and pigments regrading amber light absorptance characteristics have not yet been identified.

6.5 Conclusion

A most detailed spectral quality of photosynthesis (1-nm resolution action spectrum) was obtained using LEDs and a monochromator. Data show peak photosynthetic rates at 430 nm and 650 nm, with increased levels at 595 nm and 480 nm. Observed peaks in the blue and red wavelengths are inversely correlated to the extracted pigment absorbance spectrum. The 595-nm peak observed in this investigation and reported in other photosynthetic studies suggests that the OEC initiates the use of photosynthetic machinery in the presence of amber light.

Connecting text

Chapter 7, “Manipulating light-emitting diode spectra with optical filters to investigate lettuce”, is authored by Bo-Sen Wu and Mark Lefsrud, and it will be submitted as a manuscript for peer review (*Photochemical and Photobiological Sciences*).

In Chapter 6, a decrease in photosynthetic rate was observed at ~630 nm. In Chapter 7, this wavelength was removed with an optical filter and plant responses were investigated.

CHAPTER 7

Manipulating light-emitting diode spectra with optical filters to investigate lettuce

Abstract

The objectives of this study were to investigate plant performance under 590 nm–630 nm and 630-nm light, using manipulated phosphor-converted amber and red light- emitting diodes (LEDs). The photosynthetic rate (min) and growth of lettuce plants (*Lactuca sativa* cv. Breen) were measured at an irradiance level of 50 $W \cdot m^{-2}$ (243-267 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). To obtain the desired spectra, we outfitted existing phosphor-converted amber (602 nm) and red (633 nm) LEDs with different optical filters (shortpass and notch filters) to create a narrow spectrum (613 nm) and a double-peak spectrum (595 nm and 655 nm, hereafter referred to as 595-nm light treatment) that excluded 630-nm light. The average photosynthetic rate and biomass yields (fresh and dry mass), were highest for lettuce plants grown under the 602-nm light treatment, followed by the 595-nm, 633-nm, and 613-nm light treatments. Shifting and narrowing LED wavelengths from 602 nm to 613 nm and from 633 nm to 613 nm resulted in a biomass yield decrease of ~50 % and ~80 %, respectively. When compared to the 595-nm and 602-nm light treatments, plants treated with 630-nm light resulted in larger leaf areas, expanded plant structures, and the absence of purple coloration. We conclude that 630-nm light is necessary to reduce cell elongation, while amber light is beneficial for plant growth.

7.1 Introduction

Plant lighting experiments have shown that red (600–700 nm) light plays a critical role in short-term photosynthetic activity and long-term plant development within the photosynthetically active radiation (PAR) spectrum (400 nm–700 nm) (Goins et al., 1997; McCree, 1972a). Pioneering action spectrum and quantum yield studies using monochromatic light indicated that red light induces higher photosynthetic activity (~20 %–40 %) than other wavelengths in the PAR spectrum for typical greenhouse crops (Inada, 1976; McCree, 1972a). McCree (1972a) also determined that the highest wavelength peak was at 620 nm, with a shoulder at 670 nm in the red wavelength range. This study led to the use of red light-emitting diodes (LEDs) in plant lighting systems (Massa et al., 2008; Morrow, 2008).

Since the very first data were collected from using 660-nm LEDs for plant lighting by Bula et al. (1991), the effect of deep-red (650 nm–690 nm) LED light on plant development has been explored and evaluated. Deep-red LED light is beneficial for plant growth in terms of biomass yield (Brazaityte et al., 2006; Goins et al., 2001; Mizuno et al., 2011), yet further studies using 640-nm LEDs alone or as supplemental lighting to investigate plant responses did not show any positive effect on plant growth (Lefsrud et al., 2008; Mizuno et al., 2011; Olle and Viršile, 2013; Stutte et al., 2009). However, 640-nm LED light stimulated secondary metabolite and anthocyanin accumulation, and deep-red LEDs are still the basal component in plant LED lighting systems for plant productivity (Massa et al., 2008; Mitchell, 2015). To date, the effect of orange/red (610 nm–630 nm) LED light on typical greenhouse crops has not yet been determined.

Amber-biased (~ 590 nm–610 nm) high pressure sodium (HPS) luminaires are currently the preferred choice over LEDs in commercial greenhouse facilities, as plant productivity varies with crop choice and growth stages when plants are cultivated under LED light (Gómez et al., 2013; Olle and Viršile, 2013). In recent years, experiments that compare HPS lamps to blue/red LEDs for plant growth and yield have become one of the major foci of lighting studies (Bergstrand and Schüssler, 2013; Dueck et al., 2011; Gajc-Wolska et al., 2013; Gómez et al., 2013; Martineau et al., 2012). These studies indicate that LEDs will become the prominent

plant lighting system in horticulture, mainly because of their energy-efficient technology. Yet, according to some of the same reports, plant productivity and physiology were not significantly changed with LED lighting (Gómez et al., 2013), or were superior when grown under HPS luminaires alone (Brazaityte et al., 2006; Gajc-Wolska et al., 2013; Martineau et al., 2012). In addition, conflicting results on the effect of amber light using HPS luminaires have been reported (Dougher and Bugbee, 2001; Loughrin and Kasperbauer, 2001; Vänninen et al., 2010). Specifically, suppressed growth of some greenhouse crops, including basil (*Ocimum basilicum* L.) and lettuce (*Lactuca sativa, cv*. Grand Rapids), was observed when grown under high proportions of amber light (Dougher and Bugbee, 2001; Loughrin and Kasperbauer, 2001).

