Food safety in hydroponic systems: Crop growth and pathogen reduction with physical and biocontrol interventions

by

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B.S., University of Arizona, 2020
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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
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Abstract

Hydroponic cultivation has the potential to increase the sustainability of vegetable production through intensive production of crops, and more efficient resource use than soil-based cultivation. However, the recirculating nutrient solution that increases water use efficiency in hydroponic systems also risks the rapid spread of contaminants such as bacterial human pathogens which survive in moist or wet environments. This dissertation explores sustainable methods of hydroponic nutrient solution treatment, using UV-C irradiation or competition from beneficial bacteria, in order to mitigate food safety risks associated with hydroponic leafy green production.

The first study investigated the survival of generic *Escherichia coli* in small-scale hydroponic systems growing romaine lettuce and assessed the efficacy of ultraviolet-C (UV-C) light treatment for decontamination. The UV treatments resulted in significant average reductions of *E. coli* in the nutrient solution. However, the *E. coli* population also declined naturally over the weeks following the inoculation, independent of the UV-C treatment. Survival of *E. coli* beyond one week is limited in the nutrient solution, when not considering its potential colonization of various surfaces within the hydroponic system. UV treatment has the potential to be used as a preventative safeguard on the microbial safety of the hydroponic production system.

Having established the efficacy of UV treatment for the reduction of human pathogens in hydroponic systems, the next step was to examine the impact of the same UV treatment on plant growth in the absence of human pathogens. Nutrient solution samples were collected and analyzed for macronutrients N, P, and K before and after UV treatments, and no significant differences were found. Plant growth was assessed through measurements of height, chlorophyll

fluorescence, and SPAD value, neither of which were found to differ significantly from control groups receiving no UV treatment. The research findings contribute to the potential use of UV light in hydroponic systems to have minimal impact on the plant growth of the crops grown in the systems.

While the UV treatment effectively reduced pathogens in the recirculating nutrient solution, random samples showed that bacterial counts were higher in samples of root tissue and growing media, indicating that bacterial colonies could survive on a variety of surfaces within the system despite the UV treatment. As a result, the next study explored an alternative treatment using a plant growth-promoting bacteria product containing *Enterobacter cloacae*, *Citrobacter freundii*, *Pseudomonas putida,* and *Comamonas testosteroni* as a biocontrol agent which was incorporated into the nutrient solution and circulated to reach all surfaces within a hydroponic system growing kale microgreens. Compared to UV treatments, there were no significant differences in the reduction of *E. coli* between the biocontrol and UV systems, though the UV system did significantly reduce *E. coli* compared to the controls. No significant differences were observed in plant height, fresh weight, or SPAD value. Overall, the results indicate that UV remains a more effective broad-spectrum microbial intervention for hydroponic nutrient solution, compared to the beneficial bacteria product evaluated here.

The next study compares the effects of two commercially available plant growth-promoting bacteria (PGPB) products on the growth of hydroponic kale microgreens. Parameters measured include height, chlorophylls a and b, carotenoids, and ascorbic acid content. While the *Pseudomonas putida*-based PGPB product had a higher average height, chlorophyll a, and chlorophyll b, it was not significantly different from the *Lactobacillus casei*-based PGPB product or the control group. While the PGPB products investigated show potential for improved

crop performance, further studies using additional products, bacterial strains, and hydroponic crops will be needed to better understand the broader impacts of PGPB on parameters of crop growth.

These studies collectively demonstrate that UV-C water treatments are a safe and sustainable option to add a preventative food safety intervention to the cultivation of hydroponic leafy greens. No significant impacts on plant growth resulted from the interventions tested, but the UV water treatments did result in significant pathogen reduction. While PGPB has the potential to reach interior surfaces within hydroponic systems that remain inaccessible to UV water treatments, more research is required to evaluate and optimize its use for the biocontrol of human pathogens. Overall, UV-C water treatments remain an effective pathogen reduction strategy for use in recirculating hydroponics systems, especially when applied early in production. It is possible that future studies could explore more effective biocontrol interventions which could be added in after a UV-C treatment in order to prevent additional pathogens from colonizing the system throughout the rest of the crop growth cycle. A combination of both methods may be able to combine their respective advantages, if a PGPB product with greater biocontrol potential is able to be identified and optimized for use in hydroponic systems.

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Chapter 1 - Literature Review

The global food supply chain is increasingly vulnerable to a range of disruptions, from pandemics to climate-related events. This trend highlights the fragility of current food production systems and the need for more resilient and sustainable agricultural methods. As the world population continues to rise, it is projected that food demand will increase significantly by 2050 (van Dijk et al., 2021), placing immense pressure on existing agricultural systems. This surge in demand, coupled with concerns over environmental sustainability, resource limitations, and supply chain bottlenecks, underscores the urgency for developing alternative food production systems.

1. Hydroponics as an alternative food production system

Hydroponics is the cultivation of plants without soil, relying on a water-based nutrient solution to deliver all essential plant nutrients directly to plant roots. Hydroponics and other controlled-environment agriculture (CEA) systems have emerged as promising alternatives to traditional soil-based farming, offering the potential to produce food year-round with greater efficiency and fewer natural resource inputs (Barbosa et al., 2015). These systems reduce dependency on arable land and enable crop production in urban or otherwise non-arable regions (Gómez et al., 2019). However, several challenges remain and limit the efficacy of these systems. Leafy green vegetables account for the most cases of foodborne illness associated with fresh produce in the US (CDC, 2025), and they are also the second largest category of crops produced hydroponically (Le, Valerie, 2024). Recirculating hydroponic systems are particularly susceptible to microbial contamination due to the re-use of nutrient solutions, and ensuring the microbial safety of produce grown in these environments remains a critical concern. While hydroponic systems are most commonly in enclosed environments and therefore seen as safer

relative to traditional soil-based farms which are open to a variety of sources of contamination, contamination can still occur in hydroponic systems; such contamination could potentially be introduced by the inputs such as growing media, seeds, or nutrients (Schoch, 2022). Moreover, maintaining postharvest quality and minimizing cross-contamination during handling and storage continues to be a persistent issue in both conventional and soilless production models.

Drought conditions in the western US make water a scarce and valuable resource, and farms often find themselves competing with cities for the allocation of fresh water. A study comparing conventional outdoor lettuce cultivation to hydroponic cultivation in Arizona found that hydroponic cultivation requires on average 13 times less water to produce the same yield (Barbosa et al., 2015). Since that study was conducted in a particularly arid environment, it is possible that gains in water use efficiency may be lower in other regions. Still, this is one example of how the controlled addition of inputs (whether water or nutrients) can lead to greater resource use efficiency in hydroponic production, making it a sustainable option for intensive crop production. Despite the efficiencies offered by hydroponic production, high input costs like physical capital and high operational costs of labor and energy constrain the ability of hydroponic farms to compete with the profitability of traditional farms. Still, the US hydroponic crop market has been growing at a rate of 3.2% over the past five years and has an estimated revenue of $961.8 million for 2025 (Le, Valerie, 2024). Leafy greens including lettuce and fresh herbs are the second most common category of crops (following tomatoes) grown hydroponically in the US, accounting for approximately 20% of total hydroponic production by revenue (Le, Valerie, 2024).

2. Food Safety Concerns in Hydroponics

In 2021, an outbreak of *Salmonella* in packaged hydroponic salad greens led to 31 reported cases of illness and 4 hospitalizations (CDC, 2021). The *Salmonella* outbreak is the only one to date that is directly linked to hydroponic lettuce production but in the lettuce industry as a whole *E. coli*, including STECs, O157:H7, and other pathogenic strains is one of the biggest microbiological food safety concerns (Barril et al., 2022). Additionally, sprouts are often regarded as one of the most risky crops from a food safety perspective, due to the high number of outbreaks they have experienced in recent decades (Taormina, Peter J., 2024). Microgreens provide a generally safer alternative to sprouts, having the same crop characteristics but differing in cultivation method. Microgreens can be grown hydroponically and with ample air circulation, as opposed to the moist, enclosed sprouting drums used for sprouts (Xiao et al., 2015). Yet as with lettuce, this seemingly safer alternative has food safety risks as well, evidenced by the fact that *Listeria monocytogenes* has been detected in several instances on microgreens between 2018-2020 (Topalcengiz et al., 2024).

The environment around plant roots provides optimal conditions for many bacterial species (Vlasselaer et al., 2024), including but not limited to pathogens. This is evident in traditional soil-based cultivation as well as hydroponics, though factors such as pH and temperature of the nutrient solution can influence bacterial survival. The pH of hydroponic nutrient solution was found to have a significant impact on bacterial counts for *Salmonella enteritidis*; higher counts were observed at pHs 8 and 7 compared to 6 and 5 (Xylia et al., 2021), because the acidic environment inhibits growth of the pathogen. While it is possible to maintain pH at a slightly lower level that is still optimal for plants and may slightly inhibit bacterial growth, there is an unavoidable overlap between optimal nutrient solution temperatures for

plants and pathogens, which often is a factor contributing to the spread of plant pathogens in greenhouse hydroponic systems (Aini et al., 2024; Vargas et al., 2021).

3. Role of nutrient solution in hydroponic contamination

Water used in hydroponics commonly goes through extensive filtration and treatment. The large CEA company that was impacted by the 2021 *Salmonella* outbreak used municipal water that passed through a four-stage filtration system and was treated with UV light before being introduced to the growing reservoirs. However, the Food and Drug Administration (FDA) investigation report notes that "Once in the growing ponds, the water is not routinely disinfected or otherwise treated" (U.S. Food and Drug Administration, 2021). The FDA has recommended "operating and maintaining production ponds in a manner that does not result in the spread of pathogens to product" as a way to minimize the sources of microbial contamination in hydroponic leafy green production (U.S. Food and Drug Administration, 2021).

The plant microbiome consists of the various plant-associated microorganisms colonizing the plant itself or the area immediately surrounding the plant. The rhizospheric microbiome is often home to a range of microbes including (but not limited to) mycorrhizal fungi and bacterial species engaged in symbiotic exchanges with the root system of the host plant. Many microbes are attracted to and naturally colonize the area around plant roots, which are a source of carbon and other exudates that can serve as nutrient sources for bacteria.

Multiple vectors could lead to the contamination of hydroponically grown fresh produce, but nutrient solution is recognized as one of the primary dispersal mechanisms through which contamination comes into contact with produce (Xylia et al., 2021). Water is unlikely to be the source of this contamination, as many hydroponic farms utilize municipal water. The more likely sources of contamination are inputs such as growing media, particularly peat moss (Dankwa et

al., 2020), or seeds (Bergšpica et al., 2020). Once introduced to the system, pathogens can spread throughout recirculating nutrient solution and easily contact plants. The presence of plant pathogens has been linked with the presence, survival, and growth of foodborne pathogens on vegetables (Xylia et al., 2021). In one example, when vegetable plants were damaged by plant pathogens, the sugar exudates that leaked from the damaged tissues were suggested to serve as a rich nutrient source, enabling the growth and survival of human pathogens in the *Enterobacteriaceae* family (Wang et al., 2020).

FDA investigations into past outbreaks have also identified growth media (used for growing plants) not being properly protected from contamination sources, harvest machinery with leaking water lines and condensation build-up, an inability to exclude leaves that have contacted hydroponic nutrient solution, postharvest cooling procedures, nearby stormwater retention ponds with the potential to infiltrate the CEA facility, and inadequate cleaning and sanitation of equipment as potential sources of contamination (U.S. Food and Drug Administration, 2021).

4. Internalization of pathogens in the plants

Foodborne illness occurs when pathogens are ingested, and in the case of fresh produce, there is a concern that pathogens may internalize (enter the plant tissue rather than only being present on the surface). Studies have found that *E. coli* O157:H7 is able to internalize via the roots of intact hydroponic spinach plants and reach stem and leaf tissues (Macarisin et al., 2014). Internalization is of particular concern for production of leafy greens because postharvest decontamination steps will not be able to reach pathogens inside the plant tissue, and no kill step may be applied before consumption if such produce is uncooked. However, it is recognized that internalization is more common with injury to the roots, and in experimental settings, more

damage and more internalization were seen in plants grown in soil than in plants grown hydroponically (Macarisin et al., 2014). A study using *Salmonella enteritidis* found that internalization was more common in young hydroponic lettuce plants compared to older ones, and higher bacterial populations also resulted in higher frequencies of internalization (Xylia et al., 2021). It was also observed that nutrient solution pH values of 6, 7, and 8 corresponded to more internalization than a pH value of 5. Other studies have found that despite contamination in the water of hydroponic systems with Shiga-toxigenic *Escherichia coli* (STEC), there was no evidence of internalization into spinach, basil, or tomato plants (Wang et al., 2020).

5. UV light technology as a potential intervention to protect the safety of hydroponic systems

Since contamination introduced early in production can potentially exist both on and within plants, food safety approaches must focus on reducing chances for contamination to be introduced during production. Several treatments could be used to keep the water safe (chemical and non-thermal methods). UV-C irradiation is one technology with potential applications for both of these approaches. UV-C light is a suitable treatment for hydroponic water solutions because, unlike chemical disinfectants, it does not alter the pH or electrical conductivity (EC) of the solution. Maintaining stable pH and EC is critical for optimal nutrient availability and uptake in hydroponic systems. Thus, UV treatment offers an effective non-chemical disinfection method that preserves the chemical properties essential for plant health. It has also been used to treat the surface of fresh produce as a postharvest treatment and has been effective for crops such as blueberries, which have a relatively smooth and uniform surface which does not significantly shield pathogens from UV (Haley et al., 2023). However, postharvest UV light treatment is not ideal for crops such as romaine lettuce, which has a textured and layered surface that could

shield a variety of contaminants from irradiation. For such crops with complex physiological characteristics, postharvest interventions alone do not represent a proactive or legally recommended food safety strategy. The FDA has established guidelines that prioritize growers following good agricultural practices (GAPs) and avoiding any actions that could lead to harvesting and distributing contaminated produce (FSMA Final Rule on Produce Safety, 2024). It is key to incorporate food safety practices into every stage of cultivation, in an effort to prevent contamination from being introduced, rather than hoping to remove or inactivate it once it is in the system.

By treating the recirculating nutrient solution in hydroponic systems, it is possible to target the route through which it is predicted that hydroponic contamination is spread, ideally before it comes into contact with plant tissue. UV-C (at 254nm) treatment of water is most effective when performed at a 254nm wavelength (Haley, 2023), and it is more germicidal than UV-B (280-315nm) or UV-A (315 – 400nm) irradiation, which represent higher wavelengths. UV light is generated by gas discharge as excited electrons jump to a higher energy state and emit energy in the form of light (photons) as they return to the lower energy state. UV light is considered a physical decontamination method where mutagenesis is induced with the transfer of photochemical energy from photons to the nucleic acids within an organism, ultimately leading to cell death (Koutchma, T., 2019). The photochemical reaction is induced by wavelengths between 200 to 300 nm and proceeds with base hydration or the cycloaddition of pyrimidine or purine dimers between adjacent nucleotide bases on the DNA or RNA backbone (Patras et al., 2021). Such point mutations, also called DNA lesions, block DNA/RNA replication and transcription or lead to miscoding, eventually resulting in cell inactivation or death (Durbeej & Eriksson, 2002).

