PHOTOSYNTHETIC PHOTON FLUX DENSITIES, ANTHOCYANINS, AND POTASSIUM AFFECT LEAF EXPANSION AND GROWTH OF HYDROPONIC LETTUCE

by

PEYTON LOU PALSHA

Under the Direction of Dr. Marc W van Iersel (*in memoriam*) and

Dr. Rhuanito Soranz Ferrarezi

ABSTRACT

Crop growth is strongly affected by the amount of light captured by the canopy and light use efficiency (LUE). Increased leaf expansion and cultivar-specified photosynthetic photon flux densities (PPFD) rates from supplemental lighting can maximize biomass production and shorten the production period, essential in decreasing electricity costs in controlled environmental agriculture (CEA). Understanding the effects PPFD has on plant morphology, we conducted a study on lettuce growth under sole-source lighting, examined its impact on six different cultivars with varying leaf anthocyanin content, and investigated how increased potassium (K) concentrations influenced growth with rising daily light integrals (DLI). In the first study, changes in lettuce physiology and morphology were examined throughout a cropping cycle under various PPFDs using sole-source LED lighting. The results indicated that as PPFD increased, there was a corresponding rise in total incident light, leading to an increase in shoot dry weight under higher PPFDs. In the second study, focusing on changes in plant physiology and morphology during the cropping cycle, supplemental PPFD was applied to six lettuce cultivars, each with varying leaf anthocyanin content. Our findings suggest that a higher leaf anthocyanin content may contribute to an increase in shoot dry weight in red cultivars with rising PPFD, although this effect was prominent only in the ‘Cherokee’ cultivar, which exhibited the highest shoot dry weight among all six cultivars, including our sole green cultivar, ‘Rex’. In the final study, the use of K as a supplemental nutrient to increase lettuce growth was determined to not be an effective way to increase growth in hydroponic lettuce. Together, these studies offer further insight into how light influences plant morphology, cultivar-specific responses, and the finding that the addition of excess potassium (K) does not impact growth.

INDEX WORDS: Controlled environment agriculture, incident light, light use efficiency, daily light integral, tipburn, plant physiology

PHOTOSYNTHETIC PHOTON FLUX DENSITIES, ANTHOCYANINS, AND POTASSIUM AFFECT LEAF EXPANSION AND GROWTH OF HYDROPONIC LETTUCE

by

PEYTON LOU PALSHA

BS Horticulture, Landscape, and Turf Science, University of Arkansas, 2021

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

MASTER OF SCIENCE

ATHENS, GEORGIA

DECEMBER 2023

PHOTOSYNTHETIC PHOTON FLUX DENSITIES, ANTHOCYANINS, AND POTASSIUM AFFECT LEAF EXPANSION AND GROWTH OF HYDROPONIC LETTUCE

by

PEYTON LOU PALSHA

Major Professor:	Dr. Rhuanito Soranz Ferrarezi
Committee:	Dr. Ryan Dickson Dr. Lynne Seymour Dr. Melanie Yelton

Electronic Version Approved:
Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2023

DEDICATION

Dr. Marc van Iersel,

This work is dedicated to you because no one is more deserving of acknowledgment for guiding me through the early stages of my journey as a graduate student. You were an extraordinary man, scientist, and mentor. Without you, I would not have gone on so many adventures or learned as much as I have. You truly were the smartest, kindest, funniest, selfless, and most patient professor I've had the honor to work under. Saying goodbye to you was no easy feat, but hopefully, wherever you are, you'll be able to see all the amazing scientific accomplishments I plan to achieve. You will always have a special place in my heart, Dr. Marc. I'm grateful for every day I had with you. I look forward to seeing glimpses and reminders of you in the turtles I meet, plants I grow, and every photon of sunlight that shines down on me. I miss and love you so much.

Live long and prosper,

Peyton Lou

ACKNOWLEDGEMENTS

Kristy Palsha, you’ve always known how to calm me down and were always there to help me when I needed advice. Your perspective is consistently framed as ‘a puzzle, never a problem’, and I strive to live by those words of wisdom. There genuinely is nothing more comforting than a mother’s presence, support, advice, and love. Thank you for always believing in me, Mama. Douglas Palsha, no father has shown more pride in their child’s accomplishments like you have to mine. My wish is that you and mom always have a reason to be proud of me. Your advice to ‘just have fun’ is the reason I've created such fond memories and will continue to make many more. Thank you for always supporting me, Dad. Gracelyn Palsha, you truly are the most beloved person in my life. I wouldn’t want anyone else in the entire world to be my sister. Simply being near you heals my heart and soul like no one else can. I can’t wait to see the monumental accomplishments you achieve during our lifetime together. Thank you, G. Calvin Bryant, you’ve encouraged me to dream bigger than I ever dared before, while also supporting me in pursuing my current dream. Your loyalty has been unwavering, whether during times of great sadness or immense happiness. Because of this, I eagerly look forward to the unknown and unexpected with you by my side. Thank you, My Love. Fredrick Lewis, your support and guidance opened many doors for me in life, ultimately leading me to my dream school at The University of Georgia. I hope both my athletic and academic achievements have made you proud. Thank you, Coach Fred.

Dr. Rhuanito Ferrarezi, in our greatest time of need, you stepped up to support me and the rest of Dr. Marc van Iersel’s students in achieving our scientific dreams. We are fortunate to have a professor who genuinely wants to see us succeed. Thank you, Dr. Rhuanito. Dr. Lynne

Seymour, you have not only been my professor but also a dear friend. I’m grateful to have gotten the pleasure to know you and have learned from you. I know Dr. Marc is proud of you, just as I am. Thank you, Sensi Seymour. Dr. Melanie Yelton and Dr. Ryan Dickson, I want to express my sincere gratitude for your invaluable support as members of my committee. Your guidance has been instrumental in helping me achieve my scientific dreams, and I am truly appreciative of the kindness you've shown throughout this journey.

I want to express my heartfelt gratitude to my Plant Physiology Lab colleagues and friends: TC, Su, Kahlin, Mark, Andres, and Parker. I couldn't have completed this journey without your unwavering support. Your guidance as friends and fellow scientists has been invaluable. I am genuinely grateful for the feedback and camaraderie over the past two years, and I look forward to many more years of friendship ahead.

I extend my gratitude to the USDA-NIFA-SCRI for funding my work on ‘Lighting Approaches to Maximize Profits’ under project award number #2018-51181-28365.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction and Literature Review

Lettuce, *Lactuca sativa*, thrives in cooler climates, flourishing within the temperature range of 7-24°C (Sublett et al., 2018). Lettuce boasts an abundance of dietary fiber, antioxidants, vital vitamins like A, C, and E, and essential minerals such as calcium and iron (Sublett et al., 2018), making it a substantial addition to a health-conscious diet. In 2022 it was reported that approximately 85% of the lettuce available for consumption within the United States was domestically produced (USDA National Agricultural Statistics Service., 2023). In terms of revenue, lettuce constituted almost 20% of the $21.8 billion in cash receipts that U.S. growers earned from selling vegetables and melons in 2022 (Weber, 2023). Head lettuce production in 2022 generated total revenue of $1.33 billion, marking a 38% increase from the previous year (USDA National Agricultural Statistics Service., 2023). Lettuce cultivation is feasible throughout the year, with California and Arizona being the primary states responsible for over 98% of leaf lettuce production in the U.S. (Weber, 2023).

Nevertheless, lettuce production varies geographically based on the season within the country (Weber, 2023). Consequently, lettuce shipments from Florida and other southern states become necessary during fall and winter to address regional market shortages (Weber, 2023). In 2022, the supply chain faced challenges due to adverse weather conditions, water allocation issues, and the Impatiens Necrotic Spot Virus (INSV) (USDA National Agricultural Statistics Service., 2023). Notably, within the geographic regions of California and Arizona, epidermal blistering and peeling in select lettuce crops emerged due to prolonged freezing temperatures during December of the same year (USDA National Agricultural Statistics Service., 2023). These challenges serve as a subset of the prevailing issues that growers must grapple with in our ever-changing and uncertain climate. Producers are compelled to seek viable solutions to address these concerns. This naturally leads us to ponder: What does the future of cultivation look like?

Controlled Environment Agriculture

Over the past ten years, there has been a growing demand for locally cultivated fresh fruits and vegetables, driven by an escalating demand for produce that is accessible throughout the year (Sublett et al., 2018; Gómez et al., 2019). Controlled environments are used year-round to help grow crops during the off-season, which can help guarantee the yield and quality of crops (Duarte-Galvan et al., 2012). The current world population in 2023 has surpassed 8 billion (Warner et al., 2023), and with the world population predicted to increase and reach 9.3 billion in 2050 continuously, the Food and Agriculture Organization (FAO) estimates that 60% more food will need to be produced (Da Silva, 2012). With an increasing world population, arable land will decrease, and a potential solution to increase the global food supply could be controlled environmental agriculture (CEA) (Benke et al., 2017). CEA presents numerous production advantages. One key advantage is establishing a tailored microclimate adaptable to the grower’s specific requirements and geographical location (Duarte-Galvan et al., 2012). This level of control not only enables year-round production, potentially boosting crop yield (van Iersel & Gianino, 2017) but facilitates the anticipation of plant responses by considering various environmental factors (Gómez, C. et al., 2019). As a result, crops can thrive and contribute to local food production, regardless of location, allowing CEA to emerge as a sustainable solution.

CEA aims to reduce water, land, and energy inputs while enhancing crop quality and accelerating production timelines by providing consumers with locally grown and highly nutritious food (van Iersel & Gianino, 2017).

It is estimated that by 2025 the global investment into CEA will reach USD $172 Billion and in 2032 it is estimated to reach $270 Billion (FinancialNewsMedia.com, 2021; KD Market Insights, 2021). Despite some challenges along the way, such as costly electricity bills, CEA holds promising solutions to the global food crisis humanity faces. In CEA, one of the primary financial challenges confronting growers stems from the substantial electrical expenses incurred in sustaining year-round lighting, often in the form of supplemental or sole source Light Emitting Diodes (LED) lighting (Gianino & van Iersel 2017; Kim et al., 2022). Remarkably, the allocation for lighting costs constitutes a significant portion, ranging from 10% to 50% of the operational costs, consequently impacting the overall profitability of producers (Kim et al., 2022). In response to this financial hurdle, a pivotal focus lies in mitigating the burden of electrical costs associated with lighting by identifying the optimal target for light use efficiency that aligns with plants’ growth requirements, enabling enhanced crop development and shortened production timelines. This holds paramount importance as it promises to alleviate the financial strain of lighting expenses, thereby promoting greater economic sustainability within greenhouse production systems.

Hydroponic Growing System

Hydroponic cultivation involves soilless techniques where plant roots are positioned in a stationary or consistently aerated nutrient solution or a continuous flow or mist environment. The hydroponic technique employed in each experiment was Deep Water Culture (DWC), which, like all hydroponic systems, involves the direct delivery of nutrients and water to plant root systems. DWC facilitates water aeration through an air stone bubbling mechanism, ensuring sufficient oxygenation for root nutrient absorption. The plants are cultivated in net pots, which are upheld by a buoyant platform such as a foam board placed atop a deep reservoir. Consequently, the plant roots extend through the net pots and into the oxygenated nutrient solution. Because the plants grow within a nutrient-infused solution instead of soil, monitoring pH and electrical conductivity (E.C.) levels becomes imperative. The pH measurement is pivotal in determining the availability of essential plant elements, which can undergo shifts in composition during various stages of plant growth due to the depletion of specific nutrients (Sharma et al., 2018). Meanwhile, the E.C. measurement gauges the conductivity of the solution, thereby indicating nutrient absorption efficiency. For leafy vegetables like lettuce, maintaining an optimal pH range of 5.5 to 6.5 and an E.C. range of 1.2 to 1.8 dS m^{-1} is recommended to achieve ideal growth conditions (Sharma et al., 2018).

Cultivating crops hydroponically offers a plethora of advantages, such as diminishing soil-related pests, fungi, and diseases, since plants thrive in an aqueous nutrient solution rather than in traditional soil environments (Atmadja et al., 2017; Hamza et al., 2022). These hydroponic systems also contribute to a reduction in nutrient wastage, as precise quantities of fertilizer nutrients can be directly added to the water, effectively mitigating the risk of runoff into the surrounding ecosystem (Benke et al., 2017; Lages Barbosa et al., 2015). Furthermore, hydroponic cultivation, including DWC, significantly enhances water efficiency. Both systems predominantly employ soilless cultivation methods, resulting in potential water savings of up to

70–95%, primarily due to reduced irrigation water demand (Hamza et al., 2022; Vatistas et al., 2022). Hydroponic cultivation techniques can significantly alleviate land usage by up to 75%, which holds significance in addressing the impending arable land scarcity projected for 2050 (Hamza et al., 2022). CEA’s increasing popularity of soilless crop cultivation can be credited to its efficient resource utilization, heightened economic advantages from faster production cycles, and improved control over growing conditions (Hamza et al., 2022).

As climate change brings rising temperatures and drought, the need for water-efficient crop production is more crucial than ever (Benke et al., 2017). The reliance on natural rainfall alone can no longer suffice for successful field production, necessitating the integration of irrigation alongside nutrient supplementation (Lages Barbosa et al., 2015). Regrettably, this conventional approach often leads to wasteful water utilization during field irrigation, compounding with issues such as pesticide, fertilizer, and nutrient runoff, which carry the potential for environmental toxicity and harm (Lages Barbosa et al., 2015). To mitigate these challenges, there is a need to transition away from the sporadic occurrence of nutrient runoff stemming from excess water in traditional agricultural field settings (Benke et al., 2017). CEA, combined with hydroponic methodologies, emerges as an innovative solution to enhance water use efficiency and promote nutrient recycling sustainably (Benke et al., 2017; Vatistas et al., 2022). This strategic approach promises to minimize water inputs while concurrently addressing the issues tied to runoff, thereby presenting a more environmentally conscious path forward in crop cultivation.

Lighting in Controlled Environment Agriculture

In CEA there are two main types of growing systems used which are vertical farms and greenhouses. Vertical farming refers to crops cultivated indoors and stacked vertically, one on top of the other, to increase growing space to optimize yield per square meter of cultivation area (Vatistas et al., 2022). Vertical farms can be implemented in diverse settings, including warehouses, containers, and vacant buildings of various sizes however they are powered solely by LEDs, or light-emitting diodes, as light sources on each level (Runkle, 2017; Vatistas et al., 2022). These lights are vital in vertical farming as they provide illumination to plants grown entirely indoors under sole-source lighting (without external sunlight exposure).

In greenhouse production, plants are cultivated using a combination of natural sunlight and supplemental lighting, typically in the form of LEDs (Gianino & van Iersel, 2017). While this method doesn’t rely exclusively on sole-source lighting, it does require supplemental lighting to achieve the desired cumulative daily light integral (DLI) necessary for year around production for high quality crops (Gianino & van Iersel, 2017). The DLI is the PPFD integrated over a day which is contingent on both the intensity and duration of light received over the 24-hour cycle, known as the photoperiod (Gianino & van Iersel, 2017; Poorter et al., 2019). Many plant traits are found to be better related to DLI than to instantaneous PPFD values at any specific moment in time (Poorter et al., 2019). Supplemental lighting is crucial in locations where natural sunlight may be insufficient to meet the target DLI, which is why it is used in addition to sunlight (Poorter et al., 2019). Furthermore, supplemental lighting is necessary because natural sunlight varies throughout the day and across different seasons, therefore for efficient year-round production in greenhouses, supplemental light is often needed from late fall through early spring (Gianino & van Iersel, 2017).

LED lights are an important factor when growing plants in both types of CEA systems because they influence plant growth, however, they come with the highest expenses in terms of purchasing, installation, and operation (Runkle, 2017). These factors introduce five additional vital characteristics related to sole-source lighting, such as light quality, light uniformity, fixture efficiency, light intensity, and duration, along with considerations for cost, durability, and longevity (Runkle, 2017).

LEDs have become the preferred choice for commercial indoor farms as well as greenhouse growing. This preference stems from their numerous advantages, including a longer lifespan, low radiation, heat emission, and high efficiency and durability (Kelly et al., 2020). Light plays a pivotal role in indoor plant cultivation, serving as the primary energy source for photosynthesis and influencing plant morphology and quality through the modulation of biological signals. Fortunately, in recent years, LEDs have seen significant advancements. They have become more efficient, adjustable, and cost-effective in installation and purchase (Kelly et al., 2020).

Lighting in CEA has three dimensions that can effectively increase biomass: radiation quality, radiation quantity, and photoperiod (Kelly et al., 2020). Radiation quality is the composition of wavelengths emitted by the light or radiation source (Kelly et al., 2020). The light spectrum is a critical consideration because different wavelengths can cause different effects on plant growth. By manipulating the quality of light, which involves using specific wavelengths, we can influence characteristics such as leaf thickness, size, and color (Runkle, 2017). Radiation quantity is the number of photons striking a square meter over a specific timeframe, whether it be seconds or days, and is expressed in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, or $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ and is known as photosynthetic photon flux (PPF) (Kelly et al., 2020). This metric signifies the micromoles of photons falling upon a square meter per second and plays a pivotal role in influencing plant responses to light (Kelly et al., 2020). Photoperiod refers to the duration of light exposure plants receive over a 24-hour cycle (Kelly et al., 2020). It plays a fundamental role in regulating plant physiological processes. Additionally, the cumulative duration of light exposure during each photoperiod determines the DLI, a critical factor for optimizing biomass production. DLI depends on the intensity and duration of light exposure throughout the 24-hour cycle (Kelly et al., 2020).

Tipburn

Tipburn is a physiological disorder primarily affecting inner head vegetables like lettuce, leading to the necrosis of young leaf edges (Saure, 1998). This condition is frequently associated with calcium deficiency and is typically observed during the later stages of crop growth, particularly when growth rates are high (Saure, 1998; Ertle & Kubota, 2023). Although most studies suggest a positive correlation between increased growth rates and higher tipburn incidence, it’s important to highlight that tipburn can manifest as early as 13 days after germination, indicating it can occur at various growth stages, not solely at the end of the crop’s development (Saure, 1998; Ertle & Kubota, 2023). It is mainly seen in young leaves but there is a notable concentration of low calcium content along the leaf margins. Furthermore, external stress factors such as relative humidity, temperature, restricted transpiration rates, and light intensity can contribute to the development of tipburn (Saure, 1998). When plants are cultivated under optimal, stress-free conditions, any exposure to stress can diminish their stress tolerance and increase their susceptibility to tipburn (Saure, 1998). Ultimately, it has been observed that a combination of stressors can induce tipburn, affecting the severity of its occurrence (Saure, 1998).

Potassium

Potassium (K) physiologically and biochemically plays multiple vital roles in a plant system. Adequate amounts of K are necessary and aid in photosynthesis, stomatal regulation (Lindhauer et al., 1983; Taiz et al., 2015), enzyme activation, water, sugar, and nutrient transportation, and enhanced crop quality (Prajapati et al., 2012). K is also essential for increasing turgor pressure, which drives plant cell expansion and leaf elongation (Taiz et al., 2015). K ions activate 60 enzymes related to plant growth processes (Taiz et al., 2015). These enzymes are involved in various metabolic processes, including energy production such as adenosine triphosphate (ATP) generation (Taiz et al., 2015). The amount of potassium in the cell and its availability can also impact the activation of these enzymes.

When there is a K deficiency, plant growth processes like photosynthesis and respiration decrease due to the reduction of activating enzymes that aid in ATP generation (Prajapati et al., 2012). ATP is essential for various energy-requiring processes in plants, including transporting sugars through the phloem (Prajapati et al., 2012; Taiz et al., 2015). Since ATP is the energy used to transport sugars created through the photosynthetic process, the lack of K causes this process to break down due to the inadequate production of ATP (Prajapati et al., 2012; Taiz et al., 2015). Plants with a K deficiency can also be susceptible to water loss. K plays a vital role in stomatal guard cells since it is the primary nutrient that helps regulate the opening and closing of these pores (Prajapati et al., 2012). Guard cells regulate the exchange of oxygen, carbon dioxide, and water (Lindhauer et al., 1983). When K is available in adequate amounts, the guard cells swell with an influx of water, allowing the stomatal pore to open and release gases (Prajapati et al., 2012). However, when there is low K, the water available in the guard cells decreases, causing them to close slowly. This delayed reaction allows water vapor to be lost to the atmosphere and leads to water stress (Prajapati et al., 2012; Lindhauer et al., 1983; Taiz et al., 2015). However, plants can maintain their overall leaf growth and photosynthetic activity when exposed to water stress, but only to a certain extent (Lindhauer et al., 1983). Sufficient K is required to maintain efficient metabolic processes and water use efficiency. Water use efficiency is highest when adequate amounts of K are present to close stomatal guard cells quickly and efficiently, minimizing the amount of transpiration (Lindhauer et al., 1983). Therefore, maintaining adequate amounts of K is vital for water use efficiency, crop growth, and yield.

Photosynthetic Pigments

Photosynthetic pigments, like chlorophyll a and b, are located in chloroplasts, which absorb photons from light. The four potential fates of absorbed light by plant pigments are photochemistry, heat dissipation, fluorescence, and resonance energy transfer (Taiz et al., 2015). Photochemistry occurs when absorbed light energy, upon excitation, initiates a chemical reaction with plant pigments. This reaction involves the absorption of light photons by the pigments, leading to the release of electrons. These released electrons are then captured by other pigments that act as acceptors (Taiz et al., 2015). Heat dissipation involves converting absorbed light energy into heat when the energy level is relatively high in the excited state. This process does not result in the release of a photon (Taiz et al., 2015). Fluorescence, on the other hand, occurs when a portion of the absorbed energy is temporarily stored in an excited state and then released as a lower-energy photon when the pigment returns to its ground state. The released photon has lower energy than the absorbed energy due to a fraction of the excited energy being converted into heat before the fluorescent photon is emitted (Taiz et al., 2015). Resonance energy transfer takes place when pigments are in close proximity to one other. In this scenario, absorbed light energy is transferred from one pigment to another, exciting the subsequent pigment and initiating a cascade of energy transfer along a gradient (Taiz et al., 2015). Absorbed photons transfer the excited energy to reaction centers, which results in electron transport that produces ferredoxin, NADPH, and ATP, which provide the chemical energy required to reduce carbon dioxide and synthesize carbohydrates (Lindhauer et al., 1983; Kim & van Iersel, 2022; Lu et al., 2022). The high electron transport rates in light reaction centers are associated with rapid growth because the carbohydrates synthesized allow crops to grow faster and increase light capture (Kim & van Iersel, 2022).

Anthocyanin Pigments

Anthocyanins are a class of flavonoids that are water soluble non-photosynthetic pigments with antioxidant roles (Agati et al., 2021; Xu et al., 2018). They are synthesized in the cytoplasm and located in the vacuole lumen of the epidermal cells, which accumulate mainly in leaves and stems (Nassour et al., 2020; Xu et al., 2018). These pigments are responsible for the autumn leaf colors seen in deciduous trees and affect the light absorbed by the leaves. Red leaves absorb more green light than green leaves, resulting in lower photosynthesis rates in red leaves over green leaves (Neill et al., 2020). Lower rates of photosynthesis in plants with higher anthocyanin levels occur due to a decrease in quantum yield and light absorption since these pigments are non-photosynthetic (Neill et al., 2020).

The electron transport rate is dependent on the amount of absorbed light and the quantum yield of photosystem II, which means that if a plant has a low quantum yield of photosystem II, the absorbed light will largely dissipate as heat and not photosynthetic electron transport (Kim & van Iersel, 2022; Genty et al., 1989). This idea helps explain why some lettuce cultivars with a higher level of anthocyanin pigmentation tend to grow slower than lettuce cultivars with low anthocyanin pigments.

Under high light stress, plants increase their anthocyanin concentrations to reduce photooxidative damage (Nassour et al., 2020). Anthocyanins are also known to protect their leaves from a photoinhibition response by intercepting light energy in the vacuole that chlorophylls would have absorbed (Neill et al., 2020; Nassour et al., 2020). The interception of these photons not only reduces photoinhibition but also reduces reactive oxygen species (ROS). Xu explains that ROS is a byproduct produced under different abiotic and biotic conditions, which cause oxidative stress and damage to plant cells. With higher anthocyanin concentrations, ROS can be reduced while maintaining photosynthetic efficiency (Xu et al., 2018).

Research Objectives

The objectives of the first study aimed to investigate the impact of varying PPFDs on lettuce morphology, growth, LUE, and projected canopy size. Given the crucial role of light capture by the plant canopy and LUE in crop growth, we hypothesized that an increase in PPFD through supplemental lighting could optimize biomass production. This presumed optimization hinges on the enhancement of total incident light captured by the canopy, even at the potential cost of decreased LUE. Furthermore, we hypothesize that lettuce morphology could adapt under low PPFD conditions to enhance its light capture capabilities.

The objectives of our second study explored six different lettuce cultivars' morphological and physiological responses characterized by varying leaf anthocyanin contents. This investigation was carried out within a controlled greenhouse setting, subjecting the cultivars to diverse PPFDs provided by supplemental LED lighting. The overarching aim was to understand better how leaf anthocyanin content and PPFD variation collectively impacted each cultivar's growth, morphology, physiology, and LUE throughout an entire growing cycle. We hypothesized that cultivars displaying lower leaf anthocyanin content values would increase shoot weight and growth with increasing PPFD.

Our objectives in the third and final study were to center our investigation on the growth dynamics of hydroponic lettuce, driven by the influence K has on cellular expansion and leaf elongation to increase growth. This study was conducted within a greenhouse, with varying DLIs achieved by integrating an adaptive lighting control system (ALC). We initially hypothesized that increasing K levels in the hydroponic solution would enhance lettuce growth more effectively than DLI.

Literature Cited

Agati, G., Guidi, L., Landi, M., & Tattini, M. (2021). Anthocyanins in photoprotection: knowing the actors in play to solve this complex ecophysiological issue. New Phytologist, 232(5), 2228-2235. doi: https://doi.org/10.1111/nph.17648

Atmadja, W., Liawatimena, S., Lukas, J., Nata, E. P. L., & Alexander, I. (2017, December). Hydroponic system design with real-time OS based on ARM Cortex-M microcontroller. In IOP Conference Series: Earth and Environmental Science (Vol. 109, No. 1, p. 012017). IOP Publishing. doi: 10.1088/1755-1315/109/1/012017

Barbosa, G. L., Gadelha, F. D., Kublik, N., Proctor, A., Reichelm, L., Weissinger, E., Wohlleb, G. M., & Halden, R. U. (2015). Comparison of Land, Water, and Energy Requirements of Lettuce Grown Using Hydroponic vs. Conventional Agricultural Methods. *International journal of environmental research and public health*, *12*(6), 6879–6891. doi: https://doi.org/10.3390/ijerph120606879

Benke, K., & Tomkins, B. (2017). Future food-production systems: vertical farming and controlled-environment agriculture. Sustainability: Science, Practice and Policy, 13(1), 13-26. doi: https://doi.org/10.1080/15487733.2017.1394054

Domingues, D. S., Takahashi, H. W., Camara, C. A., & Nixdorf, S. L. (2012). Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production. Computers and Electronics in Agriculture, 84, 53-61. doi: https://doi.org/10.1016/j.compag.2012.02.006

Duarte-Galvan, C., Torres-Pacheco, I., Guevara-Gonzalez, R. G., Romero-Troncoso, R. J., Contreras-Medina, L. M., Rios-Alcaraz, M. A., & Millan-Almaraz, J. R. (2012).

Advantages and disadvantages of control theories applied in greenhouse climate control systems. Spanish Journal of Agricultural Research, 10(4), 926-938. doi: https://doi.org/10.5424/sjar/2012104-487-11

Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA)-General Subjects, 990(1), 87-92. doi: https://doi.org/10.1007/BF00032982

Gould, K. S., McKelvie, J., & Markham, K. R. (2002). Do anthocyanins function as antioxidants in leaves? Imaging of H2O2 in red and green leaves after mechanical injury. Plant, Cell & Environment, 25(10), 1261-1269. https://doi.org/10.1046/j.1365-3040.2002.00905.x

Hamza, A., Ismail, I., & Belouatek, A. (2022). Using deep water culture as one of the important hydroponic systems for saving water, mineral fertilizers and improving the productivity of lettuce crop. International Journal of Health Sciences, 6, 2311-2331. doi: https://doi.org/10.53730/ijhs.v6nS9.12932

Jayalath, T. C., & van Iersel, M. W. (2021). Canopy size and light use efficiency explain growth differences between lettuce and mizuna in vertical farms. Plants, 10(4), 704. doi: https://doi.org/10.3390/plants10040704

Kim, C., & van Iersel, M. W. (2022). Morphological and physiological screening to predict lettuce biomass production in controlled environment agriculture. Remote Sensing, 14(2), 316. doi: https://doi.org/10.3390/rs14020316

Legendre, R., & van Iersel, M. W. (2021). Supplemental far-red light stimulates lettuce growth: Disentangling morphological and physiological effects. Plants, 10(1), 166. doi: https://doi.org/10.3390/plants10010166

Lindhauer, M. G. (1983). Effect of potassium on water use efficiency. Proceedings 17th Coll. Intern. Potash Inst, 81-107.

Lu, D., Zhang, Y., Zhang, A., & Lu, C. (2022). Non-Photochemical Quenching: From Light Perception to Photoprotective Gene Expression. International journal of molecular sciences, 23(2), 687. doi: https://doi.org/10.3390/ijms23020687

Nassour, R., Ayash, A., & Al-Tameemi, K. (2020). Anthocyanin pigments: Structure and biological importance. Journal of Chemical and Pharmaceutical Sciences, 13, 45-57.