When compared to HPS luminaires and other conventional lighting sources, LEDs are advantageous because specific wavelengths may be selected and controlled (Morrow, 2008; Singh et al., 2015; Yeh et al., 2010). In particular, LED wavelengths can be selected to target specific plant physiobiological responses (Lefsrud et al., 2008; Massa et al., 2008; Olle and Viršile, 2013). However, users have limited options in terms of wavelength selection from diode manufacturers. For instance, there are only 10–15 LED nominal wavelength options in the red wavelength range from major diode manufacturers such as Cree and Philips Lumileds. Furthermore, users have limited control over existing LED light conditions (e.g. peak wavelength, spectral composition, and full width at half maximum [FWHM]) under normal operations. Although the lighting environment can be manipulated using different color LEDs, this method can only combine existing LED colors. This has led to some undesirable results, such as uneven light quality/quantity over plant surfaces and low light outputs in wavelengths of interest (Hogewoning et al., 2010a). These limitations hinder any investigations into the effect of specific narrow band wavelengths of LED light on plant growth and development.

To address these challenges, the objectives of this study were to: 1) investigate the effects of 590 nm–630 nm LED light and 630-nm LED light on photosynthetic activity and plant development at a high irradiance level, and 2) create LED spectra

(single and double-peak spectra) that are currently not available from major LED manufacturers, using optical filters. The lettuce cultivar *Lactuca sativa* cv. Breen was selected for this study as it has been used extensively for wavelength testing in the horticultural field. Although photosynthetic photon flux density (PPFD) is considered as a standard unit for plant growth, it is usually used to define photosynthetic rates rather than photomorphogenesis. Therefore, irradiance levels in this study are reported as $W \cdot m^{-2}$ (as recommended by Langhans and Tibbitts (1997) and Both et al. (2015)), and as PPFD ($\mu mol \cdot m^{-2} \cdot sec^{-1}$). The data reported herein add to our understanding of the impact of 590 nm–630 nm light on short-term photosynthetic activity and long-term plant development.

7.2 Materials and methods

Plant growth conditions and the LED lighting system

Lettuce seeds (*L. sativa* cv. Breen; pelleted MT0 OG, Johnny's Selected Seeds, Winslow, ME) were potted in 25 mm rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and placed in a growth chamber (TC30, Conviron, Winnipeg, Manitoba, Canada) for germination. Plants were kept in the chamber under cool-white fluorescent bulbs (4200 K, F72T8CW, Osram, Wilmington, MA, US) at an average irradiance level of 20 $W \cdot m^{-2}$ (equal to ~100 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) with a 16-h photoperiod. Environmental conditions in the chamber were controlled at 50 % relative humidity, with day/night temperatures of 23/21 ± 1 °C and ambient CO_2 levels. Full strength Hoagland nutrient solution was provided to the plants every other day as described by Hoagland and Arnon (1950). The young lettuce plants (2 weeks after germination), with the emergence of the fourth true leaf, were transferred to a 13-L hydroponic tank (Rubbermaid, Atlanta, GA, US) and grown under the experimental light treatments prior to measuring whole plant photosynthetic rates. Oxygen in the hydroponic tanks was provided using air pumps (Marina 200, Rolf C. Hagen Inc., Baie d'Urfé, QC, Canada).

Figure 36 illustrates the LED lighting system used in this study. Phosphor-converted (pc)-amber (LXM2-PL01, Philips Lumileds, San José, CA, USA) and red (LXM2-PD01, Philips Lumileds) LED assemblies were used as light sources. The pc-amber LED assembly was selected because its spectral composition

was similar to that of HPS luminaires. Each LED assembly was connected to a power distribution panel and powered using a DC power supply (DP832, Rigol Technologies Inc., Beaverton, OR, USA). An adjustable voltage regulator with a digital voltmeter and a 700-mA dimmable DC voltage driver (A011 FlexBlock, LED dynamics, Randolph, VT, USA) was placed between the power distribution panel and the LED assembly; this provided a constant current output while allowing for adjustments to LED light outputs. All LED assemblies were mounted on a water jacket (ST-011, Guangzhou Rantion Trading Co., Guangdong, China) and attached to lenses (25 mm focal length, No. 263, Polymer Optics, Wokingham, Berkshire, UK) that concentrated emissions from the LED assemblies into one single spot (12 mm diameter). Water was circulated at 15 °C in the water jacket behind the mounted LEDs using an Isotemp bath circulator (4100R20, Fisher Scientific, Hampton, NH, US). Two types of optical filters were used to manipulate the spectral compositions of the pc-amber and red LEDs: a 632.8-nm notch filter (25 mm diameter, #67-120, Edmund Optics, Barrington, NJ, US) and a 625-nm short pass filter (25 mm diameter, #64-604, Edmund Optics). Selection of the shortpass and notch filters was based on the requirement to decrease peak wavelengths of the red LED assemblies and to exclude 630-nm light from the pc-amber LED assemblies, respectively. Use of the notch filter eliminated overlapping wavelengths that occur when using two different color LEDs.

