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The Impact of Blue and Red LED Lighting on Biomass Accumulation, Flavor Volatile Production, and Nutrient Uptake in Hydroponically Grown Genovese Basil

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I am submitting herewith a thesis written by Hunter Albright Hammock entitled "The Impact of Blue and Red LED Lighting on Biomass Accumulation, Flavor Volatile Production, and Nutrient Uptake in Hydroponically Grown Genovese Basil." 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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The Impact of Blue and Red LED Lighting on Biomass Accumulation, Flavor Volatile Production, and Nutrient Uptake in Hydroponically Grown Genovese Basil

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Abstract

The use of light-emitting diodes (LEDs) in commercial greenhouse production is rapidly increasing due to technological advancements, increased spectral control, and improved energy efficiency. Research is needed to determine the value and efficacy of LEDs when compared to traditional lighting systems. The primary objective of this project was to establish the impact of narrow bandwidth blue(B)/red(R) LED lighting ratios on flavor volatiles in hydroponic basil (*Ocimum basilicum* var. ‘Genovese’) in comparison to non-supplemented natural light controls and traditional high-pressure sodium (HPS) lighting. Emphasis was placed on evaluating the efficacy of LED/HPS lighting sources and their impact on biomass, nutrient uptake, and flavor volatile concentrations in basil. Specific ratios of narrow-band B/R (447 nm/627 nm) LED light were used in addition to incremental daily light integrals (DLIs) to determine the impact of spectral quality and light intensity on primary and secondary metabolism of basil. Edible biomass and nutrient uptake were significantly impacted by supplemental lighting treatments and growing season. The 20B/80R LED treatment had the greatest total fresh biomass (FM) and dry biomass (DM) accumulation. Mineral analyses showed that both macro and micronutrient accumulations were impacted with supplemental lighting and across growing seasons. Many flavor volatiles varied across light treatments and showed a non-linear relationship with increasing B/R LED ratios, with the highest concentrations observed in LED ratios ranging from 20B/80R to 50B/50R. However, the concentrations of some compounds, such as methyl eugenol, were 3-4x higher in the control treatments, and decreased significantly for basil grown under supplemental lighting treatments. Every compound evaluated showed significant differences across lighting treatments and growing seasons. The results of this study show that supplemental narrow-wavelength light treatments from LED sources may be used to manipulate plant development and secondary
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CHAPTER 1: INTRODUCTION
Introduction

Supplemental lighting systems play a vital role in commercial greenhouse production. The use of light-emitting diodes (LEDs) in the horticulture industry is rapidly increasing due to continued technological advancements, increased light spectral control, improved energy efficiency, and reduction in manufacturing costs. For these reasons, research is needed to determine the efficacy of LED lights in comparison to traditional lighting systems. Early lighting sources used in horticultural applications, such as incandescent, high pressure sodium (HPS), and fluorescent, were adapted from outside industries and were not designed with the intention of growing plants. In comparison to LEDs, many of these traditional lighting systems have serious limitations and prove costly in long-term applications. Unusable wavelengths and poor energy conversion efficiencies result in wasteful energy consumption and produce vast amounts of radiant heat. In some cases, excess heat production necessitates sufficient space between the crop and lighting source to prevent crop damage. Special considerations must also be taken to dissipate the extra heat energy produced by these fixtures and ensure environmental control systems have the ability to maintain optimal growing temperatures. In addition, traditional lighting systems have limited spectral control and discharge a wide range of wavelengths that may not be useful for photosynthesis or other metabolic processes. Some UV and IR wavelengths may even harm crops under intense or prolonged exposure. Inability to manipulate the intensity of specific wavelengths and control photosynthetic photon flux density (PPFD) is another concern for traditional systems. Many HPS blubs and other types of traditional lighting cannot be easily dimmed without special design considerations and use large amounts of energy in the form of unusable wavebands and radiant heat. Traditional lighting fixtures are usually bulky, take up a lot of greenhouse space, and may reduce light intensity at the canopy level from shading effects. Additionally, these lighting
systems can become costly to operate and maintain when considering the short average lifespan of traditional bulbs and photosynthetically active radiation (PAR) output in relation to the high levels of energy they consume. Despite these limitations, HPS supplemental lighting has prevailed in the horticulture industry because of its relatively low initial cost, intense PPFD, and lack of superior alternatives. As the technology behind LED lighting systems continues to advance, commercial growers would greatly benefit from research focused on determining the efficacy of various supplemental lighting systems and which type is optimal for generalized greenhouse crop production. A comprehensive economic comparison of supplemental lighting systems among a variety of greenhouse crops would greatly benefit growers with year-round operations who wish to maintain biomass yield and sensory quality during winter months. While general information about energy consumption, energy transformation efficiency, maintenance/bulb lifespan, purchase and operational costs, and net profit is necessary to make an informed supplemental lighting purchase; the intended reasons for supplementation, individual crop requirements, and pros/cons of each lighting source should be carefully considered. Optimizing crop quality/yield and increasing profit margins are primary concerns for commercial growers; however, depending on the specific criteria that distinguishes overall quality and utilization for each crop, a substantial increase in flavor volatile content, carotenoid bioaccumulation, or nutritional value may overshadow other factors such as biomass yield. Other impacts of specific narrow-band wavelengths should also be considered in relation to beneficial physiological and morphological responses directly related to the spectral quality and PAR output of each fixture type.

Changes in overall nutritional composition/uptake should be evaluated in regard to LED supplemental lighting systems and various blue/red narrow-band wavelengths, as numerous published sources have established that the spectral quality of supplemental lighting impacts the
nutritional uptake, vigor, and sensory quality of various fruits and vegetables (Bian et al., 2015; Colquhoun et al., 2013; Fujiwara et al., 2006; Janick et al., 2015; Kopsell et al., 2011; Kopsell et al., 2014; Kopsell et al., 2015, 2017; Lefsrud et al., 2006; Li et al., 2009; Morrow, 2008; Randall et al., 2014b; Tarchoune et al., 2013; Tennessen et al., 1994; Tuan et al., 2013). Since growers now have a diverse range of supplemental LEDs with adjustable spectral qualities and intensities, understanding the various effects of this evolving technology is essential when making an informed purchasing decision. In addition to overall nutritional composition, contemporary research suggests that using specific light frequencies can impact the production of volatile organic compounds (VOCs) in a variety of fruits and flowers, as well as other secondary metabolites, flavors, aromas, and sensory-specific compounds (Barbieri et al., 2004; Colina-Coca et al., 2013; Colquhoun et al., 2013; Hu et al., 2014; Junior et al., 2011; Kim et al., 2011; Lee et al., 2005; Ochiai et al., 2014; Pripdeevech et al., 2011; Selli et al., 2014; Shumskaya et al., 2013; Tarchoune et al., 2013).

On a broad scale, this technology has the potential to improve commercial greenhouse production practices and help growers maximize profits. This specific combination of beneficial characteristics makes LED lighting systems ideal for a variety of commercial greenhouse production operations, specifically high-value greenhouse crops. For these reasons, the overall efficacy of traditional lighting systems in horticultural applications should be reviewed in comparison to modern LED lighting systems for a variety of greenhouse production schemes. Commercial producers have long sought to improve their horticultural practices from cutting costs, reducing waste, improving efficiency, and increasing overall profits. Improving modern lighting systems and application regimes will enable growers to make informed decisions when purchasing and using supplemental lights. Improving conventional horticultural practices will not only
increase profit margins for large commercial producers, but may also create the opportunity to improve the overall product quality and allow the consumer to pay less for higher quality.

One potential use for narrow-banded LED supplemental lighting is large scale greenhouse production of high-value specialty crops. The demand for fresh culinary herbs has grown tremendously over the past few decades, as many professional chefs, restaurants, and consumers want the freshest possible product for cooking and consumption. Many consumers adhere to the idea that ‘freshness’ denotes ‘quality’ when purchasing herbs and other produce. Culinary herbs are defined as herbaceous aromatic plants, which are grown and sold either fresh (e.g., cut herbs, potted plants, pastes,) or dried and packaged (Simon, 1990). Herbs are also sold wholesale for processing as ingredients in packaged and processed foods (Treadwell et al., 2007). Manufacturers, commercial kitchens, restaurants, and even high-end grocery stores desire high quality and fresh ingredients to meet this consumer demand, rather than relying on inferior dried products or extracts (Succop et al., 2004). By improving the quality of herbs being produced, growers will potentially save money, reduce wasted product, and increase net revenue while selling higher quality herbs at a lower cost to the customer.

Sweet basil (*Ocimum basilicum*) is a specific culinary herb that stands out in terms of consumer demand and potential profit. Highly valued around the world, greenhouse operations producing this crop have excellent potential for sustainable growth, expansion, and profitability (Treadwell et al., 2007). Basil is usually sold fresh for culinary uses, but it may also be easily stored by freezing, air-drying, freeze-drying, extracting essential oils, or through the manufacture of basil-related products (Raimondi et al., 2006a). While this herb stores well after drying and under refrigeration, consumers have become increasingly interested in the original source and quality of the food they consume. Top chefs and regular consumers are seeking fresher products
with high quality and best flavor. Bottom line, consumer perception of freshness is significant to overall preference, which impacts market value and the volume of product sold.

Large-scale commercial greenhouses are inclined to produce plants year-round so that they can limit downtime while continuing to acquire income from sales and maintain competitiveness in the marketplace during unfavorable seasons or periods of low demand. In some locations or unique growing situations, supplemental lighting may be required to produce quality crops during winter months through the management of lighting schedules and photoperiods. In other situations, increasing the daily light integral (DLI) and optimizing spectral quality has the potential to improve crop quality and yield.

LED lighting systems have the potential to replace traditional lighting systems in a wide range of applications while helping growers reduce costs and maintain/improve quality throughout all growing seasons. Recent research suggests that modern LED lighting systems, particularly systems providing optimized ratios of blue/red wavelengths, have a positive impact on volatile production, net biomass yield, carotenoid production, and nutritional content in basil as well as other specialty crops and high value herbs (Abney et al., 2013; Kopsell et al., 2011; Kopsell et al., 2012; Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017). For these reasons, it is advantageous to further explore the possibilities of LED lighting systems and determine how different wavelengths impact plants at a fundamental level. The horticultural application of LEDs should be researched further to establish the efficacy of using these types of systems in comparison to traditional lighting systems.

The primary focus of this project was to determine the impact of specific narrow-band B/R wavelengths (447 nm and 627 nm) from solid-state LED lighting systems on key flavor volatiles, nutrient uptake, and biomass accumulation of greenhouse hydroponic basil. The first phase of this
project investigated B/R ratios in comparison to natural light and HPS supplementation, while the second phase determined the impact of incremental daily light integral (DLI) supplements.
References A


CHAPTER 2: LITERATURE REVIEW
Basil Background

Sweet Basil belongs to the Plantae Kingdom, Magnoliophyta Phylum, Magnoliopsida Class, Lamiales Order, Lamiaceae Family, Ocimum Genus, and Basilicum Species (Deschamps et al., 2006; Klimánková et al., 2008). The genus Ocimum has more than 30 species and naturally occurs in tropical and subtropical regions (Deschamps et al., 2006). Sweet basil is an annual flowering plant that grows approximately 20-60 cm in height. Originating from India and other regions of Asia, this plant contains distinctive essential oils and has been used both fresh and dried in culinary dishes (Klimánková et al., 2008; Lee et al., 2005). Fresh basil has significant demand from both regular consumers and commercial kitchens across the globe and provides a variety of positive health benefits when consumed (Kopsell et al., 2005; Soran et al., 2009). For example, restaurants in the Southern California area commonly list basil as their most used herb and find it difficult to maintain a steady supply (Succop et al., 2004).

Basil has been used largely as a flavoring agent or spice and is cultivated globally (Claudia, 2013b). This herb has been used for a variety of purposes throughout human history, including direct consumption, cooking, and even in the manufacture of products such as essential oils, soaps, perfumes, medicines, etc. (Raimondi et al., 2006a; Succop et al., 2004). Essential oils are the most valuable commercial forms of basil and contribute flavors and aromas to a variety of products in the food and cosmetic industries (Barbieri et al., 2004; Kopsell et al., 2005). Basil produced for dried leaf markets and fresh markets rank second and third, respectively (Putievsky et al., 1999). The basil market has seen a recent shift in taste preference from Italian large leaf varieties to flavored varieties because of their unique aromas and flavor profiles, and basil consumption in the U.S. has increased about 8-fold during the period 1960 to 1996 (Klimánková et al., 2008; Sharafzadeh et al., 2011).
Large morphological and biochemical differences exist naturally among different basil cultivars due to genetic variation. Sweet basil has been broadly classified into seven different morphotypes, which include: 1) tall, slender types; 2) ‘Italian’ large-leaved types; 3) dwarf types (‘bush’ or ‘spicy globe basils’); 4) compact types (‘thai’ basils); 5.) purple types (‘purple petra’); 6) purpurascens types (‘dark opal,’ sweet purple basils); and 7) citriodorum types (flavored types) (Hussain et al., 2008). Flavor profiles, medicinal uses, customer preference, and demand/popularity vary greatly among different morphotypes (Deschamps et al., 2006; Klimánková et al., 2008; Tarchoune et al., 2013).

Traditionally, basil has been cultivated in open fields and may be subject to unfavorable environmental conditions. Harsh weather and extreme temperatures have a negative impact on basil production, specifically on the biomass yield and quality of the final product. Greenhouse hydroponic basil production provides optimal climate and fertility conditions, which has the potential to reduce variability in plant growth and development due to seasonal changes (Claudia, 2013b; Kopsell et al., 2005).

**Plant/Light Interaction**

The intensity of a light source, its duration, direction, and even spectral quality all have direct impacts on the growth of a plant (Colquhoun et al., 2013). Even though many primary and secondary metabolic pathways have been established for a variety of plant species, some metabolic pathway information is missing/unconfirmed, and the impact of specific wavelengths on secondary metabolism has not been thoroughly explored (Colquhoun et al., 2013; Shumskaya et al., 2013). In addition, the interactions between primary and secondary metabolism in response to variations
in spectral quality have not been evaluated. Further investigation of these pathway interactions may result in antagonistic or synergetic effects on a variety of important secondary products.

Almost all plants require light throughout their entire lifecycle from germination to flowering and finally seed production (Barta et al., 1992; Mccree, 1973). Plants are capable of responding to their biotic and abiotic surroundings and adapting their morphology and physiology to promote optimal growth and development (Ouzounis et al., 2015b). Light is one of the most important environmental factors and plays a major role in plant development and physiology (Bourgaud et al., 2001). Since plants are unable to move, they depend on environmental adaptability mechanisms and have diverse responses to changes in fluence rate, spectral quality, direction, and duration (Christie, 2007; Singh et al., 2015). When considering supplemental lighting systems for horticultural applications, three of the most important factors include light quantity, quality, and duration (Barta et al., 1992).

Photosynthetically active radiation (PAR) is known as the portion of the light spectrum that is most active in photosynthesis (Mccree, 1973). It ranges from 400-700 nm and is closely correlated with the visible spectrum of the human eye (Barta et al., 1992). The overall quantity of light that is provided within the PAR region is suitable for photosynthesis and primary metabolism. Light quality refers to the spectral distribution of the supplied radiation and what wavebands are produced by the lighting source. Plants have a variety of photoreceptors that accept specific wavelengths and impact biochemistry, plant shape, node length, overall development, and flowering (Goins et al., 1997). Wavelengths that do not fall within the PAR region are not particularly useful for plant photosynthesis and primary metabolism. For example, the addition of narrow-band wavelengths, such as the addition of ultraviolet (UV)-A (320-380 nm) and UV-B (280-320 nm) radiation, has been shown to influence the production and accumulation of important
secondary plant compounds used to combat light stress (Abney et al., 2013). As expected, harsh UV exposure of any type negatively impacts plant biomass in most cases (Abney et al., 2013). Infra-red (IR) wavelengths are converted into heat energy and are not useful for photosynthesis or primary/secondary metabolism (Singh et al., 2015). Light duration or photoperiod is the amount of time a crop receives light within a 24h cycle. This light may be of minimal intensity, and spectral quality is usually not a factor when supplementing photoperiods (except in some cases such as far-red wavelengths, initiating flowering in some crops, etc.). Light duration should not be confused with DLI, or the amount of light energy that is provided over a given period of time. Photoperiod primarily impacts reproductive growth and development, flowering, some other metabolic processes (Singh et al., 2015). It has been well documented that plants do not absorb all wavelengths at the same rate, and that the addition of abiotic stressors (such as light) has the potential to impact secondary metabolite production (Kopsell et al., 2005; Lichtenthaler, 1987; Mccree, 1973; Takano et al., 1996; Yeum et al., 2002; Young, 1991). Plant tissues contain a variety of pigments and photoactive compounds that require energy from specific wavebands to function (Goins et al., 1997). Fully understanding the mechanisms behind these photoreceptors, the wavebands they accept, and the impacts they have on plant development and physiology is crucial for establishing optimal supplemental LED lighting procedures with emphasis on spectral quality and improving secondary metabolite production.

The solar radiation spectrum that has the most direct impact on plant growth and development includes three parts: UV light, visible (VS) light, and IR light (Mccree, 1973). Ultraviolet-C radiation includes 200-280 nm and is harmful to plants; however, UV-C is mostly blocked by the atmosphere, and most radiation of this type does not reach the earth’s surface. Lighting sources that produce high levels UV-C radiation may be harmful to plants/animals and
should be avoided. Major industrial use for UV-C supplementation includes sterilization and disinfection processes. Ultraviolet-B radiation includes 280-315 nm and is only harmful to plants under intense applications. UV-B exposure has the potential to increase secondary metabolite production, in addition to pigment bleaching and degradation of flavor volatiles/carotenoids/other secondary metabolites at intense levels. Ultraviolet-A radiation includes 315-380 nm and has controversial impacts on plant morphology and secondary metabolite production. The range of 380-400 nm contains the transition from UV-A to the visible light spectrum. Chlorophyll and carotenoid pigments begin to absorb light within this range. Wavelengths ranging from 400-520 nm contains violet, blue, and green light. Chlorophyll pigments obtain peak energy absorption at these wavelengths and strongly influence vegetative growth and development. The range of 520-610 nm contains green, yellow, and orange wavelengths, which has limited influence on vegetative growth and development. Some studies have found that special wavebands within this range have specific impacts on plant development and secondary metabolite production, but additional research should be conducted to determine practical uses for specific wavebands. The range of 610-720 nm contains red wavelengths, and high levels of absorption occur at this range. These wavelengths strongly influence vegetative growth, photosynthesis, and reproductive growth. The range of 720-1000 nm contain far-red and infra-red wavelengths that impact germination and flowering. Wavelengths above 1000 nm contain IR radiation, which is primarily converted into heat energy. These wavebands are not particularly useful to plants for primary or secondary metabolism and are not used for photosynthesis (Abney et al., 2013; Barta et al., 1992; Briggs et al., 1999; Goins et al., 1997; Kopsell et al., 2014; Lucchesi et al., 2004; Mccree, 1973; Morrow, 2008; Tuan et al., 2013).
Advancements in LED lighting technologies have provided researchers the tools necessary to investigate individual narrow-band wavelengths and determine how plants interact with light at a fundamental level. Attempts to use colored filters on traditional lighting systems gave some insight into the impact of spectral quality on plant growth, but supplementation of specific narrow wavebands was not truly possible before LED technology was introduced. This provides an excellent opportunity to experiment with different spectral compositions to optimize plant growth and development (Singh et al., 2015). Manipulating the spectral quality of supplemental lighting may prove beneficial for growers because it would allow them to better control their crop’s physiology, morphology, and biochemistry (Caldwell et al., 2006; Kopsell et al., 2017). Some research has been published comparing and contrasting HPS and LED supplemental lighting for a variety of greenhouse production crops (Abney et al., 2013; Bian et al., 2015; Hogewoning et al., 2010b; Janick et al., 2015; Kopsell et al., 2011; Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017; Lin et al., 2013; Ouzounis et al., 2015a; Ouzounis et al., 2015b; Pimputkar et al., 2009; Randall et al., 2014a; Randall et al., 2014b; Singh et al., 2015; Tennessen et al., 1994; Tuan et al., 2013).

**Fundamentals of Secondary Metabolites**

Secondary metabolites are commonly defined organic compounds that do not directly contribute to the regular growth, development, or reproduction of a plant; however, many secondary metabolites produced in plants play an important role in environmental adaptation, overall plant fitness/vigor, and defense against biotic and abiotic stressors (Bourgaud et al., 2001). These compounds are usually classified by their biosynthetic pathway and chemical structure (Bourgaud et al., 2001). Favorable genetic traits (i.e. the ability to synthesize secondary
metabolites beneficial to survival and reproduction) have been selected through the course of evolution. Examples include fragrant volatiles and colorful pigments that attract pollinators to improve fertilization chances, metabolic compounds that alleviate or negate abiotic or biotic stressors, flavors and sugars that attract animals to improve odds of seed dispersion, and the ability to synthesize unpleasant or toxic compounds that ward off pathogens, physical damage, consumption, and/or suppress the growth of neighboring plants (Colina-Coca et al., 2013; Colquhoun et al., 2013; Junior et al., 2011; Lee et al., 2005; Pripdeevech et al., 2011; Selli et al., 2014; Tarchoune et al., 2013).

Three major secondary compound classes include phenolics, terpenoids, and alkaloids; other subclasses include specialty molecules like polyketides, fatty acids, amino acids, enzymes, carbohydrates. (Bourgaud et al., 2001). The discipline of plant chemotaxonomy has rapidly developed due to varying concentrations and prevalence of compounds within each chemical class (Shumskaya et al., 2013). Genetic lineage and botanical nomenclature are important factors when considering the three major classes of secondary metabolites, in addition to many other factors. Phenolics are common among all higher plants, for example, as they are involved with many defense mechanisms and synthesis of lignin, flavonoids, anthocyanins, tannins, etc. (Bourgaud et al., 2001; Galeotti et al., 2008). In contrast, alkaloid compounds are more narrowly distributed in the plant kingdom and are usually more species specific. Terpenoids are a diverse group of hydrocarbon-based natural products derived from isoprene units. These compounds have special physical/chemical properties in addition to low boiling points and high volatilities. In plants, terpenoids play a variety of roles such as plant hormone synthesis, attraction of pollinators, and defense against pathogens/herbivores (Bourgaud et al., 2001). Secondary metabolism is directly
linked to primary metabolism (Ashraf et al., 2013; Briggs et al., 1999; Chaves et al., 2011; Frank et al., 1996; Lichtenthaler, 1987; Young, 1991).

Many compounds from each of these metabolic classes have significant economic value because of their high biological activities in addition to low concentrations in plant material (Bourgaud et al., 2001; Frank et al., 1996). Humans have taken advantage of plant secondary metabolites for centuries, using them as healing medicines, psychotropics, flavorings, and poisons. These compounds still see common use in modern pharmaceuticals and holistic therapies (Briggs et al., 1999; Frank et al., 1996; Sandmann, 2001). In some regions such as China and India, plants have formed the foundation of sophisticated traditional medical systems practiced for thousands of years. Many pharmaceuticals in the U.S. are produced from natural products, and many other synthetic products are structural modifications of natural products (Bourgaud et al., 2001; Mares-Perlman et al., 2002; Mcquistan et al., 2012; Sugimoto et al., 2012; Yeum et al., 2002). Fransworth (1990) claims that 119 characterized drugs are still obtained commercially from higher plants, and that over 74% were found from ethnobotanical sources. Harmful/beneficial bioactivity in humans as well as acute sensitivity and potential long-term impacts should be considered in respect to this project, with emphasis on secondary plant compounds that have proven beneficial to human health and vitality.

The bioaccumulation and stability of these compounds, as well as their relative concentrations in fresh plant material determine the overall sensory experience during human consumption. Volatile flavor and aroma compounds dissipate in response to many biotic and abiotic stress factors, such as physical damage or heat energy (Barbieri et al., 2004). Extra heat energy produced by traditional lighting systems may exacerbate this phenomenon, increasing the loss of important flavor and aroma volatiles. In contrast, supplementing with specific wavelengths
from LED lighting systems can increase overall bioaccumulation of flavor volatiles by targeting photoreceptors and upregulating specific metabolic pathways as well as reducing the volatilization of key flavor and aroma compounds (Janick et al., 2015).

Plants perceive their light environment with a variety of protein pigments called photoreceptors (Fraikin et al., 2013). Various photoactive compounds, mechanisms, and metabolic pathways have been documented across many plant species. Specific wavelengths produced by LED lighting systems match the absorption spectra of key photoreceptors and can be used as supplemental lighting to facilitate growth during vegetative and reproductive stages (Randall et al., 2014b). For example, ultraviolet and blue wavelengths (365-470 nm) are absorbed by cryptochrome pigments, which impact plant morphology and high-level physiological processes (Chaves et al., 2011). Red light (620-735 nm) closely aligns with the maximum absorption for chlorophyll and matches the absorption peaks of various phytochemicals and secondary pigments (Goins et al., 1997; Pimputkar et al., 2009). Literature also suggests that the interaction between cryptochromes/phytochromes/other accessory pigments and various wavelengths are not fully understood and should be researched further (Briggs et al., 1999; Chaves et al., 2011; Fraikin et al., 2013; Goins et al., 1997; Morrow, 2008; Pimputkar et al., 2009; Tennessen et al., 1994). Other unidentified photoreceptors may be influenced by the addition of these wavelengths. Wide ranging narrow-band wavelengths should also be further investigated to determine potential impacts across many plant species, in addition to full spectrum light sources. This will provide necessary information to develop effective ‘light recipes’ that can be added for optimal growth across a variety of plant species in unfavorable growing seasons. Conflicting results have been found in relation to the suggested ratios, intensities, and DLI of narrow-band blue/red wavelengths for
optimal plant growth and secondary metabolite production, indicating a need to further investigate the interaction between supplemental lighting and plant physiology and morphology.

**Carotenoids and Chlorophyll Pigments**

Carotenoids are organic pigments that are produced by plants and algae. Over 600 carotenoids are currently known, and they are split into two classes: oxygen containing xanthophylls and oxygen lacking carotenes (Young, 1991). They belong to the category of tetraterpenoids, meaning they contain 40 carbon atoms and are built from four individual terpene units (Lichtenthaler, 1987). Their physical structure is responsible for their chemical properties and human health benefits. Their physical structure is also responsible for their color, which ranges from pale yellow, bright orange, and deep red; in general, these compounds most effectively absorb wavelengths ranging from 400-550 nm (Frank et al., 1996; Lichtenthaler, 1987; Young, 1991). These compounds are dominant pigments in a variety of plant species and serve many roles in photosynthesis and secondary metabolism. Their color is commonly masked by chlorophyll in photosynthetic tissues and may become visible in reproductive tissues such as fruits, flowers, and even tubers (Bartley, 1995). The carotenoid biosynthetic pathway serves important roles in plant biochemistry, such as photosynthesis, photoprotection, plant development, and even stress hormones. There are at least five different roles carotenoids are thought to play in photosynthesis specifically, which include light harvesting, photoprotection via triplet state chlorophyll quenching, singlet oxygen scavenging, excess energy dissipation, and structure stabilization/assembly (Frank et al., 1996). Many products of the carotenoid pathway have large biological activities and provide a variety of human health benefits (Shumskaya et al., 2013). These compounds show high antioxidant capacities and have been shown to provide positive health
benefits in humans (Kopsell et al., 2005; Kopsell et al., 2007; Lefsrud et al., 2006). Carotenoid derivatives also are active in developmental signaling and responses to abiotic/biotic stress (Croce et al., 1999).

Carotenoid pathway products usually have vivid colors and fragrant aromas, which provide a role in plant-plant signaling, mediate plant-animal interaction, and impact flavor and nutritional characteristics of food crops (Shumskaya et al., 2013). Carotenoids are primary localized on membranes of plastids, which are known to be the site of carotenoid biosynthesis (Kopsell et al., 2005; Yeum et al., 2002). Plastids are dynamic organelles that have constantly changing chemical and structural makeups, and carotenoid biosynthesis is highly dependent on these factors (Frank et al., 1996; Young, 1991). Synthesis location and plastid composition determine the destiny of metabolic products (e.g. carotenoids produced on thylakoid membranes and then used for structural components involved with photosynthesis, photoprotection, quenching, etc. in comparison to carotenoids on the envelope converting to apocarotenoids used in mediating signaling, etc.) (Shumskaya et al., 2013). Biological roles and synthesis locations of the metabolic products produced by carotenoid pathways should be further researched to determine practical techniques to enhance nutritionally important carotenoids in edible tissues. In addition, predictable control of carotenoid biosynthetic pathways will require full understanding of the various contributions each chemical component makes to overall physiology (Shumskaya et al., 2013).

PAR wavelengths are mostly absorbed by leaf tissues, and the majority of light absorption by chlorophyll pigments and quantum yield of photosynthesis occur primarily in the blue and red regions of the visible light spectrum (Kopsell et al., 2014; Morrow, 2008; Pimputkar et al., 2009). Many auxiliary pigments exist within the plant tissues and serve a variety of functions such as light harvesting and quenching, photoprotection, etc. Phytochromes are mostly red light photoreceptors
that control physiological responses via red and far-red wavelengths (Chaves et al., 2011). Cryptochromes and phototropins are both blue light receptors (Briggs et al., 2002). Cryptochromes act as signaling mechanisms that regulate circadian rhythms and prompt many physiological and morphological changes; phototropins control chloroplast movements to maximize absorption of specific wavelengths (Chaves et al., 2011; Christie, 2007; Kopsell et al., 2014).

The absorption of light by chlorophyll initiates the first steps of photosynthesis, and the photosynthetic apparatus has the ability to react to changes in environmental stimuli and light intensity/spectra (Ashraf et al., 2013; Frank et al., 1996; Goins et al., 1997). During oversaturation conditions, extra light energy must be diverted to prevent damage to vital plant systems. Carotenoid molecules are composed of long polycarbon chains which help protect the photosynthetic apparatus by quenching high energy free radicals and dissipating excess thermal energy (Croce et al., 1999).

The xanthophyll cycle is an energy reduction mechanism responsible for regulating available light energy for use in photosynthesis. When exposed to intense light, violaxanthin is rapidly and reversibly converted to zeaxanthin via antheraxanthin. Zeaxanthin can prevent photooxidative stress/lipid peroxidation and directly quench chlorophyll-excited triplet states (Croce et al., 1999). Xanthophyll carotenoid pigments, specifically zeaxanthin, can modulate blue light-dependent responses in plant and is believed to be an important photoreceptor for blue-light activated plant responses (Briggs et al., 1999).

Current literature suggests that greenhouse production with supplemental lighting would be most suited for optimization of carotenoid bioaccumulation in a variety of basil cultivars (Demmig-Adams et al., 1996; Hogewoning et al., 2010b; Kopsell et al., 2005; Kopsell et al., 2017; Lefsrud et al., 2006; Morrow, 2008; Pimputkar et al., 2009; Singh et al., 2015). Leafy specialty-
crops contain high levels of nutritionally beneficial carotenoids (Kopsell et al., 2014; Kopsell et al., 2017; Selli et al., 2014; Tyson et al., 1999). While sweet basil has the potential to accumulate high levels of nutritionally important carotenoids in field settings, identifying basil cultivars with naturally high phytonutrient levels and supplementing them with LED light in optimized greenhouse environments may have considerable human health implications.

**Volatile Organic Compounds (VOCs)**

Volatile Organic Compounds (VOCs) are defined as organic molecules that have high vapor pressure at ordinary room temperatures. Low boiling points, chemical structure, and other physical properties determine the high vapor pressures of volatile compounds (Taylor, 1996). These compounds include both naturally occurring and man-made compounds. Most scents, flavors, odors, and aromas are VOCs. While these compounds serve many uses in natural ecosystems and provide a wide range of biological impacts in minute concentrations, they may be detrimental to human health in high concentrations. However, it is difficult to ingest harmful quantities of these substances in nature because of their volatility and instability (Hu et al., 2014). The majority of VOCs found in nature are produced by plants, with isoprene being the base unit. These compounds play an important role in plant signaling, defense, pollinator attraction, etc. (Kesselmeier et al., 1999). Stabilization, volatilization, and plant-controlled emission of these compounds are directly impacted by many abiotic and biotic factors such as temperature, sunlight, turgor pressure, stomatal opening, humidity, pest and pathogen pressures, etc. (Kesselmeier et al., 1999; Klimánková et al., 2008). Emission occurs almost exclusively at the leaf’s surface through open stomata, but certain defense compounds may be released if physical damage occurs (Yousif et al., 1999). Aromatic and flavor compounds usually have low molecular weights (<300 Daltons),

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which directly contributes to their volatility along with chemical structure and high vapor pressures. Flavors affect both taste and smell, whereas fragrances (aroma) only affect smell (Fahlbusch et al., 2003).

The terms flavor and aroma tend to denote naturally occurring compounds, while fragrance typically refers to synthetic compounds (Fahlbusch et al., 2003). Some studies have been performed on basil flavor volatiles, and substantial volatile profiles have been established using a variety of analytical techniques (Bantis et al., 2016; Barbieri et al., 2004; Chalchat et al., 2008; Charles et al., 1990; Deschamps et al., 2006; Hussain et al., 2008; Klimánková et al., 2008; Kopsell et al., 2005; Olfati et al., 2012; Politeo et al., 2007; Raimondi et al., 2006b; Tarchoune et al., 2013).

However, there is limited literature that compares the efficacy of blue/red supplemental LED lighting and HPS lighting with key flavor volatiles and commercially popular basil cultivars. Since many of these compounds are directly related to the flavor and aroma of plant products, parallel sensory panels are recommended to determine impacts to human perception, bioactivity, and sensory preference, and how that relates to the manipulation of secondary metabolism in high-value specialty crops.