In order for a UV water treatment to be effective, it must be able to sufficiently penetrate the water being treated. This is usually stipulated as a required range of UV transmittance for which the device has been validated, and it is often over 80% (Atlantic Ultraviolet, 2022), meaning the water should be clear enough that 80% of UV irradiation would pass through it. Higher levels of turbidity, caused by nutrients, algae, or other microorganisms in hydroponic nutrient solution, could reduce the effectiveness of UV treatments.

While applications for human pathogen reduction in hydroponics have not been well studied, UV technology has been utilized to reduce plant pathogens which present a major threat to hydroponic operations. A higher UV dose has typically been required to inactivate the same portion of plant pathogens in hydroponic nutrient solution compared to the dose required to inactivate the same pathogens in water (Sutton et al., 2000). This was believed to be due to the higher absorbance in the solution, and the effect was more pronounced in nutrient solution taken from systems in use growing crops than in fresh batches of nutrient solution. A periodic UV-C treatment applied once weekly was shown to reduce plant pathogens by up to four log CFU (Lee et al., 2015), indicating that even infrequent treatments can be quite effective. Studies such as these set the precedent for the use of UV in hydroponic systems, enabling future research to expand and refine its applications.

UV dose is measured in millijoules per square centimeter (mJ/cm^2), and the dose increases with contact time (for the same UV irradiance). This means that in a flow-through treatment, a slower flow rate will result in a longer contact time, and thus a higher delivered dose (Patras et al., 2021). In other words, the target organism receives more UV photon energy at a slower flow rate as it is exposed to light for a longer time as compared to the faster flow rate. Also, it is important to understand that all human pathogens have defined UV sensitivity for

inactivation. The UV sensitivity of an organism is a measure of the UV dose required to inactivate it by a specified amount. If no direct measure of UV dose is available, it is possible to use the known UV sensitivity to estimate the reduction equivalent dose (RED) delivered to an organism, based on observed reductions (Patras et al., 2021). A UV dose in the range of 9.5 to 11 mJ/cm^2 is usually adequate to provide a 5-log reduction in *E. coli,* while higher doses are required for pathogens like *Salmonella* or *Listeria monocytogenes* (Patras et al., 2021).

Similar to the way UV-C irradiation disrupts the covalent bonds between pyrimidine bases in DNA, UV-C irradiation can also alter molecular chemical bonds through photochemical dissociation via excitation of electrons (Schwartzkopf et al., 1987). This phenomenon has been attributed to disruptions of nutrient levels in water that has been UV treated, including a reduction of iron in hydroponic nutrient solution (Schwartzkopf et al., 1987), and the commonly seen reduction of nitrate into nitrite in wastewater treatment (Thorn & Cox, 2012). The toxic effect of nitrite on plants and the chlorosis resulting from iron deficiency are well known, but it is unclear what potential impacts additional nutrient disruptions may have on plant growth, and thus further exploration is warranted in order to determine the suitability of UV treatments for hydroponic nutrient solution.

Additional challenges that UV treatments may face in a hydroponic setting are related to the cost of UV equipment, biofilm formation, and potential pathogen sheltering depending on treatment design. Any UV device directly integrated into a hydroponic reservoir would face the issue of not being able to affect plant tissues, hydroponic components and/or substrates, which could shelter pathogens and render the treatment ineffective as well as potentially harmful to the roots (Schwartzkopf et al., 1987). One approach to avoid this involves only treating the nutrient solution, by removing it from the system in the treatment process. However, this approach does

not account for bacterial attachment to plant roots, substrate, or other surfaces within the system, where colonies could form biofilms or otherwise survive only to proliferate after treatments.

It is possible for *E. coli* to attempt to overcome damage to DNA inflicted by UV irradiation through the activation of the SOS pathway, which enables continued DNA transcription with higher chances of error due to circumventing damaged strands (Maslowska et al., 2019). This kind of repair is likely to be utilized following UV-A or UV-B irradiation, but it is possible that bacterial populations could experience a resurgence following any UV treatment due to the activation of this pathway in surviving bacterial cells. While the cell functions that could be disrupted due to DNA transcription errors are difficult to predict, one study has found that some of the antimicrobial agents that induce the SOS pathway in Shiga toxin-producing *E. coli* also induced the expression of genes responsible for producing toxins (Kimmitt et al., 2000). This underscores the necessity to fully eradicate bacterial cells due to the possibility that once damaged, *E. coli* populations could not only experience a resurgence, but that their harmful effects on humans could potentially be amplified.

Potential applications of UV technology in hydroponics. UV treatment has the potential to be an effective and sustainable treatment that could be applied to nutrient solution during the production of hydroponic crops, such as lettuce and microgreens. Measurements of the transmittance of hydroponic nutrient solution can provide clarity on the potential effectiveness of UV treatments. It also is necessary to evaluate the impact that such treatments may have on nutrient concentrations, and plant growth, in order to determine if they are suited for this purpose. Other microbial interventions can be explored alongside UV that would be able to reach bacterial colonies attached to roots and other surfaces within the systems, and they will also need to be evaluated for impacts on plant growth. The overall goal of the Ph.D. project is to:

1) evaluate the efficacy of UV light technologies in reducing food safety risks in the hydroponic nutrient solution both for Deep Water Culture in Romaine Lettuce and Nutrient Film Technique in Microgreens and 2) determine its impact on the plant growth parameters.

Chapter 2 - Effect of Ultraviolet Water Treatment on Plant Physiology Parameters in Recirculating Hydroponic Systems

ABSTRACT

The application of ultraviolet-C (UV-C) light technologies in hydroponic production systems is gaining attention due to the potential concern of human pathogen introduction and survival in nutrient solution reservoirs. These reservoirs are rarely disinfected during or between production cycles. UV-C light treatment can be used to treat nutrient solution during the growth period and serve as a check on pathogen proliferation in the water. UV-C light has been effective at protecting the microbial safety of water systems; however, the impacts such treatments on the composition of nutrient solution and on plant physiology are underexplored.

In this study, romaine lettuce was grown in small-scale deep water culture hydroponic systems while nutrient solution was treated with UV-C light periodically during the crop growth period. Nutrient solution samples were collected and analyzed for macronutrients N, P, and K before and after UV-C treatments. Plant physiology was assessed through measurements of height, chlorophyll fluorescence, and SPAD value. The results indicated that performing a UV-C treatment (which was previously shown in Chapter 3 to effectively reduce surrogate human pathogens in recirculating hydroponic nutrient solution) has no significant impact on macronutrient levels of nutrient solution nor on the observed measures of plant growth. The research findings contribute to the potential use of UV-C light in hydroponic systems to protect safety with minimal impact on the nutritional quality of the plants grown in the systems.

INTRODUCTION

Ultraviolet light technology is an effective intervention to reduce pathogens in municipal and agricultural water (Haley, 2023) and could offer many advantages to hydroponic growers interested in a sustainable, highly effective water treatment which does not require the addition or monitoring of chemical inputs. UV-C water treatment is being explored for applications in hydroponic systems, which the Food and Drug Administration (FDA) has noted to pose a potential risk concern that hydroponic reservoirs are not routinely sanitized during or between crop growth cycles (U.S. Food and Drug Administration, 2021). Hydroponic nutrient solution is recognized as the primary route by which contamination is likely to be spread to plant tissues, as it is a very effective carrier of human pathogens once they are introduced to any part of a system (Dankwa et al., 2020). There is a need within the hydroponic industry for a food safety intervention which can be applied to recirculating water during the crop growth period. If contamination is introduced, an intervention that can be applied during crop growth could prevent the growth and spread of pathogens within the system.

The UV light spectrum is broadly divided into three categories: UV-A (315-400nm), UV-B (280-315nm) and UV-C (100-280nm). The antimicrobial efficacy of UV light is maximum at 254nm (UV-C) (Sesti-Costa et al., 2022) The microbiological efficacy of UV-C treatments of hydroponic nutrient solution has been validated, but in order to ensure the applicability of this technology it is crucial to confirm that plant growth is not negatively impacted by such treatments. Other treatments which have been studied for their potential applications during the hydroponic crop growth period, such as chemical interventions, have caused significant negative plant growth outcomes (Mensah, 2023). While UV-C disinfection is proven effective for a variety of applications, one study found evidence of morphological abnormalities in the roots of

plants grown in continuously UV-C-treated nutrient solution, as well as inhibited growth rates (Schwartzkopf et al., 1987). UV-C water treatments may have the potential to negatively impact crop growth through disruptions to the microbiome or photochemical dissociation of nutrients in the solution, underscoring the necessity of further exploring the relationship between these treatments and plant physiology parameters.

Pathogens represent only a small subset of the many microorganisms capable of colonizing the hydroponic rhizosphere, many of which are beneficial, neutral, or not fully understood in terms of their relationship with the plant (Vlasselaer et al., 2024). A robust hydroponic microbiome could have a plethora of benefits for crops, including shielding from plant and human pathogens or improved crop growth (Aini et al., 2024; Yedidia et al., 2001). It has been suggested that the reduction of bacteria in hydroponic nutrient solution may have a detrimental effect on plant growth when performed earlier in the plant growth stage, compared to a positive effect when performed in later weeks, though the implications of this observed trend are unclear (Schwartzkopf et al., 1987). Since UV-C treatments will target all microorganisms in the water indiscriminately, it is worth weighing the impact that losing the benefits of the microbiome could have on plant growth against the pathogen reduction provided by such treatments.

Compared to other kinds of UV radiation, which rely solely on the physical processes of membrane disruption and breaking single-stranded DNA, UV-C also involves a biochemical aspect. Through photochemical dissociation, also known as photolysis, UV-C irradiation can alter the chemical and physical properties of molecules when electrons become excited and able to break chemical bonds (Liu, Chao-Yun, 2019). For this reason, some speculate that UV-C water treatment within hydroponic systems may disrupt or alter macro or micro-nutrient levels.

A common concern about using ultraviolet treatment for wastewater is the potential for nitrate (NO3-) to be photolyzed into nitrite, which is highly toxic to plants; the presence of organic matter in water has also been suggested to catalyze the reduction of nitrate into forms less easily taken up by plant roots (Thorn & Cox, 2012).

Given the potential for disruptions to key plant nutrients that are vital to plant growth, this study opted to investigate changes in the three foremost plant macronutrients (nitrogen, phosphorus, and potassium) in water samples collected before and after UV-C treatments. A very early study in this field found that UV-C treatment of hydroponic nutrient solution did not significantly impact levels of potassium (K+) and nitrate in the water (Schwartzkopf et al., 1987). However, given the absence of prior literature on the effects on phosphorus, and the conflicting literature around impacts on nitrogen, evaluating these macronutrients in this study may be able to offer additional clarity.

In addition to measuring impacts on macronutrient levels, this study needed to efficiently quantify the potential impacts of UV-C water treatment on plant growth through non-destructive means throughout each trial; to accomplish this, the parameters assessed in this study were restricted to those that could be measured without removing or damaging plant tissue. Fv/Fm, a parameter of chlorophyll fluorescence, is the maximum quantum efficiency of photosystem II photochemistry (Baker, 2008). Any stress that causes inactivation damage or loss of efficiency to photosystem II will be reflected in a lower Fv/Fm value (Bartold & Kluczek, 2024; Murchie & Lawson, 2013) so it can be considered an indicator of stress. SPAD value is a measure of chlorophyll content, closely related to the nitrogen content of leaves (Xiong et al., 2015), and considered broadly to be an indicator of plant health. Fv/Fm and SPAD value considered along with plant height provide insight into the overall vitality and photosynthetic performance of the

lettuce plants, and any differences in these parameters may provide insight into which, if any, aspects of plant growth are impacted by UV-C nutrient solution treatment.

This study aimed to assess the impacts of UV-C nutrient solution treatment on the physiology of hydroponic crops by measuring the Fv/Fm, SPAD value, and plant height over time during the growth of lettuce plants grown in hydroponic systems with UV-C treated nutrient solution. It also sought to investigate the potential photochemical dissociation of key nutrients by measuring the levels of macronutrients nitrogen, phosphorus, and potassium in nutrient solution before and after each UV-C treatment. These parameters were chosen to provide insights into the impacts of integrated UV-C treatments during crop growth on overall lettuce health and physiology.

MATERIALS AND METHODS

Five replicate deep water culture hydroponic systems were assigned to each of the three treatment groups (control, three L/min UV, and six L/min UV) in a structured non-randomized arrangement for a total of fifteen systems used in each of the three repetitions of the six-week experiment. Systems were placed within eight hydroponic towers (Farm 48 and 24+ models, AeroGrow International, Inc., Boulder, Colorado) consisting of stackable frames with built-in LED light panels. All systems were housed indoors, within the Postharvest Physiology Laboratory at Kansas State University Olathe.

Hydroponic system reservoirs were filled with 8L of distilled (DI) water, and the volume was maintained with twice-weekly additions of DI water as needed throughout the growth period. The built-in, full-spectrum grow lights were set to a 13-hour photoperiod, with the target daily light integral (DLI) being 12 $mol/m^2/d$ as per the recommendation (Baker, 2008). The

lights were positioned 49 cm above the grow decks to maintain the DLI. Water pumps were tested to ensure proper functionality and flow of water throughout the systems.

Rockwool cubes were soaked for at least 15 minutes in distilled water before being placed in plastic baskets to fit in the holes in the systems' grow decks. Three lettuce (*Lactuca sativa*) 'Sparx Romaine' seeds (Johnny's Selected Seeds, Fairfield, Maine) were placed in each cube. Four cubes were then placed in each hydroponic system in an alternating pattern to maximize available space for lettuce plants as they grew, see Figure 2.1. Plants were designated with letters in order to record data at the plant level for each system.

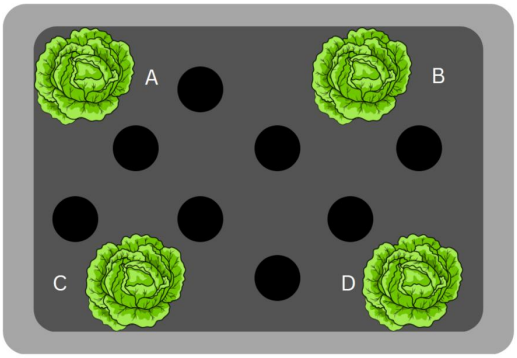

Figure 2.1 Lettuce plants were positioned in an alternating pattern within the grow deck of each system. Letters represent distinct observational units for data collection by plant.