Neill, S. O., & Gould, K. S. (2003). Anthocyanins in leaves: Light attenuators or antioxidants? Functional Plant Biology, 30(8), 865-873. doi: https://doi.org/10.1071/FP03118

Palmer, S., & van Iersel, M. W. (2020). Increasing growth of lettuce and mizuna under sole-source LED lighting using longer photoperiods with the same daily light integral. Agronomy, 10(11), 1659. doi: https://doi.org/10.3390/agronomy10111659

Prajapati, K., & Modi, H. A. (2012). The importance of potassium in plant growth–a review. Indian Journal of Plant Sciences, 1(02-03), 177-186. doi: https://doi.org/10.9790/9622-0803054452

Saure, M. C. (1998). Causes of the tipburn disorder in leaves of vegetables. Scientia Horticulturae, 76(3-4), 131-147. doi: https://doi.org/10.1016/S0304-4238(98)00153-8

Sharma, N., Tyagi, R. K., & Tyagi, S. (2018). Hydroponics as an advanced technique for vegetable production: An overview. Journal of Soil and Water Conservation, 17(4), 364-371. doi: https://doi.org/10.5958/2455-7145.2018.00056.5

Sublett, W., Barickman, T., & Sams, C. (2018). The Effect of Environment and Nutrients on Hydroponic Lettuce Yield, Quality, and Phytonutrients. Horticulturae, 4(4), 48. doi: https://doi.org/10.3390/horticulturae4040048

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant Physiology and Development (6th ed.). Sinauer Associates Incorporated.

van Iersel, M. W. (2017). Optimizing LED lighting in controlled environment agriculture. In Light Emitting Diodes for Agriculture: Smart Lighting (pp. 59-80). doi: https://doi.org/10.1007/978-981-10-5807-3_4

Vatistas, C., Avgoustaki, D. D., & Bartzanas, T. (2022). A systematic literature review on controlled-environment agriculture: How vertical farms and greenhouses can influence the sustainability and footprint of urban microclimate with local food production. *Atmosphere*, *13*(8), 1258. doi: https://doi.org/10.3390/atmos13081258

USDA National Agricultural Statistics Service. (2023, February) *Vegetables 2022 Summary*. USDA Economics, Statistics and Market Information System, usda.library.cornell.edu/.

Weber, C. (2023, May 18). *U.S. lettuce production shifts regionally by season*. USDA Economic Research Service. https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=106516 van Iersel, M. W., & Gianino, D. (2017). An adaptive control approach for light-emitting diode lights can reduce the energy costs of supplemental lighting in greenhouses. *HortScience*, *52*(1), 72-77. doi: https://doi.org/10.21273/HORTSCI11385-16

CHAPTER 2

GROWTH, MORPHOLOGY, AND PHYSIOLOGY OF HYDROPONIC LETTUCE IN RESPONSE TO PHOTOSYNTHETIC PHOTON FLUX DENSITIES UNDER SOLE-SOURCE LIGHTING[1]

---

[1] Palsha, P.L, M.W. van Iersel, R.W. Dickson, M. Yelton, L. Seymour, and R.S. Ferrarezi. To be submitted to Frontiers in Plant Science

Abstract: Crop growth is strongly affected by the amount of light captured by the canopy and light use efficiency (LUE). Increased leaf expansion and cultivar-specified photosynthetic photon flux densities (PPFD) rates from supplemental lighting can maximize biomass production and shorten the production period, essential in decreasing electricity costs in controlled environmental agriculture (CEA). The effect of different PPFDs on lettuce growth, morphology, light capture, and LUE needs to be better understood. This study documented the morphological and physiological effects of lettuce grown hydroponically at different PPFDs under sole-source lighting in controlled environments. At lower PPFD levels (201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plants increased their specific leaf area (SLA) and projected canopy size (PCS) to enhance light capture. At higher PPFD levels (333 and 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plants exhibit increased canopy overlap ratio (COR) and have higher shoot dry weights. Low and high PPFD conditions lead to a similar trend in PCS among plants. LUE did not play a significant role in determining growth. Instead, the critical factor was a strong and positive correlation between growth and total incident light (total IL) the plants received.

Introduction

Photosynthetic photon flux densities, incident light, and crop growth

Photosynthetic Photon Flux (PPF) measures the total amount of photosynthetically active radiation (PAR) or light generated within one second (Taiz et al., 2015). PAR light falls within the wavelength range of 400-700 nm and can be produced by natural sunlight or artificial lighting systems such as LEDs (Taiz et al., 2015). PPF provides information about the total light output, regardless of the photon direction or their distribution. Photosynthetic Photon Flux

Density (PPFD) quantifies the number of photosynthetically active photons that reach a one-square-meter area per second (Taiz et al., 2015). Photoperiod is the duration of light per 24-hour period, while daily light integral (DLI) is the total sum of radiation in a 24-hour period (Kelly et al., 2022). DLI is the product of PPFD and photoperiod. Crop growth is influenced by the quantity of incident light penetrating the canopy (Jayalath & van Iersel, 2021). Incident light refers explicitly to the light energy that falls onto the leaves or surfaces of plants and is typically measured in terms of PPFD, which quantifies the number of photons (particles of light) hitting a specific area over a given period (Cabrera-Bosquet et al., 2016). The total incident light (IL) a plant receives throughout a growing cycle can be ascertained by combining measurements of a plant's received PPFD with daily projections of canopy size gleaned through nondestructive digital imaging methods (Jayalath & van Iersel, 2021). For the calculation of total IL, we assumed that the amount of light reaching the plant is determined by projected canopy size (PCS) and PPFD (Kim & van Iersel, 2022). Crop growth depends on the PCS and the light use efficiency (LUE), represented as the biomass generated per unit of incident light (Jayalath & van Iersel, 2021; Legendre & van Iersel, 2021). Another critical factor influencing crop growth is LUE, which measures how efficiently crops convert incident light into plant growth and biomass production (Jayalath & van Iersel, 2021; Palmer & van Iersel, 2020). LUE is calculated by dividing the dry weight of each plant by the total IL a plant receives throughout its entire growing cycle. Evaluating the progression of PCS development and LUE is pivotal in screening crops' swift growth (Jayalath & van Iersel, 2021; Palmer & van Iersel, 2020). This concept is significant in CEA because identifying rapid early growth in a crop's growth cycle can reduce production time, leading to notable reductions in light and electrical costs (Cabrera-Bosquet et al., 2019; Jayalath & van Iersel, 2021). This acceleration shortens the duration and amount of light needed for plants to produce biomass (Kim & van Iersel, 2022).

Effect of PPFD on plant morphology, light absorption, and light use efficiency

It is crucial to understand better the significant influence of photosynthetic photon flux densities on plant morphology, light absorption, and efficiency. The quantity of light a canopy captures and its light use efficiency profoundly impact crop development (Jayalath & van Iersel, 2021). This significance is magnified in settings like controlled environment agriculture (vertical farms, greenhouses, and plant factories), where the electricity expense for lighting is substantial (Jayalath & van Iersel, 2021). Faster leaf expansion plays a pivotal role in augmenting light absorption by plants. Rapid leaf expansion and crop specific PPFD rates achieved through supplementary lighting can optimize biomass production. This synergy contributes to an increased leaf area, amplifying light captured by the canopy. Consequently, the overall crop growth is enhanced, and the production cycle is shortened, both vital for curbing electricity costs within CEA.

Knowledge gap

In the context of future CEA, there is a pressing need for research on multiple fronts. From a technological perspective, one avenue for improvement involves investigating the efficiency of LED technology to reduce energy consumption (Ji et al., 2023). Vertical farms face profitability challenges stemming from the high costs associated with electricity for lighting, maintaining the climate control system (including air conditioning and heating), and ensuring proper ventilation to manage excess humidity (Kelly et al., 2020). This is a tough market to compete in due to the high maintenance and complexity it takes to efficiently run an indoor farm while also making a profit back on initial investments (Kelly et al., 2020). Simultaneously, when focusing on plant science, there is room for enhancing yields per kilowatt-hour by fine-tuning factors such as light utilization within the canopy and optimizing leaf photosynthesis (Ji et al., 2023). To maximize crop yields, it is crucial to customize the growing environment for each type of plant. This customization should focus on optimizing photosynthetic and photochemical responses to different light levels (PPFD) (Weaver & van Iersel, 2020). This enables the effective use of LEDs for lighting control, which reduces electricity expenses by providing supplemental light based on each crop's ability to efficiently utilize light (Weaver & van Iersel, 2020).

Objective

The objective of this study was to evaluate the effects PPFD had on plant morphology, growth, and LUE in hydroponic lettuce. We hypothesized that elevating PPFD rates through sole-source supplemental lighting would increase biomass production by increasing the total incident light captured by the canopy, potentially at the expense of reduced LUE. Additionally, we aimed to explore how lettuce morphology can adapt under low PPFD conditions (201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to enhance its light capture capacity.

Materials and Methods

Experimental setup and Environmental conditions

This study was performed in a growth chamber at the University of Georgia Riverbend Greenhouse Complex (latitude: 33.9480^{o} N, longitude: 83.3773^{o} W) in Athens, GA, USA, from April 20th to May 27th, 2022. Plants were grown in a growth chamber with a maximum bench space of 1.4 m^2 (Controlled Environments LTD., Winnipeg, Manitoba, Canada). The primary lighting source for the growth chamber were sole-source LED light panels, which provided mainly white light but also contained a few red diodes (ViparSpectra, P1000-300-02, 94V) (660 nm red light, 3000K 5000K white light, and 730nm far red IR). The 3.78-liter buckets sat inside the growth chamber and had an aeration system that flowed upwards from the bottom, allowing proper airflow. The walls of the growth chamber were silver, and the window on the left door was closed, so no external light could enter once the two main doors were closed. The average temperature, vapor pressure deficit (VPD), relative humidity (RH), and ambient CO_2 in the growth chamber were 20 ± 1.4 °C, 1.2 ± 0.2 kPa, 38.0% ± 2.1, and 400 $\mu mol\ mol^{-1}$, respectively.

Seedling and transplant management

Lettuce *(Lactuca sativa)* cultivar ‘Rex’ was used in a pelleted form (Johnny’s selected seeds, Winslow, ME, USA). ‘Rex’ seeds were sowed in rockwool plugs that were 2.5 × 2.5 cm and 4 cm tall (A0 25/40; Grodan Rockwool BV, Roermond, Netherlands) for seedling production. One lettuce ‘Rex’ seed was put in each rockwool plug, and 120 plugs were seeded. The tray (1 tray of 120 plugs in each) was then put in double-stacked black rectangular plastic mesh trays (50.8 long × 12.7 wide × 5.08 cm tall) and then covered with transparent plastic domes for four days to help increase and maintain high humidity during germination. The plants were put in a walk-in vertical farm and placed on shelves under 1.1 m-long white LED light fixtures (RAY with Physiospec indoor spectrum; Fluence, USA), which provided 250μmol·m^{-2}·s^{-1} of PAR over a 16-h photoperiod. The tray was sub-irrigated daily for 5 minutes using an ebb-and-flow bench that contained a water-soluble fertilizer solution using 100 mg·L^{-1} nitrogen (N) (15N–2.2P–12.45K, Peters Excel 15–5–15 Cal-Mag Special; Everris NA, Dublin, OH, USA).

Eleven days after seeding, the 16 most uniform seedlings were transplanted into a deep-water culture hydroponic setup using 4.8 cm-tall × 4.5 cm (top-diameter) × 3.3 cm (bottom-diameter) net pots (Teku G46; Pöppelmann GmbH & Co., Lohne, Germany), then placed into individual 4.5-liter black buckets. Matching lids were placed on the buckets with a 4.1 cm hole (Lenox Bi-Metal, Bristol, PA, USA) drilled through the middle of the lid to hold each net pot and plant. Instead of employing two larger deep-water hydroponic trays, individual 3.78-liter buckets were utilized due to the airflow originating from the bottom of the growth chamber. These individual smaller buckets were essential to ensure adequate and proper airflow within the system. The same water-soluble hydroponic nutrient solution for each 3.78-liter bucket was used and was a combination of three different fertilizers: Blend 9-7-37 (Hydroponic Fertilizer; Hort Americas, Bedford, Texas, USA); $Ca(NO_3)$ (YaraTera Calcinit; Yara, Oslo, Norway); and $MgSO_4$ (EPSOTop; K+S Minerals and Agriculture GmbH, Kassel, Germany). This fertilizer blend resulted in 80 mg/L N (3 mg/L ammonium, 77 mg/L nitrate), 10 mg/L phosphorus (P), 100 mg/L K, 62 mg/L calcium (Ca), 42 mg/L magnesium (Mg), 57 mg/L Sulfur (S), 0.29 mg/L boron (B), 0.16 mg/L copper (Cu), 1.63 mg/L iron (Fe), 0.62 mg/L Manganese (Mn), 0.03 mg/L Molybdenum (Mo), and 0.62 mg/L Zinc (Zn).

Plastic tubing and air stones (Pawfly Aquarium Air Stone, Guangzhou, China) were placed in each bucket to provide proper aeration to the nutrient solution using an air pump (EcoPlus-7; Hawthorne gardening company, Vancouver, WA, USA, 0.48 bar, 3.0 A, 120V, Tube Size: 12.7 mm in diameter). The initial pH for the solution was 6.36, while the EC was 1.46 mS cm^{-1} before transplanting. We used 1M of H_3PO_4 solution to reduce the pH of the hydroponic solution between 5.5 and 6.5. The 1M H_3PO_4 solution was consistently employed during the study to maintain the pH below 6.5. However, at various points during the study, the pH levels exceeded 6.5, with the highest recorded pH reaching 7.64 before being adjusted back within the desired range.

Treatments

To investigate the influence of varying photosynthetic photon flux densities on canopy dimensions and growth, hydroponically cultivated lettuce was subjected to sole-source white LED illumination, with PPFD levels spanning 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$, within a controlled growth chamber. PPFD values were consistently maintained throughout the photoperiod, ranging from 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The different PPFDs were achieved by randomly deactivating two out of the six LED light panels. This changed how light was distributed within the growth chamber and resulted in varying PPFD values. The photoperiod was 16 hours, from 6 a.m. to 10 p.m., yielding a DLI between 11.6 to 23.8 $mol \cdot m^{-2} \cdot d^{-1}$.

Projected Canopy Size Imaging

Chlorophyll fluorescence occurs at 650-720 nm wavelength, with a peak near 690 nm (Kim and van Iersel, 2022). Chlorophyll fluorescence makes up 1-2% of absorbed light, which helps visualize the plant area only and not the background because wavelengths at 690 nm cause the plant tissue to fluoresce, which can be seen and captured on camera (Fernandez-Jaramillo et al., 2012; Kim & van Iersel, 2022). Upon transplanting, all 32 plants were individually imaged using chlorophyll fluorescence imaging (CFI) to predict PCS throughout a growing cycle. For this experiment, the same CFI system created by Kim & van Iersel (2022), was used to predict lettuce biomass accumulation and PCS (Kim & van Iersel, 2022).

The imaging system setup required a light-proof grow tent (0.6 m wide × 0.6 m long × 1.2 m tall) with a Mylar reflective interior lining on the inside sides of the tent. A monochrome camera (Chameleon® 3; FLIR, Wilsonville, OR, USA) was mounted and centered at the top of the grow tent between two blue LED light panels (Pro 650e; LumiGrow, Emeryville, CA, USA) facing downwards. The camera had a 650 to 1100 nm long-pass filter (SP700-R45X2; Midwest Optical Systems, Inc., Palatine, IL, USA). The plants growing in their buckets were lifted out of the hydroponic solution by removing the lids they were supported by and brought to the CFI station where they were placed in an empty black bucket to be imaged. The black buckets were placed in the middle and rested on a black shade cloth and were exposed to blue LED light (450–490 nm), allowing the camera to capture only the fluorescence from the chlorophyll in the plants (Kim & van Iersel, 2022). On the 11th day when plants finished germinating and were transplanted, each plant was placed in the center of the CFI station for imaging and then put back into the growth chamber in the same positions and orientation, so the planting position always remained the same. After the initial transplant and calibration images were taken, plants were imaged twice a week to determine the PCS from April 20th to May 27th, 2022, for a total of 23 days.

Data collection

Light measurements were acquired using an ePAR 400-750 nm light sensor (MQ-200X Series; Apogee Instruments, Logan, UT) which quantified the provided PPFD from the sole-source LED lights. The study originally encompassed 32 plants, however, due to growing space complications, 16 plants (half) were harvested midway through the growth cycle. Data acquired from the first harvest was excluded from the present analysis, as its primary purpose was to liberate growing space for the remaining 16 plants, mitigating overlap or crowding issues.

Twenty-three days after transplanting, on May 27th, 2022, we conducted destructive measurements on the plants which concluded the growing cycle. To identify each plants' responses to varying PPFDs, the measurements encompassed various parameters, such as the fresh and the dry weight of shoots and roots per plant. Additionally, we assessed each plant's leaf area and PCS (Topview; Aris, Eindhoven, The Netherlands). Before the destructive measurements, all plants were subjected to one last imaging session to determine the final PCS. Individual leaves were carefully separated from the plants to gauge the total leaf area and then measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE, USA). Subsequently, the shoots and roots were placed in a drying oven at 80 °C for 120 h, following which their dry weights were measured. Dry weight recordings were then utilized for a comprehensive tissue nutrient analysis performed at Waters Agriculture Labs, Camilla, GA. (Supplemental material 2.1).

Experimental design and Statistical analysis

The experiment used a completely randomized design. Each experimental unit was comprised of one plant with 16 plants used total. Six sole-source LED light panels were used and the variations in light were achieved by randomly turning off two of the six LED panels. Multiple statistical analyses were conducted using multiple regression analysis utilizing a statistical software (SigmaPlot Version 11.0; Systat software, San Jose, CA, USA). Regression analyses were used to evaluate the effects of varying PPFD on fresh and dry shoot and root weights per plant, total leaf area per plant (TLA), water content, SLA, PCS, COR, LUE, and total incident light.

Results

Dry and fresh weights

Supporting our hypothesis there was a corresponding increase in dry weights of both shoots ($p < 0.0001$) and roots ($p = 0.0045$) as the PPFD increased (Figure 2.1B). Shoot dry weight under lower PPFD conditions between 201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was 16.13% lower compared with the shoot dry weight recorded under higher PPFDs of 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Root dry weights exhibited a response pattern similar to shoot dry weights under low and high PPFD conditions. Specifically, under the lower PPFD conditions ranging from 201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$, there was a 14.7% reduction in root dry weight compared to higher PPFD conditions of 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants grown under PPFDs between 333 to 413 $\mu mol \cdot m^{-}$

$^{2}\cdot s^{-1}$ demonstrated an increase in weight, nearly doubling the shoot and root dry weights observed in comparison to their low PPFD counterparts of 201 to 292 µmol·m^{-2}·s^{-1}.

The relationship between fresh weight and varying PPFD levels exhibited a parallel relationship to the dry weight, encompassing shoots and roots (Figure 2.1A). As PPFD increased from 201 to 413 µmol·m^{-2}·s^{-1}, there was a significant increase in fresh shoot weight ($p = 0.0001$), accompanied by a corresponding increase in fresh root weight ($p = 0.0006$). Under the lower PPFD conditions ranging from 201 to 292 µmol·m^{-2}·s^{-1}, there was an 11.3% reduction in shoot fresh weight compared to higher PPFD conditions of 333 to 413 µmol·m^{-2}·s^{-1}. Root fresh weight was reduced by 14.3% under low PPFD conditions of 201 to 292 µmol·m^{-2}·s^{-1}.

Water content

The investigation of % water content with varying PPFD levels revealed a decline in water content with increasing PPFD ($p = 0.0143$) (Figure 2.2). Notably, the contrasts in water content between plants cultivated under PPFDs ranging from 333 to 413 µmol·m^{-2}·s^{-1} and those grown under PPFDs spanning 201 to 292 µmol·m^{-2}·s^{-1} were relatively subtle. A negative correlation was evident, where plants with greater shoot dry weights exhibited lower water content ($p = 0.0017$), ranging from 0.961% to 0.952% (Figure 2.3).

Leaf area and projected canopy size

When looking at the TLA and final PCS versus PPFD, we saw that the final PCS for all plants grown between 201 and 413 µmol·m-^{2}·s-^{1} all had similar final PCS ($p = 0.3721$) (Figure 2.4A). However, the TLA increased with increasing PPFD ($p = 0.0184$). Chlorophyll fluorescence imaging was used to assess the PCS of each plant throughout its growth cycle and the last image taken of each plant was the final PCS. Analyzing the relationship between TLA and the final PCS relative to PPFD, it emerged that the final PCS remained consistent across all plants cultivated within the range of 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($p = 0.3721$) (Figure 2.4A). However, in contrast, the TLA displayed a positive trend with increasing PPFD ($p = 0.0184$) (Figure 2.4A).

Specific leaf area and canopy overlap ratio

Both SLA and COR exhibited noteworthy responses when influenced by variations in PPFD (Figure 2.4B). The SLA demonstrated higher values under PPFD levels ranging from 201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$, subsequently decreasing with ascending PPFD ($p < 0.0001$) (Figure 2.4B). Conversely, plants grown under elevated PPFD levels, specifically within the range of 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$, exhibited the lowest SLA, and this decrease showed a clear negative trend as PPFD increased from 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($p < 0.0001$). Additionally, the COR of lettuce plants grown under higher PPFD values, ranging from 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$, displayed the highest COR and exhibited a significant positive linear correlation ($p = 0.0004$) (Figure 2.4B). The plants grown within the PPFD range of 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ exhibited an increased COR, suggesting the growth of leaves in a more vertically stacked and overlapping arrangement. This pattern clarifies the rationale for their smaller SLA than plants grown under lower PPFDs ranging from 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The smaller PCS observed in plants grown under higher PPFDs resulted in more leaves and reduced stretching of plant structures compared to those grown under lower PPFDs. Consequently, these plants developed thicker leaves, which, in turn, contributed to higher fresh and dry shoot weights.

Total incident light

Total incident light exhibited a positive linear correlation with increasing PPFD from 201 to 413 μmol·m^{-2}·s^{-1} ($p < 0.0001$) (Figure 2.5). The PPFD range of 333 to 413 μmol·m^{-2}·s^{-1} displayed the highest total incident light values. Analyzing shoot dry weight in relation to total incident light revealed a positive linear correlation (Figure 2.6). As SDW increased, total incident light also increased ($p < 0.0001$). Given that the highest SDWs were attained under elevated PPFD levels between 333 to 413 μmol·m^{-2}·s^{-1}, a strong positive correlation between SDW and total incident light was observed ($p < 0.0001$). The positive correlation between total incident light and the subsequent increase in SDW indicates a rise of 102% in the latter. This finding confirms that plants cultivated under higher PPFDs between 333 to 413 μmol·m^{-2}·s^{-1} received greater total incident light, leading to elevated shoot weights.

Light use efficiency

Our initial hypothesis postulated that increasing PPFD would increase growth through heightened incident light exposure despite a potential decrease in LUE. Our results, however, indicate that LUE was not a significant factor influencing growth with increasing PPFD ($p = 0.6060$) (Figure 2.7).

Discussion

Shoot fresh and dry weights increased with increasing PPFD (Figure 2.1A and 2.1B). A study by Ghorbanzadeh et al. (2021), also saw that increased growth occurred under increasing light intensity (Ghorbanzadeh et al., 2021). A potential reason for this could be that at higher light intensities there is an increase photosynthetic capacity which affects growth and biomass accumulation in plants (He et al., 2021). Root fresh and dry weight exhibited a similar response as the fresh and dry shoot weights. As PPFD increased the fresh and dry weights also increased (Figure 2.1 A and B). Because increasing PPFD increased shoot weight, it would be expected that a portion of this increased biomass would be allocated to root growth for enhanced water and nutrient absorption. An increase in root weight with increasing light intensity was also seen in another study (He et al., 2021).

Water content decreased with increasing PPFD (Figure 2.2). With increasing PPFD, the decrease in water content is most likely due to the increase in carbohydrate production due to increased photosynthesis (Proietti et al., 2023). Increasing shoot dry weight saw a decrease in water content (Figure 2.3). The increase in carbohydrate production can result in thicker leaves which may have contributed to the increase in shoot dry weight.

TLA increased with increasing PPFD while final PCS did not see any significant differences with increasing PPFD (Figure 2.4 A). The increase in TLA with increasing PPFD was most likely affected by the increase in total incident light. It was seen in a similar study that the increase in leaf area was due to the increase in total incident light (Legendre & van Iersel, 2021). The final PCS did not show significant differences in plants grown under high or low PPFDs. This suggests that toward the end of the growing cycle, plants are able to achieve similar canopy sizes. However, these differences are more pronounced in the earlier stages of growth. A similar trend is seen in another study where lettuce plants in later stages of growth began to see a decline in PCS most likely due to overlapping leaves (Kim & van Iersel 2022).

Plants that were grown under lower PPFDs had a higher SLA compared to plants grown under higher PPFDs (Figure 2.4 B). A potential reason for this could be that plants grown under low PPFDs wanted to increase incident light capture. Plants were able to alter their morphology by stretching out to capture more incident light causing them to have thinner leaves which contributed to their higher SLA. This pattern was also seen in Kim & van Iersel (2022) where higher SLA was attributed to plants having thinner leaves which allowed the lower leaves to capture enough light which contributed to the overall canopy carbon balance (Kim & van Iersel 2022). Another study saw that plants grown under lower PPFDs had longer leaves which may have been due to them extending farther to increase light capture (Legendre & van Iersel, 2021). Low SLA was seen in plants grown under higher PPFDs (Figure 2.4 B). In a previous study by Kim & van Iersel they saw that low SLA occurred due to leaves in the upper part of the plant canopy not allowing enough light to be transmitted onto the lower leaves (Kim & van Iersel, 2022).

With decreasing SLA, the COR increased (0.0004) (Figure 2.4 B). An increase in COR was seen in plants grown under higher PPFD levels (Figure 2.4 B). The observed increase can be attributed to a likely reduction in light interception by the leaves beneath the plant canopy, as they were shaded by the leaves above them. In a previous study by Legendre & van Iersel (2021) they also saw that the plants grown under higher PPFDs saw an increase of overlapping leaves and lower light interception (Legendre et al., 2021).

Since crop growth primarily hinged on the total incident light absorbed by the plants, it is essential to note again that incident light is quantified through DLI measurements in conjunction with daily measurements of PCS. In this study, plants subjected to high PPFD levels exhibited the highest incident light exposure (Figure 2.5). As total incident light increased, plants under elevated PPFD levels of 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ demonstrated higher dry weights (Figure 2.6). The strong correlation between growth and incident light stems from their growing interdependency (<0.0001). In a pervious study by Kim & van Iersel (2023), they saw that the total incident light was also positively correlated with SDW seen in green lettuce cultivars (Kim & van Iersel, 2023). Other positive correlations between total incident light and biomass have been reported in other species and studies (Christensen & Goudriaan, 1993; Legendre & van Iersel, 2021)

LUE represents the efficiency with which crops harness incident light for plant growth and biomass production. However, the observed correlation between crop growth and LUE in plants subjected to either low or high PPFD conditions during this experiment was not notably strong ($p = 0.6060$) (Figure 2.7). In a study by Kim & van Iersel (2022), they saw that LUE was not a valuable parameter when screening for rapid growth when integrated over a cropping cycle (Kim & van Iersel, 2022). A plausible explanation for this weak association may stem from the plants nearing their light saturation point.

In contrast, plants in lower light conditions adjusted their morphology to increase light capture, even though their incident light capture did not significantly increase. This change in morphology allowed them to enhance light capture in low incident light conditions while also increasing their PCS. Comparing PPFD to the final canopy size, despite not showing statistical significance (p = 0.3721), implies that plants maintain similar PCS regardless of the PPFD levels towards the end of the growing cycle. This consistency in PCS suggests that the total incident light primarily relies on PPFD rather than PCS. The reason that plants grown at a lower PPFDs can have a similar PCS as plants grown at high PPFD, despite much lower dry weight, is higher SLA. Higher SLA in plants grown under low PPFDs allowed them to increase their leaf area to capture more light. However, the consequence of having a high SLA is that the leaves became thinner, resulting in lower shoot fresh and dry weights. The COR is another contributing factor that influences PCS. Plants with higher PPFD make a more significant TLA, but the leaves largely overlap causing an increase in COR, thus not increasing PCS.

Another noteworthy observation, although not quantitatively assessed, pertains to the morphological characteristics seen in the plants. Plants subjected to higher PPFDs ranging from 333 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ visually exhibited thicker leaves with a coarser texture. Plants exposed to lower PPFDs within the range of 201 to 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$ displayed thinner leaves that exhibited greater flexibility when subjected to bending and movement during harvest. This morphological observation is consistent with the idea that under high PPFDs led to higher total incident light exposure not only increasing the COR but also could have resulted in thicker leaves, ultimately contributing to higher fresh and dry shoot weights.

Plants were grown in a controlled growth chamber and were placed in specific locations that had varying PPFDS. The diversity in the PPFD received by each plant was attained by randomly turning off two of the six light panels. Plants grown under the low PPFDs were most likely not directly under an LED light but rather off to the side and still illuminated by a neighboring light panel. This alteration affected how much light the plants received and the

PPFD values, subsequently changing the total incident light the plants received during their growing cycle, which, in turn, altered the weights and morphology. This altered distribution of light within the growth chamber led to variations in PPFD uniformity. Consequently, these variations in light distribution had an impact on many growth parameters, including weight, PCS, SLA, COR, and total incident light. It is known that the distribution of light can significantly influence the plant's growth characteristics. In this study it was seen that the amount of total incident light a plant received during the course of the growing cycle increased the biomass accumulation. It is most likely that the light variation in the growth chamber caused plants exposed to low PPFDs to change their morphology. They increased their leaf area to increase light capture, even though the amount of total IL they absorbed was lower compared to plants grown under higher PPFDs.