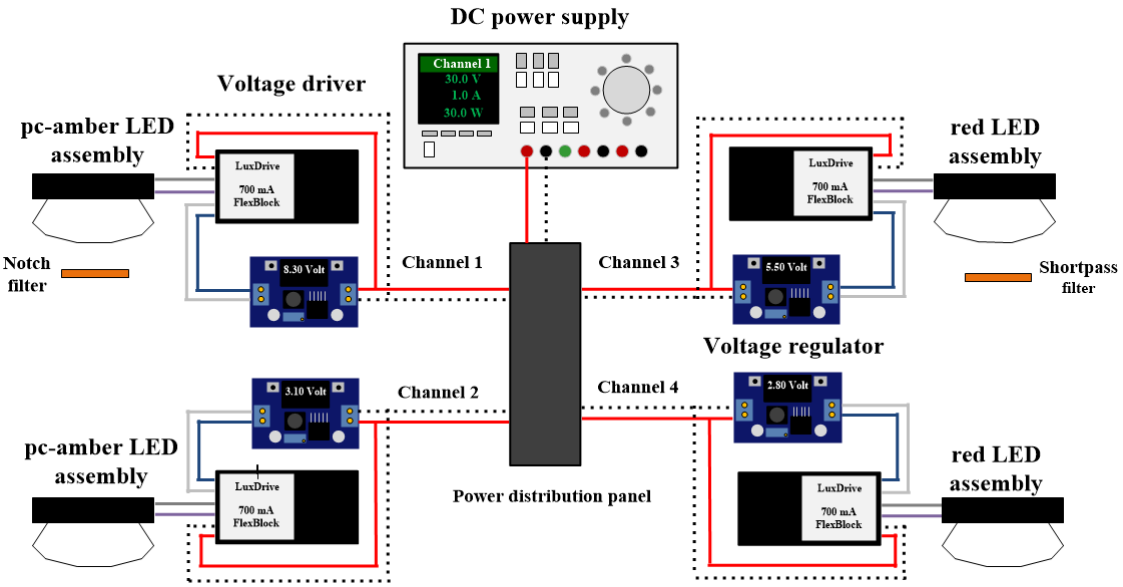

Figure 36. Simplified schematic diagram of the LED lighting system showing the power supply, power distribution panel, voltage drivers, voltage regulators, and LED assemblies.

Whole plant photosynthetic rate determination

Whole plant photosynthetic rates of the two-week old lettuce plants grown under different light treatments were carried out using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) equipped with a Whole Plant Arabidopsis Chamber (6400-17, LI-COR). The irradiance level was set to 50 $W \cdot m^{-2}$. Relative humidity (50 %) and temperature (and 23 °C) of the LI-COR environment were controlled so that the environmental conditions were the same as those in the germination growth chamber. The CO_2 concentration and flowrate in the Whole Plant Arabidopsis Chamber were set to 400 $\mu L \cdot L^{-1}$ and 400 $\mu L \cdot min^{-1}$, respectively. Photosynthetic rate measurements were repeated three times with three biological replicates. Plant leaf area was measured using ImageJ 1.48v software (NIH, Bethesda, MD, US). Imagery acquired with ImageJ software was used to determine the whole plant photosynthetic rate on a per unit leaf area basis.

Experimental setup

After emergence of the fourth true leaf, lettuce plants were transplanted to another growth chamber containing the LED lighting system. Plants were cultivated under the four following LED light treatments at an irradiance level of 50 $W \cdot m^{-2}$ for

two weeks: a pc-amber LED assembly (602 nm, 256 µmol·m^{-2}·sec^{-1}), a pc-amber assembly with the notch filter (595 nm and 655 nm, 250 µmol·m^{-2}·sec^{-1}), a red LED assembly (633 nm, 267 µmol·m^{-2}·sec^{-1}), and a red LED assembly with the shortpass filter (613 nm, 243 µmol·m^{-2}·sec^{-1}). Peak wavelengths, spectral compositions, and light intensities (irradiance levels and PPFD) of the LED light treatments were measured using a spectroradiometer (PS-300, Apogee, Logan, UT, US) (Figure 37 and Table 10).