**Essential Oils**

Essential oils have been greatly valued by humans throughout civilization and used to produce a variety of products such as fragrances, medicines, dyes, and seasonings. Most essential oils are comprised of a complex mixture of volatiles and lipids (Deschamps et al., 2006). In most cases, these VOCs exist in relatively small concentrations in comparison to the total weight of plant material and can be difficult to extract/analyze because of their volatile nature and rapid degradation (Lucchesi et al., 2004). Even though these compounds represent a very small fraction
of the plant’s overall composition, they contribute to the majority of the overall aromatic characteristics (Klimánková et al., 2008).

Different classes of chemical compounds that contribute to aroma and flavor (aromatics, terpenes, terpentes, sesquiterpenes, alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetalses, ethers, esters, etc.) vary in concentration, many of which are found in trace amounts (Pourmortazavi et al., 2007; Reverchon et al., 2006). These essential oils are usually produced in specialized structures called granular trichromes, but may be found throughout various plant parts and can be extracted from seeds, roots, flowers, petioles, stems, and leaves (Reverchon et al., 2006). In addition, plants usually partition their essential oils anatomically rather than having an equal concentration throughout the entire plant (Deschamps et al., 2006). The presence of essential oils and VOCs, as well as their chemical composition and concentrations, are directly related to the specific flavor and aroma differences among various plant parts (Klimánková et al., 2008). Changes to environmental conditions, as well as the gene expression of each cultivar can greatly impact the chemical composition of essential oils, which also impacts human sensory perception when consuming herbs or extracts (i.e. flavor and aroma) (Klimánková et al., 2008). Many common herbs and other high-value specialty crops, including sage, basil, oregano, and thyme are processed to obtain valuable essential oil products and have been associated with high antioxidant activity/human health benefits (Raimondi et al., 2006a).

**VOCs Found in Basil**

In terms of VOCs present in basil, oxygenated VOC compounds are highly aromatic and comprise the majority of fragrance as well as taste. In addition, mono-terpene hydrocarbons do not contribute much to the fragrant quality of basil’s essential oil. (Lucchesi et al., 2004) Using
headspace and solid-phase micro extraction (SPME) techniques, a group of researchers determined that linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate were the most dominant VOCs found across five separate cultivars of basil (Klimánková et al., 2008). Various research groups using various analytical methods and extraction techniques (GC-FID, GC-MS, LC-MS, HPLC, etc. combined with expeller press extraction, solvent extraction, hydrodistillation, steam distillation, low-pressure microwave extraction, simultaneous distillation-extraction (SDE) etc.) have identified at least two hundred separate volatile compounds across many basil varieties, many of which have high biological activities with strong flavors and aromas (Bantis et al., 2016; Deschamps et al., 2006; Lee et al., 2005; Soran et al., 2009; Tarchoune et al., 2013). Further research should be conducted on specific basil cultivars that have high market value to determine their overall chemical composition and flavor profiles. In addition, concentration changes in key flavor volatiles (both positive and negative) should be evaluated in comparison to human sensory experience.

While many of the pertinent flavor and aroma compounds have been identified through GC-MS analysis, little information is known about relative basil VOC concentrations in relation human bioactivity (i.e. the perception of flavor and aroma) in conjunction with LED supplemental lighting. Compounds that are important to favorable sensory perception of basil should be further investigated to determine the sensory impact of changing flavor volatile compositions through the manipulation of environmental conditions and addition of other abiotic stressors. In addition, compounds that humans perceive bitter or unfavorable should also be researched to determine how changes in overall volatile concentrations impact human sensory perception and overall flavor preference in basil. That being said, an increase in secondary metabolite production does not always result in better flavor, higher sensory quality, or improved health benefits.
Volatile Extraction Techniques

Extraction of VOC compounds, as well as essential oils from plant material can be achieved through a variety of separation techniques. Complete understanding of analytical separation methods is required for proper and thorough flavor volatile analysis via GC-MS headspace analysis (Deschamps et al., 2006; Klimánková et al., 2008; Tarchoune et al., 2013) and parallel sensory panel analysis. Even though many techniques have been developed for extracting essential oils and VOCs from plant material, only a few of these techniques are viable for laboratory research, and others on a commercial scale. When determining the most effective method for volatile extraction and analysis, many factors must be considered.

The essential oils are located within the plant tissues and structures, so mass transfer resistances and extraction efficiencies must be considered when extracting VOCs from plant tissue (Reverchon et al., 2006). In addition, paraffin contaminants and fatty acids must be considered when extracting essential oils since they may be soluble within the solution; further purification and reduction is necessary to ensure that only the desired compounds are isolated (Reverchon et al., 2006). Additional fractionations during the extraction process may be used to precipitate out undesirable waxes and fatty acids. By adjusting the pressure and temperature stages of each extraction, it is possible to remove the majority of the undesirable compounds; however, antioxidants, colors, and other compounds with similar physical properties to the solvent may remain soluble throughout the extraction process, causing a complex essential oil mixture which has many applications (Deschamps et al., 2006; Klimánková et al., 2008; Reverchon et al., 2006; Tarchoune et al., 2013). If higher quality oil is desired, the impurities may be filtered out through additional processing and separation techniques.

In comparison to the total composition of a variety of plant materials, volatiles are found
in relatively low concentrations. Lucchesi et al. (2004) found essential oil content comprised only 0.029% of the total 500 g weight of their processed basil samples. Another study found that hydrodistilled essential oil ranged from 0.5-0.8% of fresh mass across seasons and environmental variations (Hussain et al., 2008). This varying range in distillation type and analysis method necessitates further investigation that is not covered in current literature. Slightly higher concentrations (i.e. the true average VOC concentration in fresh plant material) must be considered when observing data reported in literature as well as this project for two reasons: a complete mass-transfer of the essential oil to the solvent is necessary for a true representative sample of the mean concentration, and 100% extraction efficiency is theoretically impossible, in addition to compound degradation and escape throughout processing and storage periods (Reverchon et al., 2006). Water content changes that result from storage or drying techniques should be considered, since volatile extraction quality and quantity is diminished when using material that has excess water or dried using high temperatures (Barbieri et al., 2004; Deschamps et al., 2006; Klimánková et al., 2008; Lucchesi et al., 2004; Yousif et al., 1999). It is also necessary to account for variation of VOC and essential oil concentrations throughout different parts of each basil plant, since the leaf material is noted to contain most of the essential oil compared to the other parts of the plant (Hussain et al., 2008). Since basil leaf tissue is primarily used for consumption and essential oil extraction, focus will be placed on analyzing leaf tissue concentrations as opposed to whole-plant concentrations.

As stated before, extraction techniques and solvents also play a large role in the percent yield of essential oil and volatile compounds recovered. Various extraction and separation methods should be investigated to ensure that an accurate and comprehensive VOC profile of hydroponically grown basil is established. Various methods of extraction and analysis will favor specific constituents within the plant material, providing an opportunity to further research
differences between techniques as well as separating specific constituents by combining a variety of techniques in sequence.

**Hydrodistillation**

One of the most popular techniques for extracting essential oil is hydrodistillation, in which large quantities of plant material are placed into a still and boiled for an extended period of time (Reverchon et al., 2006). In terms of quality control and analytical testing, this technique is considered the official standard method for flavor and essential oil extraction (Deschamps et al., 2006; Soran et al., 2009; Tarchoune et al., 2013). In addition, this technique is the most commonly used commercial extraction method for essential oils. Crude methods of hydrodistillation were performed in ancient Europe and Africa, but recent advances in technology have made this process feasible on an industrial scale using extremely accurate temperature and pressure control. During hydrodistillation, or specifically the Clevenger distillation method, plant material is submerged in boiling water. Various components of the essential oil mixture are extracted from the plant material, forming an azeotropic mixture with the boiling water. The steam carries these volatile compounds to the condenser, where the vapor is then cooled and returned to its liquid state. Once the mixture is cooled, the water and essential oil mixture naturally separates. The top organic layer contains essential oil along with other volatile compounds from the plant, as well as some hydrocarbons, fatty acids, esters, terpenes, etc. The aqueous layer contains water and some of the various components that were not soluble in the organic layer after temperature reduction. Such examples include the product of hydrolysis of esters, decomposed organic compounds (specifically VOCs due to heat degradation), polymerization of aldehydes, polar molecules, etc. hydrolysis of certain compounds may also impact the solubility of hydrophilic compounds within the essential
oil, creating impurities that require additional filtering and separation. Using lower pressures and
temperatures reduces the risk of decomposition and hydrolysis, which increases essential oil purity
and effect. Since VOCs are extremely volatile, care must be taken to ensure that the apparatus is
airtight, and any container storing the essential oil is fully sealed from air, humidity, light, and
high temperatures (Barbieri et al., 2004; Bayramoglu et al., 2008; Chalchat et al., 2008; Deschamps
et al., 2006; Du et al., 2015; Fahlbusch et al., 2003; Kesselmeier et al., 1999; Lucchesi et al., 2004;
Pourmortazavi et al., 2007; Reverchon et al., 2006; Soran et al., 2009; Tarchoune et al., 2013).

Even though this technique has been widely studied and very popular for extracting
essential oils, thermal degradation is a major problem since many of the compounds are damaged
or denatured when they encounter intense heat (i.e. 100 °C boiling water) (Reverchon et al., 2006).
Pressure may be increased to enhance the level of essential oil extraction. Some essential oils are
harder to extract from plants than others, as the physical and chemical properties vary greatly
among the constituents of complex essentials oils.

Steam Distillation

Steam distillation is very similar to hydrodistillation with one variation – rather than boiling
the plant material directly, steam passes through a bed of the plant material and the vapor carries
essential oils and other volatile compounds to the condenser, where the mixture is cooled. As the
mixture cools and separates, organic and aqueous layers are formed. This method is more selective
than hydrodistillation, since the material is not actually submerged into the boiling water. In
comparison to boiling water, the water vapor makes less contact with the plant material. In terms
of volume, this method extracts much less essential oil than hydrodistillation, since the water does
not come into direct contact with the plant material. Only compounds with a high level of volatility
will be carried to the condenser, while others are left behind. Other physical properties impact extraction product composition, specifically each chemical’s inherent solubility with water, molecular weight, molecular polarization, boiling point, etc. This makes the method of steam distillation effective in the extraction of highly volatile compounds with low molecular weights and high polarity (Fahlbusch et al., 2003; Junior et al., 2011). This method is suggested when the purity and quality of the essential oil mixture is vital. This method is also suggested when the essential oil has a high probability of degradation or hydrolysis, since the constituents are subjected to less water contact and relatively lower temperatures; however, this method does not completely prevent these chemical processes from occurring. Some impurities may be formed or carried into the condenser during the extraction process, but significantly less chemical interactions are expected when performing this method over hydrodistillation. Steam distillation is extremely selective in comparison to other extraction methods because of the inherent physical properties of steam, and this method is suggested if relatively pure essential oil is desired, especially if additional purification and separation is not viable (Barbieri et al., 2004; Fahlbusch et al., 2003).

**Organic Solvent Extraction**

Organic solvents have the ability to extract and recover larger percentages of essential oils in comparison to other methods. Overall, this method is used most for the pharmaceutical and cosmetic industries, especially when their products are not intended for human ingestion (Fahlbusch et al., 2003; Junior et al., 2011). Usually this method is achieved under ambient pressure, but difficult and/or selective extractions may call for an increase in chamber pressure and use of specific chemicals to facilitate the process. Grinding may or may not be used during the extraction to facilitate selective mass-transfer of important constituents. This type of chemical
extraction method may be advantageous or highly problematic, depending on desired composition of the final product as well as its intended use. In addition to efficiently extracting essential oils and volatile compounds, organic solvents also have the ability to extract a wide variety of plant compounds, such as esters, heavy terpenes, fatty acids, triglycerides, waxes, resins, and even some pigments. Less polar constituents, such as the oleoresins found in herbs, may also be extracted using solvents that match their physical and chemical properties. Examples of frequently used organic solvents are aliphatic hydrocarbons (butane, hexane, THF, etc.), short-chain alcohols (methyl alcohol, ethyl alcohol, isopropyl alcohol, etc.), acetones, and even halogenated hydrocarbons (Bayramoglu et al., 2008; Junior et al., 2011; Reverchon, 1997).

Since a variety of plant compounds are soluble in these organic solvents, care must be taken when selecting which solvent to use for extracting specific essential oils. In comparison to other methods, organic solvent extraction is difficult to control and has poor chemical selectivity. Mixtures of solvents or solvent gradients may be used to improve selectivity of organic solvents, but this technique has poor resolution and separation in comparison to other methods. The choice of solvent(s) greatly impacts the miscibility of each solute; within the complex solution varying physical properties cause interactions between each of the constituents. For this reason, chemical and physical properties of the solvent as well as the potential solute constituents must be considered before selection (Charles et al., 1990; Fahlbusch et al., 2003).

Polarity is an important factor of both the solvent and the intended solutes, as molecules with similar polarities will be soluble with one another. The solvent must also be chemically inert to the desired extraction constituents. Analysis of the final product is an ideal practice to ensure that no side reactions occurred during the extraction process and no contaminants or chemical residues are present in the essential oil mixture (Charles et al., 1990).
The increasing viscosity during extraction may also become problematic. Because of the increased solubility of waxes and other lipids in organic solvents, the mixture becomes increasingly viscous, warranting additional time and special techniques to achieve the same extraction equilibrium (Charles et al., 1990; Fahlbusch et al., 2003; Reverchon, 1997). In addition, multiple extraction sessions may be needed to fully extract all desired constituents. This requires larger volumes of solvent, which may be cost ineffective or difficult to dispose. To decrease the viscosity of an extraction solution, additional separation methods may be used, taking advantage of various chemical and physical properties of the constituents. Using a variety of methods to obtain a specific end solution is commonplace in industry.

In many cases, the solvent(s) must also be relatively non-toxic and safe to use; this is especially important in the food industry or if the finished product is intended for human use (Charles et al., 1990; Fahlbusch et al., 2003). Disposal and chemical recycling are also considerations when picking solvents for extractions. Other chemical wastes may also be created during additional separation techniques, creating the need for additional disposal procedures. Some solvents, especially those with impurities, have the potential to leave residues in the essential oil after evaporating. Additional chemicals can be added to neutralize or flush the residues, but those chemicals do not guarantee complete removal of the original chemicals and may also leave behind additional residues.

Fractional distillation is usually required to obtain extremely pure essential oils and analytical-grade standards, further increasing waste output. This processed mixture may also contain trace amounts of lipids and oleoresins as well; they may be removed with further processing depending on the desired product. This additional separation usually includes the
incorporation of alcohol(s) and/or other hydrocarbons to selectively remove targeted compounds (Cheong et al., 2011; Fahlbusch et al., 2003; Reverchon et al., 2006).

**Specialty Extraction Methods**

Simultaneous distillation-extraction (SDE) uses heat, solvents, and water vapor to extract volatiles from plant material. This method is ideal for analysis using Gas Chromatography (GC), especially when the goal is to isolate a specific volatile from a solid matrix (i.e. plant material) (Pourmortazavi et al., 2007; Reverchon et al., 2006; Soran et al., 2009). This reduces the loss of highly VOCs and prevents thermal degradation of the constituents. This particular method is not economical on an industrial scale and is mostly used in analytical lab testing. This may prove to be a valuable extraction method for fresh basil samples, in addition to using SPME and other headspace techniques. This method is especially useful when desirable volatiles are contained in a solid matrix, but can also be used to identify volatiles with higher molecular weights within solutions.

Another popular sub-technique that uses various pressures and temperatures to obtain essential oil extracts is known as supercritical fluid extraction (SFE). These techniques implement liquid gases to improve extraction (Lucchesi et al., 2004). This method of extraction is highly specific, very selective, and does not leave undesirable residues; only highly volatile and aromatic compounds are obtained using these processes (Pourmortazavi et al., 2007). Variations in pressure and temperature can be used to obtain a much wider range of selectivity. This method is also ideal for analysis of specific volatiles as well as preparation for Gas Chromatography, but will not be used for this project because of cost limitations.
Impact of Secondary Metabolic Products on Human Health

A great deal of research has been conducted on the impact that volatiles and other phytonutrients have on human health; however, literature regarding practical applications for commercial growers and consumers is somewhat limited. In addition to other various herbs, fresh basil provides a significant amount of phytonutrients essential to the human diet (Olfati et al., 2012). Increasing dietary carotenoids and other accessory pigments has the potential to improve eye health through photo-protective and antioxidant functions (Kopsell et al., 2005). Consuming a variety of fruit and vegetable crops with different combinations of carotenoid compounds was strongly associated with decreased risks of cancer and ocular disease, when compared to ingestions of monomolecular carotenoid supplements (Bourgaud et al., 2001; Hussain et al., 2008; Kopsell et al., 2005). Antioxidants consumed from natural sources have been linked with major health benefits such as reducing the effects of aging and lessening the chances of cancer, heart disease, etc. In addition, antioxidants from artificial sources have been shown to have various effects on human health, some of which were harmful (Pourmortazavi et al., 2007). By improving the nutritional quality of basil and other culinary herbs, humans would receive higher levels of these beneficial compounds at lower levels of dietary consumption. Research within our group suggests that plants have improved nutrient uptake and overall nutrition using narrow-band wavelengths from LED supplemental lighting sources (Kopsell et al., 2014); by using this rapidly evolving technology, we have the potential to improve the nutritional quality of herbs and, therefore, have a positive impact on the health of humans who consume these products.
Importance of Supplemental Greenhouse Lighting Systems

It has been well established that light is one of the most important environmental stimuli that impacts plant growth and development (Briggs et al., 2002; Christie, 2007; Kopsell et al., 2014). To satisfy growing customer demand year-round, it is necessary to optimize greenhouse lighting systems to improve production quality and lower costs. Since the amount of light varies throughout the year, supplementary lighting systems are sometimes utilized with the intention of improving quality and yield of greenhouse crops. Light is one of the most valuable factors that determines the phytonutrient content of high-value specialty crops, namely wavelength and intensity (Samuoliene et al., 2012). Traditional lighting systems used in greenhouse production vary in spectral quality and intensity, which cause differences in gene expression, plant development, and primary/secondary metabolic partitioning (Samuoliene et al., 2012).

In a controlled growing environment, such as a commercial greenhouse, supplemental lighting systems are often considered one of the most expensive initial costs, especially for large-scale operations. While some of these lighting systems are costly to maintain, they can produce a noticeable return on growth, plant development, and volatile production (Olle et al., 2013; Samuoliene et al., 2012). In order to substantially improve sensory quality and yield on a commercial scale, it is therefore beneficial to investigate different wavelengths and intensities produced by various greenhouse lighting systems to determine how current supplemental lighting systems may be improved.

Historically, HPS lamps have been the primary type of supplemental growing light used in greenhouse production, even though these systems have poor spectral quality and thermal barrier limitations (Morrow, 2008). The main reason for this choice has been availability, installation costs, and high PAR output. Even with spectral and energy consumption concerns, HPS lighting
systems were traditionally the most efficient lighting source for converting electrical energy into useable PAR wavelengths (Ouzounis et al., 2015b). In commercial practice, greenhouse crops most commonly supplement specialty-herb crops for 16-20 hours per day, and the light intensity usually ranges between 50 and 200 µmol m⁻² sec⁻¹ (Singh et al., 2015). Operational costs of traditional lighting systems, especially HPS lamps, are very expensive. In addition, these units waste large amounts of energy on heat and other wavelengths that are unusable by the crop. Much of the PAR wavelengths produced by HPS lighting fixtures fall between the yellow and orange regions, with some red between 550-650 nm, and approximately 5% in the blue region between 400-500 nm (Ouzounis et al., 2015b). Depending on the specific crop and growing conditions, low levels of blue light and other photosensitive wavelengths have the potential to limit plant growth, development, and secondary metabolism. Light distribution of LED fixtures is equal to or better than that of HPS lighting sources. They also have improved efficiency and spectral control, making LEDs a viable option for supplemental lighting in greenhouse production (Singh et al., 2015).

**LED Uses in Horticulture**

The basic definition of a light-emitting diode (LED) is a source of light produced by the movement of electrons across a two-lead semiconductor material. When sufficient voltage is applied across the leads, electrons are able to flow across the small gap and release energy in the form of photos as electrons fall into lower energy levels (Singh et al., 2015). Rather than using a filament or gas discharge, LEDs make use of solid-state lighting technology and produce light at a specific wavelength based on chemical composition of the LED mechanism (Olle et al., 2013; Samuoliene et al., 2012). This effect is called electroluminescence, and the color of the light (i.e. spectral quality) is determined by the energy band gap of the semiconductor and the corresponding energy level that is released. They inherently possess high luminous efficacy in comparison to
traditional lighting systems (Janick et al., 2015). Luminous efficacy is used to determine how well a light source produces visible light and measures the ratio of lumen output in relation to power input (Janick et al., 2015). Their lifetime can reach up to 100,000 h, in comparison to HPS lighting that is rated perform a maximum of 20,000 h under optimal conditions (Morrow, 2008). Spectral quality of HPS diminishes overtime, while LEDs experience very minimal changes to spectral quality over their life. LEDs are available in a wide variety of specific narrow-band wavelengths (usually ranging from 15-50 nm) as well as broad spectrum (white light). They may also be combined to produce various spectral qualities depending on the intended use. They range from UV-C to near-IR with half-peak bandwidths averaging from 25-50 nm on commercial units (Janick et al., 2015). LEDs do not radiate heat like traditional lamps; however, non-trivial levels of heat energy is released through the conversion of electrical energy to light energy. Therefore thermal pads or heat syncs are usually required to dissipate excess heat energy, prevent equipment malfunction, and further increase lifespan.

Over thirty years ago, the first LED research was conducted with plants. LED lighting systems were originally adapted for the space industry, but have since found many horticultural applications, both with closed-chamber environments as well as supplemental lighting in commercial greenhouse production (Morrow, 2008). LEDs currently play a variety of roles in the horticultural industry, including the use of controlled environment research, tissue culture and propagation, and addition of supplemental/photoperiod lighting regimes with specific spectra and intensities (Olle et al., 2013).

LED lighting systems also have many advantages over existing horticultural lighting systems (Janick et al., 2015). One of the primary advantages of LED lighting systems are improved spectral control. LEDs are the first lighting source to provide the capability of true spectral control,
something that has not been possible using previous lighting technologies (Morrow, 2008). This allows specific wavelengths to be matched up with plant photoreceptors and photoactive compounds to provide optimal metabolic production. Improved spectral control also enables researchers to investigate various wavelengths and determine how these wavelengths fundamentally impact plant physiology, morphology, and biochemistry.

The higher initial cost of LEDs is quickly offset by the decreased energy consumption of HPS lighting and positive yield impacts, especially as manufacturing costs continue to decrease over time. LEDs produce much less heat than traditional systems and can be calibrated to provide specific wavelengths usable by the crop; inactive and undesirable wavelengths can be targeted and eliminated from the ‘light recipe’ while intensifying beneficial wavelengths. Physical placement is not limited when using LED lighting systems, and growers can maximize the canopy’s potential to absorb light. Since LEDs give off less radiant heat than traditional lighting systems, they can be placed closer to the plants without the risk of burning or scaring while delivering the same intensity and spectral quality of light. LEDs do not radiate heat in the same way as traditional bulbs; however, the conversion of electrical to light energy produces significant amounts of heat that must be displaced with thermal pads or other cooling methods (Morrow, 2008). A total system is highly recommended throughout the literature, since the LEDs themselves require additional hardware such as cooling mechanisms, mounting brackets and stands, control/programming system, etc. Commercial systems for horticultural applications have approximately 10-1000 individual LED units, commonly with the option of blended narrow-band wavelengths or full spectrum. In addition, targeted LED mounts with plant detection software may reduce the amount of illuminated empty space (i.e. isles of greenhouse, space between ceiling and crop, etc.) in comparison to ceiling
mounted systems, further concentrating energy to the crop and reducing overall energy consumption (Janick et al., 2015).

Unlike HID lights, LEDs do not require a warm-up time and can be programmed with intricate lighting schedules and spectral adjustments without the risk of damage/lifespan reduction, such as photoperiod compensation and targeted intra-canopy lighting (Janick et al., 2015). Because they are solid-state devices, they can be easily integrated into complex digital control systems, providing researchers and producers greater control and automation power. This may facilitate the use of special lighting regimes or supplementary DLI experiments (Pimputkar et al., 2009). LEDs also have reduced operational costs because of their energy efficiency, low radiant heat output, and enhanced longevity. LEDs do not contain hazardous chemicals and can be disposed/recycled easily, making them more environmentally friendly than mercury vapor lamps. They also use direct current and operate at lower voltages, making them much more energy efficient than traditional lighting systems (Janick et al., 2015). They are inherently safer to operate because of their lower shock risk and lower operating temperatures, especially in moist greenhouse environments. Depending on the needs of the grower, light prescriptions of various narrow-band wavelengths can be used to stimulate various physiological processes, such as flowering and reproduction (Singh et al., 2015). In addition to a growing body of evidence, research from our group suggests that specific wavelength combinations can increase nutrient uptake of plants, improve overall plant health, and increase secondary metabolite production (Abney et al., 2013; Kopsell et al., 2011; Kopsell et al., 2012; Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017).

The main disadvantage of using supplemental LED lighting systems is their high initial cost in comparison to other lighting systems; however, increased LED energy efficiency has the
potential to negate this cost over their lifespan. In addition, manufacturing costs of commercial LED lighting systems has steadily decreased as lighting technologies improve, further reducing the cost for large-scale greenhouse operations (Ouzounis et al., 2015b). As previously stated, current research suggests that supplemental light with narrow-band blue/red wavelengths result in significant changes in both biomass and secondary metabolite production; however, further research should be conducted to clarify responsible mechanisms and establish overall impacts on plant biochemistry and physiology. As LED lighting technology continues to improve, these lighting systems are becoming economically feasible for large-scale commercial applications and have potential to revolutionize supplemental lighting in the horticultural industry.

**Research Objectives**

The primary objective of this research project was to determine the efficacy of supplemental LED lighting systems on hydroponically grown basil in comparison to traditional lighting systems. Quality tests were performed to determine flavor volatile concentrations, nutrient uptake, and fresh/dry yields, with the end goal of determining the optimal blue/red LED lighting ratio and optimal blue/red DLI supplementation. Overall supplemental lighting system efficacy was determined by comparing a variety of factors, including initial fixture cost, spectral output, PAR/intensity output, energy consumption, maintenance costs, modularity, intended crop, etc.

This project was split into two separate phases to answer these questions. Both phases were performed in bay 4 of the central research greenhouse, with the intention of providing reference for other research projects, large-scale commercial operations, and hydroponic herb growers. The primary objective of phase one was to determine optimal blue/red lighting ratio (in relation to biomass, nutritional value, carotenoid bioaccumulation, and flavor volatile production) and
compare that optimal ratio to traditional HPS lighting systems and natural light controls. By adding increments of blue light to phase one experimental runs (i.e. adjusting the ratio of blue/red while maintaining supplemental PAR intensity across treatments), we established the relationship between the amount of blue/red light added and secondary metabolite production. A total of nine randomized lighting treatments were used for this experiment: Natural light controls, one high pressure sodium treatment, and six LED lighting treatments with progressive ratios of blue/red (10B/90R; 20B/80R; 30B/70R; 40B/60R; 50B/50R; 60B/40R). PPFDs were maintained at 100 $\mu$mol m$^{-2}$ sec$^{-1}$, 24h per day through the duration of the experiment. Phase one consisted of six experimental runs ranging from September 2015 to July 2016.

The second phase of this project consisted of four experimental runs ranging from October 2016 to June 2016. Using the optimal blue/red ratio from phase one and similar growing parameters, four experimental runs were performed to determine the optimal DLI of supplemental lighting in comparison to non-supplemented natural light controls. A total of nine randomized lighting treatments were used for this experiment: One natural light control, two high pressure sodium lighting applications (6h and 12h starting at dusk), and six LED lighting treatments with progressive lighting applications (3h, 6h, 9h, 12h, 18h, and 24h starting at dusk). PPFDs were maintained at 100 $\mu$mol m$^{-2}$ sec$^{-1}$ for each lighting treatment for the scheduled time from seed to harvest. Based on previous research projects, we hypothesize that 20B/80R LED supplemental lighting applied 18H after dusk will result in optimal biomass production and secondary metabolite production, while maintaining all other parameters. If an increased duration of narrow-band red/blue supplementation proves useful for improving key flavor and phytonutrient concentrations, it may be beneficial for commercial growers to schedule applications of supplemental light to save
on energy while still achieving maximum benefits for whatever quality parameter is most important to their operation.

Spectral quality and PAR intensity was monitored and recorded year-round to determine changes across seasons and weather patterns in comparison to basil quality parameters. LED and HPS spectra were documented and compared to natural light controls to determine differences in key waveband intensities from supplemental/natural sources and how that impacted basil quality parameters during year-round greenhouse hydroponic basil production. To determine the overall efficacy of LED and HPS supplemental lighting across all growing seasons, a variety of quality tests were performed on basil samples which included nutrient uptake and biomass comparisons, in addition to VOC and carotenoid analysis.
References B


expression levels and carotenoid accumulation in sprouts of tartary buckwheat (Fagopyrum tataricum Gaertn.). *J Agric Food Chem* 61:12356-12361.


CHAPTER 3: SUPPLEMENTARY BLUE AND RED NARROWBAND WAVELENGTHS IMPROVE BIOMASS YIELD AND NUTRIENT UPTAKE IN HYDROponically GROWN BASIL
Abstract

Light emitting diodes (LEDs) have the ability to produce a wide range of narrow-band wavelengths with varying intensities. Previous studies have demonstrated that supplemental blue and red wavelengths from LEDs have significant impacts on plant growth, development, and morphology. Additional research is needed to determine the efficacy of blue and red LED supplemental lighting systems in comparison to traditional lighting systems, specifically in terms of yield and quality optimization for greenhouse-produced high-value specialty crops during winter months. The objective of this study was to determine the impact of LED lighting on greenhouse hydroponic basil (*Ocimum basilicum* var. ‘Genovese’) production yields in comparison to traditional lighting systems. Overall edible biomass accumulation and nutrient uptake were evaluated in response to different lighting sources. Basil was chosen because of its high demand and value among restaurants and professional chefs. A total of nine lighting treatments were used: two non-supplemented natural light controls; one high pressure sodium (HPS) treatment; and six LED treatments with progressive blue/red (B/R) ratios (10B/90R; 20B/80R; 30B/70R; 40B/60R; 50B/50R; and 60B/40R). Each supplemental lighting treatment provided 8.64 mol·m⁻²·d⁻¹ (100 µmol·m⁻²·sec⁻¹, 24 h·d⁻¹). The daily light integral (DLI) of the natural light controls averaged 9.5 mol·m⁻²·d⁻¹ during the growth period (ranging from 4 to 18 mol·m⁻²·d⁻¹). Relative humidity averaged 50%, with day/night temperatures averaging 29.4 °C/23.8 °C, respectively. All treatments were harvested 45 d after seeding. Edible biomass and nutrient uptake were significantly impacted by supplemental lighting treatments and growing season. The LED treatments had the greatest total fresh biomass (FM) and dry biomass (DM) accumulation. The LED treatment biomass was 1.3x greater than HPS, and 2x greater than the natural light control. Biomass partitioning revealed that the LED treatment had more FM and DM for the
individual main stem, shoots, and leaves of each plant at varying levels. The LED treatment also resulted in greater plant height and main stem diameter, approximately 1.4x greater than HPS treatment and 1.9x greater than the natural light treatment across all growing seasons. The HPS treatment was not significantly different than the LED treatments for many of the experiment parameters. Mineral analyses showed that both macro- and micronutrient accumulations were impacted with supplemental lighting and across growing seasons. This experiment shows that spectral quality of both supplemental sources and natural sunlight impacts the growth, development, morphology, and metabolic resource partitioning of basil. The application of LED lighting systems to supplement natural photoperiods or unfavorable growing seasons may be beneficial for improving overall biomass accumulation and nutrient accumulation in basil.

Introduction

Light intensity and spectral quality directly impact growth and development characteristics of many plant species. Various primary and secondary metabolic responses occur as light intensity and spectral quality change, such as photosynthesis, photomorphogenesis, and phototropism (Ouzounis et al., 2015b). Plants have evolved under broadband spectra and are commonly exposed to spectral variation in nature across changing weather conditions, day length, time of day, and season. Altitude impacts spectral quality and light intensity, in addition to neighboring vegetation, competition, and natural geographical layouts. Low sun angles have been associated with low red to far-red ratios (Franklin et al., 2007), while variation in cloud cover is associated with higher blue light levels and lower far-red levels (Smith, 1982). Irradiance and spectral quality are often linked, because leaves that are exposed to shade or sun spectra are also exposed to a relatively lower or higher irradiance level (Massa et al., 2008; Morrow, 2008).
Specific wavebands within the natural spectrum are directly involved with sun and shade responses of plants. Blue and red wavelengths are known to promote primary metabolic reactions such as photosynthesis, while blue and high R:FR ratios induce chloroplast development and alter density (Smith, 1982; Tlalka et al., 1999). Lack of red wavebands can reduce overall yields and photosynthetic rates (Olle et al., 2013). Morphological characteristics are directly impacted by blue/red wavelengths and low R:FR ratios, such as internode length, leaf size/number/area, node angles, secondary and tertiary branching behaviors, etc. (Briggs et al., 1999; Christie, 2007; Morrow, 2008; Smith, 1982). These types of spectral responses are regulated by pigment-proteins known as photoreceptors. Phytochromes, cryptochromes, and phototropins alter the expression of a large number of genes, and numerous spectrum-related plant responses have yet to be documented in detail (Buchanan et al., 2015; Dai et al., 2014).