Conclusions

Plant morphology at lower PPFD levels (201 to 292 $\mu mol\cdot m^{-2}\cdot s^{-1}$) had an increase in SLA and PCS to enhance light capture. At higher PPFD levels (333 and 413 $\mu mol\cdot m^{-2}\cdot s^{-1}$), plants exhibited an increase in COR and had higher fresh and dry shoot and root weights. Both low and high PPFD conditions lead to a similar trend in PCS among plants which suggests that the total IL primarily relies on PPFD rather than PCS. Plants with the highest SDW were grown under higher PPFDs and as SDW increased, total IL increased. LUE did not play a significant role in determining growth. Instead, the key factor was a strong and positive correlation between growth and total incident light the plants received.

Final Recommendations

This study showcases the importance incident light and PPFD have on growth, morphology, and biomass accumulation in lettuce. To gain deeper insights into the influence of incident light on growth, it would be valuable to explore additional cultivars to see how the morphology changes with increasing total incident light. Continuing to use PCS imaging is essential for selecting crops that will thrive efficiently in CEA as a part of the phenotype screening process. This concept is reinforced by the observation that plants tend to produce more shoot biomass with increasing total IL indicating a valuable phenotyping strategy.

We observed that the more incident light a plant received, the more biomass it created. Therefore, the recommendation for growers is to carefully consider the range of PPFD values plants are exposed to optimize total incident light for enhanced biomass production, especially under sole-source lighting conditions found in vertical farms. In theory, growers can manipulate the appearance of their lettuce by solely adjusting the PPFD to increase or decrease the total incident light their crops receive. The importance of ensuring that plants receive the correct PPFD target is essential because depending on the location under the LED light, plants can undergo changes in morphology. Growers should check both the plants that are farthest and closest to the light source to ensure that they are monitoring the light exposure of all their plants effectively. This is important for maintaining consistency and understanding how different positions relative to the light source may impact plant growth and development.

Literature Cited

Cabrera-Bosquet, L., Fournier, C., Brichet, N., Welcker, C., Suard, B., & Tardieu, F. (2016). High-throughput estimation of incident light, light interception and radiation-use efficiency of thousands of plants in a phenotyping platform. *New Phytologist*, *212*(1), 269-281. doi: https://doi.org/10.1111/nph.14027

Christensen, S.; Goudriaan, J. Deriving light interception and biomass from spectral reflectance ratio. *Remote Sens. Environ.* 1993, *43*, 87–95. doi: https://doi.org/10.1016/0034-4257(93)90066-7

Elkins, C., & van Iersel, M. W. (2020). Longer photoperiods with the same daily light integral improve growth of rudbeckia seedlings in a greenhouse. HortScience, 55(10), 1676-1682. doi: https://doi.org/10.21273/HORTSCI15200-20

Ertle, J., & Kubota, C. (2023). Reduced daily light integral at the end of production can delay tipburn incidence with a yield penalty in indoor lettuce production. *HortScience*, *58*(10), 1217-1224. doi: https://doi.org/10.21273/HORTSCI17314-23

Fernandez-Jaramillo, A. A., Duarte-Galvan, C., Contreras-Medina, L. M., Torres-Pacheco, I., de J. Romero-Troncoso, R., Guevara-Gonzalez, R. G., & Millan-Almaraz, J. R. (2012). Instrumentation in developing chlorophyll fluorescence biosensing: A review. Sensors, 12(9), 11853-11869. doi: https://doi.org/10.3390/s120911853

FinancialNewsMedia.com. (2021, October 27). *Global Controlled Environment Agriculture Market (CEA) Expected To Reach $172 Billion In 2025.* CISION PR Newswire. https://www.prnewswire.com/news-releases/global-controlled-environment-agriculture-market-cea-expected-to-reach-172-billion-in-2025-301409455.html

Ghorbanzadeh, P., Aliniaeifard, S., Esmaeili, M., Mashal, M., Azadegan, B., & Seif, M. (2021). Dependency of growth, water use efficiency, chlorophyll fluorescence, and stomatal characteristics of lettuce plants to light intensity. *Journal of Plant Growth Regulation*, *40*, 2191-2207. doi: https://doi.org/10.1007/s00344-020-10269-z

He, J., Jawahir, N. K. B., & Qin, L. (2021). Quantity of supplementary LED lightings regulates photosynthetic apparatus, improves photosynthetic capacity and enhances productivity of Cos lettuce grown in a tropical greenhouse. *Photosynthesis Research*, 1-13. doi: https://doi.org/10.1007/s11120-020-00816-w

Jayalath, T. C., & van Iersel, M. W. (2021). Canopy size and light use efficiency explain growth differences between lettuce and mizuna in vertical farms. Plants, 10(4), 704. doi: https://doi.org/10.3390/plants10040704

KD Market Insights. (2021). *Controlled Environment Agriculture Market: Global Size, growth, Trends & Outlook (2022 – 2032)*. KD Market Insights, https://www.kdmarketinsights.com, All Right Reserved 2021. https://www.kdmarketinsights.com/reports/controlled-environment-agriculture-market/2480

Kim, C., & van Iersel, M. W. (2022). Morphological and physiological screening to predict lettuce biomass production in controlled environment agriculture. Remote Sensing, 14(2), 316. doi: https://doi.org/10.3390/rs14020316

Legendre, R., Basinger, N. T., & van Iersel, M. W. (2021). Low-cost chlorophyll fluorescence imaging for stress detection. Sensors, 21(6), 2055. doi: https://doi.org/10.3390/s21062055

Legendre, R., & van Iersel, M. W. (2021). Supplemental far-red light stimulates lettuce growth: Disentangling morphological and physiological effects. *Plants*, *10*(1), 166. doi: https://doi.org/10.3390/plants10010166

Palmer, S., & van Iersel, M. W. (2020). Increasing growth of lettuce and mizuna under sole-source LED lighting using longer photoperiods with the same daily light integral. Agronomy, 10(11), 1659. doi: https://doi.org/10.3390/agronomy10111659

Proietti, S., Paradiso, R., Moscatello, S., Saccardo, F., & Battistelli, A. (2023). Light Intensity Affects the Assimilation Rate and Carbohydrates Partitioning in Spinach Grown in a Controlled Environment. Plants (Basel, Switzerland), 12(4), 804. doi: https://doi.org/10.3390/plants12040804

Rea, M. S., Brons, J., & Jarboe, C. (2023). Reducing lighting energy use in controlled agricultural environments. HortScience, 58(8), 954-961. doi: https://doi.org/10.3389/fpls.2023.1215919

Runkle, E. (2017). Technically Speaking. Sole-Source Lighting of Plants. Retrieved from: www.canr.msu.edu/floriculture/uploads/files/sole-source-lighting.pdf

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development* (No. Ed. 6). Sinauer Associates Incorporated.

Vatistas, C., Avgoustaki, D. D., & Bartzanas, T. (2022). A systematic literature review on controlled-environment agriculture: How vertical farms and greenhouses can influence the sustainability and footprint of urban microclimate with local food production. *Atmosphere*, *13*(8), 1258. doi: https://doi.org/10.3390/atmos13081258

Warner, R., Wu, B. S., MacPherson, S., & Lefsrud, M. (2023). How the distribution of photon delivery impacts crops in indoor plant environments: a review. Sustainability, 15(5), 4645. doi: https://doi.org/10.3390/su15054645

Weaver, G., & van Iersel, M. W. (2020). Longer photoperiods with adaptive lighting control can improve growth of greenhouse-grown ’Little Gem’ lettuce (Lactuca sativa). HortScience, 55(4), 573-580. doi: https://doi.org/10.21273/HORTSCI14721-19

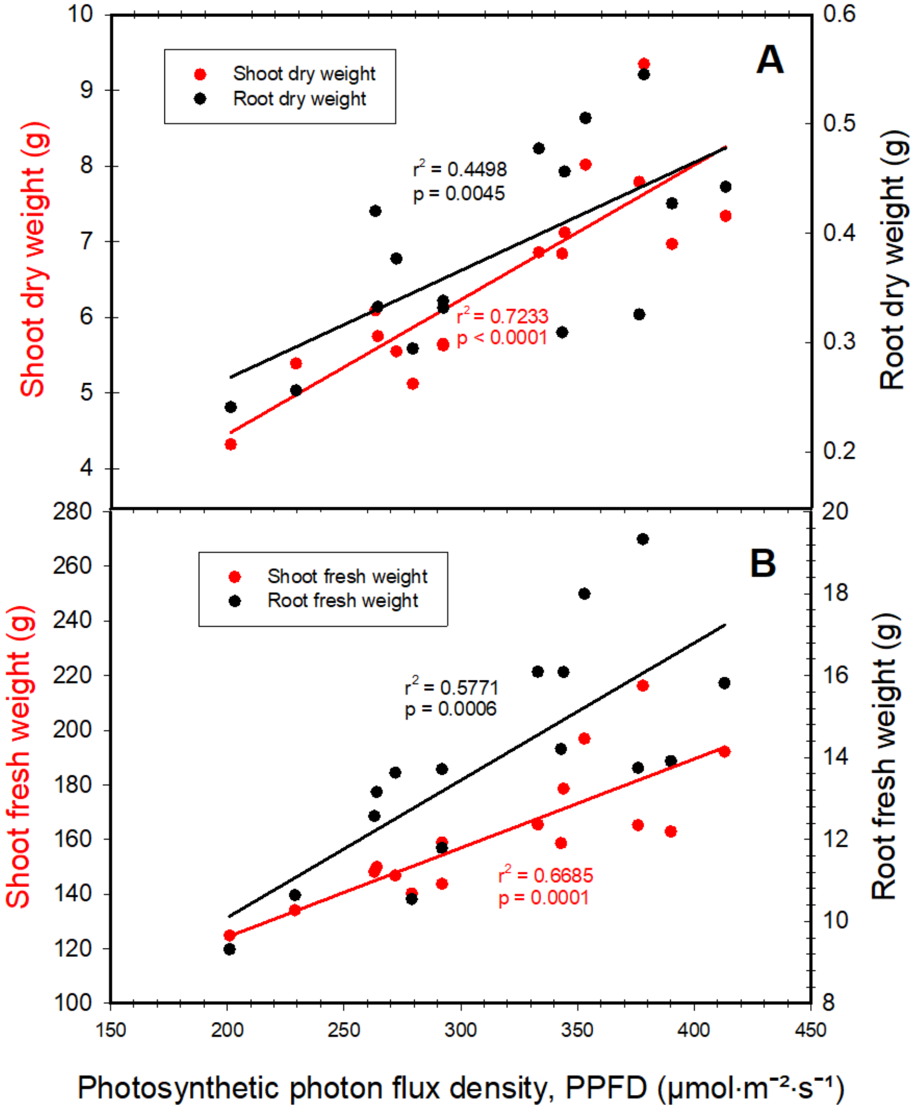


Figure 2.1: (A) Shoot fresh weight and root fresh weight, (B) shoot dry weight and root dry weight of lettuce (*Lactuca sativa* ‘Rex’) plants grown at different PPFDs in deep water culture hydroponics. Lines show multiple regression analyses’ results, indicating significant PPFD reactions. Each data point represents one plant.

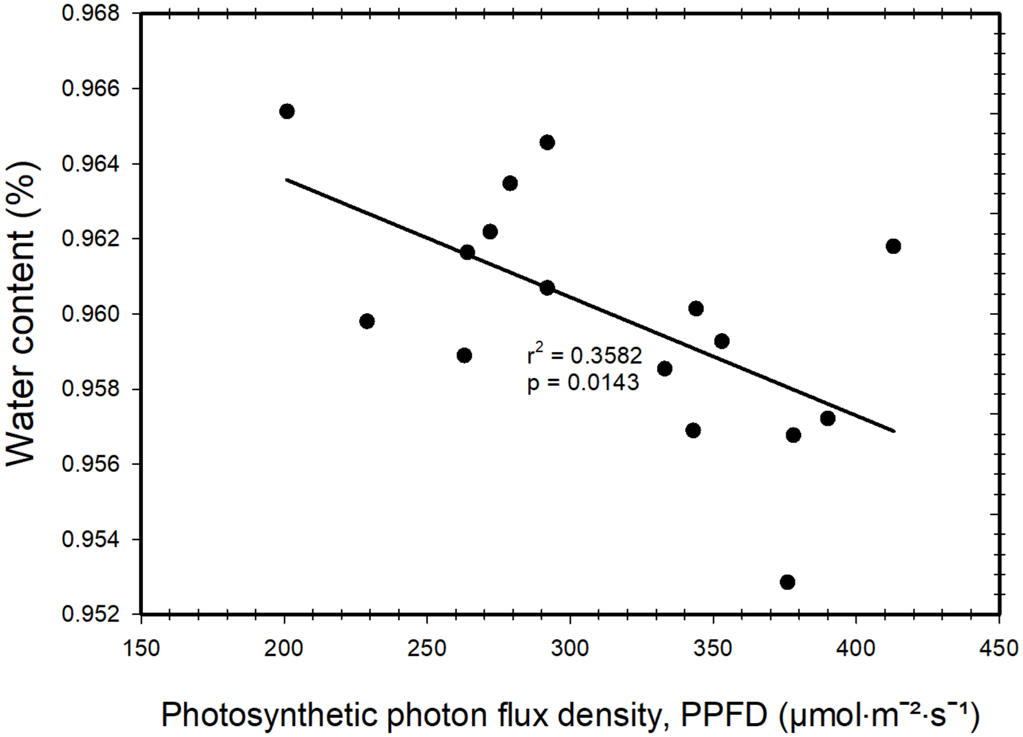


Figure 2.2: Water content of lettuce (*Lactuca sativa* 'Rex') plants grown at different PPFDs in deep water culture hydroponics. Lines show the results from multiple regression analyses, which indicated significant PPFD interactions. Each data point represents one plant.

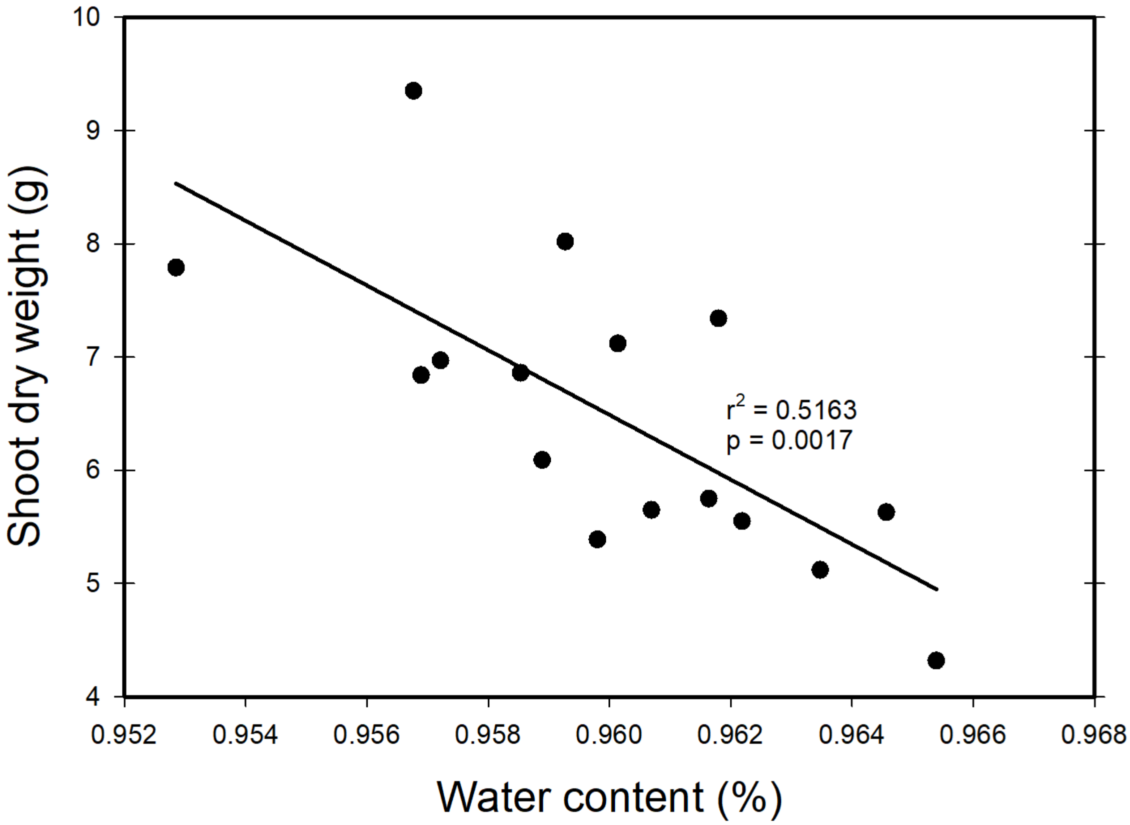


Figure 2.3: Shoot dry weight of lettuce (*Lactuca sativa* ‘Rex’) plants grown at different water contents in deep water culture hydroponics. Lines show the results from regression analyses, which indicated significant interactions between shoot dry weight and water content. Water content was calculated by subtracting fresh weight by dry weight then dividing it by fresh weight (fresh weight – dry weight / fresh weight). Each data point represents one plant.

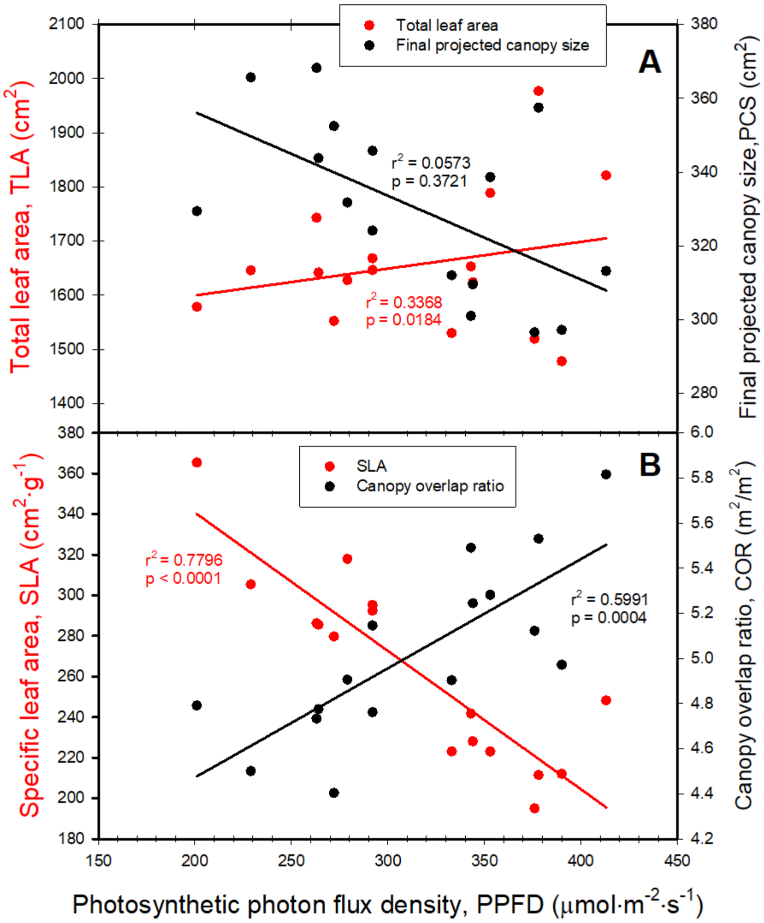


Figure 2.4: (A) Total leaf area (TLA) and final projected canopy size (PCS), (B) specific leaf area (SLA) and canopy overlap ratio (COR) of lettuce (*Lactuca sativa* 'Rex') plants grown at different PPFDs in deep water culture hydroponics. SLA was calculated by dividing the leaf area by the plant's dry weight. COR was calculated by dividing the TLA by the final projected canopy size (the final canopy size measurement was from the last image taken before harvest). Lines show the results from multiple regression analyses, which indicate significant PPFD interactions for all but the PCS.

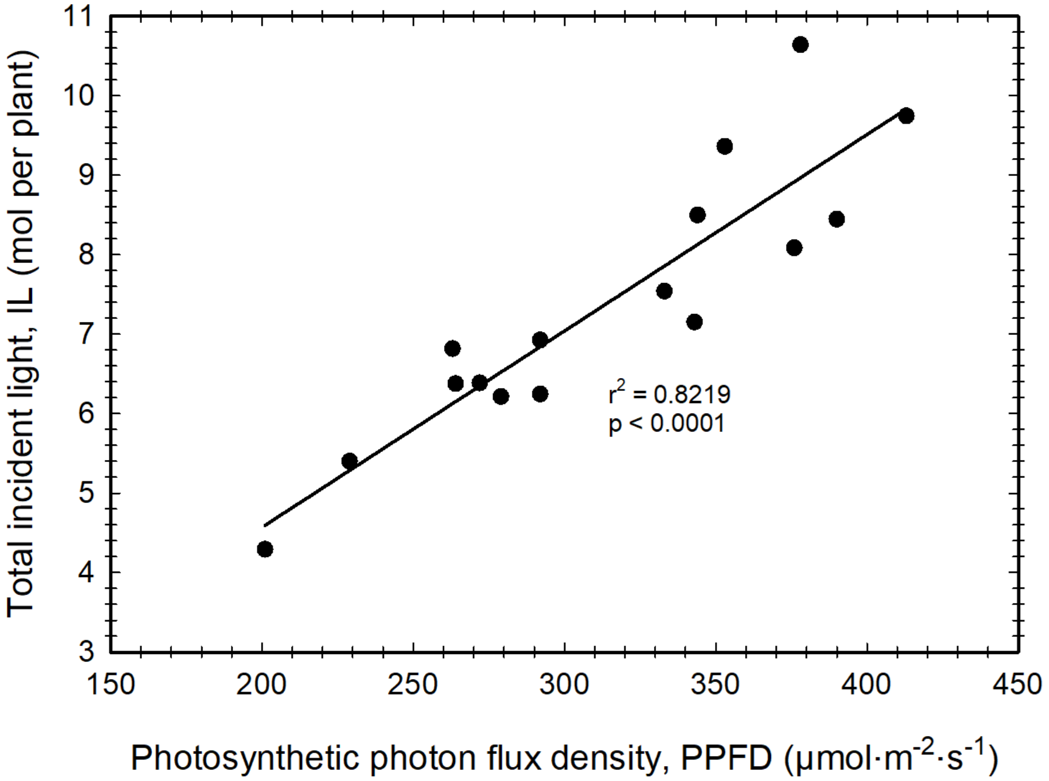


Figure 2.5: Total incident light of lettuce (*Lactuca sativa* 'Rex') plants grown at different PPFDs in deep water culture hydroponics. Total incident light was calculated by multiplying the daily light integral (DLI) and the individual projected canopy size (PCS) (∫DLI × PCS). By analyzing PCS measurements from the chlorophyll fluorescence imaging (CFI) system, we quantified the daily PCS of each plant and converted the measurements from cm^2/pixels to m^2/plant. We then multiplied the DLI by daily PCS for each plant to determine the incident light each plant received. The incident light for each day was summed to calculate the total incicent light for each plant. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents one plant.

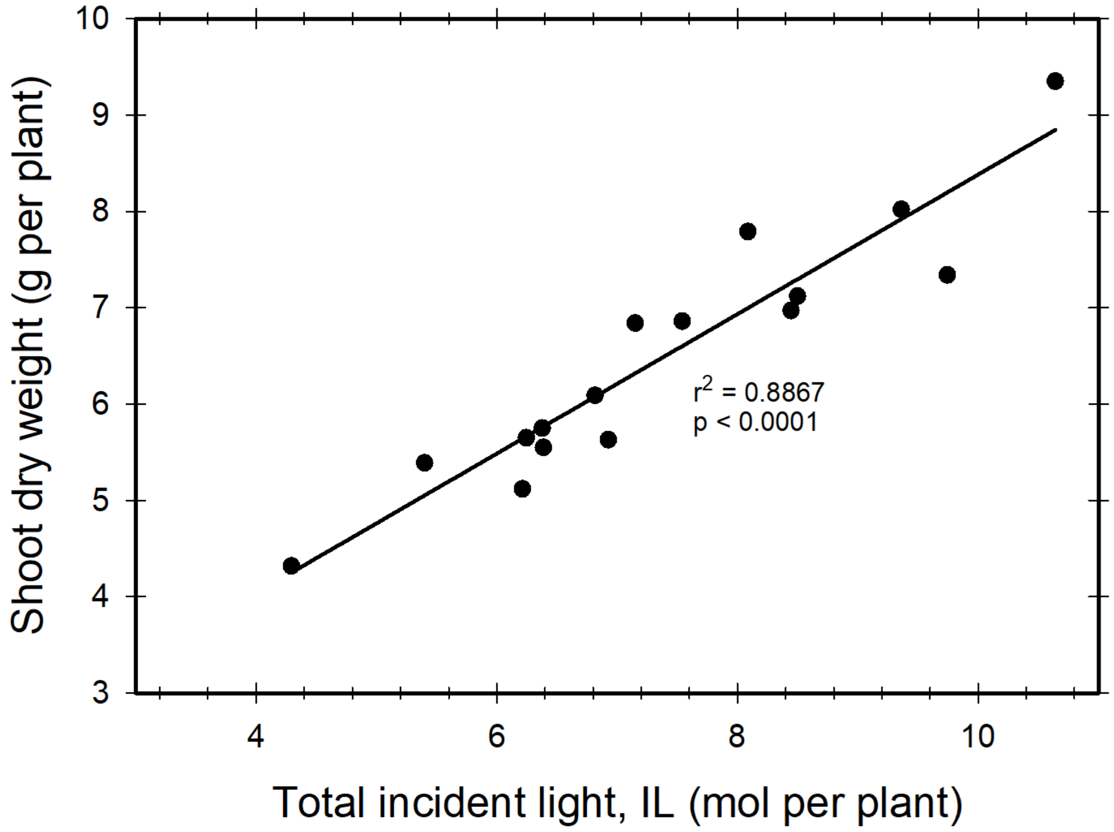


Figure 2.6: Shoot dry weight (SDW) of lettuce (*Lactuca sativa* ‘Rex’) plants grown at different total incident light levels in deep water culture hydroponics. Total incident light was calculated by multiplying DLI by the projected canopy size. Lines show multiple regression analysis results, indicating significant interactions. Each data point represents one plant.

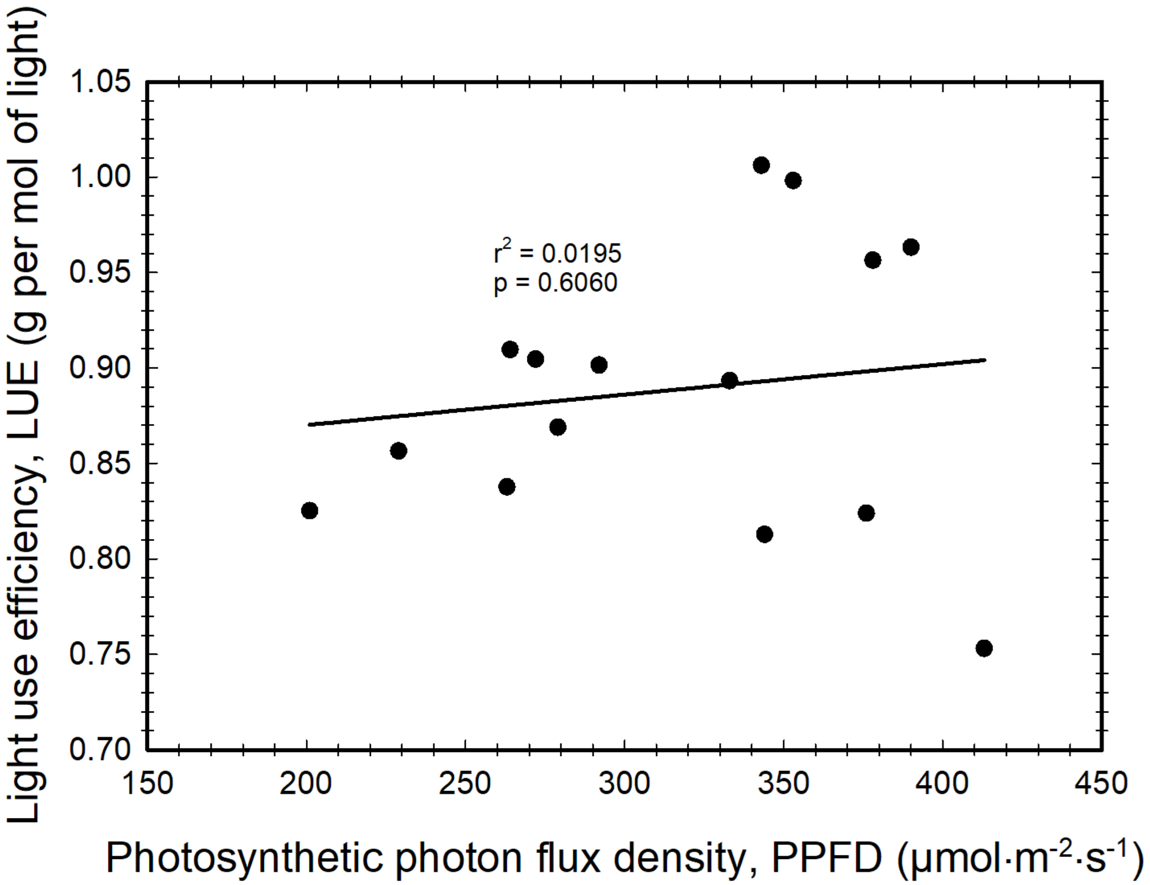


Figure 2.7: Light use efficiency (LUE) of lettuce (*Lactuca sativa* ‘Rex’) plants grown at different PPFDs in deep water culture hydroponics. LUE was calculated by dividing total plant dry weight by total IL. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents one plant.