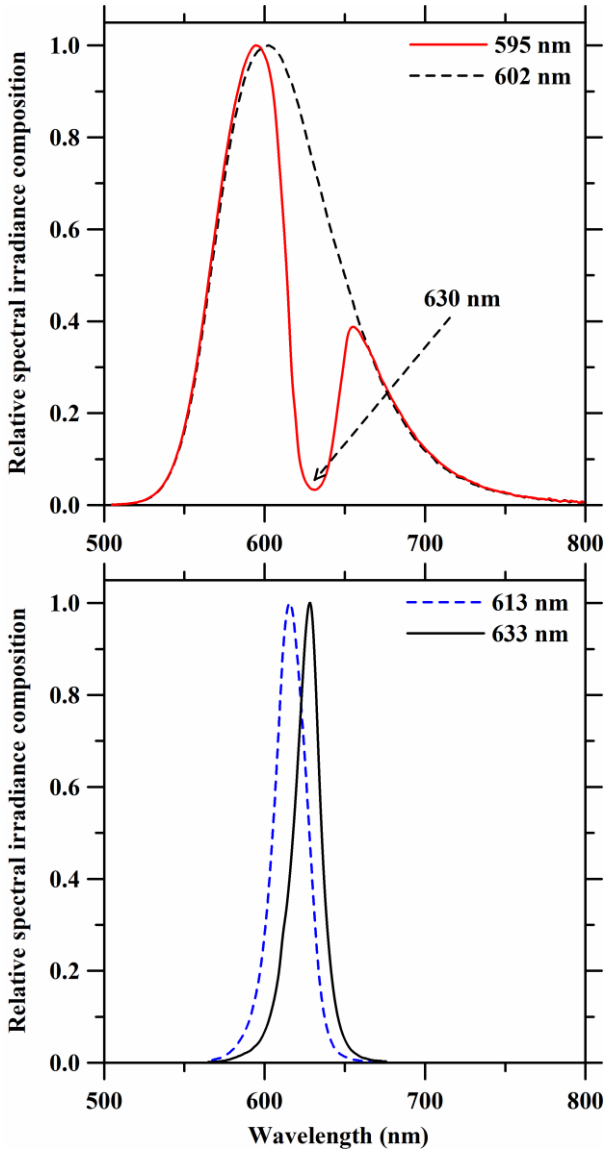

Figure 37. Relative spectral irradiance compositions of the LED light treatments. The arrow in the upper graph indicates the wavelength of the valley using the pc-amber LED assembly with the notch filter.

Table 10. Peak wavelength, full width at half maximum (FWHM), irradiance level, and photosynthetic photon flux density (PPFD) of each LED lighting system used in this study.

		Peak wavelength (nm)	FWHM (nm)	Irradiance level ($W \cdot m^{-2}$)	PPFD ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)
Pc-amber LEDs + notch filter	1st peak	595	48.90	37.5	187.5
	2nd peak	655	41.44	12.5	62.5
Pc-amber LEDs		602	74.07	50	256
Red LEDs + shortpass filter		613	22.12	50	243
Red LEDs		633	20.24	50	267

With the notch filter, the pc-amber LED spectrum was altered to narrow the 595-nm and 655-nm spectra (ratio ≅ 3: 1). This double-peak spectrum was named after its main peak (595 nm) throughout the remainder of this study. In the chamber, optical filters were secured using three-screw adjustable ring mounts (#36-605, Edmund Optics) and placed 25 mm below the amber and red LED assemblies. Cardboard covered with black plastic sheets, with a hole at the center, were placed on the ring mounts to avoid unfiltered LED spectra from reaching the plants. Between each light environment, black sheets were used as a light barrier. Due to the high irradiance levels and need for uniform light distribution over the plant surface, only one lettuce plant was placed under each light treatment. The height between the LED lights and the top of the plants were checked every three days and the LED lights were adjusted accordingly to allow the plant canopy to be exposed to the same irradiance level throughout the growth period. Differences in irradiance levels under each light treatment over the growth period were less than 2 %. Fresh full strength Hoagland solution was provided weekly. Environmental conditions (relative humidity, day/night temperature, CO_2 levels, and photoperiod) in the chamber with the LED treatments were the same as in the germination growth chamber. A timer connected to the DC power supply controlled the 16-h photoperiod.

Biomass yield and growth parameter analysis

After growing the plants for two weeks under different LED light treatments, they were harvested and sampled for biomass yield and morphological analyses,

including shoot fresh mass, dry mass, and leaf area. Biomass yield (fresh and dry mass) was determined with a balance (APX-153, Denver Instruments, Bohemia, NY, USA). To determine dry mass, plant samples were dried in an oven at 75 °C for no less than 72 h. Five biological replicates were measured under each light treatment.

Statistical analysis

Statistical analyses were performed using JMP 10 software (SAS, Cary, NC, US). Tukey-Kramer's HSD was used for multiple comparisons among spectral treatment means obtained from significant one-way analysis of variance (ANOVA) tests ($P < 0.05$).

7.3 Results

Plant growth and morphology

Lettuce plants had the largest size of plant structures when grown under the 595-nm light treatment, followed by the 602-nm, 630-nm, and 615-nm light treatments (Figure 38). Leaf-elongated lettuce (poor leaf development) was observed under the 613- and 633-nm light treatments. Differences in leaf morphology and leaf coloration were observed across all light treatments. Lettuce plants grown under the 595-nm and 602-nm light treatments had more obvious lateral veins than the 612-nm and 633-nm light treatments, and lettuce plants grown under the 602-nm light treatment were more compact than those grown under the 595-nm light treatment (Figure 38). Plants grown under the 613-nm light treatment had longer and thinner leaves compared to the 633-nm light treatment, and lettuce leaves grown under the 595- and 602-nm light treatments exhibited more curliness near the lateral veins. However, they were relatively smooth when grown under the 613-nm and 633-nm light treatments. Interestingly, purple pigmentation was only observed for lettuce leaves grown under the 602-nm light treatment (Figure 39).

