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The Impact of Genetic and Environmental Factors on Phytonutrient Concentrations within Leafy Specialty Crops in Controlled Environments

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To the Graduate Council:

I am submitting herewith a thesis written by Rosalie M. Metallo entitled "The Impact of Genetic and Environmental Factors on Phytonutrient Concentrations within Leafy Specialty Crops in Controlled Environments." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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"New knowledge is the most valuable commodity on earth."

K. Vonnegut

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Abstract

Specialty leafy greens are excellent sources of antioxidants, vitamins, and minerals. Many of these metabolites are influenced by microclimate environmental conditions and genotype. The objective of this thesis was to measure the impacts of abiotic factors on plant growth and development along with nutritional content of specialty leafy greens in controlled environments. Chapter one looks at changes in biomass and nutritional content of different microgreen cultivars grown in a greenhouse over four growing seasons. Chapter two looks at the influence of light-emitting diode (LED) treatments on the growth and nutritional content of hydroponically grown kale. The morphology and nutritional content of hydroponically grown kale plants were significantly impacted by LED treatment. LED treatments with higher proportion of blue light had significantly shorter plants and greater fresh mass (FM) as compared to all other LED treatments. Environmental and genetic factors influenced the growth and development, as well as impacted the nutritional content of the different microgreen cultivars. Brassica microgreens had the highest FM and shortest production times throughout all seasons, as compared to the herb and lettuce microgreens. Whereas, the herb microgreens had the highest concentrations of shoot tissue carotenoids and minerals, as compared to brassica and lettuce microgreens. Results from this thesis provides valuable production data for producers who grow specialty leafy greens in controlled environments. Nutritional data among microgreen species may contribute to consumer knowledge for healthy eating choices.

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Chapter One Literature Review

1.1 Introduction

The environment plays a critical role in determining plant health and development. Plants use secondary compounds to respond to stress within their local environment (Murthy et al., 2014; Ramakrishna and Ravishankar, 2011). Secondary metabolites act internally to protect plants against ultraviolet (UV) light, extreme temperatures, drought, herbivory, and insect or pathogen injury and often accumulate within shoot tissues (Chamberlain et al., 2000; Croteau et al., 2000; Hectors et al., 2014). Therefore, leafy green specialty crops can be excellent sources of secondary metabolites. Carotenoids, a class of secondary metabolites, protect against UV photodamage and can be altered through changes in light intensity and quality (Schreiner et al., 2012). Secondary metabolites and other phytonutrients benefit plant as well as human health. Strong antioxidants like anthocyanins, carotenoids, and flavonoids aid in reducing the risk of developing chronic diseases like cancer, heart disease, diabetes and cataracts (Liu, 2013).

While secondary metabolites protect against environmental stress, light is a crucial factor that promotes plant growth and development. Light is detected via photoreceptors which monitor intensity, quality, and duration to signal developmental changes or the accumulation of protective secondary metabolites to stop the over production of damaging reactive oxygen species (ROS) and photoinhibition (Kim et al., 2005; Ouzounis et al., 2015; Tyystjärvi, 2013; Figure 1.1). Therefore, the purposeful manipulation of abiotic environmental factors like light, water, and temperature to elicit

secondary metabolite accumulation can enhance phytonutrient levels in vegetables, specifically leafy greens, which directly benefit consumer health (Liu, 2013; Ramakrishna and Ravishankar, 2011). This literature review will focus on the significance of secondary metabolites to plant and human health, as well as environmental impacts on the biosynthesis of secondary metabolites. It will also discuss light and plant development to give a greater understanding of the interactions between photosynthetic and photoprotective mechanisms.

1. 2 Ecological Significance and Biosynthesis of Secondary Metabolites

Primary metabolites like carbohydrates, amino acids, and lipids are universal organic molecules that can be found across all plant families (Taiz and Zeiger, 1998; Rhodes, 1994). Primary metabolites are essential for plant growth in that they are crucial for cell wall and membrane structure, cellular respiration, cell growth and expansion, and form the basic building blocks for the synthesis of polymers and other organic compounds (Olivoto et al., 2017; Taiz and Zeiger, 1998). The highly branched biosynthetic pathways and cycles that lead to the production of primary metabolites often link to or initiate the pathways that produce secondary metabolites (Herms et al, 1992; Matsuki, 1996; Rhodes, 1994). Secondary metabolites are specialized organic compounds, which allow plants to interact with each other as well as other organisms and respond to changes within their local environment (Murthy et al., 2014; Pickett and Khan, 2016; Zhao et al., 2005).

The concentrations of both primary and secondary plant metabolites are regulated through environmental and genetic factors (Hounsome et al., 2008). Unlike primary metabolites, secondary metabolites differ among different plant families and are not directly involved in plant growth although they may be necessary during select developmental stages (Speed et al., 2015; Pichersky and Gang, 2000). However, secondary metabolites facilitate important ecological interactions and play diverse roles in protecting plant health against damaging external stimuli (Croteau et al., 2000; Mazid et al., 2011).

The amount of damaging reactive oxygen species (ROS) is primarily controlled by jasmonic acid (JA) and ethylene (ET) interactions, and these important compounds activate the biosynthesis and accumulation of secondary metabolites (Jacobo-Velázquez et al., 2015). Plants produce secondary metabolites to attract pollinators and beneficial insects and deter herbivores (Nishida, 2014; Schreiner et al., 2012). Highly specific herbivore adaptions to secondary metabolites further support the idea that they do have a significant impact on herbivore fitness and function as effective defense tools (Agrawal and Weber, 2015). They also shield sensitive tissues from harsh environmental conditions (drought, chilling, and UV light) and protect plants from pathogen or insect attack (Chamberlain et al., 2000; Croteau et al., 2000; Hectors et al., 2014). Additionally, they can act as allelochemicals, which negatively impact the germination and growth of sensitive species (Mahdavikia and Saharkhiz, 2016; Sharma et al., 2014). It is this evolutionary arms race towards reinforcing chemical defenses to protect overall plant

health that has led to the current and continued diversification of secondary metabolites in plants (Speed et al., 2015).

Terpenes, phenolic compounds, alkaloids, and sulfur (S) containing compounds, such as glucosinolates, are independent secondary metabolite groups based on their biosynthetic origins and activity (Croteau et al., 2000; Murthy et al., 2014). Terpenes are derived from the precursor isopentenyl diphosphate (IPP) and synthesized in two pathways (McGarvey and Croteau, 1995; Webb et al., 2014). Monoterpenes, diterpenes, and tetraterpenes are synthesized in the chloroplast via the methylerythritol phosphate (MEP) pathway, while sesquiterpenes and triterpenes are produced in the mevalonic acid (MVA) pathway in the cytosol (Webb et al., 2014). Alkaloids and glucosinolates are primarily derived from amino acids (Croteau et al., 2000; Liu et al., 2016). Phenolic compounds are derived from either the shikimic acid pathway or the acetate-malonate pathway (Croteau et al., 2000). The biosynthesis of secondary metabolites is predominantly constrained to specific plant tissues and occurs at pre-determined developmental stages (Pichersky and Gang, 2000). Secondary metabolite concentrations are naturally low in plants, but can fluctuate due to seasonal changes in biotic and abiotic factors, geographical differences in natural resources, and genetic variations (Ramakrishna and Ravishankar, 2011; Sampaio et al., 2016). To invoke an increase in secondary metabolite biosynthesis and accumulation, plants can be purposefully exposed to environmental stresses like UV irradiation, pathogen attack and wounding,

changes in light intensity or quality, and extreme temperatures (Edreva et al., 2008; Kopsell and Sams, 2013; Ramakrishna and Ravishankar, 2011).

1. 3 The Human Health Benefits of Phytonutrients

As the primary site for photosynthetic reactions, leaves contain a myriad of primary and secondary metabolites which are beneficial to human health (Drewnowski and Gomez-Carneros, 2000; Osorio et al., 2014). Leaves also tend to be the most nutritious plant part since they contain dietary fiber, folate, vitamin C, Fe, Zn, Ca, and Mg, in addition to a host of secondary metabolites (Pennington and Fisher, 2009). Specialty leafy vegetable crops have edible, tender foliage and can include crops like lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* var. *capitata*), collard greens (*B. oleracea*), kale (*B. oleracea* var. *sabellica*), Swiss chard (*Beta vulgaris* subsp. *vulgaris*), microgreens, and various herbs (Hochmuth and Cantliffe, 2015; Lintas, 1992). Specialty greens and herbs have formed a high-value market niche after gaining considerable attention from restaurant chefs as well as ethnic and local markets for their interesting flavors, textures, and colors (Hochmuth and Cantliffe, 2015). These distinctive qualities can be attributed to the unique phytochemical profile of the crop (Kader, 2008).

Secondary metabolites, together with primary metabolites, are responsible for imparting the characteristic flavors, aromas, and colors of fruits and vegetables that consumers and herbivores recognize (Baenas et al., 2014; Pavarini et al., 2012; Figure 1.2). Secondary metabolites also provide health benefits to consumers. For example, carotenoids are lipid-soluble orange, yellow, and red pigments in fruits and vegetables

which act as antioxidants and are precursors for provitamin A in the human diet (Hounsome et al., 2008). Secondary metabolites that have been identified in leafy green vegetables include anthocyanins, carotenoids, alkaloids, glucosinolates, phenolic acids, and flavonoids (Cavaiuolo and Ferrante, 2014; Charron et al., 2008; Khanam et al., 2012; Kopsell and Sams, 2013; Hounsome et al., 2008; Slavin and Lloyd, 2012). The secondary metabolites and other phytonutrients gained consumed in leafy vegetables are linked to the prevention of common diseases like cataracts, cardiovascular and heart disease, stroke, cancer, and diabetes (Liu, 2013). Disease prevention through the natural enhancement of phytonutrients within vegetables is a viable approach to improve consumer health since enhanced consumption of phytonutrients could be accomplished within an everyday diet (Drewnowski and Gomez-Carneros, 2000; Liu, 2013).

1. 4 The Importance of Light in Plant Development

Light is the primary source for energy in plants and drives photosynthetic reactions to create different metabolites that are important for plant growth and development (Gates et al., 1965; Taiz and Zeiger, 1998). Photosynthesis occurs in two phases; light energy is first converted into adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), and oxygen is released during the light dependent reactions (Ashraf and Harris, 2013). Then in the light-independent reactions, carbon is fixed into simple carbohydrates using the energy molecules ATP and NADPH created in the light reactions (Ashraf and Harris, 2013). Light intensity, quality, and duration are the keys factors in eliciting chemical reactions as well as controlling

metabolic pathways and developmental changes in plants (Nemhauser and Chory, 2002; Darko et al., 2014). Plant morphology, leaf shape and anatomy, photosynthetic rate, and phytochemical composition can be manipulated through changes in the light environment (Kim et al., 2005; Lefsrud et al., 2008; Li and Kubota, 2009).

Upon exposure to light, plants undergo a series of photoinduced reactions and anatomical changes following a highly regulated developmental program called photomorphogenesis (Eckardt, 2001). Chlorophyll absorbs within the visible spectrum (400 nm to 700 nm) with peak absorbance in the blue and red regions (430-450 and 640-660 nm), while secondary pigments like carotenoids have peak absorption in the blue region (380-550 nm), aiding in the total amount of light harvested and protecting photocenters from UV damage (Carvalho et al., 2011). Plants can use a variety of tools to perceive and monitor light quality and quantity like photoreceptors, the redox state of the plastoquinone pool located in the thylakoid membranes in chloroplasts, and to a lesser degree photosynthate concentrations (Kim et al., 2005; Yano and Terashima, 2001; Figure 1.3). Photoreceptors are specialized photomorphogenic pigment-proteins that contain chromophores which serve as the main site for light absorption (Darko et al., 2014; Möglich et al., 2010). The chromophore is the region where photoinduced chemical reactions occur, converting light energy into biochemical signals such as protein-protein interactions or enzyme activation to manipulate biosynthetic pathways (Kong and Okajima, 2016).

Currently, five photosensory systems have been identified and they monitor different wavelengths of light; those include phytochromes (phys), UV Resistance locus 8 (UVR8), cryptochromes (crys), phototropins (phots), and proteins in the Zeitlupe family which include Zeitlupe (ZTL), F-Box-1 (FKF1), and LOV Kelch Protein 2 (LKP2) (Christie et al., 2014; Kong and Okijima, 2016). Photoreceptors have different domains to independently bind tetrapyrrole and flavins as their chromophores: LOV (light, oxygen, or voltage) of phots, GAF (cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA) of phys, and PHR (photolyase homologous region) of crys (Briggs and Olney 2001; Christie et al., 2014; Kong and Okajima, 2016). UVR8 utilizes particular tryptophan residues for UVB absorption rather than a chromophore (Wit et al., 2016).

Crys and phys work in tandem to broaden the action spectrum of photosynthetic pigments to better coordinate photosynthetic output and development based on light conditions (Lin, 2002). Phys absorb red (P_r) (600–700 nm) and far-red (P_{fr}) (700–750 nm) wavelengths, while crys and phots are the two main photoreceptors that absorb blue light (390-500 nm) (Möglich et al., 2010). Phytochrome is a photoreversible light sensing pigment that converts between active (P_{fr}) and inactive (P_r) forms to induce seed germination, shade avoidance responses, and flowering (Briggs and Olney 2001; Wit et al., 2016). Phototropins are aptly named since they are involved in phototropisms as well as stomatal opening and chloroplastic movement in response to changing light environments and increased kinase activity (Kharshiing and Sinha 2015; Wit et al., 2016). Cryptochromes have two separate peak absorption areas in the blue

and UV-A regions respectively (Carvalho et al., 2011). They are involved in regulating the circadian clock, flowering or reproduction, and photomorphogenesis (Wit et al., 2016).

Zeitlupe group members are LOV domain, blue-light sensing proteins involved in the regulation of circadian rhythms and photoperiodic flowering (Banerjee and Batschauer, 2005; Kevei et al., 2006; Kong and Okijima, 2016; Suetsugu and Wada, 2013; Wit et al, 2016). UVR8 regulates plant developmental and protective responses to UV and absorbs light in the UV B region (280–315 nm) (Christie et al., 2014). In general, photomorphogenic responses can be induced through select photoreceptors, while others may be induced through several different light signaling pathways or receptors working antagonistically or cooperatively (Wit et al., 2016).

Recently, molecular mechanisms have linked light to hormonal responses in plants (Arsovski et al, 2012). Light induced photomorphogenesis moderates several hormonal pathways that produce gibberellins, abscisic acid, auxin, brassinosteroids, cytokinins, and ethylene (Yu et al., 2013). It has been recognized that blue light signaling via the Cryptochrome Circadian Clock 1 protein (cry1) changes the expression of Auxin Response Factor (ARF) genes to directly act on modifying the expression of auxin (Arsovski et al., 2012). The Constitutive photomorphogenesis 1 with the Long Hypocotyl 5 (Cop1-HY5 complex) and the phytochrome interacting factors (PIFs) pathways are the main light signaling pathways downstream from photoreceptors that connect light signaling and hormonal responses (Lau and Deng, 2010; Yu et al., 2013). HY5 competes with PIFs for the same binding sites and works with COP1 to suppress

photomorphogenesis in darkness (Ang et al., 1998; Delker et al., 2014; McNellis et al., 1994). Light encourages the buildup of HY5 proteins to promote photomorphogenesis by limiting the accumulation of COP1 in the nucleus (Yu et al., 2013). Additionally, light triggers the rapid degradation of PIFs and successively increases the biosynthesis of photosynthetic pigments (Liu et al., 2013). PIFs are able to act as repressors or promoters for the synthesis of photosynthetic pigments and chloroplast development (Liu et al., 2013). The effects of the light regulated control of hormones and photomorphogenesis is apparent in the dramatic changes that occur to seeds and seedlings during early growth (Arsovski et al., 2012; Lau and Deng, 2010).

1. 5 Light Stress, Photoinhibition and Its Influence on Plant Growth

Light-harvesting systems, composed mainly of chlorophylls and carotenoids, capture radiant energy and transfer it to the reaction centers of photosystem I (PSI) and photosystem II (PSII) within the thylakoid membrane (Yamori, 2016; Figure 1.4). Photoreceptors and photosynthetic machinery within leaves often have the difficult task of harmonizing incoming light conditions with the requirements for metabolism (Kim et al., 2005; Mohr, 1994). The amount of light considered excessive for the photosynthetic apparatus of a leaf depends on compounding environmental conditions like drought or high temperature, the ability of photoprotective systems to work efficiently, irradiance level, and genetic influences like plant species (Ort, 2001). Low light conditions limit photosynthetic rate via low photonic energy input to the photosystems, while high light conditions can saturate the photosystems, which may cause photoinhibition of PSII and

subsequently reduce photosynthetic outputs (Takahashi and Badger, 2011; Yamori, 2016).

Photoinhibition is the inactivation of photosynthetic machinery or competence due to irreversible photooxidative damage to PSII which has been associated with excess light absorption by the manganese cluster within the oxygen-evolving complexes (OEC) (Kato et al., 2003; Lichtenthaler and Burkart, 1999; Takahashi and Badger, 2011). Reactive oxygen species (ROS) are produced within the electron transport chains (ETCs) of both photosystems during light reactions (Gururani et al., 2015). The ROS produced during light stress inhibit PSII leading to the damage of PSI, which has less efficient repair mechanisms when electrons from PSII exceed the capacity of PSI electron acceptors (Yamori, 2016). When light energy entering PSII is not fully utilized, singlet chlorophyll is converted to deleterious triplet chlorophyll, which can convert oxygen (O_2) into the ROS singlet oxygen ($^1O_2$) (Ksas et al., 2015; Pospíšil, 2016).

During electron transport, ROS can be formed through the single-electron reduction of oxygen (O_2) to form superoxide anion radical singlet oxygen ($^1O_2$) and through the two-electron oxidation of water (H_2O) to form hydrogen peroxide (H_2O_2), which is then reduced to hydroxyl radicals (HO) and singlet oxygen ($^1O_2$) (Gururani et al., 2015; Pospíšil, 2016). When ROS concentrations are maintained at low levels under moderate stress, they serve as signaling molecules to activate acclimation responses to stress and programmed cell death (Pospíšil, 2016). Left unchecked by scavenging systems, ROS cause significant damage to biological systems due to oxidation of nucleic

acids, lipids, membranes, and proteins (Bartwal et al., 2013). Hydroxyl radicals (OH) are the primary ROS responsible for the oxidation of proteins and peroxidation of lipids, damaging important proteins near PSII like D1 and proteins in the Light Harvesting Complex II (LHCII) subunit (Pospíšil and Yamamoto, 2016; Yoshioka-Nishimura, 2016).

Photodamage occurs before photoinhibition and also results in a depression in photosynthesis, but an important difference in this stage is that PSII is able to recover from damage using innate repair strategies (Yamamoto et al., 2014). Plants have evolved complex photoprotection mechanisms to avoid or negate the effect of photoinhibition that include leaf and chloroplastic movement, structural changes to the thylakoid membrane, ROS scavenging systems, non-photochemical quenching (NPQ) of chlorophyll fluorescence, photorespiration, and cyclic electron flow (CEF) around PSI (Nath et al., 2013; Takahashi and Badger, 2011; Yoshioka-Nishimura, 2016). In the early stages of high light damage to the PSII-LHCII complex, tolerance mechanisms concentrate on replacing damaged DI proteins (Yamamoto, 2016). Both lipophilic and hydrophilic antioxidant compounds and enzymes scavenge for ROS to protect against excessive oxidative damage (Bartwal et al., 2013). Carotenoids, which are lipophilic molecules, function as antenna pigments within light-harvesting complexes to reduce photodamage caused by the triplet state of chlorophyll molecules (Bian et al., 2015).

Other antioxidant scavenging systems include glutathione, ascorbate, tocopherol, and their associated enzymes such as superoxide dismutase, catalase, and peroxidase (Tripathy and Oelmüller, 2012). NPQ is activated though a change in pH via

protonation of antenna components within light harvesting complexes or based on the activity of xanthophyll cycles (Ruban, 2016). LHCII proteins within thylakoids will form reversible aggregates that work to dissipate excessive light energy through the xanthophyll cycle as heat via NPQ (Pospíšil and Yamamoto, 2016). Under high light, epoxidized xanthophylls are de-epoxidized and then return to their original epoxidized forms via non-radiative heat dissipation, facilitating the release of excess energy from the light harvesting complex (Latowski et al., 2011; Niyogi et al., 1997).