CHAPTER 3

LEAF ANTHOCYANIN CONCENTRATION AND PHOTOSYNTHETIC PHOTON FLUX DENSITIES: BIOMASS ACCUMULATION OF SIX LETTUCE CULTIVARS OVER A GROWING CYCLE[2]

---

[2] Palsha, P.L, M.W. van Iersel, R.W. Dickson, M. Yelton, L. Seymour, and R.S. Ferrarezi. To be submitted to Frontiers

Abstract: Anthocyanins are secondary metabolites classified as water-soluble, non-photosynthetic pigments with the potential ability to shield chloroplasts from excess light energy. The effect anthocyanins have on lettuce growth, morphology, and light use efficiency (LUE) must be better understood. This study investigated the morphological and physiological responses of six lettuce cultivars with different leaf anthocyanin content grown in a greenhouse under different supplemental photosynthetic photon flux densities (PPFD). Cultivars ‘Cherokee’, ‘Teodore’, ‘Rex’, and ‘Rouxai’, decreased their specific leaf area (SLA) with increasing PPFD, respectively. We observed that by increasing PPFD, growth in cultivars with higher leaf anthocyanin content (‘Cherokee’ and ‘Rouxai’) increased with increasing PPFD. LUE is an important physiological parameter that affects biomass accumulation which can be seen in cultivars ‘Cherokee’, ‘Rex’, ‘Teodore’, and ‘Rouxai’, which had the highest LUE and shoot weight, respectively. Future studies require more frequent periodic destructive dry weight measurements to accurately quantify changes in LUE across the entire crop cycle.

Introduction

Anthocyanins

Anthocyanins belong to the flavonoid subgroup and are responsible for the red, blue, and purple coloration observed in plants (Oliveira et al., 2020; Liakopoulos et al., 2006). Anthocyanins represent the largest group of water-soluble natural phenolic compounds and are generally present in the form of anthocyanin glycosides and acylated anthocyanins (Assefa et al., 2021; Oliveira et al., 2020; Liakopoulos et al., 2006).

Anthocyanins are water-soluble pigments that do not contribute to photosynthesis. Chloroplasts harness light energy to drive photosynthetic electron flow. As photosystems reach their light saturation threshold, any surplus radiation is dissipated as heat through a mechanism referred to as the xanthophyll cycle (Liakopoulos et al., 2006). The xanthophyll cycle is an essential photoprotective response to light saturation (Liakopoulos et al., 2006). This process reduces the availability of photons necessary for photosynthesis, ultimately impeding crop growth (Liakopoulos et al., 2006). When leaves receive more light than can be used in photochemistry, there is a decline in photosynthesis due to a decline in quantum efficiency called photoinhibition (Gould, 2004). When photoinhibition is severe, chloroplasts generate reactive oxygen species (ROS), which can destroy the thylakoid membranes, damage DNA, and denature proteins associated with electron transport in photosynthesis (Gould, 2004). Nevertheless, several studies suggest that foliar anthocyanins do serve a photoprotective function (Liakopoulos et al., 2006). They have also been shown in many species to reduce the frequency and severity of photoinhibition (Gould, 2004).

Anthocyanin concentrations differ not only among lettuce varieties but also between cultivated types (commercial and traditional) and their wild relatives (Medina-Lozano et al., 2021). Due to these differences, the effect environmental factors have on growth can be enhanced when these phenolic compounds are present because they protect the leaves from excess radiation without compromising photosynthesis (Medina-Lozano et al., 2021).

Objectives

The objectives of this study were to investigate the morphological and physiological responses of six different lettuce cultivars characterized by varying leaf anthocyanin contents. We hypothesized that cultivars with lower leaf anthocyanin content values will increase shoot weight with increasing PPFD levels.

Materials and Methods

Seedling and transplant management

For seedling production, all six lettuce cultivars were sown in rockwool plugs measuring 2.5 × 2.5 × 4 cm (A0 25/40; Grodan Rockwool BV, Roermond, Netherlands) on February 13th, 2023. Prior to seeding, the rockwool plugs were thoroughly saturated with water. Subsequently, a single lettuce seed was placed into each rockwool plug, with 120 plugs constituting one tray. Six trays, one tray for each cultivar was seeded. After seeding all six trays, the rockwool was misted again to ensure adequate moisture for seed germination. These rockwool plugs were then placed in double-stacked, black, rectangular plastic mesh trays measuring 50.8 × 12.7 × 5.08 cm. The seeded trays were transferred to a walk-in vertical farm and positioned on shelves beneath 1.1 m-long white LED light fixtures (RAY with Physiospec indoor spectrum; Fluence Bioengineering, Austin, TX, USA), delivering a photosynthetically active radiation (PAR) of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during a 16-hour photoperiod. Transparent plastic domes were utilized to cover the trays for the first four days, creating an environment conducive to maintaining high humidity during germination.

Daily sub-irrigation for 5 minutes was performed using an ebb-and-flow bench, with a water-soluble fertilizer solution containing 100 $mg \cdot L^{-1}$ nitrogen (N) (15N–2.2P–12.45K, Peters

Excel 15–5–15 Cal-Mag Special; Everris NA Inc, Dublin, OH, USA). Eleven days after seeding on February 13th, 2023, we selected the most uniform seedlings from each cultivar on February 24th, 2023. These seedlings were randomly transplanted among light treatments into a deep-water culture hydroponic system using 36 net pots (4.8 cm height × 4.5 cm top diameter × 3.3 cm in bottom diameter) (Teku G46; Pöppelmann GmbH & Co., Lohne, Germany). Foam lids were utilized to accommodate the plants and net pots within each tray. These lids featured 36 holes, evenly spaced 12.90 square cm apart, and each hole had a width of 4.1 cm (Lenox Bi-Metal, Bristol, PA, USA). Each hole held a net pot containing a plant in rockwool. Subsequently, they were placed into 144-liter black square trays measuring 1.2 × 1.2 m × 10 cm. To enable flotation in the deep-water tray, a square foam board was placed on top of the trays.

A consistent water-soluble hydroponic nutrient solution was utilized for all trays and replications. This nutrient solution was formulated as a combination of various fertilizers: Blend 9-7-37 Hydroponic Fertilizer (Hort Americas, Bedford, Texas, USA); $Ca(NO_3)$ (YaraTera Calcinit; Yara, Oslo, Norway); and $MgSO_4$ (EPSOTop; K+S Minerals and Agriculture GmbH, Kassel, Germany). To ensure proper aeration of the fertilizer solution, plastic tubing and six air stones (Pawfly Aquarium Air Stone, Guangzhou, China) were placed within each tray. An air pump (EcoPlus-7; Hawthorne gardening company, Vancouver, WA, USA, 0.48 bar, 3.0 A, 120V, Tube Size: 12.7 mm in diameter). was employed to facilitate the circulation of air and maintain optimal oxygen levels in the nutrient solution. The initial pH for the solution was 6.53, while the electrical conductivity (EC) was 1.22 mS/cm. We used 2M of H_3PO_4 solution to reduce the pH of the hydroponic solution between 5.5 and 6.5. The 2M H_3PO_4 solution was used throughout the study to keep the pH from going above 6.5, but multiple times throughout the study, the pH was between 6.78 to 7.20, with the highest pH recorded at 7.30. The temporary increase in pH may have caused an increase in tipburn symptoms, however tipburn incidence for this study was not measured.

Growing and environmental conditions

Plants were grown in a glass covered greenhouse at the University of Georgia Riverbend Greenhouse Complex (lat. 33°57’26.676” N, long. 83°22’36.48” W) in Athens, USA, from February 13th to March 22nd, 2023 (37 days after germination, DAG). This study involved a total of 216 plants, where one tray with 36 plants represented one block. In total, there were six blocks in this study. Each block consisted of six different PPFD light treatments per tray. To prevent bias and ensure that the effects of different cultivars were evenly assessed in each lighting treatment, one plant from each of the six cultivars was randomly assigned to each light treatment and replication. As a result, there was one representative of each of the six cultivars in each light treatment. The average daily temperature, relative humidity, and vapor pressure deficit (VPD) during the entirety of this study was 20.1° C ± 1.45, 62.2% ± 13.6, and 0.89 ± 0.27 kPa, respectively.

Treatments

Six lettuce (*Lactuca sativa*) cultivars: ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova® Red Batavia,’ and ‘Salanova® Hydroponic Red Batavia’ (Johnny’s Selected Seeds, Winslow, Maine, USA) were chosen for their distinctive characteristics, encompassing variations in morphology, anthocyanin content, and pigmentation. All six cultivars subjected to diverse

PPFDs provided by supplemental LED lighting throughout the entire growing cycle. The collected PPFD measurements were grouped into six lighting treatments, each comprising six plants. These groupings were based on the similarity of PPFD levels, resulting in six lighting treatments with an average maximum supplemental PPFD at 142, 196, 245, 302, 306 and 372 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Figure 3.1). Consequently, the supplemental PPFD values had a DLI range of 5.36 to 24.11 $mol \cdot m^{-2} \cdot d^{-1}$, without considering the DLI received from natural sunlight since that was extra. By taking into consideration both the supplemental LED DLI and sunlight DLI, the averaged total DLI values throughout the experiment were 12.2, 15.3, 18.6, 21.4, 21.7, and 25.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Supplemental material 3.1). As a result, the total DLI received from the sunlight and LEDs exceeded the provided supplemental DLI values from only LEDs (Supplemental material 3.1)

Lighting conditions and measurement setup

On February 21st, 2023, three days prior to transplanting, six 1.2-meter-long LED light fixtures were set up directly over the central portion of each bench (Arize Element Top Light PPR; Current, Montreal, Canada). This deliberate arrangement was designed to introduce natural variations in PPFD, with the aim of achieving a supplemental PPFD of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the highest lighting treatment (Arize Element Top Light PPR; Current, Montreal, Canada). To ensure that the LED fixtures primarily provided supplemental lighting to the bench they were positioned above, we installed aluminum reflectors on the sides of each LED fixture. Each LED light fixture illuminated two trays positioned side by side on a single bench. Above the LED fixtures was a 70% shade net that was used to reduce the amount of sunlight intensity entering the greenhouse. Between two trays we placed one photosynthetically active radiation (PAR) sensor (SQ500-SS; Apogee Instruments). The PAR sensor was used to monitor the intensity of light the LEDs fixtured gave off. Two additional PAR sensors were placed above the LED fixtures but below the 70% shade net to measure the sunlight intensity entering the greenhouse which was recorded and used to calculate weather conditions. All quantum sensors were connected to a datalogger (CR1000; Campbell Scientific, Logan, UT, USA) to control the dimmable LED drivers but for this experiment the dimmable LEDs were not used and were kept at a constant maximum power PPFD threshold.

To measure the PPFD provided by the LED fixtures at full power for each of the 36 holes per tray we affixed 36 interconnected diodes to a foam board. Each diode was placed in the same location each of the 36 plants would grow. With this, we were able capture the PPFD measurements from all 36 planting locations at once, managed through a proprietary software (LoggerNet program version 4.1; Cambell Scientific, Logan, Utah, USA). These measurements were obtained at night, exclusively under supplemental lighting conditions. Throughout the entire 16-hour photoperiod, the supplemental LED fixtures remained consistently powered, ensuring uniform lighting conditions for the study.

The supplemental LED fixtures did not adjust to increasing or decreasing sunlight throughout the day and was kept at a constant target level of 400 ± 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$. As the distance from the LED fixture increased, the supplementary PPFD values decreased (Figure 3.2).

Data collection

On March 22nd, 2023 at 9:30 pm, 26 days after transplanting and on the night before the destructive harvest, we assessed the quantum yield of photosystem II (Φ_{PSII}). This assessment was performed using a pulse-amplitude modulated (PAM) fluorometer (Mini-PAM II; Heinz Walz, Effeltrich, Germany). We recorded the Φ_{PSII} measurements from the upper-most fully expanded leaves from every plant under each lighting treatment for all three blocks. These Φ_{PSII} measurements were acquired exclusively under supplemental lighting at night. To identify each cultivar's morphological responses to varying supplemental PPFDs, the measurements taken encompassed various parameters such as the shoots and roots dry weight per plant, and leaf area for each plant in two out of three blocks. Just prior to the leaf area measurements, all plants were imaged individually in a multispectral imaging system to determine leaf anthocyanin content before proceeding with destructive measurements (Topview, Aris, Eindhoven, The Netherlands). To gauge total leaf area, individual leaves were carefully separated from each plant and then measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE, USA). Subsequently, the shoots and roots were placed in a drying oven at 80 °C for 7 days, following which their dry weights were measured. SLA was calculated using dry shoot weight and total leaf area (SLA; total leaf area/ dry shoot weight). LUE was calculated by first determining the amount of shoot dry weight produced in square meters. Next, we calculated the total amount of light energy provided over the entire growing period on the same square meter. By dividing the total grams of shoot dry weight by the total moles of light energy, we were able to calculate LUE (Jin et al., 2020).

Experimental design and Statistical analysis

The experimental framework employed a randomized complete block design encompassing six lighting treatments within a single block. The experimental unit was made up of 36 plants per block with six blocks total which resulted in 216 total plants. To investigate the influence of varying PPFDs on canopy dimensions and growth, hydroponically cultivated lettuce was subjected to supplemental LED illumination, with the PPFD levels from the LEDs spanning 93 to 418.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Subsequent analyses were performed via multiple regression analysis utilizing a statistical software (SigmaPlot 11.0; Systat software, San Jose, CA, USA). This method aimed to discern any potential interaction effects PPFD had on various parameters, including dry shoot and root weights per plant, leaf area per plant, SLA, and LUE.

Results

Shoot dry weight

‘Cherokee’ exhibited the highest shoot dry weight at 24.7%, with ‘Rex’ demonstrating the second-highest dry shoot weight only 4.3% behind ‘Cherokee’ (Figure 3.1A). Conversely, Teodore’ and ‘Rouxai’ exhibited the third and fourth-highest shoot dry weights, with ‘Teodore’ having 2.6% more dry shoot weight than ‘Rouxai’ and being 1.2% behind ‘Rex’ (Figure 3.1A). ‘Salanova® Red Batavia’ and ‘Salanova® Hydroponic Red Batavia’ recorded the lowest dry weights among the cultivars with only a 0.68% difference between them, respectively (Figure 3.1A). When examining the relationship between dry shoot weight and PPFD, we observed that ‘Cherokee’ exhibited the highest dry shoot weight, which also increased significantly with rising PPFD ($p < 0.0001$) (Figure 3.1B). Similarly, ‘Rex’ ($p = 0.0287$) and ‘Rouxai’ ($p = 0.0111$) displayed a comparable trend, showing increased dry shoot weights as PPFD levels increased

(Figure 3.1B). The increasing PPFD values did not have a significant effect on ‘Teodore’ ($p = 0.8669$) (Figure 3.1B). As noted above, ‘Salanova® Red Batavia’ and ‘Salanova® Hydroponic Red Batavia’ had the lowest shoot dry weights among all the cultivars, respectively, and they also did not show a statistically significant increase in shoot dry weight with increasing PPFD values ($p = 0.0509$) ($p = 0.1060$) respectivly (Figure 3.1B).

Anthocyanin content index versus PPFD

‘Rouxai’ exhibited the highest leaf anthocyanin content at 23%, followed by ‘Cherokee’ at 19.8% and ‘Salanova® Hydroponic Red Batavia’ at 19.1% with the second and third-highest values (Figure 3.2A). Conversely, ‘Teodore’ and ‘Salanova® Red Batavia’ had the third and fourth-highest leaf anthocyanin content at 18.1% and 16.5%, respectively (Figure 3.2 A). ‘Rex’ recorded the lowest leaf anthocyanin content (2.9%) among the cultivars, attributed to its status as the only green cultivar (Figure 3.2A). ‘Salanova® Hydroponic Red Batavia’ and ‘Salanova® Red Batavia’ both exhibited an increase in leaf anthocyanin content with rising PPFD levels ($p < 0.0001$ and $p = 0.0001$, respectively) (Figure 3.2B). Interestingly, even though ‘Rex’ was a green cultivar, it also displayed an increase in leaf anthocyanin content with increasing PPFD ($p = 0.0005$) (Figure 3.2B). leaf anthocyanin content index did not increase with increasing PPFD for ‘Cherokee’ ($p = 0.0561$), ‘Teodore’ ($p = 0.0743$), and ‘Rouxai’ ($p = 0.5865$) (Figure 3.2B).

Light use efficiency versus PPFD

The three cultivars that exhibited the highest LUE at 23.6%, 21%, and 19.7% were ‘Cherokee’, ‘Rex’, and ‘Teodore’, respectivly (Figure 3.3 A). The fourth-ranking cultivar for

LUE was ‘Rouxai’ at 16.9% (Figure 3.3A). ‘Salanova® Hydroponic Red Batavia’ and ‘Salanova® Red Batavia’ consistently demonstrated the lowest LUE values at 9.6% and 9.1%, respectively (Figure 3.3 A). In the evaluation of LUE versus PPFD, all cultivars exhibited a significant trend ($p > 0.05$) (Figure 3.3B). With increasing PPFD, LUE in all cultivars decreased: ‘Cherokee’ ($p = 0.0397$), ‘Rex’ ($p = 0.0031$), ‘Teodore’ ($p = 0.0008$), ‘Rouxai’ ($p = 0.0081$), ‘Salanova® Red Batavia’ ($p = 0.0028$), and ‘Salanova® Hydroponic Red Batavia’ ($p = 0.0032$) (Figure 3.3B). Ultimately, LUE was highest for all cultivars when exposed to lower PPFDs compared to higher PPFDs (Figure 3.3B).

Specific leaf area versus PPFD

‘Salanova® Red Batavia’ and ‘Salanova® Hydroponic Red Batavia’ exhibited the two highest SLA values among all the cultivars at 24% and 19.3%, respectively (Figure 3.4A). ‘Cherokee’, ‘Teodore’, ‘Rex’, and ‘Rouxai’ shared the second-highest SLA values at 15.3%, 14.3%, 13.2%, and 13%, respectively (Figure 3.4A).While ‘Salanova® Red Batavia’ ($p = 0.1262$) and ‘Salanova® Hydroponic Red Batavia’ ($p = 0.2100$) exhibited the highest SLA values, this was attributed to their lower shoot dry weights. ‘Cherokee’ ($p = 0.0381$), ‘Teodore’ ($p = 0.0034$), ‘Rex’ ($p < 0.0001$), and ‘Rouxai’ ($p < 0.0001$) all exhibited a reduction in SLA in response to increasing PPFD levels.

PSII Chlorophyll fluorescence versus PPFD

Chlorophyll fluorescence decreased as PPFD levels increased across all cultivars, ‘Cherokee’ ($p < 0.0001$), ‘Teodore’ ($p < 0.0001$), ‘Rex’ ($p < 0.0001$), ‘Rouxai’ ($p < 0.0001$), ‘Salanova® Red Batavia’ ($p < 0.0001$), and ‘Salanova® Hydroponic Red Batavia’ ($p < 0.0001$) (Figure 3.11). All six cultivars had higher chlorophyll fluorescence values when grown under lower PPFDs compared to when they were grown under high PPFDs.

Discussion

Regarding cultivar differences in weights, ‘Cherokee’, ‘Rex’, ‘Teodore’, and ‘Rouxai’ exhibited the highest shoot dry weights, respectively (Figure 3.3). Previous studies have reported that higher anthocyanin levels result in lower biomass when compared to green plants (Kim & van Iersel, 2022). Because anthocyanins are non-photosynthetic pigments, they reduce the fraction of absorbed photons that can be used in the light reactions of photosynthesis which leads to reduction in biomass accumulation (Kim & van Iersel, 2022). However, the only green cultivar in this study was ‘Rex’ which had the lowest leaf anthocyanin content, but it had the second highest shoot dry weight while ‘Cherokee’ had the highest shoot dry weight (Figure 3.3). In a study by Kim & van Iersel (2022), they saw that red cultivars had less dry weight compared to green cultivars but in this study the red cultivar ‘Cherokee’ had a higher shoot dry weight than our only green cultivar ‘Rex’. One possible explanation is that, although 'Cherokee' exhibited the second-highest leaf anthocyanin content, it also could have displayed higher chlorophyll levels towards the center of the leaves, with the primary anthocyanin concentration predominantly localized at the edges. However, chlorophyll content (CCI) was not measured during this study.

With increasing PPFD the shoot dry weights for ‘Cherokee’, ‘Rex’, and ‘Rouxai’ increased (Figure 3.4). This increase in shoot dry weight was most likely due to the increase in PPFD the plants received which caused an increase in photosynthesis activity (Elkins & van

Iersel, 2020). When PPFD conditions increase, the PSII reaction centers close to minimize photodamage. This protective mechanism results in a greater dissipation of absorbed light energy as heat (non-photochemical quenching, NPQ) (Lu et al., 2022).

Previous studies have shown that red cultivars had less dry weight compared to green cultivars (Kim & van Iersel, 2022), but in this study the cultivar ‘Cherokee’ had a higher shoot dry weight than our only green cultivar ‘Rex’. A theorized possible explanation is that, although ‘Cherokee’ exhibited the second-highest leaf anthocyanin content, it also could have displayed higher chlorophyll levels towards the center of the leaves, with the primary leaf anthocyanin content predominantly localized at the edges. However, leaf chlorophyll content was not measured during this study which was a limitation seen.

We hypothesized that cultivars with lower leaf anthocyanin content would increase shoot weight with increasing PPFD. The cultivars with the three lowest leaf anthocyanin content values were ‘Rex’ (2.9%), ‘Salanova® Red Batavia’ (16.5%), and ‘Teodore’ (18.1%), respectivly. The cultivars with the three highest leaf anthocyanin content values were ‘Rouxai’ (23.3%), ‘Cherokee’ (19.8%), and ‘Salanova® Hydroponic Red Batavia’ (19.1%), respectivly. While ‘Rex’ was the only green lettuce cultivar used, we saw that all red lettuce cultivars except ‘Cherokee’ that had low leaf anthocyanin content did not increase as much shoot dry weight as ‘Rex’, the only green cultivar (Figure 3.5). Studies on various lettuce cultivars have consistently shown that the presence of anthocyanins can hinder growth (Kim & van Iersel, 2022). This reduction in growth is most likely attributed to the diminished availability of photons for photosynthesis due to the presence of anthocyanins (Kim & van Iersel, 2022).

The two cultivars, ‘Rouxai’, and ‘Cherokee’, exhibited an increase in growth with increasing PPFD which is important because they are the cultivars with the 1st and 2nd highest leaf anthocyanin content. ‘Rex’ had the lowest leaf anthocyanin content and the second highest shoot dry weight only 2% less than ‘Cherokee’. It is known that under high light stress, plants can increase their anthocyanin concentrations to mitigate photooxidative damage (Nassour et al., 2020). This could potentially explain the observed increase in growth associated with high leaf anthocyanin content values, suggesting their potential role in reducing photooxidative damage.

‘Salanova® Hydroponic Red Batavia’ and ‘Salanova® Red Batavia’ had the lowest shoot dry weights but the highest SLA (Figure 3.9). The higher SLA observed in these two cultivars can be primarily attributed to their low shoot weight. This increase in SLA is likely due to thinner leaves, a characteristic also observed in plants grown under 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a similar study by Bhuiyan & van Iersel (2021). However, this high SLA for ‘Salanova® Hydroponic Red Batavia’ and ‘Salanova® Red Batavia’ was not influenced significantly by the PPFD. respectively. Regarding cultivar differences in SLA, all cultivars, except for ‘Salanova® Hydroponic Red Batavia’ and ‘Salanova® Red Batavia’, showed a reduction in SLA with increasing PPFD (Figure 3.10). Other studies also show that with increasing PPFD a reduction in SLA is seen (Jin et al., 2023). At lower PPFDs, an increase in SLA is observed, likely facilitating greater light absorption to enhance photosynthesis (Kim et al., 2019).

The cultivar differences for LUE showed that ‘Cherokee’, ‘Rex’, ‘Teodore’, and ‘Rouxai’ had the highest LUE, respectively (Figure 3.7). Increasing PPFD decreased the LUE for all cultivars (Figure 3.8). In a previous study by Kim & van Iersel (2022), they found that the LUE of red cultivars was lower than that of green cultivars due to an inhibitory effect of anthocyanins on photosynthesis (Kim & van Iersel, 2022). This study saw that ‘Cherokee’, a red cultivar, had on average a higher LUE compared to the green cultivar ‘Rex’, however both decreased with increasing PPFD. The reason LUE for all cultivars decreased with increasing PPFD is most likely due to the quantum yield of PSII (ΦPSII) decreasing with increasing PPFD (Geoffrey & van Iersel, 2020). Light is used more efficiently to drive photosynthesis at lower PPFDs which is why plants have a lower LUE under higher PPFDs (Geoffrey & van Iersel, 2020; Palmer & van Iersel, 2020).

With increasing PPFD, the ΦPSII for all cultivars decreased (Figure 3.11). The highest ΦPSII among all cultivars was observed under lower PPFD conditions. This phenomenon can be attributed to the rising PPFD, which causes the closure of a large portion of the reaction centers in photosystem II (PSII) thus preventing them from accepting additional excited energy photons (Elkins & van Iersel, 2020, TC & van Iersel 2021). This trend aligns with findings from a study by Elkins and van Iersel (2020), where an increase in PPFD corresponded to a decrease in ΦPSII.

Towards the conclusion of the study, a limitation in the pH of the nutrient solution rose to 7.30, surpassing the optimal pH range recommended for hydroponic systems, which is typically between 5.5 and 6.5. Although the pH was readjusted back into the optimal recommended range, it is plausible that it still contributed to the observed tipburn symptoms. Regrettably we didn’t document tipburn incidence during this study. If this study was replicated it would be worth recording tip burn incidence in each cultivar to determine if leaf anthocyanin content plays a role in reducing tipburn symptoms.

Conclusions

Our objectives for this study were to investigate the morphological and physiological responses of six lettuce cultivars with different leaf anthocyanin content grown in a greenhouse under different supplemental PPFDs. We observed an increase in plant shoot dry weight for red lettuce cultivars, particularly in ‘Cherokee’ and ‘Rouxai’, under rising PPFD. Notably, these two cultivars also exhibited the highest leaf anthocyanin content. Our results suggest that higher leaf anthocyanin content may allow red cultivars to increase shoot dry weight with increasing PPFD to an extent, but this was only seen in the cultivar ‘Cherokee’.

Future studies

This study revealed notable cultivar differences, prompting the need for future investigations to investigate deeper into the role that anthocyanins play in plant growth. While the increase in leaf anthocyanin content with increasing PPFD was primarily observed in red cultivars like ‘Salanova® Red Batavia’ and ‘Salanova® Hydroponic Red Batavia’, exploring research methods to increase the leaf anthocyanin content using light could be valuable for future investigations in enhancing these phenolic pigments. For future studies, it would also be beneficial to use an automated lighting control (ALC) system to maintain a target daily light integral (DLI) rather than supplying supplemental PPFD to determine if DLI plays a role in how leaf anthocyanin content affects plant morphology and growth.