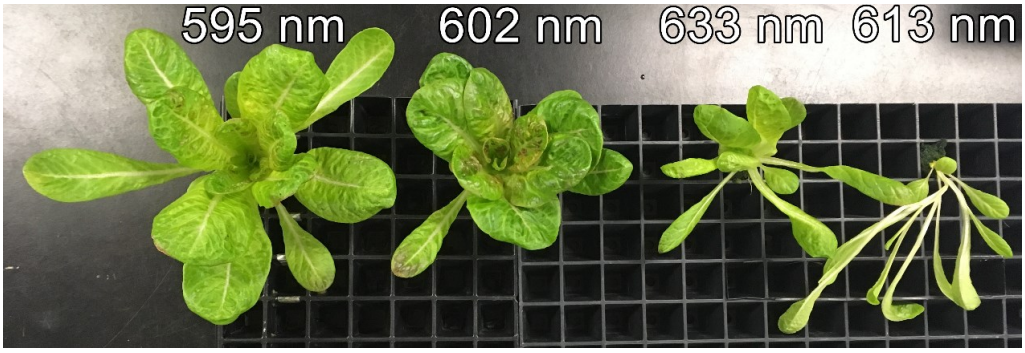

Figure 38. Plant morphology of lettuce (*Lactuca sativa, cv*. Breen) grown under 595-nm, 602-nm, 633-nm, and 613-nm LED light treatments (from left to right) for two weeks. Wavelength values in the figure indicate the peak wavelengths of each light treatments. Each grid measured 30 mm^2.

Figure 39. Representative pigmentation of a lettuce (*Lactuca sativa, cv*. Breen) leaf grown under 602-nm LED light treatment for two weeks.

Photosynthetic rates and biomass yield

Table 11 shows whole plant photosynthetic rates and biomass data for the lettuce plants grown under the 595-nm, 602-nm, 613-nm, and 633-nm LED light treatments. The highest average photosynthetic rate was obtained with the 602-nm light treatment, followed by the 595-nm, 633-nm, and 613-nm light treatments. Fresh and dry mass were higher for lettuce plants grown under the 602-nm LED

light treatment, followed by the 595-nm, 633-nm and 613-nm light treatments. The same trend was observed for photosynthetic rate, while differences in biomass between some specific light treatments were significant. The biomass yield observed under the 602-nm LED light treatment was nearly fourfold higher than the lowest biomass yield observed under the 613-nm light treatments, whereas the difference in photosynthetic rate between the 602-nm and 613-nm LED light treatments was only ~20 %. It is important to note that shifting the LED wavelength from 633 nm to 613 nm, and from 602 nm to 613 nm, resulted in a biomass yield decrease of approximately ~50 and ~80 %, respectively. Although plants grown under the 602-nm LED light treatment had the highest photosynthetic rates and fresh/dry mass, the largest leaf area was observed for lettuce plants grown under the 595-nm light treatment. Figure 40 highlights the comparative data for whole photosynthetic rates and fresh mass of the lettuce plants grown under all four different LED light treatments.

Table 11. Photosynthetic rates (n = 3) and biomass (n = 5) (mean ± standard deviation) of lettuce (*Lactuca sativa, cv*. Breen) plants grown under different LED light treatments. Letters indicate significant differences ($P \leq 0.05$)

Peak wavelengths of LEDs (nm)	Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	Shoot fresh mass (g)	Shoot dry mass (g)	Leaf area (cm^2)
595	2.66 ± 0.18^{ab}	27.16 ± 5.97^{c}	1.16 ± 0.20^{e}	419.5 ± 230.7^{g}
602	2.87 ± 0.12^{a}	27.12 ± 5.37^{c}	1.16 ± 0.21^{e}	400.7 ± 132.5^{g}
613	2.31 ± 0.03^{b}	5.76 ± 3.51^{d}	0.25 ± 0.17^{f}	136.6 ± 44.1^{h}
633	2.34 ± 0.19^{ab}	11.99 ± 3.07^{d}	0.50 ± 0.15^{f}	220.3 ± 90.3^{h}

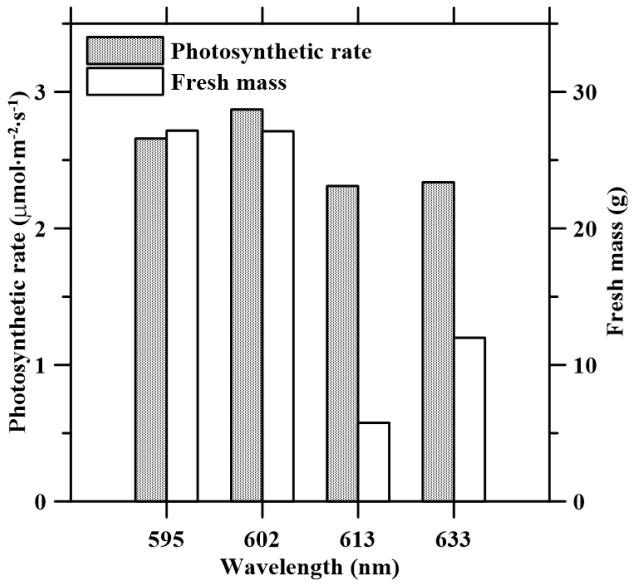

Figure 40. Comparative data of whole photosynthetic rates and shoot fresh mass for lettuce plants (*Lactuca sativa, cv*. Breen) grown under different LED light treatments.