Supplemental lighting systems play a vital role in plant research and greenhouse production. Research on spectral responses of various plant species has been limited by technology and the inability to accurately provide narrow-band wavelengths (Janick et al., 2015). Before the advent of LEDs, special filters were used to alter the spectral quality broad-spectrum growth lamps with varied success. LEDs provide researchers the opportunity to determine how light quality fundamentally impacts plant growth and development (Massa et al., 2008). In addition, the use of LEDs in horticulture industries has dramatically increased due to continued technological advancements, increased spectral control, improved energy efficiency, and reduction in manufacturing costs (Bantis et al., 2016; Hogewoning et al., 2010b; Massa et al., 2008; Singh et al., 2015). LED and high pressure sodium (HPS) lamps offer many advantages over one another, and full-scope efficacy comparison is needed to determine the efficacy of LED lights in comparison to traditional lighting systems.
As the technology behind LED lighting systems continues to advance, commercial growers would greatly benefit from research focused on determining the efficacy of various supplemental lighting systems and which type is optimal for generalized greenhouse crop production (Singh et al., 2015). A comprehensive economic comparison of supplemental lighting systems among a variety of greenhouse crops would greatly benefit those involved with the horticulture industry and provide them with useful information when purchasing supplemental lighting systems. Radiant heat from HPS systems should also be considered, in addition to any beneficial heat energy gained from these lighting systems during winter months that may help offset heating cost. While general information about energy consumption, energy transformation efficiency, maintenance/bulb lifespan, purchase and operational costs, net profit, etc., is necessary to make an informed supplemental lighting purchase, the intended reasons for supplementation and individual crop requirements should be carefully considered.

On a broad scale, LED technology has the potential to improve commercial greenhouse production yields and maximize profits (Janick et al., 2015). This specific combination of beneficial characteristics makes LED lighting systems ideal for a variety of commercial greenhouse production operations. Optimizing yield and profit are primary concerns for commercial growers; however, a substantial increase in nutritional value may improve marketability and health benefits of certain crops. Specific characteristics may become significant while overshadowing other factors such as net biomass yield and nutritional value. Other impacts of supplemental lighting should also be considered, such as desirable physiological and morphological responses directly related to the spectral quality and photosynthetic photon flux density (PPFD) of each fixture type. For example, some crops may benefit from morphology
manipulations such as increased canopy densities and edible biomass production, changes in node angles, intermodal spacing, etc.

Changes in overall nutritional composition/uptake should be evaluated in regard to LED supplemental lighting systems and various narrow-band wavelengths, as numerous published sources have established that the spectral quality of supplemented light impacts the nutritional uptake and vigor of various fruits and vegetables (Bian et al., 2015; Colquhoun et al., 2013; Fujiwara et al., 2006; Janick et al., 2015; Kopsell et al., 2011; Kopsell et al., 2014; Kopsell et al., 2015, 2017; Lefsrud et al., 2006; Li et al., 2009; Morrow, 2008; Randall et al., 2014b; Tarchoune et al., 2013; Tennessen et al., 1994; Tuan et al., 2013). Since growers now have a diverse range of supplemental LEDs with various spectral qualities and intensities to choose from, it is critical to fully understand various impacts this evolving technology may have across a variety of crops. Different wavelength combinations and intensities (commonly known as ‘light recipes’) may drastically impact various greenhouse crops and should be evaluated further.

One potential use for narrow-banded LED supplemental lighting is large scale greenhouse production of high-value specialty crops. The demand for fresh culinary herbs has grown tremendously over the past few decades. Culinary herbs are defined as herbaceous aromatic plants, which are grown and sold either fresh (cut herbs, potted plants, pastes, etc.) or dried and packaged (Simon, 1990). Herbs are also sold wholesale for processing as ingredients in packaged and processed foods (Treadwell et al., 2007). Manufacturers, commercial kitchens, restaurants, and even high-end grocery stores desire high quality and fresh ingredients to meet this consumer demand, rather than relying on inferior dried products or extracts (Succop et al., 2004). Sweet basil (Ocimum basilicum) is a specific high-value culinary herb that stands out in terms of consumer demand and potential profit. Greenhouse operations growing this highly valuable crop
have excellent potential for sustainable growth, expansion, and profitability, especially in fresh market and high-quality scenarios (Raimondi et al., 2006b; Rakocy et al., 2004; Treadwell et al., 2007; Treadwell et al., 2011).

Large-scale commercial greenhouses are inclined to produce year-round so that they can limit downtime while continuing to acquire income from sales and maintain competitiveness in the marketplace during unfavorable seasons or periods of low demand. In some locations or unique growing situations, supplemental lighting may be required to produce quality crops during winter months through the management of lighting schedules and photoperiod. In other situations, increasing the daily light integral (DLI) and optimizing the spectral quality has the potential to improve biomass production and shorten growth periods (Gouvea et al., 2012; Janick et al., 2015; Samuoliene et al., 2012). LED lighting systems have the potential to replace traditional lighting systems in a wide range of applications while helping growers reduce costs and still maintaining/improving quality throughout all growing seasons. Current research data suggests that modern LED lighting systems, particularly systems providing optimized ratios of blue/red wavelengths, have a positive impact on primary and secondary metabolism, net biomass yield, and nutritional content in basil as well as other specialty crops and high value herbs (Abney et al., 2013; Colquhoun et al., 2013; Hogewoning et al., 2010b; Kopsell et al., 2011; Kopsell et al., 2012; Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017; Samuolienė et al., 2009; Sharafzadeh et al., 2011; Tuan et al., 2013; Wink, 2010). For these reasons, it is advantageous to further explore the possibilities of LED lighting systems and determine how different wavelengths impact plants at a fundamental level.

The primary objective of this project was to determine the impact of specific narrow-band blue/red wavelengths (447 nm/627 nm) from solid-state LED lighting systems on the biochemistry
and physiology of greenhouse hydroponic basil (*Ocimum basilicum* var. ‘Genovese’). Quality tests were performed in order to determine how these wavelengths impacted edible biomass yield and nutrient uptake. Emphasis was placed on determining the optimal blue/red ratio in comparison to HPS lighting. The Genovese variety of sweet basil was specifically chosen because of its unique flavor profile, high market demand, and preference among professional chefs. Number of shoots/leaves in addition to fresh (FM)/dry (DM) biomasses were recorded to determine morphological impacts of blue/red supplemental lighting.

**Materials and Methods**

This project was conducted at The University of Tennessee Institute of Agriculture (UTIA) in Knoxville, TN, USA (35°56'44.5"N, 83°56'17.3"W). Growing dates for these six experimental runs occurred from August 2015 to June 2016 and have been labeled as growing seasons.

**Cultural Techniques and Environmental Growing Conditions**

‘Genovese’ pesto basil seeds (*Ocimum basilicum* var. ‘Genovese’; Johnny’s Select Seeds, Winslow, ME) were germinated in peat moss based cubes (Park’s Bio Dome Sponges, Hodges, SC) at 83 °C and 95% RH. After 2 weeks, seedlings were transplanted into 5x5 cm plastic pots using 1 part peat moss to 3 parts perlite potting mix. Relative humidity during the growth period averaged 55%. Day temperatures averaged 29.4 °C, while night temperatures averaged 23.8 °C. The daily light integral (DLI) of the natural light controls averaged 9.5 mol·m⁻²·d⁻¹ during the growth period (ranging from 4 to 18 mol·m⁻²·d⁻¹). Specific growing parameters for each of the seasons may be found in Table 3.1, Appendix C.
Emphasis was placed on investigating biomass yield and nutrient uptake in response to specific ratios of narrow-band blue/red (447nm/627nm) LED light. A total of nine lighting treatments were added immediately after seedling transplant: two non-supplemented natural light controls, one HPS treatment, and six LED treatments with progressive B/R ratios [10B/90R; 20B/80R; 30B/70R; 40B/60R; 50B/50R; and 60B/40R (Orbital Technologies, Madison, WI)]. Each supplemental lighting treatment provided 8.64 mol·m$^{-2}$·d$^{-1}$ (100 µmol·m$^{-2}$·sec$^{-1}$, 24 h·d$^{-1}$). The two natural light controls were placed at opposite corners of the greenhouse to determine changes in spectral quality and intensity throughout the growing periods. A randomized complete block design was used for this experiment, and lighting treatments were randomized after each experimental run to account for variations in natural light intensity and spectral quality across growing seasons and greenhouse production area. Basil plants were grown in ebb and flow hydroponic systems and watered for 5 min each day with full strength general-mix nutrient solution; the fertility regime was kept constant across the duration of all seasons. Total growth time lasted approximately 45 d across all six experimental runs. Like many commercial basil growing operations, harvest occurred as the first signs of change from vegetative to reproductive growth were observed; care was taken to ensure each growing season was harvested at similar growth stages, and representative samples were taken from each of the two plants within each sub-rep. Each of the nine treatments consisted of 36 plants, for 324 plants per experimental run (season), and 1,944 total basil plants for phase one of this experiment. Sub-reps consisted of two plants each to improve statistical power and decrease variance due to uncontrollable factors.
Mineral Extraction

To determine nutrient uptake changes in basil plants exposed to supplemental lighting, samples were analyzed for nutrient content. Air-dried samples were ground into a fine power using a Magic Bullet blender. 0.5 grams (±0.01) of ground plant material was weighed into 15-ml sterile plastic centrifuge test tubes and labeled. An Ethos 1112 microwave digestion unit was used to process the basil samples. Samples were microwaved for 30 min at 150 ºC, then cooled for an additional 30 min. A 9.9 ml of ICP matrix solution (2% nitric acid, 0.5% hydrochloric acid, 97.5% RO water) was placed into 15-ml sterile test tubes. A disposable 1-ml plastic pipette was used to add 0.1 ml of the acid digested sample mixture to the 9.9-ml ICP matrix solution. This mixture was then thoroughly shaken to ensure that the acid was uniformly distributed within the matrix. An Agilent 7500 Series ICP-MS was used to determine the mineral content of each of the samples ((Barickman et al., 2013).

Statistical Analyses

A Randomized Complete Block Design was used for this experiment. All data sets were analyzed by GLM and Mixed Model Analysis of Variance procedures using the statistical software SAS (version 9.4, SAS Institute, Cary, NC). Design and Analysis macro created by Dr. Arnold Saxton (DandA.sas) was utilized in addition to Tukey’s adjustment, regression analysis, and univariate/normalization procedures to provide additional statistical insights on the complete data set. Treatments were separated by least significant difference (LSD) at $\alpha=0.05$. 
Results

Biomass parameters and nutrient concentrations were evaluated in this experiment, many of which were significantly influenced by growing season, lighting treatment, and season*treatment interactions. Data presented includes plant weights (g), heights/diameters (cm), and mineral concentrations (µg·g⁻¹ DM).

Biomass

Total FM was significantly impacted by season (P≤0.0001; F=381.30), lighting treatment (P≤0.0001; F=34.53), and season*treatment interactions (P≤0.0001; F=9.56). The June growing season produced the highest biomass, while the lowest was produced in the November/December growing seasons. Lighting treatment 40B/60R produced the highest biomass across all seasons, which was significantly higher than HPS and natural light controls.

Leaf FM was significantly impacted by season (P≤0.0001; F=316.21), lighting treatment (P≤0.0001; F=25.35), and season*treatment interactions (P≤0.0001; F=6.28). The June growing season produced significantly higher biomass than any other growing season. Lighting treatment means for all LED treatments were not significantly different, but were all significantly higher (approximately 2x) than HPS and natural light controls.

Shoot FM was significantly impacted by season (P≤0.0001; F=205.03), lighting treatment (P≤0.0001; F=31.92), and season*treatment interactions (P≤0.0001; F=12.64). The June growing season produced the highest shoot FM, while the September month produced the least amount of shoot FM. FM values progressively increased across growing seasons, from approximately 1 g in September to almost 11 g in June. Lighting treatment significantly impacted shoot growth, in which the 40B/60R treatment had the highest fresh weight biomass in comparison to other LED
treatments and natural light controls. The HPS and natural light controls were all significantly lower than all LED treatments.

Main Stem FM was significantly impacted by season ($P \leq 0.0001; F=362.70$), lighting treatment ($P \leq 0.0001; F=28.73$), and season*treatment interactions ($P \leq 0.0001; F=9.12$). Again, the June growing season gave the highest stem FM, and the lowest was observed during the fall/winter months. Stem FM increased 4x from the September to June growing season. LED treatments 10B-40B showed the highest stem FM, with the HPS and natural light controls significantly lower.

Total plant DM followed a similar pattern to FM and was significantly impacted by season ($P \leq 0.0001; F=241.11$), lighting treatment ($P \leq 0.0001; F=25.96$), and season*treatment interactions ($P \leq 0.0001; F=7.14$). June showed the highest total plant DM accumulation, almost 6x higher than the November growing season. LED treatments did not show significant DM differences, but all were significantly higher than HPS and natural light controls across all growing seasons.

Leaf DM was significantly impacted by season ($P \leq 0.0001; F=245.08$), lighting treatment ($P \leq 0.0001; F=25.79$), and season*treatment interactions ($P \leq 0.0001; F=6.53$). June showed the highest leaf DM accumulation in comparison to other growing seasons. Winter seasons experienced a 3x decrease in comparison to the optimal June growing season. The lighting treatments 30B/70R and 40B/60R produced the highest leaf DM, which was approximately 1.5 grams higher than the HPS and natural light controls.

Shoot DM was significantly impacted by season ($P \leq 0.0001; F=73.30$), lighting treatment ($P \leq 0.0001; F=14.12$), and season*treatment interactions ($P \leq 0.0001; F=4.70$). June and April were the best growing seasons, with 10x increases over the September growing season. LED lighting treatments varied in significance, but showed separation from HPS and natural light controls.
Main stem DM was also significantly impacted by season (P≤0.0001; F=215.31), lighting treatment (P≤0.0001; F=17.74), and season*treatment interactions (P≤0.0001; F=6.15). April and June showed the highest main stem DM accumulation, with approximately 6x increase over the September growing season. LED treatments did not separate, but all showed significance over HPS and natural light controls.

**Physical Counts and Biometrics**

Total leaf counts were significantly impacted by season (P≤0.0001; F=732.46), lighting treatment (P≤0.0001; F=17.90), and season*treatment interactions (P≤0.0001; F=3.18). The highest number of leaves was observed during spring growing seasons, a 7x increase over winter growing seasons. LED lighting treatments also had impacts on total leaf number, but significance varied. LED treatment counts were significantly higher than HPS and natural light controls.

Main stem leaf counts were significantly impacted by season (P≤0.0001; F=33.76), lighting treatment (P≤0.0001; F=5.17), and season*treatment interactions (P≤0.0001; F=6.27). April showed significantly higher stem counts in comparison to all other seasons, which were not statistically different. LED treatments had some impact on main stem leaf counts, and the highest number of leaves occurred on 40B/60R; however, most of the LED treatments were not significantly different than natural light controls and HPS treatments.

Side shoot leaf counts were significantly impacted by season (P≤0.0001; F=96.45), lighting treatment (P≤0.0001; F=11.16), and season*treatment interactions (P≤0.0116; F=2.11). April had significantly higher side shoot leaf counts than any of the other growing seasons, while winter months showed lower shoot counts. 50B/50R gave the highest side shoot leaves of any other LED treatment, but many of the LED treatments were not significantly different. HPS and natural light
treatments had much lower side shoot leaf counts in comparison to LED treatments, many of which were not statistically different.

Total shoot counts were significantly impacted by season ($P \leq 0.0001$; $F = 124.68$), lighting treatment ($P \leq 0.0001$; $F = 11.28$), and season*treatment interactions ($P \leq 0.0003$; $F = 2.26$). April showed the highest total shoot counts, $3x$ increase over late fall total shoot counts. LED lighting treatments made some impact on total shoot counts, but were not statistically different than HPS and natural light controls; however, natural light control treatments were significantly different from all LED treatments.

Plant height was significantly impacted by season ($P \leq 0.0001$; $F = 232.41$), lighting treatment ($P \leq 0.0001$; $F = 46.13$), and season*treatment interactions ($P \leq 0.0001$; $F = 4.97$). Heights during September and April growing seasons were significantly increased over winter seasons. LED lighting treatments showed increased heights over natural light controls, but were similar to HPS lighting treatments. The lowest observed heights were natural light controls, which were $33\%$ shorter than any of the LED treatments or HPS treatment.

Stem diameter (at ground level) was significantly impacted by season ($P \leq 0.0001$; $F = 434.39$), lighting treatment ($P \leq 0.0001$; $F = 58.79$), and season*treatment interactions ($P \leq 0.0001$; $F = 7.24$). The June growing season had the largest stem diameters, while the winter growing seasons had significantly smaller stem diameters. Lighting treatment made a significant impact on stem diameter, with 40B/60R being the optimal treatment. Natural light controls and HPS treatments had significantly smaller stem diameters, with the far regions of LED treatments being equal to natural light control values.
Minerals

Tissue boron (B) was significantly impacted by season (P≤0.0001; F=83.49), lighting treatment (P≤0.0001; F=8.62), and season*treatment interactions (P≤0.0001; F=2.60). Plants grown in December had significantly higher B concentrations in comparison to all other seasons. 50B/50R had the optimal B concentrations. Most of the LED treatments separated from HPS and natural light controls.

Tissue sodium (Na) was significantly impacted by season (P≤0.0001; F=78.74) and season*treatment interactions (P≤0.0001; F=3.65), but not by lighting treatment (P=0.3168; F=1.17). Plants grown in fall and early winter months had significantly higher Na concentrations, while spring seasons had varying Na concentrations. There were no statistically significant Na impacts observed among lighting treatments.

Tissue magnesium (Mg) was significantly impacted by season (P≤0.0001; F=6.41), lighting treatment (P≤0.0001; F=4.21), and season*treatment interactions (P≤0.0001; F=3.43). Seasonal concentrations varied, but showed moderately significant decreases during winter months. Mg concentrations in LED treatments were somewhat elevated in comparison to HPS and natural light controls; however, there was variation across treatments, and many did not statistically separate.

Tissue phosphorous (P) was significantly impacted by season (P≤0.0001; F=56.92), lighting treatment (P=0.0044; F=2.88), and season*treatment interactions (P=0.0006; F=2.06). Spring seasons accumulated the most P in comparison to fall and winter growing seasons. There were slightly elevated levels of P in 20B/80R treatment and decreased levels in one of the natural light controls; however, none of the treatments statistically separated from the group, with the exception of the two previously mentioned treatments.
Tissue potassium (K) was significantly impacted by season (P≤0.0001; F=8.54) and season*treatment interactions (P≤0.0001; F=3.07), but not by lighting treatment (P=0.3931; F=1.06). Plants grown in fall and early winter months had significantly higher K concentrations, while spring seasons had varying K concentrations. There were no statistically significant K impacts observed among lighting treatments.

Tissue sulfur (S) was significantly impacted by season (P≤0.0001; F=17.09) and season*treatment interactions (P≤0.0001; F=2.33), but not by lighting treatment (P=0.0009; F=3.43). Plants grown in fall and early winter months had significantly higher S concentrations, while spring seasons had varying S concentrations. There were no statistically significant S impacts observed among lighting treatments, with the exception of one natural light control which separated from the optimal 50B/50R.

Tissue calcium (Ca) was significantly impacted by season (P≤0.0001; F=83.20), lighting treatment (P≤0.0001; F=4.60), and season*treatment interactions (P≤0.0001; F=3.38). Winter seasons accumulated the most Ca in comparison to fall and spring growing seasons. Some varied impacts were observed across lighting treatments. There were slightly elevated levels of Ca in 10B/90R and 50B/50R treatments and decreased levels in one of the natural light controls.

Tissue manganese (Mn) was significantly impacted by season (P≤0.0001; F=6.05) and season*treatment interactions (P=0.0002; F=2.15), but not by lighting treatment (P=0.7407; F=0.64). Plants grown in early fall and spring months had significantly higher Mn concentrations, while December showed the lowest levels. There were no statistically significant Mn impacts observed among lighting treatments.

Tissue iron (Fe) was significantly impacted by season (≤=0.0001; F=11.17), but not by season*treatment interactions (P=0.1953; F=1.21) or lighting treatment (P=0.8490; F=0.51).
Plants grown in early fall and spring months had significantly higher Fe concentrations, while winter seasons had varying Fe concentrations. There were no statistically significant Fe impacts observed among lighting treatments.

Tissue copper (Cu) was significantly impacted by season ($P=0.0021; F=5.04$), but not by season*treatment interactions ($P=0.8356; F=0.77$) or lighting treatment ($P=0.2791; F=1.23$). Plants grown in early fall and spring months had somewhat higher Cu concentrations, but significance was varied across winter and spring months. There were no statistically significant Cu impacts observed among lighting treatments.

Tissue zinc (Zn) was significantly impacted by season ($P\leq0.0001; F=24.38$), but not by season*treatment interactions ($P=0.1449; F=1.26$) or lighting treatment ($P=0.4999; F=0.92$). Plants grown in early fall and spring months had significantly higher Zn concentrations, while winter seasons had varying Zn concentrations. There were no statistically significant Zn impacts observed among lighting treatments.

Tissue molybdenum (Mo) was significantly impacted by season ($\leq0.0001; F=416.44$), but not by season*treatment interactions ($P=0.9899; F=0.34$) or lighting treatment ($P=0.3293; F=1.15$). Plants grown in spring months had significantly higher Mo concentrations, while fall and winter seasons had lower Mo concentrations. There were no statistically significant Mo impacts observed among lighting treatments.

**Discussion**

LED lighting systems have the potential to revolutionize the horticulture industry, but need a thorough efficacy comparison with traditional lighting systems such as HPS lamps. The spectral quality of light emitted by these fixtures amounts to the relative intensity and distribution of
different wavelengths emitted by a lighting source and perceived by photoreceptors then utilized by other photoactive compounds in plant tissues (Gouvea et al., 2012; Massa et al., 2008; Samuoliene et al., 2012). Light intensity and quality are two of the most important factors affecting plant metabolism (Briggs et al., 2002; Briggs et al., 1999; Smith, 1982). Varying ratios of red to far-red wavelengths have direct impact on germination and flowering (Chaves et al., 2011). Blue wavelengths can impact phototropism, stem elongation, stomatal mechanisms, and many secondary metabolic processes (Christie, 2007; Massa et al., 2008). Edible biomass yield and nutritional uptake are the result of the genetic expression promoted by various environmental factors (Gouvea et al., 2012; Massa et al., 2008; Pimputkar et al., 2009; Samuoliene et al., 2012). Optimizing environmental conditions (i.e. light intensity and spectral quality) may have the ability to significantly manipulate primary metabolic pathways as well as impact edible biomass accumulation and improve nutrient uptake.

As expected, supplemental lighting treatments significantly impacted total plant FM across all seasons. The lighting treatment 40B/60R produced the highest total FM across all lighting treatments and was significantly higher than HPS and natural light controls (Fig. 3.1). Total plant FM average was 49 g in comparison to the natural light control average, which was 24 g. Chlorophyll and carotenoid pigments are highly effective at absorbing light at the supplementary wavelengths provided by the LED treatments, which explains the dramatic increase in biomass accumulation. The most dramatic increase was found under LEDs during winter months. HPS lighting treatment did not statistically separate from either of the natural light controls for total plant FM. Most recent papers suggest the use of approximately 640 nm (Darko et al., 2014; Lefsrud et al., 2008; Lefsrud et al., 2006; Samuoliene et al., 2013; Samuoliene et al., 2012; Samuolienė et al., 2009) or 660 nm (Li et al., 2009; Lin et al., 2013; Olle et al., 2013; Singh et al., 2015) for the
cultivation of many greenhouse crops. Red light alone did not have significant increases in biomass or growth/development impacts across these studies, but red and blue combinations of supplemental lighting showed increased biomass yields and photo synthetic rates (Christie, 2007; Hogewoning et al., 2010b; Smith, 1982).

Leaf, shoot, and main stem FM followed similar patterns, but revealed various impacts to primary metabolic resource partitioning and morphology (Fig. 3.2-3.4). Leaf FM (i.e. edible biomass) for LED treatments were all statistically higher than HPS and natural light controls (Fig. 3.2). Natural light controls and HPS treatments did not statistically separate from each other. Edible biomass was significantly improved for the optimal LED lighting treatment 40B/60R compared to the natural light control average (30.88 grams per plant vs. 15.5 grams per plant). Reductions in fresh edible biomass suggest that light quality has the ability to alter growth, decrease the mean weight of edible biomass fresh of basil, and lower market value. This reduction in growth may have been caused by lack of total PPFD, lack of specific wavelengths that were necessary for optimal production of primary and secondary metabolites, or most likely a combination of both.

Shoot FM showed dramatic impacts across lighting treatments (Fig. 3.3). All LED treatments were statistically higher than the natural light controls and HPS treatment. The natural light controls did not separate from the HPS treatment. The optimal LED treatment (40B/60R) yielded 8 grams of side shoots per plant, in comparison to the natural light control average of 2.5 grams per plant. Higher levels of blue light significantly decreased shoot biomass in comparison to other LED treatments with lower levels of blue wavelengths. Exposure to only red light has resulted in plant elongation and reduction of total biomass of lettuce and other greenhouse crops (Christie, 2007; Hogewoning et al., 2010b; Li et al., 2009; Lin et al., 2013; Samuoliene et al., 2012;
Smith, 1982). Blue wavelengths are necessary for leaf expansion and have also been shown to improve biomass production (Li et al., 2009; Lin et al., 2013; Samuoliene et al., 2012).

Stem FM were significantly impacted by lighting treatment (Fig. 3.4). LED supplemented treatments showed significant increases in main stem FM. Treatments with blue ratios between 10B-40B were highest, and treatments with increased blue wavelengths showed slight reductions in main stem fresh weight. Optimal LED treatment showed significant increases over the natural light control average (11.9 grams per plant vs. 6.3 grams per plant).

Total plant DM had a similar pattern with total FM, with 40B/60R producing the most biomass (4.9 g per plant). High levels of blue light reduced the total dry plant weight, and 60B/40R had the lowest biomass in comparison to other LED treatments (Fig. 3.5). All LED treatments were significantly higher than the natural light controls and HPS treatment. HPS treatment and natural light controls did not statistically separate. The optimal LED ratio produced an approximately 2x increase over the average of the natural light controls (4.9 g per plant vs. 2.4 grams per plant).

Biomass partitioning revealed that leaf, side shoot, and main stem DM were all significantly impacted by lighting treatment (Fig. 3.6). In addition, their percentage of total weight of each partitioned weight was significantly impacted (Fig. 3.13). High levels of both blue and red wavelengths slightly reduced leaf DM, with 30B/70R having the optimal leaf DM. The same can be said about side shoot and main stem DM; however, optimal biomass was produced under 40B/60R. Natural light controls produced significantly less biomass across all three parameters, and the HPS treatment did not significantly improve natural light control weights. Leaf DM for the optimal treatment was 2.95 g per plant, while leaf DM for the natural light controls was 1.29 g per plant.
Analysis of physical count data indicated that total number of shoots per plant and total number of leaves per plant were significantly impacted by lighting treatments (Fig. 3.7). Total number of shoots was increased for the LED treatments with varying significance. Natural light controls and HPS treatment did not separate from each other. The optimal LED treatment had approximately 1.5x total shoots in comparison to the natural light control average. Total number of leaves was significantly impacted by LED treatments, with 50B/50R being the optimal treatment. Low levels of blue wavelengths showed significantly less total leaves than the optimal LED treatment. Natural light controls were not significantly different than the HPS treatment.

The ratio of side shoot leaves to main stem leaves showed some variance across lighting treatments (Fig. 3.11). The 50B/50R treatment produced the highest number of total leaves in addition to the highest number of side shoot leaves and main stem leaves, but percentage was not significantly different. Side shoot leaves consisted of approximately 65-80% of the total leaf counts across all treatments. Side shoot leaf counts and main stem leaf counts varied across LED treatments, and showed some statistical separation from HPS and natural light controls.

Lighting treatments significantly impacted plant height. LED lighting treatment 40B/60R gave the tallest plant averages (28.8 cm), with many of the LED treatments within 2-3 cm of the optimal B/R ratio. Plants from the natural light controls and HPS treatments were significantly shorter than plants treated with LED lights, which is consistent with a basil study conducted by Carvalho (2016). The optimal LED treatment was significantly taller than the natural light control average. Main stem diameter averages followed the same pattern as heights, with 40B/60R being the optimal LED treatment. Many of the LED treatments do not separate from one another, but they are both significantly wider in diameter than the natural light controls. HPS treatment averages did not significantly differ from many of the LED treatments, but was narrower than the
optimal LED treatment (5.1 cm vs. 4.0 cm). Other notable observations included changes in intermodal length, crotch angles, leaf area and thickness, visible trichromes, pigmentation changes, etc.

Light treatment primarily impacted macronutrient concentrations. Tissue P concentrations were highest in the 20B/80R treatment, with elevated levels shown in all LED treatments and HPS treatment. Kopsell et al. (2013) showed similar results when analyzing nutrient uptake in sprouting broccoli microgreen shoot tissues (*Brassica oleacea* var. italica). The natural light controls had slightly lower levels of P; natural light control two was statistically lower than the optimal LED treatment. Tissue Ca concentrations were significantly impacted, with 10B/90R being the optimal lighting treatment. Levels vary among LED treatments and separation does not exist between many of the LED treatments and the HPS treatment. Natural light controls show lower Ca levels, but with varying levels of significance. Tissue S concentrations were highest in the 50B/50R treatment, with varying significance among LED treatments. Natural light controls and HPS show similar levels to many of the LED treatments. Magnesium levels showed some variance across lighting treatments, with 50B/50R being the optimal treatment. None of the LED treatments statistically separated for Mg, but the optimal treatment was significantly higher than the natural light controls and HPS treatment.

The only micronutrient that was impacted by lighting treatment was B, which showed elevated levels in the LED treatments (Table 3.2). 50B/50R had the highest levels of B (66.7 µg·g⁻¹ DM), with many of the LED treatments within 5 µg·g⁻¹ DM of the optimal LED treatment. Natural light controls and HPS treatments were significantly lower than the LED optimal treatment, and the natural light control treatments averaged 51.2 µg·g⁻¹ DM. The nutrients K, Cu, Mn, Fe, Na, Zn, and Mo were not statistically different across lighting treatments. Boron is
primarily used for cell division and cell wall synthesis. Sub-optimal levels of B can reduce uptake of P and K levels, both of which were reduced under natural light controls and increased under LED treatments. Previous studies from our group (Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017) showed that overall nutrient concentrations were increased using supplemental blue/red wavelengths. Many of the macronutrients followed similar patterns to these previous studies. Some of the micronutrients did not show significant improvements with LED lights when analyzed across all six growing seasons; however, when analyzed individually, each of the six seasons showed significant differences among almost all macro and micronutrients. In addition, the winter months showed the lowest uptake values, specifically in the natural light treatments that did not receive supplemental light.

Overall, LED lights provided optimal results for both fresh/dry edible biomass yield and improved the uptake of many macro and micro nutrients when compared to HPS and natural light controls. For all parameters considered in this study, the optimal ratio of blue/red supplemental lighting is between 20B/80R to 40B/60R. Narrow-band wavelengths within the solar spectrum have direct impacts on plant metabolic processes (Colquhoun et al., 2013; Olle et al., 2013). In this efficacy comparison, LEDs have surpassed traditional lighting systems. That being said, one major factor that influences biomass yield is temperature. The HPS lamps provide large amounts of heat energy that may impact photosynthetic rates and, therefore, impact net biomass accumulation as well as the production of other primary and secondary metabolites. Since our research greenhouse has advanced temperature controls, ambient growing temperatures were kept nearly consistent across growing seasons (within 1-2 °C). For commercial growers located in cold environments or that have harsh winter months, traditional lighting sources may offset heating costs due to extra radiant heat energy. Depending on the operation size and many other factors, this may dissuade
potential LED customers from making a purchase. That said, HPS and other traditional lighting sources use vast amounts of energy in comparison to the heat and IR wavelengths that are produced. In most cases, natural gas and propane are much cheaper than electricity and both are inherently more efficient at conversion to useable heat energy. It is possible that light directed on the canopy and IR wavelengths may have other significant impacts on plant metabolism, rather than ambient temperature, which may benefit certain parameters that are important for yield or sensory quality. Other wavelengths within the HPS spectrum (broad-range spectral quality) may benefit growth and provide additional wavelengths to accessory pigments. Overall morphology impacts should be considered, as increases in leaf area or node angles may prove significant for increasing overall photosynthetic rates, absorption of light energy, and customer appeal. For these reasons, further efficacy comparison between HPS and LED lighting systems should be conducted on a variety of parameters to determine economically favorable practices.

Results from this study support the growing body of literature that detail photomorphogenic responses, biomass increases and nutrient uptake impacts by exposure to specific blue and red wavelengths from LED lighting. These results suggest that manipulation of spectral quality and the addition of specific narrowband wavelengths impact plant morphology and resource partitioning for primary metabolism. In addition, this study shows that biomass partitioning ratios vary as a response to altered spectral qualities. Supplementary blue wavelengths have been shown to trigger a wide range of metabolic responses in plants. Manipulating light quality through LED supplementation may be a viable means to improve edible biomass yields, nutrient uptake, and overall plant quality.
References C


composition of Cucumis sativus grown under different combinations of red and blue light.