A number of photoprotective mechanisms have been developed by plants for avoidance of high light, repair of essential photosynthetic components, and ROS sequestration systems to prevent photoinhibition (Porcar-Castell, et al., 2014; Tyystjärvi, 2013). Leaf morphology and anatomy can also change in relation to light conditions. Sun leaves tend to be thicker and smaller as compared to the wide, thin blades of shade leaves (see appendix) (Kim et al., 2005). Photoinhibition is most likely to take place within the first leaf layers since this region has the greatest exposure to incoming solar radiation (Pinto et al., 2011). Plants are able to alter the leaf angle, curl leaves, or convert to C4 or Crassulacean Acid Metabolism (CAM) to avoid ROS production (Gowik and Westhoff, 2011; Mittler, 2002). Chlorophyll congregate at the cell walls parallel to the direction of incoming light to avoid excessive light and maximize CO_2 absorption via larger intracellular air spaces (Harada et al., 2003; Takahashi and Badger, 2011). Thylakoids undergo stacking and unstacking along with shrinkage and swelling in response to high light situations to support the quick replacement of damaged proteins,

in particular the degradation of impaired D1 proteins by FtsH protease (Yamamoto, 2016; Yoshioka-Nishimura, 2016).

1. 6 Lighting Options in Controlled Environments

The use of supplemental or sole-source artificial lighting in controlled environments is often necessary to ensure normal and dynamic plant growth (Darko et al., 2014). Artificial lighting within controlled environments must supply plants with energy to fuel photosynthesis and signal developmental changes over the entire growth cycle (Darko et al., 2014; Massa et al., 2015). Several lighting options are available for use in controlled environments. The most common artificial lighting sources used for plant growth include metal-halide (MH) lamps, high pressure sodium (HPS) lamps, incandescent lighting, fluorescent lighting, and light emitting diodes (LED) (Massa et al., 2015; Wheeler, 2008). Fluorescent lighting, incandescent lighting, and MH and HPS lighting all produce excess radiant heat and have reduced lifespans as compared to LED lighting (Singh et al., 2015). While MH lamps and HPS lighting have some of the highest PAR efficiency rates among other common lighting sources, LED lighting has the maximum PAR efficiency as well as the ability to target specific wavelengths intensity (Darko et al., 2014).

LEDs are a solid-state lighting device that uses a chip as a diode to generate photons of light at varying wavelengths (Singh et al., 2015; Figure 1.5). As a lighting option, LEDs are energy efficient, low density, can maintain a continuous light output over several years, and do not contain harmful chemicals (Bula et al., 1991; Morrow,

2008; Olle and Viršile, 2013). They also emit low heat radiation and can provide high light intensities, while allowing wavelengths to be changed throughout the growing period (Darko et al., 2014; Sabzalian et al., 2014; Sandahl et al., 2013). Currently, LED technologies are looking to improve intensity as well as longevity of the commercial bars and panels. Future challenges include adapting LED technologies to suit existing production systems and reducing investment or installation costs for LED lighting systems. Currently, LED technologies offer a more precise way to measure or control the impacts of light quality, intensity, and duration on plant growth and development in research or commercial applications.

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Appendix A

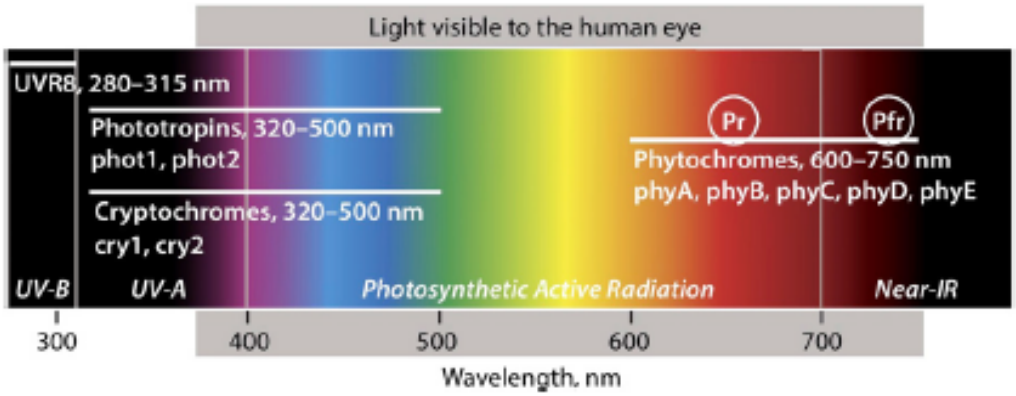

Figure 1.1 The main photoreceptors in plants and their associated absorption spectrums (Ouzounis et al., 2015).

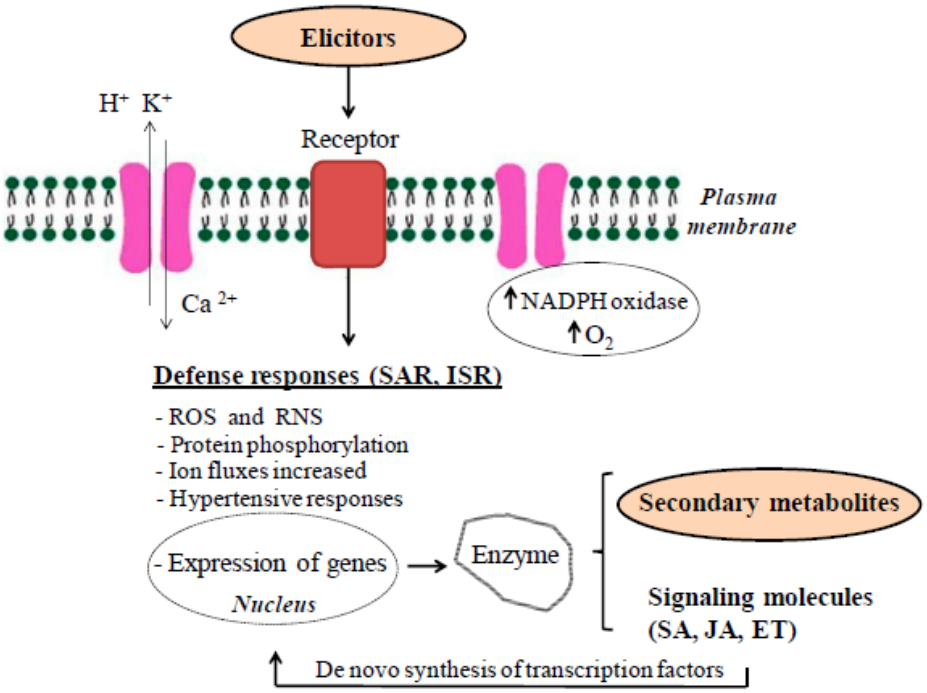

Figure 1.2 Overview of how elicitors regulate gene expression to influence the production of secondary metabolites and signaling molecules (Baenas et al., 2014).

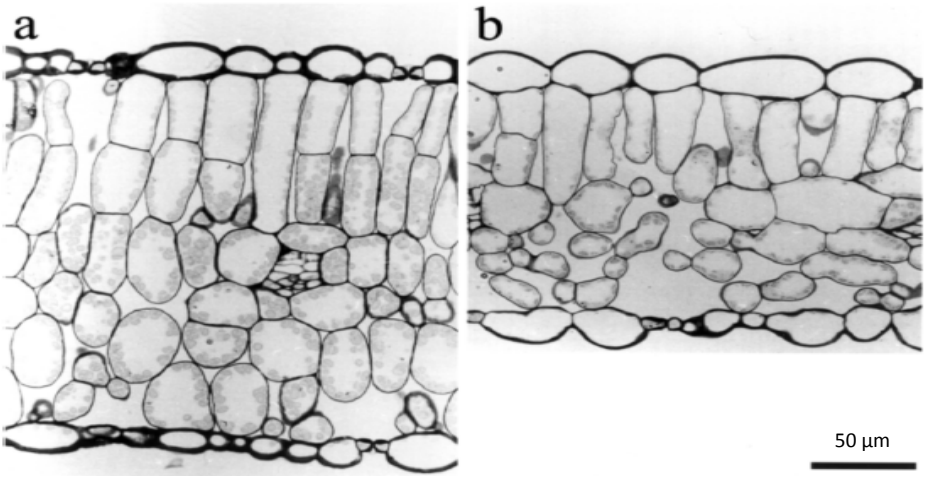

Figure 1.3 Cross-sectional light micrographs of (a) sun (360 µmol·m^{-2}·s^{-1}) and (b) shade (60 µmol·m^{-2}·s^{-1}) leaves of *C. album* (Yano and Terashima, 2001).

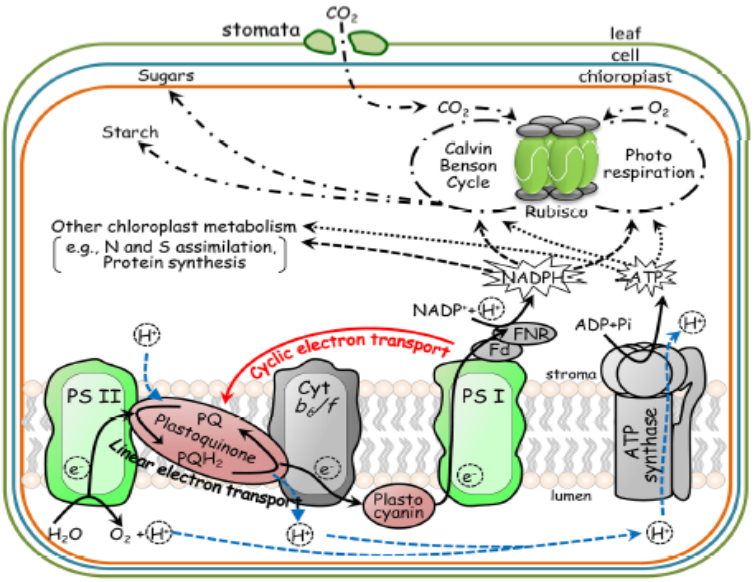

Figure 1.4 Illustration of C3 photosynthetic reactions and electron transport within the thylakoid membrane (Yamori, 2016).

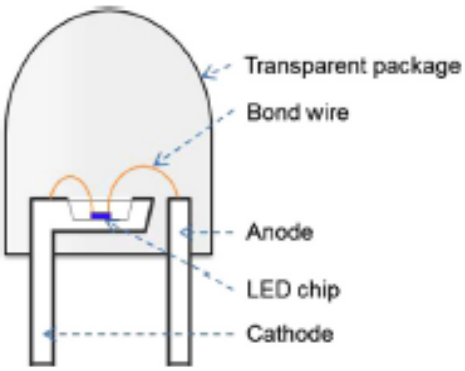

Figure 1.5 Diagram of internal structure of LED (Singh et al., 2015).

Chapter Two Screening of Microgreen Brassica, Herb, and Lettuce Cultivars over Different Environments for Biomass and Nutritional Quality Parameters

Abstract B

Microgreens are a relatively new and diverse specialty leafy greens category. Reports have suggested microgreens have more concentrated nutritional content as compared to their mature counterparts. Shoot metabolite concentrations can be modulated by microclimate environmental conditions and genotype. The objective of this thesis was to measure changes in biomass and nutritional content of fifteen commercially-grown microgreen cultivars in a greenhouse over four growing seasons in Knoxville, TN in 2016. Microgreen cultivars were divided into three groups: brassica, herb, and lettuce. The cultivars were grown in solid-bottom plastic germination trays (26 x 52 x 3 cm) using a soilless peat mix (Fafard germination mix 59-69%; Agawam, MA) and misted daily. All aboveground fresh mass (FM) was harvested after the microgreen cultivars reached the first to second true leave stage. Environmental and genetic factors influenced the growth and nutritional content of the different microgreen cultivars. Brassica microgreens had the highest FM as well as the shortest production and germination times throughout all seasons, as compared to the herb and lettuce microgreens. The herb microgreens had the highest concentrations of shoot tissue carotenoids and minerals, but the longest germination times as compared to brassica and lettuce microgreens. Information on the nutritional content of different microgreen cultivars may improve consumer knowledge, leading to healthier options. Cultivation data on each microgreen cultivar groups over the course of a year can benefit microgreen producers and home growers.

2. 1 Introduction

Microgreens have been grown commercially since the 1980s and found major success among innovative restaurants and health conscious consumers (Bliss, 2014; Kaiser and Ernst, 2012). The classification of a crop as a microgreen is based on its size and maturity. Microgreens are immature plants that are 2-7 cm in height, typically harvested after 7 to 14 d depending on species, and sold or consumed with the stem and fully developed cotyledon leaves attached (Xiao et al., 2012). Microgreens are commonly grown in greenhouses or controlled environments, with or without artificial lights, to protect the sensitive young plants from harsh environmental conditions (Di Gioia et al., 2016; Murphy et al., 2010).

Many different types of herbs, tender annuals, and vegetables can be grown as microgreens due to the broad classification of this specialty leafy crop. Members of the *Brassicaceae*, *Asteraceae*, *Chenopodiaceae*, *Lamiaceae*, *Apiaceae*, *Amarillydaceae*, *Amaranthceae*, *Fabaceae*, and *Cucurbitaceae* are popularly used for microgreens due to their fast growing time, unique seedling characteristics, seed availability, and simple germination/cultivation needs (Kyriacou et al., 2016).

During the early stages of development, secondary metabolites are crucial to protect the vulnerable seedling from pathogens, herbivory, or harsh environmental factors (Bourgaud et al., 2011; Chacón et al., 2013). Secondary metabolites that are initially concentrated within the endosperm or cotyledon are most likely contributions from the mother plant; secondary metabolites are produced *de novo* within the various

developing tissues of seedlings (Chacón et al., 2013). Many of the phytochemicals present in vegetables are strong antioxidants that can protect consumer health via bioactive mechanisms that quench free radicals which can cause oxidative stress as well as deoxyribonucleic acid (DNA) and membrane damage throughout the body (Chu et al., 2002). The synergistic defense roles of the different antioxidants and other phytochemicals reduce the risk for the development of chronic diseases like cardiovascular disease, cancer, and diabetes (Chu et al., 2002; Dillard and German, 2000; Liu, 2013; Van Duyn and Pivonka, 2000).

Microgreens are nutrient-dense vegetables with a host of bioactive compounds and usually have higher levels of phytonutrients as compared to their mature forms (Vaštakaitė and Viršlė, 2015; Xiao et al., 2012). Microgreens contain essential minerals along with antioxidants and other phytonutrients like ascorbic acid (vitamin C), phylloquinone (vitamin K), tocopherols (vitamin E), glucosinolates and carotenoids (Kopsell and Sams, 2013; Sun et al., 2013; Xiao et al., 2016; Xiao et al., 2014). The objective of this study was to conduct a genetic screening of 15 commercially grown microgreen cultivars for cultivation requirements, biomass and nutritional content and to measure changes in quality parameters over four growing seasons in a protected environment. Expanding the available information about the concentration of phytonutrients in microgreen shoot tissue within three different cultivar groups will improve consumer and producer knowledge concerning nutritional content.

2. 2 Materials and Methods

Plant Production and Growing Conditions

A total of fifteen cultivars were grown in a greenhouse at ambient light and temperature conditions over the course of four seasons categorized as winter, spring, summer, and fall (Table 2.1). The cultivars were grown using a soilless peat mix (Fafard germination mix 59-69%; Agawam, MA) in solid-bottom plastic germination trays (26 x 52 x 3 cm) and misted daily using a fine spray nozzle head (7.5 $L \cdot min^{-1}$). The cultivar groups represented three categories of leafy specialty microgreens: lettuce, herb, and brassica (Table 2.1; Johnny's Selected Seeds, Winslow, ME). The various groups of cultivars were selected to give a more inclusive comparison of the different growth habits and nutrition elements of different microgreen crops as well as their commercial popularity. The experiment was arranged in a randomized complete block design, with four replications. The main effects of cultivars were evaluated over four consecutive growing seasons (Table 2.2).

All aboveground fresh mass (FM) from the microgreens was harvested after the cultivars reached the first to second true leave stage. If the average microgreen height per individual block was above 2 cm (± 0.5 cm), shoot tissues were cut and collected using electric hand held shears (model GSN30; Black+Decker Inc., New Britain, CT). If the average microgreen height per individual block was ≤ 2 cm (±0.5 cm), shoot tissues were cut and collected using small hand shears. Height (cm), FM ($g \cdot plant^{-1}$), and dry mass (DM) ($g \cdot plant^{-1}$) data were collected at harvest. Fresh microgreen tissue was stored at -

20 ±1˚C prior to sample analyses. A 20-g sample of frozen tissue was freeze-dried (model 6 L FreeZone; LabConCo, Kansas City, MO) at -25 ± 1 ˚C for 48 h.

Shoot Tissue Pigment Extraction

The procedure from Kopsell et al. (2012) was used for pigment extraction. A 0.1-g (± 0.05 g) sample of freeze-dried tissue was weighed out into glass culture tubes (16 x 100 mm) and then rehydrated with 0.8 mL of ultrapure water for 10 min, and then 2.5 mL of tetrahydrofuran (THF) was added to begin extraction. After rehydration, 0.8mL of the internal standard ethyl-β-8'-apo-carotenoate (Sigma-Aldrich, St. Louis, MO) was added to measure the efficiency of the extraction process. Samples were homogenized using a drill press set at 540 rpm in a Potter-Elvehjem (Kontes, Vineland, NJ) tissue grinding tube using twenty insertions. After homogenization, the sample was placed in a centrifuge for 5 min at 500 g_n. The supernatant was removed and the sample pellet was then re-suspended in 2 mL THF and homogenized with the same extraction technique for a total of three extractions until the supernatant was colorless. The collected total supernatant was then reduced to 0.5 mL using nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA). Acetone was added to the concentrated supernatant bringing it up to the final volume of 5 mL. A 2 mL portion of the solution was filtered through a 0.2-µm polytetrafluoroethylene (PTFE) filter (Model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE) using a 5-mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and collected into amber crimp-top vials for high-performance liquid chromatography (HPLC) analysis.

Shoot Tissue Pigment HPLC Analysis

Pigments were separated using an Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA). Separation of chemically similar pigments was accomplished using an analytical scale (4.6 mm i.d. x 250 mm) 5 µm, 200 Å polymeric RP-C_{30} column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, PA). The thermostatted compartment column set at 30 ˚C was equipped with a 5-µm guard cartridge (4.0 mm i.d. x 10 mm) and holder (ProntoSIL). All separations were carried out isocratically using a binary mobile phase of 11% methyl tert-butyl ether (MTBE), 88.99% methanol (MeOH), and 0.01% 44rimethylamine (TEA) (v/v/v). Eluted compounds from a 10 µL injection detected pigments at 453 (carotenoids and internal standard), 652 [chlorophyll a (Chl *a*)], and 665 [chlorophyll *b* (Chl *b*)] nm. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignments for pigments were performed by matching retention times and line spectra obtained from the photodiode array detection using the external standards [α-carotene (AC), antheraxanthin (ANT), β-carotene (BC), Chl *a*, Chl *b*, lutein (LUT), neoxanthin (NEO), violaxanthin (VIO), and zeaxanthin (ZEA) from ChromaDex Inc., Irvine, CA].

Shoot Tissue Mineral Element Analysis

The procedure from Barickman et al. (2013) was used for mineral element analyses from freeze-dried tissue. A 0.2-0.5 g (± 0.05 g) subsample of ground, freeze-dried shoot tissue was combined with 10 mL HNO_3 (70%). Samples were sealed in a

closed vessel microwave digestion system (ETHOS series, Milestone Inc., Shelton, CT). The digestion procedures used follow those for organically based matrices (U.S. Environmental Protection Agency, 1996). The digested samples were then diluted with 2% HNO_3/ 0.5% HCl (v/v), and elemental measurements were conducted using an Agilent 7500 ce ICP-MS system (Agilent Technologies). The ICP-MS system had an octapole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and a CETAC (ASX-510, CETAC Inc., Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity (Li, Y, Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solution containing 10 $\mu g \cdot L^{-1}$ of Li, Y, Tl, Ce, and Co in a 2% HNO_3/0.5% HCl (v/v) matrix.

Shoot Tissue Glucosinolate Extraction

The procedure from Charron et al. (2004) was used for glucosinolate extraction from freeze-dried tissue and analysis. A 0.2 g (± 0.05 g) sample of freeze dried tissue was combined with 1 mL benzyl GS solution (1mM), to act as the internal standard. 2.0 mL MeOH and 0.1 mL barium-lead acetate (0.6 M) were then added to the sample in a culture tube (16 x 100 mm) and shaken at 60 rpm for 60 min Each tube was then centrifuged at 2000 g_n for 20 min at 22 °C. A 0.5 mL aliquot of the supernatant was then combined with a 1 mL column that consisted of 0.3mL DEAE Sephadex A-25 (Sigma-Aldrich). The sample was then desulfated using the procedure by Raney and McGregor (1990).