7.4 Discussion

Photosynthetic rates

In this study, we noted the highest photosynthetic rates when plants were grown under 595-nm and 602-nm LED light, compared to 613-nm and 633-nm LED light. These data are not consistent with previous findings on action spectra observed by McCree (1972a) and Inada (1976), as both reported higher photosynthetic rates at 620 nm than at 600 nm. When comparing these earlier works to the current study, the main differences with respect to light properties are the light intensities and FWHMs used. The light intensity that was used to construct the action spectra in earlier studies is relatively low (~less than 150 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) (Inada, 1976; McCree, 1972a; Nelson and Bugbee, 2014), whereas in this study, approximately ~250 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ was used for all LED light treatments examined. Our data indicate that at a higher light intensity, photosynthetic activity does not follow the findings of McCree (1972a) and Inada (1976). This is supported by Bugbee (2016), who similarly stated that using these early action spectrum data to predict whole plant photosynthesis may not be as appropriate when using higher light intensities.

The high photosynthetic rates recorded at 595 nm and 602 nm were also inconsistent with the extracted pigment absorbance spectra, which were low in the

580–600 nm wavelengths. Within this range, absorbance percentages of the major pigments, such as chlorophylls *a* and *b* measured in acetone, are relatively lower than those of the blue and red wavelengths (Taiz and Zeiger, 2002). Similar studies with green (500–600 nm) light, which displays a pigment absorbance that is similar to that of amber light, have shown that supplementing 550-nm light with strong white light results in higher photosynthetic efficiencies than 680-nm light (Terashima et al., 2009), and that 532-nm light penetrates deeper into the leaf tissues in *Antirrhinum majus* L. (snapdragon) when compared to 488-nm or 650-nm light (Brodersen and Vogelmann, 2010). This difference is mostly due to scattering effects that allow green light to drive photosynthesis in the lower chloroplasts when the green light penetrates leaf tissues. Based on the literature, it is still not clear if amber light exerts an effect on leaf tissues that is similar to that of 532-nm or 550-nm light (Brodersen and Vogelmann, 2010; Terashima et al., 2009), yet our data indicate that the pc-amber LED light across the 500–800 nm range can induce higher whole plant photosynthetic rates than narrow orange/red light (600–650 nm). This may be caused by deeper penetration into leaves and by a wavelength interaction effect between green, amber, and red light that is similar to the interaction effect of 550-nm LED light and white light reported by Terashima et al. (2009). Nevertheless, how the light absorptance profile and internal plant anatomy contribute to this phenomenon merits further investigation.

Plant morphology and biomass yield

Higher fresh mass, dry mass, and leaf areas were found for lettuce plants grown under the 595-nm and 602-nm LED light treatments when compared to the 613-nm and 633-nm LED light treatments. For the amber wavelengths (595-nm and 602-nm), similar plant productivity was reported using HPS luminaires with and without sunlight for greenhouse-grown tomato (*Solanum lycpersicum* 'Komeett' F_1 and 'Starbuck' F_1) (Gajc-Wolska et al., 2013) and lettuce (*L. sativa* var. *capitata*) plants (Martineau et al., 2012). However, suppressed growth of lettuce (*L. sativa, cv.* Grand Rapids) grown under HPS luminaires was reportedly caused by a high portion of amber light (Dougher and Bugbee, 2001). If we compare the light intensities used in these studies, we can see that a maximum irradiance level threshold strongly influenced plant growth under amber light. On the other hand, studies that reported a

positive effect on plant growth occurred with lower light intensities of HPS luminaires (80-170 μmol·m^{-2}·sec^{-1}) (Gajc-Wolska et al., 2013; Martineau et al., 2012). The light intensity used in this study fell between the light intensities reported in the studies mentioned above, which was approximately ~250 μmol·m^{-2}·sec^{-1}. Therefore, when comparing data from these published studies and the current study, there is an indication that within the amber wavelength range, plants respond differently according to the irradiance levels of amber light used. Low irradiance levels of amber light will result in higher plant productivity, and these data support those reported by Gajc-Wolska et al. (2013) and Martineau et al. (2012). In contrast, high irradiance levels of amber light will lead to suppressed plant growth and to defense or interference of primary metabolism (Dougher and Bugbee, 2001; Loughrin and Kasperbauer, 2001; Vänninen et al., 2010).

Unlike the 595-nm and 602-nm LED light treatments, lettuce leaf elongation and poor leaf development was observed when plants were grown under the 613- and 633-nm LED light treatments. This suggests that the 613-nm and 633-nm LED light treatments affected plant morphology in a way that is similar to that of deep-red light (Heo et al., 2002; Johkan et al., 2010). Furthermore, although the 613-nm and 633-nm LED light treatments induced photosynthetic rates approximately 20 % less than those of the 595-nm and 602-nm LED light treatments, biomass yield decreased by approximately 50 % and 80 % when shifting LED wavelengths from 633 nm to 613 nm and 602 to 613 nm, respectively. This indicates that lettuce growth was strongly influenced by peak wavelengths of these LED light treatments at high irradiance levels. However, these data are not consistent with conclusions made by Cope et al. (2014) and Johkan et al. (2012), who found that wavelength has a much smaller effect on plant growth rates than light intensity. The lack of accordance with our findings might be due to differences in PPFD levels and wavelengths used in these studies. We studied wavelengths and light intensities between 595–633 nm and 240–260 μmol·m^{-2}·sec^{-1}, respectively, whereas Cope et al. (2014) and Johkan et al. (2012) reported results using white light and 510–530 nm LED light spectra, between 200–500 μmol·m^{-2}·sec^{-1} and 100–300 μmol·m^{-2}·sec^{-1}, respectively. This result reinforces the statement that under higher irradiance levels, plants respond differently and that predictions for plant growth and development that

are based on the early findings for quantum yield may be inappropriate (Bugbee, 2016; Inada, 1976; McCree, 1972a).