### Appendix C

**Table 3.1** Environmental conditions during growing periods.

<table>
<thead>
<tr>
<th></th>
<th>September</th>
<th>November</th>
<th>January</th>
<th>March</th>
<th>April</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Day Temp (°C)</td>
<td>27.33°C</td>
<td>26.84°C</td>
<td>25.83°C</td>
<td>25.85°C</td>
<td>26.95°C</td>
<td>27.84°C</td>
</tr>
<tr>
<td>Average Night Temp (°C)</td>
<td>23.61°C</td>
<td>20.62°C</td>
<td>19.11°C</td>
<td>21.01°C</td>
<td>21.94°C</td>
<td>22.56°C</td>
</tr>
<tr>
<td>Average Relative Humidity</td>
<td>55%</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>60%</td>
</tr>
<tr>
<td>Average Daily Light Integral (DLI) (mol m$^{-2}$ d$^{-1}$)</td>
<td>9.81</td>
<td>3.26</td>
<td>4.65</td>
<td>8.62</td>
<td>11.99</td>
<td>14.55</td>
</tr>
<tr>
<td>Average Day Length (h)</td>
<td>12.98</td>
<td>10.35</td>
<td>9.93</td>
<td>11.65</td>
<td>13.20</td>
<td>14.27</td>
</tr>
<tr>
<td>Average Natural Blue (447nm) Intensity at Noon (µmol·m$^{-2}$·s$^{-1}$)</td>
<td>138</td>
<td>122</td>
<td>103</td>
<td>121</td>
<td>133</td>
<td>145</td>
</tr>
<tr>
<td>Average Natural Red (627nm) Intensity at Noon (µmol·m$^{-2}$·s$^{-1}$)</td>
<td>148</td>
<td>132</td>
<td>108</td>
<td>135</td>
<td>144</td>
<td>156</td>
</tr>
</tbody>
</table>
Light Treatment Effects on Biomass

**Figure 3.1** Influence of LED treatments on total fresh mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 3.2** Influence of LED treatments on fresh leaf mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 3.3 Influence of LED treatments on fresh shoot mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 3.4 Influence of LED treatments on fresh stem mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 3.5 Influence of LED treatments on total dry mass of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).

Figure 3.6 Influence of LED treatments on total dry mass partitioning and biomass partitioning of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).
Figure 3.7 Influence of LED treatments on total shoot and total leaf counts of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 3.8 Influence of LED treatments on main stem and side shoot leaf counts of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 3.9 Influence of LED treatments on plant height of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).

Figure 3.10 Influence of LED treatments on main stem diameter of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).
Figure 3.11 Influence of LED treatments on main stem and side shoot leaf counts of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).

Figure 3.12 Influence of LED treatments on total shoot and total leaf counts of hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).
Figure 3.13 Influence of LED treatments on dry total plant weight of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 3.14 Visual representation of LED lighting impacts on morphology of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Light Treatment Effects on Nutrient Concentration

Table 3.2 Influence of LED treatments on macronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>S</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED (10B/90R)</td>
<td>11810a</td>
<td>73810a</td>
<td>13780b</td>
<td>5088ab</td>
<td>7885ab</td>
</tr>
<tr>
<td>LED (20B/80R)</td>
<td>11930a</td>
<td>72200a</td>
<td>13180b</td>
<td>4945ab</td>
<td>7511abc</td>
</tr>
<tr>
<td>LED (30B/70R)</td>
<td>11300b</td>
<td>68450a</td>
<td>12760abc</td>
<td>4917ab</td>
<td>7258abc</td>
</tr>
<tr>
<td>LED (40B/60R)</td>
<td>11670a</td>
<td>73790a</td>
<td>13010abc</td>
<td>5211a</td>
<td>7773ab</td>
</tr>
<tr>
<td>LED (50B/50R)</td>
<td>11450a</td>
<td>73950a</td>
<td>13511a</td>
<td>5212a</td>
<td>8092ab</td>
</tr>
<tr>
<td>LED (60B/40R)</td>
<td>11720a</td>
<td>73910a</td>
<td>13010abc</td>
<td>5143a</td>
<td>7680abc</td>
</tr>
<tr>
<td>Natural Light Control 1</td>
<td>10360a</td>
<td>71200a</td>
<td>11540bc</td>
<td>4714b</td>
<td>7029bc</td>
</tr>
<tr>
<td>Natural Light Control 2</td>
<td>10240b</td>
<td>72670a</td>
<td>11270c</td>
<td>4493b</td>
<td>6723c</td>
</tr>
<tr>
<td>High Pressure Sodium</td>
<td>10780ab</td>
<td>75740a</td>
<td>11940abc</td>
<td>4660ab</td>
<td>6964bc</td>
</tr>
</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg g⁻¹ DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).

Table 3.3 Influence of LED treatments on micronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>B</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
<th>Na</th>
<th>Zn</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED (10B/90R)</td>
<td>63.21b</td>
<td>32.61a</td>
<td>158.2a</td>
<td>167.2a</td>
<td>594.3a</td>
<td>73.13a</td>
<td>0.325a</td>
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<tr>
<td>LED (20B/80R)</td>
<td>62.53b</td>
<td>19.81a</td>
<td>146.1a</td>
<td>154.7a</td>
<td>530.6a</td>
<td>64.04a</td>
<td>0.367a</td>
</tr>
<tr>
<td>LED (30B/70R)</td>
<td>57.09bcd</td>
<td>25.17a</td>
<td>155.1a</td>
<td>165.9a</td>
<td>530.1a</td>
<td>67.33a</td>
<td>0.345a</td>
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<tr>
<td>LED (40B/60R)</td>
<td>65.76b</td>
<td>20.52a</td>
<td>157.4a</td>
<td>154.3a</td>
<td>516.1a</td>
<td>67.81a</td>
<td>0.402a</td>
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<tr>
<td>LED (50B/50R)</td>
<td>66.67a</td>
<td>25.69a</td>
<td>158.1a</td>
<td>161.7a</td>
<td>565.8a</td>
<td>73.55a</td>
<td>0.386a</td>
</tr>
<tr>
<td>LED (60B/40R)</td>
<td>62.19bcd</td>
<td>18.34a</td>
<td>155.8a</td>
<td>164.3a</td>
<td>490.8a</td>
<td>66.36a</td>
<td>0.431a</td>
</tr>
<tr>
<td>Natural Light Control 1</td>
<td>53.12c</td>
<td>22.85a</td>
<td>153.2a</td>
<td>147.1a</td>
<td>533.5a</td>
<td>71.14a</td>
<td>0.452a</td>
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<tr>
<td>Natural Light Control 2</td>
<td>49.44d</td>
<td>21.12a</td>
<td>155.3a</td>
<td>150.8a</td>
<td>558.2a</td>
<td>63.29a</td>
<td>0.462a</td>
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<tr>
<td>High Pressure Sodium</td>
<td>52.77cd</td>
<td>17.06a</td>
<td>150.9a</td>
<td>151.3a</td>
<td>573.9a</td>
<td>66.75a</td>
<td>0.431a</td>
</tr>
</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg g⁻¹ DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).
### Seasonal Effects on Nutrient Concentration

#### Table 3.4 Influence of growing season on macronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Growing Season</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>S</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>10140&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73150&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10710&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5407&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7640&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>November</td>
<td>10846&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76830&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13090&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4425&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7534&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>January</td>
<td>8147&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77890&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15580&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4385&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7273&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>March</td>
<td>10320&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75190&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7461&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5173&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6559&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>April</td>
<td>13610&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68620&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13890&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4836&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7691&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>June</td>
<td>14440&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65430&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15280&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4362&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7914&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*

#### Table 3.5 Influence of growing season on micronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Growing Season</th>
<th>B</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
<th>Na</th>
<th>Zn</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>57.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>183.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>696.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>November</td>
<td>38.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>128.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>710.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.138&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>January</td>
<td>83.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>140.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>761.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>March</td>
<td>64.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>151.4&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>125.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>502.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.57&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>April</td>
<td>46.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>164.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>221.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>82.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>June</td>
<td>64.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>193.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>363.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*
CHAPTER 4: NARROW BAND BLUE AND RED LED SUPPLEMENTS IMPACT KEY FLAVOR VOLATILES IN HYDROPONICALLY GROWN BASIL
Abstract

The use of light-emitting diodes (LEDs) in commercial greenhouse production is rapidly increasing due to technological advancements, increased spectral control, and improved energy efficiency. Research is needed to determine the value and efficacy of LEDs in comparison to traditional lighting systems. The objective of this study was to establish the impact of narrow bandwidth blue(B)/red(R) LED lighting ratios on flavor volatiles in hydroponic basil (*Ocimum basilicum* var. ‘Genovese’) in comparison to non-supplemented natural light controls and traditional high-pressure sodium (HPS) lighting. ‘Genovese’ basil was chosen because of its high market value and demand among professional chefs. Emphasis was placed on investigating concentrations of important flavor volatiles in response to specific ratios of narrow-band blue/red (447 nm/627 nm) LED light. A total of nine lighting treatments were used: two non-supplemented natural light controls; one HPS treatment; and six LED treatments with progressive B/R ratios as: 10B/90R; 20B/80R; 30B/70R; 40B/60R; 50B/50R; and 60B/40R. Each supplemental lighting treatment provided 8.64 mol m\(^{-2}\) d\(^{-1}\) (100 µmols m\(^{-2}\) sec\(^{-1}\), 24 h per day). The daily light integral (DLI) of the natural light controls averaged 9.5 mol m\(^{-2}\) d\(^{-1}\) during the growth period (ranging from 4 to 18 mol m\(^{-2}\) d\(^{-1}\)). Relative humidity averaged 50%, with day/night temperatures averaging 29.4 °C/23.8 °C, respectively. Basil plants were harvested 45 d after seeding, and flavor volatile profiles were obtained by GC-MS. Flavor volatile concentrations varied significantly among lighting treatments. Many compounds showed a non-linear relationship with increasing B/R LED ratios, with the greatest bioaccumulation observed in LED ratios ranging from 20B/80R to 50B/50R. However, the concentrations of some compounds, such as methyl eugenol, were 3-4x higher in the control treatments, and decreased significantly for basil grown under supplemental lighting treatments. Every compound evaluated showed significant differences across lighting treatments.
and growing seasons. Maximum concentrations for each compound varied among lighting treatments, but most were highest under 20B/80R to 50B/50R. The results of this study show that supplemental narrow-wavelength light treatments from LED sources may be used to manipulate plant development and secondary metabolism. The application of LED lighting systems to supplement natural DLI has great potential for improving overall flavor quality of basil and other high-value specialty herbs.

**Introduction**

Plants have a variety of receptors that sense environmental conditions and biotic pressures. They have the ability to sense and respond to specific narrow-band wavelengths within the ambient spectrum, ranging from UV-C (260 nm) to far-red (720-780 nm) regions (Carvalho et al., 2016; Galvao et al., 2015). Changes in light intensity and spectra quality directly impact plant growth throughout development, from germination, seedling establishment, transplanting, vegetative growth, transition to flowering, and even secondary metabolite production as well as adaptation mechanisms in response to biotic and abiotic stress (Fraikin et al., 2013; Galvao et al., 2015).

Secondary metabolites contain a wide range of chemical compound classes that serve many functions in plants, most of which involve adaptation to environmental stress (Bourgaud et al., 2001). In plant tissues, the synthesis of secondary metabolites are significantly impacted by environmental conditions in addition to many physiological, biochemical, and genetic factors (Bourgaud et al., 2001; Lefsrud et al., 2008). Light intensity and spectral quality are two of the most influential factors on secondary metabolism (Kopsell et al., 2005; Kopsell et al., 2014), and changes in light intensity and spectral quality directly impact plant physiology and biochemistry (Bian et al., 2015; Colquhoun et al., 2013).
Plants use specialized pigment-proteins called photoreceptors, which react to changes in light intensity and spectra, promoting physiological and biochemical changes that allow the plant to better adapt to the surrounding environment, thus enhancing chances of survival/reproduction (Galvao et al., 2015). Responses from environmental stressors (i.e. changes in light intensity and spectra) prompt a diverse range of photomorphogenic responses across many plant species (Carvalho et al., 2016; Massa et al., 2008; Montgomery, 2016). The stimulation of light sensors, such as phytochromes, cryptochromes, and phototropins, has been shown to up and down regulate metabolic pathways that directly influence plant growth, development, secondary metabolism, and physiology (Briggs et al., 1999; Christie, 2007; Galvao et al., 2015). Cryptochromes and phototropins primarily respond to blue wavelengths, while phytochromes typically respond to red and far-red wavelengths (Fraikin et al., 2013). Changes in specific blue wavelengths that target phototropins or cryptochromes have the ability to impact primary and secondary metabolism, volatile production, carotenoid and chlorophyll pigment bioaccumulation, circadian rhythms, stomatal opening/closing, intermodal length, leaf area and thickness, and intracellular structure configurations/positioning (Abney et al., 2013; Briggs et al., 2002; Briggs et al., 1999; Christie, 2007; Frank et al., 1996; Kopsell et al., 2013; Lichtenthaler, 1987). Changes in specific blue wavelengths that target phytochromes impact germination rates, vegetative and reproductive growth/development, leaf size/thickness, phenolic and antioxidant pathways, etc. (Li et al., 2009; Olle et al., 2013).

Both blue (B) and red (R) wavelengths evaluated in this study also match the absorption spectrums of chlorophyll and carotenoid compounds, which have direct impacts on photosynthesis and promote significant increases in overall biomass accumulation across a variety of plant species (Olle et al., 2013). Because of their role in photosynthesis and primary metabolism, B and R
wavelengths have the greatest influence on plant growth and development (Bantis et al., 2016; Darko et al., 2014; Massa et al., 2008). In addition, secondary metabolic pathways may be influenced by the supplementation of specific B and R wavelengths (Carvalho et al., 2016; Colquhoun et al., 2013). These various metabolic pathways may act independently or be interconnected with primary metabolism, which has the potential to impart a variety of antagonistic and synergistic effects on metabolic products that have sensory/nutritional value for humans (Deschamps et al., 2006; Kesselmeier et al., 1999; Lee et al., 2005). To date, limited information is available detailing the relationship between spectral quality and the underlying mechanisms that promote changes in physiology and secondary metabolism, specifically in high-value specialty crops (Bantis et al., 2016; Deschamps et al., 2006; Kopsell et al., 2005).

LED lighting provides researchers the opportunity to investigate how specific wavelengths fundamentally impact plant metabolism and development (Darko et al., 2014). Purposefully manipulating environmental conditions or abiotic stressors (i.e. the addition of specific wavelengths) to promote increases in secondary metabolic production has been shown to increase the nutritional value of specialty herb crops and benefit overall consumer health (Bantis et al., 2016; Kopsell et al., 2015, 2017; Lefsrud et al., 2006).

Exposure to UV light and specific B wavelengths have resulted in higher concentrations of favorable flavor volatiles in many high-value crops such as mint (*Mentha piperita*) (Hikosaka et al., 2010; Lucchesi et al., 2004; Treadwell et al., 2011), thyme (*Thymus vulgaris*) (Lee et al., 2005), strawberries (*Fragaria × ananassa*) (Colquhoun et al., 2013), chives (*Allium fistulosum*) (Abney et al., 2013), and basil (*Ocimum basilicum*) (Bantis et al., 2016; Carvalho et al., 2016; Chalchat et al., 2008; Deschamps et al., 2006; Hussain et al., 2008; Klimánková et al., 2008; Kopsell et al., 2005; Lee et al., 2005; Loughrin et al., 2003). Narrow-band supplements to natural light spectra
can improve the sensory quality of various fruit, vegetable, and herb crops through the modulation of pathways that produce specific flavor volatiles and other secondary metabolic products (Bourgaud et al., 2001; Carvalho et al., 2016; Colquhoun et al., 2013; Loughrin et al., 2001). A recent report indicates that basil plants grown under various narrow-band wavelengths had significant concentration changes in specific volatile classes and that LED narrow-band lighting may manipulate specific secondary metabolic pathways of basil under certain conditions (Carvalho et al., 2016). Another study indicated that B and R light significantly increased a variety of volatile fatty-acid derivatives, volatile phenylpropanoids/benzenoids, and volatile terpenes in pre/post-harvest tea leaves, including key flavor volatiles such as linalool, eugenol, 2-phenylethanol, etc. (Fu et al., 2015), all of which share similar pathways across species and prove relevant for the flavor and aroma of basil.

In comparison to other herbs, basil contains a wide variety of compounds that are nutritionally significant to humans, including flavonoids, carotenoids, volatile compounds, etc. (Bourgaud et al., 2001; Hussain et al., 2008). In previous surveys, basil has been reported as having one of the highest antioxidant concentrations in comparison to other popular herbs and spices (Kopsell et al., 2005; Lee et al., 2005; Politeo et al., 2007), increasing potential health benefits for consumers.

Basil is a highly-valued, annual culinary and medicinal herb that has a complex aroma profile preferred by top restaurants and professional chefs. Basil is rich in antioxidants and phenolic compounds that play roles in human health (Kim et al., 2016; Kopsell et al., 2005; Kopsell et al., 2013). The Genovese cultivar was specifically chosen because of its popularity and highly desirable flavor/aroma profiles that have extensive use in a wide variety of culinary dishes and manufacturing productions (i.e. essential oils, soaps, etc.). To date, only a few studies have been
conducted using a supplemental LED lighting source to influence basil sensory quality and volatile organic compound (VOC) flavor profiles (Bantis et al., 2016).

The primary objective of this study was to determine the impact of specific B/R supplemental wavelengths on the production of key flavor volatiles and overall sensory quality of basil. Our goal was to determine the optimal ratio of narrow-band B/R wavelengths for volatile concentration increases and overall highest quality product. This study demonstrates that narrow-bandwidth illumination from LED lighting sources may be used to manipulate secondary metabolism and influence the production of important flavor volatiles in basil and other high-value herb crops. In addition, this study shows the efficacy of improving basil crop quality in commercial production operations using blue/red supplemental wavelengths in comparison to using traditional lighting systems or natural light.

Materials and Methods

This project was conducted on The University of Tennessee, Knoxville, Agriculture Campus. All experimental replications were performed in Central Greenhouse (Knoxville, TN, USA, 35°56'44.5"N, 83°56'17.3"W). Growing dates for these six experimental replications occurred from August 2015 to June 2016 and have been labeled as growing seasons.

Cultural Techniques and Environmental Growing Conditions

‘Genovese’ pesto basil seeds (O. basilicum var. ‘Genovese’; Johnnny’s Select Seeds, Winslow, ME) were germinated in peat moss based cubes (Park’s Bio Dome Sponges, Hodges, SC) at 83°C and 95% RH. After two weeks, seedlings were transplanted into 5x5 cm plastic pots using peat moss and perlite based potting mix. Relative humidity during the growth period
averaged 55%. Day temperatures averaged 29.4°C, while night temperatures averaged 23.8°C. The daily light integral (DLI) of the natural light controls averaged 9.5 mol m⁻² d⁻¹ during the growth period (ranging from 4 to 18 mol m⁻² d⁻¹). Specific growing parameters for each of the seasons may be found in Table 4.1, Appendix D.

Emphasis was placed on investigating concentrations of important flavor volatiles in response to specific ratios of narrow-band blue/red (447nm/627nm) LED light. A total of nine lighting treatments were added immediately after seedling transplant: Two non-supplemented natural light controls, one HPS treatment, and six LED treatments with progressive B/R ratios as: 10B/90R; 20B/80R; 30B/70R; 40B/60R; 50B/50R; and 60B/40R (Orbital Technologies, Madison, WI). Each supplemental lighting treatment provided 8.64 mol m⁻² d⁻¹ (100 µmols m⁻² sec⁻¹, 24 hours per day). A randomized complete block design was used for this experiment, and lighting treatments were randomized after each experimental run to account for variations in natural light throughout the greenhouse bay and growing seasons. Basil plants were grown in ebb and flow hydroponic systems and were watered for 5 minutes each day with full strength general-mix nutrient solution, and the fertility regime was kept constant across the duration of all seasons. Total growth time lasted approximately 45 days among the 6 experimental runs. Like many commercial basil growing operations, harvest occurred as the first signs of change from vegetative to reproductive growth were observed. Care was taken to ensure that plants during each growing season were harvested at similar growth stages, and representative samples were taken from each of the two plants within each sub-rep. Each of the nine treatments consisted of 36 plants, with 324 plants per experimental run (season) and 1,944 total basil plants for phase one of this experiment. Sub-reps consisted of two plants each to improve statistical power and decrease variance due to uncontrollable factors.
**GC-MS Headspace Volatile Analysis**

Three g of fresh plant material (two basil plants per sample, 1.5 g of representative material from each plant) were placed in 20-mL borosilicate glass vials then immediately sealed and placed onto an Network Headspace Sampler (Agilent G1888, Santa Clara, CA, USA). Fresh and fresh frozen samples were tested to determine differences in volatile profiles based on storage type. Samples were heated to 80 °C for 10 min and pressurized with Helium (Air Gas, analytical purity) to 95.21 kPa for 1 min. The tube was then vented for 1 min into the headspace transfer line (110 °C) and injected (port at 250 °C) into the GC (Agilent Technologies 6890N Network GC System). The volatiles were separated by an HP-5MS capillary column (5%-Phenyl)-methylpolysiloxane, length: 30m, ID: 0.250 mm, film thickness: 1µm, Agilent Technologies) using analytical purity Helium carrier gas at 95.21 kPa, constant pressure. At the start of data acquisition, temperature was held at 40 °C for 5 min, ramped-up from 40 °C to 250 °C at 5 °C per min, then held constant for the duration of the run. Total run time was 70 min, including post-run and cool-down phases. After sample separation and column elution, the analytes were passed through a mass selective detector (Agilent Technologies 5973 Network Mass Selective Detector) at 250 °C and collected over the course of the sample run. The transfer line, ion source, and quadrupole temperatures were 250 °C, 230 °C, and 170 °C, respectively. The full scan mass range was set to 40-550 m/z (threshold: 150).

Agilent ChemStation was used for data collection and processing. Calibration curves were previously established using analytical standards found in basil and shown in the literature to be important for human sensory perception. Over 200 separate compounds were identified throughout the course of this project, but emphasis will be placed on key flavor compounds that have been calibrated to our GC-MS and HP-5MS column using analytical standards (Sigma-Altech, St.
Louis, MO) to determine leaf tissue concentrations of key VOCs on a fresh plant weight basis. The MS spectra from analytical standards and fresh samples were compared to NIST, ADMIS, and a basil reference library created from calibrated analytical standards to confirm peak identity and retention times. MassHunter Workstation Software Version B.06.00 (Agilent Technologies, Inc., 2012) was used to automatically integrate peaks. Relative peak areas were automatically adjusted based on analytical standards and multiple library references.

**Statistical Analyses**

A Randomized Complete Block Design was used for this experiment. All data sets were analyzed by GLM and Mixed Model Analysis of Variance procedures using the statistical software SAS (version 9.4, SAS Institute, Cary, NC). Design and Analysis macro (DandA.sas) was utilized in addition to Tukey’s adjustment, regression analysis, and univariate/normalization procedures to provide additional statistical insights on the complete data set. Treatments were separated by least significant difference (LSD) at \( \alpha = 0.05 \). Due to the overwhelming number of compounds analyzed, only statically significant separations were reported from this study. Concentration changes among growing seasons and light treatments were investigated. Key volatiles were analyzed and presented on a fresh mass (FM) basis in comparison to micro molar calibration curves created from analytical standards. All volatile concentrations units are reported in micromolarity of analyte concentration per g of fresh leaf tissue (\( \mu \text{M} \cdot \text{g}^{-1} \) FM) to most accurately represent VOC emissions from the collected headspace sample above fresh plant tissues under specific reproducible analytical conditions.
Results

The following volatiles were analyzed for this experiment because of their relative abundance and importance in sensory perception: (S)-(−)-Limonene; (R)-(−)-Limonene; 1,3,6-Octatriene, 3,7-dimethy-, (Z); 1,3,6-Octatriene, 3,7-dimethy-, (E); Methyl Eugenol; Linalool; α-Humulene; Hexanal; Estragole; 1-Hexanol; α-Pinene; β-Pinene; Phenly-2-Ethanol, and Eucalyptol. Many of these compounds were significantly influenced by growing season, lighting treatment, and season*treatment interaction (Appendix D, Fig. 4.1-4.21).

(S)-(−)-Limonene concentrations were significantly impacted by season (P≤0.0001; F=52.86), treatment (P≤0.0001; F=14.03), and season*treatment interaction (P≤0.0001; F=2.32). Winter treatments were most significantly impacted by the addition of supplemental lighting, while the limonene concentrations during the early fall and late spring season were significantly lower. 40B/60R was significantly higher than any of the other treatments. The natural light controls had significantly lower concentrations of limonene than the LED or HPS treatments.

The changes in (R)-(−)-Limonene concentrations similar to its enantiomer. There were still significant differences in season (P≤0.0001; F=115.43), treatment (P≤0.0001; F=14.90), and season*treatment interaction (P=0.0050; F=1.76). Early fall seasons contained significantly higher concentrations than any of the other growing seasons. Late spring had the lowest concentration of any season. Significant differences exist between LED lighting treatments and natural light controls, and the means of the HPS control and other LED treatments were not well separated from the other LED treatments.

1,3,6-Octatriene, 3,7-dimethy-, (E) showed significant season (P≤0.0001; F=40.80) and treatment (P≤0.0001; F=5.99) differences. The early spring season had the highest concentrations (4x over any other season), while the late spring season had the lowest concentrations. Most LED
treatments were significantly higher than the natural light controls, but they did not separate well from one another. The natural light controls had the lowest concentration averages, 2x less than the average LED treatment. 1,3,6-Octatriene, 3,7-dimethy-, (Z) did not show any significant impacts across season, treatment, or season*treatment interaction.

Methyl Eugenol showed significant impacts in season (P≤0.0001; F=23.48), treatment (P≤0.0001; F=14.14), and season*treatment interaction (P≤0.0001; F=2.37). Mid-late winter seasons were significantly lower than all other growing seasons. Natural light controls show significantly different methyl eugenol concentrations than any other treatment (with the exception of 10B/90R). The natural light controls show significant increases of methyl eugenol over LED treatments and HPS treatments. 50B/50R showed the lowest concentrations of methyl eugenol overall.

Linalool concentrations were significantly impacted by season (P≤0.0001; F=28.24), treatment (P≤0.0001; F=16.52), and season*treatment interaction (P=0.0398; F=1.48). Early fall and late winter showed the highest concentrations of linalool, while late spring had the lowest concentrations, all of which were significantly different. All LED treatments were significantly higher than natural light controls, with 30B/70R and 40B/60R being the optimal LED treatments. HPS treatment concentrations fell between the LED and controls, not separating significantly from either group.

α-Humulene concentrations were significantly impacted by season (P≤0.0001; F=34.34), treatment (P≤0.0001; F=12.29), and season*treatment interaction (P≤0.0001; F=3.20). There was a great deal of concentration variation among seasons, with early fall being significantly highest and late spring being significantly lowest. All LED treatments were significantly higher than the HPS and natural light controls; none of the LED treatments separated.
Hexanal concentrations were significantly impacted by season ($P \leq 0.0001; F=76.86$), treatment ($P \leq 0.0001; F=4.12$), and season*treatment interaction ($P=0.0002; F=2.14$). Early fall showed the highest levels of hexanal, with all other treatments being significantly lower. None of the other growing seasons showed significant changes in concentration. Some of the LED treatments were significantly higher than natural light controls, but they did not separate well.

Estragole concentrations were significantly impacted by season ($P \leq 0.0001; F=36.57$) and season*treatment interaction ($P=0.0017; F=1.89$), but did not show significant impacts across treatments ($P=0.1127; F=1.64$). Mid-winter had the highest levels of estragole, while late spring showed significantly lower levels than all the other seasons.

$\alpha$-Pinene concentrations were significantly impacted by season ($P \leq 0.0001; F=56.98$), treatment ($P \leq 0.0001; F=9.46$), and season*treatment interaction ($P \leq 0.0010; F=1.96$). The highest concentrations were found in early fall, while the lowest concentrations were found in late spring, both significantly different than any other growing season. All LED treatments were significantly higher than the natural light controls. HPS treatment concentration was not significantly different than the controls, and did not separate from some of the LED treatments.

$\beta$-Pinene concentrations were significantly impacted by season ($P \leq 0.0001; F=42.03$), treatment ($P \leq 0.0001; F=10.25$), and season*treatment interaction ($P=0.0017; F=1.90$). The highest concentrations were found in early fall, while the lowest concentrations were found in late spring; and late spring had significantly lower concentrations, almost 5x difference. All LED treatments were significantly higher than the natural light controls. HPS treatment was not significantly different than the controls, and did not separate well from some of the LED treatments. The LED treatments show a regression trend, with 40B/60R being the optimal ratio for pinene intensity.
Phenyl-2-Ethanol concentrations were significantly impacted by season ($P \leq 0.0001; F=19.72$), treatment ($P \leq 0.0001; F=4.38$), but not by season*treatment interaction ($P=0.1028; F=1.32$). Early fall concentrations were significantly higher than any other season. The best LED ratio separated from the natural light controls, but overall the LED treatment and natural light treatments did not separate.

Eucalyptol showed the greatest impacts in comparison to any other compound evaluated in this experiment. Overall concentrations were significantly impacted by season ($P \leq 0.0001; F=47.47$), treatment ($P \leq 0.0001; F=16.20$), and season*treatment interaction ($P=0.0007; F=2.00$). Early fall concentrations were significantly higher than winter and spring seasons. Optimal LED concentrations were significantly higher than the natural light controls. Natural light control concentrations all separated from LED treatments, but did not separate from the HPS control.

**Discussion**

No matter the intended use, sweet basil is highly appreciated for its aroma and flavor. High yields and overall quality are critical foundations of successful commercial growing operations, and a variety of quality parameters have been established for the flavor and aroma of many high-value herbaceous crops. The delicate flavor and aroma of basil is a result of the specific and complex ratios of chemical compounds produced through primary and secondary metabolic processes directly related to environmental conditions and genetic makeup (Lachowicz et al., 1996; Ouzounis et al., 2014; Samuolienė et al., 2009).

The demand for variety and improvement in basil flavor profiles have led to numerous breeding strategies, resulting in many distinct varieties with complex flavor profiles. Advances in GC-MS analysis and LED technologies have allowed for simple and efficient quantification of
basil flavor volatiles; concentrations of these key flavor volatiles that are impacted by various narrow-band wavelengths may now be explored in-depth. The impacts of environmental stressors, growing season, and cultivar chemotype are reflected in VOC profiles, which is a direct result of changes to primary and secondary metabolism. Many studies have focused on achieving optimal yield, flavor, and aroma in basil, but only a handful demonstrate changes to biomass and flavor volatiles in response to the ambient light spectrum and supplemental narrow-band wavelengths (Loughrin et al., 2001; Loughrin et al., 2003; Morrow, 2008; Ouzounis et al., 2014; Pimputkar et al., 2009; Singh et al., 2015). Understanding the mechanism behind resource partitioning of secondary compounds based on changes to light intensity and wavelength will add to the limited knowledge of this subject area and provide detailed supplemental lighting strategies for commercial producers with the intention of optimizing yield and flavor.

Spectral quality and light intensity will change significantly across growing seasons (Banthorpe et al., 1971; Barta et al., 1992; Briggs et al., 1999; Goins et al., 1997; Olle et al., 2013; Ouzounis et al., 2015b; Samuoliene et al., 2013; Smith, 1982; Tennessen et al., 1994; Wink, 2010). Because primary and secondary metabolism are directly linked to the intensity and spectral quality of available light, seasonal differences are expected for biomass yield and the production of secondary metabolites from these fluctuations in light quality/intensity (Banthorpe et al., 1971; Carvalho et al., 2016; Kang et al., 2009; Olle et al., 2013; Sugimoto et al., 2012; Wink, 2010); however, few studies have determined the relationship between specific B/R wavelengths, key flavor volatiles in basil, and impact of growing season in addition to supplemental lighting treatments (Buchanan et al., 2015; Wink, 2010). This idea was further investigated using data collected from various instruments and sources throughout the greenhouse and laboratory to determine the impact of season on flavor volatile production. To ensure an accurate and effective
comparison was performed across all growing seasons, lighting treatment randomization and environmental measurements (DLI, natural light spectra, temp/humidity, etc.) were recorded to provide grounds for data normalization to further reduce variability due to greenhouse shading or changes to the solar spectrum throughout the experiment. Reference data on environmental conditions and cultural practices is presented in Table 4.1 in Appendix D and the materials/methods section, respectively. Significant concentration differences were observed for all evaluated compounds across a variety of growing seasons (early September to late June). These seasons were specifically chosen to determine the efficacy and overall impact of LED lighting on key flavor volatiles during fall/winter months with decreased light intensity and spectral quality.

As expected, supplemental lighting most significantly increased overall biomass production and collective terpenoid concentrations during winter months; however, specific compounds had various significant seasonal effects. Overall terpenoid compound concentrations were slightly less during winter months, but supplemental lighting treatments significantly improved those concentrations in comparison to natural light controls during those winter months. Key flavor volatiles in basil as well as specific classes of secondary metabolites had opposing changes in concentration across growing seasons and followed some general trends that coincide with many studies that investigate the impact of abiotic factors (i.e. climate and light) on crop production and physiology (Briggs et al., 2002; Carvalho et al., 2016; Choi et al., 2015; Colquhoun et al., 2013; Dai et al., 2014; Goins et al., 1997; Gouvea et al., 2012; Houle et al., 2015; Jamieson et al., 2012; Kang et al., 2009; Kopsell et al., 2017; Lange et al., 2000; Leonard et al., 2010; Lin et al., 2013; Loughrin et al., 2003; Mccree, 1973; Morrow, 2008; Olle et al., 2013; Ouzounis et al., 2015b; Pimputkar et al., 2009; Singh et al., 2015; Smith, 1982). Even though greenhouses protect from winter weather and poor climate conditions, outside environmental conditions have
significant influence on crop production quality and yields. Some factors that may impact volatile concentrations across growing seasons include: reduced natural light intensity, poor spectral quality, increased cloud cover, day/night temperature reductions and fluctuations, and changes in relative humidity.