Shoot Tissue Glucosinolate HPLC Analysis

Extracted desulfoglucosinolates were separated using an HPLC unit with a photodiode array detector (1100 series, Agilent Technologies), using a reverse-phase 250 x 4.6 mm i.d., 5 µm Luna C_{18} column (Phenomenex Inc., Torrance, CA) at a wavelength of 230 nm. The temperature of the column was set at 40 °C with a flow rate of 1 mL·min^{-1}. The gradient elution parameters were set to 100% water for 1 min, followed by a 15 min linear gradient set to 75% water: 25% acetonitrile. Solvent levels were then held constant for 5 min and then returned to 100% water for the final 5 min. Identification of desulfoglucosinolates took place using a comparison of retention times of authentic standards or previously reported results (Hansen et al., 1995; Kushad et al., 1999).

Non-structural Water Soluble Carbohydrate Extraction

Nonstructural water soluble carbohydrates were extracted from kale tissues based on the methods of Muir et al. (2009) and Thavarajah et al. (2016), with slight modifications. Microgreen fresh tissue was ground using a pestle and mortar for homogenous sub-samples. A 0.1-g sub-sample of ground, freeze-dried tissue will be extracted in a 15 mL test tube by adding 2 mL of RO water which was heated to 80 °C, vortexed, and then shaken for 15 min at 200 rpm. Samples will then be centrifuged at 4400 rpm for 20 min. A 1.0-mL aliquot of the supernatant will then be transferred into a new 15 mL test tube and placed into a stream of N gas until it is reduced to 0.5 ml. Once

dried, samples will be re-dissolved in 2.5 mL of RO water. Samples will then be put through a 0.2 µm syringe filter and collected in a 2 mL HPLC vial for analysis.

Soluble Sugars HPLC Analysis

Separation parameters and carbohydrate quantification will be carried out with authentic standards using an HPLC with an evaporative light scattering (ELS) detector (Agilent Technologies). The ELS detector had an N gas flow rate of 1.6 ml, evaporative gas temperature of 80 °C, and a nebulizer gas temperature at 50 °C. Chromatographic separations were achieved using a Rezex RCM Monosaccharide Ca+2 (8%) 300 x 7.8mm i.d., 8 µm analytical scale column (Phenomenex, Torrance, CA, USA) which allows for effective separation of chemically similar compounds. The column was equipped with a Carbo-Ca 4 x 3.0 mm i.d. security guard cartridge and holder (Phenomenex), and was maintained at 80 °C using a heated column compartment. All separations will be carried out isocratically using a mobile phase of 100% RO water. The flow rate was 0.6 mL per min, with a run time of 15 min, followed by a 2 min equilibration prior to the next injection. Data for eluted compounds were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment values for sucrose, glucose, and fructose were performed by comparing retention times from the ELS detector using external standards (Sigma-Aldrich, St. Louis, MO).

Statistical Analyses

A randomized complete block design was used (Figure 2.1). Data sets were analyzed by GLM procedure using statistical software (version 9.4; SAS Institute, Cary,

NC). Treatment means were separated by least significant difference (LSD) at α=0.05. Plant height means were separated by Duncan's Multiple Range Test at α=0.05. Data were analyzed by season, species, and cultivar. Microgreen shoot tissue pigments, shoot tissue mineral elements, and carbohydrate data are presented on a DM basis.

Season one is winter, season two is spring, season three is summer, and season four is fall. Species one is *Brassica* cultivars (red cabbage, Kogane Chinese cabbage, Champion collards, Red Giant mustard, and Hong Vit radish), species two is herb cultivars (Genovese basil, Italian Large Leaf basil, Calypso cilantro, Grosfruchtiger fennel, and Giant of Italy parsley), and species three is lettuce cultivars (Buttercrunch lettuce, Carioca lettuce, Red Sails lettuce, Vulcun lettuce, Winter Density lettuce) (Table 2.1).

2. 3 Results

Environmental data

The photosynthetically active radiation (PAR) levels averaged 161 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 368 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 467 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 417 $\mu mol \cdot m^{-2} \cdot s^{-1}$; no shade system was used (Table 2.2). Daily light integral (DLI) values averaged 14 $mol \cdot m^{-2} \cdot d^{-1}$, 32 $mol \cdot m^{-2} \cdot d^{-1}$, 40 $mol \cdot m^{-2} \cdot d^{-1}$, and 36 $mol \cdot m^{-2} \cdot d^{-1}$ for winter, summer, spring, and fall seasons, respectively (Table 2.2; Figure 2.2; Figure 2.3). The relative humidity (RH) levels averaged 44%, 50%, 57%, and 64% for winter, spring, summer, and fall seasons, respectively (Table 2.2; Figure 2.3). Average air temperatures were 20 °C, 26 °C, 25 °C, and 29 °C for winter, spring, summer, and fall seasons, respectively (Table 2.2; Figure 2.3). Fall had the lowest temperature variance between high and low average

temperatures, while summer had the highest temperature variance (Table 2.2; Figure 2.3). Values for PAR, DLI, RH, and average air temperatures had the greatest values during the summer and lowest values during the winter (Table 2.2). Values for PAR, DLI, RH, and average temperature levels generally increased from winter to fall (Table 2.2).

Brassica, Herb, and Lettuce Microgreen Cultivar Morphology

The germination time and total production time (germination to harvest) were significantly different for season, cultivar, and season x cultivar interactions for all microgreens. Shoot FM was significantly different for all microgreens for season and cultivar; brassica and lettuce cultivars had season x cultivar interactions for FM, while herbs did not. Shoot DM was significantly different for cultivar. Plant height was significantly different for season and cultivar interactions for all microgreens. Herb microgreens had season x cultivar interactions for plant height, while brassica and lettuce microgreens did not. Shoot tissue %moisture for all microgreens was significantly different for cultivar and season x cultivar interactions. Shoot tissue %DM was significantly different for all microgreens for cultivar and cultivar x season interactions. Brassica and herb microgreens had significant differences in shoot tissue %moisture and DM for season, while lettuce did not.

Brassica, herb, and lettuce microgreens had the fastest germination time and total production time in the summer and fall seasons (Table 2.3; Figure 2.4; Figure 2.5). Overall, brassica had the shortest germination and total production time followed by lettuce and then herb microgreens (Table 2.3; Figure 2.4). Brassicas has the greatest

plant heights across all seasons, while lettuce and herbs had similar plant height values in winter and summer (Table 2.4). All microgreens had the shortest production time in the summer and fall (Table 2.3; Figure 2.5). Shoot FM and DM for brassica microgreens increased in the spring and decreased in the winter (Table 2.5; Figure 2.6; Figure 2.7). Shoot FM for herb microgreens increased in the winter and decreased in the spring (Table 2.5; Figure 2.6). Shoot DM for herb microgreens remained relatively constant throughout all seasons (Table 2.5; Figure 2.7). Shoot FM and DM for lettuce microgreens increased in the summer and decreased in the spring (Table 2.5; Figure 2.6; Figure 2.7). Brassica microgreens had the greatest DM compared to all other microgreens (Figure 2.7). Shoot tissue %moisture increased for brassica and herb microgreens in the winter and decreased in the spring (Table 2.5). Shoot tissue %moisture for lettuce microgreens increased in the fall and decreased in the spring (Table 2.5). Shoot tissue %DM for all microgreens increased in the spring, while it decreased for brassica and herb microgreens in the winter and decreased in the fall for lettuce microgreens (Table 2.5).

Brassica, Herb, and Lettuce Microgreen Cultivar Shoot Pigments

β-carotene for brassica and lettuce microgreens was significantly different for season, cultivar, and season x cultivar interactions. β-carotene for herb microgreens was significantly different for season and season x cultivar interactions, but not for cultivar. Zeaxanthin for brassica, herb, and lettuce microgreens was significantly different for season, cultivar and season x cultivar interactions. Lutein for brassica and lettuce microgreens was significantly different for season, cultivar and season x cultivar

interactions. Lutein for herb microgreens was significantly different for season and season x cultivar interactions, but not for cultivar. Antheraxanthin for brassica, lettuce, and herb microgreens was significantly different for season, but not cultivar and season x cultivar interactions. Neoxanthin was significantly different for brassica and lettuce microgreens for season, cultivar, and season x cultivar interactions. Neoxanthin was significantly different for herb microgreens for season and season x cultivar interactions, but not cultivar. Violaxanthin was significantly different for brassica microgreens for season, cultivar, and season x cultivar interactions. Violaxanthin was significantly different for herb microgreens for season and season x cultivar interactions, but not for cultivar. Violaxanthin was significantly different for lettuce microgreens for season, cultivar, but not season x cultivar interactions. Total carotenoids for brassica and lettuce microgreens was significantly different for season, cultivar, and season x cultivar. Total carotenoids for brassica microgreens were significantly different for season and season x cultivar interactions, but not for cultivar.

β-carotene increased for all microgreens in the summer, while NEO increased for all microgreens in the fall (Table 2.6; Figure 2.8). Zeaxanthin increased in the winter for herb microgreens, summer for brassica microgreens, and fall for lettuce microgreens (Table 2.6). Lutein increased in the winter for lettuce microgreens, in the summer for brassica microgreens, and in the fall for herb microgreens (Table 2.6; Figure 2.8). Antheraxanthin increased for herb microgreens in the winter, in the summer for brassica microgreens, and lettuce microgreens in the fall (Table 2.6). Violaxanthin

increased for herb in the summer and for brassica and lettuce microgreens in the fall (Table 2.6).

Chlorophyll *a* concentrations were significantly different for brassica, herb, and lettuce microgreens for season, cultivar, and season x cultivar interactions. Chlorophyll *b* concentrations were significantly different for brassica, herb, and lettuce microgreens for season, cultivar, and season x cultivar interactions. Total chlorophyll concentrations were significantly different for brassica, herb, and lettuce microgreens for season, cultivar, and season x cultivar interactions.

Total carotenoid content in shoot tissue increased for herb and lettuce microgreens in the fall, whereas brassica microgreens had increased total carotenoid levels in the summer (Table 2.6; Figure 2.9). In general, ZEA, LUT, and ANT levels increased for brassica microgreens in the summer (Table 2.6; Figure 2.8). Herb microgreens had increased levels of ZEA and ANT in the winter, while lettuce microgreens had increased levels of ZEA, ANT, and VIO in the fall (Table 2.6). Chl *a*, Chl *b*, and total chlorophyll concentrations in herb and lettuce microgreens increased in the fall (Table 2.7). For brassica microgreens, chl *a* and total chlorophyll concentrations were increased in the fall, while chl *b* concentrations were increased in the winter (Table 2.7).

Brassica, Herb, and Lettuce Microgreen Cultivar Carbohydrates

Sucrose concentrations in brassica, herb, and lettuce microgreens were significantly different for season, cultivar, and season x cultivar interactions. Glucose

concentrations in herb microgreens were significantly different for season, cultivar, and season x cultivar interactions. Glucose concentrations in brassica microgreens were significantly different for season and cultivar, but not season x cultivar interactions. Glucose concentrations in lettuce microgreens were significantly different for season x cultivar interactions and cultivar, but not season. Fructose concentrations in brassica, herb, and lettuce microgreens were significantly different for season, cultivar, and season x cultivar interactions.

Sucrose, fructose, and glucose concentrations fluctuated according to cultivar and season (Table 2.8). Sucrose content in shoot tissue increased in the winter for herb and lettuce microgreens, while sucrose increased in the spring for brassica microgreens (Table2.8; Figure 2.11; Figure 2.12). Sucrose in shoot tissue decreased in the fall for herb microgreens, while sucrose decreased in the summer for brassica and lettuce microgreens (Table 2.8; Figure 2.10; Figure 2.11; Figure 2.12). Glucose in shoot tissue increased in the summer for brassica and lettuce microgreens, while glucose increased in the spring for herb microgreens (Table 2.8; Figure 2.10; Figure 2.11; Figure 2.12). Glucose in shoot tissue decreased in the fall for lettuce microgreens, while glucose decreased in the winter for brassica and herb microgreens (Table 2.8; Figure 2.10; Figure 2.11; Figure 2.12). Fructose in shoot tissue increased in the fall for herb and lettuce microgreens, while fructose increased in the fall for brassica microgreens (Table2.8; Figure 2.10; Figure 2.11; Figure 2.12). Fructose in shoot tissue decreased in the winter

for brassica microgreens, while fructose decreased in the spring for herb and lettuce microgreens (Table 2.8; Figure 2.10; Figure 2.11; Figure 2.12).

Brassica, Herb, and Lettuce Microgreen Cultivar Minerals

Tissue Ca was significantly different for brassica and lettuce microgreens for season, cultivar, and season x cultivar interactions. Tissue Ca was significantly different for herb microgreens for season and cultivar, but not season x cultivar interactions. Tissue K was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue K was significantly different for lettuce microgreens for season and season x cultivar interactions, but not for cultivar. Tissue Mg was significantly different for brassica microgreens for season and season x cultivar interactions, but not for cultivar. Tissue Mg was not significantly different for herb microgreens for season, cultivar, and season x cultivar interactions. Tissue Mg was significantly different for lettuce microgreens for season, but not for cultivar and season x cultivar interactions. Tissue P was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue P was significantly different for lettuce microgreens for season and cultivar, but not season x cultivar interactions. Tissue S was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue S was significantly different for lettuce microgreens for season and cultivar, but not season x cultivar interactions.

Macronutrients within microgreen shoot tissues fluctuated according to season and cultivar. Ca increased in brassica and herb microgreens in the winter, whereas Ca decreased in the fall for both microgreens (Table 2.9). Ca increased in lettuce microgreens in the summer, whereas Ca decreased in the spring (Table 2.9). K increased for all microgreens in the winter, while K decreased for brassica and lettuce microgreens in the fall and herb microgreens in the spring (Table 2.9). Mg increased for herb microgreens in the winter, brassica microgreens in the spring, and lettuce microgreens in the summer (Table 2.9). Mg decreased in the fall for brassica and herb microgreens, whereas Mg decreased in the winter for lettuce microgreens (Table 2.9). P increased for brassica and herb microgreens in the winter and decreased in the fall (Table 2.9). P increased in lettuce microgreens in the summer and decreased in the spring (Table 2.9). S increased for brassica and herb microgreens in the winter and decreased in the summer, whereas S increased for lettuce microgreens in the summer and decreased in the spring (Table 2.9).

Tissue B was significantly different for brassica microgreens for season, cultivar, and season x cultivar interactions. Tissue B was significantly different for herb microgreens for season, but not for cultivar and season x cultivar interactions. Tissue B was significantly different for lettuce microgreens for season and cultivar, but not for season x cultivar interactions. Tissue Cu was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue Cu was significantly different for lettuce microgreens for season and cultivar, but not for season

x cultivar interactions. Tissue Fe was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue Mn was significantly different for brassica and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue Mn was significantly different for lettuce microgreens for season and season x cultivar interactions, but not for cultivar. Tissue Mo was significantly different for brassica, lettuce, and herb microgreens for season, cultivar, and season x cultivar interactions. Tissue Se was significantly different for brassica microgreens for season, cultivar, and season x cultivar interactions. Tissue Se was significantly different for herb microgreens for season, but not for cultivar and season x cultivar interactions. Tissue Se was significantly different for lettuce microgreens for season and season x cultivar interactions, but not for cultivar. Tissue Zn was significantly different for brassica microgreens for season, cultivar, and season x cultivar interactions. Tissue Zn was significantly different for herb microgreens for season, but not for cultivar or season x cultivar interactions. Tissue Zn was not significantly different for lettuce microgreens for season, cultivar, and season x cultivar interactions.

Micronutrients within microgreen shoot tissue fluctuated according to season and cultivar. B increased in brassica and herb microgreens in the winter, whereas B increased in lettuce microgreens in the spring. B decreased in all microgreens in the fall (Table 2.10). Cu increased for all microgreens in the winter and decreased for brassica and herb microgreens in the summer and lettuce microgreens in the fall (Table 2.10). Fe increased for all microgreens in the summer and decreased for all microgreens in the

winter (Table 2.10). Mn increased for all microgreens in the winter and decreased for all microgreens in the fall (Table 2.10). Mo increased for brassica and herb microgreens in the winter and increased for lettuce microgreens in the summer (Table 2.10). Mo decreased for herb and lettuce microgreens in the spring and decreased for brassica microgreens in the fall (Table 2.10). Se and Zn increased for all microgreens in the winter (Table 2.10). Se decreased for all microgreens in the fall (Table 2.10). Zn decreased for brassica and herb microgreens in the summer and decreased for lettuce microgreens in the spring (Table 2.10).

2. 4 Discussion

Growth and Morphology of brassica, herb, and lettuce microgreens

Overall, brassica microgreens had the shortest germination time and total production time over all seasons, followed by lettuce and then herb microgreens. Germination time can be attributed to several environmental factors such as soil and air temperature, RH, as well as water, O_2:CO_2, and light levels. Seed dormancy is regulated through hormonal changes in Abscisic acid (ABA) or Gibberellic acid (GA) levels within embryonic tissue, which is triggered by environmental signals to promote or delay germination (Corbineau et al., 2014; Nonogaki, 2014). While higher temperatures can speed up germination, soil media temperatures in excess of 30 °C can reduce germination by inducing seed dormancy and limiting radicle growth as studied in brassica and lettuce crops (Derkx and Karssen, 1993; Elson et al., 1992; Kondra, 1983; Kristie et al., 1981). Brassica, herb, and lettuce microgreens had the shortest

germination time in the spring and fall seasons, which is consistent with previous studies since summer had the maximum temperature ranges, while winter had the lowest temperature ranges.

Total production time was shortest for all microgreens in the fall whereas it was the longest in the winter. Shoot FM varied according to season and cultivar. Brassica microgreens had the greatest FM in the spring and fall, whereas herb microgreens had the greatest FM in the fall and lettuce microgreens had the greatest FM in the summer. In general, all microgreens had delayed germination and production times as well as reduced FM during the conditions of the winter season. A previous study reported the most suitable irradiation for optimal microgreen production ranges from 320–440 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with no significant influence on plant growth factors from higher light treatments (Samuolienė et al., 2013). These findings support the relationship between reduced microgreen growth and PAR differences as these conditions changed between winter and all other seasons in this study. Plant height was increased for brassica microgreens in the spring, while plant height increased in the winter for herb and lettuce microgreens. Hypocotyl length could increase as a result of lower radiation levels which would trigger hypocotyl elongation in response to decreased light intensity as supported by previous microgreen studies (Carvalho and Folta, 2014; Evans et al., 1965; Samuolienė et al., 2013; Vaštakaitė and Viršlė, 2015).

Shoot DM increased in brassica and lettuce microgreens in the winter, whereas DM increased in herbs in the fall. In contrast, DM was reported to increase when lettuce

roots in a hydroponic system were exposed to warmer daytime and nighttime temperatures (Thompson and Langhans, 1998). The DM of plant shoot tissue typically fluctuates in connection with light intensity and quality, which can increase CO_2 assimilation subsequently impacting metabolic and photosynthetic activity (Gerardeaux et al., 2009; Peterson and Zelitch, 1982; Samuoliené et al., 2013). Additionally, DM is linked to K uptake, which supports the results of this study in that K concentration in leaves and DM of microgreens increased simultaneously during the winter (Gerardeaux et al., 2009; Marschner et al., 1996; Tiwari et al., 1982). Shoot tissue %moisture of brassica and herb microgreens increased in the winter, whereas shoot tissue %moisture of lettuce microgreens increased in the fall.

Shoot Tissue Pigments in Brassica, Herb, and Lettuce Microgreens

β-carotene increased in the shoot tissue of all microgreens in the summer and fall seasons, respectively. LUT increased in the shoot tissue of brassica microgreens in the summer, herb microgreens in the fall, and lettuce microgreens in the winter. ZEA increased in the shoot tissue of brassica microgreens in the summer, herb microgreens in the fall, and lettuce microgreens in the winter. ZEA and LUT concentrations in microgreen shoot tissue increased in the summer and fall for brassica and herb microgreens and lettuce microgreens, respectively. NEO increased for all microgreens in the fall. ANT increased in the fall for herb and lettuce microgreens, while ANT increased in the summer for brassica microgreens. VIO increased in the fall for brassica and lettuce microgreens, while VIO increased in the summer for herb microgreens. Total carotenoid

content increased in the summer for brassica microgreens and in the fall for both herb and lettuce microgreens. Herb microgreens had the highest concentration of total carotenoids followed by brassica and then lettuce microgreens.