Blocking 630-nm light resulted in the largest leaf areas (~ 5 % larger) and different leaf coloration responses when compared to the 595-nm and 602-nm LED light treatments. It has been previously demonstrated that sole or supplemental 650–660 nm light enhances fresh/dry mass gain and leaf expansion in lettuce plants (Johkan et al., 2010; Shimizu et al., 2011; Son and Oh, 2013). The effect of 630-nm light alone on lettuce growth or leaf expansion has not yet been reported. However, it has been explored in pea seedlings (*Pisum sativum* L.) (Wu et al., 2007), *Protea cynaroides* L. (Wu and Lin, 2012), and poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) (Islam et al., 2012). When comparing the morphology of lettuce plants grown under the 602-nm and 633-nm LED light treatments (Figure 38), plants grown under the 602-nm light treatment displayed normal morphology with a compact architecture, while plants grown under the 613-nm and 633-nm light treatments prompted stem elongation with poor leaf development. Eliminating 630-nm light from the pc-amber spectrum (595-nm light treatment) resulted in plants with expanded structures and larger leaf areas when compared to the 602-nm light treatment. This suggests that the presence of 630-nm light impacts plant growth and morphology, unlike 650-nm light alone, which should not have any significant morphological impact (Bula et al., 1991). These results show that removing or adding individual wavelengths of light can have positive, negative or neutral effects on plant growth and can also impact plant architecture.

Purple coloration was observed in leaves grown under the 602-nm LED light treatment, but not when grown under 595-nm LED light treatment that blocked 630-nm light via the notch filter. Red and purple coloration in fruit and leaves is typically due to anthocyanin, a polyphenolic pigment (Cheng et al., 2014; Swain, 1976). Anthocyanin accumulation in lettuce plants can be induced by supplementing 373-nm, 455-nm, 460-nm, 476-nm, 505-nm, 658-nm, and 660-nm light with different light sources such as HPS luminaires, solar light, and white fluorescent bulbs (Li and Kubota, 2009; Owen and Lopez, 2015; Samuolienė et al., 2012). However, anthocyanin accumulation mechanisms and interactions with light signal

transduction pathways are not yet understood (Samuolienė et al., 2012). In the present study, we observed that 630-nm light may impact purple coloration in lettuce leaves. More validation with other greenhouse crops could clarify the effect of the 630-nm light on morphology and purple coloration in lettuce plants.

Photosynthetic rates and biomass yield

Although the observed differences for whole plant photosynthetic rates and biomass yields for lettuce plants grown with different LED light treatments were not proportional, both sets of data showed quadratic response curves with respect to the LED wavelengths (Figure 40). Because of instrument and chamber size limitations, two-week old plants were used for whole plant photosynthetic rate analyses. As plants grow under LED lighting, different photosynthetic rates are induced and plant architecture (e.g. leaf area) is impacted. During long-term plant cultivation, this would result in different light interception efficiencies for plants grown under different light treatments over the plant's lifespan (Evers et al., 2009). In this study, we observed differences in biomass yield (50 %–80 %) between different LED treatments, even though a constant irradiance level was provided over the plant canopies. These differences may be due to interaction effects between plant architecture, light interception, and wavelength. For instance, if lettuce plants are grown under 595-nm and 613-nm light, the former wavelength induces higher photosynthetic rates and results in faster leaf expansion rates, resulting in different light interceptions despite having the same irradiance level over the plant canopies for both light treatments. Over time, differences in plant growth would become greater between plants, because of the total energy received by their leaves. Lastly, the leaf elongation induced by the 613-nm LED light treatment may have influenced light interception differences, resulting in the observed difference in biomass yield.

We have demonstrated the importance of wavelength on plant growth and development using optical filters that manipulate LED spectra. Currently, lighting studies on plant performance mainly use single wavelength LEDs or mixed wavelength treatments created with standard LED wavelengths for plant growth (e.g. 460 nm and 655 nm). This is similar to a bottom-up approach, in which narrow LED FWHM (~20–30 nm) can allow for the construction and optimization of light

recipes for plant growth. However, typical LED spectra can cover a range of over 50 nm and even as great as 100 nm for some “narrow” spectrum LEDs. Data collected from the 613-nm and 633-nm LED light treatments show that wavelength strongly affects plant biomass yield production and morphology. In this scenario, using typical LED spectra would still result in wavelength interaction effects on plant development and consequently, lead to wrong (or partially wrong) conclusions if only considering their peak wavelengths. This opens the discussion for needing narrower spectrum lighting systems, such as laser diodes for plant lighting experiments. Data obtained using the notch filter in this study highlights that eliminating a wavelength that has negative effects on plant growth can possibly promote plant development. This method is similar to a top-down approach that has not been reported in plant lighting studies. Ultimately, optimal light recipes for plant growth can be created by removing specific wavelengths. This represents a new method that has not been applied to the LED lighting field. Further applications in plant lighting studies using this approach would be of interest.