Many of the compounds explored in this study include abundant terpenes and phenols found in edible tissues from basil that profoundly impact human sensory experience (i.e. flavor and aroma). They are produced by subsidiaries of the mevalonate and non-mevalonate pathways and originally derived from geranyl pyrophosphate. Almost all compound concentrations were significantly impacted by growing season, lighting treatment, and seasons*treatment interactions. Concentrations are presented in μM of analyte per g FM sample to accurately and efficiently represent headspace emissions based on serial-diluted calibrated curves from analytical standards that ranged from 0.01 μM to 100 mM in concentration.

(S)-(−)-Limonene and (R)-(+)−Limonene leaf tissue concentrations showed significant differences between supplemental lighting treatments and natural light controls, but did not separate among themselves (Fig. 4.1 and 4.2). (S)-(−)-Limonene and (R)-(+)−Limonene concentrations differed by a factor of 7-10x, with (R)-(+)−Limonene being the more intense flavor volatile in terms of both analytical response and human sensory perception (Chalchat et al., 2008; Larsen et al., 2000). (S)-(−)-Limonene is said to have an orange like aroma, while (R)-(+)−Limonene has a stronger lemon/citrus aroma (Larsen et al., 2000); both compounds possess similar bioactivities. The combination of these two limonene compounds, in addition to other volatile compounds, contributes to overall citrus flavor; changes in the relative concentrations of these flavor volatiles determine the “type” of citrus flavor that is perceived by humans as well as the intensity. The optimal LED treatment for increasing both limonene compound concentrations was
40B/60R, and changes to the relative concentration ratios of (S)-(−)-Limonene and (R)-(+)−Limonene were similar across all lighting treatments, which demonstrates that blue/red light treatments significantly increase concentrations of (S)-(−)-Limonene and (R)-(+)−Limonene, but do not modify the ratio of (S)-(−)-Limonene and (R)-(+)−Limonene produced. This suggests that the B/R wavelengths impact secondary pathways upstream from the R/S conformation-decision point and produce higher volumes of both compounds, rather than preferentially synthesizing one over the other.

(S)-(−)-Limonene concentrations were significantly highest during the winter growing seasons (January-March) (Fig. 4.11). Average natural DLIs were much lower during this time in comparison to other growing seasons, which suggests that the supplemental B/R wavelengths have significant impacts when natural sources lack sufficient DLI or spectral quality. November had the significantly lowest concentration of overall terpenoid compounds across all harvest seasons, which may be explained by the high temperatures/light intensity during the first stage of development and decreasing temperatures/natural light intensity as the growing season progressed into late fall. Day/night temperature averages were maintained throughout all growing seasons due to an automated environmental control system in the research greenhouse where this set of experiments were conducted. Greenhouses reduce temperature variation across growing seasons, but it is nearly impossible for any type of environmental control system to completely eliminate variations in humidity and temperature across all growing seasons. Therefore, temperature variations are expected within these results, but do not provide basis for the significance between LED treatment concentrations and HPS treatment concentrations. It is also possible that secondary metabolic carryover effects were experienced from the seedling stage into later growth periods. Multiple studies have found that specific wavelength, light intensities, and temperature variations
at the seedling stage can impact secondary metabolism and resource partitioning for specific
classes of terpenoids at later stages of development (Bantis et al., 2016; Carvalho et al., 2016; Jishi
et al., 2016; Massa et al., 2008; Matsuda et al., 2016; Randall et al., 2014b).

(R)-(−)-Limonene did not follow the same trend across growing seasons as its enantiomer
(Fig. 4.12); ratios of R/S limonene varied across all growing seasons and did not follow any
patterns. The average ratio of R/S was 10:1 across growing seasons and showed variance among
seasons. In addition, total concentrations of both compounds did not follow any logical
physiological-based pattern across growing seasons. This is somewhat unexpected since both
limonene compounds are so closely related in terms of chemical structure, biosynthesis,
bioactivity, and importance in flavor for basil/other herbaceous and citrus crops. This suggests an
internal mechanism shift or pathway up/down regulation at the specific point in the metabolic
pathway that determines the R/S conformation, as opposed to explanations based on physical
chemistry. On its own, passive volatilization does not explain the differences in limonene
compound concentrations, as emission would theoretically be similar for both; unless some type
of specific mechanism or active process is emitting one volatile over the other as a result of
environmental changes, such as plant defense, metabolic regulation in reaction to abiotic stress,
pollinator attraction, and/or plant-plant signaling. September showed the highest concentrations of
(R)-(−)-Limonene, a 6x increase over June, which was the lowest concentration observed across
all growing seasons.

1,3,6-Octatriene, 3,7-dimethy-,(Z) and 1,3,6-Octatriene, 3,7-dimethy-, (E) were measured
across lighting treatments (Fig. 4.3), both of which exhibit an herbaceous and terpene-based aroma.
Limited information was availability regarding sensory perception of these compounds,
concentrations within basil, the impact of LED lighting treatments on these compounds, or any
combination of these areas of study. The (Z) conformation did not show any significant changes across lighting treatments, but the (E) conformation did show significant differences between the 40B/60R treatment and the HPS treatment/natural light controls. Approximately 2x concentration increase was observed for the (E) conformation between the best LED treatment (40B/60R) and the natural light controls. The concentrations of the 40B/60R treatment was significantly higher than the HPS treatment, and the HPS treatment did not separate from the other LED treatments and natural light controls. The (E) conformation of 1,3,6-Octatriene showed seasonal concentration changes, but the (Z) conformation did not show any significant concentration changes across season (Figures 4.13 and 4.14). Octatriene followed the same total terpenoid concentration patterns for (R)-(+) L-limonene across growing seasons. September had the highest concentrations, 6x higher than the June growing season, which had the lowest concentration of any growing season observed.

Methyl eugenol showed an inverse relationship to LED lighting treatments and natural light controls in comparison to other key flavor volatiles evaluated in this study (Fig 4.4). Many of the compounds evaluated show a regression relationship between the LED lighting treatments peaking around 40B/60, and natural light controls showing the lowest concentrations. The opposite is true for methyl eugenol, as the natural light controls show the highest concentrations in comparison to any other treatment, and the 40B/60R had the lowest concentrations.

Methyl eugenol has a strong, spicy, herbaceous aroma that greatly contributes to the flavor and aroma of basil. This compound also has valuable medicinal properties and many human health benefits (Lee et al., 2005). It can be described as having a clove-like flavor and is used extensively in the pharmaceutical and cosmetic industries. It has also been described as an antioxidant, antimitagenic, antigenotoxic, anti-inflammatory, and even has the potential to reduce the recurrence
of certain cancers (Carvalho et al., 2016). Methyl eugenol shows an inverse relationship with recorded DLI and available light intensity, which suggests that as light intensity increases and blue/red wavelength supplements are added, methyl eugenol concentrations decrease. Methyl eugenol may be released through volatilization in higher qualities due to changes in light intensity and spectral quality because of its high boiling point and vapor pressure. It is also important to note that methyl eugenol is the only phenolic compound in comparison to the other terpene based compounds evaluated in this study. Methyl eugenol is derived from a separate secondary metabolic pathway (may be derived from a variety of pathways but dominant biosynthesis occurs from the precursor amino acid l-tyrosine and is converted into a variety of eugenol conformations through multi-step biosynthesis) in comparison to the other terpene based compounds created from the isoprenoid pathway, which suggests that spectral quality has an impact on resource partitioning for secondary metabolism and specifically tyrosine/enzymes that are used to synthesize eugenol and other phenolic compounds in this pathway. These results are consistent with similar studies that investigated the impact of LED lighting on flavor volatiles and resource partitioning of other secondary metabolites (Carvalho et al., 2016; Colquhoun et al., 2013; Loughrin et al., 2003; Samuoliene et al., 2013). Overall concentrations of terpene-based compounds showed concentration increases with the addition of B wavelengths (peaking around 40B/60R), while methyl eugenol and other phenylpropanoid compounds generally showed concentration decreases with the addition of supplementary B light, which is consistent with results from a similar study (Carvalho et al., 2016). Many studies have shown how UV, far-red, and specific B/R wavelengths impact plant development, morphology, and secondary metabolite production including carotenoids, accessory pigments, hormones, and flavonoids (Olle et al., 2013); however, very few studies have related specific wavelengths to changes in terpenes and phenylpropanoids that are
crucial for aroma, flavor, and overall sensory quality of high-value herbaceous crops (Bantis et al., 2016). Because methyl eugenol concentrations have such a large impact on flavor and aroma perception of basil, further studies should be conducted to determine the specific mechanisms that are involved in the synthesis of this compound and how blue/red wavelengths impact this process. The benefits (i.e. increase of other terpenoids that are important to flavor) that result from the addition of supplementary wavelengths may outweigh the net loss of methyl eugenol and other phenolic compounds that are also important to flavor. It is important to note that methyl eugenol can be somewhat toxic at levels found in naturally occurring sources; reducing the amount of methyl eugenol or other somewhat toxic/unpalatable compounds give growers the opportunity to create designer flavors while optimizing the health benefits of their crop (Fahlbusch et al., 2003; Reverchon, 1997). The biological activities, sensory impacts, and overall health benefits of each key flavor/aroma compound should be investigated to determine which compounds are most important for basil quality and human sensory perception; this information may be used to determine the optimal spectrum and intensity of supplemental light treatments to improve flavor and aroma profiles of basil and other high-value greenhouse crops.

Methyl Eugenol concentrations across all lighting treatments showed significant decreases during March and April growing seasons (Fig 4.14). All other growing seasons (Sept., Nov., Jan., and Jun.) showed elevated concentrations, all of which were approximately significantly higher than the March/April growing seasons. Since there is less ambient natural light and reduced spectral quality at the start of the March/April growing seasons, the production of all secondary metabolic products are expected to slightly decrease; however, these results suggest that either a lack of overall intensity from natural sources or a specific wavelength that was provided by natural light and not supplemented in the B/R regiment (i.e. variations of other B/R, yellows, greens,
oranges, etc.) is responsible for the drastic changes in methyl eugenol concentration. Results from lighting treatment effects suggest that lower light intensities are responsible. Two natural lighting treatments were used to determine the impact of fluence and spectral differences in opposite sides of the greenhouse, and the lower intensity control consistently had higher levels of methyl eugenol in comparison to the higher natural light controls across all six growing seasons. This suggests that light-independent (i.e. dark) reactions and photoperiod/DLI changes may also have influence on secondary metabolic partitioning. While basil does not exhibit photoperiodic responses towards reproductive growth, many plants have a variety of metabolic processes that are regulated by light intensity and specific wavelengths, all of which may have an impact on quality (Banthorpe et al., 1971; Buchanan et al., 2015; Ouzounis et al., 2015a). It is also possible that the blue/red treatments had a much more pronounced impact on monoterpene synthesis during winter/early spring months from reduced day length and light intensity/spectral quality, which diverted resources away from the pathway that synthesizes eugenol compounds. Eugenol, iso-eugenol, methyl eugenol, and several other isomeric compounds should be further investigated to determine the impact of wavelength and intensity on the ratios of these specific compounds and/or overall concentrations of this class of metabolic products in comparison to other classes, since they all have impacts on flavor and aroma in basil. Secondary metabolic partitioning in high-value herbaceous crops should also be further explored to determine physiological and biochemical changes in response to various wavelengths and intensities of supplemented light.

Patterns with linalool (Fig. 4.5 and 4.15) concentration changes were consistent with other terpenoids in this as well as other studies involving basil, mint, and other high-value crops (Carvalho et al., 2016; Colquhoun et al., 2013; Galeotti et al., 2008; Lee et al., 2005). Linalool is a critical component in the overall flavor of basil and many other herbaceous crops. The aroma of
linalool can be described as sweet and floral, very similar to the aroma of fruity-pebbles cereal. It has been shown to possess antioxidant and anti-inflammatory properties in addition to numerous other health benefits (Carvalho et al., 2016; Randall et al., 2014b). Concentrations peaked around 40B/60R, and the optimal lighting treatment did not separate from other LED lighting treatments. All LED treatments were significantly different than natural light controls; the optimal LED treatment showed a significant increase over the natural light controls.

α-Humulene is one of the primary chemical constituents that results in the flavor and aroma of flowering cones of the hops plant and many other herbaceous crops. This compound and its reaction products are essential for brewing processes and is responsible for the “hoppy” flavor and aroma in beer and other fermented products. This sesquiterpene can be found in many other plants in the Lamiaceae family and has relative impacts on flavor and aroma in many basil cultivars/varieties (Lee et al., 2005). All LED treatments were significantly higher than the natural light controls and HPS treatment; however, none of the LED treatments separated from one another, and the controls/HPS treatment did not separate from each other (Fig. 4.6 and 4.16). Volatile emission from LED treatments was approximately 10 µM/g FM higher than controls and HPS.

Linalool and α-humulene concentrations showed significance across growing seasons, with the highest concentrations in September, March, and April. The lowest concentrations for both isoprenoid compounds were found in June. Since they are both created with fundamental isoprene units and found in the same pathway, seasonal concentration similarities may be expected, dependent on a wide range of factors. Concentration levels between the two were found approximately 10:1 linalool:α-humulene and remained constant across all seasons, with less than 5% variance across all seasonal concentration ratios. This is expected, since linalool is a
monoterpenes and α-humulene is a sesquiterpene, both of which are synthesized in the same metabolic pathway. These results further suggest that terpene biosynthesis is increased with the addition of blue/red supplemental light, while other compounds (such as methyl eugenol) are decreased under the same conditions. This specific pathway and/or other secondary metabolic pathways that have great relevance for commercial producers and improve sensory quality should be further explored.

Hexanal is considered an aliphatic aldehyde and is used extensively in the food industry to produce fruity flavors. It has a fruity herbaceous odor that also resembles fresh cut grass. LED treatments showed some difference between controls, but concentrations were relatively low and lighting treatments did not make a significant impact (Fig. 4.7). The most noticeable change is on natural light 2 control, which was placed in one corner of the greenhouse with partial afternoon shade as opposed to full afternoon shade. Less than sufficient light intensity/spectral quality caused a substantial decrease in hexanal concentrations, but supplementing with B/R light vs. HPS treatments did not significantly increase hexanal concentrations. Further research should be conducted with regard to hexanal and other volatile compounds that are used extensively for their aromatic properties.

α-pinene and β-pinene are two compounds that have significant influence on the flavor and aroma of basil – both of which are impacted by the total concentration of all pinene compounds present as well as the concentration ratio of different pinene isomers in relation to one another. Pinene compounds are primarily found in pine resin, and they are one of the most abundant terpenoids in nature (Noma et al., 2010). These compounds have high biological activities in mammals (both beneficial medical properties as well as moderate toxicity levels) and strongly repel insects. Independent of lighting treatment, slightly higher levels of β-pinene were observed
in comparison to α-pinene (i.e. ratio of α/β) (Fig. 4.8 and 4.9). These ratios remained consistent across lighting treatments but were significantly different across season. For both isomers, optimal concentration peaked between lighting treatments 40B/60R and 60B/40R. All LED lighting treatment concentrations were significantly higher than the natural light controls for both pinene compounds. The HPS treatment concentrations did not statically separate from the natural light controls or lesser LED treatments, but the optimal LED treatment concentrations were significantly higher than the HPS treatment concentrations for both compounds.

These results are consistent with our ongoing conclusion that B/R wavelengths increase the production volume of monoterpenes while maintaining consistent ratios of isomer products, conformations, and analogs for each monoterpenic product. Further statistical analysis should be performed to determine if significant differences exist among these specific chemicals and broad chemical classes; this may require the implementation of metabolomics software and genomic analysis (Bourgaud et al., 2001; Carvalho et al., 2016; Colquhoun et al., 2013; Sugimoto et al., 2012) and may provide valuable insight on secondary metabolism. However, this is outside the scope of this project and may be explored in the future. Various studies that have investigated narrow-band wavelengths in relation to pinene isomers, monoterpenic hydrocarbon compounds, and broad secondary metabolic resource partitioning (i.e., phenylpropanoids, sesquiterpenoids, other isoprenoids, etc.) are congruent with the findings of this study (Carvalho et al., 2016; Colina-Coca et al., 2013; Colquhoun et al., 2013; Darko et al., 2014; Deschamps et al., 2006; Du et al., 2015; Fu et al., 2015; Klimánková et al., 2008; Loughrin et al., 2001; Loughrin et al., 2003; Olle et al., 2013; Ouzounis et al., 2014; Samuoliene et al., 2013; Selli et al., 2014; Tarchoune et al., 2013).
Eucalyptol, or 1,8-cineole, is another monoterpenoid that is closely related in both chemical/physical properties and structure to pinene, limonene, and other secondary metabolites produced from isoprene sub-units (Buchanan et al., 2015). Eucalyptol is the primary VOC that influences flavor in a variety of basil cultivars. Eucalyptol is the most abundant flavor volatile found in basil leaf tissues, accounting for over 50% of GC-MS response area in a variety of studies (Bantis et al., 2016; Carvalho et al., 2016; Claudia, 2013a; Klimánková et al., 2008; Lee et al., 2005; Loughrin et al., 2003; Tarchoune et al., 2013). It has a spicy, energizing, camphoraceous aroma with a cooling mint-like taste. Biological activity related to this compound is extremely high, in addition to moderate toxicity levels at relatively low concentrations. For these reasons, this compound is used in extremely low concentrations when manufacturing food products and cosmetics.

In this study, eucalyptol concentrations in LED lighting treatments were significantly higher than the natural light controls (Fig. 4.10). HPS treatment showed some separation for optimal LED treatments, but not full separation from other LED treatments and the natural light controls. 40B/60R produced the highest eucalyptol concentrations, with nearly 1 mM emission per gram of fresh leaf tissue. Estragole, (R)-(+) -limonene, α-pinene, β-pinene, phenyl-2-pthanol, and eucalyptol showed similar concentration patterns across growing seasons (Fig. 3.17, 3.18, 3.19, 3.20, and 3.21). While the specific concentrations of the individual compounds found in plant tissue varied greatly, they all followed approximate concentration ratios as growing seasons progressed. September showed the highest concentrations of these compounds, while November and June showed the lowest concentrations. This may be explained through established physiological responses across growing season in addition to volatilization at higher growing temperatures, up/down regulation of specific volatile biosynthesis in response to variation in solar
spectra/intensity, and secondary metabolic resource partitioning. The ratios of two important flavor compounds, \( \alpha \)-pinene and \( \beta \)-pinene, varied slightly across growing seasons progressed, which suggests an up/down regulation at this point in the metabolic pathway requiring further investigation. In addition, specific volatiles were impacted at varying levels during early fall/late spring months, with specific sesquiterpenoids and other phenols being impacted the most during months with poor spectral quality and lower intensities. In general, monoterpenes were found in higher concentrations using LED supplemental lighting, and concentrations were significantly increased during winter months and seasons with low-moderate lighting conditions with unfavorable spectral quality. Seasonal variations were observed for many of the compounds evaluated, and the results of this study demonstrate that the use of LED lighting may be used to supplement natural photoperiods with the intention of optimizing sensory quality.

Overall, eucalyptol, (R)-(+) -limonene, linalool, and methyl eugenol showed the highest calibrated concentration abundance in basil for this experiment, approximately 1-3 mM/ g FM headspace emission across a variety of seasons and treatments. All other flavor volatiles were significantly lower, within the range of 1.0 mM/g FM to 0.01 \( \mu \)M/g FM headspace emission. Based on GC-MS analysis, this study demonstrates that supplemental narrow-band wavelengths have the ability to alter secondary metabolism resource partitioning in basil, specifically isoprenoids and phenol groups. Some significant differences between HPS lighting and LED supplements were observed for specific compounds, but many were not significantly different. There is no question that supplemental lighting increases biomass and the concentrations of many important flavor volatiles in basil. While some concentrations of flavor volatiles were not significantly impacted across LED/HPS lighting, other factors may be involved with the final supplemental lighting purchase, such as energy efficiency, initial cost, and/or specific uses such as the desire to
manipulate VOC concentrations in herbaceous crops to create designer flavor profiles. Considerations involved with evaluating the efficacy of HPS lighting and LED lighting to manipulate secondary metabolism also include the end goal of the crop and financial-sustainability. All of the other compounds evaluated in this study did not show significant concentration differences across season, treatment, or season*treatment interaction and were not reported. Plans for additional research are being implemented to test a variety of basil cultivars in order to determine additional impacts of narrow-band wavelengths of supplemental lighting on secondary metabolism, specifically flavor and aroma volatiles that have a direct impact on basil quality and favorable sensory perception.

For most of the compounds evaluated, individual LED lighting treatments did not statistically separate, but showed significant concentration increases over HPS treatments and natural light controls. 40B/60R treatment consistently resulted the highest concentrations (with the exception of methyl eugenol and hexanal). Many of the LED treatments statistically separated from natural light controls and HPS supplements. All key flavor volatiles evaluated had significant impacts on emission concentrations across various lighting treatments. While it was not measured in this experiment, LED treated basil samples had advanced levels of lignification and were very difficult to process in comparison to natural light and HPS samples, further suggesting that primary and secondary metabolic pathways partitioned resources differently when exposed to B/R wavelengths.

Overall, lighting treatments were shown to have significant benefits for the manipulation of flavor volatiles, since the intensity of supplemented light was approximately 1/10 to 1/20 of the available natural light (depending on growing season and weather conditions). This confirms that the spectral quality of light has a substantial impact on flavor volatiles and sensory qualities of
basil in addition to other high-value herbaceous crops. This also indicates that supplemental blue/red wavelengths have direct impacts on secondary metabolite partitioning, necessitating further exploration to determine specific mechanisms and impacts of various supplemental wavelengths.
References D


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# Appendix D

**Table 4.1** Environmental conditions during growing periods.

<table>
<thead>
<tr>
<th></th>
<th>September</th>
<th>November</th>
<th>January</th>
<th>March</th>
<th>April</th>
<th>June</th>
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<td>10/14/15-</td>
<td>12/16/15-</td>
<td>2/10/16-</td>
<td>3/11/16-</td>
<td>5/16/16-</td>
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<tr>
<td><strong>Average Day Temp (°C)</strong></td>
<td>27.33</td>
<td>26.84°C</td>
<td>25.83°C</td>
<td>25.85°C</td>
<td>26.95°C</td>
<td>27.84°C</td>
</tr>
<tr>
<td><strong>Average Night Temp (°C)</strong></td>
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<td>20.62°C</td>
<td>19.11°C</td>
<td>21.01°C</td>
<td>21.94°C</td>
<td>22.56°C</td>
</tr>
<tr>
<td><strong>Average Relative Humidity</strong></td>
<td>55%</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>Average Daily Light Integral (DLI) (mol m$^{-2}$ d$^{-1}$)</strong></td>
<td>9.81</td>
<td>3.26</td>
<td>4.65</td>
<td>8.62</td>
<td>11.99</td>
<td>14.55</td>
</tr>
<tr>
<td><strong>Average Day Length (h)</strong></td>
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<td>10.35</td>
<td>9.93</td>
<td>11.65</td>
<td>13.20</td>
<td>14.27</td>
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<td><strong>Average Natural Blue (447nm) Intensity at Noon (µmol·m$^{-2}$·s$^{-1}$)</strong></td>
<td>138</td>
<td>122</td>
<td>103</td>
<td>121</td>
<td>133</td>
<td>145</td>
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<tr>
<td><strong>Average Natural Red (627nm) Intensity at Noon (µmol·m$^{-2}$·s$^{-1}$)</strong></td>
<td>148</td>
<td>132</td>
<td>108</td>
<td>135</td>
<td>144</td>
<td>156</td>
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Impact of Light Treatment on Key Flavor Volatiles

**Figure 4.1** Influence of LED treatments on (S)-(−)-Limonene concentrations (µM·g−1 FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 4.2** Influence of LED treatments on (R)-(+)–Limonene concentrations (µM·g−1 FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 4.3 Influence of LED treatments on 1,3,6-Octatriene, 3,7-dimethy-, (E) concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 4.4 Influence of LED treatments on Methyl Eugenol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 4.5** Influence of LED treatments on Linalool concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 4.6** Influence of LED treatments on α-Humulene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 4.7** Influence of LED treatments on Hexanal concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 4.8** Influence of LED treatments on α-Pinene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 4.9** Influence of LED treatments on β-Pinene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 4.10** Influence of LED treatments on Eucalyptol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Seasonal Impacts on Key Flavor Volatiles

**Figure 4.11** Influence of season on (S)-(−)-Limonene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 4.12** Influence of season on (R)-(+)−Limonene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 4.13 Influence of season on 1,3,6-Octatriene, 3,7-dimethy-,(E) concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 4.14 Influence of season on Methyl Eugenol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 4.15 Influence of season on Linalool concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

![Linalool Concentrations in Hydroponic Basil Across Growing Seasons](image)

**Figure 4.15** Influence of season on Linalool concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 4.16 Influence of season on α-Humulene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

![α-Humulene Concentrations in Hydroponic Basil Across Growing Seasons](image)
Figure 4.17 Influence of season on Estragole concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 4.18 Influence of season on α-Pinene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 4.19 Influence of season on β-Pinene concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 4.20 Influence of season on Phenyl-2-Ethanol concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 4.21 Influence of season on Eucalyptol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
CHAPTER 5: DAILY LIGHT INTEGRAL IMPACTS BIOMASS ACCUMULATION AND NUTRIENT UPTAKE IN HYDROTONICALLY GROWN BASIL
Abstract

Light quantity, quality and duration are three primary factors that impact plant growth and development. Light-emitting diodes (LEDs) have the ability to manipulate each of these parameters and allow commercial growers to optimize biomass yield and plant quality throughout growing seasons. Many studies have evaluated the impact of spectral quality and minimum daily light integral (DLI) requirements for specialty crops. However, an in-depth efficacy comparison of progressive incremental DLIs using LED and high-pressure sodium (HPS) sources is needed to determine optimal lighting durations for a variety of crops, specifically for improving yield and quality of greenhouse produced high-value specialty crops during winter months. The objective of this study was to determine the impact of progressive incremental DLIs on greenhouse hydroponic basil (*Ocimum basilicum* var. ‘Genovese’) production using broad spectrum HPS lamps and blue (B)/red (R) narrowband wavelengths from LED lighting systems. Overall edible biomass accumulation and nutrient uptake were evaluated. A total of nine lighting treatments were used: one non-supplemented natural light control, two HPS treatments with DLIs as 6 h and 12 h, and six 20B/80R LED treatments with progressive DLIs as 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h. Each supplemental lighting treatment provided 100 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}\). The DLI of the natural light control averaged 9.5 \(\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}\) during the growth period (ranging from 4 to 18 \(\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}\)). Relative humidity averaged 50%, with day temperatures averaging 29.4 °C and night temperatures averaging 23.8 °C. All treatments were harvested 45 d after seeding. Edible biomass accumulation and nutrient uptake were significantly impacted by supplemental lighting treatments and growing season. The 6 h HPS treatment had the highest total biomass accumulation, both in fresh (FM) and dry biomass (DM); the 9 h LED treatment produced the lowest FM and DM averages across all seasons. The 18 h LED treatment produced the highest FM and DM of any other LED treatment.
but was not statistically separate from the optimal HPS treatment. The natural light control produced moderate FM and DM values, surpassing four of the LED treatments. January growing season produced the lowest FM and DM values in comparison to November, which had the highest. Mineral analysis revealed that both macro and micronutrient accumulation was impacted with incremental DLI supplements across growing seasons and lighting type. This experiment shows that the spectral qualities and DLIs of both supplemental sources and natural sunlight have varying levels of impact on the growth and development of basil. This study demonstrates that LED and HPS lighting systems both have merits for optimizing biomass accumulation and nutrient uptake in basil; however, both biomass optimization and nutrient tissue concentration increases are highly dependent on proper cultural practices and a variety of other factors.

**Introduction**

Plants have the ability to perceive and respond to variety of environmental stimuli in order to improve their chances of survival and reproduction. Light is one of the most important factors that impacts plant growth, development and morphology (Briggs et al., 2002; Moe et al., 2006). A sophisticated network of photoreceptors is responsible for sensing and reacting to changes in spectral quality, fluence rate, and duration (Briggs et al., 1999; Christie, 2007; Hutchinson et al., 2012; Oh et al., 2009; Runkle et al., 2006; Smith, 1982). Unfavorable environmental conditions can dramatically impact the response of these photoreceptors and prompt other undesirable morphological and physiological responses (Chaves et al., 2011; Fraikin et al., 2013). Many specialty crop producers use controlled environments to improve overall yields and quality. Low light intensity and poor spectral quality during winter months forces some growers to provide supplemental lighting in order to achieve successful year-round production and maintain crop
quality. Supplemental lighting regimes have been suggested for these situations in order to satisfy daily light integral (DLI) crop requirements and provide sufficient spectral quality for a variety of supplemental lighting sources (Currey et al., 2015; Fausey et al., 2005; Faust et al., 2005; Garland et al., 2010; Sinclair et al., 1989; Warner et al., 2003; Warner et al., 2005). In commercial production, many growers use high pressure sodium (HPS) or light-emitting diode (LED) supplements spanning 12-24 h per day, with intensities commonly ranging from 100-200 µmols m$^{-2}$sec$^{-1}$ for high-value specialty crops (Randall et al., 2014b; Sinclair et al., 1989). HPS lighting systems have been the predominant choice for commercial operations, but LEDs may prove advantageous for optimizing growth and development characteristics for controlled environment production (Randall et al., 2014b).

Daily light integral is known as the cumulative amount of photosynthetically active photons that are received by the plant’s canopy in a 24 h period (Fausey et al., 2005). Photosynthesis is known to quickly increase asymptotically with the detection of light until it reaches its specific saturation limit, and many responses are species specific and dependent on spectral quality (Faust et al., 2005; Garland et al., 2010). DLI requirements vary considerably across species, but it has been generalized that most perennial crops require 10-16 mol m$^{-2}$d$^{-1}$ to satisfy quality standards (Faust et al., 2005). Previous studies on a variety of herbaceous crops have revealed that the relationship between DLI and biomass accumulation (DM) is mostly linear until approximately 20 or 30 mol m$^{-2}$d$^{-1}$ (higher DLIs for a wide range of other species such as C4 crops) (Faust et al., 2005; Warner et al., 2003). Another study observed linear increases in biomass (DM), plant quality and inflorescence number on a variety of herbaceous crops with increasing DLI from 5-20 mol m$^{-2}$d$^{-1}$ (Fausey et al., 2005; Frąszczak et al., 2009; Moccaldi et al., 2007; Runkle et al., 2006). That being said, the southeast region receives anywhere from 2-15 mol m$^{-2}$d$^{-1}$
throughout fall and winter months (Korczynski et al., 2002). This amount may be further reduced by poor weather conditions or cloud cover. Greenhouse covers (glass, plastic, etc.) as well as structural supports decrease natural DLIs, up to 60% in some cases (Frąszczak et al., 2009; Hutchinson et al., 2012; Moccaldi et al., 2007). For these reasons, optimal DLI requirements may not be satisfied during unfavorable growing seasons without supplements.

Spectral quality of the DLI supplement is a substantial factor, as plants have various photomorphogenic responses based on photoreceptors and other photoactive compounds that react to specific wavelengths within the spectrum (Carvalho et al., 2016). This is one major benefit of LED lighting in comparison to HPS and other traditional lighting systems, as the intensity/spectral output can be easily managed. In theory, specific narrow-band wavelength supplements can be used to target photoreceptors with the intention of manipulating primary and secondary metabolism, inducing phototropism and promoting desirable growth/development characteristics (Carvalho et al., 2016; Goins et al., 1997; Haque et al., 2015; Massa et al., 2008). Natural spectral quality and intensity can be measured in real-time and utilized to provide optimal quantity, quality and duration of supplemental light for a variety of crop across all growing seasons. This can be achieved without wasting energy on unusable wavelengths or heat energy that may be produced by broad-spectrum lighting sources.

For this reason, LED lighting systems have the potential of significantly reducing energy costs of commercial producers in the horticulture industry. It should be noted that in-depth efficacy comparisons between both types of lighting sources should be performed to determine the economic feasibly and production quality of LED and HPS lighting systems in relation to non-supplemental greenhouse environments across a wide range of crops, specifically during winter months or unfavorable growing seasons (Ouzounis et al., 2015b; Pimputkar et al., 2009). It is well
known that LEDs produce significantly less radiant heat energy than HPS lighting systems. Most of the radiant heat energy produced by HPS lamps are directed at the crop canopy’s surface, while LED heat is mostly radiated through thermal sinks and directed away from the canopy’s surface. Consequently, the ambient temperature of the greenhouse may be impacted depending on the design of the heating system, outside temperatures and distance between the crop and lighting source. HPS lighting sources may contribute significantly more radiant heat energy to the canopy’s surface temperature, which may prove useful during cold months by increasing photosynthetic rates in comparison to plants without that additional heat. It may conversely prove harmful for plans with low leaf temperature requirements, especially during summer months with high light intensities and ambient temperatures, resulting in heat stress and photodamage. Adjustments to ambient air temperature may be required to keep leaf temperatures consistent across day and night periods, requiring additional ventilation strategies or specific on/off schedules. For crops with lower leaf temperature requirements, LED lighting may prove useful for the management of desired humidity, temperatures and DIF values (Frąszczak et al., 2009; Moccaldi et al., 2007; Runkle et al., 2006). A few other factors that may influence the efficacy comparison between these two lighting sources include: broad vs. narrow spectra and their ability to target key photoreceptors, accessory pigments and photosynthetic systems that use these specific wavelengths, changes in sensory quality, desirable morphological impacts, local energy costs in comparison to environmental conditions and natural solar spectra, total cost of each lighting type and number of lights purchased, spectral quality in comparison to intensity output and broad/specific crop requirements.