Literature Cited

Assefa, A. D., Hur, O. S., Hahn, B. S., Kim, B., Ro, N. Y., & Rhee, J. H. (2021). Nutritional metabolites of red pigmented lettuce (*Lactuca sativa*) germplasm and correlations with selected phenotypic characters. Foods, 10(10), 2504. doi: https://doi.org/10.3390/foods10102504

Bhuiyan, R., & Van Iersel, M. W. (2021). Only extreme fluctuations in light levels reduce lettuce growth under sole source lighting. Frontiers in Plant Science, 12, 619973. doi: https://doi.org/10.3389/fpls.2021.619973

Chen, Z., Shah Jahan, M., Mao, P., Wang, M., Liu, X., & Guo, S. (2021). Functional growth, photosynthesis, and nutritional property analyses of lettuce grown under different temperature and light intensity. The Journal of Horticultural Science and Biotechnology, 96(1), 53-61. doi: https://doi.org/10.1080/14620316.2020.1807416

Da Silva, J. G. (2012). Feeding the world sustainably. United Nations. https://www.un.org/en/chronicle/article/feeding-world-sustainably

Elkins, C., & van Iersel, M. W. (2020). Longer photoperiods with the same daily light integral improve growth of rudbeckia seedlings in a greenhouse. HortScience, 55(10), 1676-1682. doi: https://doi.org/10.3390/plants9091172

Ertle, J., & Kubota, C. (2023). Reduced daily light integral at the end of production can delay tipburn incidence with a yield penalty in indoor lettuce production. HortScience, 58(10), 1217-1224. doi: https://doi.org/10.21273/HORTSCI17314-23

Giampieri, F., Cianciosi, D., Alvarez-Suarez, J.M., Quiles, J.L., Forbes-Hernández, T.Y., Navarro-Hortal, M.D., Machì, M., Casanova, M.R.D.J.P., Espinosa, J.C.M., Chen, X., &

Zhang, D., (2023). Anthocyanins: what do we know until now?. Journal of Berry Research, (Preprint), 1-6. doi: http://doi.org/10.3233/JBR-220087

Gould, K. S. (2004). Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. Journal of Biomedicine and Biotechnology, 2004(5), 314. doi: https://doi.org/10.1155/S1110724304406147

Jin, W., Ji, Y., Larsen, D. H., Huang, Y., Heuvelink, E., & Marcelis, L. F. (2023). Gradually increasing light intensity during the growth period increases dry weight production compared to constant or gradually decreasing light intensity in lettuce. Scientia Horticulturae, 311, 111807. doi: https://doi.org/10.1016/j.scienta.2022.111807

Jin, W., Formiga Lopez, D., Heuvelink, E., & Marcelis, L. F. (2023). Light use efficiency of lettuce cultivation in vertical farms compared with greenhouse and field. Food and Energy Security, 12(1), e391. doi: https://doi.org/10.1002/fes3.391

Kelly, N., Choe, D., Meng, Q., & Runkle, E. S. (2020). Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Scientia Horticulturae, 272, 109565. doi: https://doi.org/10.1016/j.scienta.2020.109565

Kim, C., & van Iersel, M. W. (2022). Morphological and physiological screening to predict lettuce biomass production in controlled environment agriculture. Remote Sensing, 14(2), 316. doi: https://doi.org/10.3390/rs14020316

Kim, J. K., Kang, H. M., Na, J. K., & Choi, K. Y. (2019). Changes in growth characteristics and functional components of (*Lactuca indica L.*) 'Sunhyang' baby leaf vegetable by light

Intensity and cultivation period. Horticultural Science and Technology, 37(5), 579-588. doi: https://doi.org/10.7235/HORT.20190058

Liakopoulos, G., Nikolopoulos, D., Klouvatou, A., Vekkos, K. A., Manetas, Y., & Karabourniotis, G. (2006). The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). Annals of botany, 98(1), 257-265. doi: https://doi.org/10.1093/aob/mcl097

Lu, D., Zhang, Y., Zhang, A., & Lu, C. (2022). Non-photochemical quenching: from light perception to photoprotective gene expression. International journal of molecular sciences, 23(2), 687. doi: https://doi.org/10.3390/ijms23020687

Lu, Y., Zhang, M., Meng, X., Wan, H., Zhang, J., Tian, J., Hao, S., Jin, K., & Yao, Y. (2015). Photoperiod and shading regulate coloration and anthocyanin accumulation in the leaves of malus crabapples. Plant Cell, Tissue and Organ Culture (PCTOC), 121, 619-632. doi: https://doi.org/10.1007/s11240-015-0733-3

Medina-Lozano, I., Bertolín, J. R., & Díaz, A. (2021). Nutritional value of commercial and traditional lettuce (Lactuca sativa L.) and wild relatives: Vitamin C and anthocyanin content. Food Chemistry, 359, 129864. doi: https://doi.org/10.1016/j.foodchem.2021.129864

Oliveira, H., Correia, P., Pereira, A. R., Araújo, P., Mateus, N., de Freitas, V., Oliveira, J., & Fernandes, I. (2020). Exploring the applications of the photoprotective properties of anthocyanins in biological systems. International journal of molecular sciences, 21(20), 7464. doi: https://doi.org/10.3390/ijms21207464

Saure, M. C. (1998). Causes of the tipburn disorder in leaves of vegetables. Scientia horticulturae, 76(3-4), 131-147. https://doi.org/10.1016/S0304-4238(98)00153-8

Song, J., Huang, H., Song, S., Zhang, Y., Su, W., & Liu, H. (2020). Effects of photoperiod interacted with nutrient solution concentration on nutritional quality and antioxidant and mineral content in lettuce. Agronomy, 10(7), 920. doi: https://doi.org/10.3390/agronomy10070920

Steyn, W. J., Wand, S. J. E., Holcroft, D. M., & Jacobs, G. J. N. P. (2002). Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytologist, 155(3), 349-361. doi: https://doi.org/10.1046/j.1469-8137.2002.00482.x

Weaver, G., & van Iersel, M. W. (2020). Longer photoperiods with adaptive lighting control can improve growth of greenhouse-grown ’Little Gem’ lettuce (*Lactuca sativa*). HortScience, 55(4), 573-580. doi: https://doi.org/10.21273/HORTSCI14721-19

Kim, J. K., Kang, H. M., Na, J. K., & Choi, K. Y. (2019). Changes in growth characteristics and functional components of Lactuca indica L.‘Sunhyang’ baby leaf vegetable by light Intensity and cultivation period. Horticultural Science and Technology, 37(5), 579-588.

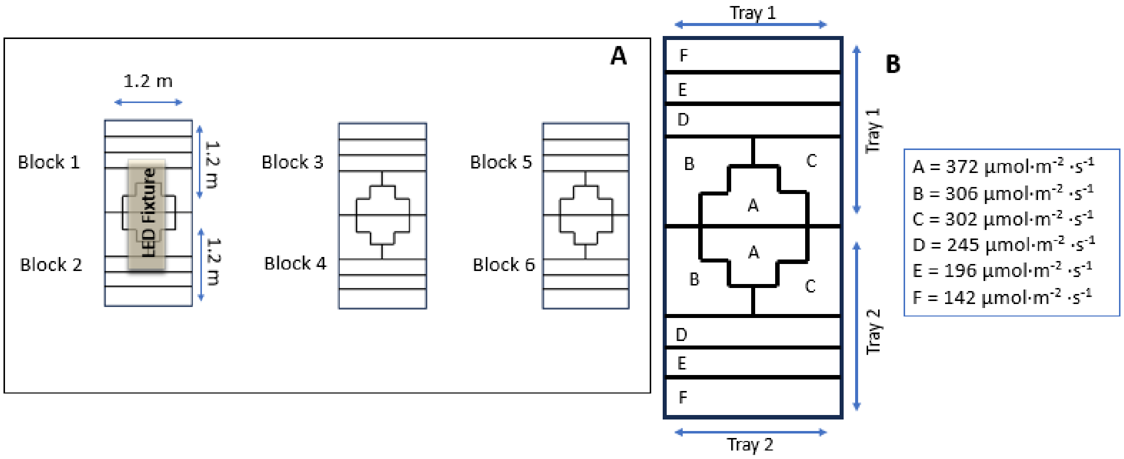
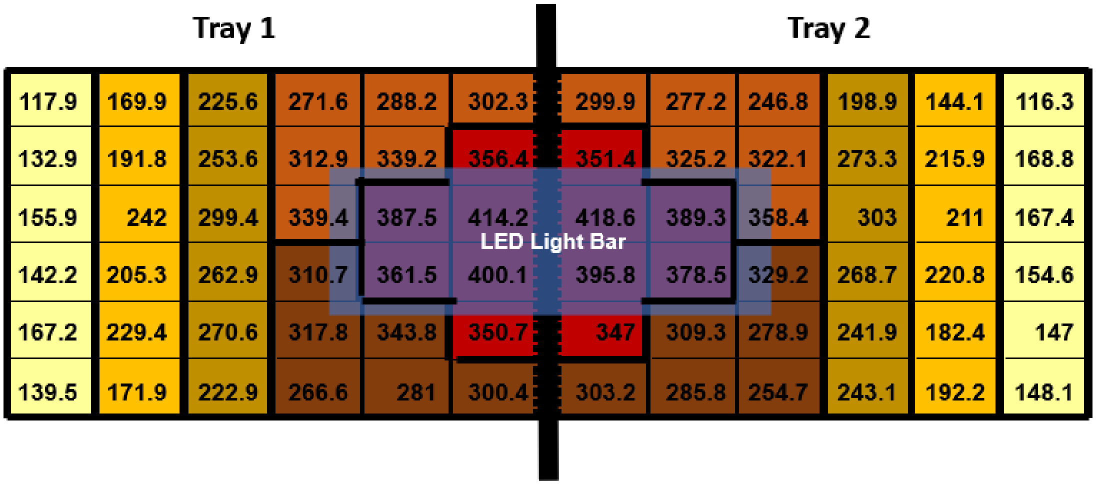


Figure 3.1: Illustration of the greenhouse setup: (A) Three benches are arranged in the greenhouse, each holding two growing trays. Positioned at the center over each bench, a single Light Emitting Diode (LED) lighting fixture provides supplemental lighting for both trays on the bench, resulting in a total of six trays in use. (B) Within each tray, there are six sections, categorized based on the similarity of supplemental light received from the LED fixture, creating distinct light treatments. Each of these six lighting treatments includes six planting holes with similar PPFDs.



Tray 1						Tray 2					
117.9	169.9	225.6	271.6	288.2	302.3	299.9	277.2	246.8	198.9	144.1	116.3
132.9	191.8	253.6	312.9	339.2	356.4	351.4	325.2	322.1	273.3	215.9	168.8
155.9	242	299.4	339.4	387.5	414.2	418.6	389.3	358.4	303	211	167.4
142.2	205.3	262.9	310.7	361.5	400.1	395.8	378.5	329.2	268.7	220.8	154.6
167.2	229.4	270.6	317.8	343.8	350.7	347	309.3	278.9	241.9	182.4	147
139.5	171.9	222.9	266.6	281	300.4	303.2	285.8	254.7	243.1	192.2	148.1

Figure 3.2: Heat map of the photosynthetic photon flux density (PPFD) lighting treatments from the supplemental LED fixture is provided. The farther away from the LED light bar the plants were, the less supplemental light was provided to the plants. Plants directly under the LED fixture had high supplemental PPFD values. The six groups of six plants were grouped by tray based on their similarity in PPFD light measurements to create the six light treatments.

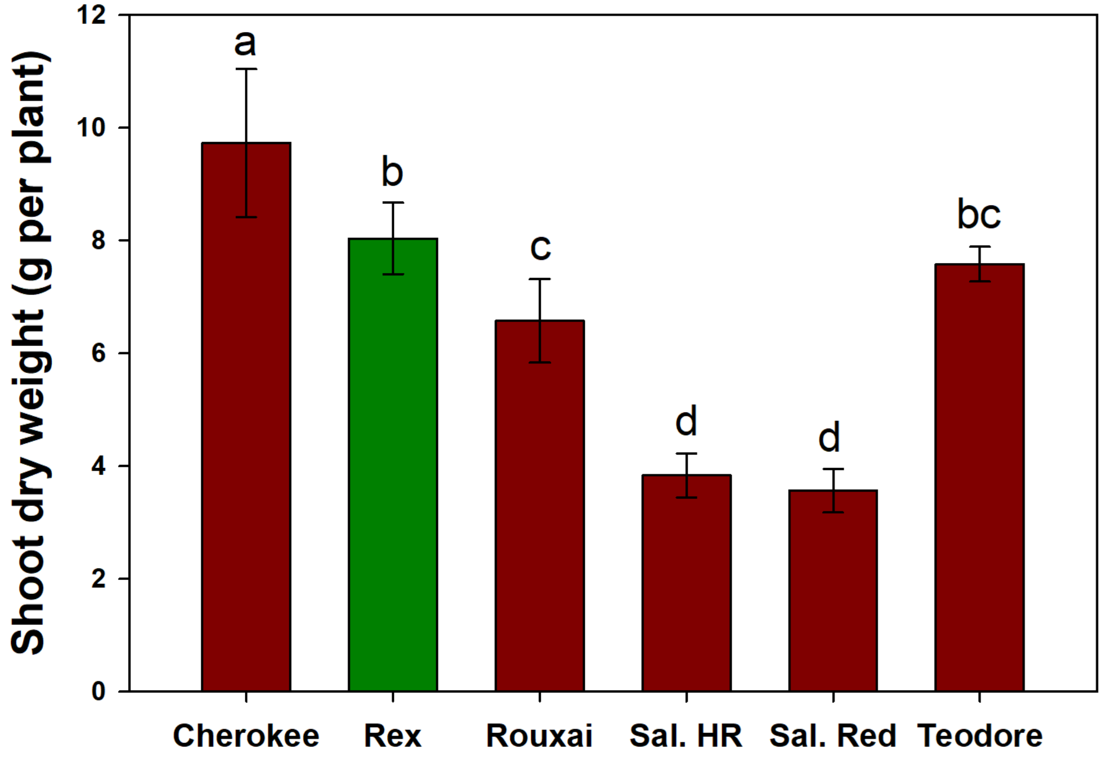


Figure 3.3: Shoot dry weight differences among cultivars of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’). All cultivars grown under different supplemental photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Bars with different letters denote a significant difference between cultivars. Bars with the same letter denote no significant difference between cultivars. ($p = 0.05$, $n = 6$). Green bars indicate green cultivars while red bars indicate red cultivars. Error bars represent standard error.

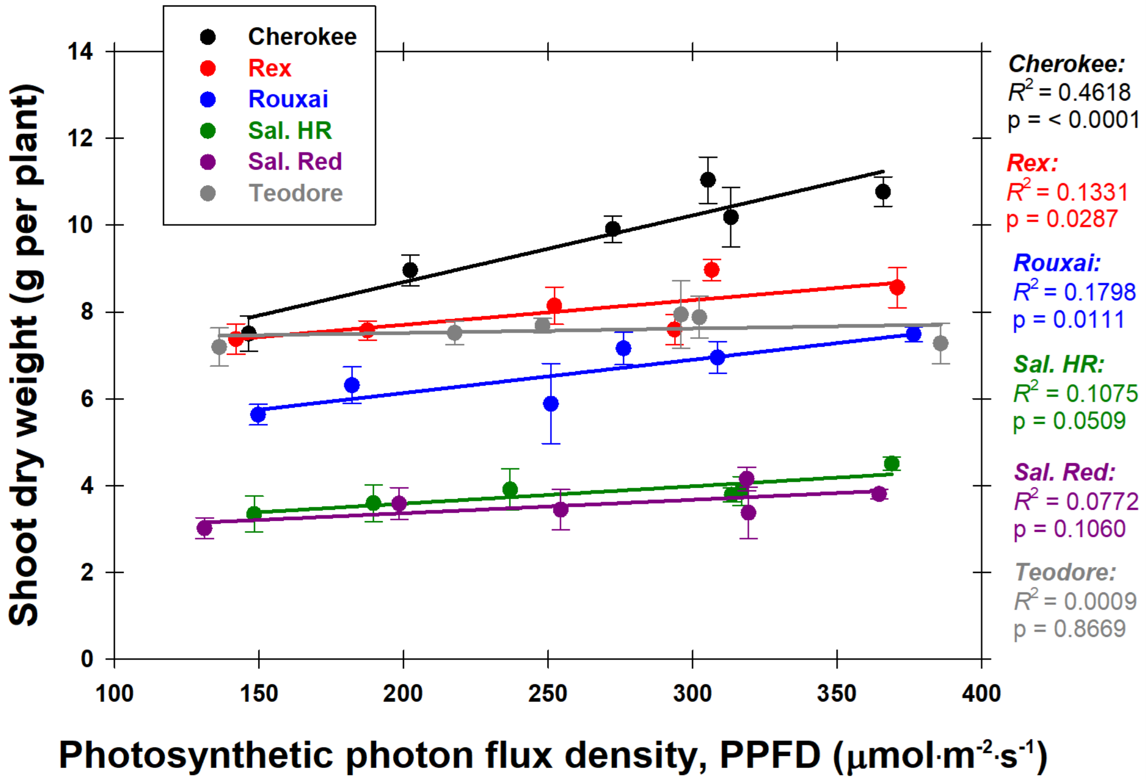


Figure 3.4: Shoot dry weight (of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’) plants grown under different photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents the average of six plants. Error bars represent standard error.

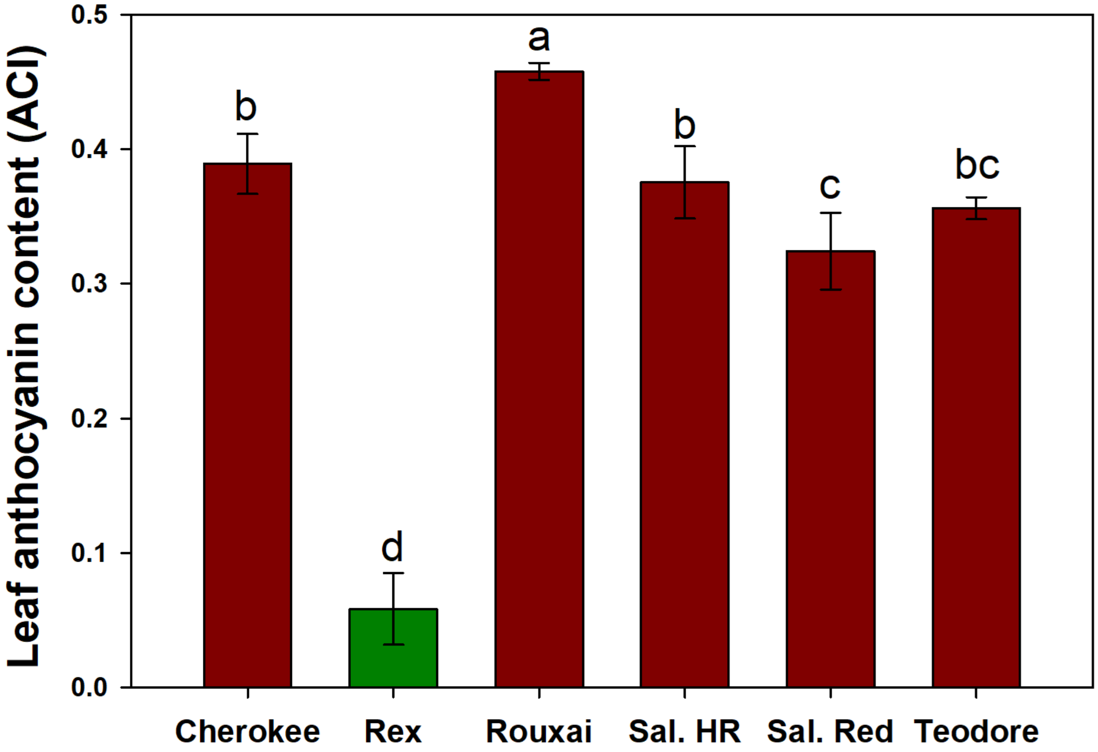


Figure 3.5: Anthocyanin content index differences among cultivars of lettuce (*Lactuca sativa* 'Rex', 'Cherokee', 'Rouxai', 'Teodore', 'Salanova Red Batavia', and 'Salanova Hydroponic Red Batavia'). All cultivars grown under different supplemental photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Bars with different letters denote a significant difference between cultivars. Bars with the same letter denote no significant difference between cultivars. ($p = 0.05$, $n = 6$). Each data point represents the average of six plants. Green bars indicate green cultivars while red bars indicate red cultivars. Error bars represent standard error.

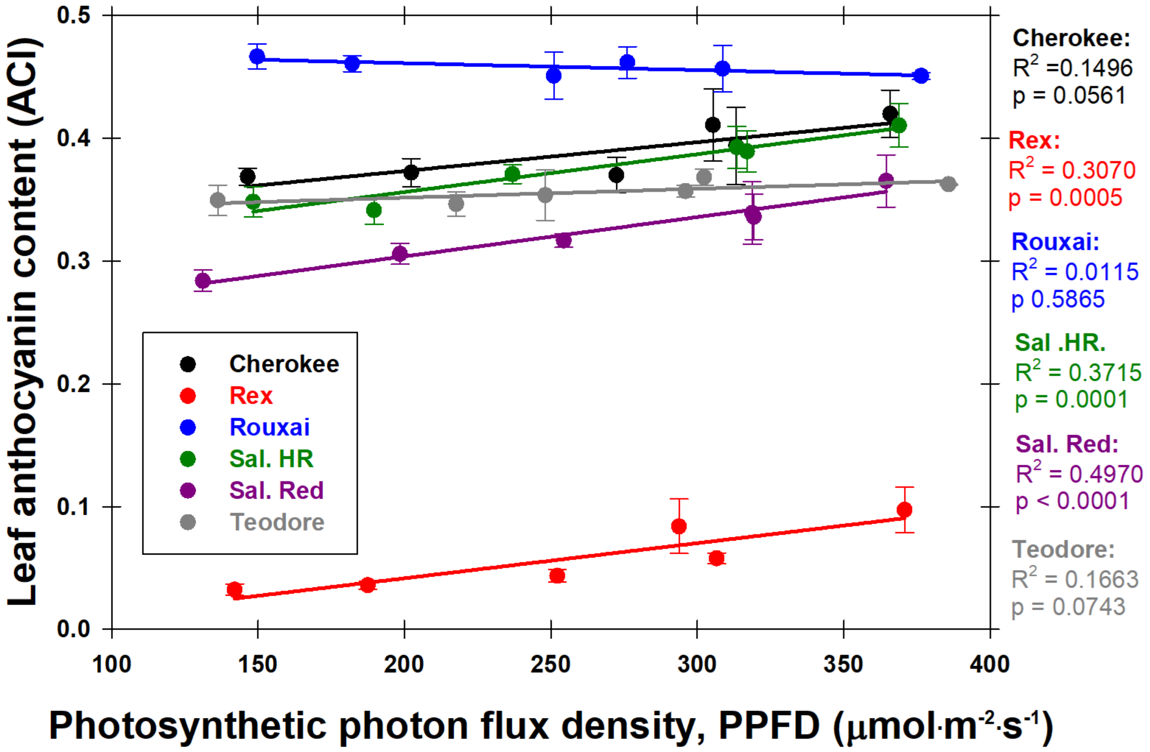


Figure 3.6: Leaf anthocyanin content of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’) plants grown under different photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents the average of six plants. Error bars represent standard error.

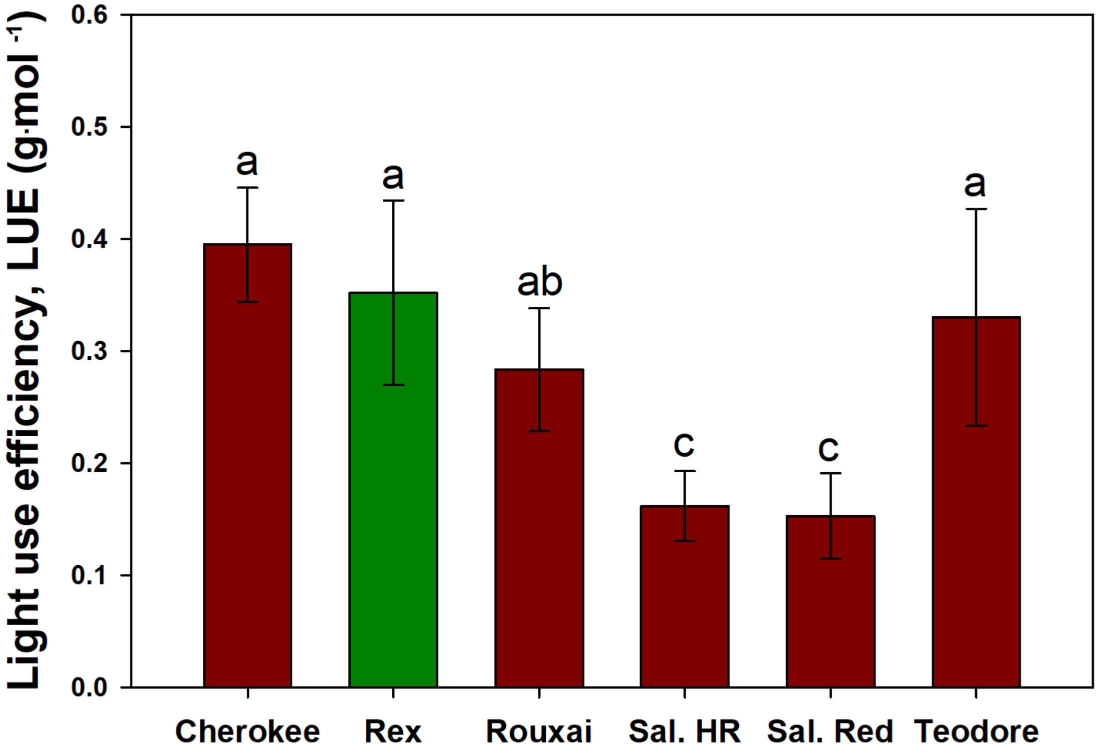


Figure 3.7: Light use efficiency differences among cultivars of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’). All cultivars grown under different supplemental photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Bars with different letters denote a significant difference between cultivars. Bars with the same letter denote no significant difference between cultivars. (p = 0.05, n = 6). Each data point represents the average of six plants. Green bars indicate green cultivars while red bars indicate red cultivars. Error bars represent standard error.

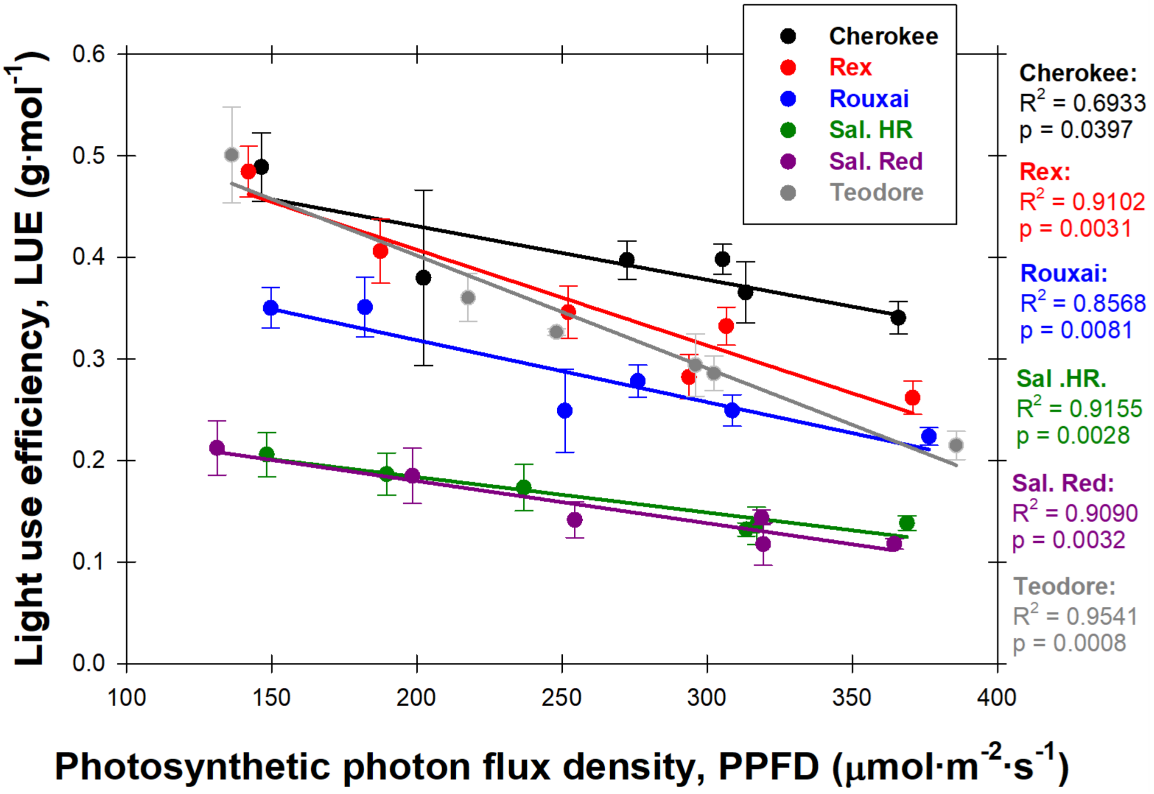


Figure 3.8: Light use efficiency (LUE) of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’) plants grown under different photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. LUE was calculated by dividing total plant dry weight by light integral. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents the average of six plants. Error bars represent standard error.

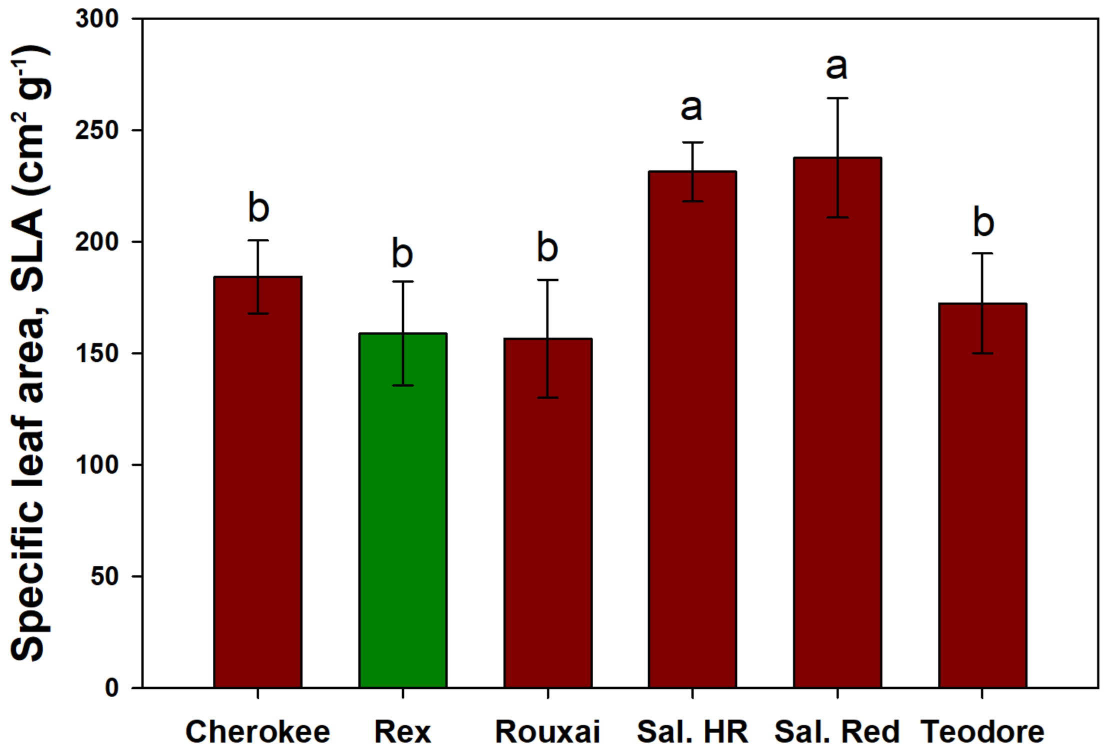


Figure 3.9: Specific leaf area differences among cultivars of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’). All cultivars grown under different supplemental photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Bars with different letters denote a significant difference between cultivars. Bars with the same letter denote no significant difference between cultivars. (p = 0.05, n = 6). Each data point represents the average of six plants. Green bars indicate green cultivars while red bars indicate red cultivars. Error bars represent standard error.