Once approximately 80% of the seeds had germinated (on average day 8 of the experiment), an 8.88% stock solution of Hydro-Gro Leafy fertilizer containing 4.30% N, 9.30% P205, 35% K20, 3.9% Mg, 200 mg/kg B, 105 mg/kg Cu, 2100 mg/kg Fe, 190 mg/kg Mn, 42 mg/kg Mo, and 210 mg/kg Zn (CropKing Inc., Lodi, Ohio) and a 6.97% stock solution of calcium nitrate fertilizer containing 15.5% N and 19.0% Ca were then added to each reservoir until the electrical conductivity (EC) was between 1.0 and 1.2 mS/cm. The pH was adjusted to the range of 5.7-5.9 using 1% potassium hydroxide (to increase pH) and 1% sulfuric acid (to reduce pH). When true leaves emerged in 80% of the seedlings (on average day 16 of the

experiment), the EC was maintained to the range of 1.6-2.0 mS/cm by adding additional fertilizer. The EC and pH levels were adjusted twice per week and maintained throughout the rest of the lettuce growth period.

UV-C light treatments were performed three times over the six-week growth period on days 7, 21, and 35. The treatment was performed using a Minipure MIN-1 ultraviolet chamber (Atlantic Ultraviolet Corporation, Hauppauge, New York) connected via silicone tubing to a P12A peristaltic pump (Across International, Livingston, New Jersey). Systems were assigned to two treatment groups based on the flow rate used to perform the treatment, in addition to a control group in which systems had nutrient solution pumped out in the same process while the UV-C light remained off. A rate of six L/ minute corresponded to a comparatively low UV-C treatment level, and three L/ minute corresponded to a comparatively high treatment level in order to achieve a longer contact time as the nutrient solution passed through the UV-C chamber. The flow rates were determined based on the water volume to be treated (eight L) and the flow rate capacity of the UV-C device.

The nutrient solution was pumped out of each hydroponic reservoir at a rate corresponding to its designated treatment level, passed through the UV-C chamber for treatment, and ultimately deposited in a five-gallon bucket. This was to ensure the total volume of water in the reservoir would pass through the UV-C chamber (single pass), as opposed to experimental designs that rely on incorporating a UV-C treatment within the systems' built-in pump (recirculating treatment) (Giuseppina Pennisi et al., 2020). The direction of the pump was reversed, so the nutrient solution contained in the bucket was pumped back into its reservoir.

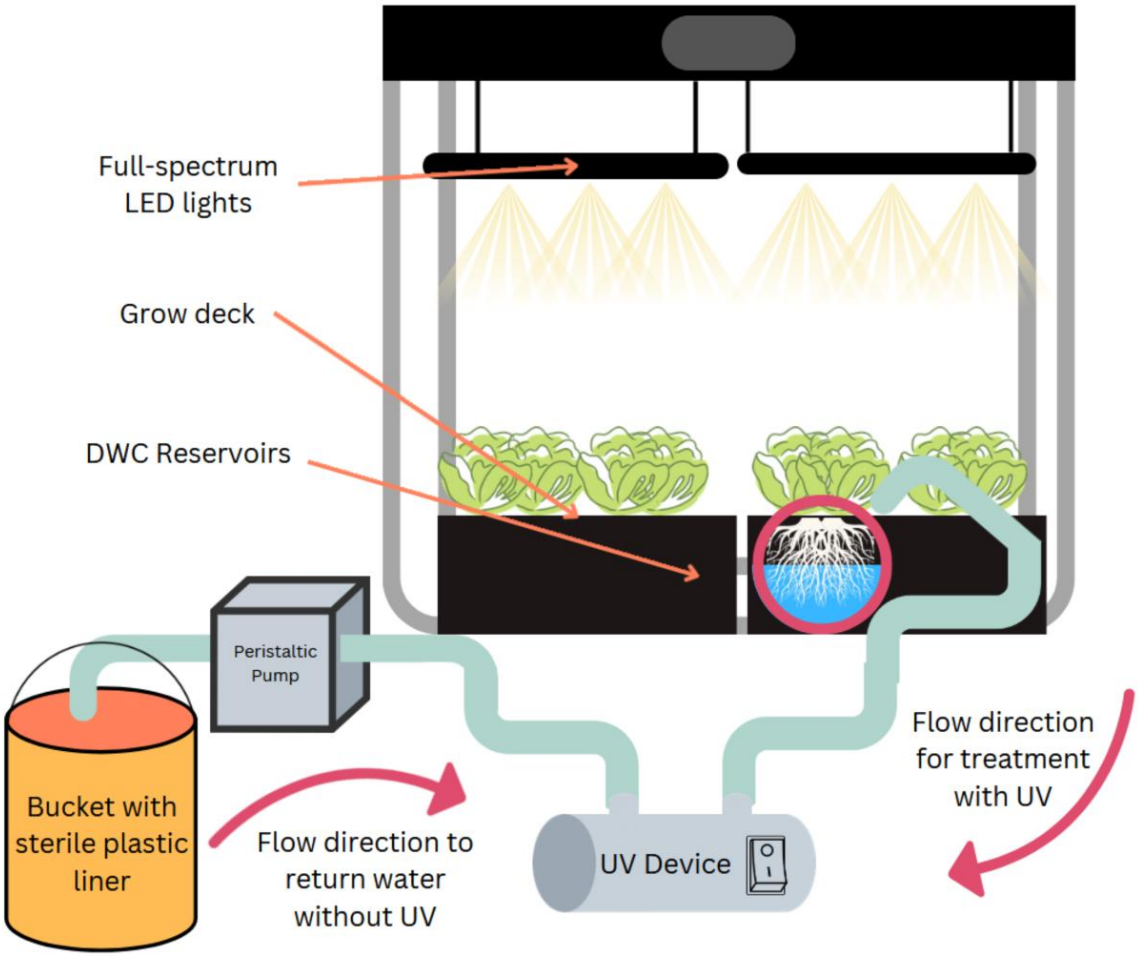

Figure 2.2 UV-C treatments were performed by pumping all nutrient solution out of the reservoir, through a UV-C chamber, and collecting it in a bucket lined with a sterile plastic bag. The UV-C device was then turned off, and nutrient solution was pumped back into the reservoir.

Before and after each UV-C treatment, a 50ml sample of nutrient solution was collected and sent to the Kansas State University Agronomy Soil Testing laboratory for analysis of the total nitrogen and phosphorous content by potassium persulfate digestion, and the potassium content by inductively coupled plasma atomic emission spectroscopy.

Plant growth parameters. Once seeds had germinated and were tall enough to be measured (approximately 10 days after germination), plant height was recorded twice per week. Height was recorded at the apex of the shoot. As soon as leaves were large enough to fill the

measurement area of the meter, the SPAD value was recorded twice per week using a chlorophyll meter (SPAD-502Plus, Konica Minolta Inc, Osaka, Japan). For each plant, a reading was taken from two leaves of approximately medium size and age, and the average of those two readings was recorded. When leaves were large enough to support the weight of the clips without being damaged, twice-weekly Fv/Fm measurements were taken with a handheld chlorophyll fluorometer (OS30p+, Opti-Sciences Inc., Hudson, New Hampshire).

Data analysis. A Kruskal-Wallis test was selected due to the distribution of residuals requiring a non-parametric test to evaluate statistical differences between UV-C treatment groups for dependent variables height, SPAD value, chlorophyll fluorescence, and nutrient solution total nitrogen, total phosphorus, and potassium. A Levene's test was performed first, which verified a sufficient homogeneity of variance in all variables to proceed with the Kruskal-Wallis test. Data was analyzed using R version 4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Height was recorded in cm from approximately day 26 until the conclusion of each trial, providing approximately 96 data points per system across three repetitions of the experiment. There was no statistically significant difference in average heights of plants by treatment group ($P > 0.05$).

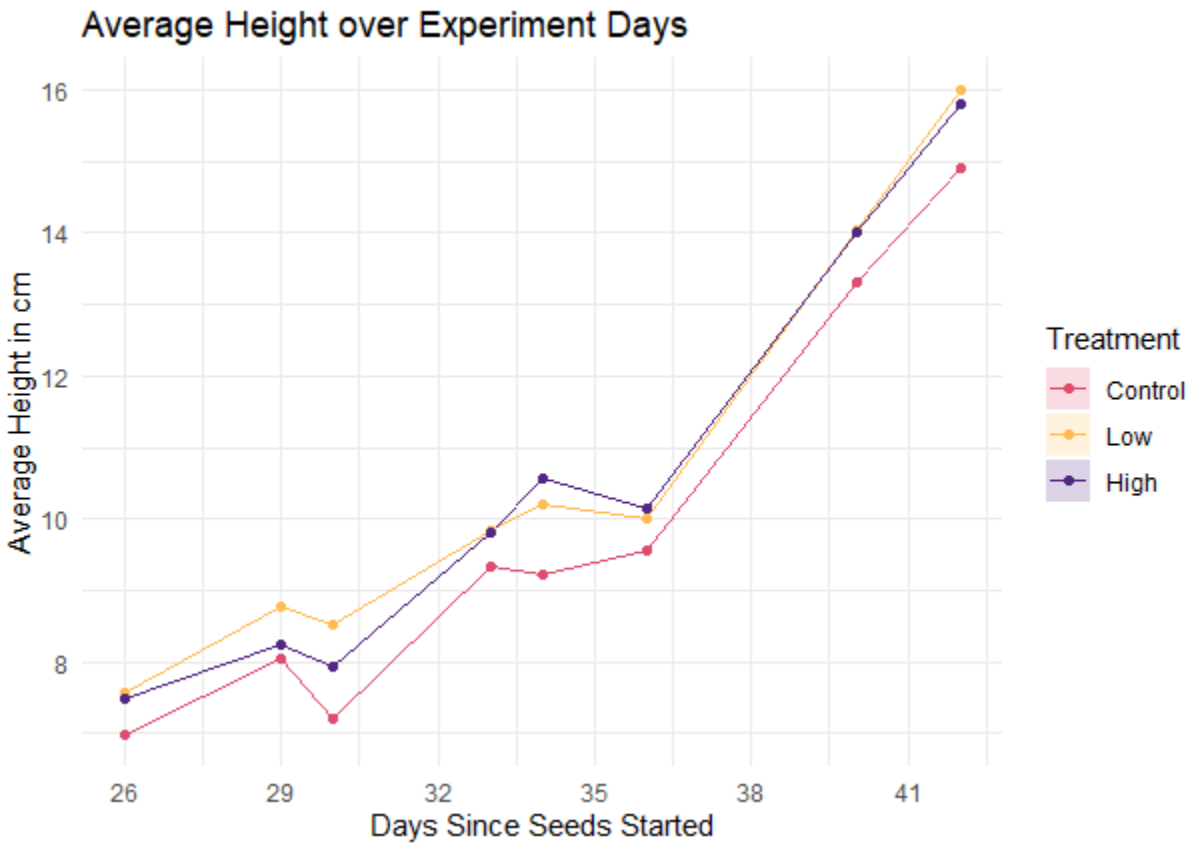

Figure 2.3 Lettuce height, measured in cm twice per week during the six-week growth period, increased over time with no differences between treatment groups.

There were no observed differences in average SPAD value among the three treatments (control, low UV dose, and high UV dose), shown in figure 2.4.

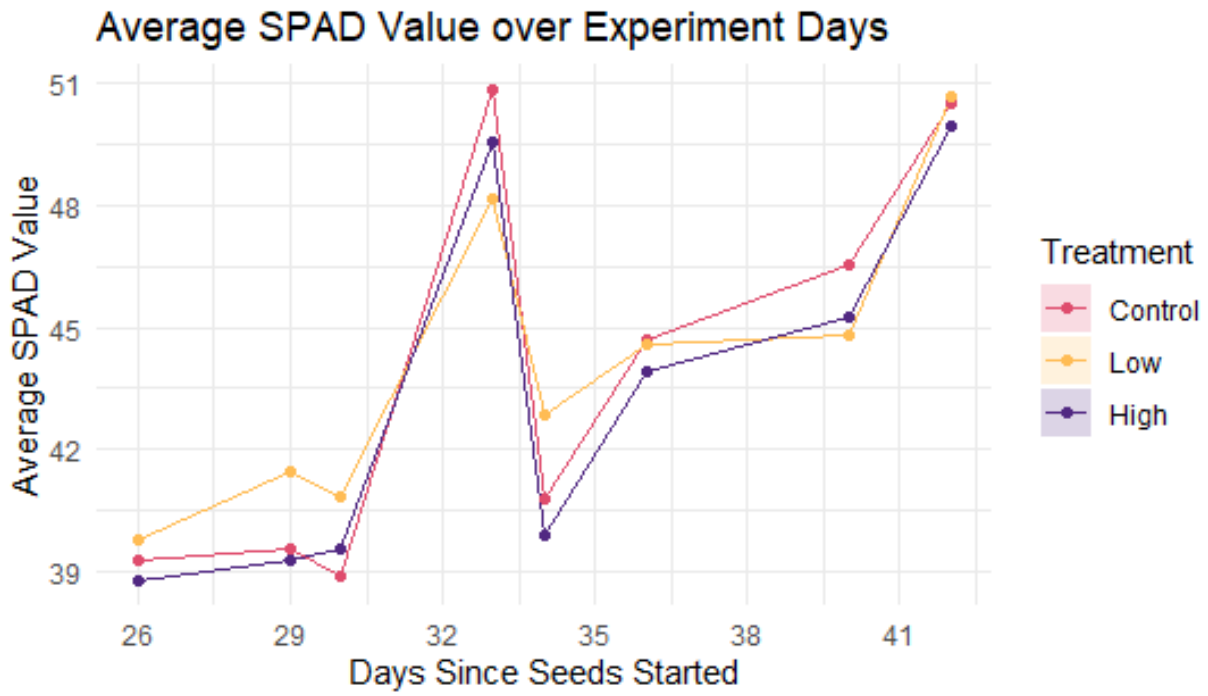

Figure 2.4 Average SPAD value trended upward over the six-week crop growth period, but fluctuated as leaves aged and new leaves emerged, with no differences between treatment groups.

There was no statistically significant difference ($P>0.05$) in average chlorophyll fluorescence between samples taken from systems receiving either treatment and samples taken from the control systems.