LED lighting systems have the potential to replace traditional lighting systems in a wide range of applications. Studies suggest modern LED lighting systems, particularly systems
providing optimized ratios of blue and red wavelengths, promote desirable morphological
characteristics in addition to positively impacting primary and secondary metabolism, net biomass
yield and nutritional content in basil (*Ocimum basilicum*). as well as other specialty crops and high
value herbs (Abney et al., 2013; Chong et al., 2014; Colquhoun et al., 2013; Currey et al., 2012;
Currey et al., 2015; Fausey et al., 2005; Faust et al., 2005; Hogewoning et al., 2010b; Hutchinson
et al., 2012; Kopsell et al., 2011; Kopsell et al., 2012; Kopsell et al., 2013; Kopsell et al., 2014;
Kopsell et al., 2015, 2017; Matsuda et al., 2016; Poel et al., 2017; Randall et al., 2014b; Samuolienė
et al., 2009; Sharafzadeh et al., 2011; Tuan et al., 2013; Wink, 2010; Yousif et al., 1999). For these
reasons, it is advantageous to further explore the possibilities of LED lighting systems and
determine how different wavelengths impact plant growth and development at a fundamental level.

The primary objective of this project is to determine the impact of specific narrow-band
blue/red wavelengths (447 nm/627 nm) from solid-state LED lighting systems on the physiology
of greenhouse hydroponic basil (*O. basilicum* var. ‘Genovese’); specifically, how these
wavelengths impact edible biomass yield and nutrient uptake across incremental DLI increases
from LED supplemental sources in comparison to HPS supplemental sources and natural light
controls. The Genovese cultivar of sweet basil was specifically chosen because of its unique flavor
profile, high market demand and preference among professional chefs. Fresh mass (FM) and dry
mass (DM) were evaluated and quality tests were performed to determine impacts of blue/red
supplemental lighting. If an increased duration of our previously established narrow-band blue/red
supplementation is useful for improving edible biomass for high-value specialty crops, it may be
very beneficial to commercial herb production.
**Materials and Methods**

This project was conducted at The University of Tennessee Institute of Agriculture (UTIA) in Knoxville, TN, USA (35°56'44.5"N, 83°56'17.3"W). Growing dates for these six experimental runs occurred from October 2016 to June 2017 and have been labeled as growing seasons.

**Cultural Techniques and Environmental Growing Conditions**

‘Genovese’ basil seeds (Johnny’s Select Seeds, Winslow, ME) were germinated in peat moss based cubes (Park’s Bio Dome Sponges, Hodges, SC) at 83 °C and 95% RH. After 2 weeks, seedlings were transplanted into 5 x 5 cm plastic pots using peat moss and perlite based potting mix. Relative humidity during the growth period averaged 55%. Day temperatures averaged 29.4 °C, while night temperatures averaged 23.8 °C. The DLI of the natural light controls averaged 9.9 mol·m⁻²·d⁻¹ during the growth period (ranging from 4 to 20 mol·m⁻²·d⁻¹). Specific growing parameters for each of the seasons may be found in Table 4.1, Appendix C.

Emphasis was placed on investigating biomass yield and nutrient uptake in response to specific DLI using narrow-band blue/red (447nm/627nm) LED light. A total of nine lighting treatments were added immediately after seedling transplant: one non-supplemented natural light control, two HPS treatments with DLIs as 6 h and 12 h, and six 20B/80R LED treatments with progressive DLI as 3 h, 6 h, 9 h, 12 h, 18 h and 24 h (Orbital Technologies, Madison, WI). The 20B/80R LED lighting ratio was specifically chosen for this experiment because of its prevalence in recent literature; it was also deemed optimal for a variety of growth and development parameters from a previous experiment in this thesis (CH 2). Each supplemental lighting treatment provided 100 µmol·m⁻²·sec⁻¹. Supplemental lighting regimes for all treatments were initiated each day 1 h before sunset, which is commonplace for commercial herb production. A randomized complete
block design was used for this experiment, and lighting treatments were randomized after each experimental run to account for variations in natural light intensity and spectral quality across growing seasons and the greenhouse production area. Basil plants were grown in ebb and flow hydroponic systems and watered for 5 min each day with full strength general-mix nutrient solution; the fertility regime was kept constant across the duration of all seasons. Total growth time lasted approximately 45 d across all 6 experimental runs. Like many commercial basil growing operations, harvest occurred as the first signs of change from vegetative to reproductive growth were observed; care was taken to ensure each growing season was harvested at similar growth stages, and representative samples were taken from each of the two plants within each sub-rep. Each of the nine treatments consisted of 36 plants, for 324 plants per experimental run (season), and 1,296 total basil plants for phase two of this experiment. Sub-reps consisted of two plants each to improve statistical power and decrease variance due to uncontrollable factors.

**Mineral Extraction**

To determine nutrient uptake changes in basil plants across supplemental lighting treatments and growing seasons, samples were analyzed for macro and micronutrient content. Air-dried samples were ground into a fine power using a Magic Bullet blender (MBR1101, Homeland Housewares). 0.5 g ($\pm 0.01$) of ground plant material was weighed into 15-ml sterile plastic centrifuge test tubes. An Ethos 1112 microwave digestion unit was used to process the basil samples. Samples were microwaved for 30 min at 150 °C, then cooled for an additional 30 min. A 9.9 ml of ICP matrix solution (2% nitric acid, 0.5% hydrochloric acid, 97.5% RO water) was placed into 15-ml sterile test tubes. A disposable 1-ml plastic pipette was used to add 0.1 ml of the acid digested sample mixture to the 9.9-ml ICP matrix solution. This mixture was then thoroughly
shaken to ensure that the acid was uniformly distributed within the matrix. An Agilent 7500 Series ICP-MS was used to determine the mineral content of each of the samples (Barickman et al., 2013).

**Statistical Analyses**

A Randomized Complete Block Design was used for this experiment. All data sets were analyzed by GLM and Mixed Model Analysis of Variance procedures using the statistical software SAS (version 9.4, SAS Institute, Cary, NC). Design and Analysis macro, created by Dr. Arnold Saxton (DandA.sas), was utilized in addition to Tukey’s adjustment, regression analysis and univariate/normalization procedures to provide additional statistical insights on the complete data set. Treatments were separated by least significant difference (LSD) at $\alpha=0.05$.

**Results**

Biomass parameters and nutrient concentrations were evaluated in this experiment, many of which were significantly influenced by growing season, lighting treatment and season by treatment interactions. Data presented include plant weights (g), heights/diameters (cm) and mineral concentrations ($\mu$g·g$^{-1}$ DM).

**Biomass**

Total FM was significantly impacted by season ($P=0.0001; F=138.36$) and lighting treatment ($P=0.0001; F=62.23$), but did not show significant season*treatment interactions ($P=0.1235; F=9.62$). The November growing season produced the highest biomass, while the lowest was produced in the January growing season (Fig. 5.3). HPS lighting treatments 6 h and 12 h produced the highest biomass across all growing seasons (Fig. 5.1). The optimal LED treatment
was 18 h and did not statistically separate from the HPS treatments; it was only 3 g less on average than the 6 h HPS treatment. The natural light control averaged 43 g FM per plant in comparison to the optimal LED treatment, which averaged 53 g FM per plant. Across all seasons, the lowest FM per plant was produced under the 9 h LED treatment.

Total plant DM followed a similar pattern to FM and was significantly impacted by season (P=0.0001; F=144.24) and lighting treatment (P=0.0001; F=61.21), but not by season*treatment interactions (P=0.2651; F=7.88). The November growing season again produced the highest DM, while the lowest DM was produced in the January growing season (Fig. 5.4). HPS lighting treatments 6 h and 12 h produced the highest DM across all growing seasons averaging 6.8 g and 5.9 g DM per plant respectively (Fig. 5.2). The optimal LED treatment was 18 h and did not statistically separate from the HPS treatments; it was only 0.21 g less on average than the 6 h HPS treatment. The natural light control produced 5.2 g DM in comparison to the optimal LED treatment, which was 6.4 g. The lowest DM was again produced under the 9 h LED treatment.

**Minerals**

Tissue boron (B) was significantly impacted by season (P=0.0001; F=62.03), lighting treatment (P=0.0001; F=5.32) and season*treatment interactions (P=0.0027; F=2.14). Plants grown in January had significantly higher B concentrations in comparison to all other seasons (Table 5.5). The 9h LED treatment had optimal B concentrations across all growing seasons (89.2 µg·g⁻¹ DM), while the lowest was observed under the 6 h LED treatment (64.25 µg·g⁻¹ DM) (Table 5.3).

Tissue sodium (Na) was significantly impacted by season (P=0.0001; F=84.89), lighting treatment (P=0.0002; F=4.13) and season*treatment interactions (P=0.0001; F=3.48). Plants
grown in the November season had significantly higher Na concentrations, while spring seasons had lower Na concentrations (Table 5.5). The lowest levels were observed in many treatments during the March growing season. The optimal treatment observed across all growing seasons was 6 h LED (278.49 µg·g⁻¹ DM), while the lowest was 6 h HPS (205.25 µg·g⁻¹ DM). The natural light control fell between the optimal and lowest treatments (Table 5.3).

Tissue magnesium (Mg) was significantly impacted by season (P=0.0001; F=11.78), lighting treatment (P=0.0118; F=2.25) and season*treatment interactions (P=0.0283; F=1.70). Seasonal concentrations varied, but showed moderately significant decreases during the March growing season (Table 5.4). Mg concentrations in 18 h LED treatment and the 6 h HPS treatments were found to be elevated (7.716 mg·g⁻¹ DM and 7.709 mg·g⁻¹ DM) in comparison to HPS and natural light controls; however, there was variation across treatments, and many did not statistically separate (Table 5.2).

Tissue phosphorous (P) was significantly impacted by season (P=0.0001; F=47.30) and lighting treatment (P=0.0035; F=3.00), but did not show season*treatment interactions (P=0.1051; F=1.42). January and June seasons accumulated the most P (9.571 mg·g⁻¹ DM and 9.162 mg·g⁻¹ DM) in comparison to the March growing season (6.524 mg·g⁻¹ DM) (Table 5.4). There were slightly elevated levels of P in the 12 h LED treatment across all seasons and decreased levels in the 6 h LED treatment (8.998 mg·g⁻¹ DM and 7.496 mg·g⁻¹ DM); however, most of the treatments did not statistically separate from the group, with the exception of the two previously mentioned treatments (Table 5.2).

Tissue potassium (K) was significantly impacted by season (P=0.0001; F=8.82) and season*treatment interactions (P=0.0043; F=2.05), but not by lighting treatment (P=0.0531; F=2.21). Plants grown in January had significantly higher K concentrations, while spring seasons
had varying K concentrations (Table 5.4). There were no statistically significant K impacts observed among lighting treatments (Table 5.2).

Tissue sulfur (S) was significantly impacted by season (P=0.0001; F=57.33), lighting treatment (P=0.0001; F=5.91) and season*treatment interactions (P=0.0001; F=2.95). Plants grown in January and June had significantly higher S concentrations, while fall and spring seasons had varying S concentrations (Table 5.4). There were no statistically significant S impacts observed among lighting treatments, with the exception the highest treatments (24 h LED and 6 h HPS) and the lowest treatment (6 h LED) (Table 5.2).

Tissue calcium (Ca) was not significantly impacted by season (P=0.0768; F=2.32), lighting treatment (P=0.0636; F=1.89) or season*treatment interactions (P=0.1134; F=1.40) (Table 5.4, 5.2).

Tissue manganese (Mn) was significantly impacted by season (P=0.0001; F=37.62), lighting treatment (P=0.0359; F=2.12) and season*treatment interactions (P=0.0024; F=2.16). Plants grown in January showed the highest Mn concentrations, while all other months were significantly lower (Table 5.5). There were no statistically significant Mn impacts observed among lighting treatments with the exception of 12 h LED and 6 h LED (127.01 µg·g⁻¹ DM in comparison to 104.13 µg·g⁻¹ DM) (Table 5.3).

Tissue iron (Fe) was significantly impacted by season (P=0.0001; F=42.11), lighting treatment (P=0.0005; F=3.74) and season*treatment interactions (P=0.0116; F=1.87). Plants grown in January had significantly higher Fe concentrations, while the November growing season had the lowest concentrations (Table 5.5).

Tissue copper (Cu) was significantly impacted by season (P=0.0001; F=37.24), lighting treatment (P=0.0040; F=2.96) and season*treatment interactions (P=0.0019; F=2.20). Plants
grown in November had somewhat higher Cu concentrations, but significance varied across winter and spring months (Table 5.5). There were no statistically significant Cu impacts observed among lighting treatments, but LED treatments showed elevated levels in comparison to the natural light controls and HPS treatments (Table 5.3).

Tissue zinc (Zn) was significantly impacted by season (P=0.0001; F=7.21) and lighting treatment (P=0.0106; F=3.74), but no significant season*treatment interactions (P=0.1026; F=1.42) were observed. Plants grown in November had significantly higher Zn concentrations, while winter and spring seasons had varying Zn concentrations (Table 5.5). There were no statistically significant Zn impacts observed among lighting treatments with the exception of the 9 h LED treatment and the natural light control (60.21 µg·g⁻¹ DM vs. 51.33 µg·g⁻¹ DM) (Table 5.3).

**Discussion**

Three of the primary environmental factors that influence plant growth and development are light quality, quantity and duration (Colquhoun et al., 2013; Galvao et al., 2015; Ouzounis et al., 2015b; Samuoliene et al., 2012; Smith, 1982). Improving cultural practices and optimizing environmental conditions such as light intensity, spectral quality and photoperiod may provide significant benefits in terms of yield, quality and overall profit margins for commercial growers (Randall et al., 2014b) Because light is critical for successful plant growth and development, supplemental lighting plays a major role in the horticulture industry (Janick et al., 2015). Traditional lighting systems, such as HPS, has an unchangeable broadband spectrum, while LEDs have the ability to produce easily adjustable narrowband wavelengths (Jishi et al., 2016; Morrow, 2008; Singh et al., 2015). Determining the efficacy and economic feasibility of these types of
lighting systems in comparison to spectral quality and intensity across a wide variety of crop species would benefit commercial growers producing high-value specialty crops.

Using growth chambers and greenhouses, a large number of spectral distributions have been applied to many crop species in order to determine the impacts of LEDs and fundamentally understand the impact of different wavelengths on plant growth and development (Bantis et al., 2016; Carvalho et al., 2016; Frąszczak et al., 2009; Goins et al., 1997; Haque et al., 2015; Hogewoning et al., 2010a; Hogewoning et al., 2010b; Kopsell et al., 2013; Kopsell et al., 2014; Lin et al., 2013; Martineau et al., 2012; Massa et al., 2008; Matsuda et al., 2016; Moccaldi et al., 2007; Oh et al., 2009; Olle et al., 2013; Ouzounis et al., 2015a; Petersen et al., 2010; Singh et al., 2015; Tennessen et al., 1994). As optimal wavelengths are further investigated for greenhouse crops, focus should be placed on DLI requirements in comparison to natural solar spectra and photoperiods. While outdoor DLIs can exceed 50 mol m\(^{-2}\) d\(^{-1}\) in some parts of the continental US, the DLI delivered to greenhouse bench crops is highly variable and typically ranges from 1 to 25 mol m\(^{-2}\) d\(^{-1}\) (Chong et al., 2014; Faust et al., 2005). Depending on season, location and weather conditions, these values may be even lower. Photoperiods (or day length) across the country range from less than 8 h per day to upwards of 16 h per day during the summer solstice (Fausey et al., 2005; Faust et al., 2005; Korczynski et al., 2002). Failure to meet DLI crop requirements throughout the year will reduce overall productivity and potentially limit growing seasons. In addition, lack of natural light intensity and spectral quality will prevent optimal plant growth and development (Janick et al., 2015). Optimizing environmental conditions in greenhouse production (specifically light intensity, spectral quality and duration) has the potential to manipulate primary metabolic pathways as well as impact edible biomass accumulation and improve nutrient uptake (Massa et al., 2008; Ouzounis et al., 2014).
On average, DM was approximately 10-12% FM across all seasons and treatments. FM and DM showed significant impacts among growing seasons and lighting treatments, namely patterns that resulted from inherent characteristics of each lighting system, supplemental lighting treatment (amount of progressive DLI increment), spectral quality variations and natural DLI provided across growing seasons (Fig. 5.1-5.6). Specific LED lighting regimes (LED 18 h and/or LED 24 h) resulted in the highest FM during November, March and May growing seasons. In contrast, the HPS 6 h and 12 h lighting treatments overwhelmingly produced the highest biomass during the January growing seasons, 8 g higher than any other treatment during that month. It was interesting that the 6 h HPS lighting systems provided the most significant benefit to FM and DM during January, considering that the DLI supplement was ¼ the amount provided by the 24 h LED treatment (Fig. 5.5). Across all seasons, the 12 h HPS treatment had 2.5 g less than the 6 h HPS treatment, but those averages were not statistically separate. The LED treatments also saw a slight decrease from the optimal 18 h LED treatment to the 24 h LED treatment (approximately 6 g less). While DLI was a significant factor, these results suggest that other factors had influence on biomass production other than DLI alone. The FM and DM increases may have resulted from elevated leaf temperatures from the radiant heat produced by HPS lamps. Since the supplemental light treatments were initiated 1 h before sunset, a boost of radiant heat energy from HPS lighting systems during winter nights may have increased photosynthetic rates and kept light-dependent reactions active throughout the night period, resulting in significantly higher FM and DM for the January growing season. In comparison, some blue and red wavelength LED treatments were sufficient at improving FM and DM during all evaluated seasons, but less significant impacts were observed during winter months in comparison to the HPS treatments. This may have occurred because supplemental LED sources compensated for the natural solar spectrum and its lack of
these specific wavelengths. Blue and red wavelengths are critical for successful plant development, and lighting sources missing these wavelengths commonly see reductions in FM/DM in addition to significant morphology impacts (Bantis et al., 2016; Ouzounis et al., 2015b). Excess radiant heat produced from HPS sources during warm growing seasons may explain why FMs and DMs were not as pronounced during these seasons, as heat stress is known to reduce biomass and induce thermomorphogenic responses. Excess IR wavelengths may have hindered optimal photosynthetic rates, in addition to variance in DIF values between experimental treatments that may have influenced FM/DM. This may also explain why some visible signs of photodamage and oxidative stress were observed in the May growing season under the HPS treatments, while natural light controls during colder months showed reductions in biomass, intermodal length, leaf area, etc. Overall, the impact of the HPS treatment became more significant as day length decreased. Thermomorphogenic and photomorphogenic responses both appear to influence hydroponic basil FM and DM.

In the November and March seasons, increasing DLI directly correlated with higher FM and DM (Fig. 5.1). January and May seasons showed interesting relationships; the addition of progressive DLI supplements to natural photoperiods did not result in a linear increase in FM, as the 6 h and 9 h LED treatments resulted in the lowest FM/DM across all seasons. In addition, the 18 h treatment was the optimal LED treatment across all seasons in terms of FM and DM. Natural light treatments showed significantly lower FM and DM during winter growing seasons, but values increased during November and May seasons and strongly correlated with DLI provided from the natural solar spectrum. HPS and some of the LED treatments surpassed the FM and DM values across all seasons. This suggests that spectral quality and supplementation schedule optimization are important factors to consider when maximizing yield, in addition to the total DLI received by
a crop. DLI is an important factor in greenhouse production, but choice of lighting type and spectral quality has significant impact on biomass yields, all of which is highly dependent on growing season and specific greenhouse crop.

Temperature and light-induced stomatal regulation may have been a significant factor for biomass production in this experiment because of its significant role in primary metabolism, transpiration and gas exchange. While temperature variation is known to induce stomatal opening, one important factor that regulates stoma position is blue and red wavelengths (Briggs et al., 1999). At low PPFD (15 to 30 µmol·m\(^{-2}·s^{-1}\)), blue light will induce stomatal opening with red light being ineffective; as PPFD increases, stomatal opening is consistently higher for blue light than red light under concurrent PPFD, making the process more sensitive to blue wavelengths (Briggs et al., 1999; Matsuda et al., 2016; Olle et al., 2013; Ouzounis et al., 2014). In addition, stomatal conductance in leaves subjected to blue and red wavelengths were shown to be higher than in leaves subjected to only blue or red wavelengths, suggesting a synergistic action of stomatal regulation. It is worth noting that this increase in stomatal conductance under blue wavelengths may result from additive or synergistic effects with red light that was possible from morphogenic responses that include increased stomatal density, width aperture length, etc. that resulted from exposure to blue wavelengths (Buchanan et al., 2015; Chong et al., 2014; Dai et al., 2014; Tuan et al., 2013). Transpiration, gas exchange and water uptake all have direct impacts on plant growth and development in addition to biomass accumulation and nutrient content. Increased transpiration rates allow more nutrients to be absorbed by the plant. Increased water uptake is an important driver of photosynthesis and respiration, both of which are responsible for FM and DM. Higher levels of transpiration increase the amount of CO\(_2\) that passes through the stoma, resulting in higher carbon-fixation rate (i.e. photosynthetic rate) and increases in net biomass accumulation. It is likely
that a combination of these factors resulted in varying FM, DM and nutrient concentrations interactions between treatment and season.

As expected, supplemental lighting treatments significantly impacted basil FM and DM across all seasons (Fig. 5.1, 5.2). Various ratios of blue and red supplementary wavebands can impact biomass accumulation, morphology, physiology and nutrient uptake (Bantis et al., 2016; Buchanan et al., 2015; Carvalho et al., 2016; Currey et al., 2015; Darko et al., 2014; Janick et al., 2015; Kim et al., 2016; Kopsell et al., 2015; Lin et al., 2013; Matsuda et al., 2016; Olle et al., 2013; Ouzounis et al., 2014; Ouzounis et al., 2015b). The lighting treatment 6 h HPS produced the highest total biomass across all growing seasons evaluated and was significantly higher than the natural light controls and LED treatments. The 12 h HPS treatment did not statistically separate from the 6 h HPS treatment and was 2.4 g FM less on average than the optimal treatment. HPS treatment (6 h) was deemed optimal for FM and DM production in comparison to other lighting treatments, and this was most likely a result of radiant heat energy that raised leaf tissue temperatures and increased photosynthetic rates. The optimal LED treatment was 18 h, which was 3.6 g FM less on average than the 6 h HPS treatment and did not statistically separate. Across all seasons, the natural light control achieved higher FM and DM than four of the six LED treatments. The 9 h LED treatment produced the lowest FM (25.2 g) in comparison to the optimal LED treatment FM (52.4 g). Across all growing seasons, incremental DLI supplements were not directly correlated with increased FM or DM. That being said, November growing season averages were dramatically offset from the increased 3-12 h LED treatments in comparison to all other seasons (Fig. 5.5 and 5.6). This may be explained from previously discussed factors that have significant impacts on plant growth and development, such as photomorphogenic responses, variation in spectral quality across growing seasons, thermal differences between HPS/LED treatments and
effects of spectral quality on photosynthetic and respiration rates. Because the DLI supplements were applied 1 h before sunset, primary metabolic rates may have been significantly impacted as photosystems remained fully-active for some of the optimal treatments after sunset. Based on day length calculations, 18 h LED treatment resulted in a ‘24 h photoperiod’ for the basil crop, while the 9 h LED treatment resulted in a ‘15 h photoperiod.’ 24 h LED treatment may have provided above-optimal DLI levels or blue/red wavelengths that negatively impacted biomass production in comparison to the 18 h LED treatment. One study found that 500 μmol·m⁻²·sec⁻¹ for 16 hours per day produced optimal edible biomass production (Beaman et al., 2009), while others found that oversaturation resulted in significant decreases in edible biomass. In addition, the 24 h LED treatment provided an additional 2.1 mol·d⁻¹ of blue/red wavelengths during the morning hours as compared to the 18 h treatment, which shut off around sunrise. The generally established positive linear correlation between DLI and net biomass accumulation did not hold true for this experiment. Keeping photosystems active 24 h per day without oversaturating them may prove useful for optimizing FM while saving energy costs. Sub-optimal DLI, insufficient spectral quality and poor photoperiod schedules have been found to reduce biomass accumulation in a variety of plant species (Briggs et al., 1999; Goins et al., 1997; Moe et al., 2006; Runkle et al., 2006; Smith, 1982). As follows, the 9 h LED treatment’s low FM and DM averages may have resulted from lack of light quality, quantity and duration in comparison to other lighting treatments. Growing season had significant impacts on both FM and DM (Fig. 5.3, 5.4). January produced the lowest FM/DM values 31.2 g/ 3.81 g), while November had the highest (57.5 g/ 6.95 g). DLI shows moderately strong correlation to biomass accumulation across growing seasons, but it is apparent that other factors have significance.
Natural day length varied from 9.9 h to 13.5 h across this experiment (Table 5.1), while supplemental photoperiods ranged from 11.9 h to 24 h per day. In most cases, treatments that maintained natural day length schedules (November and May) in addition to treatments that extended the natural day length to between 18 h to 24 h per day resulted in the highest FM and DM averages. Because photosystems were kept active 24 h per day, light-dependent reactions were able to occur nonstop (all while light-independent reactions were free to occur). Plants have evolved with circadian rhythms and the ability to adapt their metabolism and physiology based on environmental cues (Darko et al., 2014). Maintaining a natural day length (i.e. 9.9 h to 13.5 h) may provide useful for synchronous light/dark reactions and increased biomass accumulation, given sufficient DLI and spectral quality requirements are met. These results suggest that light schedule and exposure length to supplemental lighting may be equally important as DLI for biomass accumulation. It is possible that overstimulation of blue wavelengths significantly impacted the circadian rhythms and primary metabolism of basil plants in this experiment. It would be beneficial to evaluate incremental DLI supplements starting at sunrise as compared to this experiment in order to determine which is more impactful in terms of biomass accumulation, physiology and morphology. Because day length and DLI do not share a direct relationship, exploration of equal DLIs and spectral quality over varying simulated day lengths in greenhouse settings (i.e. 200 µmol·m⁻²·sec⁻¹ for 12 h per day vs. 100 µmol·m⁻²·sec⁻¹ for 24 h per day) would be useful for determining the relationship between DLI, spectral quality and photoperiod.

Interactions between light treatment and growing season significantly impacted many of the nutrient concentrations evaluated, namely elevated macronutrient concentrations found 12-24 h LED treatments in the January growing season. This likely occurred because plants grown in January had lower FM averages in general, while the 12-24 h LED treatments in this season
resulted in some of the highest biomasses for that growing period. Because of the increased biomass, additional macronutrient concentrations are needed to satisfy nutrient requirements and facilitate healthy plant growth and development. Relatively low macro and micronutrient concentrations were observed in the natural light control, especially in the January growing season.

Growing season significantly impacted nearly all nutrient concentrations evaluated in this study (Table 5.3 and 5.4). Tissue P concentrations were highest in the 12 h LED treatment, with elevated levels shown in most LED treatments and HPS treatments. The natural light controls had slightly lower levels of P, but the lowest concentration was observed in the 6 h LED treatment. Tissue Ca concentrations were significantly impacted, with 12 h LED again being the optimal lighting treatment. Levels vary among LED treatments and separation does not exist between many of the LED treatments and the HPS treatment. The 6 h LED treatment showed lower Ca levels, but with varying levels of significance. Tissue S concentrations were highest in the 24 h LED and 6 h HPS treatments, with varying significance among other LED treatments. The 6 h LED again had the lowest concentration. Basil Mg levels showed some variance across lighting treatments. None of the LED treatments statistically separated from the rest of the group. Micronutrients that were impacted by lighting treatment were B, Zn, Fe and Na, which showed elevated levels in the LED treatments in comparison to HPS and natural light controls (Table 5.2). The LED treatments between 6 h and 24 h had the most significant micronutrient concentrations in comparison to the natural light control. Previous studies from our group (Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017) showed that nutrient uptake was impacted using supplemental blue/red wavelengths, following similar patterns to those observed in this study between comparable HPS treatments, LEDs treatments and natural light controls.
Overall, HPS lights provided optimal results for both fresh/dry edible biomass yield, while 9 h to 18 h LED treatments significantly increased the uptake of many macro and micronutrients when compared to HPS and natural light controls. For all parameters considered in this study, the optimal supplemental DLI ranged 4.2-6.3 mol\(\text{d}^{-1}\). Lighting type was a significant factor in terms of biomass accumulation and nutrient uptake. In this efficacy comparison, LEDs and HPS have proven their merits, but both systems have limitations. Additional efficacy comparisons between HPS and LED lighting systems should be conducted on a variety of parameters to determine economically favorable practices. This study supports the rapidly growing body of literature that details biomass accumulation and nutrient uptake impacts by manipulating light intensity, spectral quality and duration. Altering DLI and spectral quality through LED supplementation has significant implications for improving edible biomass yields, nutrient uptake and overall plant quality.
References E


# Appendix E

## Table 5.1 Environmental conditions during growing periods.

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<td>27.81</td>
<td>27.62</td>
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</tr>
<tr>
<td>Average Night Temp (°C)</td>
<td>22.31</td>
<td>20.11</td>
<td>21.83</td>
<td>22.43</td>
</tr>
<tr>
<td>Average Relative Humidity</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
</tr>
<tr>
<td>Average Daily Light Integral (DLI) (mol m(^{-2}) d(^{-1}))</td>
<td>5.66</td>
<td>4.81</td>
<td>8.95</td>
<td>12.77</td>
</tr>
<tr>
<td>Average Day Length (hours)</td>
<td>10.35</td>
<td>9.90</td>
<td>11.68</td>
<td>13.46</td>
</tr>
<tr>
<td>Average Natural Blue (447nm) Intensity at Noon (µmol m(^{-2}) s(^{-1}))</td>
<td>127</td>
<td>111</td>
<td>124</td>
<td>138</td>
</tr>
<tr>
<td>Average Natural Red (627nm) Intensity at Noon (µmol m(^{-2}) s(^{-1}))</td>
<td>133</td>
<td>112</td>
<td>133</td>
<td>145</td>
</tr>
</tbody>
</table>
Figure 5.1 Influence of progressive DLI increments on total plant fresh mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 5.2 Influence of progressive DLI increments on total plant dry mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Seasonal Impact on Biomass Accumulation

**Figure 5.3** Influence of progressive DLIs increments on total fresh mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 5.4** Influence of progressive DLIs increments on total dry mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 5.5 Influence of progressive DLIs increments on total plant fresh mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 5.6 Influence of progressive DLIs increments on total plant fresh mass of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
### Table 5.2 Influence of LED treatments on macronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>S</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED 3H</td>
<td>8472&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>53710&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16450&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4482&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>702&lt;sup&gt;2b&lt;/sup&gt;</td>
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<td>49830&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15740&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4160&lt;sup&gt;c&lt;/sup&gt;</td>
<td>654&lt;sup&gt;3b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LED 9H</td>
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<td>53030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17880&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4976&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>712&lt;sup&gt;1b&lt;/sup&gt;</td>
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<td>48967&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18370&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5374&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>758&lt;sup&gt;0b&lt;/sup&gt;</td>
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<td>LED 18H</td>
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<td>48810&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17090&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4969&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>771&lt;sup&gt;6a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LED 24H</td>
<td>7989&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>47150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16920&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5547&lt;sup&gt;a&lt;/sup&gt;</td>
<td>755&lt;sup&gt;3b&lt;/sup&gt;</td>
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<tr>
<td>HPS 6H</td>
<td>7844&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>45980&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17470&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5628&lt;sup&gt;a&lt;/sup&gt;</td>
<td>771&lt;sup&gt;0a&lt;/sup&gt;</td>
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<td>17430&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4732&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>734&lt;sup&gt;8b&lt;/sup&gt;</td>
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<td>Natural Light Control</td>
<td>7585&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46490&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16430&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>691&lt;sup&gt;4b&lt;/sup&gt;</td>
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</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*

### Table 5.3 Influence of LED treatments on micronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>B</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
<th>Na</th>
<th>Zn</th>
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<td>179.8&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>LED 6H</td>
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<td>28.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168.6&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>LED 9H</td>
<td>74.90&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>28.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>187.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>60.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LED 12H</td>
<td>89.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>173.8&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
<td>LED 18H</td>
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<td>24.71&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>164.7&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>237.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>LED 24H</td>
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<td>21.84&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
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<td>205.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>110.5&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>236.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>51.32&lt;sup&gt;b&lt;/sup&gt;</td>
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*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*
Seasonal Impact on Nutrient Concentrations

**Table 5.4** Influence of growing season on macronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Growing Season</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>S</th>
<th>Mg</th>
</tr>
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<tr>
<td>November</td>
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<td>48980&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16190&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3737&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7913&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>January</td>
<td>9571&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53880&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17390&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7061&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>March</td>
<td>6525&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45530&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17310&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>May</td>
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<td>17440&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5574&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7556&lt;sup&gt;ab&lt;/sup&gt;</td>
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*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*

**Table 5.5** Influence of growing season on micronutrient mineral concentrations of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

<table>
<thead>
<tr>
<th>Growing Season</th>
<th>B</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
<th>Na</th>
<th>Zn</th>
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<td>November</td>
<td>67.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>141.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>325.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>January</td>
<td>97.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>March</td>
<td>75.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>102.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>175.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.67&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>May</td>
<td>65.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.95&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>198.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.11&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>

*All concentrations are presented in micrograms per gram dry plant weight (µg·g<sup>-1</sup> DM). Mean values represent 2 plants per replication and 6 replications per treatment. Values were analyzed using Tukey’s protected LSD, and those followed by the same letter are not significantly different (α=0.05).*
CHAPTER 6: DAILY LIGHT INTEGRAL IMPACTS FLAVOR VOLATILES IN HYDROPONICALLY GROWN BASIL
Abstract

Plants have the ability to respond to a wide range of intensities and narrowband wavelengths from the solar spectrum. While many studies have shown that light emitting diode (LED) supplementation is useful for high-value specialty crop production, research is needed to determine the value and efficacy of LEDs in comparison to traditional lighting systems, with emphasis placed on determining the impact of spectral distribution and daily light integral (DLI) on secondary metabolism and flavor volatile production. The objective of this study was to establish the effects of progressive DLIs using LED and high-pressure sodium (HPS) supplementation on key flavor volatiles in hydroponic basil (*Ocimum basilicum* var. ‘Genovese’). A total of nine lighting treatments were used: one non-supplemented natural light control, two HPS treatments with DLIs as 6 h and 12 h, and six 20B/80R LED treatments with progressive DLIs as 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h. Each supplemental lighting treatment provided 100 µmols·m⁻²·sec⁻¹. The daily light integral (DLI) of the natural light control averaged 9.9 mol·m⁻²·d⁻¹ during the growth period (ranging from 4 to 20 mol·m⁻²·d⁻¹). Relative humidity averaged 50%, with day/night temperatures averaging 29.4 °C/23.8 °C, respectively. Basil plants were harvested 45 d after seeding, and flavor volatile profiles were obtained by GC-MS. Variations in spectral quality and DLI across growing season were compared to DLI supplements in order to determine the impact of LEDs and HPS when used to enhance the solar spectrum under greenhouse conditions. Flavor volatile concentrations varied significantly among seasons and lighting treatments. All of the compounds evaluated in this study showed significant concentration differences across lighting treatments and growing seasons. Many compounds showed a non-linear relationship as DLIs increased, with the greatest flavor volatile concentrations observed in LED ratios ranging from 12-24 h and the 6 h HPS treatment. Across all compounds evaluated, the 6 h HPS lighting treatment
produced comparable volatile organic compound (VOC) concentrations to the 24 h LED treatment, suggesting that DLI is only one of the many factors that drives secondary metabolism resource allocation. Further, concentrations of some compounds, such as methyl eugenol, were 3–4x higher in the 3 h LED treatment and decreased significantly for basil subjected to higher DLI increments. As DLI supplements were increased, secondary metabolism partitioning was significantly impacted; compared to the 3 h LED treatment, the 24 h LED treatment commonly showed significant increased mono and diterpenes, with reduced sesquiterpenes and phenols. The results of this study show that using LEDs to supplement natural photoperiods has the potential to manipulate secondary metabolism and flavor volatile production.