Carotenoids are generally known for their strong antioxidant and ROS scavenging capabilities, but vary in their primary protective roles for human health (Brazaitytė et al., 2015). β-carotene, LUT, and ZEA are the three of the most abundant carotenoids in human blood serum and can be found in green leafy vegetables (Chung et al., 2009; McDevitt et al., 2005; Ribaya-Mercado and Blumberg, 2004). Carotenoids decrease the risk of developing chronic diseases like cataracts, age-related macular degeneration, and certain cancers (Mayne, 1996; Rao and Rao, 2007). Carotenoids are lipid-soluble nutrients that diffuse through the mucosal lining of the intestine via bile and fat micelles; they are stored in adipose tissue and independently accumulate in different regions throughout the body (Furr and Clark, 1997; Nagao, 2011; Parker, 1996; Rao and Rao, 2007). β-carotene is the primary source of dietary vitamin A, which is important for proper growth and development along with eye health and functions as pro-vitamin A in the human diet (Burri, 1997; WHO, 2009). Vitamin A deficiency causes night-blindness, stunted growth, anemia, and reduced immune system resistance to infection, as well as xerophthalmia, which is a disease that causes blindness in children (WHO, 2009).

While the function of BC as pro-vitamin A aids in preventing blindness, LUT and ZEA are the primary xanthophyll pigments that collect within the macula (Beatty et al., 1999). LUT and ZEA accumulate within the retinal region of the eye and protect against

macular degradation as well as reduce the risk of developing cataracts (Beatty et al., 1999; Mayne, 1996). LUT and ZEA are thought to work in two ways to protect tissues within the eye; they filter blue light to reduce UV damage and provide antioxidant activity to scavenge free radicals to lessen tissue damage from light and metabolic activity within the retinal and macular tissues (Krinsky and Johnson, 2005; Johnson, 2014; Ribaya-Mercado and Blumberg, 2004). Increased consumption and subsequently accumulation of LUT and ZEA within the macula has been linked to slowing the development of macular degradation (Berg and Lin, 2014; Koushan et al., 2013).

β-carotene responded similarly for all microgreens within the screening, while ZEA+LUT content responded differently according to cultivar and season. Carotenoid concentrations are highly impacted by environmental growing conditions along with genotype since the concentrations commonly differ by plant species and cultivar (Brazaitytė et al., 2015; Czeczuga, 1987; Kopsell et al., 2004). Carotenoids are an integral part of the LHC and actively function as antioxidants within biological systems. Therefore, their content can fluctuate with changes in light quality, quantity, and quality (Cazzonelli, 2011; Kopsell and Sams, 2013; Yamori, 2016). Previous studies have shown moderate light radiation, generally below 520 and 540 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increased chlorophyll and secondary metabolite production in microgreens (Brazaitytė et al., 2015; Samuolienė et al., 2013). β-carotene and ZEA+LUT content in shoot tissue of all microgreens increased in connection with the increased PAR levels in the summer and fall which suggests additional light stress placed on microgreens in the summer and fall

could up-regulate carotenoid production to protect sensitive shoot tissue from light damage and photoinhibition.

Non-Structural, Water Soluble Carbohydrates in Brassica, Herb, and Lettuce Microgreens

Water soluble carbohydrate concentrations in microgreen shoot tissue differed by cultivar and season. Brassica microgreens had the highest glucose concentration in shoot tissue, while herb microgreens had the second highest glucose concentration in shoot tissue. Lettuce microgreens had higher sucrose and fructose concentrations than glucose concentrations in shoot tissue. Recent studies have measured non-structural, water soluble carbohydrates in brassica microgreens with concentrations varying by cultivar (Bulgari et al., 2016; Samuolienė et al., 2013; Vaštakaitė and Viršlė, 2015). Altering sugar content through seasonal changes in non-structural, water soluble carbohydrate concentrations in microgreen shoot tissue can potentially influence consumer preference.

Macro- and Micro-Nutrients in Brassica, Herb, and Lettuce Microgreens

Ca increased in the shoot tissue of brassica and herb microgreens in the winter and decreased for brassica and herb microgreens in the fall. Ca increased in the shoot tissue of lettuce microgreens in the summer and decreased in the spring. K increased in the shoot tissue of all microgreens in the winter and decreased for brassica and lettuce microgreens in the fall, while K decreased in lettuce microgreens in the summer. Mg decreased in the shoot tissue of brassica and herb microgreens in the fall. Mg decreased

in the shoot tissue of lettuce microgreens in the winter. Mg increased in the spring for brassica microgreens, in the winter for herb microgreens, and in the summer for lettuce microgreens. B increased in shoot tissue of brassica and herb microgreens in the winter, while B increased in the spring for lettuce microgreens. Cu increased for all microgreens in the winter. B decreased in the shoot tissue of all microgreens in the fall. Fe decreased in the shoot tissue of all microgreens in the winter and increased in all microgreens in the summer. Zn increased in the shoot tissue of all microgreens in the winter, while it decreased in the spring for lettuce microgreens and in the summer for brassica and herb microgreens. Se and Mg increased for all microgreens in the winter.

Fe, Mg, K, Cu, Mn, S, and Zn are important mineral nutrients involved in the photosynthetic process as integral constituents of molecules or chemical reactions and influence biomass accumulation in plants (Ericsson, 1995; Wilson, 1988). Microgreen shoot tissue had the highest mineral concentrations of Fe, Mg, and Zn. The general concentration of minerals within the shoot tissue of all cultivars in this study agrees with the ranges of previous studies (Kopsell and Sams, 2013; Pinto et al., 2015; Weber, 2017). While the physical properties of media control nutrient availability, a previous study reported minimal impact of media on mineral concentrations in lettuce microgreens (Pinto et al., 2015). In contrast, another study found that media significantly impacted mineral accumulation in brassica microgreens. They reported increased mineral concentrations for microgreens grown in peat-based media (Di Gioia et al., 2016). The increased mineral concentrations of microgreens grown in peat-based media can be

attributed to the existing nutrient charge and pH level of commercially available peat-based growing media. Also, minerals within the municipal water supply could have increased the content of minerals within the microgreens shoot tissue.

Roots provide anchorage to support aboveground biomass and are important for facilitating the transport of water, mineral nutrients, and metabolites to actively growing shoot tissue. Additionally, they produce hormones and other signaling molecules to help coordinate shoot metabolic activity and biomass accumulation as well as the production of secondary metabolites in response to environmental stress (Beveridge, 2000; Galen et al., 2007; Mansoorkhani et al., 2014). Plant species have diverse root morphology patterns that can influence their competition for mineral nutrients and other resources in soil (Gross et al., 1992). Seasonal temperature changes which increase soil media temperature can promote root growth up to a certain point, subsequently increasing mineral uptake, after which root growth is restricted (Cumbus and Nye, 1985; Kaspar and Bland, 1992; McMichael and Burke, 1998).

Fruits and vegetables are a major source of dietary minerals, fiber, and vitamins (Dias, 2012; Pennington and Fisher, 2009). Mineral malnutrition is an issue that impacts developing and industrialized nations; insufficient dietary Fe, Zn, and Se are the three most common mineral deficiencies worldwide (Pinto et al., 2015; WHO, 2004). Herb microgreens had the highest concentrations of Zn, Se, and Fe in addition to B and Cu compared to brassica and lettuce microgreens. In contrast, brassica microgreens had the highest

concentrations of Mn. Ca and K are important minerals for human development (WHO, 2004). Ca increased in shoot tissue of brassica and herb microgreens in the winter, while it increased for lettuce microgreens in the summer. Whereas K was increased in all microgreen shoot tissue in the winter. Various minerals like B, Ca, Fe, Zn, Cu, Se, and Mo have been previously identified in shoot tissue of microgreens at similar concentrations (Kopsell and Sams, 2013; Pinto et al., 2015; Weber, 2017). Generally, the microgreens in this screening have greater concentration of micronutrients as compared to macronutrients in shoot tissue (Table 2.9; Table 2.10).

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Appendix B

Table 2.1 Fifteen commercially grown microgreens used in the cultivar screening. The microgreens are divided into three groups; brassica, herb, and lettuce.

Commercial Name	Scientific Name		Seed Source	Location
	Family	Genus and Species		
Red Cabbage	Brassicaceae	Brassica oleracea L. var. *capitata rubra*	Johnny's Selected Seeds	Winslow, ME
Kogane Chinese Cabbage	Brassicaceae	*Brassica rapa* L. ssp. *pekinensis*	Johnny's Selected Seeds	Winslow, ME
Champion Collards	Brassicaceae	*Brassica oleracea* L. var. *acephala*	Johnny's Selected Seeds	Winslow, ME
Red Giant Mustard	Brassicaceae	*Brassica juncea* L.	Johnny's Selected Seeds	Winslow, ME
Hong Vit Radish	Brassicaceae	*Raphanus sativus* L.	Johnny's Selected Seeds	Winslow, ME
Genovese Basil	Lamiaceae	*Ocimum basilicum* L.	Johnny's Selected Seeds	Winslow, ME
Italian large Leaf Basil	Lamiaceae	*Ocimum basilicum* L.	Johnny's Selected Seeds	Winslow, ME
Calypso Cilantro	Apiaceae	*Coriandrum sativum* L.	Johnny's Selected Seeds	Winslow, ME
Grosfruchtiger Fennel	Apiaceae	*Foeniculum vulgare* L.	Johnny's Selected Seeds	Winslow, ME
Giant of Italy Parsley	Apiaceae	*Petroselinum crispum* L.	Johnny's Selected Seeds	Winslow, ME
Buttercrunch lettuce	Asteraceae	*Lactuca sativa* L.	Johnny's Selected Seeds	Winslow, ME
Carioca Lettuce	Asteraceae	*Lactuca sativa* L.	Johnny's Selected Seeds	Winslow, ME
Red Sails Lettuce	Asteraceae	*Lactuca sativa* L.	Johnny's Selected Seeds	Winslow, ME
Vulcun Lettuce	Asteraceae	*Lactuca sativa* L.	Johnny's Selected Seeds	Winslow, ME
Winter Density Lettuce	Asteraceae	*Lactuca sativa* L.	Johnny's Selected Seeds	Winslow, ME

Table 2.2 Greenhouse environmental data collected over four seasons in Knoxville, TN in 2016; PAR ($\mu mol \cdot m^{-2} \cdot s^{-1}$), daily light integral (DLI) ($mol \cdot m^{-2} \cdot d^{-1}$), relative humidity (RH) (%), maximum and minimum RH (%), temperature (°C), and maximum and minimum temperature (°C). [a]

season	PAR	DLI	RH	MAX. RH	MIN. RH	AVG. TEMP.	MAX. TEMP.	MIN. TEMP.
winter	161	14	44	80	7	20	31	8
spring	368	32	50	83	7	26	37	15
summer	467	40	57	97	17	25	38	11
fall	417	36	64	93	34	29	39	19

[a] mean values collected during winter (January-February), spring (March-April), summer (May-July), and fall (August-September) of 2016 in Knoxville, TN, USA.

Block 2	Block 4
Buttercrunch lettuce	Calypso cilantro
Champion collards	red cabbage
Grosfruchtiger fennel	Italian Large Leaf basil
Carioca lettuce	Kogane Chinese cabbage
Winter Density lettuce	Grosfruchtiger fennel
Red Giant mustard	Carioca lettuce
Italian Large Leaf basil	Winter Density lettuce
Kogane Chinese cabbage	Red Sails lettuce
Red Sails lettuce	Red Giant mustard
Genovese basil	Genovese basil
Giant of Italy parsley	Buttercrunch lettuce
Hong Vit radish	Giant of Italy parsley
Vulcan lettuce	Champion collards
red cabbage	Vulcan lettuce
Calypso cilantro	Hong Vit radish

Block 1	Block 3
red cabbage	Red Giant mustard
Genovese basil	Italian Large Leaf basil
Red Sails lettuce	Vulcan lettuce
Red Giant mustard	Genovese basil
Hong Vit radish	red cabbage
Giant of Italy parsley	Calypso cilantro
Calypso cilantro	Hong Vit radish
Champion collards	Winter Density lettuce
Buttercrunch lettuce	Grosfruchtiger fennel
Vulcan lettuce	Buttercrunch lettuce
Winter Density lettuce	Red Sails lettuce
Grosfruchtiger fennel	Carioca lettuce
Carioca lettuce	Champion collards
Italian Large Leaf basil	Giant of Italy parsley
Kogane Chinese cabbage	Kogane Chinese cabbage

Figure 2.1 Randomized complete block (RCB) design for microgreen cultivar screening.

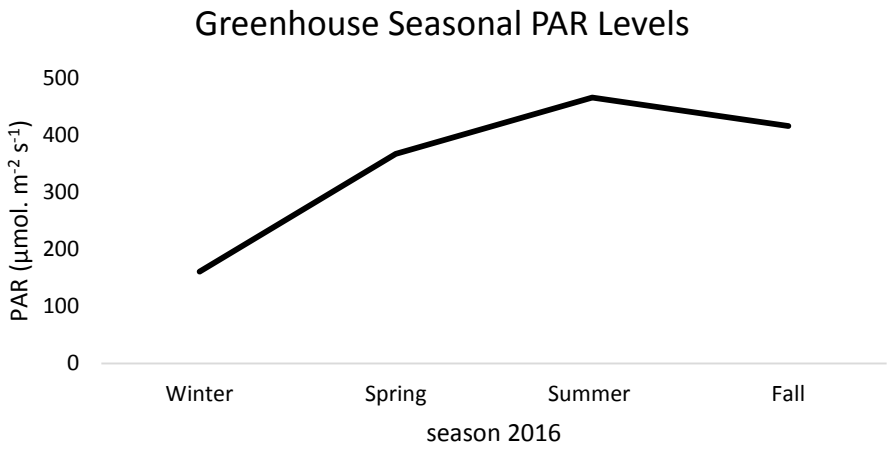

Figure 2.2 Seasonal photosynthetically active radiation (PAR) ($\mu mol \cdot m^{-2} \cdot s^{-1}$) levels in a greenhouse in 2016.

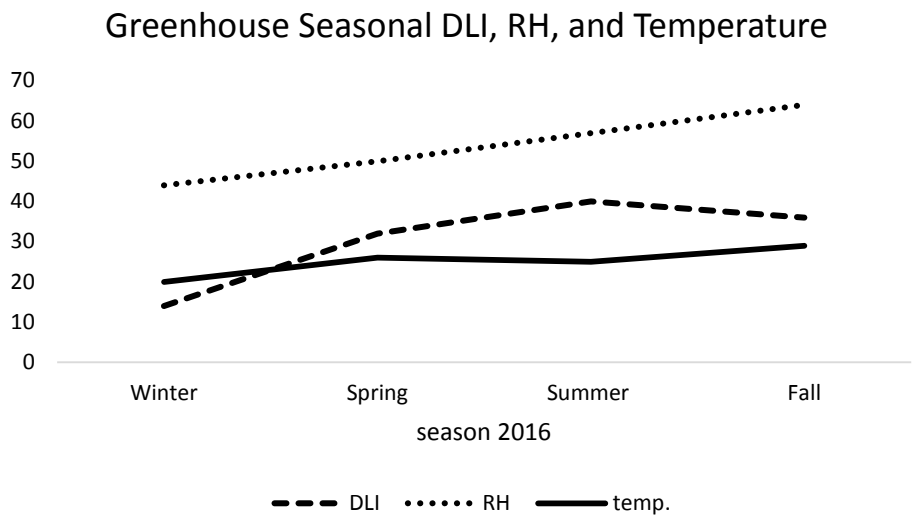

Figure 2.3 Greenhouse seasonal daily light integral (DLI) (mol·m^{-2}·d^{-1}), relative humidity (%RH), and average temperature (temp.) (°C) in 2016.

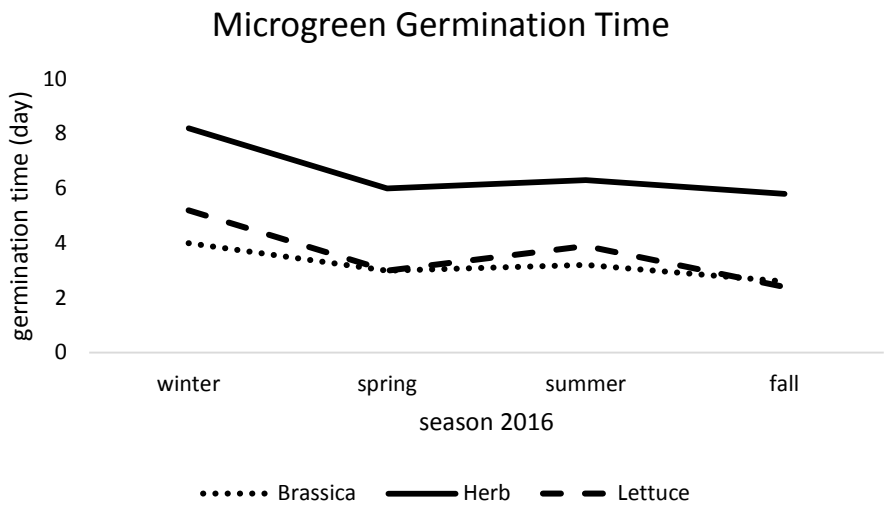

Figure 2.4 Seasonal germination time (day) for brassica, herb, and lettuce microgreens.

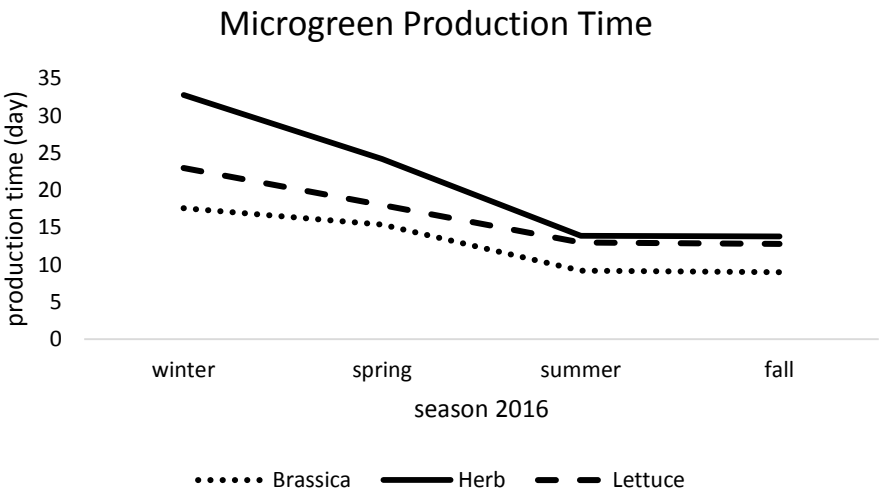

Figure 2.5 Seasonal production time (day) for brassica, herb, and lettuce microgreens.

Table 2.3 Seasonal germination time (day) and total production time (day) of brassica, herb, and lettuce microgreens. [a]

species	germination time (day)	total production time (day)
winter		
brassica	4 [C]	18 [C]
herb	8 [A]	33 [A]
lettuce	5 [B]	23 [B]
LSD ∝=0.5	0.00	0.00
spring		
brassica	3 [B]	15 [C]
herb	6 [A]	24 [A]
lettuce	3 [B]	18 [B]
LSD ∝=0.5	0.00	0.00
summer		
brassica	3 [C]	9 [C]
herb	6 [A]	14 [A]
lettuce	4 [B]	13 [B]
LSD ∝=0.5	0.27	0.44
fall		
brassica	3 [B]	9 [C]
herb	6 [A]	14 [A]
lettuce	2 [C]	13 [B]
LSD ∝=0.5	0.00	0.00

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

Table 2.4 Seasonal plant height (cm) of brassica, herb, and lettuce microgreens. [a]

species	plant height (cm)
winter	
brassica	4.69 [A]
herb	3.81 [B]
lettuce	3.81 [B]
spring	
brassica	5.20 [A]
herb	3.20 [B]
lettuce	2.42 [C]
summer	
brassica	4.49 [A]
herb	3.56 [B]
lettuce	3.39 [B]
fall	
brassica	4.66 [A]
herb	3.42 [B]
lettuce	3.08 [C]

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means were separated using a Duncan's Multiple Range Test. Means with the same uppercase letter are not statistically different (α =0.05).