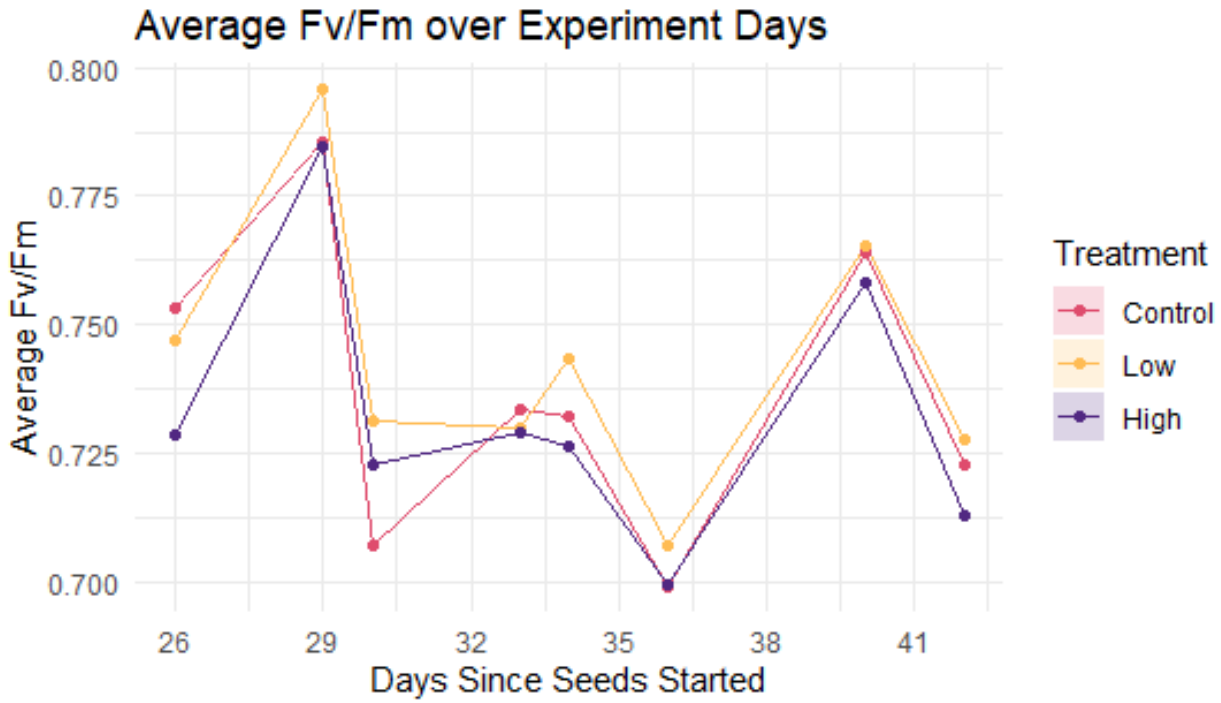

Figure 2.5 Average Fv/Fm fluctuated over the crop growth period but tended to remain low, with no differences between treatment groups.

Throughout the experiment, F_V/F_M values fell below 0.75 on average on all but two observation days. These low values are considered indicators of moderate to severe plant stress (Lee et al., 2014). All *P*-values are greater than 0.05, indicating that UV treatment at any level does not have a statistically significant impact on plant height, SPAD value, or chlorophyll fluorescence.

Table 2.1 Kruskal-Wallis Rank Sum Tests assessing the impact of treatment group on plant growth parameters height, SPAD value, and chlorophyll fluorescence

Dependent Variable	*P*-value
Height	0.27
SPAD Value	0.57
Chlorophyll Fluorescence	0.28

In the nutrient water samples, analysis of the data revealed that there were no significant differences in total nitrogen, total phosphorus, or potassium levels following UV treatments. Levels of each nutrient increased over time and coincided with the addition of nutrient stock solutions as the plants developed.

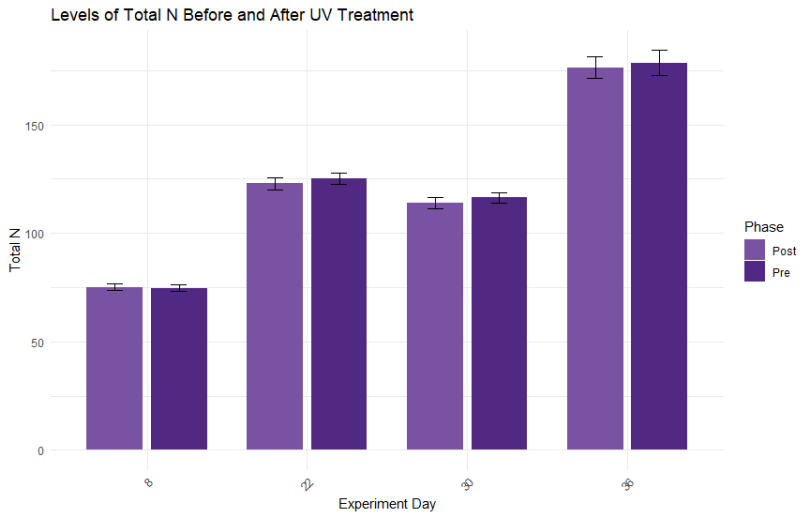

Figure 2.6. Levels of N in the nutrient solution increased over the six week crop growth period as liqid fertilizer solution was added to increase the nutrient solution electrical conductivity, but N levels were unaffected by UV-C treatments

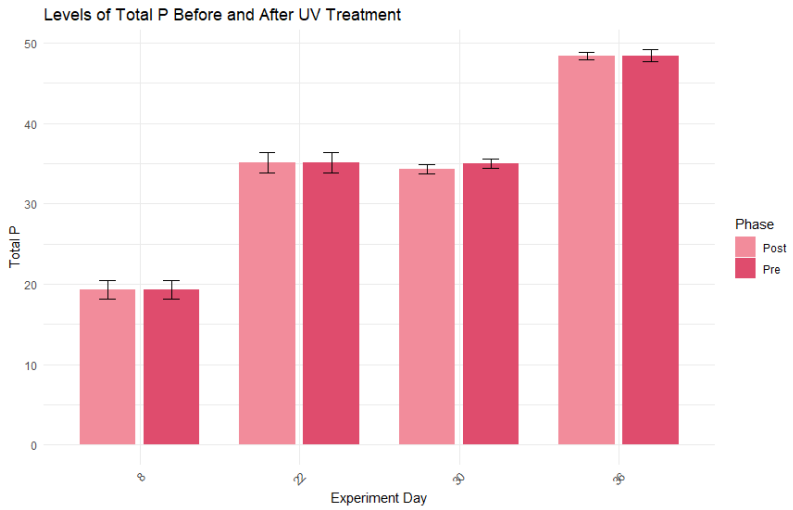

Figure 2.7. Levels of P in the nutrient solution increased over the six week crop growth period as liqid fertilizer solution was added to increase the nutrient solution electrical conductivity, but P levels were unaffected by UV-C treatments

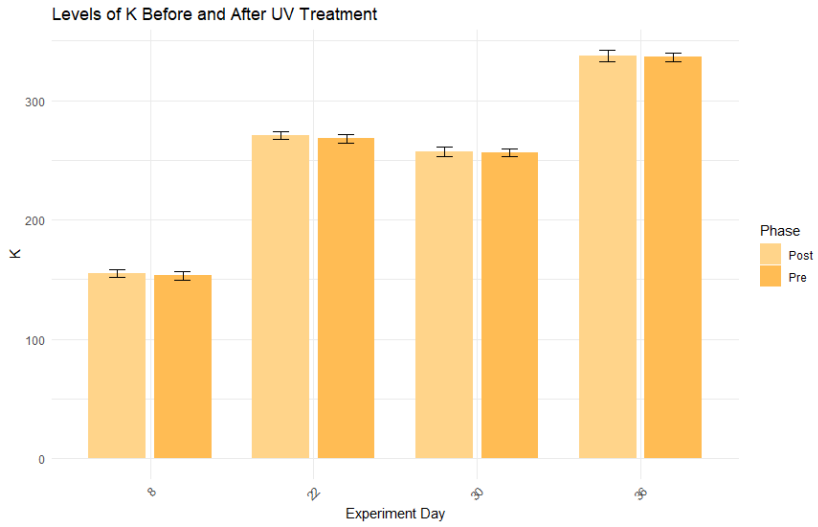

Figure 2.8 Levels of K in the nutrient solution increased over the six week crop growth period as liqid fertilizer solution was added to increase the nutrient solution electrical conductivity, but K levels were unaffected by UV-C treatments

DISCUSSION

The goal of this study was to evaluate the impact of a UV-C nutrient solution on several plant physiology parameters, as well as the three major macronutrient levels in the nutrient

solution, over the entire growth period of romaine lettuce. In order to evaluate the findings, an estimate of the normal range of values for physiological parameters height, SPAD value, and F_v/F_m was obtained by consulting various other published studies, which often provided values observed in healthy control plants in addition to those undergoing various treatments or induced sources of stress (Chowdhury et al., 2024; Galieni et al., 2016; Lee et al., 2014; Maleki, M. et al., 2012; Wang et al., 2024). The values for the healthy control plants from such studies formed the baseline for comparison used in this study. Overall, the heights and SPAD values observed in this study were found to be approximately normal for romaine lettuce, however the unusually low F_v/F_m values could be considered an indicator of plant stress.

The slight reduction in growth rate seen around day 30 may be attributed to the opening of leaves as they began to splay outward rather than growing directly upward around that experiment day. Literature is limited on UV-C treatments applied to hydroponic systems, but one study reported significantly stunted growth and reduced in lettuce following continuous UV-C water treatment in a nutrient film technique hydroponic system (Schwartzkopf et al., 1987).

A study on lettuce intercropping in controlled environments reported that SPAD value increased in early stages of lettuce growth and then plateaued or decreased over time (Wang et al., 2024), which was similar to the findings of this trial. A different study sought to establish how SPAD values in lettuce plants vary between health control plants and plants experiencing various kinds of stress, and reported that control plants had average SPAD values ranging from 20-40 (Galieni et al., 2016). The chlorophyll content of leaves increases as they mature, meaning that younger leaves will have lower SPAD values compared to older leaves (Maleki, M. et al., 2012). Since this project endeavored to sample leaves of intermediate age, young leaves would have been sampled initially, and as new leaves emerged it would eventually become necessary to

sample leaves younger than those originally sampled, leading to the apparent increase, decline, and increase again in SPAD value over time.

Observed F_v/F_m values fluctuated in a normal pattern between 0.70 – 0.80. Other studies have reported that the average F_v/F_m for lettuce grown under normal conditions is 0.83, and values below indicate that the plant is experiencing stress (Lee et al., 2014). While the values observed in this study do appear lower than normal for lettuce, there were no other clear physiological signs indicating plant stress. Some factors that can negatively impact the F_v/F_m ratio include light quality and intensity (Chen et al., 2022), water stress (Ibaraki & Murakami, 2007), high plant density (Luo et al., 2011), more nitrate than ammonium nitrogen (Roosta, 2024), and limited dissolved oxygen (Roosta, 2024). Future studies could explore dissolved oxygen levels along with specific components of nitrogen, and test different plant density schemes, in order to provide further insight into the low observed F_v/F_m values.

Previous research has also found that ferric (Fe_3^+) ions significantly decreased in hydroponic nutrient solution after UV-C treatment (Liu, Chao-Yun, 2019; Schwartzkopf et al., 1987). Iron is a crucial plant micronutrient, and supplementation of iron is associated with higher yields, higher chlorophyll fluorescence, and higher leaf chlorophyll content in lettuce (Kleiber et al., 2023). Iron levels in the nutrient solution were not measured directly in this study, though plants were not visually observed to show common symptoms of iron deficiency (such as chlorosis or stunted growth).

Future studies could benefit from testing individual components of N like nitrate, nitrite, and ammonium individually. While measuring the total N was an efficient way to capture all nitrogen in the system, it may have been more relevant to see if there were differences over time in levels of nitrite and nitrate, particularly if there was any photochemical dissociation of nitrate

into nitrite or ammonium. There is an opportunity for future studies to examine different components of nitrogen over time and following UV-C within the system, as well as iron, and to examine nutrients within the lettuce tissue after harvest.

CONCLUSION

Ultimately, the lack of significant differences in all parameters measured in this study indicate that UV-C treatment likely would not have a negative impact on crop growth. These findings suggest that UV-C does not negatively impact lettuce height, chlorophyll fluorescence, leaf chlorophyll content, or levels of the macronutrients nitrogen, phosphorus, and potassium in the nutrient solution. The use of UV-C light systems could therefore address multiple concerns within hydroponic systems, including human and plant pathogen proliferation, without sacrificing plant health.

Chapter 3 - [1]Effect of Ultraviolet Water Treatment on Survival and Proliferation of *E. coli* in Recirculating Hydroponic Systems

ABSTRACT

Hydroponic nutrient solution can serve as a distribution mechanism for human pathogens to spread throughout an indoor farm or greenhouse. However, many hydroponic leafy green farms do not include water treatment during the crop growth period. Contamination can be introduced to recirculating nutrient solution through numerous routes, including seeds, growing media or substrate, and worker contact. In leafy green production, it is common for the nutrient solution to inadvertently contact produce during harvest or packaging. Therefore, it is critical that such water be free of human pathogens. Ultraviolet (UV) treatment of water could enable nutrient solution disinfection without disrupting production or introducing new chemical inputs to the nutrient solution.

This study investigated the survival of generic *Escherichia coli* (*E. coli*) in hydroponic systems and assessed the efficacy of a 254 nm UV-C light treatment for decontamination. Romaine lettuce was grown directly from seed in the hydroponic system for six weeks. *E. coli* was artificially inoculated into hydroponic systems at 6.02 average log CFU/ml. UV treatments of water were performed every two weeks. Hydroponic controls grew plants but received no UV treatment, and non-hydroponic controls neither grew plants nor received treatment. Nutrient solution was pumped out of the reservoirs through a UV chamber and then back into the

[1] This chapter is based on "Effect of Ultraviolet Water Treatment on Survival and Growth of *Escherichia coli* in Recirculating Hydroponic Systems" by Markanna Moore, Teng Yang, Majid Jaberi Douraki, Cary Rivard, Eleni Pliakoni, Londa Nwadike, and Manreet Bhullar, 2025. *Journal of Food Protection*, Volume 88, Issue 9. Copyright 2025 by the authors.

reservoirs. The efficacy of the treatment in terms of bacterial reduction in log CFU per ml was assessed.

The UV-C treatments resulted in significant ($P < 0.001$) average reductions of 1.4-1.5 $\log_{10}$ CFU/ml of *E. coli* in the nutrient solution. However, the *E. coli* population declined naturally over the weeks following the inoculation, independent of the UV-C treatment. Survival of *E. coli* beyond one week is limited in the nutrient solution, particularly if it is not able to colonize various surfaces within the hydroponic system. Still, UV-C treatment has the potential to be used as a preventative safeguard for the microbial safety of the hydroponic production system by reducing contamination within the nutrient solution during crop growth. This can help maintain the safety of hydroponically-grown fresh produce and reduce the likelihood of future outbreaks

INTRODUCTION

Lettuce and herbs account for approximately 20% of the US hydroponic industry by revenue, with other prominent crops including tomatoes, cucumbers, peppers, and strawberries (Le, Valerie, 2024). Hydroponic cultivation offers advantages in resource use efficiency, illustrated by the finding that lettuce grown hydroponically uses, on average, approximately 13 times less water compared to traditional outdoor cultivation methods (Barbosa et al., 2015). Many of the efficiencies attributed to hydroponic cultivation can be linked to the densely-planted protected and controlled environments housing the soilless systems that use a water-based nutrient solution to grow plants. However, hydroponic growers face a variety of unique challenges, including greater energy needs than many traditional farms, significant start-up costs, and the potential for plant or human pathogens to contaminate an entire crop rapidly via shared recirculating nutrient solution.