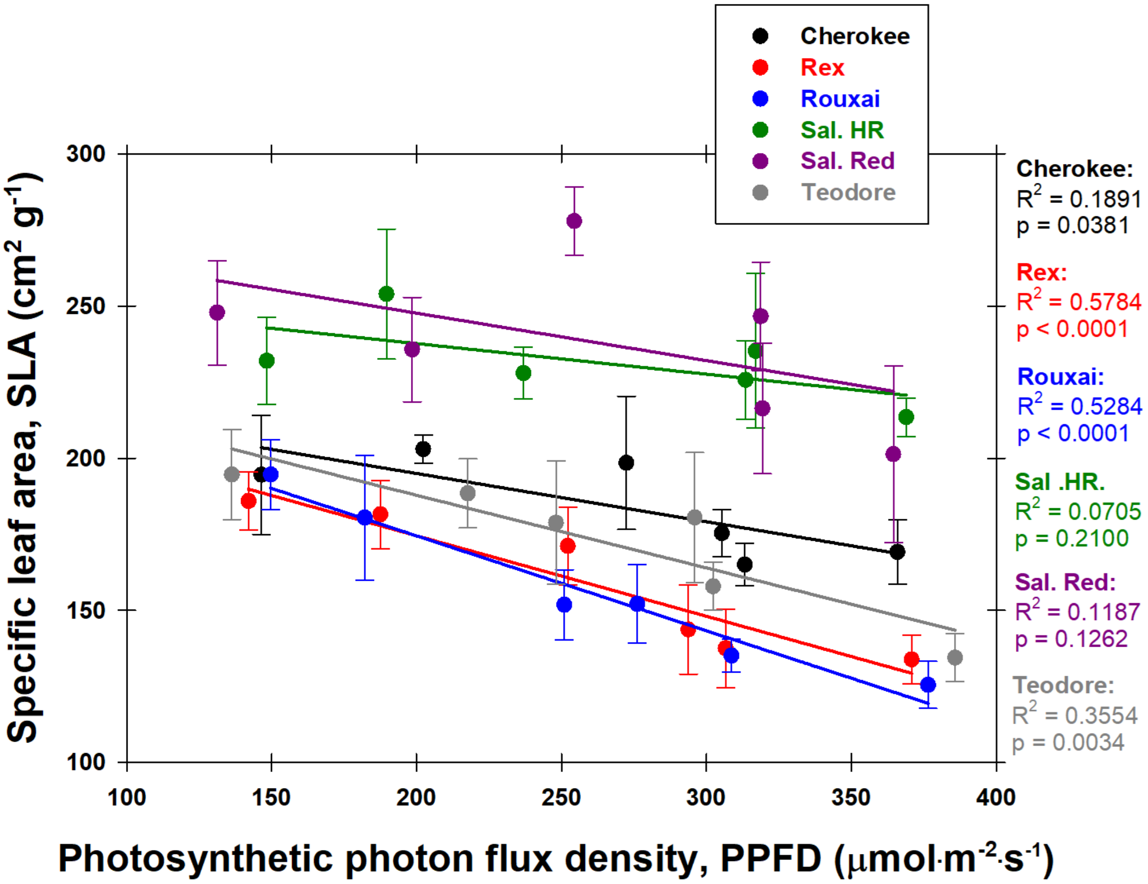


Figure 3.10: Specific leaf area (SLA) of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Baltiva,’ and ‘Salanova Hydroponic Red Baltiva’) plants grown under different photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. SLA was calculated by dividing total leaf area by shoot dry weight. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents the average of six plants. Error bars represent standard error.

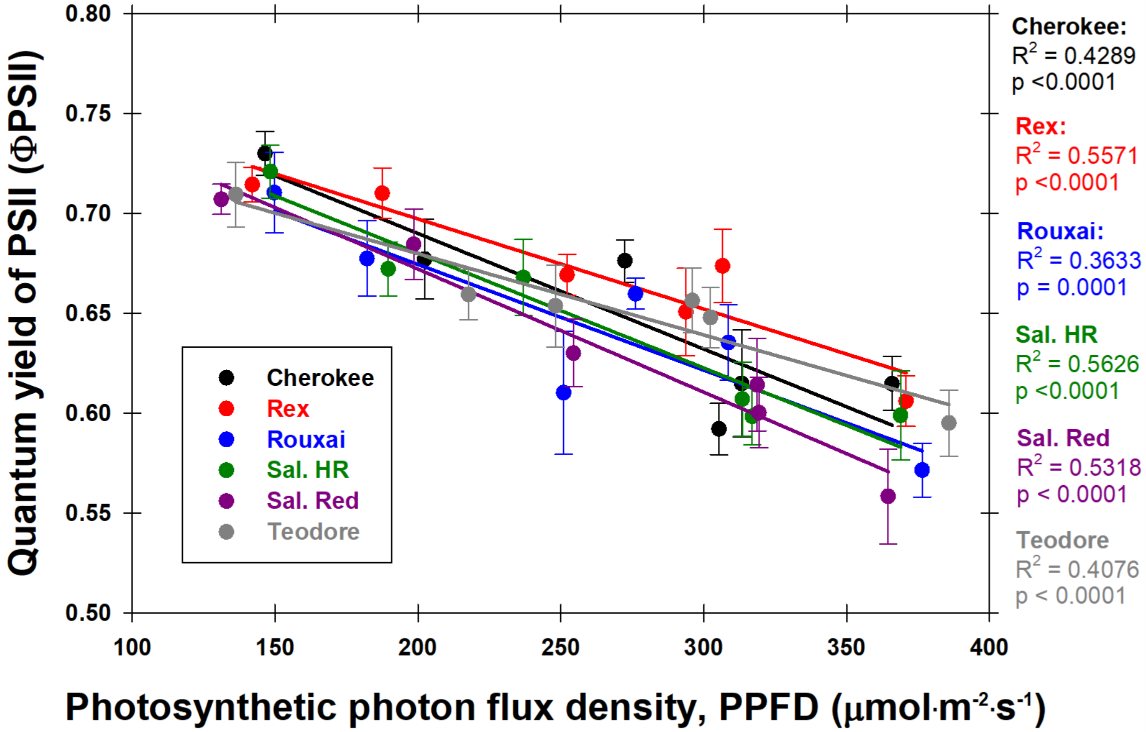


Figure 3.11: Chlorophyll fluorescence of lettuce (*Lactuca sativa* ‘Rex’, ‘Cherokee’, ‘Rouxai’, ‘Teodore’, ‘Salanova Red Batavia’, and ‘Salanova Hydroponic Red Batavia’) plants grown under different photosynthetic photon flux densities (PPFDs) in deep water culture hydroponics. Lines show multiple regression analysis results, indicating significant PPFD interactions. Each data point represents the average of six plants. Error bars represent standard error.

CHAPTER 4

POTASSIUM NUTRITION RECOMMENDATIONS: MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES OF HYDROPONIC LETTUCE GROWN IN VARYING POTASSIUM CONCENTRATIONS AND UNDER DIFFERENT DAILY LIGHT INTEGRALS[3]

---

[3] Palsha, P.L, M.W. van Iersel, R.W. Dickson, M. Yelton, L. Seymour, and R.S. Ferrarezi. To be submitted to Frontiers

Abstract: We investigated the growth dynamics of hydroponic lettuce, driven by the influence potassium (K) has on crop growth. The objective of this study was to determine if increased K concentrations under different DLIs in a hydroponic system will increase growth in greenhouse lettuce. This study was conducted within a controlled glass greenhouse environment, with varying daily light integrals (DLIs) achieved by integrating an adaptive lighting control (ALC) system over a 16-hour photoperiod. We used three K treatments of 200, 400, or 600 mg·L^{-1} K and six DLI lighting treatments from 11.1, 12.9, 14.6, 15.9, 16.9, and 17 mol·m^{-2} ·d^{-1}.We found that increasing K did not increase shoot dry weight, leaf area, or specific leaf area with increasing DLI. While K and DLI had an interacting effect on root dry weight fraction, leaf chlorophyll content, and quantum yield of PSII, the K treatments did not increase or decrease with increasing DLI. The influencing factor was DLI, which led to an increase in shoot dry weight and leaf area, while a decrease in SLA was observed with increasing DLI. Ultimately, the addition of supplemental concentrations of K did not enhance the growth of lettuce, nor did these effects show any increase with increasing DLI.

Introduction

CEA lighting

In controlled environmental agriculture (CEA), the conventional role of natural sunlight is either replaced or supplemented by electric lighting with the purpose of securing adequate photosynthesis levels and fostering optimal growth (Palmer and van Iersel, 2020). The extraordinary capabilities of programmable control systems, combined with the integration of dimmable light-emitting diodes (LEDs), empower the precise regulation of horticulture lighting’s timing and intensity (Palmer and van Iersel, 2020). LED lighting has gained popularity due to its high efficiency and its unique feature of dimmability, which allows for precise control (Weaver & van Iersel, 2020; Nelson & Bugbee, 2014). Utilizing a dimmable LED lighting system within greenhouses serves the purpose of regulating the photosynthetic photon flux density (PPFD) to which a plant is exposed to and aids in achieving a target daily light integral (DLI) by the end of the photoperiod.

Automated lighting control system

Dimmable LED lights can be interfaced with quantum sensors and control systems, enabling adaptive lighting control (ALC) (Weaver & van Iersel, 2020). ALC provides a constant adjustment to the supplemental lighting to ensure that the combined DLI from sunlight and supplemental lighting reaches a predefined DLI threshold. If the DLI from sunlight alone exceeds this predetermined threshold, the supplemental lights are turned off to reduce electricity costs. Lighting recommendations for greenhouse crops recommend the minimum DLI be consistently met to ensure adequate growth is met (Mosharafian et al., 2021).

The process starts with the quantum sensor measuring sunlight intensity in PPFD, which is then sent to the data logger. The data logger calculates the amount of PPFD needed from the LEDs to reach the desired plant DLI threshold (Weaver & van Iersel, 2020). The ALC system ensures precise light adjustments based on changing sunlight levels, with the LEDs brightening or dimming in response. It’s important to note that this control is contingent upon the actual sunlight received by the plants, facilitating an adaptable and responsive lighting environment that can reach the programmed target DLI (Palmer & van Iersel, 2020; Weaver & van Iersel, 2020).

Importance of K

Potassium (K) is an essential macronutrient and is the most abundant inorganic cation in plant tissue (Wang et al., 2013; Jordan-Meille & Pellerin, 2008; Xu et al., 2020). As a macronutrient, K is needed in larger quantities for adequate plant growth and development (Taiz et al., 2015). K affects most biochemical and physiological processes which are essential for enzyme activation, photosynthesis, sugar transportation, stomatal regulation, protein and starch synthesis, and stress resistance (Wang et al., 2013; Jordan-Meille & Pellerin, 2008; Prajapati & Modi, 2012). K reduces abiotic and biotic stresses and can be used to improve plant stress responses (Wang et al., 2013). K is also essential for increasing turgor pressure, which drives plant cell expansion and leaf elongation (Taiz et al., 2015).

Potassium has a crucial and complex role in photosynthesis. It can activate enzymes and is involved in the production of adenosine triphosphate (ATP), which is an energy-rich molecule created from sunlight, carbon dioxide, and water (Prajapati & Modi, 2012). ATP is the primary source of energy for most chemical reactions in plants. K ions help maintain the electrical balance at the site of ATP production, which directly affects the rate of photosynthesis, ATP production, and respiration (Prajapati & Modi, 2012). When a plant lacks sufficient K, its photosynthesis and ATP production rates decrease, slowing down plant growth (Prajapati & Modi, 2012). Additionally, K-deficient plants experience an increase in respiration rates, which further reduces their growth (Prajapati & Modi, 2012). In essence, K plays a vital role in maintaining plant energy production and growth.

Potassium is also used to transport sugars created in photosynthesis. Sugars are carried through the phloem, where they can be used or stored. This transportation process relies on energy in the form of ATP. If a plant lacks sufficient K, its ATP levels decrease, leading to a breakdown in sugar transportation (Prajapati & Modi, 2012). This, in turn, causes the rate of photosynthesis to decrease because photosynthates accumulate in the leaves (Prajapati & Modi, 2012). The symptoms of a deficiency of K have been reported in soybeans, and maize, where plants have a reduced stature and canopy size as well as a reduction in number of leaves and leaf area of those leaves (Oosterhuis et al., 2014).

Tipburn

Tipburn is a physiological disorder usually seen in leaves of vegetable plants like lettuce (Saure, 1998). Tipburn symptoms are irreversible and can cause entire yield losses for growers. These marketable losses are caused by a localized Calcium (Ca+) macronutrient deficiency that causes necrosis on the younger tip of leaves (Saure, 1998). Tipburn occurs mainly in the late stages of lettuce growth when the rate of growth rapidly increases, thus causing the limited transportation of Ca+ to the younger expending leaves (Saure, 1998; Kirkby & Pilbeam, 1984). Excess K can impact the movement of Ca+ ions within leaves, leading to reduced absorption through the roots (Sustr et al., 2019; Kirkby & Pilbeam, 1984). This creates a competitive scenario between Ca+ and K, hindering efficient nutrient uptake of Ca which could cause tipburn in lettuce (Sustr et al., 2019). It is also seen that plants grown under high light intensity have caused severe tipburn or an increased risk of tipburn (Saure, 1998). Plants grown in greenhouses are affected earlier and to a greater extent compared to plants grown in field production even though they receive nearly half the amount of radiation (Saure, 1998).

Objectives

The objective of this study was to determine if increased K concentrations under different DLIs in a hydroponic system will increase growth in greenhouse lettuce. We hypothesize that increasing the K concentration in hydroponic nutrient solutions will enhance lettuce growth, and this effect will further intensify with higher DLI. We also hypothesize that increasing K concentrations will increase the chance of tipburn symptoms.

Materials and Methods

Growing conditions

Plant cultivation took place at the University of Georgia Riverbend Greenhouse Complex, Athens, USA (latitude 33°57’26.676” N, longitude 83°22’36.48” W), from June 9th to July 10th, 2023. Seedlings were transplanted and grown in the glass greenhouse on June 21st, 2023. The average daily temperature, relative humidity (RH), and vapor pressure deficit (VPD) during the entirety of this study was 26.2° C ± 1.15, 87.1% ± 3.3, and 0.44 ± 0.11 kPa, respectively.

This study was conducted within a glass-enclosed greenhouse comprising nine benches, each measuring 2.4 m long and 1.2 m wide. The layout of these benches followed a 3 × 3 configuration, as illustrated in Figure 4.1. Each individual bench was furnished with two deep water trays, each measuring 1.2 by 1.2 meters with a depth of 10 cm, resulting in a total of 18 trays distributed across six designated blocks within the greenhouse. Planting density was 25 plants/m^2. Each tray held 144 L of treatment fertilizer solution and water. The entirety of this study involved a total of 648 individual plants, with each tray serving as an experimental block, and a total of 18 trays utilized.

Nine, 1.2-meter-long dimmable LED light bars (Arize Element Top Light PPR; Current, Montreal, Canada) were centered between two trays and hung 1.4 m above the shared benches. To optimize the light distribution from the LED bars, aluminum reflectors were attached around the edges of each LED fixture. A 70% shade net was installed above the LED fixtures to reduce the amount of sunlight entering the glass greenhouse. An extended PAR sensor (ePAR, covering 400-750 nm range) (SQ-610-SS; Apogee Intruments) and PAR sensor (SQ500-SS; Apogee Instruments) connected to a data logger, (CR1000; Cambell Scientific, Logan, UT, USA) was used to monitor and log the amount of sunlight received by the plants. All the PAR sensors were placed above the LED fixtures and below the 70% shade net, to be able to accurately record the light measurements.

The datalogger was used to monitor the combined PAR of both sunlight and target extended DLI (eDLI) of 17 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ at the end of the 16-hour photoperiod. The maximum DLI threshold of 17 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ was selected based on the recommendation provided by (Weaver & van Iersel, 2020) and is known as an ideal target DLI for lettuce (Mosharafian et al., 2021). The datalogger controlled and adjusted the output of the LED fixtures in real-time, continuously adapting to sunlight intensity variations throughout the day. This adjustment was achieved by delivering a 0 to 10-V direct current dimming (DC) signal, which was regulated using a four-channel analog output module (SDMAO4A; Campbell Scientific, Logan, UT, USA) (Weaver & van Iersel, 2020).

Lighting conditions

One day before transplanting on June 20st, 2023, supplemental greenhouse lights were deliberately positioned to create variations in light intensity. In each supplementary lighting treatment, a maximum DLI of 17 $\mathrm{mol \cdot m^{-2} \cdot d^{-1}}$ was provided by the LED lights and sunlight. The maximum output of the LED light bars used in this experiment targeted an intensity of 400 ± 34 $\mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$, and hence, a DLI of at least 17 $\mathrm{mol \cdot m^{-2} \cdot d^{-1}}$ could be reached if there was a total absence of sunlight. These measurements were recorded directly beneath the center of two trays using a quantum sensor, with the maximum PPFD reading recorded at 434 $\mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$, while the average was 393 $\mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$.

To measure the supplementary light conditions, we set up a system with 36 diodes attached to a foam board and this configuration allowed us to efficiently capture all 36 light measurements simultaneously. The diodes were interconnected and controlled using proprietary software (LoggerNet 4.1, Cambell Scientific, Logan, Utah, USA) and measured using a hand-held quantum sensor (MQ-500; Apogee Instruments, Logan, UT, USA). The quantum sensor allowed to measure the PPFD provided by the LED fixtures when they were at maximum power. Importantly, these measurements were obtained at night, exclusively under supplemental lighting conditions.

At the end of the study, we calculated the supplemental DLI provided during each day by subtracting the total DLI from the DLI of sunlight (Table 4.1). Using the PPFD ratios above, we were able to calculate the DLI for each lighting treatment received from the supplemental lighting. For replication purposes, we aimed to calculate the average total DLI for each lighting treatment in the study. This involved adding the DLI received from sunlight to the supplemental DLI of each lighting treatment which resulted in the average total DLI.

Hydroponic Potassium formulation

There were three K hydroponic nutrient solution treatments that were randomly allocated to the 18 trays in a completely randomized block. Each tray was filled with either 200, 400, or 600 mg·L^{-1} K hydroponic solution for 6 replications each. The three fertilizer treatments differ both in concentration and ratio of potassium nitrate (Table 4.1). In treatment K 200 mg·L^{-1}, the nutrient solution contained 57.49 mg·L^{-1} NO_3^- and 161.5 mg·L^{-1} K, while in K 400 mg·L^{-1} treatment, the levels were 128.8 mg·L^{-1} NO_3^- and 361.5 mg·L^{-1} K. For K treatment 600 mg·L^{-1}, the nutrient solution included 199.88 mg·L^{-1} NO_3^- and 561.5 mg·L^{-1} K. The initial pH of each K treatment solution ranged from 6.39 to 6.48, with electrical conductivity (EC) values ranging from 1.37 to 2.52. The K treatment solutions were 200 mg•L^{-1} K: (pH = 6.48, EC = 2.52), 400: (pH = 6.42, EC =1.99), 600: (pH = 6.39, EC = 1.37). To achieve a desired pH range of 5.5 to 6.5, we employed a 1M NaOH solution for pH adjustment. Approximately one week before the harvest, we observed an increase in pH, prompting us to make corrections using a 2M H_3PO_4 solution.

Treatments

Hydroponically cultivated lettuce was subjected to six different DLIs of 11.1, 12.9, 14.6, 15.9, 16.9, 17 $mol \cdot m^{-2} \cdot d^{-1}$ achieved by using an ALC system (sunlight + LED) and was also subjected to three different K concentrations of 200, 400, and 600 ppm. To calculate the DLI, we measured the PPFDs from the supplemental lighting across six different treatments, each offering varying light intensities at the beginning of the study. We then calculated the average PPFD ratios between these six treatments by dividing the DLI each light treatment received from the supplemental LED fixture by the highest supplemental DLI (17 $mol \cdot m^{-2} \cdot d^{-1}$) resulting in ratios of 0.41, 0.59, 0.76, 0.89, 0.99, 1.0, respectively (Table 4.1). The 36 collected light measurements were grouped into six treatments based on their similarity in PPFD light levels. This grouping resulted in six distinct lighting treatments, each with an average maximum supplemental PPFD of 162, 233, 300, 353, 390, and 393 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Seedling management

The lettuce cultivar ‘Casey’ (*Lactuca sativa*) (Johnny’s Selected Seeds, Winslow, Maine, USA) was selected due to its disease resistance, strong root system, and exceptional heat tolerance, making it an ideal choice for summer greenhouse growing conditions. For seedling production, around 1000 pelleted seeds were sown in rockwool plugs measuring 2.5 × 2.5 × 4 cm (A0 25/40; Grodan Rockwool BV, Roermond, Netherlands). Prior to seeding, these rockwool plugs were thoroughly saturated with water then a single lettuce seed was placed within each rockwool plug (120 plugs were arranged per tray). A total of eight trays were seeded, and the rockwool was misted once more to ensure adequate moisture for seed germination. Transparent plastic domes were used to cover the trays for the initial four days, creating a conducive environment for sustaining high humidity levels during the germination phase to ensure the seeds did not dry out.

The seeded rockwool plugs were placed within double-stacked, black, rectangular plastic mesh trays measuring 50.8 × 12.7 × 5.08 cm in height. These trays were double-stacked to prevent seedling roots from growing through and into the black felt fabric lining the ebb-and-flow shelves inside the walk-in vertical farm. Subsequently, these trays were transferred to the walk-in vertical farm and positioned on shelves located beneath 1.1 m-long white LED light fixtures (RAY with Physiospec indoor spectrum; Fluence Bioengineering, Austin, TX, USA). These light fixtures provided a photosynthetically active radiation (PAR) of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during a 16-hour photoperiod. Daily ebb-and-flow sub-irrigation was performed for 5 minutes, utilizing a water-soluble fertilizer solution using a 15N–2.2P–12.45K, containing 100 $mg \cdot L^{-1}$ Nitrogen (N) (Peters Excel 15–5–15 Cal-Mag Special; Everris NA Inc, Dublin, OH, USA).

Transplant management

Twelve days after the initial seedling on June 9th, we carefully selected the most uniform seedlings for further experimentation. These selected seedlings were randomly distributed among various light treatments and transplanted into a deep-water culture hydroponic system utilizing 36 net pots on June 21st, 2023. These net pots had dimensions measuring 4.8 cm in height, 4.5 cm in top diameter, and 3.3 cm in bottom diameter (Teku G46; Pöppelmann GmbH & Co., Lohne, Germany).

To create an appropriate environment for the plants and their net pots within each tray, we utilized foam lids that ensured buoyancy within the deep-water hydroponic system. To achieve this, we positioned a square foam board with dimensions of 1.2 × 1.2 m × 1.3 cm in thickness on the top of the trays. These foam lids were equipped with 36 evenly spaced holes, each 12.9032 square centimeters apart, and each hole had a diameter of 4.2 cm (Lenox Bi-Metal, Bristol, PA, USA specifications). Each of these holes held a net pot containing a single plant grown in rockwool. Subsequently, these prepared setups were placed into black square trays measuring 1.2 × 1.2 m × 10 cm in depth.

To ensure adequate aeration and oxygenation of the nutrient solution, clear plastic tubing 0.64 cm-diameter and six 2 cm-wide air stones (Pawfly Aquarium Air Stone, Guangzhou, China) were positioned within each tray. The circulation of air and maintenance of optimal oxygen levels within the nutrient solution were facilitated by the use of an air pump (EcoPlus-7; Hawthorne gardening company, Vancouver, WA, USA, 0.48 bar, 3.0 A, 120V, Tube Size: 12.7 mm in diameter).

Measurements

Seventeen days after transplanting, CO_2 assimilation using a gas analyzer (CIRAS-4; PP Systems, Amesbury, MA, USA) was collected on the upper-most fully expanded leaf of one randomly selected plant from each of the six lighting treatments in two replications for each K treatment . The leaf CO_2 assimilation measurements were obtained exclusively under the illumination of supplemental lighting without sunlight at 9:30 pm on July 8th, 2023, at 9:45 pm. On July 9th, 2023, the leaf chlorophyll content was taken from two plants from each of the six lighting treatments in all blocks using a leaf chlorophyll meter (CCM-200 plus; Apogee Instruments). We also assessed the quantum yield of photosystem II (Φ_{PSII}) on July 9th, 2023, at

9:45 pm taken exclusively under the illumination of supplemental lighting without sunlight. This assessment was performed using a pulse-amplitude modulated (Mini-PAM) fluorometer (Mini-PAM II; Heinz Walz, Effeltrich, Germany). We recorded the Φ_{PSII} measurements for two randomly selected plants from each lighting treatment in three blocks. This data collection took place the night before the destructive harvest on July 9th, 2023, which marked the end of the growth cycle.

On July 10th, 2023, we conducted various assessments on two randomly selected plants from each of the six lighting treatments across three replications. These assessments included individual imaging of two randomly selected plants from each lighting treatment in 3 replications to determine projected canopy size (PCS), accomplished using a multispectral imaging system (Topview, Aris, Eindhoven, The Netherlands). To assess total leaf area, we separated individual leaves from the same two randomly selected plants and measured them using a leaf area meter (LI-3100; LI-COR, Lincoln, NE, USA). Subsequently, the shoots and roots for the same two plants were weighed for fresh weights then placed in a drying oven at 80 °C for 96 hours (4 days), following which their dry weights were measured.

Petiole sap samples were obtained from additional plants within each lighting treatment. This involved extracting sap from two fully grown leaf petioles by squeezing them through cheesecloth using a garlic press. A small quantity of the collected sap was then analyzed for K+. For the remaining plants in each lighting treatment and K treatment across all replications, we measured the average fresh shoot and root weights. These plants were also placed in a drying oven at 80 °C for 144 hours (6 days) to determine their dry weights.

The fresh and dry shoot and root weights were divided by the number of plants in each lighting treatment to calculate the average weights per plant within each treatment.

Specific leaf area (SLA) was calculated using dry shoot weight and total leaf area (total leaf area/ dry shoot weight).

Tipburn

On July 10th, before the initial harvest, it is noteworthy that although we did not formally record tipburn incidence among light treatments, tip burn incidence was recorded for K treatments.

Experimental design and Statistical analysis

The experiment was arranged on a randomized complete block design with six lighting treatments in each block. The experimental unit is six plants. Based on the configuration of six plants per block, resulting in a total of 36 plants per block when considering the 6 plants multiplied by 6 blocks. This experimental design encompasses 18 treatments, which comprise six lighting treatments combined with three K levels

Subsequent analyses were conducted using multiple regression analysis using a statistical software (SigmaPlot 11.0, Systat software, Inc., San Jose, CA, USA). To identify any interaction effects between DLI and K concentrations, we used a multiple stepwise regression analysis with backward selection in a statistical software (JMP Pro 17; SAS Institute, Cary, NC, USA) at a significant level of 0.05. Significant levels of 0.05 were considered statistically significant while non-significant components in the regression equation were eliminated using stepwise selection. This method was applied to investigate potential interaction effects of DLI and K on various parameters, including shoot fresh weight per plant, shoot dry weight per plant, root dry weight per plant, root dry weight fraction, leaf area per plant (LA), SLA, PCS, PSII, CO^2 assimilation, K+ petiole sap concentration, chlorophyll content, and tipburn.

Results

Fresh and dry weights

The average shoot fresh weight (SFW) of each plant increased with increasing DLI ($p < 0.0001$) (Figure 4.2). While each K treatment may have seen a different increase in shoot fresh weight (g/plant), this was not attributed to the K treatment but rather DLI. Similarly, for shoot dry weight (SDW) it was seen that increasing DLI from 11.1 to 17 $mol \cdot m^{-2} \cdot d^{-1}$ increased the average plant weight ($p < 0.0001$) (Figure 4.3) and K did not affect the SDW. The average root dry weight (RDW) of each plant increased with increasing DLI ($p = 0.0002$) (Figure 4.4) and K treatment for 200, 400, and 600 $mg \cdot L^{-1}$ K was not a contributing factor to this increase in RDW. The root dry weight fraction (RDWF), which was calculated by dividing the root dry weight by total plant dry weight. RDWF was affected by DLI and K ($p = 0.0007$) but with increasing DLI none of the K treatments increased or decreased (Figure 4.5).

Leaf area, specific leaf area, and projected canopy size

Total LA increased with increasing DLI while all K did not have an interacting effect on LA ($p = 0.0374$). LA increased from 1934 to 2031 cm^2/plant when the DLI increased from 11.1 to 17 $mol \cdot m^{-2}\ d^{-1}$ (Figure 4.6). With increasing K, the total LA did not change. SLA, which is calculated by dividing SDW by LA, decreased with increasing DLI ($p = 0.0001$) (Figure 4.7). SLA decreased from 568 to 449 $cm^2 \cdot g^{-1}$ with increasing DLI from 11.1 to 15.9 $mol \cdot m^{-2} \cdot d^{-1}$ while an increase in DLI from 16.9 to 17 $mol \cdot m^{-2} \cdot d^{-1}$ saw a decrease in SLA from 480 to 443 $cm^2 \cdot g^{-1}$ (Figure 4.7). SLA did not change with increasing K. PCS for all plants were similar in size and decreased with increasing DLI ($p = 0.0659$) (Figure 4.8). K did not change the PCS and was not significant.