7.5 Conclusion

Lettuce plant growth and photosynthetic performance were investigated when grown under amber and orange/red LED light treatments with spectra manipulated by optical filters at the same irradiance level. Importantly, this study indicates that under high irradiance levels, we should no longer refer to early quantum yield and photosynthetic rate findings when predicting plant growth and development. It also highlights the necessity of higher wavelength resolution for plant performance investigations. Amber (590–610 nm) and orange/red (610–630 nm) light play important roles in plant growth and development, however, more validation is needed to understand the specific effects of these wavelengths.

CHAPTER 8

General summary

8.1 General conclusion

The development of novel, high-intensity LEDs across the full visible spectrum has allowed for in-depth exploration of the impact of specific wavelengths on plant photosynthesis and cultivation. However, the uncertainty of using LEDs for plant cultivation still exists and prohibits the adoption of LEDs in the horticultural industry, even with great efforts made toward determining the optical plant-growing spectrum. Evaluating plant performance using LED luminaires requires a better understanding of both photosynthesis and complex LED technology. Early spectral photosynthesis data and the reliability of LED spectral properties have been misinterpreted. Moreover, increases in light intensity have increased concerns about eye safety. The objective of this research was to provide in depth information to the LED user in the industry, and to explore how plant productivity could be improved using this technology.

This work began with an evaluation of human photobiological eye safety with LED lights. We provided a guideline for selecting eyewear protection, aimed at LED users. The test irradiance level was 1000 $W \cdot m^{-2}$ (equivalent to ~5000 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), which would be applicable to all electrical lighting environments that use LED light. Spectral data revealed that transmitted spectra exhibited spectrum shifts, or an alteration in the bimodal distribution. This alteration might change the original spectrum to a narrower spectrum light that could compromise eye safety. In compliance with these data and international standards, we recommend that consumers and workers who use horticultural lighting select welding goggles or polarized glasses to limit any possible ocular impact. Sunglasses and safety goggles would not be advised as protection, particularly if infrared radiation was used.

Using the test apparatus, developed for the LED photo-biological safety evaluation, plant responses under monochromatic light were investigated at different light intensities (20-500 $W \cdot m^{-2}$, equivalent to 100-2500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$), through optical and spectral control. With this apparatus and different monochromatic light conditions, we discovered that (1) shade plants may be, “blue light-spot plants”, which may be used to mediate photo-damage better than sun plants. This mediation of photo-damage may occur by sensing blue light intensity; and (2) light directionality strongly affects light profiles in leaves and subsequently influences photosynthetic efficiency.

Further investigation into leaf optical properties showed that tomato and lettuce leaves emitting green light from 490-–600 nm, peaked at 530 nm when using a 410-nm LED assembly. To the best of our knowledge, the 530-nm spectrum has not been reported in earlier publications. Our data implies that the green coloration of leaves is not only from reflected green light, but it also comes from green light re-emission. In addition, the 530-nm spectrum was not influenced by irradiated time; rather, it was impacted by leaf water content and the 530-nm spectrum was highest in dried leaves.

We have performed the most detailed spectral quality of photosynthesis analysis known to date, with a 1-nm resolution action spectrum at 30 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ for tomato and lettuce plants. Both species had action spectrum curves with two distinct peaks at 430 nm and 650 nm, with shoulders at 480 and 595 nm. Our results show that there is a strong difference between the spectral quality of photosynthesis and individual wavelengths with light intensity effects. Furthermore, a reverse relationship was observed between the action spectrum and extracted pigment absorbance spectrum. For the 595-nm peak, we found that photo-damage efficiency, which strongly associates with OEC absorptance, is in accordance with the spectral photosynthetic data in the green light region (500–600 nm). Therefore, we hypothesize that chlorophyll is an important light energy dissipater and an absorber, and that 595-nm light imitates photosynthetic machinery through the OEC.

When the spectral photosynthetic data revealed a valley at 630 nm, we further investigated how 630-nm light might affect lettuce plant growth. We outfitted existing pc-amber (602 nm) and red (633 nm) LEDs with different optical filters (shortpass and notch filters) to create a narrow spectrum (613 nm) and a double-peak spectrum (595 nm and 655 nm; referred to as 595-nm light treatment in Chapter 7) that excluded 630-nm light. Blocking out 630-nm light led to larger leaf areas, expanded plant structures, and the absence of purple coloration. We propose that removing certain wavelengths that have negative effects on plant growth will allow researchers to prompt plant growth and alter plant architecture in future experiments.

8.2 Further suggested studies

The following recommendations are based on data compiled over the course of this research and are offered as possible future studies in this field:

1. The effect of light directionality on pigment absorbance.
2. The impact of 595-nm light paired with other wavelengths on plant growth.
3. The absorptance of OEC (or Mn).
4. Further investigation into the 530-nm emission spectrum and its applications in plant growth monitoring.
5. The blue light impacts on sun and shade plants at biochemical and molecular level.

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