Hydroponically grown produce has traditionally been perceived as safer due to the protected growing environment, creating the impression of reduced opportunities for contamination from wildlife or soil (*Is CEA a Safer Way to Grow and Process RTE Vegetables? | Food Safety*, n.d.; Schoch, 2022). Outbreaks associated with field-grown lettuce are fairly common in the US, as evidenced by the recent 2024 outbreak of *E. coli* O157:H7 in romaine lettuce that led to 89 documented cases, the 2022 outbreak of *E. coli* O121:H19 in romaine lettuce, or the four separate outbreaks of various pathogens in salad greens in 2021 (Program, 2025). While the risks associated with salad greens in the US are widely covered in the media, the FDA has specifically excluded hydroponic lettuce labeled as "indoor grown" from consumer warnings in outbreak investigation reports in the past (*FDA In Brief*, 2019), which may have further contributed to a consumer perception of safety. However, studies have found both *Salmonella* and *L. monocytogenes* on tissue samples of lettuce grown hydroponically and suggested that similar risks may exist for conventional and hydroponic crop growth methods (Mohammad et al., 2022).

This risk may have been slow to enter the public consciousness due to the historical lack of an outbreak linked to hydroponic production. Yet, in 2021, hydroponic salad greens were implicated in an outbreak of *Salmonella* Typhimurium that led to 31 documented illnesses across four US states (Schoch, 2022). The Food and Drug Administration (FDA) was unable to determine the vector through which contamination was introduced, but the agency noted several trends in the hydroponic industry with concerning implications for food safety, including that "once in the growing ponds, the water is not routinely disinfected or otherwise treated" (U.S. Food and Drug Administration, 2021), and that some portions of lettuce heads contact hydroponic nutrient solution during the harvest process.

The sources of contamination are not well understood, but it is clear that once foodborne pathogens enter a recirculating hydroponic system, water acts as "a carrier, but not a source" of microbial contamination (Dankwa et al., 2020). Suspected sources of contamination in hydroponic systems include substrates, particularly peat moss, or other improperly stored growing media (Dankwa et al., 2020). Once in the water, pathogens can form biofilms and remain persistently attached to surfaces within the system, including plant roots, substrates, or mechanical parts.

If this recirculating water contacts the edible portion of plants that are commonly consumed raw, it could potentially lead to an outbreak of foodborne illness. When ingested, pathogens such as *Salmonella* and *E. coli* can cause gastrointestinal symptoms including nausea, diarrhea, vomiting, and fever, or in severe cases, may lead to complications including meningitis or organ damage, which could be deadly (CDC, 2025). In the US, assorted strains of Shiga toxin-producing *E. coli* are estimated to cause 265,000 illnesses and 100 deaths each year (NCDPH, 2019). With a common goal of reducing foodborne outbreaks, hydroponic growers can contribute to this goal with preventative measures that aim to ensure harvested produce is free of contamination.

Along with *Salmonella* and *Listeria monocytogenes*, *E. coli* is considered one of the three major foodborne pathogens of public health significance (Covney, 2025). In recent years, various strains of *E. coli* have increased in prevalence in terms of foodborne disease outbreaks associated with fresh produce, especially leafy greens (Barril et al., 2022). *E. coli* is also known for its widespread use in water quality testing and its prevalence as a potential contaminant in agricultural settings (Haley, 2023). These factors contributed to the choice of *E. coli* in this study (represented by the surrogate strain 25922).

Previous studies have examined the growth of pathogens in a hydroponic system or nutrient solution over short intervals (Shaw et al., 2016) or tested for the presence of coliform bacteria in hydroponic inputs and water (Dankwa et al., 2020). One study focused on the survival of *Salmonella enteritidis* each week up to 21 days after inoculation in hydroponic nutrient solutions ranging from pH values of five through eight (Xylia et al., 2021), but did not include a quantitative description of the population. There is a research gap examining the survival and growth of human pathogens, quantified in log CFU, at regular intervals throughout the entire growth cycle of a hydroponic crop. This information is essential for identifying and developing strategies to mitigate the food safety risks associated with hydroponic practices.

UV light technology is an intervention commonly used to sanitize water. Several research studies have reported the antimicrobial efficacy of UV treatment on fresh produce and in agricultural water (Haley, 2023; Haley, Pliakoni, et al., 2023; Haley, Zhao, et al., 2023). The Food Safety Modernization Act's Produce Safety Rule establishes standards for the microbial quality of agricultural water used in the production of fresh produce (FSMA Final Rule on Pre-Harvest Agricultural Water, 2024), which leads many growers to explore options to treat their agricultural water in order to remain in compliance. While chemical treatments like chlorine remain the most common in the industry, they may adversely affect water and soil quality and require routine monitoring (Adhikari et al., 2020). Studies have found that UV-C treatment presents a promising alternative form of treatment for agricultural water from a variety of sources, including pond water, with the only limitation being a slight reduction in efficacy as water turbidity increases (Adhikari et al., 2020). The reduction potential of UV-C treatments of hydroponic nutrient solution warrants additional exploration to assess the applicability in controlled environment agriculture settings.

Studies have also indicated that frequent (especially continuous) UV-C treatments may have an adverse effect on lettuce growth (Schwartzkopf et al., 1987), so this experiment aimed to take a conservative approach to applying UV-C irradiation in choosing 14-day intervals for treatments. Furthermore, some experimental designs have provided continuous treatment by incorporating a UV-C device with the pumps found in the system reservoirs (Perez et al., 2024); considering that this approach does not ensure equal treatment of the full volume of water contained in a reservoir, this study chose to instead pump all water out as it was treated, and then pump it back into the reservoir. This was done to ensure the total volume of water in the reservoir would be confirmed to pass through the UV-C chamber.

While previous studies have examined short-term pathogen survival in hydroponic systems, there are critical gaps in understanding the long-term survival and quantification of specific human pathogens throughout the entire crop growth cycle, and the effectiveness of UV-C treatment as a practical disinfection method. This study seeks to evaluate the long-term survival of a surrogate strain of *E. coli* in hydroponic nutrient solution through samples taken twice weekly over the entire growth period of hydroponically grown lettuce. The study also assessed the efficacy of UV-C treatments of the contaminated nutrient solution and its impact on bacterial survival during the crop growth period.

MATERIALS AND METHODS

A total of 12 hydroponic systems were used, with four replicate systems assigned to each of the three treatment groups (control, three L/min UV-C, and six L/min UV-C) in a structured non-randomized manner. *E. coli* was added to the reservoir of each system and *E. coli* survival was assessed in log CFU/ml through samples taken twice weekly from the day seeds are directly sown in the system to the harvest six weeks later. The quantity of *E. coli* in these systems was

compared to the quantity in non-hydroponic control systems without any plants or power connected to the pumps. Samples were also used to measure the absorbance and transmittance of the nutrient solution at 254 nm. The experiment was repeated three times, with each trial lasting six weeks from the day seeds were sown to the day of harvest. Trials utilized six commercially available hydroponic towers (Farm 48 and 24+ models, AeroGrow International, Inc., Boulder, Colorado) consisting of a stackable frame with built-in lights, and space for two hydroponic systems, each treated as an independent experimental unit. Systems were comprised of reservoirs with pumps that circulated nutrient solution and delivered it directly to rockwool cubes, which are a hydroponic substrate made from fibers of molten rock, in the top grow deck (see figures 3.1 and 3.2). The built-in light systems were adjusted to the same height to eliminate model differences in the units.

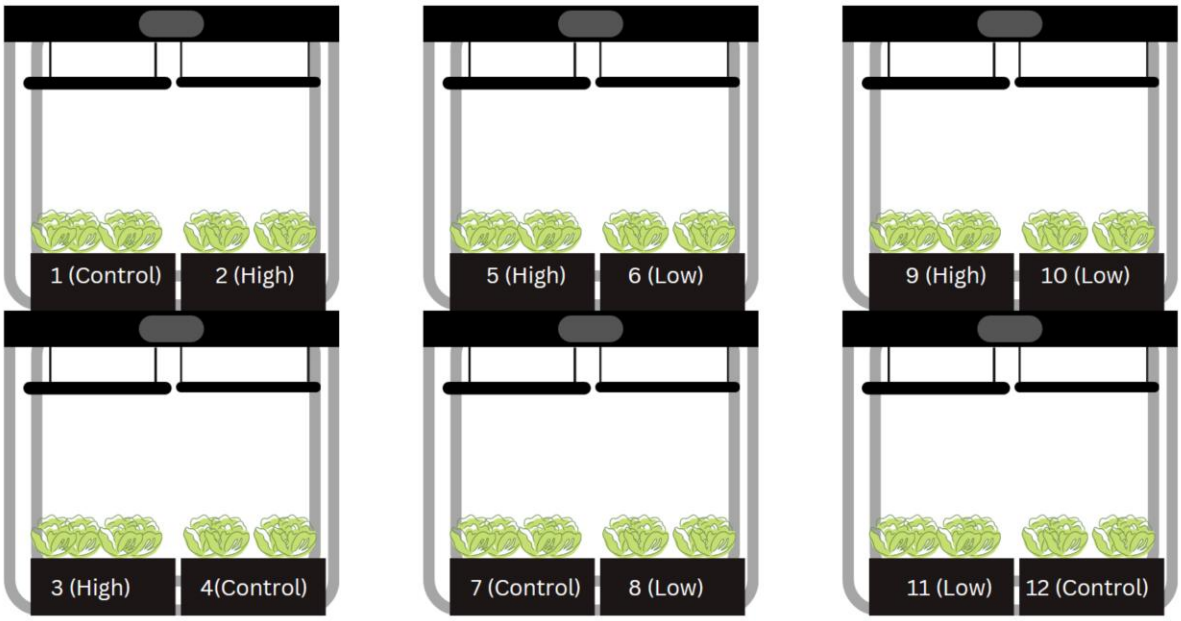

Figure 3.1. NFT systems were arranged in a structured non-randomized design with respect to assigned treatment groups, split between the upper and lower levels of three towers.

Growing conditions. On the first day of each trial, each system (including two additional static controls not growing lettuce) was filled with eight L of distilled water. Distilled water was used due to preliminary experiments showing that *E. coli* failed to survive at detectable levels in systems using the local municipal tap water. The full-spectrum grow lights were set to a 13-hr photoperiod, with the average daily light integral being 12 mol/m^2/d as per the recommendation (Giuseppina Pennisi et al., 2020). The lights were positioned 49 cm above the grow decks to maintain the same daily light integral across the growing systems.

To ensure that *E. coli* was not present in the hydroponic systems prior to inoculation, an IDEXX Colilert test (IDEXX Laboratories, Inc., Westbrook, Maine) was performed using a composite 100 ml sample of water taken from each system. Rockwool cubes were soaked for at least 15 mins in distilled water before being placed in plastic baskets to fit in the holes in the systems' grow decks. Three Lettuce (*Lactuca sativa*) 'Sparx Romaine' seeds (Johnny's Selected Seeds, Fairfield, Maine) were placed in each cube. Four cubes were then placed in each hydroponic system in an alternating pattern to maximize available space for lettuce plants as they grew, see Figure 3.2.

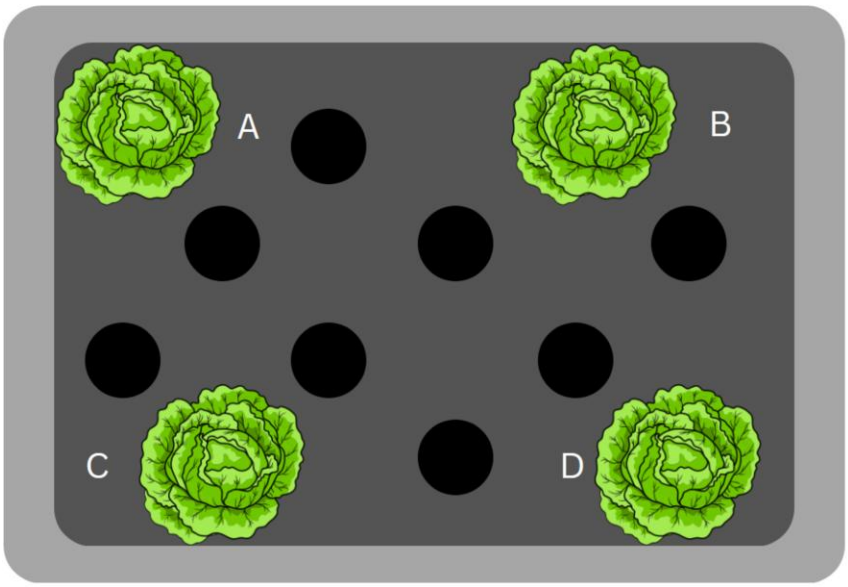

Figure 3.2. Lettuce plants were positioned in an alternating pattern within the grow deck of each system.

Culture preparation. *E. coli* ATCC On the first day of each trial, each system (including two additional static controls not growing lettuce) was filled with eight L of distilled water. Distilled water was used due to preliminary experiments showing that *E. coli* failed to survive at detectable levels in systems using the local municipal tap water. The full-spectrum grow lights were set to a 13-hr photoperiod, with the average daily light integral being 12 mol/m^2/d as per the recommendation (Giuseppina Pennisi et al., 2020). The lights were positioned 49 cm above the grow decks to maintain the same daily light integral across the growing systems.

To ensure that *E. coli* was not present in the hydroponic systems prior to inoculation, an IDEXX Colilert test (IDEXX Laboratories, Inc., Westbrook, Maine) was performed using a composite 100 ml sample of water taken from each system. Rockwool cubes were soaked for at least 15 mins in distilled water before being placed in plastic baskets to fit in the holes in the systems' grow decks. Three Lettuce (*Lactuca sativa*) 'Sparx Romaine' seeds (Johnny's Selected Seeds,

Fairfield, Maine) were placed in each cube. Four cubes were then placed in each hydroponic system in an alternating pattern to maximize available space for lettuce plants as they grew, see Figure 3.2.