Table 2.5 Fresh mass (FM) (g·plant^{-1}), dry mass (DM) (g·plant^{-1}), %moisture, and %dry mass of shoot tissue of brassica, herb, and lettuce microgreens. [a]

species	FM (g·plant^{-1})	DM (g·plant^{-1})	% Moisture	% DM
		winter		
brassica	141.28 ^{A}	6.96 ^{A}	94.71 ^{A}	5.29 ^{C}
herb	58.83 ^{C}	4.51 ^{C}	91.51 ^{C}	8.49 ^{A}
lettuce	78.10 ^{B}	5.53 ^{B}	92.80 ^{B}	7.20 ^{B}
LSD ∝=0.5	4.62	0.80	0.73	0.73
		spring		
brassica	164.42 ^{A}	13.59 ^{A}	90.81 ^{B}	9.19 ^{B}
herb	39.92 ^{C}	4.36 ^{B}	87.17 ^{C}	12.83 ^{A}
lettuce	59.27 ^{B}	3.90 ^{B}	92.57 ^{A}	7.43 ^{C}
LSD ∝=0.5	10.95	0.78	0.9	0.9
		summer		
brassica	152.86 ^{A}	10.89 ^{A}	92.36 ^{B}	7.64 ^{B}
herb	52.01 ^{C}	4.57 ^{C}	90.39^{C}	9.61 ^{A}
lettuce	87.07 ^{B}	5.62 ^{B}	93.13 ^{A}	6.87 ^{C}
LSD ∝=0.5	10.5	0.78	0.68	0.68
		fall		
brassica	155.89 ^{A}	10.53 ^{A}	93.19 ^{A}	6.81 ^{B}
herb	51.38 ^{C}	4.08 ^{C}	91.19 ^{B}	8.81 ^{A}
lettuce	77.67 ^{B}	4.82 ^{B}	93.50 ^{A}	6.50 ^{B}
LSD ∝=0.5	7.33	0.65	0.63	0.63

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

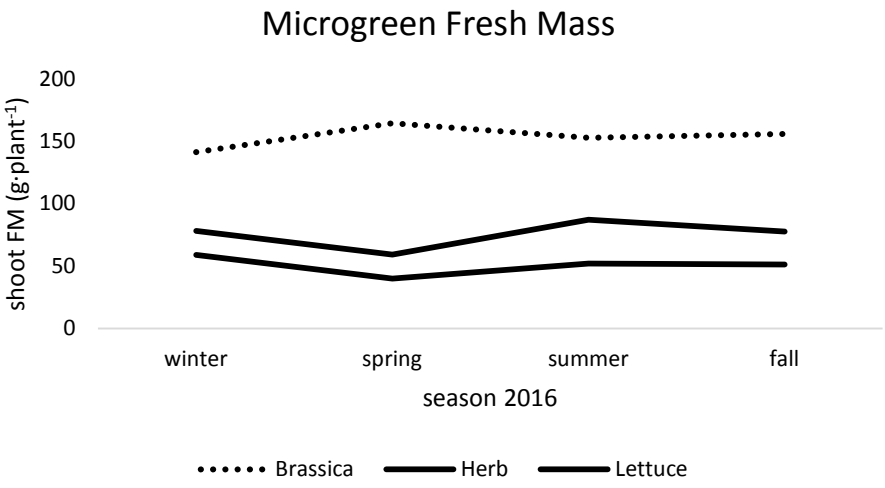

Figure 2.6 Seasonal FM (g·plant^{-1}) for brassica, herb, and lettuce microgreens.

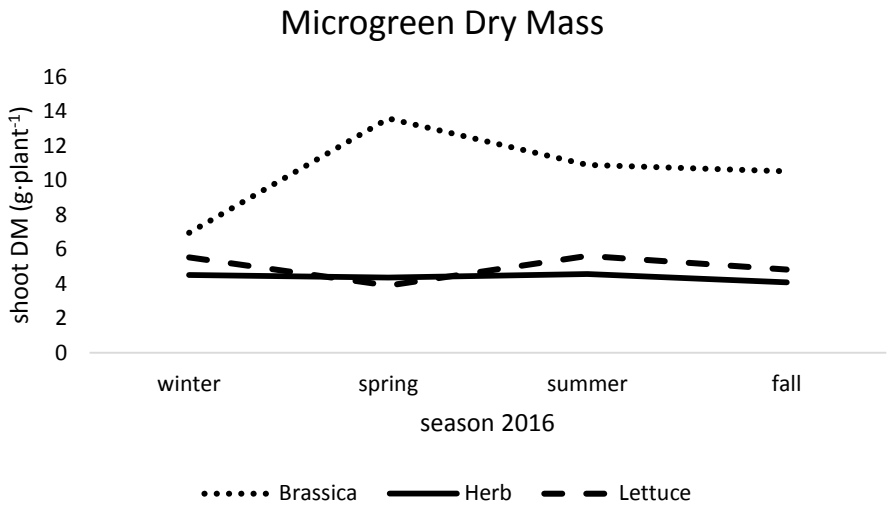

Figure 2.7 Seasonal DM (g·plant^{-1}) for brassica, herb, and lettuce microgreens.

Table 2.6 Seasonal changes in shoot carotenoid concentrations (mg·g^{-1} DM) in brassica, herb, and lettuce microgreens. [a]

species	β-carotene	Zeaxanthin	Lutein	Antheraxanthin	Neoxanthin	Violaxanthin	Total Carotenoids
				mg·g^{-1} DM			
winter							
brassica	0.235 ^{A}	0.014 ^{B}	0.517 ^{A}	0.032 ^{B}	0.193 ^{A}	0.149 ^{B}	1.140 ^{A}
herb	0.184 ^{B}	0.023 ^{A}	0.255 ^{C}	0.170 ^{A}	0.140 ^{C}	0.164 ^{A}	0.935 ^{B}
lettuce	0.231 ^{A}	0.008 ^{C}	0.434 ^{B}	0.017 ^{C}	0.163 ^{B}	0.109 ^{C}	0.961 ^{B}
LSD ∝=0.5	0.012	0.003	0.023	0.013	0.008	0.010	0.046
spring							
brassica	0.207 ^{A}	0.003 ^{C}	0.412 ^{A}	0.028 ^{B}	0.134 ^{A}	0.128 ^{B}	0.913 ^{A}
herb	0.162 ^{B}	0.021 ^{A}	0.225 ^{C}	0.139 ^{A}	0.117 ^{B}	0.145 ^{A}	0.809 ^{B}
lettuce	0.168 ^{B}	0.010 ^{B}	0.331 ^{B}	0.014 ^{C}	0.115 ^{B}	0.089 ^{C}	0.727 ^{C}
LSD ∝=0.5	0.013	0.002	0.022	0.007	0.010	0.007	0.052
summer							
brassica	0.299 ^{B}	0.015 ^{A}	0.557 ^{A}	0.038 ^{B}	0.277 ^{A}	0.223 ^{B}	1.408 ^{B}
herb	0.360 ^{A}	0.018 ^{A}	0.541 ^{A}	0.129 ^{A}	0.297 ^{A}	0.347 ^{A}	1.692 ^{A}
lettuce	0.279 ^{B}	0.017 ^{A}	0.428 ^{B}	0.095 ^{A}	0.239 ^{B}	0.240 ^{B}	1.298 ^{B}
LSD ∝=0.5	0.032	0.004	0.059	0.036	0.029	0.033	0.138
fall							
brassica	0.271 ^{B}	0.006 ^{C}	0.516 ^{B}	0.014 ^{C}	0.309 ^{B}	0.225 ^{C}	1.340 ^{B}
herb	0.338 ^{A}	0.017 ^{B}	0.661 ^{A}	0.023 ^{B}	0.384 ^{A}	0.293 ^{B}	1.716 ^{A}
lettuce	0.261 ^{B}	0.025 ^{A}	0.345 ^{C}	0.171 ^{A}	0.268 ^{C}	0.318 ^{A}	1.387 ^{B}
LSD ∝=0.5	0.018	0.001	0.025	0.006	0.020	0.016	0.080

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

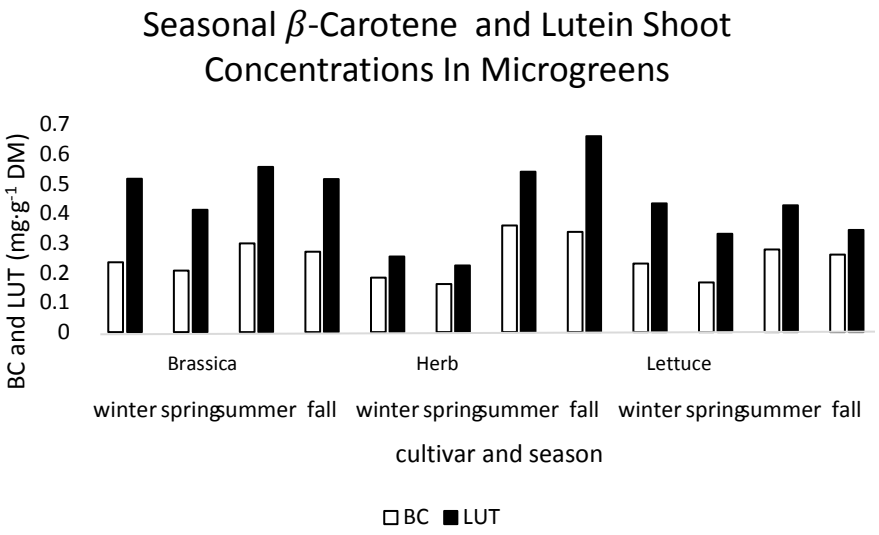

Figure 2.8 Seasonal BC and LUT concentrations (mg·g^{-1} DM) in shoot tissue of brassica, herb, and lettuce microgreens.

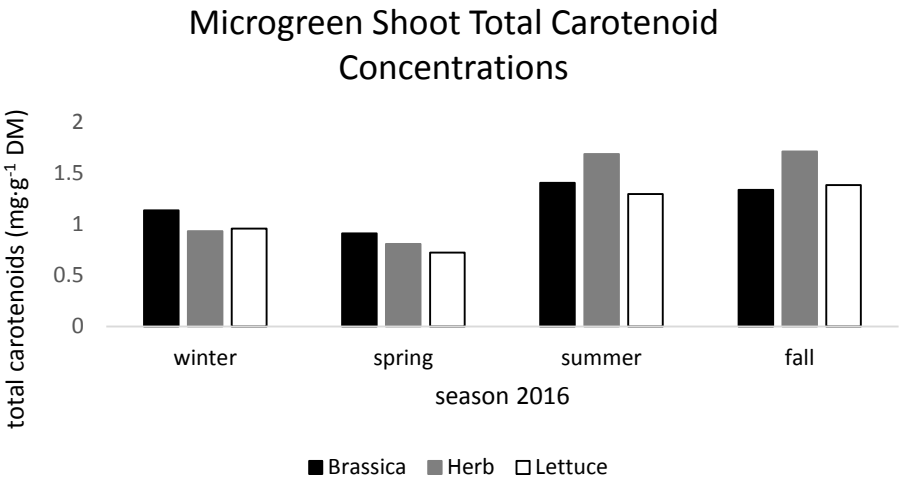

Figure 2.9 Seasonal total carotenoid concentrations (mg·g^{-1} DM) in shoot tissue of brassica, herb, and lettuce microgreens.

Table 2.7 Seasonal changes in chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), and total chlorophyll (*Chl*) concentrations ($mg \cdot g^{-1}$ DM) in shoot tissue of brassica, herb, and lettuce microgreens. [a]

species	Chl *a*	Chl *b*	Total Chl
	$mg \cdot g^{-1}$ DM		
winter			
brassica	4.71 [B]	1.42 [B]	6.13 [B]
herb	3.91 [C]	1.21 [C]	5.12 [C]
lettuce	5.42 [A]	1.60 [A]	7.01 [A]
LSD ∝=0.5	0.36	0.07	0.41
spring			
brassica	3.26 [B]	1.08 [B]	4.34 [B]
herb	3.32 [B]	1.05 [B]	4.36 [B]
lettuce	4.22 [A]	1.17 [A]	5.39 [A]
LSD ∝=0.5	0.33	0.09	0.42
summer			
brassica	4.82 [C]	1.35 [C]	6.17 [C]
herb	5.36 [B]	1.50 [B]	6.86 [B]
lettuce	6.29 [A]	1.67 [A]	7.95 [A]
LSD ∝=0.5	0.52	0.11	0.63
fall			
brassica	5.01 [B]	1.36 [B]	6.37 [B]
herb	4.61 [C]	1.28 [C]	5.89 [C]
lettuce	5.86 [A]	1.58 [A]	7.44 [A]
LSD ∝=0.5	0.27	0.07	0.33

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

Table 2.8 Seasonal changes in non-structural, water soluble carbohydrate concentrations (mg·g^{-1} DM) in shoot tissue of brassica, herb, and lettuce microgreens. [a]

species	sucrose	glucose	fructose
	mg·g^{-1} DM		
winter			
brassica	8.16 ^{C}	16.62 ^{A}	12.72 ^{B}
herb	13.65 ^{B}	12.96 ^{B}	12.99 ^{B}
lettuce	19.48 ^{A}	12.78 ^{B}	14.42 ^{A}
LSD ∝=0.5	0.84	0.74	0.40
spring			
brassica	13.64 ^{B}	22.35 ^{A}	13.29 ^{A}
herb	12.93 ^{B}	18.04 ^{B}	11.69 ^{B}
lettuce	16.26 ^{A}	11.09 ^{C}	12.34 ^{AB}
LSD ∝=0.5	1.83	2.29	1.42
summer			
brassica	4.71 ^{B}	24.07 ^{A}	16.16 ^{A}
herb	4.80 ^{B}	14.98 ^{B}	13.86 ^{B}
lettuce	12.13 ^{A}	12.88 ^{B}	16.09 ^{A}
LSD ∝=0.5	1.57	2.69	1.79
fall			
brassica	4.85 ^{B}	20.47 ^{A}	14.22 ^{C}
herb	4.56 ^{B}	17.55 ^{B}	16.38 ^{B}
lettuce	14.60 ^{A}	14.39 ^{C}	17.92 ^{A}
LSD ∝=0.5	1.65	1.10	1.52

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

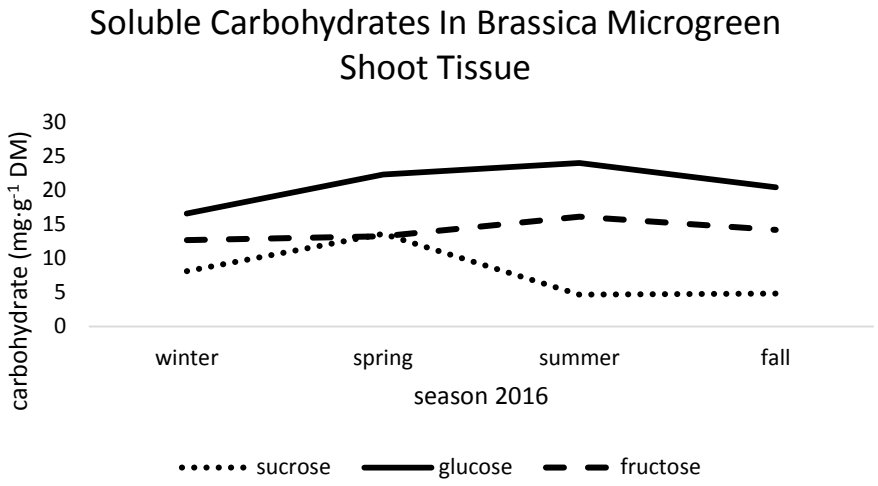

Figure 2.10 Seasonal changes in sucrose, glucose, and fructose concentrations (mg·g^{-1} DM) in shoot tissue of brassica microgreens.

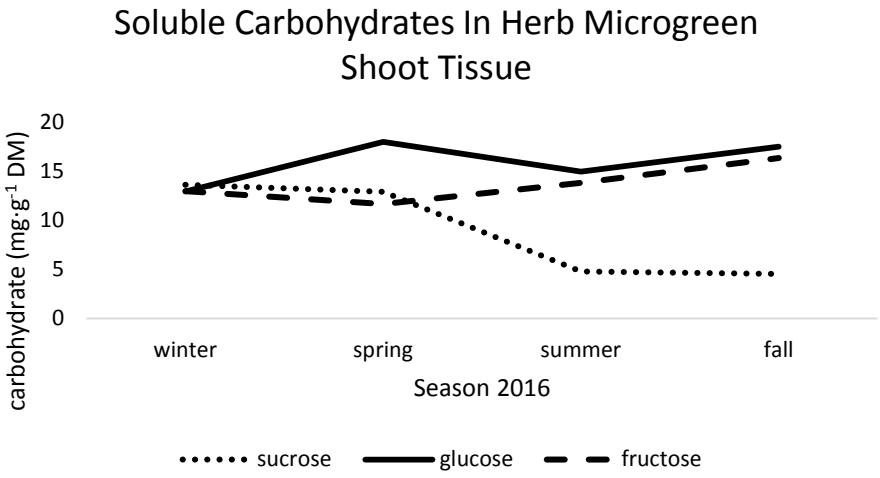

Figure 2.11 Seasonal changes in sucrose, glucose, and fructose concentrations (mg·g^{-1} DM) in shoot tissue of herb microgreens.

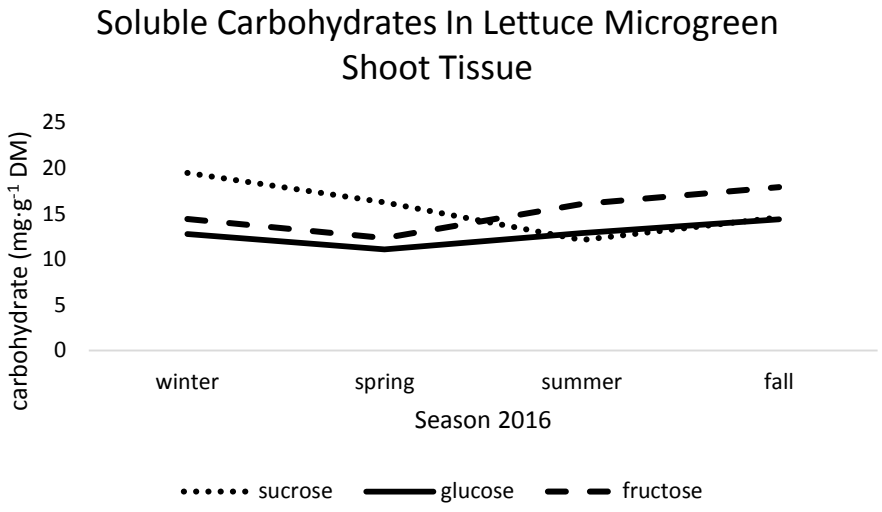

Figure 2.12 Seasonal changes in sucrose, glucose, and fructose concentrations (mg·g^{-1} DM) in shoot tissue of lettuce microgreens.

Table 2.9 Seasonal changes in macronutrient concentrations (% DM) in shoot tissue of brassica, herb, and lettuce microgreens. [a]

species	Ca	K	Mg	P	S
			%DM		
			winter		
brassica	2.12 [A]	5.07 [A]	0.61 [B]	0.73 [A]	2.35 [A]
herb	1.92 [A]	5.47 [A]	0.76 [A]	0.68 [A]	0.81 [B]
lettuce	1.17 [B]	6.30 [A]	0.35 [C]	0.46 [B]	0.51 [C]
LSD ∝=0.5	0.29	1.28	0.11	0.16	0.18
			spring		
brassica	1.79 [A]	3.44 [B]	0.64 [A]	0.54 [A]	1.62 [A]
herb	1.37 [B]	2.50 [C]	0.64 [A]	0.38 [B]	0.45 [B]
lettuce	1.02 [C]	3.85 [A]	0.38 [B]	0.36 [B]	0.28 [C]
LSD ∝=0.5	0.16	0.39	0.11	0.05	0.10
			summer		
brassica	0.99 [B]	4.02 [A]	0.45 [B]	0.34 [C]	0.24 [C]
herb	1.50 [A]	3.37 [B]	0.72 [A]	0.42 [B]	0.43 [B]
lettuce	1.65 [A]	3.02 [B]	0.66 [A]	0.51 [A]	1.57 [A]
LSD ∝=0.5	0.29	0.64	0.11	0.08	0.15
			fall		
brassica	0.75 [C]	3.31 [A]	0.35 [B]	0.22 [C]	0.36 [C]
herb	0.10 [B]	3.21 [A]	0.51 [A]	0.31 [B]	0.51 [B]
lettuce	1.24 [A]	2.60 [B]	0.53 [A]	0.38 [A]	1.31 [A]
LSD ∝=0.5	0.13	0.35	0.05	0.04	0.14

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

Table 2.10 Seasonal changes in micronutrient concentrations (µg·g^{-1} DM) in shoot tissue of brassica, herb, and lettuce microgreens. [a]

species	B	Cu	Fe	Mn	Mo	Se	Zn
			µg·g^{-1} DM				
winter							
brassica	17.03 ^{B}	41.40 ^{B}	176.98 ^{A}	151.66 ^{C}	1.84 ^{A}	0.30 ^{C}	79.15 ^{B}
herb	26.61 ^{A}	63.25 ^{A}	271.62 ^{A}	220.18 ^{B}	1.15 ^{B}	0.41 ^{A}	96.67 ^{A}
lettuce	16.84 ^{B}	61.84 ^{A}	276.59 ^{A}	299.47 ^{A}	0.06 ^{C}	0.35 ^{B}	66.27 ^{B}
LSD ∝=0.5	5.59	8.64	108.04	23.61	0.15	0.05	14.97
spring							
brassica	16.35 ^{A}	38.49 ^{C}	203.10 ^{B}	94.43 ^{C}	0.84 ^{A}	0.14 ^{B}	61.75 ^{A}
herb	18.15 ^{A}	44.13 ^{B}	524.30 ^{A}	116.42 ^{B}	0.22 ^{B}	0.18 ^{AB}	60.20 ^{A}
lettuce	17.77 ^{A}	51.58 ^{A}	344.80 ^{AB}	162.96 ^{A}	0.05 ^{C}	0.23 ^{A}	53.05 ^{B}
LSD ∝=0.5	1.87	4.83	237.92	19.07	0.15	0.05	5.11
summer							
brassica	12.41 ^{AB}	23.57 ^{AB}	1069.10 ^{A}	80.02 ^{A}	0.22 ^{C}	0.14 ^{A}	44.58 ^{B}
herb	13.71 ^{A}	27.72 ^{A}	1207.70 ^{A}	66.19 ^{A}	0.59 ^{B}	0.18 ^{A}	59.84 ^{A}
lettuce	10.85 ^{B}	22.23 ^{B}	952.40 ^{A}	70.82 ^{A}	0.79 ^{A}	0.13 ^{A}	59.18 ^{A}
LSD ∝=0.5	2.04	4.58	269.80	14.42	0.18	0.06	9.38
fall							
brassica	12.30 ^{B}	30.70 ^{A}	885.50 ^{A}	65.43 ^{A}	0.14 ^{C}	0.08 ^{A}	64.16 ^{A}
herb	13.61 ^{A}	29.96 ^{A}	793.90 ^{A}	47.48 ^{C}	0.89 ^{A}	0.03 ^{A}	60.13 ^{A}
lettuce	10.59 ^{C}	20.88 ^{B}	886.90 ^{A}	57.76 ^{B}	0.52 ^{B}	0.02 ^{A}	60.49 ^{A}
LSD ∝=0.5	1.23	2.78	210.95	7.38	0.29	0.07	5.32

[a] mean values represent four replications with four blocks per treatment. The main effects of cultivars were evaluated over four consecutive growing seasons. Means with the same uppercase letter are not statistically different (α =0.05).