Quantum yield of photosystem II and CO_2 assimilation

The quantum yield of PSII was affected by DLI and K ($p = 0.0011$) however quantum yield of PSII for all K treatments did not increase or decreased with increasing DLI from 11.1 to 17 $mol \cdot m^{-2} \cdot d^{-1}$ (Figure 4.9). DLI had an interacting effect on CO_2 assimilation rate which increased with increasing DLI ($p < 0.0001$) (Figure 4.10).

Petiole K+ concentration and leaf chlorophyll content

K and DLI both had an interacting effect on petiole K+ concentration (0.0186) (Figure 4.11). Petiole K+ concentration for all K treatments did not increase or decrease with increasing DLI. The leaf chlorophyll content had an interacting effect with DLI and K ($p = 0.0061$) (Figure 4.12). Leaf chlorophyll content did not increase or decrease with increasing DLI.

Tipburn

Unfortunately, tipburn was only recorded for the K treatments and not among the DLI treatments which was a mistake made during the harvest (Figure 4.13). At the 200 $mg \cdot L^{-1}$ K treatment level, tipburn incidence was minimal at 0.92%. For the 400 mg·L^{-1} K treatment, the tipburn incidence increased to 4.62%, and the highest tipburn incidence was observed at 600 mg·L^{-1} K treatment, reaching 9.72%.

Discussion

It has been stated that when K is supplied in deficient amount, plants see a decrease in biomass accumulation (Jordan-Meilla & Pellerin, 2008). In theory supplementing excess K to plants should increase growth since a deficiency in K causes biomass accumulation to decrease (Jordan-Meilla & Pellerin, 2008). However, K did not affect the SFW or SDW but rather the increase was due to increasing DLI (Figure 4.2). This increase in SFW and SDW was most likely due to the increased PPFD the plants received which caused an increase in photosynthesis activity. Increasing DLI has been seen in other studies to increase the fresh and dry shoot weight of lettuce cultivars (Kelly et al. 2020). When PPFD conditions increase, the PSII reaction centers close to minimize photodamage. This protective mechanism results in a greater dissipation of absorbed light energy as heat (non-photochemical quenching, NPQ). Consequently, a reduced proportion of the absorbed light energy remains in an excited state, which leads to a decline in PSII efficiency under conditions of high DLI.

In previous studies it was seen that an increase in PPFD decreased the PSII due to the partially closed PSII reaction centers and upregulation of NPQ (Elkins & van Iersel, 2020). In this study, the quantum yield of PSII did not decrease or increase with increasing DLI even though there was an interacting effect between DLI and K on the quantum yield of PSII (Figure 4.9).

With increasing DLI we saw an increase in CO_2 assimilation (Figure 4.10). This also was most likely due to the increase in PPFD the plants received to increase DLI. Some studies have also seen an increase in CO_2 assimilation due to increasing PPFD (Weaver & van Iersel, 2019; Zhen & van Iersel, 2017). Therefore, the observed increase in CO_2 assimilation with rising DLI could potentially be attributed to plants allocating more carbon resources towards biomass production. This reasoning could also explain why SFW and SDW had a positive relationship with increasing DLI while K did not influence SFW and SDW.

We saw that the RDW increased with increasing DLI (Figure 4.4). The increase in DLI was most likely due to the increase in PPFD, which primarily led to an increase in growth like SFW and SDW, however it could also explain the increase in RDW too. As biomass production increased, it would be expected that a portion of this increased biomass would be allocated to root growth for enhanced water and nutrient absorption. While K did not affect RDW, it was seen in apple root stock seedlings, that an excess of K and a deficiency of K both saw a decrease in root development and growth (Xu et al., 2020). However, with increasing DLI there was no increase or decrease in RDWF (root dry weight/ total plant dry weight) (Figure 4.5). Other studies have seen a reduction in RDWF in hydroponic lettuce but in this study, we did not see that effect (Lin et al., 2018).

With increasing DLI we saw an increase in LA (Figure 4.6). As the DLI increased, the LA increased also which can most likely be attributed to the increasing DLI. Increasing DLI has been seen to increase LA in lettuce more than kale (Baumbauer et al., 2019). Under low DLI conditions a reduction in leaf area and growth was seen in cucumber seedlings (Kitaya et al., 1998). Both of these studies showed similar results to our study where under low DLI plants had a lower LA and under high DLIs plants had a higher LA.

SLA decreased with increasing DLI (Figure 4.7). A similar trend was observed in a study looking at increasing PPFDs on lettuce which saw the SLA decreasing with increasing PPFD (Jayalath & van Iersel, 2021). The reduction in SLA can be attributed to the increasing DLI. As DLI increases, leaves can become thicker. By using the PCS imaging system, we were also able to observe that leaves began to overlap under high DLI, as the PCS decreases with increasing DLI (Figure 4.8).

Unfortunately, tipburn symptoms were only recorded for the K treatments on the day of harvest, presenting a limitation. Nevertheless, it was observed that tipburn increased with rising K levels (Figure 4.13). One potential reason for this observed phenomenon could be that an excess of potassium may influence the movement of Ca+ ions within leaves, resulting in reduced absorption through the roots (Sustr et al., 2019; Kirkby & Pilbeam, 1984). This establishes a competitive scenario between Ca+ and K, hindering the effective nutrient uptake of Ca+ and potentially contributing to tipburn in lettuce (Sustr et al., 2019).

For leaf petiole K concentration, none of the K treatments showed an increase with rising DLI (Figure 4.11). In a study by Gent (2014), he saw that the potassium levels in plants increased with increasing daily integrated irradiance and plants also exhibited a greater increase in K concentration when harvested in the afternoon rather than the morning (Gent, 2014). However, our study did not observe the same trend. A potential explanation for these differing results may be attributed to the extended harvest period, encompassing both morning and afternoon sessions, potentially affecting the leaf petiole K concentrations.

Having high leaf chlorophyll content can increase the CO_2 assimilation rate and potentially growth rate of plants (Jayalath & van Iersel, 2021), however, in this study the leaf chlorophyll content did not increase or decrease with increasing DLI among any of the K treatments (Figure 4.12). A different trend is seen in Begonia where there is a small decrease in leaf chlorophyll content with increasing DLI (Krishna & van Iersel, 2004). It was seen that the CO_2 assimilation rate increased with increasing DLI (Figure 4.10). Other studies have also shown that elevated DLI correlates with an increased CO_2 assimilation rate and enhanced growth in tomato plants (Huber et al., 2021). This aligns with our findings, as we observed the highest growth under high DLIs in our study.

Conclusions

The objective of this study was to determine if increased K concentrations in a hydroponic solution will increase growth in greenhouse lettuce grown under varying DLIs. The increase in K did not affect the SDW, LA, or SLA with increasing DLI. There was an interacting effect between K and DLI on RDWF, leaf chlorophyll content, and quantum yield of PSII, the K treatments did not increase or decrease with increasing DLI. The increase in plant growth was due to the increase in DLI as evidenced by increases in SFW, SWD, and LA with increasing DLI. While elevated K levels appear to have minimal or no beneficial effects, adverse outcomes, such as an increase in tipburn, are observed with higher K fertilization. Future studies are required to investigate whether the interaction between increasing light intensity and higher K concentrations influences tipburn in lettuce. Ultimately this study suggests that potassium is not a useful way of increasing growth in hydroponic lettuce.

Literature Cited

Baumbauer, D. A., Schmidt, C. B., & Burgess, M. H. (2019). Leaf lettuce yield is more sensitive to low daily light integral than kale and spinach. HortScience, 54(12), 2159-2162. doi: https://doi.org/10.21273/HORTSCI14288-19

Elkins, C., & van Iersel, M. W. (2020). Longer photoperiods with the same daily light integral improve growth of rudbeckia seedlings in a greenhouse. HortScience, 55(10), 1676-1682. doi: https://doi.org/10.3390/plants9091172

Gent, M. P. (2014). Effect of daily light integral on composition of hydroponic lettuce. HortScience, 49(2), 173-179. doi:10.21273/HORTSCI.49.2.173

Huber, B. M., Louws, F. J., & Hernández, R. (2021). Impact of different daily light integrals and carbon dioxide concentrations on the growth, morphology, and production efficiency of tomato seedlings. Frontiers in Plant Science, 12, 615853. doi: https://doi.org/10.3389/fpls.2021.615853

Jayalath, T. C., & van Iersel, M. W. (2021). Canopy size and light use efficiency explain growth differences between lettuce and mizuna in vertical farms. Plants, 10(4), 704. doi: https://doi.org/10.3390/plants10040704

Jordan-Meille, L., & Pellerin, S. (2008). Shoot and root growth of hydroponic maize (*Zea mays L.*) as influenced by K deficiency. Plant and Soil, 304(1-2), 157-168. doi: https://doi.org/10.1007/s11104-007-9534-8

Kelly, N., Choe, D., Meng, Q., & Runkle, E. S. (2020). Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod. Scientia Horticulturae, 272, 109565. doi: https://doi.org/10.1016/j.scienta.2020.109565

Kirkby, E. A., & Pilbeam, D. J. (1984). Calcium as a plant nutrient. Plant, Cell & Environment, 7(6), 397-405. https://doi.org/10.1111/j.1365-3040.1984.tb01429.x

Kitaya, Y., Niu, G., Kozai, T., & Ohashi, M. (1998). Photosynthetic photon flux, photoperiod, and CO2 concentration affect growth and morphology of lettuce plug transplants. HortScience, 33(6), 988-991. doi: https://doi.org/10.21273/HORTSCI.33.6.988

Lin, K., Huang, Z., & Xu, Y. (2018). Influence of light quality and intensity on biomass and biochemical contents of hydroponically grown lettuce. HortScience, 53(8), 1157-1163. doi: https://doi.org/10.21273/HORTSCI12796-17

Mosharafian, S., Afzali, S., Weaver, G. M., van Iersel, M., & Velni, J. M. (2021). Optimal lighting control in greenhouse by incorporating sunlight prediction. Computers and Electronics in Agriculture, 188, 106300. doi: https://doi.org/10.1016/j.compag.2021.106300

Nelson, J. A., & Bugbee, B. (2014). Economic analysis of greenhouse lighting: light emitting diodes vs. high intensity discharge fixtures. PloS one, 9(6), e99010. doi: https://doi.org/10.1371/journal.pone.0099010

Nemali, K. S., & van Iersel, M. W. (2004). Acclimation of wax begonia to light intensity: Changes in photosynthesis, respiration, and chlorophyll concentration. Journal of the American Society for Horticultural Science, 129(5), 745-751. doi: https://doi.org/10.21273/JASHS.129.5.0745

Palmer, S., & van Iersel, M. W. (2020). Increasing growth of lettuce and mizuna under sole-source LED lighting using longer photoperiods with the same daily light integral. Agronomy, 10(11), 1659. doi: https://doi.org/10.3390/agronomy10111659

Prajapati, K., & Modi, H. A. (2012). The importance of potassium in plant growth–a review. Indian Journal of Plant Sciences, 1(02-03), 177-186. doi: https://doi.org/10.9790/9622-0803054452

Saure, M. C. (1998). Causes of the tipburn disorder in leaves of vegetables. Scientia horticulturae, 76(3-4), 131-147. doi: https://doi.org/10.1016/S0304-4238(98)00153-8

Sustr, M., Soukup, A., & Tylova, E. (2019). Potassium in root growth and development. Plants, 8(10), 435. doi: https://doi.org/10.3390/plants8100435

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant Physiology and Development (6th ed.). Sinauer Associates Incorporated.

Torres, A. P., & Lopez, R. G. (2011). Photosynthetic daily light integral during propagation of *Tecoma stans* influences seedling rooting and growth. HortScience horts, 46(2), 282-286. doi: https://doi.org/10.21273/HORTSCI.46.2.282

Weaver, G., & van Iersel, M. W. (2020). Longer photoperiods with adaptive lighting control can improve growth of greenhouse-grown 'Little Gem' lettuce (Lactuca sativa). HortScience, 55(4), 573-580. doi: https://doi.org/10.21273/HORTSCI14721-19

Wang, M., Zheng, Q., Shen, Q., & Guo, S. (2013). The critical role of potassium in plant stress response. International journal of molecular sciences, 14(4), 7370-7390. doi: https://doi.org/10.3390/ijms14047370

Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., ... & Jiang, Y. (2020). Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. Frontiers in Plant Science, 11, 904. doi: https://doi.org/10.3389/fpls.2020.00904

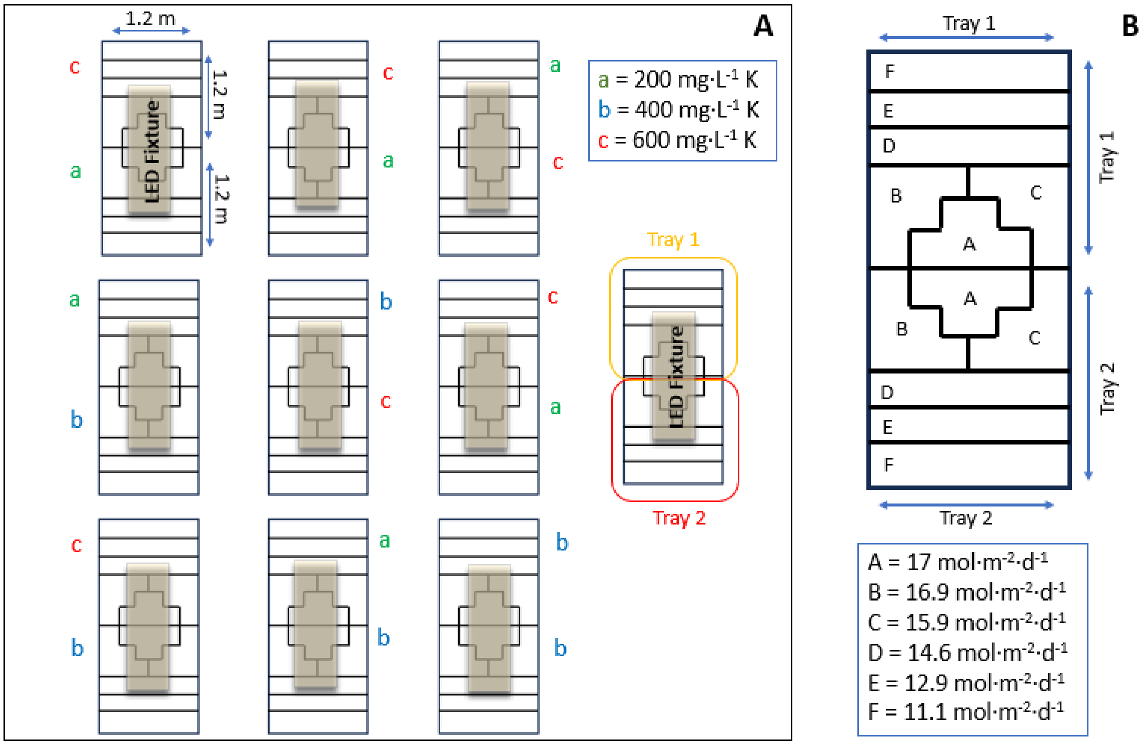


Figure 4.1: (A) Two growing trays were placed on each bench and 9 benches total were used. In the center of each tray is one Light Emitting Diode (LED) lighting fixture which provided supplemental lighting to both trays on each bench. There were 18 trays total which were randomly filled with either 200, 400, or 600 mg·L^{-1} K hydroponic solution. (B) The daily light integral (DLI) averages received from the supplemental LED fixtures were provided for each lighting treatment. The lighting treatments were divided into six sections based on their similarity of received supplemental light which created the six light treatments.

Table 4.1: Total DLI calculations (Sun + LED) using the ALC system. DLI received each day from the sunlight and the supplemental LED fixtures each day is averaged over a total of nineteen days. Averaged total DLI received in each light treatment is calculated for each of the six lighting treatments.

Day	DLI (Sun + LED) mol·m^{-2}·d^{-1}	DLI (Sun) mol·m^{-2}·d^{-1}	DLI (LED) mol·m^{-2}·d^{-1}	DLI received (mol·m^{-2}·d^{-1})											
				A		B		C		D		E		F	
				1.00		0.99		0.89		0.76		0.59		0.41	
				LED DLI	Total DLI	LED DLI	Total DLI	LED DLI	Total DLI	LED DLI	Total DLI	LED DLI	Total DLI	LED DLI	Total DLI
1	17.0	7.0	10.0	10.0	17.0	9.9	16.9	8.9	15.9	7.6	14.6	5.9	12.9	4.1	11.1
2	17.0	7.2	9.8	9.8	17.0	9.7	16.9	8.7	15.9	7.5	14.7	5.8	13.0	4.0	11.2
3	17.0	8.7	8.3	8.3	17.0	8.2	16.9	7.4	16.1	6.3	15.0	4.9	13.6	3.4	12.1
4	17.0	9.0	8.0	8.0	17.0	8.0	16.9	7.2	16.1	6.1	15.1	4.7	13.7	3.3	12.3
5	17.0	8.7	8.3	8.3	17.0	8.2	16.9	7.4	16.1	6.3	15.0	4.9	13.6	3.4	12.1
6	17.0	7.8	9.2	9.2	17.0	9.2	16.9	8.2	16.0	7.0	14.8	5.4	13.2	3.8	11.6
7	17.0	3.0	14.0	14.0	17.0	13.9	16.9	12.5	15.5	10.7	13.7	8.3	11.2	5.8	8.7
8	17.0	8.1	8.9	8.9	17.0	8.9	16.9	8.0	16.0	6.8	14.9	5.3	13.3	3.7	11.7
9	17.0	6.8	10.2	10.2	17.0	10.1	16.9	9.1	15.9	7.8	14.6	6.0	12.8	4.2	11.0
10	17.0	6.8	10.2	10.2	17.0	10.1	16.9	9.1	15.9	7.7	14.6	6.0	12.8	4.2	11.0
11	17.0	5.6	11.4	11.4	17.0	11.3	16.9	10.2	15.8	8.7	14.3	6.7	12.3	4.7	10.3
12	17.0	4.1	12.9	12.9	17.0	12.8	16.9	11.5	15.6	9.8	13.9	7.6	11.7	5.3	9.4
13	17.0	7.1	9.9	9.9	17.0	9.8	16.9	8.8	15.9	7.5	14.7	5.8	12.9	4.1	11.2
14	17.0	6.1	10.9	10.9	17.0	10.8	16.9	9.7	15.8	8.3	14.4	6.4	12.5	4.4	10.6
15	17.0	7.3	9.7	9.7	17.0	9.7	16.9	8.7	15.9	7.4	14.7	5.7	13.0	4.0	11.3
16	17.0	7.7	9.3	9.3	17.0	9.2	16.9	8.3	16.0	7.1	14.8	5.5	13.2	3.8	11.5
17	17.0	4.6	12.4	12.4	17.0	12.3	16.9	11.1	15.7	9.5	14.1	7.3	11.9	5.1	9.7
18	17.0	8.9	8.1	8.1	17.0	8.0	16.9	7.2	16.1	6.1	15.1	4.8	13.7	3.3	12.2
19	17.0	8.8	8.2	8.2	17.0	8.1	16.9	7.3	16.1	6.3	15.1	4.8	13.6	3.4	12.2
Averaged DLI Received mol·m^{-2}·d^{-1}				10.0	17.0	9.9	16.9	8.9	15.9	7.6	14.6	5.9	12.9	4.1	11.1

Table 4.2: Potassium (K) nutrient formulation for the three K treatments of 200, 400, and 600 mg·L^{-1} K using KNO_3^-.

Total K mg·L^{-1}	N mg·L^{-1}	NO_3^- mg·L^{-1}	NH_4^+ mg·L^{-1}	P mg·L^{-1}	K mg·L^{-1}	Ca mg·L^{-1}	Mg mg·L^{-1}	S mg·L^{-1}	B mg·L^{-1}	Cu mg·L^{-1}	Fe mg·L^{-1}	Mn mg·L^{-1}	Mo mg·L^{-1}	Zn mg·L^{-1}
200	149	139	10	31	200	24	32	0.25	1.8	0.02	1.8	0.1	0.02	0.2
400	212	202	10	31	400	24	32	0.25	1.8	0.02	1.8	0.1	0.02	0.2
600	283	274	10	31	600	24	32	0.25	1.8	0.02	1.8	0.1	0.02	0.2

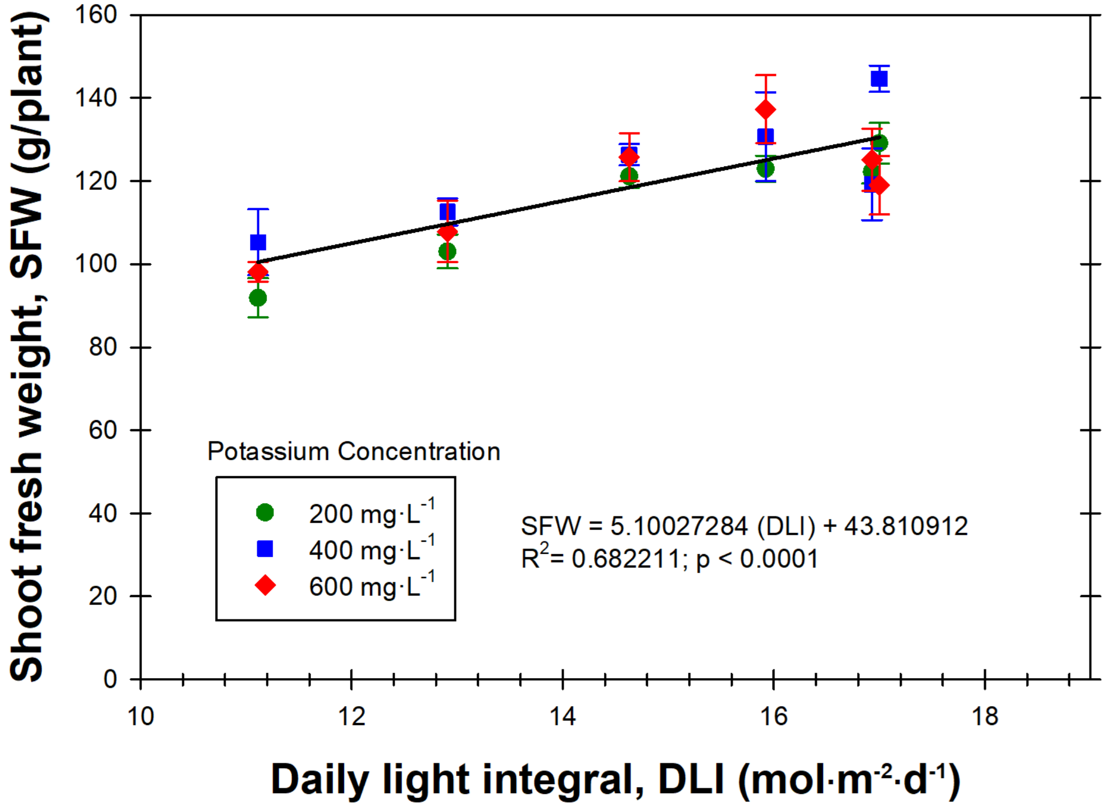


Figure 4.2: Shoot fresh weight (SFW) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

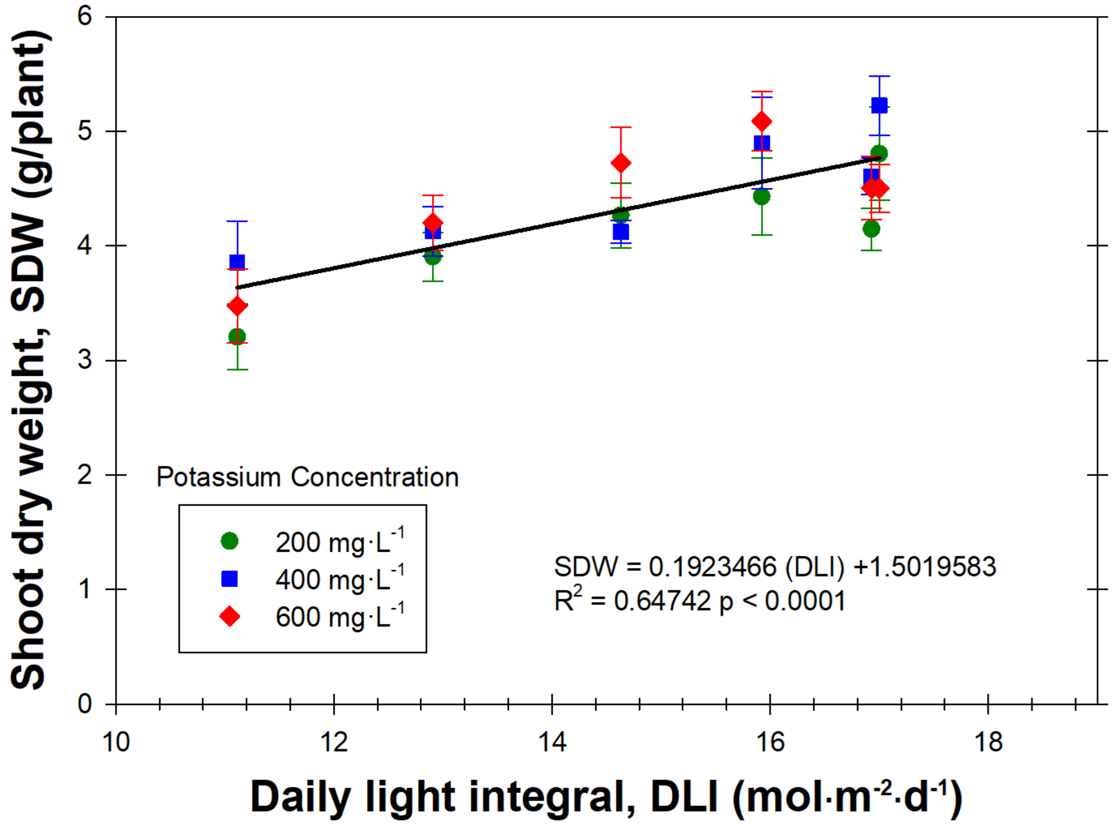


Figure 4.3: Shoot dry weight (SDW) of lettuce (*Lactuca sativa* ‘Casey’) plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

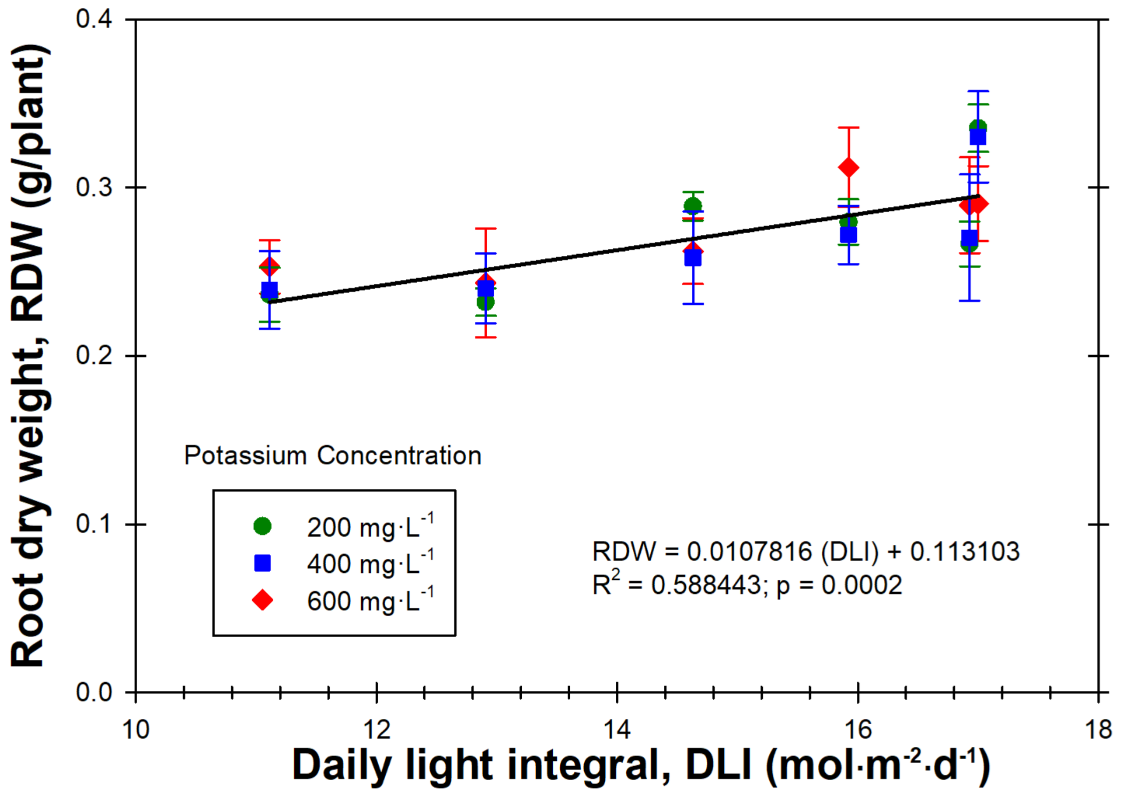


Figure 4.4: Root dry weight (RDW) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