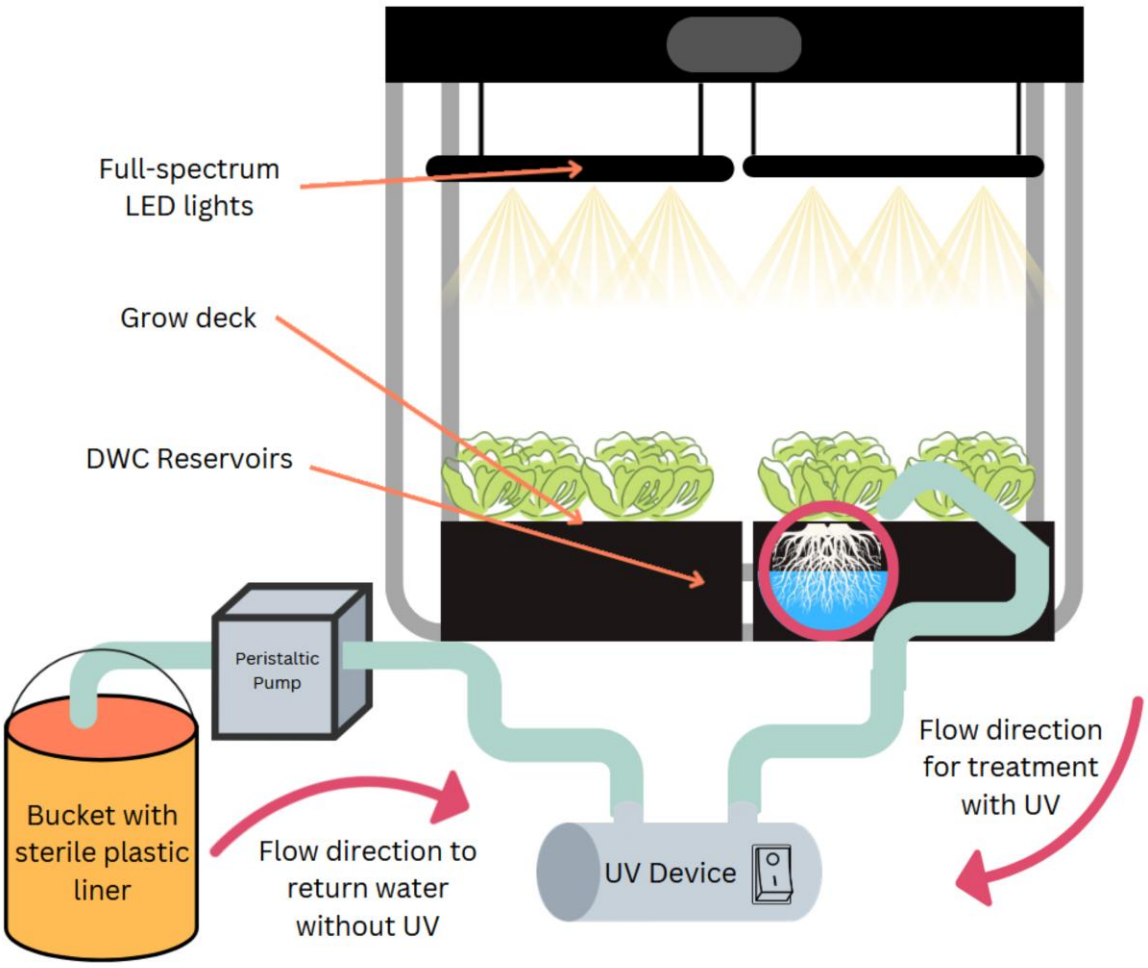

Figure 3.3. UV-C treatments were performed by pumping all nutrient solution out of the reservoir, through a UV-C chamber, and collecting it in a bucket lined with a sterile plastic bag. The UV-C device was then turned off, and nutrient solution was pumped back into the reservoir.

The nutrient solution was pumped out of each hydroponic reservoir at a rate corresponding to its designated treatment level, passed through the UV-C chamber for treatment, and ultimately deposited in a sterile Whirlpak bag inside of a five-gallon bucket, as seen in

Figure 3.3. The direction of the pump was then reversed, so the nutrient solution contained in the Whirlpak bag was pumped back into its reservoir.

Sample collection. Nutrient solution reservoir volume was maintained at eight L with the addition of distilled water as needed. Twice per week, water was thoroughly mixed before a 35 ml sample was collected. Ten ml of the sample was reserved for making serial dilutions which were used for spread plating and microbial enumeration. Concurrently, 25 ml were used for testing pH and EC. Initial samples taken following inoculation of the nutrient solution required three dilutions in order to produce a countable range of colonies via spread plating. However, lower dilutions were used after the initial inoculation as bacterial populations were no longer detected at high levels in nutrient solution over time. After the second trial, samples of rockwool measuring approximately one cubic cm were taken from nine random systems, homogenized in 20 ml of 0.1% BPW, and spread plated for comparison to water samples.

Bacterial enumeration. Petri dishes containing 18 ml of Tryptic Soy Agar with 80µg/ml rifampicin were used in bacterial enumeration via spread plating. From each serial dilution, 0.1 ml was spread plated in duplicate and incubated at 35°C for 24 ± 2 hrs. After incubation, colonies were counted (within a range of 25-250 colonies per plate) to determine the average log CFU/ml of the sample.

Data Analysis. Colony forming units of *E. coli* were $\log_{10}$ transformed prior to analysis for each day that samples were collected over the six-week growth periods. A Friedman test was performed to assess differences in bacterial survival between hydroponic systems and non-hydroponic systems excluding experiment days where reinoculation or treatment took place. The Friedman test was chosen due to the necessity for a non-parametric test because of repeated measures, and the violated assumption of normally distributed residuals ruling out ANOVA

models. A Kruskal-Wallis test was used to assess the impact of the UV-C treatments on log CFU of *E. coli* with the interaction of two factors: phase (the temporal status of being pre-or post-UV-C treatment) and treatment group (three L/min, six L/min, and control). Following the Kruskal-Wallis test, a Dunn post hoc test with Bonferroni corrections for multiple comparisons was conducted to examine pairwise differences between groups within each phase. Data was analyzed using R version 4.3 (R Foundation for Statistical Computing, Vienna, Austria.).

RESULTS AND DISCUSSION

This study aimed to examine the efficacy of a 254 nm UV-C nutrient solution treatment in terms of bacterial reduction, to assess the survival of *E. coli* over six weeks of lettuce growth, and to evaluate the absorbance and transmittance of hydroponic nutrient water during the same time period. Water samples were collected twice weekly as well as pre and post UV-C treatments. These samples were used to test absorbance and transmittance as well as to create serial dilutions for spread plating. This resulted in 15 total observations per system, per six-week trial.

In 216 total observations (each of the 12 systems was sampled before and after the nine treatments taking place throughout three trials) averaged by treatment group, UV-C treatments were found to significantly reduce the observed bacterial populations in the nutrient solution. Pre-treatment bacteria levels (shown in purple in figure 3.6) were approximately 4.5 log CFU. Post treatment bacteria levels (shown in yellow in figure 3.6) for both UV-C treatments were significantly different from the post treatment levels in control systems. There was no significant reduction of *E. coli* in CFU/ml in control systems (pre and post comparison; no UV-C treatment).

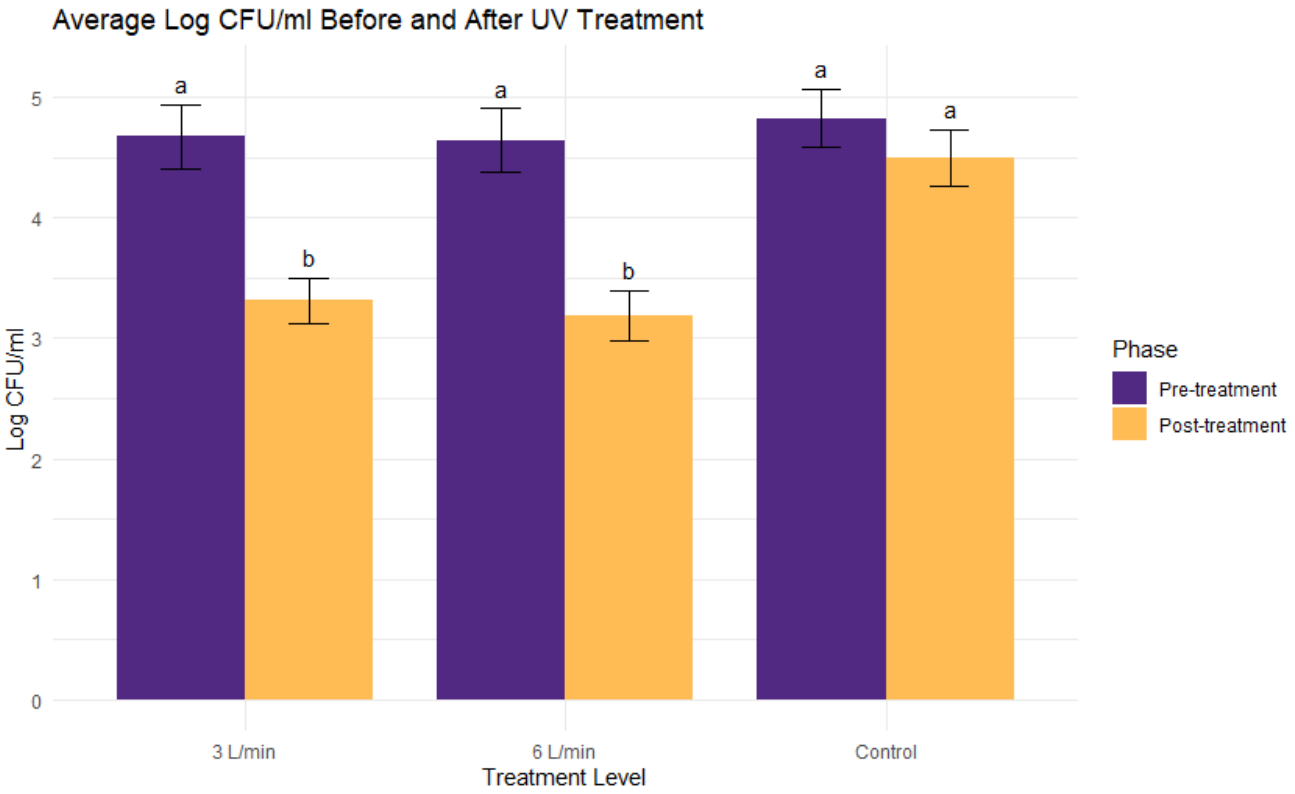

Figure 3.4 Before UV-C treatment, hydroponic nutrient solution in systems from all treatment groups contained on average just under five log CFU/ml of E. coli, and after UV-C treatment, levels were significantly reduced by approximately 1.4 log CFU/ml in solution

There was a significant average reduction of 1.36 ± 0.19 log CFU/ml in the nutrient solution following the three L/min UV-C treatment, and an average reduction of 1.46 ± 0.21 log CFU/ml following the six L/min UV-C treatment. The difference in post treatment log CFU/ml was not significant between the six L/min and three L/min treatments. The Kruskal-Wallis test indicated that the UV-C treatment had a significant ($P < 0.001$) effect on log CFU/ml in at least one group. To explore which group(s) had significant differences, a post hoc test was conducted.

Table 3.1 Kruskal-Wallis rank sum test results for log CFU/ml by treatment and phase indicate a statistically significant difference

chi-squared	75.415

df	5
p-value ****	7.62E-15

As seen in Table 3.2, samples from systems receiving the six L/min or the three L/min UV-C treatment had significantly ($P < 0.001$) fewer log CFU/ml after treatment compared to samples from the control systems. No significant differences existed between the two UV-C treatment groups before or after treatments.

Table 3.2 Results of Dunn post hoc test with Bonferroni corrections for multiple comparisons, examining pairwise differences between groups within each phase, indicating significant differences for both treatment groups compared to the post-treatment control groups.

Pairwise Comparison	Z	Adjusted p-value	Significance
Control post-treatment – six L/min post-treatment	**4.28**	**2.76E-04**	***
6 L/min pre-treatment – six L/min post-treatment	-5.26	2.18E-06	****
Control post-treatment –three L/min post-treatment	**4.32**	**2.37E-04**	***
3 L/min pre-treatment –three L/min post-treatment	-5.33	1.50E-06	****

The UV-C treatment provided significant reductions in sample log CFU compared to control system samples, but there were no significant differences between the six L/min and three L/min treatment levels, indicating the flow rate did not greatly impact the effectiveness of the treatment at the same UV transmittance level. The average reductions of 1.36 and 1.46 log CFU/ml still may be considered low compared to other interventions targeting water, considering that a 6-log reduction is the standard requirement for a safe disinfection (Sommer et al., 2000),

but the comparatively low observed reductions may be attributed partly to the design and procedure of the treatment.

UV-C disinfection works by inducing the formation of pyrimidine dimers, which interfere with the ability of microorganisms to replicate DNA (Silva et al., 2013). The efficacy of UV-C treatments can be impacted by factors including the phase of growth of microorganisms being treated, any repair mechanisms employed by the microorganism, water characteristics that may increase absorbance and scattering of UV light, or weakened lamp performance over time (Silva et al., 2013). Flow dynamics within the UV-C reactor can also impact the efficacy of the treatment, with turbulent flow usually improving the device performance (Koutchma et al., 2004).

Another approach which may increase the efficacy of treatments could include treating the solution multiple times or in a recirculating fashion. However, there is some evidence to suggest that continuous UV-C treatment of nutrient solution may be harmful to plant growth (Schwartzkopf et al., 1987). Consequently, there is an opportunity for future studies to explore various rates of UV-C treatment in order to find a frequency that maximizes bacterial reduction without harm to plants.

In order to compare *E. coli* survival over time in hydroponic systems and non-hydroponic controls, observations on treatment days were excluded to reduce interference from reinoculations and treatments. This resulted in a total of 378 observations over three trials, with 324 from the 12 hydroponic systems and 54 from the two non-hydroponic controls. Mean values are graphed in Figure 3.5 with standard error bars.

Bacterial populations in the hydroponic systems declined over time before recovering, and though efforts were made to exclude reinoculations from this analysis, it is possible that

lingering impacts of reinoculation may contribute to the recovery of populations illustrated in Figure 3.5. The Friedman rank sum test comparing the average log CFU/ml of nutrient solution samples from the hydroponic systems (excluding samples taken within a day of reinoculation or treatment) with average log CFU/ml of samples taken from the non-hydroponic controls produced a p-value of 0.004. This indicates significantly (**) higher log CFU/ml in the nutrient solution of the non-hydroponic control systems.