Chapter Three The Impact of Duration and Light Quality of Narrow-Band Wavelength LEDs on Biomass, Root and Shoot Morphology, and Nutritional Quality of Hydroponically Grown Kale

Abstract C

Kale and other leafy greens are important sources of dietary vitamins, minerals, and antioxidants that benefit consumer health. Specialty greens can be grown in controlled environments which often require the use of supplemental or sole-source lighting like light-emitting diodes (LEDs). Little is known about the interactions between light quality and duration and phytonutrient concentration in hydroponically-grown young leafy greens. The objective of this thesis was to measure the impacts of different LED treatments on plant growth and development along with nutritional content of hydroponically grown 'Premier' kale. Kale were grown in growth chambers at 22 ℃ under LED panels at an average light intensity of 250 $\pm$ 10 µmol·m^{-2}·s^{-1} (Orbital Technologies, Madison, WI) in 10 L tubs containing a ½ strength Hoagland's nutrient solution and watered daily using DI water (Hoagland's #2 solution; Hoagland and Arnon, 1950). Treatments included: 1) white LED for 37 d; 2) 5% B/95% R for 37 d; 3) 20% B 80%/R for 37 d; 4) 20% B/80% R for 25 d; 5) 20% B/80% R for 20 d; 6) 20% R/80% B for 15 d Kale were harvested after 37 days. The phytonutritional concentrations as well as the morphology of hydroponically grown kale plants were significantly impacted by LED treatment. LED treatments with more blue light had significantly shorter plants, lower Xanthophyll pigment concentrations in shoot tissue and greater root dry mass (DM) as compared to all other LED treatments. Results from this thesis may give producers who grow specialty leafy greens or transplants in controlled environments valuable information on the interactions between LED treatment and plant growth and nutrition.

3. 1 Introduction

The light environment influences critical developmental and phytochemical pathways in plants. Specialized pigment-proteins called photoreceptors are able to perceive incoming solar radiation to signal developmentally appropriate photomorphogenic responses to help plants adapt to changes in their light environment (Kong and Okajima, 2016; Montgomery, 2016). These light signals can prompt a diverse range of developmental responses such as germination, cotyledon expansion, chloroplast development, stem elongation, root and leaf growth, along with senescence and flowering (Kim et al., 2005; Montgomery, 2016). Photoreceptors are able to sense the intensity of light and signal chloroplast movement and gene expression accordingly (Li et al., 2009). Cryptochromes and phototropins are blue and UV-A light photoreceptors, while phytochromes are red and far red light photoreceptors (Dai Yin and Hong Xuan, 2010).

Plant responses to blue light from cryptochromes or phototropins include circadian rhythms, phototropism, stomatal opening, compact growth, and the intracellular positioning of chloroplasts to increase light absorption (Christie, 2014; Johkan et al., 2010; Lin, 2002; Wollaeger and Runkle, 2014). Green light can reverse the effects of blue light on stomatal opening (Frechilla et al., 2000). Plant responses to red light from phytochromes include shade avoidance, cell elongation, seed germination, reproductive development, and the development of a greater leaf surface area (Franklin and Quail, 2010; Lin, 2002; Pierik and de Wit, 2013). Light emitting diodes (LEDs) allow

for the specific targeting of wavelengths, high intensity, and the unique combination of blue, red, yellow, orange, ultra-violet (UV), and far-red light (Darko et al., 2014). Red light (650-665 nm) satisfies the peak absorption spectrums of chlorophyll and phytochromes, while blue light normalizes the developmental responses triggered by signals from phototropins and cryptochromes (Darko et al., 2014). Red and blue wavelengths provide targeted energy to pigments (chlorophyll and secondary pigments or receptors) involved in photosynthetic CO_2 fixation and basic metabolism; therefore red and blue wavelengths have the greatest influence on plant growth and development (Bantis et al., 2016; Chen et al., 2014; Figueroa et al., 1995; Massa et al., 2015; Muneer et al., 2014).

In addition to influencing plant growth and development, the light environment is able to signal photoreceptors to adjust the accumulation or allocation of different pigments and other photoprotective molecules in response to changes in light quality and intensity (Ouzounis et al., 2015). Carotenoids are associated with proteins in chloroplasts, where they act as accessory pigments to transfer a broader range of spectral energy to chlorophyll to promote photosynthesis (Cazzonelli, 2011; Khoo et al., 2011). To ensure that incoming solar energy does not damage photosynthetic apparatus within chloroplasts, carotenoids quench triplet chlorophyll, release excess energy through non-photochemical quenching (NPQ) via the xanthophyll cycle, and scavenge radical oxygen species (ROS) in cooperation with other antioxidants like ascorbate and tocopherols (Cazzonelli, 2011). These strong antioxidants protect both plants and

consumers by quenching free radicals that can damage cell membranes and proteins, aiding in the prevention of cancer as well as other chronic diseases (Bartwal et al., 2013; Slavin and Lloyd, 2012). Purposeful manipulation of the light environment to promote the accumulation of carotenoids and other antioxidants can increase the nutritional value of specialty leafy greens, benefiting consumer health (Kopsell et al., 2016; Liu, 2013; Mozian, 2000).

Vegetables within the *Brassica* genus often contain glucosinolates (GS) within shoot tissue (Wu et al., 2009). GS concentration is highly impacted by environmental conditions and can change depending on variety, climate, type of cultivation, type of tissue, developmental stage, and fertility (Johnson, 2002a; Navarro et al., 2011; Wu et al., 2009). GS can be hydrolyzed by either myrosinase in *Brassica* or β-thioglucosidases in gut bacteria to form different breakdown products like indoles and isothiocyanates (ITC) and, to a lesser degree, nitriles (Navarro et al., 2011). GS can help protect against the development of certain cancers and other diseases primarily through their breakdown products, especially ITC (Fahey et al., 2012; Keck and Finley, 2004; Podsędek, 2007). ITCs can induce apoptosis and immobilize the cell cycle, preventing and limiting carcinogenesis in animal models or *in vitro* (Johnson, 2002b; Villarreal-García and Jacobo-Velázquez, 2016; Wu et al., 2009; Zhang, 2010).

Kale (*Brassica oleracea* var. *acephala*) is a member of the Cruciferous family, which is composed of distinctly unique plants that are used around the world in mild-weathered regions for different economic purposes (Clark, 2007; Stewart and

McDougall, 2012; Warwick and Gugel, 2009). Cruciferous vegetables within the Brassicaceae family include varieties of broccoli (*B. oleracea* var. *italica*), cabbage (*B. oleracea* var. *capitata*), kale, Brussels sprout (*B. oleracea* var. *gemmifera*) and cauliflower (*B. oleracea* var. *botrytis*) (Podsędek, 2007). Kale contains a diversity of nutrients including flavonoids, glucosinolates, and carotenoids; many of which can be manipulated through changes in the environment (Bourgaud et al., 2011; Schmidt et al., 2010; Taiz and Zeiger, 1998). Previous studies have looked at the impact of LED lighting within the early developmental stages of leafy vegetables, whereas this study examines the impact of LED lighting at a mature growth stage (Bian et al., 2015; Kim et al., 2005; Kobayashi et al., 2013; Lefsrud et al., 2008; Lin et al., 2013; Martineau et al., 2012). The objective of this study was to examine the impact of duration and light quality of narrow-band wavelength LEDs on the biomass, root and shoot morphology, and nutritional quality of hydroponically grown 'Premier' kale.

3. 2 Materials and Methods

Plant Production and Growing Conditions

'Premier' kale (*B. oleracea* var. *acephala*; Johhny's Selected Seeds, Winslow, ME) was hydroponically grown in Oasis® Horticubes® for 37 d after germination (Smithers-Oasis Company, Kent, OH). The 'Premier' kale cultivar was selected due to its prostrate leaf angle and compact growth habit. Kale seeds were presoaked in deionized (DI) water for 24 h in the dark and then germinated in a low temperature refrigerated incubator (Thermal Fisher Scientific, Waltham, MA) at 24±1 °C for 72 h in the dark. The

germinated seeds were then transferred to growth chambers at 22±1 °C (Model E15; Conviron, Winnipeg, Manitoba, Canada). Three chambers divided into half sections to create a total of six chambers were used to match the number of light treatments (Figure 3.1).

They were given a 7 d acclimation period within the growth chambers in solid bottom trays (26 x 52 x 6 cm) filled with DI water. Plants in the treatment 1 group were acclimated under white LEDs, while all other treatments were acclimated under 5% Blue (470 nm) 95% Red (627 nm) LED panels at an average light intensity of 250±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Orbital Technologies, Madison, WI). After the 7 d acclimation period during which they were watered daily using DI water, seedlings were then transplanted into 10 L tubs containing a ½ strength Hoagland's nutrient solution and watered daily using DI water (Hoagland's #2 solution; Hoagland and Arnon, 1950). After transplanting, plants were grown using a 37 d production cycle under different LED light treatments. Except for the white LED treatment, all other treatments were exposed to 5% Blue/95% Red LED before the individual treatment was applied to the kale at their respective treatment duration time.

Treatments included: 1) white LED for 37 d; 2) 5% B/95% R for 37 d; 3) 20% B 80%/R for 37 d; 4) 20% B/80% R for 25 d; 5) 20% B/80% R for 20 d; 6) 20% R/80% B for 15 d (Figure 3.1). All LED treatments had an intensity of 250±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$; percentages indicate the contribution of blue (B) and red (R) light to total intensity. A randomized, complete block design was used (Figure 3.1). Plants were grown for a total

of 40 d and harvested 30 d after transplanting. Plant height (cm), leaf length and width (cm), shoot fresh mass (FW; g), root fresh mass (FM; g), shoot dry mass (DM; g), and root dry mass (DM; g) data were collected post-harvest. Microgreen shoot tissue was analyzed for carbohydrate, mineral, and carotenoid concentrations. Fresh shoot tissue samples were stored at -20 °C (±1 °C) for later use.

Shoot Tissue Pigment Extraction

Kale fresh tissue was freeze-dried (model 6 L FreeZone; LabConCo, Kansas City, MO) at -25 ˚C. The procedure from Kopsell et al. (2012) was used for pigment extraction from freeze-dried tissue. A 0.1 g (± 0.05 g) sample of frozen tissue was weighed out into glass culture tubes (16 x 100 mm) and then rehydrated with 0.8 mL of ultrapure H_2O for 10 min, and then 2.5 mL of tetrahydrofuran (THF) was added to the sample. Following rehydration, 0.8 mL of the internal standard ethyl-β-8'-apo-carotenoate (Sigma-Aldrich, St. Louis, MO) was added to measure the efficiency of the extraction process. The sample was homogenized using a drill press set at 540 rpm in a Potter-Elvehjem (Kontes, Vineland, NJ) tissue grinding tube using twenty insertions. After homogenization, the sample was placed in a centrifuge for 5 min at 500 g_n. The supernatant was extracted and the sample pellet was then re-suspended in 2 mL THF and homogenized with the same extraction technique for a total of three extractions until the supernatant was colorless. The collected total supernatant was then reduced to 0.5 mL using nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA). Acetone was added to the concentrated supernatant bringing it up to the final volume of 5 mL. A 2 mL portion was filtered

through a 0.2 µm polytetrafluoroethylene (PTFE) filter (Model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE) using a 5-mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and collected into brown crimp-top vials to prepare the extracted solution for high-performance liquid chromatography (HPLC) analysis.

Shoot Tissue Pigment HPLC Analysis

Pigments were separated using an Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA). Separation of chemically similar pigments was successfully completed using an analytical scale (4.6 mm i.d. x 250 mm) 5 µm, 200 Å polymeric RP-C30 column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, PA). The thermostatted compartment column maintained a temperature of 30 ˚C. It was equipped with a 5 µm guard cartridge (4.0 mm i.d. x 10 mm) and holder (ProntoSIL). All separations were completed isocratically using a binary mobile phase of 11% methyl tert-butyl ether (MTBE), 88.99% MeOH, and 0.01% triethylamine (TEA) (v/v/v). Eluted compounds from a 10 µL injection were detected pigments at 453 (carotenoids and internal standard), 652 [chlorophyll *a* (Chl *a*)], and 665 [chlorophyll *b* (Chl *b*)] nm. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignments for pigments were performed by matching retention times and line spectra obtained from the photodiode array detection using the external standards [antheraxanthin (ANT), β-carotene (BC), Chl *a*, Chl *b*, lutein (LUT), neoxanthin (NEO), violaxanthin (VIO), zeaxanthin (ZEA) from ChromaDex Inc., Irvine, CA].

Shoot Tissue Glucosinolate Extraction

The procedure from Charron et al. (2004) was used for glucosinolate extraction from freeze-dried tissue and analysis. A 0.2 g (± 0.05 g) sample of freeze dried tissue was combined with 1 mL benzyl GS solution (1mM), to act as the internal standard. 2.0 mL MeOH and 0.1 mL barium-lead acetate (0.6 M) were then added to the sample in a culture tube (16 x 100 mm) and shaken at 60 rpm for 60 min. Each tube was then centrifuged at 2000 g_n for 20 min at 22 °C. A 0.5 mL aliquot of the supernatant was then be combined with a 1 mL column that consisted of 0.3 mL DEAE Sephadex A-25 (Sigma-Aldrich). The sample was then desulfated using the procedure by Raney and McGregor (1990).

Shoot Tissue Glucosinolate HPLC Analysis

Extracted desulfoglucosinolates were separated using an HPLC unit with a photodiode array detector (1100 series, Agilent Technologies), using a reverse-phase 250 x 4.6 mm i.d., 5 µm Luna C18 column (Phenomenex Inc., Torrance, CA) at a wavelength of 230 nm. The temperature of the column was set at 40 ˚C with a flow rate of 1 mL min^{-1}. The gradient elution parameters were set to 100% water for 1 min, followed by a 15 min linear gradient set to 75% water: 25% acetonitrile. Solvent levels were then held constant for 5 min and then returned to 100% water for the final 5 min. Identification of desulfoglucosinolates took place using a comparison of retention times of authentic standards or previously reported results (Hansen et al., 1995; Kushad et al., 1999).

Soluble Sugars Extraction

Nonstructural water soluble carbohydrates were extracted from kale tissues based on the methods of Muir et al. (2009) and Thavarajah et al. (2016), with slight modifications. A 0.1 g sub-sample of ground, freeze-dried tissue was extracted in a 15 mL test tube by adding 2 mL of RO water which was heated to 80 °C. Samples were shaken for 15 min at 300 rpm then vortexed. The tubes were centrifuged at 4400 rpm for 20 min and then a 1.0 mL aliquot of the supernatant was transferred into a new 15 mL test tube. Samples were reduced to dryness under a stream of N gas. Extracts were rehydrated to 2.5 ml with RO water. Samples were put through a 0.2 µm filter and collected in a 2 mL vial for high performance liquid chromatography (HPLC) analysis.

Soluble Sugars HPLC Analysis

Separation parameters and carbohydrate quantification were done using an HPLC unit (Agilent 1200 series; Agilent Technologies, Santa Clara, CA) with an evaporative light scattering (ELS) detector (1290 Infinity II; Agilent Technologies). The ELS detector had an N gas flow rate of 1.6 $L \cdot min^{-1}$, evaporative gas temperature at 80 °C, and a nebulizer gas temperature at 50 °C. Chromatographic separations were achieved using a Rezex RCM Monosaccharide Ca^{+2} (8%) 300 x 7.8mm i.d., 8 μm analytical scale column (Phenomenex, Torrance, CA), which allowed for effective separation of chemically similar carbohydrate compounds. The column was equipped with a Carbo-Ca 4 x 3.0 mm i.d. security guard cartridge and holder (Phenomenex), and was maintained at 80 °C using a heated column compartment. All separations were achieved

isocratically using a mobile phase of 100% RO water. The flow rate was set at 0.6 $mL \cdot min^{-1}$, with a run time of 20 min, followed by a 2 min equilibration prior to the next injection. Eluted compounds from a 5.0 µL injection were detected and the data collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment for individual carbohydrates were performed by comparing retention times from the ELS detector using external standards of fructose, glucose, and sucrose (Sigma-Aldrich, St. Louis, MO).

Statistical Analyses

Data sets were analyzed by GLM procedure using statistical software (version 9.4; SAS Institute, Cary, NC). Treatment means were separated by least significant difference (LSD) at $\alpha=0.05$. Kale shoot tissue pigments, shoot tissue glucosinolates, mineral elements, and carbohydrate data are presented on a DM basis.

3. 3 Results

Hydroponically Grown Kale Root Morphology

Hydroponic kale root tissue FM was not influenced by LED treatments ($P=0.13$; $F=1.85$), while the root tissue DM was different among LED treatments ($P=0.02$; $F=3.24$). The 80%B/20%R (20 d) LED treatment had the highest root tissue FM, while the 5%B/95%R (37 d) LED treatment had the lowest (Table 3.1; Figure 3.2). All 80%B/20%R LED treatments had a higher root tissue DM as compared to the 5%B /95%R (37 d) LED treatment and the white (37 d) LED (Table 3.1; Figure 3.3). Overall, the 80%B/20%R (20

d) LED treatment had the highest root tissue DM, while the 5%B/95%R (37 d) LED treatment had the lowest mean root tissue DM (Table 3.1; Figure 3.3).

Hydroponically Grown Kale Shoot Morphology

The average shoot FM did not differ between LED treatments (P=0.49; F=0.88) (Table 3.2; Figure 3.4). Plant height was influenced by LED treatment (P=0.01; F=14.27) (Table 3.2; Figure 3.5). The white (37 d) LED treatment had the tallest plants compared to all other LED treatments, while the 80%B/20%R (25 d) LED treatment had the shortest plants (Table 3.2; Figure 3.5). All plants treated with blue narrow band wavelength LED were shorter compared to the white (37 d) and 5%B/95%R LED treatments respectively (Table 3.2; Figure 3.5). There was no difference in leaf length (P=0.79; F=0.48), but leaf width was impacted by LED treatment (P=0.03; F=2.51) (Table 3.2; Figure 3.6). The 5%B/95%R (37 d) LED treatments had the greatest leaf width, while the white (37 d) LED treatment has the smallest leaf width (Table 3.2, Figure 3.6).

Chlorophyll Fluorescence

Chlorophyll minimum fluorescence (F_o) (P=0.01; F=5.71), variable fluorescence (F_v) (P=0.01; F=13.60), and chlorophyll maximum fluorescence (F_m) (P=0.01; F=12.76), were all influenced by LED treatment, while maximum quantum yield (F_v/F_m) (P=0.06; F=2.20) was not (Table 3.3). The white (37 d) LED treatment had the highest F_o and the 80%B/20%R (20 d) LED treatment had the lowest F_o (Table 3.3). The 80%B/20%R (37 d) LED treatment had the highest F_v and F_m, while the 80%B/20%R (20 d) LED treatment had the lowest F_v and F_m (Table 3.3).