Introduction

Sweet basil (*Ocimum basilicum* L.) is a very popular commercially grown culinary herb that is valued for its complex flavor and aroma profile. Basil is rich in health-beneficial compounds such as antioxidants, carotenoids, volatile organic compounds (VOC), flavonoids, and phenols (Kopsell et al., 2005; Lee et al., 2005; Politeo et al., 2007). Providing optimal environmental conditions and light requirements are necessary for sufficient year-round crop quality and growth. Because quality is vital for the success of greenhouse operations, controlled environmental agriculture and supplemental lighting are commonly implemented to improve yields and crop quality (Janick et al., 2015). As new lighting technologies continue to evolve, research should be focused on determining optimal lighting quality, quantity, and duration for basil and other high-value specialty crops under greenhouse conditions.

Daily light integral (DLI) is known as the cumulative amount of photosynthetically active photons that are received by the plant’s canopy in a 24 h period (Fausey et al., 2005). DLI
requirements show considerable variation across species, but it has been generalized that most perennial crops require 10-16 mol·m⁻²·d⁻¹ to satisfy quality standards (Faust et al., 2005). Many studies have determined the optimal DLI for basil production is between 12-20 mol·m⁻²·d⁻¹ (Bantis et al., 2016; Carvalho et al., 2016; Olle et al., 2013; Singh et al., 2015). Other studies observed linear increases in biomass (DM), plant quality, and inflorescence number on a variety of herbaceous crops with increasing DLI from 5-20 mol·m⁻²·d⁻¹ (Fausey et al., 2005; Frąszczak et al., 2009; Moccaldi et al., 2007; Rundle et al., 2006). The southeast region receives anywhere from 2-15 mol·m⁻²·d⁻¹ throughout fall and winter months (Korczyński et al., 2002). This amount may be further reduced by sub-optimal weather conditions or cloud cover. Greenhouse covers (glass, plastic, etc.) as well as structural supports decrease natural DLIs and spectral quality, up to 60% in some cases (Frąszczak et al., 2009; Hutchinson et al., 2012; Moccaldi et al., 2007). For these reasons, optimal DLI requirements may not be achieved during a large portion of the year.

DLI variation can have significant impacts on plant morphology and secondary metabolism (Currey et al., 2015; Goins et al., 1997; Haque et al., 2015; Hutchinson et al., 2012; Poel et al., 2017; Sinclair et al., 1989). Plants exposed to high light intensities or specific narrow-band wavelengths promote short and long-term metabolic responses, such as xanthophyll cycle reaction rates, non-photochemical quenching, photorespiration, and antioxidant capacities (Ashraf et al., 2013; Croce et al., 1999; Frank et al., 1996; Li et al., 2009; Namitha et al., 2010; Young, 1991). Optimal light intensities and spectral distributions have proven useful for increasing terpenoid and phenylpropanoid production in a variety of specialty crops (Carvalho et al., 2016; Colquhoun et al., 2013; Jamieson et al., 2012; Namitha et al., 2010; Petersen et al., 2010). Some studies have indicated that plants exposed to extreme irradiances or specific narrow-band wavelength supplements have reduced secondary metabolite production, which suggests negative impacts on
sensory quality (Azari et al., 2010; Christiaens et al., 2014; Dai et al., 2014; Darko et al., 2014; Garland et al., 2010; Hikosaka et al., 2010). While many studies have been performed on the impacts of blue and red wavelengths from LED sources, the mechanistic aspects of LED lighting and the impact of narrow-band wavelengths on gene regulation are not well understood (Bantis et al., 2016; Janick et al., 2015; Wink, 2010).

Because of their role in photosynthesis and primary metabolism, blue and red wavelengths have the greatest influence on plant growth and development (Bantis et al., 2016; Darko et al., 2014; Massa et al., 2008). Primary and secondary metabolism are both directly impacted by light quality, quantity, and duration (Carvalho et al., 2016; Galvao et al., 2015). Photosynthetic and secondary metabolic processes are often modified in plants that are grown under sole source artificial lighting, because many traditional lighting sources do not usually mimic the spectral quality and intensity of sunlight (Massa et al., 2008; Smith, 1982; Tennessen et al., 1994). Some argue that plants have evolved under sunlight and required broadband spectrum for optimal plant growth and development, while others suggest that providing narrowband blue (B)/red(R) wavelengths is advantageous for increased biomass production and secondary metabolism (Azari et al., 2010; Bantis et al., 2016; Chong et al., 2014; Currey et al., 2012; Galvao et al., 2015; Janick et al., 2015; Jishi et al., 2016; Kopsell et al., 2013; Samuolienė et al., 2009; Tennessen et al., 1994). LEDs have the potential to cover fluence and spectral quality requirements of a wide range of valuable greenhouse crops while allowing specific wavelengths to be enhanced or reduced, thus optimizing light quality and modifying the production of primary and secondary metabolic products (Darko et al., 2014). This may be specifically useful for greenhouse production operations located in areas with variable solar spectra and poor weather conditions.
Narrow-band supplements to natural light spectra and fluence can improve the sensory quality of herb crops through the regulation of specific metabolic pathways that produce key flavor volatiles and other secondary metabolic products (Abney et al., 2013; Bantis et al., 2016; Bourgaud et al., 2001; Carvalho et al., 2016; Colquhoun et al., 2013; Darko et al., 2014; Hussain et al., 2008; Janick et al., 2015; Kopsell et al., 2005; Kopsell et al., 2012; Kopsell et al., 2013; Kopsell et al., 2014; Kopsell et al., 2015, 2017; Lefsrud et al., 2008; Lefsrud et al., 2006; Loughrin et al., 2001; Martineau et al., 2012; Morrow, 2008; Olle et al., 2013; Ouzounis et al., 2014; Ouzounis et al., 2015a; Ouzounis et al., 2015b; Pimputkar et al., 2009; Poel et al., 2017; Singh et al., 2015; Tarchoune et al., 2013). A recent report indicates that basil (*O. basilicum*) plants grown under blue, red, and white narrow-band wavelengths had significant concentration changes in specific volatile classes and that LED narrow-band lighting may manipulate specific secondary metabolic pathways of basil under certain conditions (Carvalho et al., 2016). In addition to using monochromatic blue or red supplements, synergetic effects have been observed when using optimal ratios of both in combination with natural sunlight (Darko et al., 2014; Ouzounis et al., 2015b).

Many studies have observed the impact of LED lighting on basil (Bantis et al., 2016; Carvalho et al., 2016; Chalchat et al., 2008; Deschamps et al., 2006; Fahlbusch et al., 2003; Klimánková et al., 2008; Lee et al., 2005; Olle et al., 2013; Petersen et al., 2010; Politeo et al., 2007; Pourmortazavi et al., 2007; Putievsky et al., 1999; Singh et al., 2015; Tarchoune et al., 2013; Treadwell et al., 2011; Yousif et al., 1999), but few studies have compared sensory quality and biomass production in comparison to various blue/red ratios and DLI increments across growing seasons (Darko et al., 2014; Janick et al., 2015).

The primary objective of this study was to determine the impact of progressive DLIs on the production of key flavor volatiles in hydroponically grown sweet basil. To date, only a few
studies have been performed using blue/red supplemental LED and HPS lighting sources to manipulate secondary metabolism and sensory quality of basil (Bantis et al., 2016). Providing narrow-bandwidth illumination from LED lighting sources may be used to manipulate secondary metabolism and influence the production of important flavor volatiles in basil and other high-value herb crops. In addition, this study provides a thorough efficacy comparison of blue/red supplemental wavelengths and HPS traditional lighting systems that may be used to improve overall basil crop quality in commercial production operations.

Materials and Methods

This project was conducted on The University of Tennessee, Knoxville (35°56'44.5"N, 83°56'17.3"W). Growing dates for these six experimental replications occurred from October 2016 to June 2017 and have been labeled as growing seasons.

Cultural Techniques and Environmental Growing Conditions

‘Genovese’ basil seeds (*O. basilicum* var. ‘Genovese’; Johnny’s Select Seeds, Winslow, ME) were germinated in peat moss based cubes (Park’s Bio Dome Sponges, Hodges, SC) at 83 ℃ and 95% RH. After 2 weeks, seedlings were transplanted into 5x5 cm plastic pots using peat moss and perlite based potting mix. Relative humidity during the growth period averaged 55%. Day temperatures averaged 29.4 ℃, while night temperatures averaged 23.8 ℃. The daily light integral (DLI) of the natural light controls averaged 9.9 mol·m⁻²·d⁻¹ during the growth period (ranging from 4 to 20 mol·m⁻²·d⁻¹). Specific growing parameters for each of the seasons may be found in Table 6.1, Appendix F.
Emphasis was placed on investigating biomass yield and nutrient uptake in response to specific DLIs using narrow-band B/R (447nm/627nm) LED light. A total of nine lighting treatments were added immediately after seedling transplant: one non-supplemented natural light control, two HPS treatments with DLIs as 6 h and 12 h, and six 20B/80R LED treatments with progressive DLIs as 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h (Orbital Technologies, Madison, WI). The 20B/80R LED lighting ratio was specifically chosen for this experiment because of its prevalence in recent literature; it was also deemed optimal for a variety of growth and development parameters from a previous experiment in this thesis (CH 3). Each supplemental lighting treatment provided 100 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1} \). Supplemental lighting regimes for all treatments were initiated each day one hour before sunset, which is commonplace for commercial herb production. A randomized complete block design was used for this experiment, and lighting treatments were randomized after each experimental run to account for variations in natural light intensity and spectral quality across growing seasons and the greenhouse production area. Basil plants were grown in ebb and flow hydroponic systems and watered for 5 min each day with full strength general-mix nutrient solution; the fertility regime was kept constant across the duration of all seasons. Each growth period lasted approximately 45 d across all six experimental runs. Like many commercial basil growing operations, harvest occurred as the first signs of change from vegetative to reproductive growth were observed. Care was taken to ensure each crop was harvested by replication at similar growth stages, and representative samples were taken from each of the two plants within each sub-replicate. Each of the nine treatments consisted of 36 plants, for 324 plants per experimental run (season), and 1,296 total basil plants for phase two of this experiment. Sub-replications consisted of two plants each to improve statistical power and decrease variance due to uncontrollable factors.
GC-MS Headspace Volatile Analysis

Three g of fresh plant material (two basil plants per sample, 1.5 g of representative material from each plant) were placed in 20-mL borosilicate glass vials then immediately sealed and placed onto a Network Headspace Sampler (Agilent G1888, Santa Clara, CA, USA). Fresh and fresh frozen samples were tested to determine differences in volatile profiles based on storage type. Samples were heated to 80 ºC for 10 min and pressurized with Helium (Air Gas, analytical purity) to 95.22 kPa for 1 min. The tube was then vented for 1 min into the headspace transfer line (110 ºC) and injected (port at 250 ºC) into the GC (Agilent Technologies 6890N Network GC System). The volatiles were separated by an HP-5MS capillary column (5%-Phenyl)-methylpolysiloxane, length: 30m, ID: 0.250 mm, film thickness: 1µm, Agilent Technologies) using analytical purity Helium carrier gas at 90.32 kPa, constant pressure. At the start of data acquisition, temperature was held at 40 ºC for 5 min, ramped-up from 40 ºC to 250 ºC at 5 ºC per min, then held constant for the duration of the run. Total run time was 70 min, including post-run and cool-down phases. After sample separation and column elution, the analytes were passed through a mass selective detector (Agilent Technologies 5973 Network Mass Selective Detector) at 250 ºC and collected over the course of the sample run. The transfer line, ion source, and quadrupole temperatures were 250 ºC, 230 ºC, and 170 ºC, respectively. The full scan mass range was set to 40-550 m/z (threshold: 150).

Agilent ChemStation was used for data collection and processing. Calibration curves were previously established using analytical standards found in basil and shown in the literature to be important for human sensory perception. Over 200 separate compounds were identified throughout the course of this project, but emphasis was placed on key flavor compounds that have been calibrated to our GC-MS and HP-5MS column using analytical standards (Sigma-Altech, St.
Louis, MO) to determine leaf tissue concentrations of key VOCs on a fresh plant weight basis. The MS spectra from analytical standards and fresh samples were compared to NIST, ADMIS, and a basil reference library created from calibrated analytical standards to confirm peak identity and retention times. MassHunter Workstation Software Version B.06.00 (Agilent Technologies, Inc., 2012) was used to automatically integrate peaks. Relative peak areas were automatically adjusted based on analytical standards and multiple library references.

**Statistical Analyses**

A Randomized Complete Block Design was used for this experiment. All data sets were analyzed by GLM and Mixed Model Analysis of Variance procedures using the statistical software SAS (version 9.4, SAS Institute, Cary, NC). Design and Analysis macro (DandA.sas) was utilized in addition to Tukey’s adjustment, regression analysis, and univariate/normalization procedures to provide additional statistical insights on the complete data set. Treatments were separated by least significant difference (LSD) at $\alpha=0.05$. Over 200 compounds were identified using GC-MS analysis, and approximately 20 key flavor compounds were statistically analyzed based on literature review and previous research. Concentration changes among growing seasons and light treatments were investigated. Key volatiles were analyzed and presented on a fresh mass (FM) basis in comparison to micro molar calibration curves created from analytical standards. Unless otherwise stated, all volatile concentration units are reported in micromolarity of analyte concentration per g of fresh leaf tissue ($\mu\text{M} \cdot \text{g}^{-1} \text{FM}$) to most accurately represent VOC emissions from the collected headspace sample above fresh plant tissues under specific reproducible analytical conditions.
Results

The following volatiles were analyzed for this experiment because of their relative abundance and importance in sensory perception: (S)-(−)-Limonene; (R)-(+)−Limonene; 1,3,6-Octatriene, 3,7-dimethy-, (Z); 1,3,6-Octatriene, 3,7-dimethy-, (E); Methyl Eugenol; Linalool; α-Humulene; Hexanal; Estragole; 1-Hexanol; α-Pinene; β-Pinene; Phenly-2-Ethanol, and Eucalyptol. Many of the compound concentrations evaluated were significantly influenced by growing season, lighting treatment, and season*treatment interaction (Fig 6.1-6.28).

(S)-(−)-Limonene concentrations were significantly impacted by season (P≤0.001; F=416.52) and treatment (P≤0.001; F=6.75), but not by season*treatment interaction (P=0.1197; F=1.39). The May growing season produced the highest concentrations, while concentrations during the November growing season were the lowest. The natural light control did not statistically separate from the optimal LED treatments (12-24 h) and the HPS (6 and 12 h) treatments. The 6 h HPS treatment had the highest concentrations across all seasons, while the 6 H LED treatment had the lowest (Fig. 6.1, 6.17).

(R)-(+)−Limonene concentrations showed a similar pattern to its enantiomer. There were significant differences in season (P≤0.001; F=50.06), treatment (P≤0.001; F=8.98), and season*treatment interaction (P=0.001; F=2.32). March and May growing seasons had significantly higher (R)-(+)−Limonene concentrations in comparison to the November and January seasons. The 6 h HPS treatment produced the highest R-Lim concentrations, while the 6 h LED treatment produced the lowest (R)-(+)−Limonene concentrations. The natural light control showed significantly higher concentrations in comparison to the 6 h LED treatment, but did not separate from any of the other treatments (Fig. 6.2, 6.18). Plants grown in January under the 3 h LED
treatment showed the lowest concentrations of any treatment or season combination. The ratio of 
(R)-(+-)-Limonene to (S)-(+-)-Limonene was consistent across all light treatments (Fig. 6.3).

1,3,6-Octatriene, 3,7-dimethy-, (E) showed significant impacts in season (P≤0.001; 
F=44.48), treatment (P≤0.001; F=9.03), and season*treatment interaction (P=0.005; F=2.03). March had the highest concentrations, while November and May had the lowest concentrations 
(Fig. 6.19). LED lighting significantly increased concentrations during the winter months, but 
concentrations were not significantly improved during the November and May growing seasons. Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any 
treatment or season combination. LED treatments 12-24 h and the 6 h HPS treatment produced the 
highest concentrations, while the 6 h LED and natural light treatments had the lowest 
concentrations (Fig. 6.4). 1,3,6-Octatriene, 3,7-dimethy-, (Z) did not show any significant impacts 
across season, treatment, or season*treatment interaction.

Methyl Eugenol concentrations showed significant impacts across seasons (P≤0.001; 
F=6.94) and treatments (P≤0.001; F=3.50), but did not show any significant season*treatment 
interactions (P=0.1055; F=1.41). The November growing season produced the highest ME 
concentrations, while the May season showed the lowest concentrations (Fig. 6.20). The natural 
light control did not separate from the HPS or LED treatments (Fig. 6.5). The 3 h LED treatment 
produced the highest methyl eugenol concentrations in comparison to all other treatments. Plants 
grown in January under the 3 h LED treatment showed the highest concentrations of any treatment 
or season combination.

Linalool concentrations were significantly impacted by season (P≤0.001; F=85.27), 
treatment (P≤0.001; F=14.08), and season*treatment interaction (P=0.001; F=3.32). Plants grown 
in January under the 3 h LED treatment showed the lowest concentrations of any treatment or
season combination. The 12-24 h LED and 6 h HPS treatments provided the highest linalool concentrations, and these impacts were most drastic during the January growing season. Many of the lowest linalool concentrations were observed during the November growing season, especially the 3-6 h LED treatments. The March and May growing seasons had significantly higher linalool concentrations, while the November growing season showed the lowest concentrations (Fig. 6.21). The 6 h HPS treatment produced the highest linalool concentrations across all growing seasons, but did not statistically separate from the 12-24 h LED treatments or the natural light control (Fig. 6.6).

α-Humulene concentrations were significantly impacted by season ($P \leq 0.001; F=11.40$), but not by treatment ($P=0.064; F=1.89$) or season*treatment interactions ($P=0.378; F=1.70$). The May growing season produced the highest concentrations, and November produced the lowest (Fig. 6.22). While light treatment did not show significance within our confidence intervals, the natural light treatment produced the highest concentrations and showed significant separation from the lowest treatment, which was 6 h LED (Fig. 6.7).

Hexanal concentrations were significantly impacted by season ($P \leq 0.001; F=40.98$) and treatment ($P=0.001; F=3.47$), but did not show significant season*treatment interactions ($P=0.073; F=1.50$). March and May growing seasons showed the highest concentrations, while the November growing season showed the lowest. The 6 h HPS and 18 h LED treatments produced the highest concentrations, while the 3-6 h LED treatments produced the lowest (Fig. 6.8).

α-Pinene concentrations were significantly impacted by season ($P \leq 0.001; F=56.25$), treatment ($P \leq 0.001; F=11.19$), and season*treatment interaction ($P \leq 0.001; F=3.32$). Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. The March growing season produced the highest concentrations in
comparison to the lowest concentrations produced in November months (Fig. 6.24). The 6 h LED treatment produced the lowest concentrations across all growing seasons, but all LED treatments showed significant improvement during the spring months compared to late fall and early winter months. Overall, the 6 h HPS treatment produced the highest concentrations and was comparable to the 12-24 h LED treatments (Fig. 6.9). The optimal LED treatment for production was 24 h supplementation.

β-Pinene concentrations were significantly impacted by season ($P \leq 0.001; F=53.81$), treatment ($P \leq 0.001; F=11.59$), and season*treatment interaction ($P \leq 0.001; F=3.24$). As with the enantiomer, plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. The highest concentrations were found in the March growing season, while the lowest concentrations were found in the November growing season (Fig. 6.25). The 6 h LED treatment produced the lowest concentrations across all growing seasons, but especially during the November and January growing seasons. The 6 h HPS treatment consistently produced higher concentrations across all growing seasons in comparison to the optimal LED treatment, which was 24 h supplementation (Fig. 6.10). The total concentration increases of pinene compounds were consistent across all lighting treatments; the ratio of pinene isomers did not fluctuate across all lighting treatments (Fig. 6.11 and 6.12).

Estragole concentrations were significantly impacted by season ($P \leq 0.001; F=19.63$), lighting treatment ($P=0.013; F=2.48$), and season*treatment interaction ($P \leq 0.001; F=2.53$). Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. The highest concentrations were found under November and January seasons with 3-6 h LED light. May had consistent statistically similar concentrations as November, and the 24 h LED treatment provided the highest concentrations across all treatments (Fig. 6.13).
Eucalyptol showed the greatest impacts in comparison to any other compound evaluated in this experiment. Overall concentrations were significantly impacted by season (P≤0.001; F=140.71), treatment (P≤0.001; F=11.59), and season*treatment interaction (P≤0.001; F=2.80). The May growing season produced the highest concentrations, while the November season produced the lowest concentrations (Fig. 6.26). Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. The 18-24 h LED treatments during the May growing season showed significant concentration increases in comparison to any other season or treatments evaluated. The 24 h LED treatment and 6 h HPS treatment produced the highest eucalyptol concentrations across all growing seasons, while the 3-6 h LED treatments produced the lowest (Fig. 6.14). The 12 h HPS treatment produced lower concentrations than the 6 h HPS treatment, which has been consistent across all of the other volatiles evaluated in this study.

Discussion

Plants use a variety of photoreceptors and other photoactive compounds to sense light quality, quantity and duration. They have the ability to respond to narrow-band wavelengths within the ambient spectrum, ranging from UV-C (260 nm) to far-red (720-780 nm) regions (Carvalho et al., 2016; Galvao et al., 2015). Changes in light intensity, spectral quality, and duration directly impact secondary metabolism and flavor metabolite production (Fraikin et al., 2013; Galvao et al., 2015). Secondary metabolites contain a wide range of chemical compound classes that serve many functions in plants, most of which involve environmental stressors adaptation (Bourgaud et al., 2001). Light intensity and spectral quality are two of the most influential factors on secondary metabolism (Kopsell et al., 2005; Kopsell et al., 2014), and changes in light intensity and spectral
quality directly impact plant physiology and biochemistry (Bian et al., 2015; Colquhoun et al., 2013).

Spectral quality and fluence from sunlight change significantly across growing seasons (Banthorpe et al., 1971; Barta et al., 1992; Briggs et al., 1999; Goins et al., 1997; Olle et al., 2013; Ouzounis et al., 2015b; Samuoliene et al., 2013; Smith, 1982; Tennessen et al., 1994; Wink, 2010). Because primary and secondary metabolism are directly related to the intensity and spectral quality of available light, seasonal differences are expected for biomass yield and the production of secondary metabolites from these fluctuations in light quality/intensity (Banthorpe et al., 1971; Carvalho et al., 2016; Kang et al., 2009; Olle et al., 2013; Sugimoto et al., 2012; Wink, 2010). Few studies have determined the relationship between specific B/R wavelengths, key flavor volatiles in basil, and the impact of growing season in addition to supplemental lighting treatments (Buchanan et al., 2015; Wink, 2010). Reference data on environmental conditions and cultural practices is presented in Table 6.1 in Appendix F and the materials/methods section, respectively. Significant concentration differences were observed for all evaluated compounds across growing seasons (November 2016 to May 2017). These seasons were specifically chosen to determine the efficacy and overall impact of LED lighting on key flavor volatiles during fall/winter months with decreased light intensity and spectral quality.

‘Genovese’ basil is highly valued for its aroma and flavor profile. Maintaining optimal yields and overall quality during winter months is critical for successful commercial growing operations, and a variety of lighting regimes and other cultural techniques have been established to improve quality parameters that determine the flavor and aroma of many high-value herbaceous crops. The delicate flavor and aroma of basil is a result of the complex ratios of chemical compounds produced through primary and secondary metabolic processes directly impacted by
environmental conditions and genetic makeup (Lachowicz et al., 1996; Ouzounis et al., 2014; Samuolienė et al., 2009).

Advances in GC-MS analysis and LED technologies have allowed for simple and efficient quantification of basil flavor volatiles. The impacts of environmental stressors, growing season, and cultivar chemotype are reflected in VOC profiles, which is a direct result of changes to primary and secondary metabolism. Many studies have focused on achieving optimal yield, flavor, and aroma in basil, but only a handful demonstrate changes to biomass and flavor volatiles in response to the ambient light spectrum and supplemental narrow-band wavelengths in comparison to human sensory perception (Loughrin et al., 2001; Loughrin et al., 2003; Morrow, 2008; Ouzounis et al., 2014; Pimputkar et al., 2009; Singh et al., 2015). Understanding the mechanism behind metabolic partitioning of secondary compounds based on changes to light intensity, spectral quality, and DLI will add to the limited knowledge of this subject area and provide detailed supplemental lighting strategies for commercial producers with the intention of optimizing yield and flavor.

Many of the compounds evaluated in this study include abundant terpenes and phenols found in edible tissues from basil that directly impact human sensory experience (i.e. the perception of flavor and aroma). These secondary metabolites are produced by subsidiaries of the mevalonate and non-mevalonate pathways and originally derived from geranyl pyrophosphate (Bourgaud et al., 2001; Petersen et al., 2010). Many compounds were found in preliminary analysis, but emphasis was placed on compounds that most profoundly impacted human sensory perception and were found in high relative abundance in comparison to all VOCs identified in this study. Almost all compound concentrations were significantly impacted by growing season, light treatment, and seasons by light treatment interactions.
(S)-(−)-Limonene and (R)-(+)−Limonene concentrations showed significant differences across supplemental lighting treatments (Fig 6.1, 6.2), but the ratio of (S)-(−)-Limonene to (R)-(+)−Limonene did not significantly change across treatments (Fig. 6.3). On average, (S)-(−)-Limonene and (R)-(+)−Limonene concentrations differed by a factor of 10x, with (R)-(+)−Limonene showing higher analytical response and human sensory perception (Chalchat et al., 2008; Larsen et al., 2000). (S)-(−)-Limonene is said to have an orange-like aroma, while (R)-(+)−Limonene has a stronger lemon/citrus aroma (Larsen et al., 2000); both compounds possess similar bioactivities (Chalchat et al., 2008; Larsen et al., 2000). The combination of these two limonene compounds contributes to overall citrus flavor in basil and other crops. Supplemental lighting provided the most improvement during November, when DLI was the lowest of all four growing seasons. Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. The optimal LED treatment for increasing both limonene compound concentrations was the 6 h HPS treatment. The HPS treatments provided relatively higher concentrations during the winter months in comparison to the May growing season, most likely because of radiant heat energy produced by the HPS lamps. The lowest concentrations for the two compounds were produced by the 6 h LED treatment across all seasons. The 24 h LED treatment produced the highest concentrations across all LED treatments, and the 12-24 h LED treatments did not separate from the 6 h HPS treatment. Changes to the relative concentration ratios of (S)-(−)-Limonene and (R)-(+)−Limonene were consistent across all lighting treatments, which demonstrates that B/R LED treatments and HPS treatments significantly increase concentration of all limonene compounds, but does not modify the ratio of (S)-(−)-Limonene to (R)-(+)−Limonene produced. This suggests that both DLI and B/R wavelengths provided impact secondary pathways upstream from the R/S conformation-decision point and produce higher volumes of both
compounds, rather than preferentially synthesizing one over the other. As expected, supplemental lighting most significantly increased overall biomass production and collective terpenoid concentrations during winter months; however, specific compounds had various significant seasonal effects. The HPS treatments were overwhelmingly beneficial for improving limonene concentrations during winter months, while the LED treatment was more useful during November and May growing seasons. Overall terpenoid compound concentrations were slightly less during the January growing season, but supplemental lighting treatments significantly improved those concentrations during winter months. Key flavor volatiles in basil, as well as specific classes of secondary metabolites had opposing changes in concentration across growing seasons and followed some general trends that coincide with many studies that investigate the impact of abiotic factors (i.e. climate and light) on crop production and physiology (Briggs et al., 2002; Carvalho et al., 2016; Choi et al., 2015; Colquhoun et al., 2013; Dai et al., 2014; Goins et al., 1997; Gouvea et al., 2012; Houle et al., 2015; Jamieson et al., 2012; Kang et al., 2009; Kopsell et al., 2017; Lange et al., 2000; Leonard et al., 2010; Lin et al., 2013; Loughrin et al., 2003; Mccree, 1973; Morrow, 2008; Olle et al., 2013; Ouzounis et al., 2015b; Pimputkar et al., 2009; Singh et al., 2015; Smith, 1982). These patterns also align well with biomass accumulation from chapter four as well as photoperiod and suggested DLI requirements for basil. Even though greenhouses protect from winter weather and poor climate conditions, other environmental conditions and abiotic factors have significant influence on crop production quality and yields. Some previously discussed factors that may impact volatile concentrations across growing seasons include: reduced natural light intensity, poor spectral quality, increased cloud cover, day/night temperature reductions and fluctuations, and changes in humidity.
(S)-(-)-Limonene concentrations were significantly highest during May growing season (Fig. 6.17). Average natural DLI was highest during this time in comparison to other growing seasons, which suggests that the fluence rate has significant impacts when natural sources lack sufficient DLI or spectral quality. November had the significantly lowest concentration of limonene compounds across all lighting treatments, which may be explained by the high temperatures/light intensity during the first stage of development and decreasing temperatures/natural light intensity as the growing season progressed into late fall. Day/night temperature averages were maintained throughout all growing seasons due to an automated environmental control system in the research greenhouse where this set of experiments was conducted. Greenhouses reduce temperature variation across growing seasons, but it is nearly impossible for any type of environmental control system to completely eliminate variations in humidity and temperature across all growing seasons. Therefore, temperature variations are expected within these results, but do not provide basis for the significance between LED treatment concentrations and HPS treatment concentrations. Exact amount of DLI received by each lighting treatment does not follow a logical trend, as the 6 h HPS treatment is comparable to the 12-24 h LED treatment, further indicating that spectral quality plays a key factor. Stomatal regulation may have played a factor, as B/R wavelengths and fluence rates can induce stomatal opening as well as increase transpiration and carbon-fixation rates (Carvalho et al., 2016; Colquhoun et al., 2013).