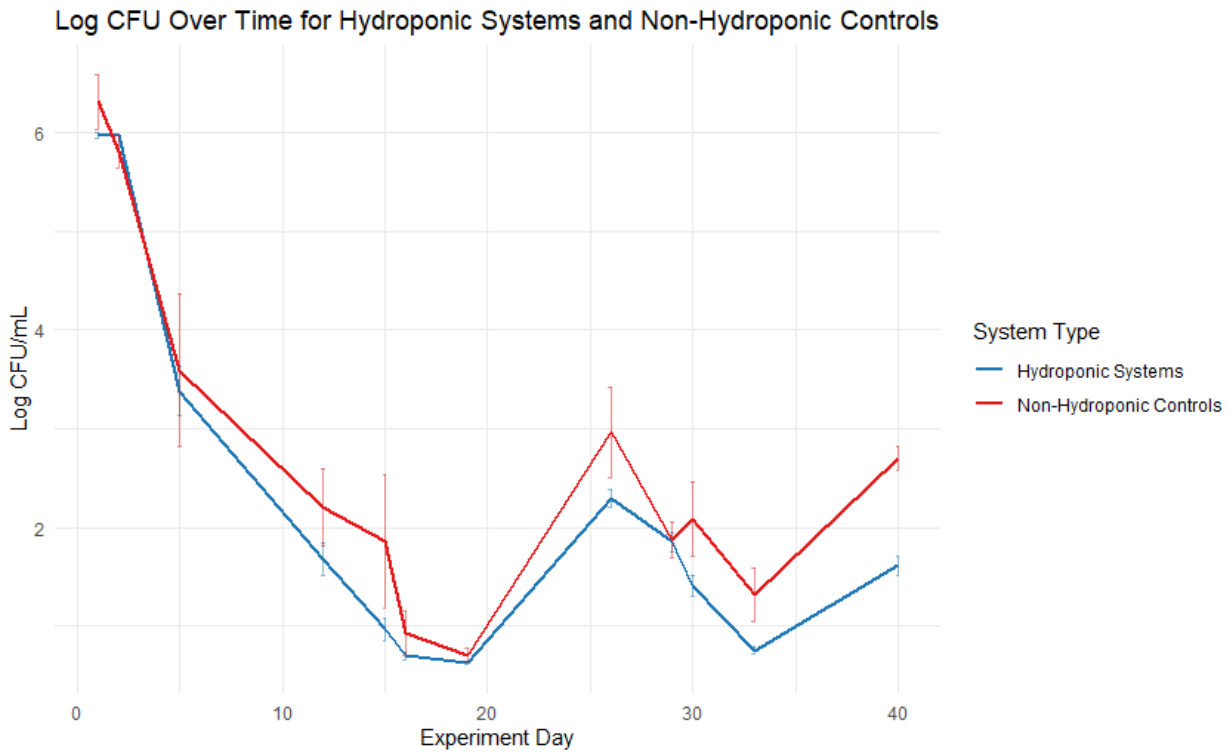

Figure 3.5 . Bacterial survival declined over time for hydroponic systems and non-hydroponic controls, but the decline was significantly greater in hydroponic systems. Data excludes observations from days with reinoculation or UV-C treatment.

The decline over time in bacterial populations may be in part due to the lack of nutrient sources in the DI water during the period before seeds had germinated, when bacterial access to carbon sources and micronutrients would have been severely limited. Another study found that

Shiga-toxin producing *E. coli* levels increased in plain distilled water as well as a hydroponic fertilizer solution during the first 24 hrs after inoculation, but decreased in fertilizer solutions which had been used for four weeks in a hydroponic system growing basil (Shaw et al., 2016). The different temporal scales and seed propagation methods used in the current study may explain the different phenomenon observed for the non-hydroponic controls, since in this experiment bacteria was added at the same time seeds were sown.

Additionally, bacterial survival over time was significantly greater in the non-hydroponic control systems. It is possible that the lower pH values maintained in hydroponic systems or competition from the natural microbiome of the lettuce rhizosphere inhibited *E. coli* survival in later crop growth stages, as the presence of native microorganisms has been linked with greater inactivation over time of *E. coli* in water (John & Rose, 2005).

During the lettuce growth period, the transmittance of the hydroponic nutrient solution at 254nm decreased as the absorbance increased (Figure 3.4). This is expected given the inverse relationship of the two parameters, but the overall trend is reflective of the nutrient solution becoming more turbid over time. Furthermore, transmittance was confirmed via a linear mixed-effects model to have a statistically significant ($|T| > 4$) negative impact (estimate of -1.04) on the log reduction in CFU/ml resulting from treatments.

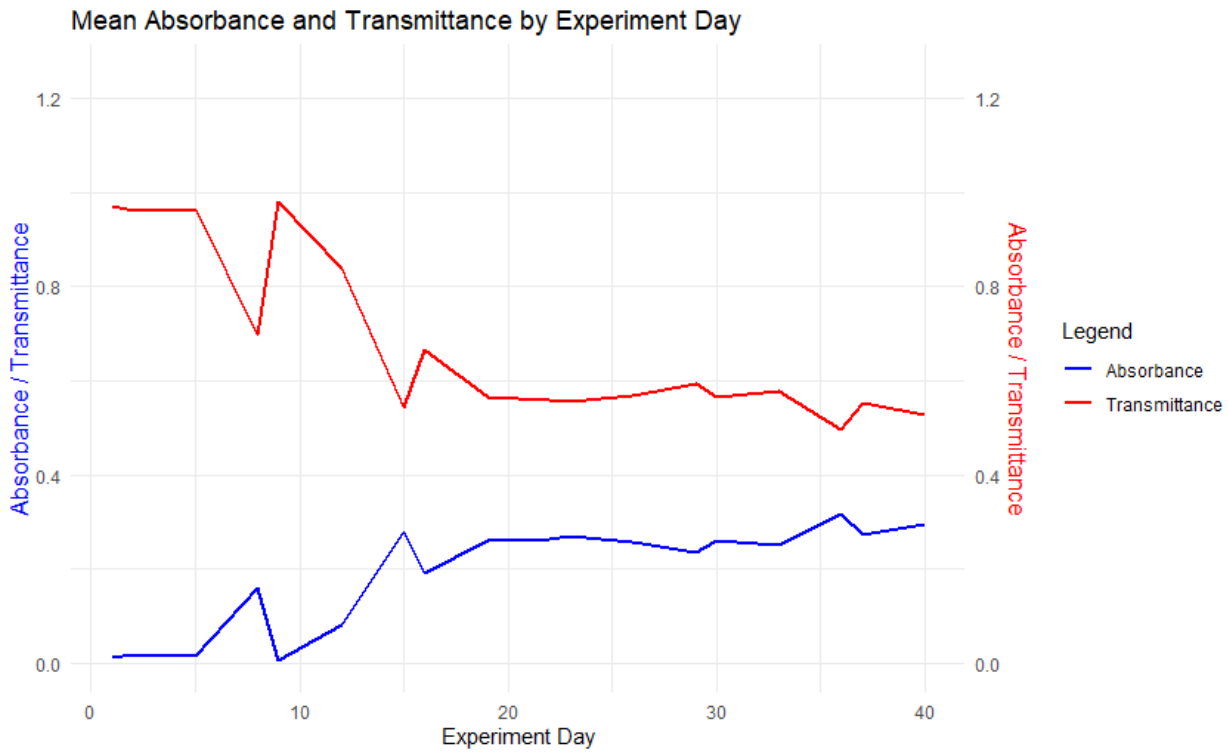

Figure 3.6 Absorbance of the nutrient solution increased over time for all systems as transmittance declined.

Based on observed reductions following the treatments, and a previous study's estimate of *E. coli* having a D_{10} value of approximately three mJ/cm^2 (Bhullar et al., 2019), the dosages received during the UV-C treatments are estimated to be approximately 4.08 to 4.38 mJ/cm^2. These lower doses are supported by the fact that a dose of 6.6 mJ/cm^2 was validated by the manufacturer to inactivate 99% of *E. coli* for the MIN-1 (Atlantic Ultraviolet, 2022), and the reductions observed in this study were much lower. This discrepancy between the device specifications and our observed reductions may be due in part to decreasing UV transmittance over time as nutrient levels and overall turbidity of the water increased (Figure 3.4).

The decrease over time in the UV transmittance of the nutrient solution could be caused by increasing levels of nutrients and naturally occurring microorganisms, which are often a water

quality concern in hydroponic systems and can contribute to the clogging of the pumps or hoses (Jafari et al., 2024). The transmittance, on average, fell below the UV-C device manufacturer recommended level of 85% (Atlantic Ultraviolet, 2022) within the first two weeks of crop growth; this may help to explain the relatively low reductions from UV-C treatments, and the UV-C dose estimates being lower than those claimed by the manufacturer. Previous studies using UV-C treatments on agricultural water found low transmittance and high turbidity of the water to be the primary limiting factors for the efficacy of the treatment (Adhikari et al., 2020).

The difficulties presented by the water transmittance do not imply that UV-C treatments are working differently in hydroponic nutrient solution, but rather they may reflect challenges unique to the limited scale of systems and treatments in this study. It is worth noting that the UV-C device used in this study was quite small, in accordance with the small-scale hydroponics systems being used. A larger UV-C device may be able to overcome the slightly occluded water and achieve greater reductions through higher dosage delivery.

The UV-C nutrient solution treatment in this research may not address microbial attachment via biofilms to plant roots, surfaces within the hydroponic system, or the rockwool growing media. Taking a random sample of rockwool at harvest revealed that log CFU were on average 1.37 logs higher in rockwool samples than in water samples taken at the same time. Even if the UV-C device was integrated directly into the nutrient solution reservoir, the root mass would obstruct the device's ability to contact all surfaces in the reservoir, potentially providing shelter for the bacteria.

It may be possible to overcome the challenge of internal bacterial attachment with the use of a sanitizer added to the nutrient solution reservoir, which would theoretically contact all interior surfaces that could be contaminated via nutrient solution. However, most of the products

currently available to growers have phytotoxic effects. Sanidate 12.0, a PAA-based sanitizer, has been shown to reduce *Salmonella* by 5.6 log CFU in a hydroponic reservoir, but at the cost of significantly reduced lettuce fresh weight and chlorophyll content at harvest (Mensah, 2023). Other commercial sanitizers in that study were ultimately excluded from nutritional analysis because all seedlings had died within a few days of application. Treatments with such pronounced negative impacts on plant growth are not viable solutions for the industry.

CONCLUSION

Future studies could explore additional alternatives to chemical interventions, one of which may be using bacterial competition to reduce human pathogens in the nutrient solution. Biocontrol products such as *Trichoderma* spp. are commonly used in commercial hydroponic settings to antagonize plant pathogens (Dankwa et al., 2020). It is possible similar products could be used to control the incidence of human pathogens in hydroponic systems, with the same principle of antagonistic competition from bacterial species that are neutral or beneficial to plants and humans. These could potentially be applied following UV-C treatments, in order to inhibit future bacterial growth after a highly effective initial UV-C treatment to address any contamination from the early stages of production. More research is needed to determine the potential efficacy of biocontrol products in comparison to physical interventions like UV, or chemical interventions.

UV-C treatments are desirable for many growers, particularly those interested in organic production, because they completely circumvent the need to choose disinfecting agents from a very limited list and carefully ensure that chlorine levels in any preharvest water align with the limits considered safe for drinking water . If UV-C treatments also do not negatively impact crop growth parameters, they may have an advantage over chemical interventions in hydroponic

nutrient solution. Further research is recommended to investigate the impact of this UV-C treatment on plant growth, and possibly to determine how impactful the native microbiome is for both plant growth and biocontrol of pathogenic microbes. UV-C treatments have proven their effectiveness in reducing human pathogens in drinking and agricultural water, and this study suggests they can also provide a convenient and safe way to integrate a needed disinfection step during the growth period of hydroponic crops.

Chapter 4 - Comparing the Pathogen Reduction Potential of UV Water Treatment with Competition from Beneficial Bacteria

ABSTRACT

Recirculating nutrient solutions in hydroponic systems can serve as a transmission route whereby pathogenic bacteria that cause foodborne illness can quickly spread at any stage of production. For this reason, it is desirable to develop a method of reducing potential contamination in nutrient films on a continuous basis during the crop growth period. The physical and chemical interventions tested for this purpose to date have been broad-spectrum and often achieve a negative or neutral effect on plant growth. Furthermore, the naturally occurring hydroponic microbiome includes a wide range of species which have complex and sometimes synergistic relationships with the plant. Certain biocontrol products are used in hydroponic systems as a preventative measure against plant pathogens work through competition. Commercially available Plant Growth-Promoting Bacteria (PGPB) products make similar claims that competition from these "beneficial" bacteria to reduce populations of undesirable organisms in the system.

This study tests the efficacy of a commercially available hydroponic PGPB product as a biocontrol agent against the human pathogen *Escherichia coli* in small-scale Nutrient Film Technique (NFT) systems growing kale microgreens. Reductions in the pathogen log CFU/ml were compared with an ultraviolet (UV-C) water treatment previously demonstrated to have neutral impacts on plant growth, and control systems receiving neither UV nor biocontrol interventions. In addition to *E. coli* levels present in the nutrient solution, plant height was recorded twice per week. Fresh weight, SPAD value, and *E. coli* levels on samples of root tissue

and growing media were measured upon harvest of the microgreens. There were no significant differences in the reduction of *E. coli* between the biocontrol and UV systems, though the UV system did significantly reduce *E. coli* compared to the controls. No significant differences were observed in plant height, fresh weight, or SPAD value. Overall, the results indicate that UV remains a more effective broad-spectrum microbial intervention for hydroponic nutrient solution, compared to the beneficial bacteria product evaluated here.

INTRODUCTION

Products known as sprouts and microgreens are typically both seedlings of a variety of plant species, desirable for the concentrated phytochemicals they possess and their novel culinary applications. Microgreens began their rise to popularity in the 1980s among high-end chefs in San Francisco, California, where they were originally used in a decorative capacity, but have since gained popularity in juices, smoothies, and salads due to their high levels of bioactive compounds and vitamins (Singh et al., 2024). For decades, sprouts have been considered among the most high-risk fresh produce due to their implication in numerous outbreaks of major foodborne illness due to *Salmonella* and *E. coli* O157:H7 (Taormina, Peter J., 2024). The primary distinguishing feature between sprouts and microgreens is the method of cultivation used to produce them. Microgreens have the potential to serve as a safer alternative to sprouts, as microgreens are usually grown in open air conditions rather than in the warm, moist conditions of sprouting drums which are optimal for bacterial growth (Xiao et al., 2015). Microgreens can also be grown hydroponically, allowing intensive cultivation without the use of soil.

Hydroponic cultivation has many advantages in terms of enhancing food safety, because of the controlled conditions which limit the opportunities for contamination to be introduced. Despite the perception of greater food safety, hydroponic cultivation is not completely free of

risk. *Listeria. monocytogenes* has been detected in several instances on microgreens and shoots between 2018 and 2020 (Topalcengiz et al., 2024). Studies have found that spinach plants grown in hydroponic nutrient solution inoculated with *E. coli* experienced higher levels of bacterial internalization compared to seedlings grown in inoculated soil (Warriner et al., 2003). It was speculated that this could be due to the comparative ease of access to roots provided by the water, and due to greater competition from the soil microflora which could restrict root colonization by *E. coli*.

The Food and Drug Administration (FDA) has identified one of the primary shortcomings of preventative food safety practices in the hydroponic industry to be the lack of disinfection or treatment of the recirculating nutrient solution once it is added to a system (U.S. Food and Drug Administration, 2021). This is one of the challenges the industry is currently trying to address by finding new disinfection methods that can be applied to the nutrient solution while crops are actively growing, without compromising plant growth. Various chemical interventions have negative impacts on crop growth (Mensah, 2023). Research by our team has indicated that UV treatments of hydroponic nutrient solutions during the growth period effectively reduced *E. coli* by 1.36 to 1.46 log CFU/ml (Moore et al., 2025), and have no detectable impact on crop physiological parameters including plant growth, chlorophyll content, and chlorophyll fluorescence in romaine lettuce.