Shoot Chlorophyll and Pigment Concentrations

Chlorophyll *a* (Chl *a*) (P=0.92; F=0.29), Chl *b* (Chl *b*) (P=0.99; F=0.11), and total Chl (P=0.95; F=0.23) concentrations in kale shoot tissue did not significantly differ among LED treatments (Table 3.4). Total carotenoid concentrations within kale shoot tissue were not altered by LED treatment (P=0.62; F=0.72) (Table 3.5). β-carotene (BC) (P=0.96; F=0.21), lutein (LUT) (P=0.72; F=0.57), neoxanthin (NEO) (P=0.12; F=1.91), and violaxanthin (VIO) (P=0.59; F=0.75) concentrations within kale shoot tissue were not influenced by LED treatment (Table 3.5). Zeaxanthin (ZEA) (P=0.01; F=13.19) and antheraxanthin (ANT) (P=0.01; F=11.64) concentrations within kale shoot tissue were different among LED treatments (Table 3.5).

The white (37 d) LED treatment had the highest ZEA and ANT concentrations in shoot tissue (Table 3.5). The 80%B/20%R (20 d) LED treatment had the lowest concentrations of ZEA and ANT within shoot tissue (Table 3.5). The 5%B/95%R (37 d), 80%B/20%R (20 d), and 80%B/20%R (15 d) had lower ANT concentrations in shoot tissue as compared to all other LED treatments (Table 3.5). The 80%B/20%R (37 d) had the highest concentration of NEO, while the 80%B/20%R (20 d) had the lowest concentration of NEO in shoot tissue (Table 3.5). The shoot tissue concentrations of pigments involved in the Xanthophyll Cycle (ZEA+ANT/ZEA+ANT+VIO) were significantly altered by LED treatment (P=0.02; F=3.13) (Table 3.6; Figure 3.7). The white (37 d) LED treatment had the highest concentration of Xanthophyll Cycle pigments compared to all

other LED treatments, while the 80%B/20%R (15 d) had the lowest (Table 3.6; Figure 3.7).

Shoot Glucosinolate Concentrations

Iberin (P=0.45; F=0.98), Progoitrin (P=0.84; F=0.41), Sinigrin (P=0.43; F=1.02), Erucin (P=0.55; F=0.81), Indole glucosinolates (P=0.33; F=1.20) and Aliphatic glucosinolates (P=0.72; F=0.57) were not significantly influenced by LED treatments (Table 3.7). Total GS concentrations (P=0.70; F=0.61) were not significantly influenced by LED treatment (Table 3.7). While there were no significant differences in GS concentrations among LED light treatments, total GS concentrations within kale shoot tissue were increased under the 5%B/95%R (37 d) LED treatment as compared to all other treatments (Table 3.7; Figure 3.8).

Shoot Non-Structural, Water-Soluble Carbohydrate Concentrations

Sucrose (P=0.81; F=0.45) and glucose (P=0.12; F=1.94) concentrations within hydroponic kale shoot tissue did not differ among LED treatments (Table 3.8). Fructose concentration in hydroponic kale shoot tissue was influenced by LED treatment (P=0.03; F=3.04) (Table 3.8; Figure 3.9). The 5%B/95%R (37 d) LED treatment had the lowest fructose concentration compared to all other LED treatments (Table 3.8; Figure 3.9).

3.4 Discussion

Root and Shoot Morphology and LED Treatment

While the root and shoot FM were not different among LED treatments, the root DM, plant height, and leaf width of the hydroponically grown kale were significantly

influenced by the LED treatments. Although not significant, the 5%B/95%R (37 d) LED treatment had decreased root and shoot FM compared to all other LED treatments. Furthermore, the 5%B/95%R (37 d) LED treatment also had the lowest root DM. The root DM for kale was increased for all blue LED treatments as compared to the red and white LED treatments and is consistent with the results of other studies that use hydroponically grown lettuce (Johkan et al., 2010; Kobayashi et al., 2013; Martineau et al., 2012). There was greater metabolite partitioning into roots under the blue LED treatments, causing a variation in root DM which is consistent with other studies (Samuolienė et al., 2010; Tripathy and Brown, 1995). Blue light, especially within the UV spectrum, triggers drought avoidance strategies via PHOT1 (phototropin-1), which promotes the vertical growth of roots away from the soil surface (Galen et al., 2007; Yokawa et al., 2014). Blue light causes greater biomass accumulation, promotes vegetative growth, and delays leaf senescence which in turn could lead to greater metabolite partitioning to roots (Hogewoning et al., 2010; Johkan et al., 2010; Wang et al., 2016).

Kale height was significantly decreased under all blue LED treatments as compared to the white and red LED treatments, with the shortest plants under the 80%B/20%R (25 d) LED treatment. Compact growth or reduced height is common among plants exposed to blue light (Johkan et al., 2010). Blue light, which is intercepted via cryptochrome photoreceptors in shoot tissue, signals Cryptochrome Circadian Clock 1 (CRY1) to upregulate gene expression within the Gibberellic Acid (GA) biosynthetic

pathway, and as a result blue light causes compact growth and reduced stem elongation in *Brassica spp.* (Chatterjee et al., 2006; Olszewski et al., 2002). While leaf length was not significantly different, leaf width was influenced by the LED treatment. Leaf width was increased under the blue LED treatments as compared to the white LED treatment. The effect of blue light on leaf morphology and area is dependent on age, species and cultivar. The increase in leaf width would aid in more efficient light harvesting to compensate for the decrease in chlorophyll shoot content under certain blue light treatments (Lin et al., 2013; Wang et al., 2015).

Shoot Pigments and LED Treatment

Chlorophyll has maximum energy absorption within the red and blue wavelengths, with red light having the highest quantum yield (Hogewoning et al., 2012; McCree, 1972). Chlorophylls can move and concentrations can fluctuate based on duration and light quality (Kopsell et al., 2016; Lefsrud et al., 2008; Johkan et al., 2010). In kale shoot tissue, the Chl *a*, Chl *b*, and Total Chl concentrations were not significantly different between LED treatments. While not significant, total Chl concentration was increased under the 80%B/20%R (37 d) LED treatment. Nonetheless, the blue LED treatments with reduced duration had decreased levels of chlorophyll. Studies have reported an increase in chlorophyll content under blue LED in lettuce and Chinese cabbage (*B. campestris*) (Johkan et al., 2010; Olle and Viršile, 2013; Wang, 2016). In contrast, other studies have reported a decrease in chlorophyll concentration in kale and sprouting broccoli (*B. oleacea* var. *italica*) under blue LED treatment (Kopsell and

Sams, 2013; Lefsrud et al., 2008). Blue narrow-band LED wavelengths could alter chlorophyll concentrations as the plants adjust chlorophyll quality and content to match the reduced photosynthetic efficiency of the blue light environment.

Carotenoid concentrations varied with LED treatment in kale shoot tissue. Previous studies have reported a positive relationship between blue LED treatments and increased carotenoid concentration in leafy greens (Bian et al., 2015; Johkan et al., 2010; Lefsrud et al., 2008, Kopsell and Sams, 2013; Son and Oh, 2013). These findings are consistent with the 80%B/20%R (15 d) LED treatment in the current study which had the highest total carotenoid concentration as compared to all other treatments. This suggests that a blue LED light treatment approximately 15 d before harvest may improve carotenoid concentrations within shoot tissue. While BC and LUT concentrations within kale shoot tissue were not significantly different between LED treatments, the 5%B/95%R (37 d) LED treatment had the highest BC and LUT concentrations which is consistent with the findings of other studies (Wu et al., 2007; Lefsrud et al., 2008).

Lutein and BC are important in preventing cataracts and other eye diseases as well as lung cancer and cardiovascular disease (Bian et al., 2015). Increasing the innate concentrations of LUT and BC within kale shoot tissue through the use of a 5%B/95%R LED treatment could yield added nutritional value which in turn could benefit consumer health. All blue LED treatments had lower concentrations of Xanthophyll Cycle (ZEA+ANT+VIO) pigments as compared to all other treatments. Xanthophylls are

responsible for non-photochemical quenching (NPQ) of excess energy within light harvesting antennae proteins to prevent the formation of surplus reactive oxygen species (ROS), which can ultimately lead to PSII damage and photoinhibition (Demmig-Adams and Adams, 1996; Jahns and Holzwarth, 2012; Latowski et al., 2011). Thus, the blue LED treatments did not contribute additional stress to PSII light harvesting complexes, promoting effective photosynthetic energy collection.

Chlorophyll Fluorescence and LED Treatment

Chlorophyll minimum fluorescence (F_o), variable fluorescence (F_v), and chlorophyll maximum fluorescence (F_m) values decreased with decreasing duration of the blue LED treatment. Since F_o, when all PSII reaction centers are theoretically open, suggests that kale plants were not photosynthetically stressed under the blue LED treatments (Hazrati et al., 2016; Kooten and Snel, 1990). Maximum quantum yield (F_v/F_m) was consistent among LED treatments. The F_v/F_m values for all LED treatments are within the previous reported ranges for ideal levels for most plant species which is typically 0.83 (Allen et al., 1997; Hogewoning et al., 2012; Johnson et al., 1993; Maxwell and Johnson, 2000; Son and Oh, 2013). This suggests that the blue LED pre-harvest treatments did not place additional stress on plant photosynthetic activity or limit non-photochemical energy dissipation (Demmig-Adams et al., 1996; Maxwell and Johnson, 2000). With reduced stress placed on photosynthetic systems, plants are better able to carry out photosynthetic reactions and increase metabolic activity.

Glucosinolate Concentrations and LED Treatment

Glucosinolates are S-containing compounds that are characteristically produced by the *Brassica* and *Allium* families (Tsao et al., 2002). When plants are wounded by herbivory or pathogens, GS are released into the surrounding air and plant tissue when they react with myrosinase enzymes which are differentially compartmentalized within specialized vacuoles in plant cells (Charron et al., 2005; Halkier and Gershenzon, 2006; Wittstock and Burow, 2010; Figure 3.10). Glucosinolates have been used effectively as soil bio-fumigant agents in novel applications (Tsao et al., 2002). Glucosinolates are influenced by environmental and genetic factors. For example, Indole GS are influenced by environmental conditions, while aliphatic GS are influenced via genetic factors (Keck and Finley, 2004). Glucosinolates act as important anti-carcinogenic agents within the human diet, reducing the risk of developing certain cancers like lung, prostate or colon cancers (Johnson, 2002b; Keck and Finley, 2004; Navarro et al., 2011).

As a group, kale has a varied GS profile which can be attributed to the diverse genetic background of this group (Carlson et al., 1987; Traka and Mithen, 2009). Iberin, Sinigrin, Progoitrin, Erucin, Indole, and Aliphatic GS were identified in hydroponic kale shoot tissue, which is consistent with other studies (Carlson et al., 1987; Charron et al., 2005; Nilsson et al., 2006). The 5%B/95%R (37 d) LED treatment had the highest total, Indole, and Aliphatic GS concentrations in kale shoot tissue as compared to all other treatments. There have been contrasting reports on light and GS concentrations. Previous studies have reported a null effect of LED treatment on GS content in kale baby

greens, while others have found blue LED increased GS in broccoli microgreens (Lefsrud et al., 2008; Kopsell and Sams, 2013). These results suggest using a 5%B/95%R LED treatment can cause an increase in GS concentrations in kale. Differences in developmental stage and cultivar could contribute to differences in light treatment effect.

Non-Structural, Water-Soluble Carbohydrates and LED Treatment

Light duration and quality is able to influence soluble carbohydrate content in plants by altering CO_2 assimilation (Eckstein et al., 2012; Li et al., 2010; Roitsch, 1999; Rosa et al., 2009; Samuolienė et al., 2010). Sucrose, glucose and fructose are the major transportable sugars plants shuttle to sink tissues like mature leaves, flowers, and seeds via phloem tissue (Taiz and Zeiger, 1998). Sucrose can be broken down to form fructose and glucose which are involved in various catabolic reactions (Lemoine et al., 2013; Rosa et al., 2009; Taiz and Zeiger, 1998). Fructose is involved in osmoprotection and secondary metabolite synthesis (Bogdanović et al., 2008; Rosa et al., 2009). Glucose levels can be used to signal ROS concentrations and stress (Bogdanović et al., 2008; Koch, 2004). Glucose, sucrose, and fructose are the main nonstructural, water soluble carbohydrates found in brassica shoot tissue (Ayaz et al., 2006; King et al., 1997).

Kale shoot tissue with a lower concentration of sucrose and a higher concentration of fructose is consistent with other studies (Ayaz et al., 2006; Nilsson et al., 2006). While sucrose and glucose concentrations did not differ between LED treatments, fructose concentrations were influenced by LED treatment. The 5%B/95%R

(37 d) LED treatment had the lowest fructose concentration as compared to all other LED treatments. All blue LED treatments had overall increased levels of sucrose, glucose, and fructose compared to all other treatments, which is consistent with other studies (Terfa et al., 2012). Increasing soluble sugar levels in plants can lead to an increased metabolic activity and the generation of amino acids, cellulose, and lipids (Eckstein et al., 2012). Soluble sugars are also involved in the generation and detoxification pathways for ROS, which are produced in response to stress (Couée et al., 2005). Elevated levels of soluble sugars within kale shoot tissue suggest increases photosynthetic activity or source to sink partitioning under the blue LED treatments.

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Appendix C

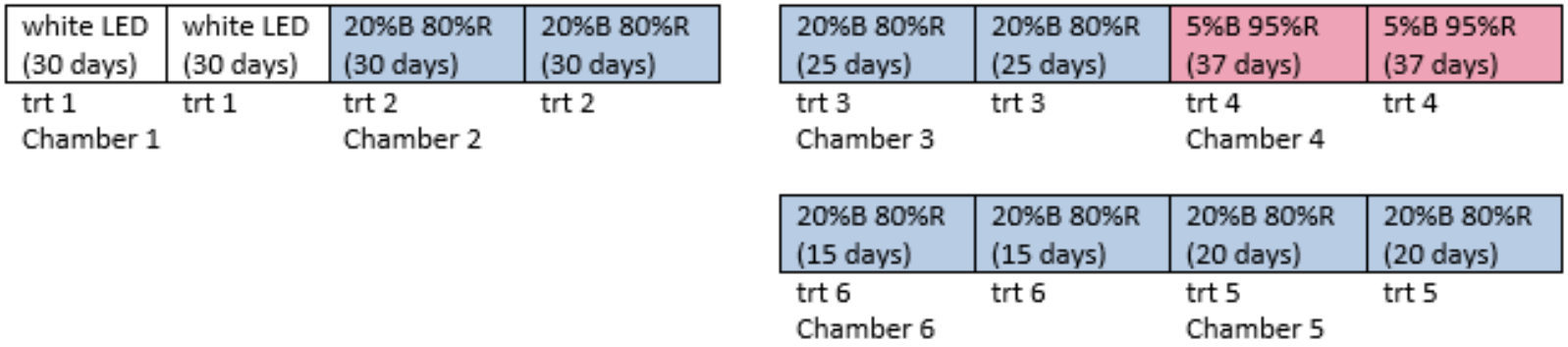

Figure 3.1 Randomized complete block (RCB) design for hydroponic 'Premier' kale LED study; three growth chambers which were divided into six half sections with two blocks per treatment.

Table 3.1 Impact of LED treatments on root tissue fresh (FM; g·plant^{-1}) and dry mass (DM; g·plant^{-1}) of hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	root FM (g·plant)	root DM (g·plant^{-1})
white (37 days)	29.72	1.65 [AB]
5%B 95%R (37 days)	19.98	1.29 [B]
20%B 80%R (37 days)	33.44	2.07 [A]
20%B 80%R (25 days)	29.61	2.09 [A]
20%B 80%R (20 days)	36.09	2.14 [A]
20%B 80%R (15 days)	31.77	1.84 [A]
LSD ∝ = 0.5	NS	0.53

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

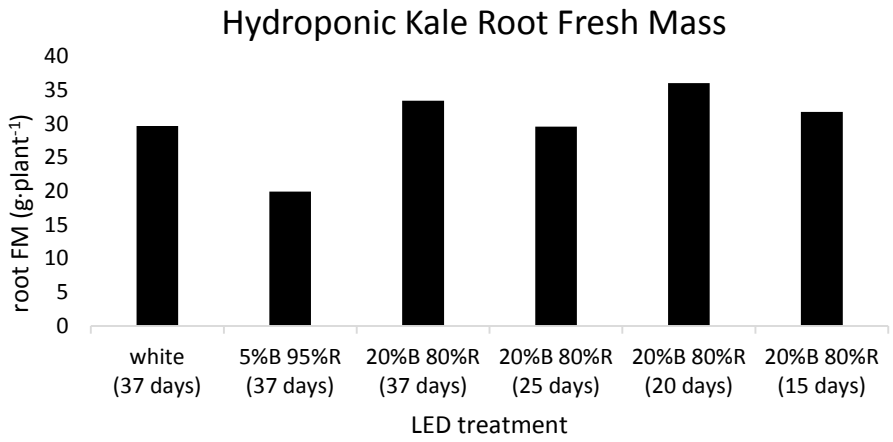

Figure 3.2 The influence of LED treatment on root fresh mass ($g \cdot plant^{-1}$) for hydroponically grown kale.

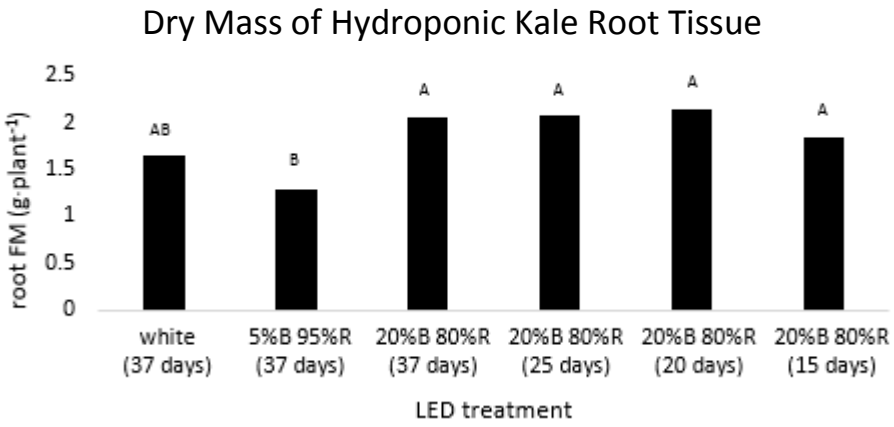

Figure 3.3 The influence of LED treatment on root tissue dry mass (g·plant^{-1}) for hydroponically grown kale. Means with the same uppercase letter are not statistically different (α =0.05).