R)-(+-) LImonene did not follow the same trend across growing seasons as its enantiomer; ratios of R/S limonene varied across all growing seasons and did not follow any patterns (Fig. 6.18). The average ratio of R/S was 10:1 across all growing seasons and showed upwards of 25% variance among seasons. In addition, total concentrations of both compounds did not follow any logical physiological-based pattern across growing seasons, photoperiod, or DLI increment. This
is somewhat unexpected since both limonene compounds are so closely related in terms of chemical structure, biosynthesis, bioactivity, and importance in flavor for basil/other herbaceous and citrus crops. Biomass parameters from CH 4 show moderate correlation to the concentration values in this study, further suggesting that primary and secondary metabolism are directly related. These results also suggest an internal mechanism shift or pathway up/down regulation at the specific point in the metabolic pathway that determines the R/S conformation, as opposed to explanations based on physical chemistry. On its own, passive volatilization or even elicitation does not explain the differences in limonene compound concentrations, as emission would theoretically be similar for both, unless some type of specific mechanism or active process is emitting one volatile over the other as a result of environmental changes, such as plant defense, metabolic regulation in reaction to abiotic stress, pollinator attraction, and/or plant-plant signaling. 1,3,6-Octatriene, 3,7-dimethy-, (Z) and 1,3,6-Octatriene, 3,7-dimethy-, (E) concentrations were measured across lighting treatments, both of which exhibit an herbaceous and terpene-based aroma. Limited information was availability regarding sensory perception of these compounds, concentrations within basil, the impact of LED lighting treatments on these compounds, or any combination of these areas of study. The (Z) conformation did not show any significant changes across lighting treatments, but the (E) conformation did show significant differences between the optimal 6 h LED treatment and the lowest concentrations observed, which was observed in the 6 h LED treatment (Fig. 6.4). This further proves the impact of spectral quality in comparison to DLI. The natural light control was comparable to the lowest treatment and was decreased during November, as expected. Additional experiments should be performed to determine the metabolic pathway responsible for the production of these compounds in addition to the sensory and bioavailability impacts. If (Z) concentration changes cannot be inducted using B/R wavelengths,
a specific photoreceptor or enzyme may be responsible for the biosynthesis of one conformation over the other (i.e. manipulation to metabolic allocation). The (E) conformation showed seasonal concentration changes, but the (Z) conformation did not show any significant concentration changes across season. Octatriene followed the same total terpenoid concentration patterns for R-Lim across growing seasons. March had the highest concentrations, while November had the lowest concentrations.

Methyl eugenol showed an inverse relationship to increasing DLI increments from LED sources in comparison to other key flavor volatiles evaluated in this study. Many of the compounds evaluated show a regression relationship between the increasing DLI treatments, with concentrations optimizing between 12-24 h LED and 6 h HPS treatments; the opposite is true for methyl eugenol, as the 3 h LED treatment showed the highest concentrations in comparison to any other treatment (Fig. 6.5) The HPS treatments produced the lowest methyl eugenol concentrations of any treatment. The November growing season produced the highest concentrations, while the May season produced the lowest concentrations. Interactions occurred where high and low DLIs were provided; concentration amounts followed a general positive trend as DLI was decreased.

Methyl eugenol has a strong, spicy, herbaceous aroma that greatly contributes to the flavor and aroma of basil. This compound also has valuable medicinal properties and many human health benefits (Lee et al., 2005). It can be described as having a clove-like flavor and is used extensively in the pharmaceutical and cosmetic industries. Methyl eugenol shows an inverse relationship with recorded DLI and available light intensity, which suggests that as light intensity increases and blue/red wavelength supplements are added, methyl eugenol concentrations decrease. The natural light treatment had lower concentrations than the 3 h LED supplement, demonstrating that spectral quality has significant impacts on secondary metabolism. Methyl eugenol may be released through
volatilization in higher qualities due to increases in light intensity and specific wavelengths because of its high boiling point and vapor pressure. The HPS treatments produced significant amounts of radiant heat energy, which may further impact the volatilization process and reduce methyl eugenol concentrations as observed in this experiment. It is also important to note that methyl eugenol is the only phenolic compound in comparison to the compounds evaluated in this evaluation, all of which are terpene based. Methyl eugenol is derived from a separate secondary metabolic pathway in comparison to terpene based compounds created from the isoprenoid pathway. This suggests that DLI and spectral quality have significant impacts on resource partitioning for the specific pathway that is used to synthesize eugenol and other related phenolic compounds. These results are consistent with similar studies that investigated the impact of LED lighting on flavor volatiles and resource partitioning of other secondary metabolites (Carvalho et al., 2016; Colquhoun et al., 2013; Loughrin et al., 2003; Samuoliene et al., 2013). Overall concentrations of terpene-based compounds showed concentration increases with the addition of B/R wavelengths (3 h LED supplement), while methyl eugenol and other phenylpropanoid compounds generally showed concentration decreases with the addition of supplementary B/R light and HPS light, which is consistent with results from a similar study (Carvalho et al., 2016). Very few studies have related specific wavelengths to changes in terpenes and phenylpropanoids that are crucial for aroma, flavor, and overall sensory quality of high-value herbaceous crops (Bantis et al., 2016). Because methyl eugenol concentrations have such a large impact on flavor and aroma perception of basil, further studies should be conducted to determine the specific mechanisms that are involved in the synthesis of these compounds and how B/R wavelengths impact this process. The biological activities, sensory impacts, and overall health benefits of each key flavor/aroma compound should be analyzed against the total concentration of compounds
evaluated in order to determine which are most important for basil quality and human sensory perception.

Linalool concentration patterns were consistent with other terpenoids in this, as well as other studies involving basil and other high-value crops (Carvalho et al., 2016; Colquhoun et al., 2013; Galeotti et al., 2008; Lee et al., 2005). Linalool is a critical component in the overall flavor of basil and many other herbaceous crops. The aroma of linalool can be described as sweet and floral, very similar to that of fruity-pebbles. It has been shown to possess antioxidant and anti-inflammatory properties in addition to numerous other health benefits (Carvalho et al., 2016; Randall et al., 2014b). Concentrations peaked using the 6 h HPS treatment, and the optimal lighting treatment did not separate from the natural light controls or the 12-24 h LED treatment (Fig. 6.6). The 3-6 h LED treatments had the lowest linalool concentrations in comparison to any other treatment, and the 3 h LED treatment in January produced the lowest concentration of any season or treatment combination. Because the 24 h LED treatment and the 6 h HPS treatment did not separate, it is obvious that DLI is not the only factor that is involved with secondary metabolism resource allocation, specifically the production of linalool and other terpene compounds.

α-Humulene is one of the primary constituents that produce the flavor and aroma of flowering cones of the hops plant and many other herbaceous crops. This compound and its many isomer products are essential for pollinator attraction and is responsible for the “hoppy” flavor and aroma in beer and other fermented products. This sesquiterpene can be found in many other plants in the Lamiaceae family and has relative impacts on flavor and aroma in many basil cultivars/varieties (Lee et al., 2005). The natural light control produced the highest concentrations of α-humulene in comparison to any other lighting treatment, which was the opposite effect of all other compounds evaluated in this study (Fig. 6.7). The optimal LED supplement was 9 h, while
the lowest concentrations were observed in the 6 h LED treatment. This suggests that sesquiterpene production is reduced under increasing DLI and spectral quality manipulations.

Linalool and α-humulene concentrations showed significant differences across growing seasons, with the highest concentrations produced in March (Fig. 6.21, 6.22). The lowest concentrations for both isoprenoid compounds were found in November, specifically under the 6 h LED treatment. Since they are both created with fundamental isoprene units and found in the same pathway, seasonal concentration similarities may be expected, dependent on a wide range of factors. Concentration levels between the two were found approximately 10:1 linalool:α-humulene and remained constant across all seasons, with less than 5% variance across all seasonal concentration ratios. This is expected, since linalool is a monoterpene and α-humulene is a sesquiterpene, both of which are synthesized in the same metabolic pathway. These results further suggest that terpene biosynthesis is increased with the addition of blue/red supplemental light, while other compounds (such as methyl eugenol) are decreased under the same conditions.

Hexanal is considered an aliphatic aldehyde and is used extensively in the food industry to produce fruit flavors. It has an herbaceous odor that also resembles fresh cut grass. LED treatments showed some difference between controls; but concentration differences were relatively small, and lighting treatments did not make a significant impact (Fig. 6.9). The 3-6 h LED treatments had the lowest hexanal concentrations, while all other LED/HPS treatments were comparable to the natural light control and did not show separation.

α-pinene and β-pinene are two compounds that have significant influence on the flavor and aroma of basil. Sensory quality is directly impacted by the total concentration of all pinene compounds present as well as the concentration ratio of different pinene isomers in relation to one another. Pinene compounds are primarily found in pine resin and are one of the most abundant
terpenoids in nature (Noma et al., 2010). These compounds have high biological activities in mammals (both beneficial medical properties as well as moderate toxicity levels) and strongly repel insects. Independent of lighting treatment, slightly higher levels of β-pinene were observed in comparison to α-pinene (i.e. ratio of α/β). For both isomers, optimal concentration peaked at the 6 h HPS treatment. The 12-24 h LED treatments were comparable to the 6 h HPS treatment, while the lowest concentrations were observed in the 6 h LED treatment (Fig. 6.9, 6.10). Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. Both compounds showed similar concentration patterns across growing seasons and showed little variance.

These results are consistent with our ongoing conclusion that variation in DLI and supplemental B/R wavelengths increase the production of monoterpane hydrocarbons while maintaining consistent ratios of isomer products, conformations, and analogs for each monoterpane product (Fig. 6.11). Further studies should be performed to determine if significant differences exist among these specific compounds and broad chemical classes; this may require the implementation of metabolomics software and genomic analysis (Bourgaud et al., 2001; Carvalho et al., 2016; Colquhoun et al., 2013; Sugimoto et al., 2012), but will provide valuable insight on secondary metabolism. Various studies that have investigated narrow-band wavelengths in relation to pinene isomers, monoterpane hydrocarbon compounds, and broad secondary metabolic resource partitioning (i.e., phenylpropanoids, sesquiterpenoids, other isoprenoids, etc.) are congruent with the findings of this study (Carvalho et al., 2016; Colina-Coca et al., 2013; Colquhoun et al., 2013; Darko et al., 2014; Deschamps et al., 2006; Du et al., 2015; Fu et al., 2015; Klimánková et al., 2008; Loughrin et al., 2001; Loughrin et al., 2003; Olle et al., 2013; Ouzounis et al., 2014; Samuoliene et al., 2013; Selli et al., 2014; Tarchoune et al., 2013).
Eucalyptol, or 1,8-cineole, is another monoterpenoid that is closely related in both chemical/physical properties and structure to pinene, limonene, and other secondary metabolites produced from isoprene sub-units (Buchanan et al., 2015). Eucalyptol is the primary VOC that influences flavor in a variety of basil cultivars. Eucalyptol is the most abundant flavor volatile found in basil leaf tissues, accounting for over 50% of GC-MS response area in a variety of studies (Bantis et al., 2016; Carvalho et al., 2016; Claudia, 2013a; Klimánková et al., 2008; Lee et al., 2005; Loughrin et al., 2003; Tarchoune et al., 2013). It has a spicy, energizing, camphoraceous aroma with a cooling mint-like taste. Biological activity related to this compound is extremely high, in addition to moderate toxicity levels at relatively low concentrations. For these reasons, this compound is used in extremely low concentrations when manufacturing food products and cosmetics.

In this study, the highest eucalyptol concentrations were observed under the 6 h HPS treatment and were comparable to 12-24 h LED treatments. The lowest concentrations were produced under the 3-6 h treatments, and the natural light control concentrations were comparable to the average of the best and worst LED treatment. Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. Overall, eucalyptol, limonene, linalool, and methyl eugenol showed the highest calibrated concentration abundance in basil for this experiment, approximately 1-3 mM/ g FM headspace emission across a variety of seasons and treatments. All other flavor volatiles were significantly lower, within the range of 1.0 mM/g FM to 0.01 µM/g FM headspace emission. R-Lim, α-pinene, β-pinene, phenyl-2-ethanol, and eucalyptol showed similar concentration patterns across growing seasons. While the specific concentrations of the individual compounds found in plant tissue varied greatly, they all followed approximate concentration ratios as growing seasons progressed. March showed the
highest concentrations of these compounds, while November showed the lowest concentration averages of each. This may be explained through established physiological responses across growing season in addition to volatilization at higher growing temperatures, up/down regulation of specific volatile biosynthesis in response to variation in solar spectra/intensity, and secondary metabolic resource partitioning. The ratios of two important flavor compounds, α-pinene and β-pinene, varied slightly across growing seasons, which suggests an up/down regulation at this point in the metabolic pathway requiring further investigation; however, because this was seasonal variation, many factors can explain the shifts in metabolic resource partitioning. In addition, specific volatiles were impacted at varying levels during early fall/late spring months, generally with sesquiterpenoids and phenols being positively impacted the most during months with poor spectral quality and low DLIs. In most cases, monoterpenes were found in higher concentrations using LED supplemental lighting. When using supplemental lighting, monoterpane and diterpene concentrations were significantly increased during seasons with low-moderate sunlight intensity and spectral quality, namely the January growing season.

Key flavor compounds showed concentration variations across growing season and incremental DLI supplements. The results of this study demonstrate that the use of LED lighting may be used to supplement natural photoperiods with the intention of optimizing sensory quality. Light supplements used to enhance natural spectral quality or optimize DLI requirements have direct impacts on secondary metabolite partitioning at varying levels, which necessitates further exploration to determine specific mechanisms and impacts of supplemental wavelengths. In general, monoterpane and diterpene compound concentrations were increased under B/R wavelengths and HPS lighting sources, while sesquiterpenes and phenolics showed significant decreases when exposed to intense DLI supplements. Recommended lighting type and DLI
supplement are highly variable and dependent on solar spectral quality/intensity/duration, crop species DLI requirement, time of year, and desired sensory quality characteristics. Regardless of DLI, supplemental narrow-band wavelengths and traditional HPS lighting systems have both proven their ability to positively impact secondary metabolism resource partitioning in hydroponic basil production.
References F


Kopsell, D.A., C.E. Sams, T.C. Barickman, and R.C. Morrow, 2014. Sprouting Broccoli Accumulate Higher Concentrations of Nutritionally Important Metabolites under Narrow-


Appendix F

Table 6.1 Environmental conditions during growing periods.

<table>
<thead>
<tr>
<th>Growing Period</th>
<th>November</th>
<th>January</th>
<th>March</th>
<th>May</th>
</tr>
</thead>
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<tr>
<td>Average Day Temp (°C)</td>
<td>28.88</td>
<td>27.81</td>
<td>27.62</td>
<td>28.03</td>
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<tr>
<td>Average Night Temp (°C)</td>
<td>22.31</td>
<td>20.11</td>
<td>21.83</td>
<td>22.43</td>
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<tr>
<td>Average Relative Humidity</td>
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<td>50%</td>
<td>55%</td>
<td>55%</td>
</tr>
<tr>
<td>Average Daily Light Integral (DLI) (mol·m⁻²·d⁻¹)</td>
<td>5.66</td>
<td>4.81</td>
<td>8.95</td>
<td>12.77</td>
</tr>
<tr>
<td>Average Day Length (hours)</td>
<td>10.35</td>
<td>9.90</td>
<td>11.68</td>
<td>13.46</td>
</tr>
<tr>
<td>Average Natural Blue (447nm) Intensity at Noon (µmol·m⁻²·s⁻¹)</td>
<td>127</td>
<td>111</td>
<td>124</td>
<td>138</td>
</tr>
<tr>
<td>Average Natural Red (627nm) Intensity at Noon (µmol·m⁻²·s⁻¹)</td>
<td>133</td>
<td>112</td>
<td>133</td>
<td>145</td>
</tr>
</tbody>
</table>
Impact of Light Treatment on Key Flavor Volatiles

**Figure 6.1** Influence of LED treatments on (S)-(−)-Limonene concentrations (\(\mu\text{M} \cdot \text{g}^{-1} \text{FM}\)) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.2** Influence of LED treatments on (R)-(+)-Limonene concentrations (\(\mu\text{M} \cdot \text{g}^{-1} \text{FM}\)) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.3 Influence of LED treatments on (S)-(−)-Limonene and (R)-(+)−Limonene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 6.4 Influence of LED treatments on 1,3,6-Octatriene, 3,7-dimethy-, (E) concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 6.5** Influence of LED treatments on Methyl Eugenol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.6** Influence of LED treatments on Linalool concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 6.7** Influence of LED treatments on \( \alpha \)-Humulene concentrations \((\mu M \cdot g^{-1} \text{ FM})\) of hydroponically grown ‘Genovese’ basil \((\textit{Ocimum basilicum} \text{ var. ‘Genovese’})\).

**Figure 6.8** Influence of LED treatments on Hexanal concentrations \((\mu M \cdot g^{-1} \text{ FM})\) of hydroponically grown ‘Genovese’ basil \((\textit{Ocimum basilicum} \text{ var. ‘Genovese’})\).
**Figure 6.9** Influence of LED treatments on α-Pinene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.10** Influence of LED treatments on β-Pinene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.11 Influence of LED treatments on $\alpha$-Pinene (blue) and $\beta$-Pinene (orange) concentrations ($\mu$M·g$^{-1}$ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 6.12 Influence of LED treatments on the distribution of $\alpha$-Pinene (blue) and $\beta$-Pinene (orange) concentrations ($\mu$M·g$^{-1}$ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 6.13** Influence of LED treatments on Estragole concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.14** Influence of LED treatments on Eucalyptol concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.15 Influence of LED treatments on all compound concentrations (µM·g⁻¹ FM) evaluated in hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.16 Influence of LED treatments on all compound concentrations (μM·g⁻¹ FM) evaluated in hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).

Seasonal Effects on Key Flavor Volatiles
Figure 6.17 Influence of season on (S)-(-)-Limonene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 6.18 Influence of season on (R)-(+) -Limonene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 6.19** Influence of season on 1,3,6-Octatriene, 3,7-dimethy-, (E) concentrations ($\mu$M·g$^{-1}$ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.20** Influence of season on Methyl Eugenol concentrations ($\mu$M·g$^{-1}$ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
**Figure 6.21** Influence of season on Linalool concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

**Figure 6.22** Influence of season on α-Humulene concentrations (µM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.23 Influence of season on Estragole concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 6.24 Influence of season on α-Pinene concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.25 Influence of season on β-Pinene concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).

Figure 6.26 Influence of season on Eucalyptol concentrations (μM·g⁻¹ FM) of hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.27 Influence of growing season on all compound concentrations (μM·g⁻¹ FM) evaluated for hydroponically grown ‘Genovese’ basil (*Ocimum basilicum* var. ‘Genovese’).
Figure 6.28 Influence of growing season on all compound concentrations (µM·g⁻¹ FM) evaluated in hydroponically grown ‘Genovese’ basil (Ocimum basilicum var. ‘Genovese’).
CHAPTER 7: CONCLUSION
LED lighting systems have the ability to revolutionize the horticulture industry and the results of these experiments show that optimizing environmental conditions (i.e. light intensity and spectral quality) significantly impacts primary and secondary metabolism. As expected, supplemental lighting treatments significantly improved total plant FM across all seasons. The most dramatic increase was found under LEDs during winter months. HPS lighting treatment did not statistically separate from either of the natural light controls for total plant FM. Leaf, shoot, and main stem FM followed similar patterns, but revealed various impacts to primary metabolic resource partitioning and morphology. Leaf FM (i.e. edible biomass) for LED treatments were all statistically higher than HPS and natural light controls. Natural light controls and HPS treatments did not statistically separate from each other. Edible biomass was significantly improved for the optimal LED lighting treatment 40B/60R compared to the natural light control average (30.88 grams per plant vs. 15.5 grams per plant). Reductions in fresh edible biomass suggest that light quality has the ability to alter growth, decrease the mean weight of edible biomass fresh of basil, and lower market value. This reduction in growth may have been caused by lack of total PPFD, lack of specific wavelengths that were necessary for optimal production of primary and secondary metabolites, or most likely a combination of both. Light treatment primarily impacted macronutrient concentrations. Tissue P concentrations were highest in the 20B/80R treatment, with elevated levels shown in all LED treatments and HPS treatment. The natural light controls had slightly lower levels of P; natural light control two was statistically lower than the optimal LED treatment. Tissue Ca concentrations were significantly impacted, with 10B/90R being the optimal lighting treatment. Natural light controls show lower Ca levels, but with varying levels of significance. Tissue S concentrations were highest in the 50B/50R treatment, with varying significance among LED treatments. Natural light controls and HPS show similar levels to many
of the LED treatments. Magnesium levels showed some variance across lighting treatments, with 50B/50R being the optimal treatment. None of the LED treatments statistically separated for Mg, but the optimal treatment was significantly higher than the natural light controls and HPS treatment. The only micronutrient that was impacted by lighting treatment was B, which showed elevated levels in the LED treatments. 50B/50R had the highest levels of B (66.7 µg·g⁻¹ DM), with many of the LED treatments within 5 µg·g⁻¹ DM of the optimal LED treatment. Natural light controls and HPS treatments were significantly lower than the LED optimal treatment, and the natural light control treatments averaged 51.2 µg·g⁻¹ DM. The nutrients K, Cu, Mn, Fe, Na, Zn, and Mo were not statistically different across lighting treatments. Overall, LED lights provided optimal results for both fresh/dry edible biomass yield and improved the uptake of many macro and micro nutrients when compared to HPS and natural light controls. For all parameters considered in this study, the optimal ratio of blue/red supplemental lighting is between 20B/80R to 40B/60R. The results from chapter three support the growing body of literature that detail photomorphogenic responses, biomass increases and nutrient uptake impacts by exposure to specific blue and red wavelengths from LED lighting. These results suggest that manipulation of spectral quality and the addition of specific narrowband wavelengths impact plant morphology and resource partitioning for primary metabolism. In addition, this chapter shows that biomass partitioning ratios vary as a response to altered spectral qualities. Supplementary blue wavelengths have been shown to trigger a wide range of metabolic responses in plants. Manipulating light quality through LED supplementation may be a viable means to improve edible biomass yields, nutrient uptake, and overall plant quality.

To date, only a few studies have evaluated the impact of environmental stressors, growing season, and variety in basil VOC profiles, and changes to cultivation practices may directly result
in changes to primary and secondary metabolism. Many studies have focused on achieving optimal yield, flavor, and aroma in basil, but only a handful demonstrate changes to biomass and flavor volatiles in response to the ambient light spectrum and supplemental narrow-band wavelengths. Spectral quality and light intensity will change significantly across growing seasons. Because primary and secondary metabolism are directly linked to the intensity and spectral quality of available light, seasonal differences are expected for biomass yield and the production of secondary metabolites from these fluctuations in light quality and intensity. Specific B/R wavelengths, key flavor volatiles in basil, and impact of growing season in addition to supplemental lighting treatments were explored in chapter four.

Supplemental lighting most significantly increased overall biomass production and collective terpenoid concentrations during winter months; however, specific compounds had various significant seasonal effects. Overall terpenoid compound concentrations decreased during winter months, but supplemental lighting treatments significantly improved those concentrations in comparison to natural light controls during those winter months. Many of the compounds explored in this study include abundant terpenes and phenols found in edible tissues from basil that profoundly impact human sensory experience (i.e. flavor and aroma). Almost all compound concentrations were significantly impacted by growing season, lighting treatment, and seasons*treatment interactions. (S)-(−)-Limonene and (R)-(+)−Limonene leaf tissue concentrations showed significant differences between supplemental lighting treatments and natural light controls. (S)-(−)-Limonene and (R)-(+)−Limonene concentrations differed by a factor of 7-10x, with (R)-(+)−Limonene being the more intense flavor volatile in terms of both analytical response and human sensory perception. The optimal LED treatment for increasing both limonene compound concentrations was 40B/60R, and changes to the relative concentration ratios of (S)-(−-
Limonene and (R)-(+-) Limonene were similar across all lighting treatments, which demonstrates that blue/red light treatments significantly increase concentrations of (S)-(+-) Limonene and (R)-(+-) Limonene, but do not modify the ratio of (S)-(+-) Limonene and (R)-(+-) Limonene produced. Average natural DLIs were much lower during this time in comparison to other growing seasons, which suggests that the supplemental B/R wavelengths have significant impacts when natural sources lack sufficient DLI or spectral quality. November had the lowest concentration of overall terpenoid compounds across all harvest seasons, which may be explained by the high temperatures/DLI during the first stage of development and decreasing temperatures/DLI as the growing season progressed into late fall. \( \alpha \)-pinene and \( \beta \)-pinene are two compounds that have significant influence on the flavor and aroma of basil. Independent of lighting treatment, slightly higher levels of \( \beta \)-pinene were observed in comparison to \( \alpha \)-pinene (i.e. ratio of \( \alpha / \beta \)). These ratios remained consistent across lighting treatments but were significantly different across season. For both isomers, optimal concentration peaked between lighting treatments 40B/60R and 60B/40R. All LED lighting treatment concentrations were significantly higher than the natural light controls for both pinene compounds. The HPS treatment concentrations did not statically separate from the natural light controls or lesser LED treatments, but the optimal LED treatment concentrations were significantly higher than the HPS treatment concentrations for both compounds.

Overall, eucalyptol, (R)-(+-)-limonene, linalool, and methyl eugenol showed the highest calibrated concentration abundance in basil for this experiment, approximately 1-3 mM/ g FM headspace emission across a variety of seasons and treatments. All other flavor volatiles were significantly lower, within the range of 1.0 mM/g FM to 0.01 \( \mu \)M/g FM headspace emission. Based on GC-MS analysis, this study demonstrates that supplemental narrow-band wavelengths have the ability to alter secondary metabolism resource partitioning in basil, specifically isoprenoids and
phenol groups. Some significant differences between HPS lighting and LED supplements were observed for specific compounds, but many were not significantly different. There is no question that supplemental lighting increases biomass and the concentrations of many important flavor volatiles in basil. While some concentrations of flavor volatiles were not significantly impacted across LED/HPS lighting, other factors may be involved with the final supplemental lighting purchase, such as energy efficiency, initial cost, and/or specific uses such as the desire to manipulate VOC concentrations in herbaceous crops to create designer flavor profiles. Considerations involved with evaluating the efficacy of HPS lighting and LED lighting to manipulate secondary metabolism also include the end goal of the crop and financial-sustainability. A variety of basil cultivars will be tested in the future in order to determine the impact of narrow-band wavelengths on the secondary metabolism of basil, specifically flavor and aroma volatiles that have a direct impact on quality and favorable sensory perception. All key flavor volatiles evaluated in chapter four had significant impacts on emission concentrations across various lighting treatments. This confirms that the spectral quality of light has a substantial impact on flavor volatiles and sensory qualities of basil in addition to other high-value herbaceous crops. This also indicates that supplemental blue/red wavelengths have direct impacts on secondary metabolite partitioning, necessitating further exploration to determine specific mechanisms and impacts of various supplemental wavelengths.

In terms of interaction between incremental DLI and growing season, macronutrient concentrations found in 12-24 h LED treatments in the January growing season were optimal. Growing season significantly impacted most nutrient tissue concentrations evaluated in this study. Tissue P concentrations were highest in the 12 h LED treatment, with elevated levels shown in most LED treatments and HPS treatments. The natural light controls had slightly lower levels of
P, but the lowest concentration was observed in the 6 h LED treatment. Tissue Ca concentrations were significantly impacted, with 12 h LED again being the optimal lighting treatment. Levels vary among LED treatments and separation does not exist between many of the LED treatments and the HPS treatment. The 6 h LED treatment showed lower Ca levels, but with varying levels of significance. Tissue S concentrations were highest in the 24 h LED and 6 h HPS treatments, with varying significance among other LED treatments. The 6 h LED again had the lowest concentration. Basil Mg levels showed some variance across lighting treatments. None of the LED treatments statistically separated from the rest of the group. Micronutrients that were impacted by lighting treatment were B, Zn, Fe and Na, which showed elevated levels in the LED treatments in comparison to HPS and natural light controls (Table 5.2). The LED treatments between 6 h and 24 h had the most significant micronutrient concentrations in comparison to the natural light control.

Overall, HPS lights provided optimal results for both fresh/dry edible biomass yield, while 9 h to 18 h LED treatments significantly increased the uptake of many macro and micronutrients when compared to HPS and natural light controls. For all parameters considered in this study, the optimal supplemental DLI ranged 4.2-6.3 mol·d\(^{-1}\). Lighting type was a significant factor in terms of biomass accumulation and nutrient uptake. In this efficacy comparison, LEDs and HPS have proven their merits, but both systems have limitations. Additional efficacy comparisons between HPS and LED lighting systems should be conducted on a variety of parameters to determine economically favorable practices. Chapter five supports the rapidly growing body of literature that details biomass accumulation and nutrient uptake impacts by manipulating light intensity, spectral quality and duration. Altering DLI and spectral quality through LED supplementation has significant implications for improving edible biomass yields, nutrient uptake and overall plant quality.
In chapter six, \( \alpha \)-pinene and \( \beta \)-pinene varied significantly across DLI supplements. Sensory quality is directly impacted by the total concentration of all pinene compounds present as well as the concentration ratio of different pinene isomers in relation to one another. Pinene compounds are primarily found in pine resin and are one of the most abundant terpenoids in nature. For both isomers, optimal concentration peaked at the 6 h HPS treatment. The 12-24 h LED treatments were comparable to the 6 h HPS treatment, while the lowest concentrations were observed in the 6 h LED treatment (Fig. 6.9, 6.10). Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. Both compounds showed similar concentration patterns across growing seasons and showed little variance.

These results are consistent with our ongoing conclusion that variation in spectral quality and light intensity alter the production of monoterpene hydrocarbons while maintaining consistent ratios of isomer products, conformations, and analogs for each monoterpene product. Further studies should be performed to determine if significant differences exist among these specific compounds and broad chemical classes.

The highest eucalyptol concentrations were observed under the 6 h HPS treatment and were comparable to 12-24 h LED treatments. The lowest concentrations were produced under the 3-6 h treatments, and the natural light control concentrations were comparable to the average of the best and worst LED treatment. Plants grown in January under the 3 h LED treatment showed the lowest concentrations of any treatment or season combination. Overall, eucalyptol, limonene, linalool, and methyl eugenol showed the highest calibrated concentration abundance in basil for this experiment, approximately 1-3 mM/ g FM headspace emission across a variety of seasons and treatments. All other flavor volatiles were significantly lower, within the range of 1.0 mM/g FM
to 0.01 µM/g FM headspace emission. limonene, α-pinene, β-pinene, phenyl-2-ethanol, and eucalyptol showed similar concentration patterns across growing seasons. While the specific concentrations of the individual compounds found in plant tissue varied greatly, they all followed approximate concentration ratios as growing seasons progressed. March showed the highest concentrations of these compounds, while November showed the lowest concentration averages of each. This may be explained through established physiological responses across growing season in addition to volatilization at higher growing temperatures, up/down regulation of specific volatile biosynthesis in response to variation in solar spectra/intensity, and secondary metabolic resource partitioning. The ratios of two important flavor compounds, α-pinene and β-pinene, varied slightly across growing seasons, which suggests an up/down regulation at this point in the metabolic pathway requiring further investigation; however, because this was seasonal variation, many factors can explain the shifts in metabolic resource partitioning. In addition, specific volatiles were impacted at varying levels during early fall/late spring months, generally with sesquiterpenoids and phenols being positively impacted the most during months with poor spectral quality and low DLIs. In most cases, monoterpenes were found in higher concentrations using LED supplemental lighting. When using supplemental lighting, monoterpene and diterpene concentrations were significantly increased during seasons with low-moderate sunlight intensity and spectral quality, namely the January growing season.

Key flavor compounds showed concentration variations across growing season and incremental DLI supplements. The results of chapter six demonstrate that the use of LED lighting may be used to supplement natural photoperiods with the intention of optimizing sensory quality. Based on the results of these experiments, the recommendation for basil growers is to use 20B/80R LED supplemental lighting at 100-200 µmol·m²·s⁻¹ for 18 h per day. HPS lights are beneficial
during winter months, while LED lights promote the most biomass accumulation and volatile production during summer months. Light supplements used to enhance natural spectral quality or optimize DLI requirements have will direct impacts on secondary metabolite partitioning at varying levels, and this concept necessitates further exploration to determine specific mechanisms and impacts of supplemental wavelengths on plant physiology and biochemistry. In general, monoterpane and diterpene compound concentrations were increased under B/R wavelengths and HPS lighting sources, while sesquiterpenes and phenolics showed significant decreases when exposed to intense DLI supplements. Recommended lighting type and DLI supplement are highly variable and dependent on solar spectral quality/intensity/duration, crop species DLI requirement, time of year, and desired sensory quality characteristics. Regardless of DLI, supplemental narrow-band wavelengths and traditional HPS lighting systems have both proven their ability to positively impact secondary metabolism resource partitioning in hydroponic basil production.
VITA

Hunter Albright Hammock was born in Nashville, TN to the parents of Harry and Tami Hammock. He attended Cumberland Heights Elementary School in Clarksville, TN. He then attended Cheatham Middle School and Cheatham County Central High School, both located in Ashland City, TN. After his high school graduation, he continued his college undergraduate education at The University of Tennessee, Knoxville where he studied Chemistry and Psychology. During his time as an undergraduate, he completed over 240 hours of community service and was chapter president of NSLS UTK, a leadership organization that grew to over 2,400 active members during his tenure. During his senior year, he worked in Dr. Carl Sams’ plant physiology lab and became excited about using his organic and analytical chemistry background in combination with his newfound passion for plant sciences to improve current agriculture practices and provide sustainable food sources for future generations. He obtained a BS degree from the University of Tennessee, Knoxville in May 2015 in Chemistry and Psychology. He accepted a graduate research assistantship at the University of Tennessee, Knoxville in Plant Sciences to study the impact of LED lighting on high value specialty crop production and basil flavor volatile compositions. Hunter will graduate with a MS degree in Plant Sciences in May 2018. He will be continuing his research and pursuing a Ph.D. at the University of Tennessee, Knoxville under the direction of Dr. Carl Sams.