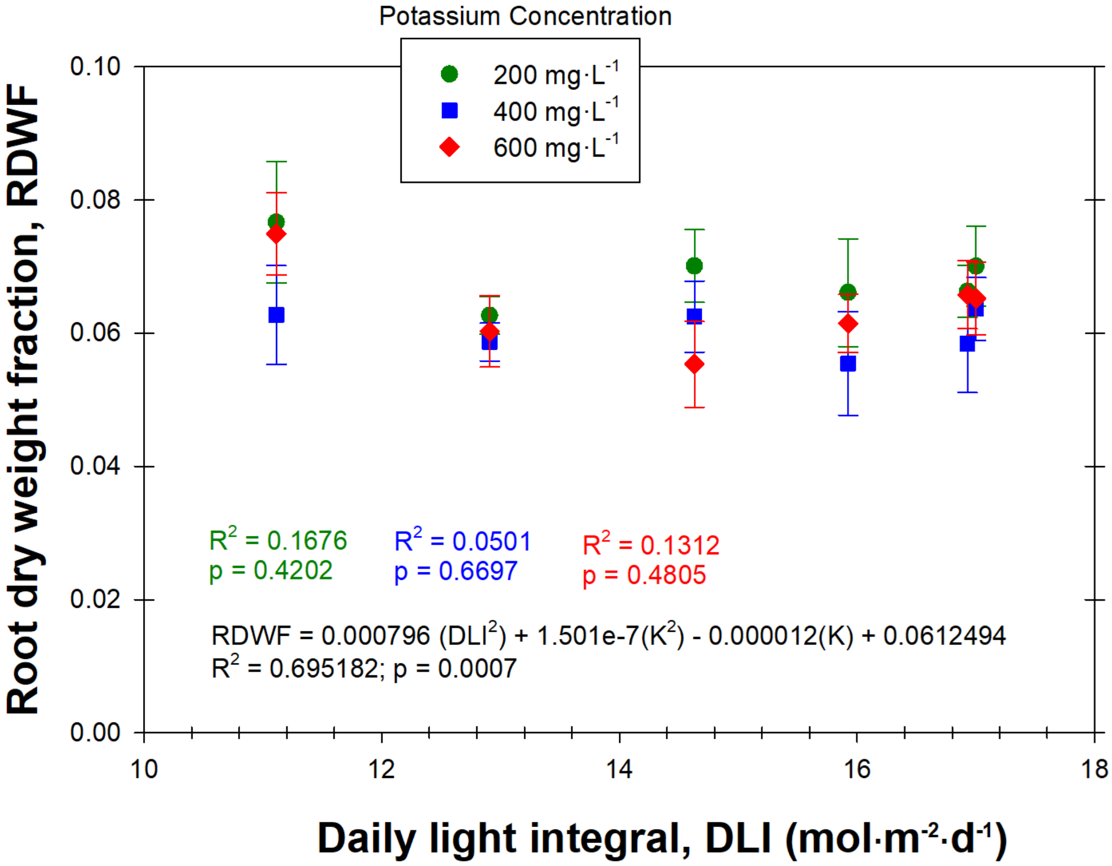


Figure 4.5: Root dry weight fraction (RDWF) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Results from multiple regression analyses indicated no significant DLI × K interaction ($p > 0.05$) and lines are not presented. Each data point represents the average of three plants. RDWF was calculated by dividing RDW from total plant dry weight. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

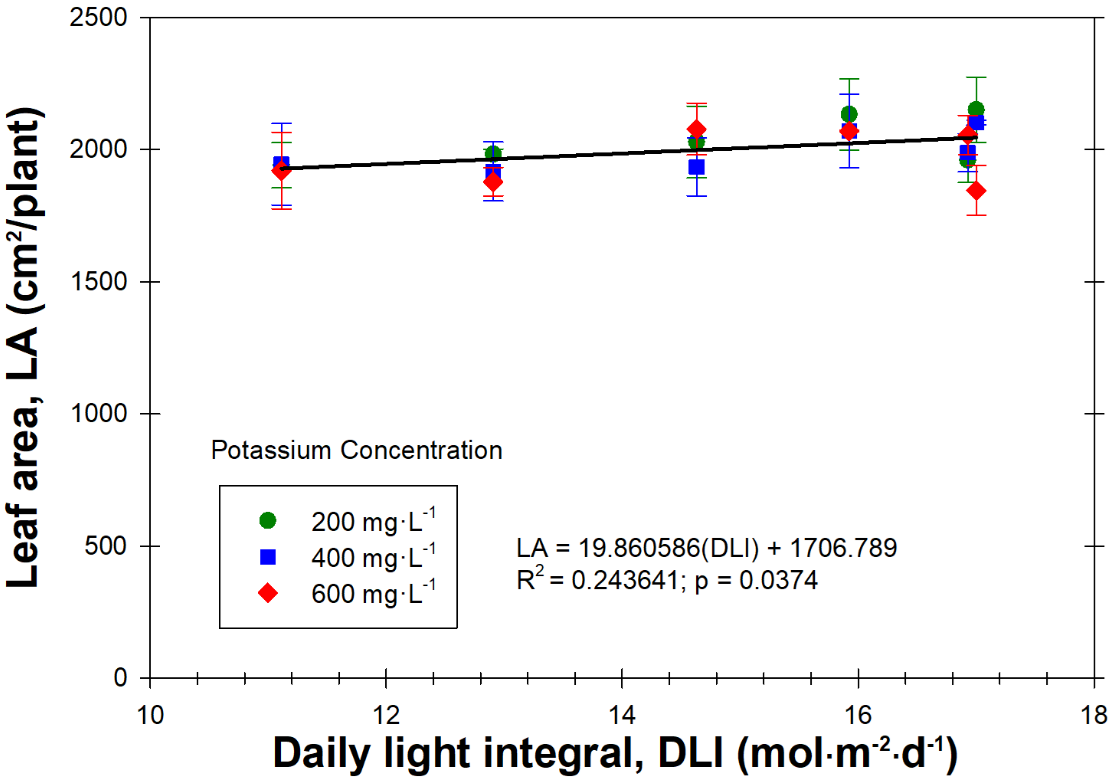


Figure 4.6: Leaf area (LA) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

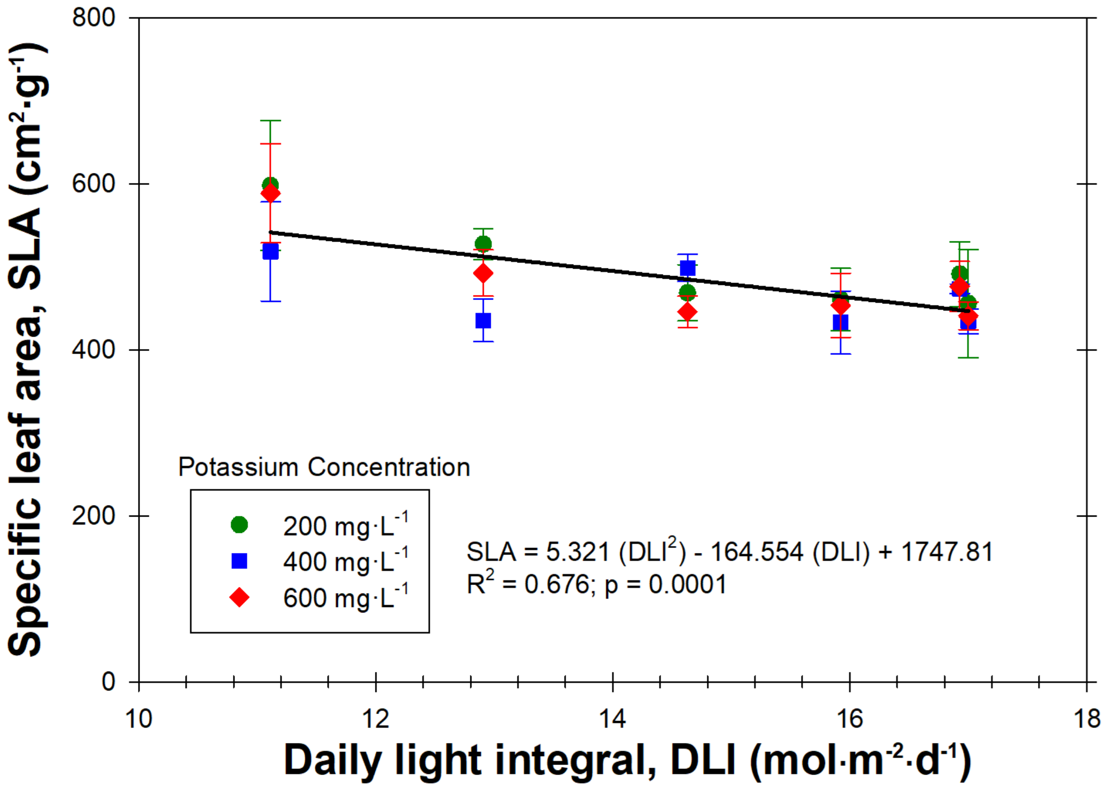


Figure 4.7: Specific leaf area (SLA) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. SLA was calculated by dividing LA by SDW. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

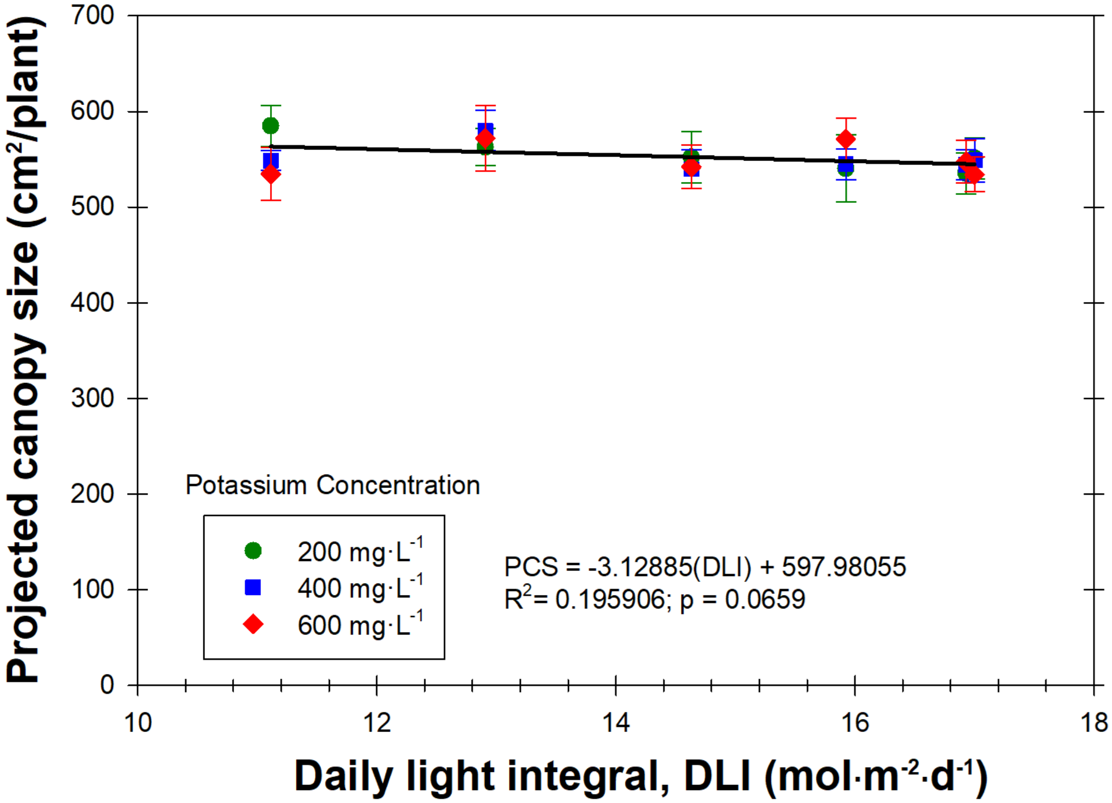


Figure 4.8: Projected canopy size (PCS) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Line shows the results from multiple regression analyses, which indicated only a significant DLI interaction. K and DLI × K interaction were not significant. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

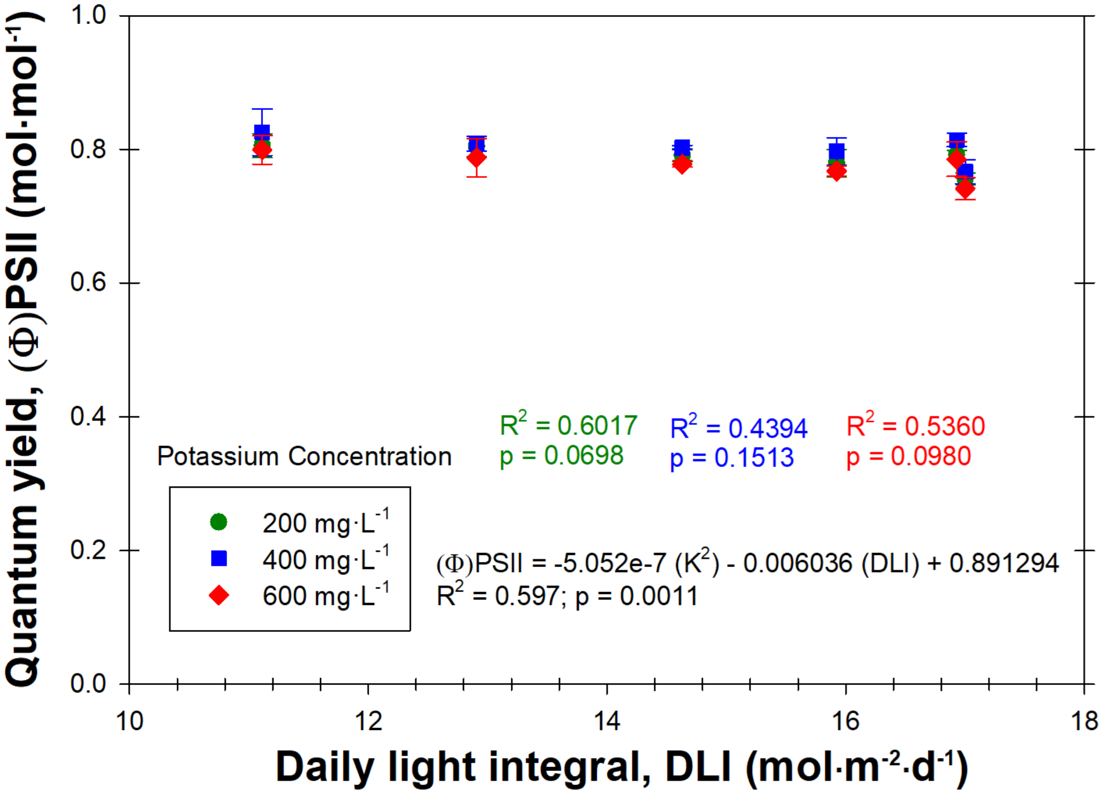


Figure 4.9: Quantum yield of photosystem II (Φ_{PSII}) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Results from multiple regression analyses indicated no significant DLI × K interaction ($p > 0.05$) and lines are not presented. Each data point represents the average of three plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

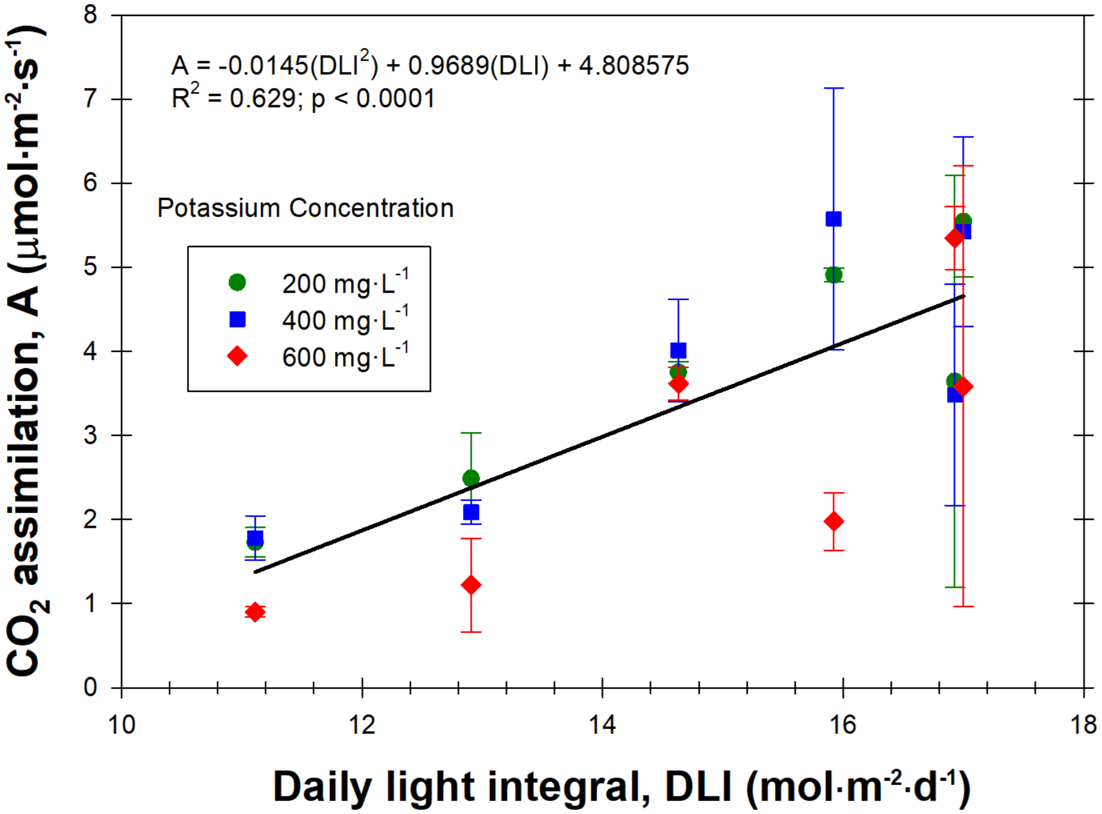


Figure 4.10: Net CO_2 assimilation rate (A) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Black line show the results from multiple regression analyses, which indicated significant DLI effect. K and DLI × K interaction were not significant. Each data point represents the average of three plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

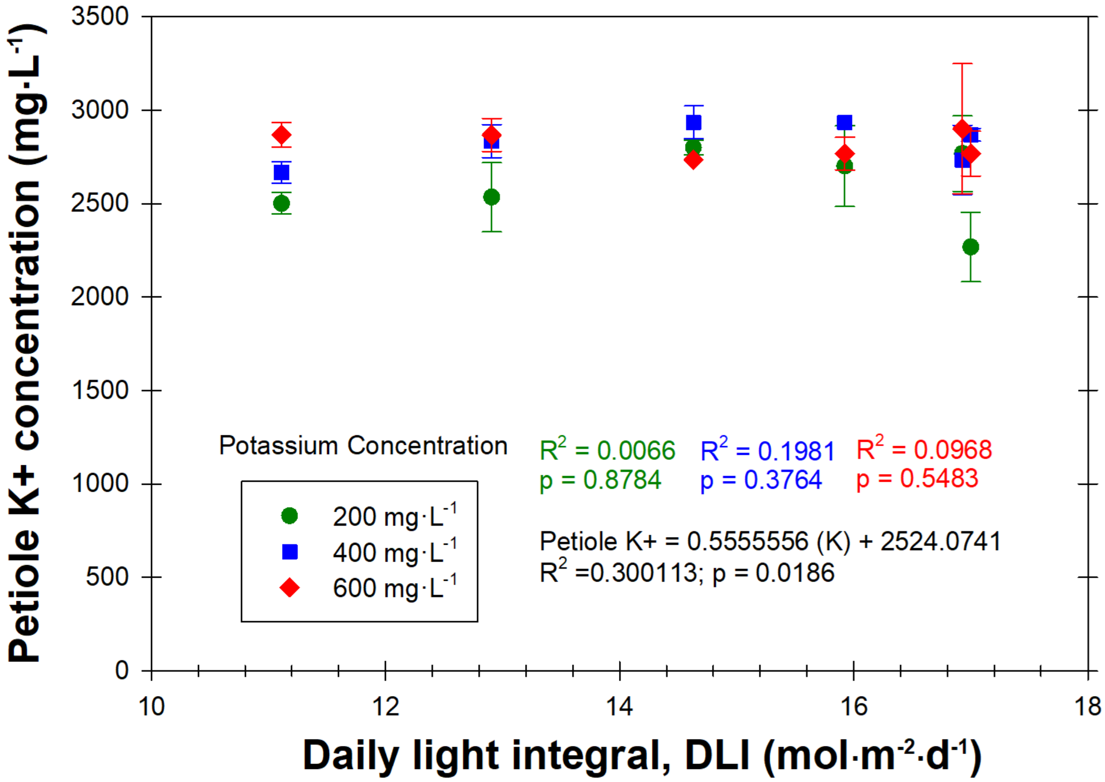


Figure 4.11: Petiole K + concentration of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Results from multiple regression analyses indicated no significant DLI × K interaction (p > 0.05) and lines are not presented. Each data point represents the average of three plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

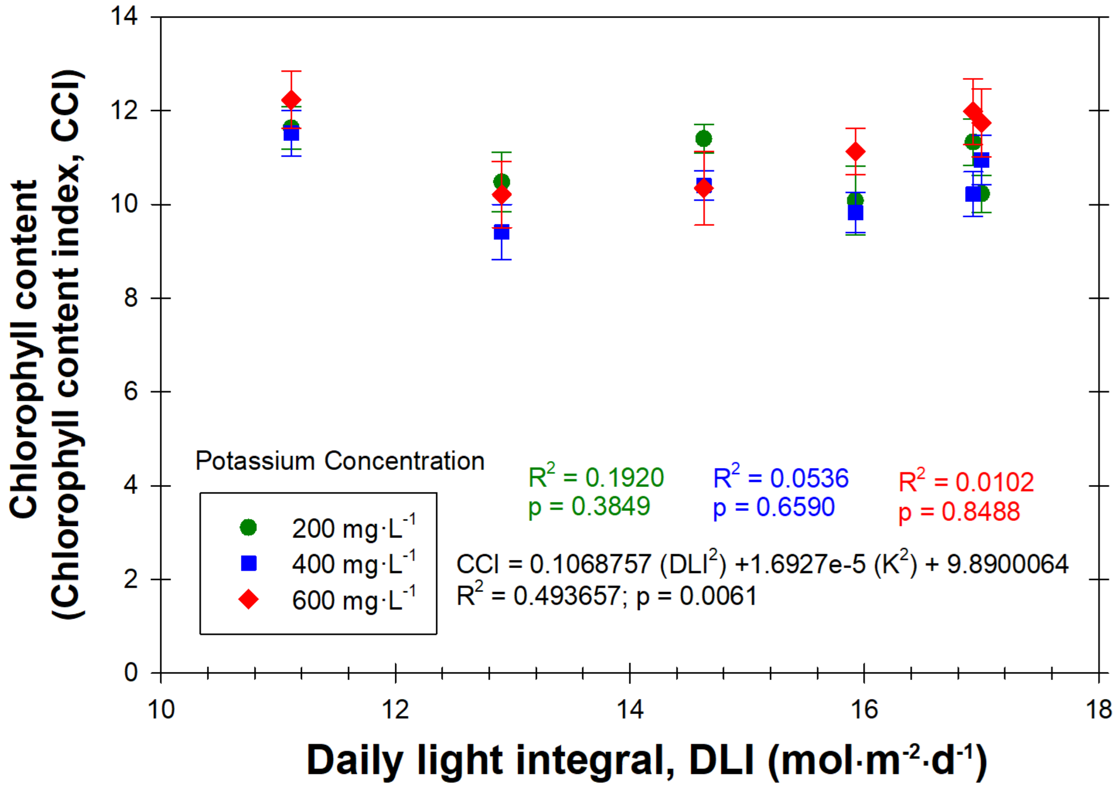


Figure 4.12: Chlorophyll content index (CCI) of lettuce (*Lactuca sativa* 'Casey') plants grown at six different daily light integrals (DLI) and three different K concentrations in hydroponics solution. Results from multiple regression analyses indicated no significant DLI × K interaction. Each data point represents the average of six plants. Datapoints and error bars indicate the average and standard error at each DLI × K concentration.

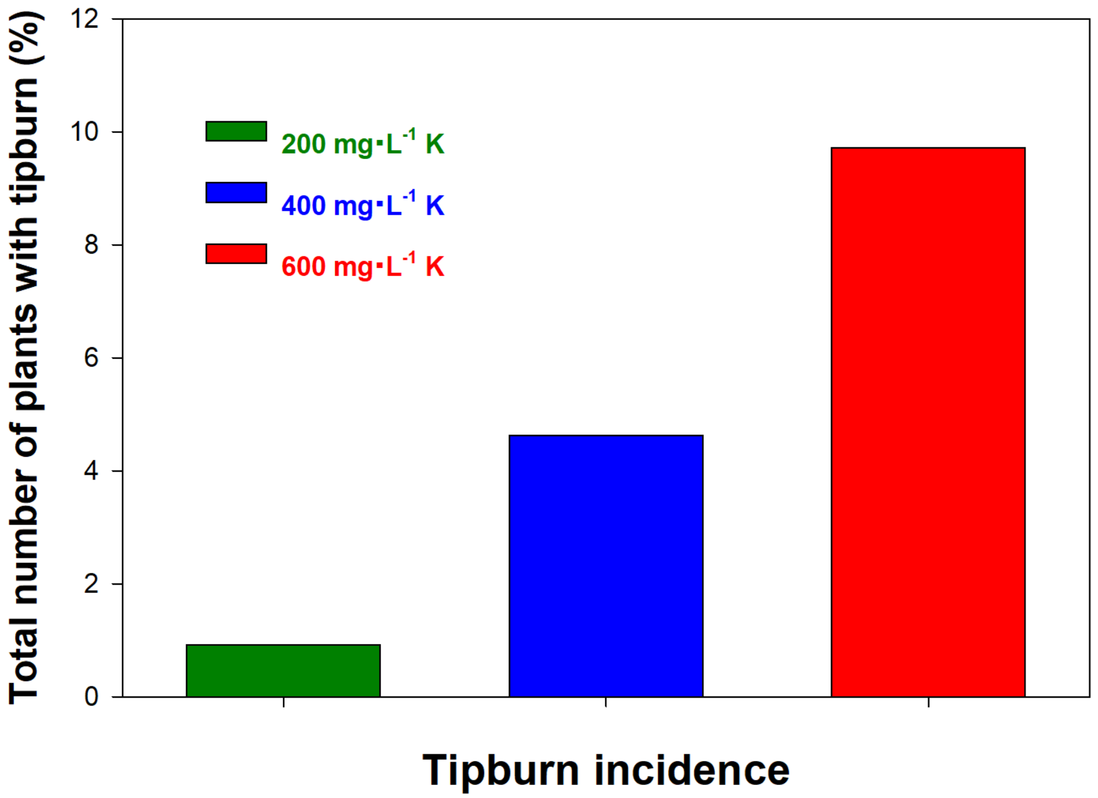


Figure 4.13: Tipburn incidence by K treatment. Each tipburn plant represents one plant with tipburn symptoms at the end of the study before the harvest. Each bar on the graph represents the percentage of plants with tipburn symptoms out of a total of 216 plants for each K treatment.

CHAPTER 5

CONCLUSIONS

Using projected canopy size (PCS) imaging, our findings illustrate that plant morphology grown under lower photosynthetic photon flux density (PPFD) levels (201 to 292 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increased their specific leaf area (SLA) and PCS to enhance light capture. At higher photosynthetic photon flux density (PPFD) levels (333 and 413 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plants experienced an increase in their canopy overlap ratio (COR). Simultaneously, under these elevated PPFD conditions, there was a corresponding increase in the total incident light (IL) plants received. Plants with the highest shoot dry weights were grown under higher PPFDs and as shoot dry weight increased, total IL increased. Both low and high PPFD conditions lead to a similar trend in PCS among plants which suggests that the total IL primarily relies on PPFD rather than PCS. Our findings suggest that light use efficiency (LUE) did not play a significant role in determining growth. Instead, the key factor was a strong and positive correlation between growth and total incident light the plants received.

The investigation of morphological and physiological responses in six lettuce cultivars, each with varying leaf anthocyanin content and grown under different supplemental PPFDs, revealed an interesting trend. Plant shoot dry weight increased for red lettuce cultivars, particularly in ‘Cherokee’ and ‘Rouxai,’ as PPFD levels increased. These two cultivars also displayed the highest leaf anthocyanin content, respectively. Our findings suggest that a higher leaf anthocyanin content may contribute to an increase in shoot dry weight in red cultivars with rising PPFD, although this effect was prominent only in the ‘Cherokee’ cultivar, which exhibited the highest shoot dry weight among all six cultivars, including our sole green cultivar, ‘Rex’.

Using an adaptive lighting control (ALC) system to reach a target daily light integral (DLI) we were able to better understand the effect supplemental potassium (K) has on lettuce growth. Our results suggest that the increase in K did not affect the shoot dry weight, leaf area, or specific leaf area of plants with increasing DLI. The increase in plant growth was due to the increase in DLI as evidenced by increases in shoot fresh weight, shoot dry weight, and leaf. While elevated K levels appear to have minimal or no beneficial effects, adverse outcomes, such as an increase in tipburn symptoms are observed with higher K fertilization. Ultimately this study suggests that K is not a useful way of increasing growth in hydroponic lettuce.

Collectively, these studies contribute valuable insights into optimizing lettuce growth, encompassing light manipulation, cultivar-specific responses, and nutrient dynamics. Future research should further explore the intricate interplay between PPFD, leaf anthocyanin variations, and nutrient availability to refine strategies for enhancing lettuce productivity in controlled environments to maximize profits for growers.