Table 3.2 Influence of LED treatments on shoot fresh mass (g·plant^{-1}), plant height (cm), and leaf length and width (cm) of hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	shoot FM (g·plant^{-1})	plant height (cm)	leaf length (cm)	leaf width (cm)
white (37 days)	45.61	25.10 [A]	14.15	10.92 [B]
5%B 95%R (37 days)	37.86	22.83 [B]	13.94	12.03 [A]
20%B 80%R (37 days)	42.76	19.61 [C]	14.25	11.47 [AB]
20%B 80%R (25 days)	41.79	19.04 [C]	14.03	11.96 [A]
20%B 80%R (20 days)	44.25	19.58 [C]	13.64	11.47 [AB]
20%B 80%R (15 days)	41.15	21.03 [C]	14.33	12.33 [A]
LSD ∝ = 0.5	NS	1.74	NS	0.89

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

Table 3.3 Impact of LED treatments on chlorophyll minimum fluorescence (F_o), variable fluorescence (F_v), chlorophyll maximum fluorescence (F_m), and maximum quantum yield (F_v/F_m) of hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	F_o	F_v	F_m	F_v/F_m
white (37 days)	52.69 [A]	201.28 [AB]	253.97 [A]	0.79
5%B 95%R (37 days)	47.25 [B]	175.06 [D]	222.31 [CD]	0.79
20%B 80%R (37 days)	52.22 [A]	207.44 [A]	259.61 [A]	0.80
20%B 80%R (25 days)	48.47 [B]	186.75 [C]	235.22 [BC]	0.79
20%B 80%R (20 days)	45.11 [B]	172.06 [D]	217.17 [D]	0.79
20%B 80%R (15 days)	46.92 [B]	191.22 [BC]	238.28 [B]	0.80
LSD $\propto$ = 0.5	3.56	10.60	13.11	NS

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

Table 3.4 Influence of LED treatment on shoot tissue chlorophyll concentrations ($mg \cdot g^{-1}$ DM) in hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	Chl *a*	Chl *b*	Total Chl	*a* to *b* ratio
		$mg \cdot g^{-1}$ DM		
white (37 days)	12.62	3.68	16.30	3.42
5%B 95%R (37 days)	13.12	3.68	16.80	3.56
20%B 80%R (37 days)	13.71	3.85	17.57	3.56
20%B 80%R (25 days)	12.99	3.65	16.64	3.56
20%B 80%R (20 days)	11.77	3.52	15.28	3.34
20%B 80%R (15 days)	11.76	3.73	15.49	3.15
LSD $\propto$ = 0.5	NS	NS	NS	

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

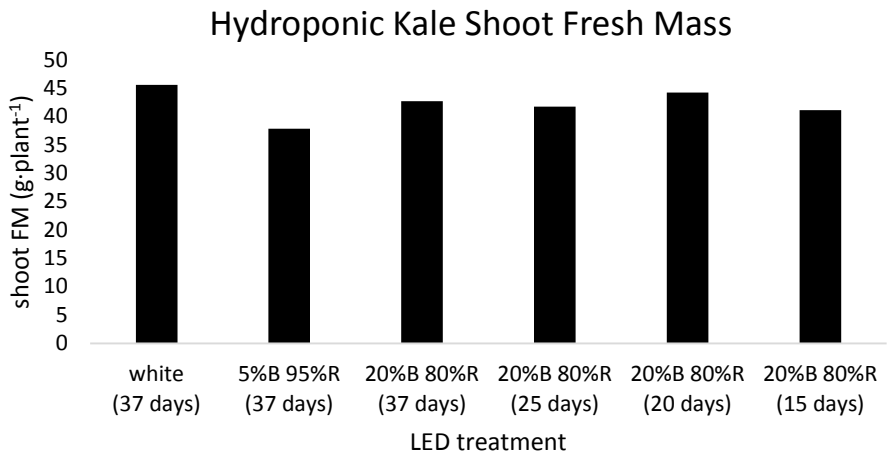

Figure 3.4 The influence of LED treatment on shoot fresh mass ($g \cdot plant^{-1}$) for hydroponically grown kale.

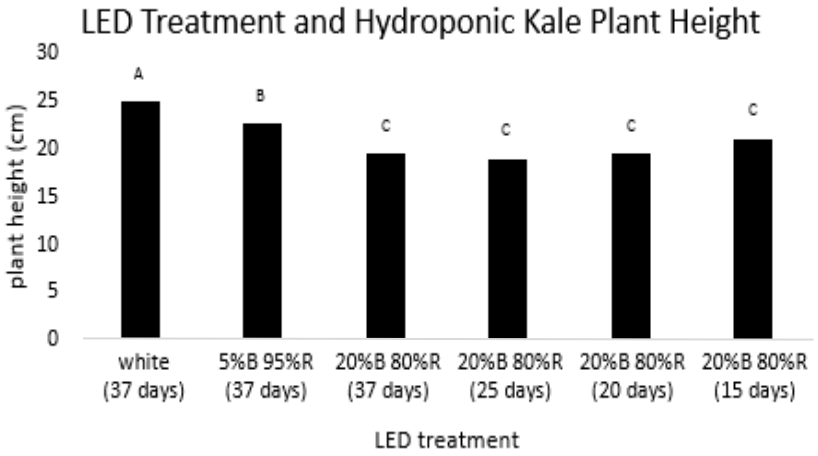

Figure 3.5 The influence of LED treatment on plant height (cm) for hydroponically grown kale. Means with the same uppercase letter are not statistically different (α =0.05).

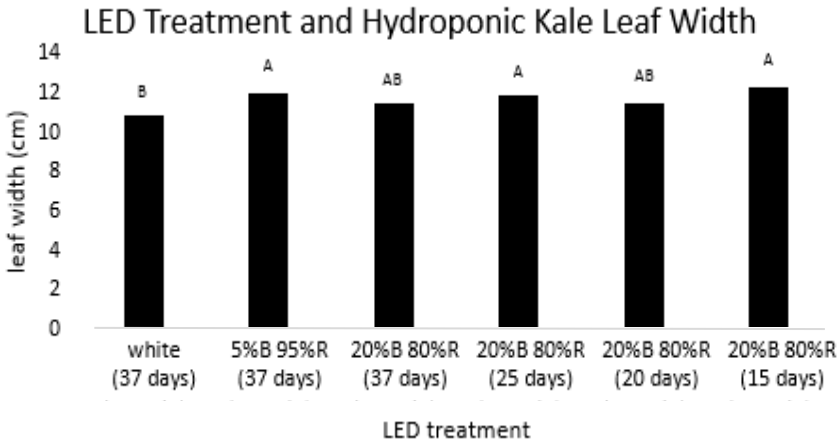

Figure 3.6 The influence of LED treatment on leaf width (cm) for hydroponically grown kale. Means with the same uppercase letter are not statistically different (α =0.05).

Table 3.5 Influence of LED treatment on shoot tissue carotenoid pigment concentrations (mg·g^{-1} DM) in hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	β-carotene	Zeaxanthin	Lutein	Antheraxanthin	Neoxanthin	Violaxanthin	Total Carotenoids
				mg·g^{-1} DM			
White (37 days)	0.78	0.029 ^{A}	1.37	0.09 ^{A}	0.67 ^{A}	0.43	3.38
5%B 95%R (37 days)	0.82	0.007 ^{B}	1.45	0.03 ^{B}	0.57 ^{AB}	0.47	3.35
20%B 80%R (37 days)	0.78	0.005 ^{B}	1.36	0.04 ^{B}	0.68 ^{A}	0.41	3.26
20%B 80%R (25 days)	0.76	0.005 ^{B}	1.32	0.04 ^{B}	0.50 ^{AB}	0.52	3.14
20%B 80%R (20 days)	0.70	0.003 ^{B}	1.23	0.03 ^{B}	0.44 ^{B}	0.43	2.84
20%B 80%R (15 days)	0.79	0.004 ^{B}	1.40	0.03 ^{B}	0.59 ^{AB}	0.73	3.55
LSD $\propto$=0.5	NS	0.008	NS	0.02	0.20	NS	NS

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity. NS = not significant

Table 3.6 Influence of LED treatment on Xanthophyll Cycle shoot tissue pigment concentrations ($mg \cdot g^{-1}$ DM) in hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	ZEA+ANT+VIO	ZEA+ANT/ZEA+ANT+VIO
	$mg \cdot g^{-1}$ DM	
white (37 days)	0.55	0.22 [A]
5%B 95%R (37 days)	0.52	0.14 [AB]
20%B 80%R (37 days)	0.46	0.10 [B]
20%B 80%R (25 days)	0.56	0.11 [B]
20%B 80%R (20 days)	0.46	0.08 [B]
20%B 80%R (15 days)	0.77	0.07 [B]
LSD $\propto$ = 0.5	NS	0.09

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

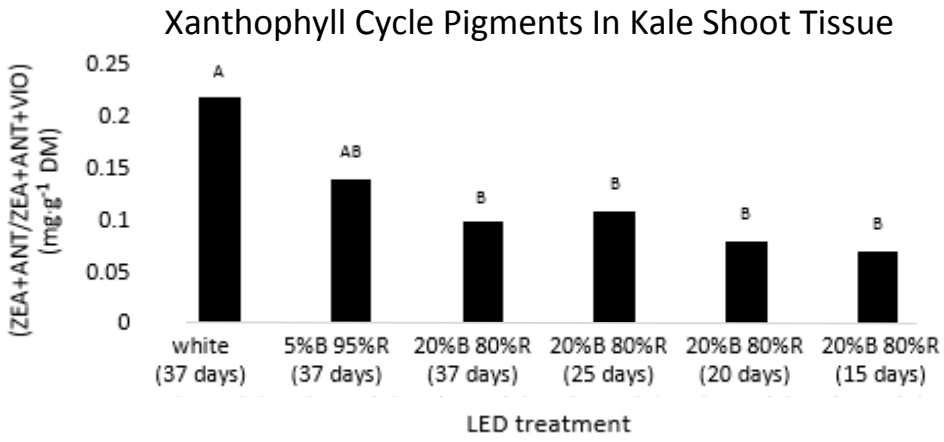

Figure 3.7 The influence of LED treatment on Xanthophyll Cycle pigment (ZEA+ANT/ZEA+ANT+VIO) concentrations ($mg \cdot g^{-1}$ DM) in kale shoot tissue. Means with the same uppercase letter are not statistically different (α =0.05).

Table 3.7 Influence of LED treatment on shoot tissue glucosinolate concentrations (mg·g^{-1} DM) in hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	Iberin	Sinigrin	Progoitrin	Erucin	Aliphatic glucosinolates	Indole glucosinolates	Total Glucosinolates
				mg·g^{-1} DM			
white (37 days)	0.54	0.09	1.05	3.09	4.76	0.18	4.94
5%B 95%R (37 days)	0.77	BD	0.8	4.70	6.30	0.40	6.70
20%B 80%R (37 days)	1.28	0.20	1.27	3.40	6.15	0.14	6.29
20%B 80%R (25 days)	0.38	0.14	1.74	3.52	5.78	0.07	5.84
20%B 80%R (20 days)	0.15	BD	1.30	2.31	3.76	0.11	3.88
20%B 80%R (15 days)	BD	BD	1.69	2.96	4.66	0.36	5.02
LSD ∝=0.5	NS	NS	NS	NS	NS	NS	NS

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant
BD = below detection

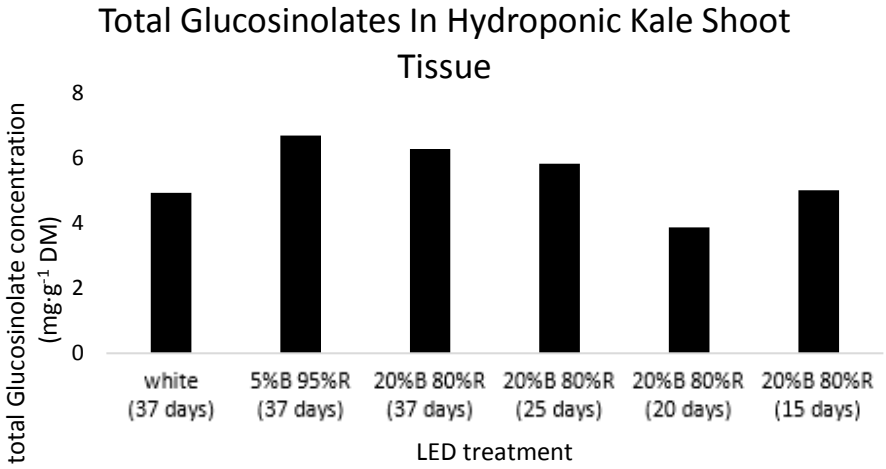

Figure 3.8 The influence of LED treatment on total glucosinolate concentration ($mg \cdot g^{-1}$ DM) in hydroponic kale shoot tissue.

Table 3.8 Influence of LED treatment on sucrose, glucose and fructose concentrations (mg·g^{-1} DM) in the shoot tissue of hydroponically grown 'Premier' kale (*B. oleracea* var. *Acephala*). [a]

LED treatment	sucrose	glucose	fructose
		mg·g^{-1} DM	
white (37 days)	0.103	12.42	12.13 [A]
5%B 95%R (37 days)	0.102	11.66	6.83 [B]
20%B 80%R (37 days)	0.102	13.61	12.47 [A]
20%B 80%R (25 days)	0.103	14.51	12.66 [A]
20%B 80%R (20 days)	0.103	18.26	13.60 [A]
20%B 80%R (15 days)	0.103	15.48	10.95 [A]
LSD ∝ = 0.5	NS	NS	4.00

[a] mean values represent two replications of six plants per block treatment for each of the three complete experimental runs. Means with the same uppercase letter are not statistically different (α =0.05). All LED treatments have an intensity of 250±10 µmol·m^{-2}·s^{-1}; percentages indicate the contribution of red (R) and blue (B) light to total intensity.
NS = not significant

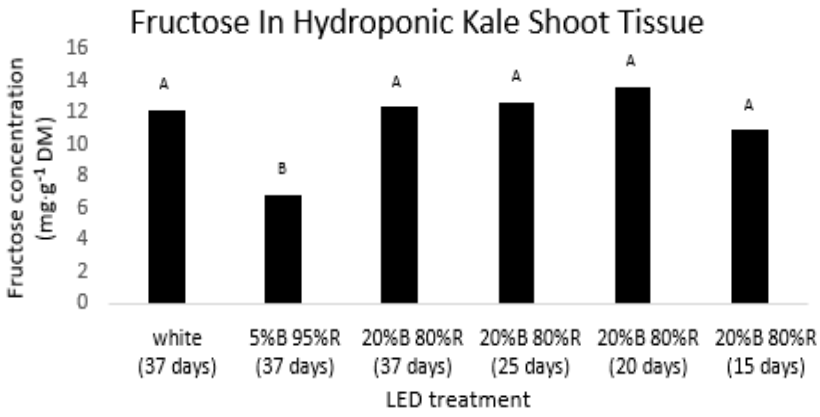

Figure 3.9 The influence of LED treatment on fructose concentrations ($mg \cdot g^{-1}$ DM) in hydroponic kale shoot tissue. Means with the same uppercase letter are not statistically different (α =0.05).

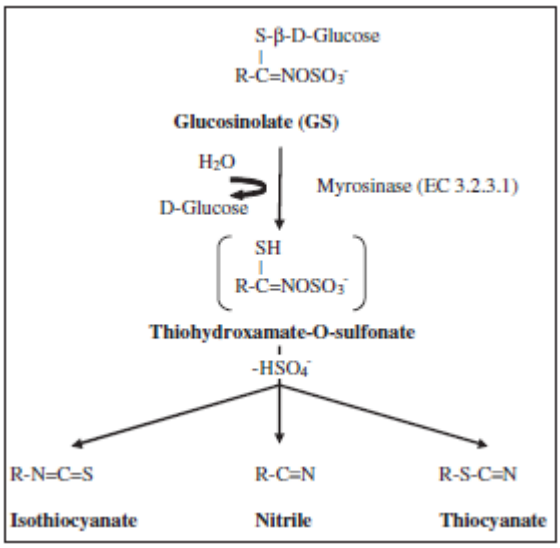

Figure 3.10 Bioactivation pathway for glucosinolates and products (Keck and Finley, 2004).

Chapter Four Conclusions

4.1 Screening of Microgreen Brassica, Herb, and Lettuce Cultivars over Different Environments for Biomass and Nutritional Quality Parameters

There is an expansive range of herbs, vegetables, and annual species that can be grown as microgreens. The genetic diversity of microgreen crops can cause differences in crop growth, nutritional content, and yield. Additionally, genotype can influence how the different plant species interact and respond to changing environmental conditions. Genetic variability within the cultivars that were screened in this study contributed to the diverse phytochemical, visual, and physical traits of each microgreen species group as well as regulated how each cultivar responded to environmental conditions throughout the different seasons.

Cultivars had diverse mineral accumulation rates and phytonutrient contents throughout the four seasons. Germination time and production time was influenced by genotype and seasonal environmental conditions. The herb microgreens had the longest germination and production times as compared to lettuce and brassica cultivars of which the brassica microgreens had the shortest germination and production times. Generally, herb microgreens had higher mineral and carotenoid concentrations with brassica microgreens having the second highest carotenoid concentrations, while lettuce had greater water soluble carbohydrate concentrations. Brassica microgreens consistently had the highest FM, while herb microgreens had the lowest throughout all seasons.

Environmental factors like light, water, humidity, and temperature varied according to season and significantly impacted microgreen growth and development. Light quality and temperature played a significant role in shaping plant metabolic activity and morphology throughout the different seasons. Temperatures should remain as constant as possible throughout the growing period to promote uniform germination and growth; especially cooler nighttime temperatures which can trigger secondary dormancy or delay germination microgreens. Temperatures between 20-30 °C had the greatest impact on promoting microgreen growth, while moderate PAR levels between 250-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ tended to benefit metabolite and biomass accumulation in microgreens. Future studies are needed to document the impacts on nutritionally important metabolites of seasonal spectral differences in greenhouse produced specialty crops.

The spring and fall seasons were optimal for general microgreen growth and development. Carotenoid concentrations in all microgreen cultivars tended to increase in the summer and fall. Glucose and fructose tended to increase in microgreen shoot tissue in the summer and fall, while sucrose increased in the winter and spring. Micronutrient concentrations in shoot tissue tended to increase in the winter, while macronutrient concentrations had a more diverse accumulation pattern based on cultivar and season. Microgreens prefer warmer growing conditions with high humidity and moderate PAR levels. With the genetic diversity of commercial microgreen crops, future studies are needed to analyze the nutritional content of different microgreen

cultivars. Additionally, future studies can investigate the impact of LED treatments on different microgreen cultivars in hydroponic, compost+peat based media, and peat-based media production systems. Since microgreens are a short-cycle crop with the potential to secure high market prices, a complete economic analysis of microgreen production in greenhouses could benefit producers seeking to grow them commercially.

4.2 Impact of Duration and Light Quality of Narrow-Band Wavelength LEDs on Biomass, Root and Shoot Morphology, and Nutritional Quality of Hydroponically Grown Kale

Light plays a major role in plant growth and development by triggering the up or down regulation of internal signaling pathways to alter metabolic and hormone pathways to match environmental conditions. Hydroponically grown leafy greens can be successfully grown in controlled environments using artificial lighting. The impacts of LED lighting on leafy greens have been primarily investigated in lettuce crops, but more information is needed on a larger range of specialty leafy greens like kale, collard greens, and cabbages. Root development is essential to promoting healthy plant growth via efficient water, metabolite, and nutrient translocation to sink tissues. Light can influence root development through the partitioning of metabolites between root and shoot tissues. Blue LED treatments improved the FM and DM of hydroponic kale roots. Hydroponically grown plants offer a more convenient method to study root morphology and development. More studies are needed on the impacts of LED lighting on radical and root development in specialty greens and transplants.

Additionally, light quality, quantity, and duration can alter plant morphology, biomass accumulation, along with primary metabolism and secondary metabolite levels in shoot tissue of leafy greens. Previous studies have established the use of LED treatments to influence primary and secondary metabolite levels in shoot tissue of specialty greens. The blue LED treatments increased carbohydrate and chlorophyll concentrations, decreased plant height, as well as decreased xanthophyll cycle pigments. In contrast, the red LED treatment increased GS concentrations and plant height of hydroponic kale. Blue LED treatments promoted vegetative growth and biomass accumulation in root and shoot tissues in hydroponic kale. It also down-regulated the xanthophyll cycle which protects light harvesting centers from photodamage, indicating the blue LED treatments did not place additional stress on photosynthesis. Specific wavelengths of red and blue light can impact plant growth and development through the modulation of metabolic and hormonal pathways via signals from photoreceptor pigments. The results of this study connect the changes in metabolic pathways and root and shoot morphology in hydroponic kale greens to narrow-band red and blue wavelengths.

The consistent FM values of hydroponic kale across all LED treatments could signify that other factors besides light within the genotype or environment of kale play a greater role in controlling biomass accumulation. Contrasting results within this study with other specialty green LED studies could be attributed to differences in maturity, genotype, cultivar, and cultivation method. Alternating the quality and duration of red

and blue narrow band wavelengths can influence the morphology and phytonutrient content of hydroponic kale. Future studies are needed to determine the most effective developmental stage to apply an LED treatment. For example, a study could investigate the influence of a blue LED treatment applied just after germination, as compared to a blue LED treatment applied just before harvest. An additional study could look at the influence of a sole source blue or red LED pre-harvest treatment on metabolite concentrations along with root and shoot morphology for a screening of specialty leafy green cultivars.

Vita

Rosalie M. Metallo was born July 12, 1993 and grew up in Lindenhurst, IL. She has loving parents and a twin sister. From a young age, she developed an interest in the natural world through time spent gardening and camping with her family. She graduated from the College of Lake County in July 2013 with an Associates in Applied Sciences Degree. At Illinois State University, she was able to gain experience in scouting and biological controls, greenhouse environmental regulation, and different agricultural production systems. She majored in Agronomy and minored in Biological Sciences at Illinois State University where she graduated in May 2015 with a Bachelor in Science Degree. Rosalie continued her studies at the University of Tennessee where she researched the impacts of environmental conditions on plant growth and development along with the nutritional content of different specialty greens grown in controlled environments. She received a Master in Science Degree in Plant Science with a minor in Entomology from the University of Tennessee in August 2017. Rosalie looks forward to a career working within the Agriculture Industry in Research and Development where she can help strengthen food security as well as advance the progress of agricultural development nationally and globally.