Chemical interventions and physical interventions (such as Ultraviolet Light (UV)) target any and all microorganisms in the nutrient solution indiscriminately. While they are very effective at reducing pathogen inoculum, they could have non-target effects on other microorganisms existing in the nutrient solution, many of which benefit plant growth and health (Vlasselaer et al., 2024). This natural microbiome, which is present in all hydroponic systems,

can also contain plant pathogens, which can easily spread throughout a crop via shared recirculating nutrient solution. Many biocontrol products exist to combat such plant pathogens, relying on the principle of bacterial competition as well as other antagonistic strategies to do so (Junaid et al., 2013).

In addition to those that help control plant pathogens, other bacterial products are available that seek to amplify the beneficial and plant-growth-enhancing properties of various microbes in the rhizospheres of hydroponic crops. One of these, Mammoth P, also claims it can "shield the plant rhizosphere by outcompeting potentially harmful pathogenic microbes". It contains four different beneficial bacterial strains: *Citrobacter freundii, Enterobacter cloacae, Pseudomonas putida,* and *Comamonas testosterone* (Baas et al., 2016). Of these bacteria, certain species of *Pseudomonas* have been identified as promising candidates for biocontrol applications specifically in hydroponics (Vlasselaer et al., 2024), though the main function of the Mammoth P consortium is to increase mobilization of phosphorus for plant uptake.

While separate examples exist showing the efficacy of microbial inoculants to combat plant pathogens and to enhance plant growth outcomes, it is not clear if a biocontrol product could simultaneously improve plant growth while limiting the growth of human pathogens. This study aimed to establish a baseline for the pathogen reduction potential of a commercially available PGPB product when compared to a UV intervention by recording the average log CFU/ml of a surrogate strain of *E. coli* in hydroponic nutrient solution in systems receiving both kinds of treatments to the average log CFU/ml of the nutrient solution in control systems. In addition to the reduction potential of the treatments, the study sought to compare the effects of both types of treatments on plant health through measurements of height and SPAD value.

MATERIALS AND METHODS

Three replicate NFT hydroponic systems were assigned to each of the three treatment groups (control, UV, and biocontrol) in a structured non-randomized manner, resulting in a total of nine systems used in each of the three repetitions of the 11-day experiment. The nine systems were placed within five hydroponic towers (Farm 48 and 24+ models, AeroGrow International, Inc., Boulder, Colorado) consisting of stackable frames with built-in full-spectrum LED light panels. The layout of the systems can be seen in Figure 4.1. All systems were housed indoors, in the BSL2 Fresh Produce Safety Laboratory at Kansas State University Olathe.

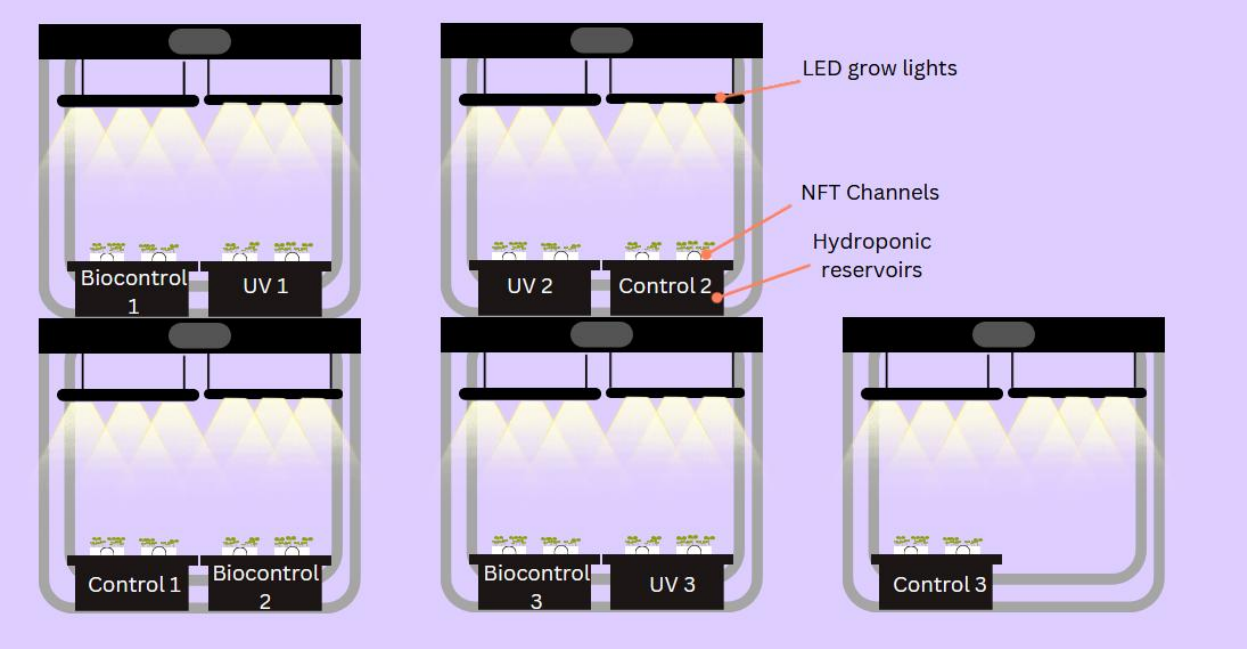

Figure 4.1 The nine NFT hydroponic systems used in the experiment were arranged in a structured non-randomized design with regards to treatment group assignments and the upper and lower levels of the towers in which they were housed.

Preparation of Hydroponic Systems. Nine NFT desktop systems (CropKing Inc., Lodi, Ohio) were filled with eight liters of distilled water and equipped with adjustable flow rate pumps (Vivosun, Ontario, California). The NFT systems were plugged in and water flow through

the channels was visually confirmed. Pumps were taped to ensure they stayed set to an average flow rate of 823 ml/min. Systems were positioned perpendicularly inside AeroGarden Farm 24+ towers, and lighting panels were adjusted to a height of 49cm above the reservoir lids. NFT Systems were plugged into timers which allowed them to run for one minute every two hours, from 8 am to 8 pm every day in accordance with the recommendation to keep the BioStrate moist but not wet (*Ultimate Guide*, n.d.). Each tower was plugged in and lights were set to a 14-hour photoperiod from 7 am to 9 pm to achieve a target Daily Light Integral of 12 mol/m^2/d. BioStrate growing media (CropKing Inc., Lodi, Ohio) was cut into strips that filled one channel evenly, wetted in the reservoir of the system in which they were to be placed, and shaped with two small parallel folds down the center to act as a channel to reduce the displacement of seeds due to water flow. Mesh screens were installed at the draining end of each channel to prevent seeds and other debris from washing into the reservoir.

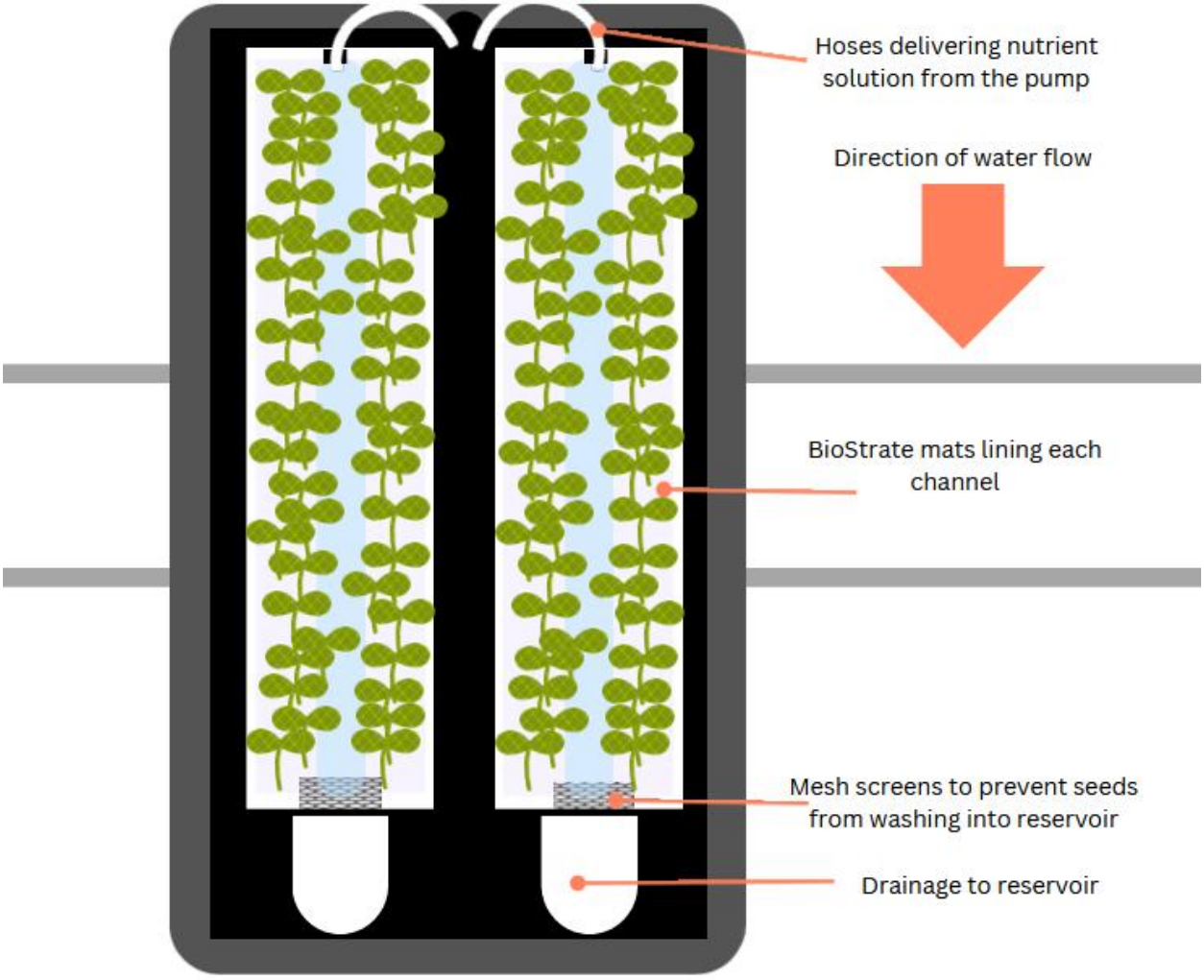

Figure 4.2 One hydroponic system as viewed from above, showing two NFT channels lined with BioStrate and the approximate positioning of kale seedlings.

Growing conditions. Hydro-Gro Leafy fertilizer with 4.30% N, 9.30% P205, 35% K20, 3.9% Mg, 200 mg/kg B, 105 mg/kg Cu, 2100 mg/kg Fe, 190 mg/kg Mn, 42 mg/kg Mo, and 210 mg/kg Zn (CropKing Inc., Lodi, Ohio) and calcium nitrate fertilizer with 15.5% N and 19.0% Ca were mixed with distilled water to create stock solutions according to manufacturer recommendations, which were added to the reservoirs to adjust EC within the range of 1.2 - 1.6. Additionally, pH was raised with 1% KOH and lowered with 1% H_2SO_4 to the range of 5.5 -

6.0. 1.7 g (± 0.09 g) of kale (*Brassica napus*, 'Red Russian') microgreen seeds (Johnny's Selected Seeds, Fairfield, Maine) were scattered across the BioStrate mat uniformly, avoiding only the center portion between folds.

***E. coli* Culture Preparation**. A 100 ml composite sample was collected from all systems and used to perform an IDEXX Colilert test, which tests for the presence of coliforms (and *E. coli* specifically), in order to ensure none was present prior to inoculation. A 48 hr subculture of rifampicin resistant *E. coli* (ATCC# 25922) was washed, harvested, and 8 ml of the resulting inoculum was added to all of the systems to achieve an initial concentration of approximately 6 log CFU/ml, confirmed by plating dilutions of the inoculum. Pre-treatment water samples of 10ml were collected after thoroughly mixing the nutrient solution of the reservoirs. Serial dilutions were made from the samples, and were subsequently spread plated onto tryptic soy agar with 80 µg/ml rifampicin (Remel, Inc.).

Biocontrol Treatment. After the *E. coli* had been added to all reservoirs, 1.27 ml of Mammoth P (Growcentia, Inc., Loveland, Colorado) was added to the 8L of distilled water in each system assigned to the biocontrol group, according to manufacturer recommendations. The same quantity of Mammoth P was added every seven days, once again in accordance with the manufacturer recommended usage.

UV Treatment. UV treatments were performed at 6 L/min, pumping water out of the NFT reservoir, through the UV chamber, and into a sterile whirlpak bag to ensure the full volume of water receives the UV treatment. The pump direction was reversed to return nutrient solution to the reservoir, keeping the UV lamp on. Following each treatment, hose ends were sterilized with 70% ethanol, and 6 L of DI water was pumped through the system to clean it. No sanitizers were used in order to prevent the potential of introducing residual sanitizers into the

reservoir, and thereby adversely impacting bacterial populations. Post treatment samples of 10ml were collected and plated.

Data Collection. In addition to the pre and post treatment samples on the first day of the experiment, water samples were taken four times per system over each of the three repetitions of the 11-day experiment. Samples were used to make serial dilutions and spread plated. Plant height and nutrient solution pH and EC were recorded three times per week during the 11-day growth period.

Harvesting Method. Water pumps were unplugged, and 10ml nutrient solution samples were collected from the reservoirs and used to make serial dilutions which were spread plated. All microgreen plants were harvested from both channels of each system by cutting the shoots with scissors. The total fresh weight was recorded by channel using an Ohaus CS200P balance. Three random samples of individual plants from each channel were selected, and the SPAD value of each was measured.

An approximately 1in×1in square of BioStrate with root tissue was cut from the bottom right corner of each channel using sterile scissors and forceps. Samples were placed in whirlpak bags filled with 20 ml 0.1% BPW and homogenized for 30 seconds. Serial dilutions were made from the bags and spread plated. Hydroponic systems were disinfected with a minimum of 200 ppm chlorine between each repetition of the 11-day trial and thoroughly rinsed to remove any chlorine residue.

Data analysis. A Kruskal-Wallis test was performed to assess the impact of the treatment group (biocontrol, UV, or control) on log CFUs. Upon obtaining a significant treatment effect, a Dunn's post-hoc test with Bonferroni correction was performed to determine which pairwise comparisons yielded significant differences. Other variables were analyzed with a one-way

ANOVA when the model yielded residuals that were sufficiently normal in distribution, otherwise Kruskal-Wallis was employed. Data was analyzed using R version 4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

The Krusk al-Wallis test indicated that the treatment group had a significant ($p < 0.05$) impact on log CFU/ml. The Dunn's post hoc test revealed significant differences between UV and control groups as well as biocontrol and UV groups, but upon Bonferroni correction, only the UV and control difference remained significant.

Table 4.1 Dunn's Post-Hoc Test on Water Sample log CFU/ml

Pairwise Comparison	Mean difference	p-value (unadjusted)
Biocontrol-Control	-0.30	1.00
Biocontrol-UV	2.28	0.03
Control-UV	2.58	0.02

Plant height values were on average observed to be higher for UV systems and lower for biocontrol systems, but there were no statistically significant differences in height between any of the treatment groups.

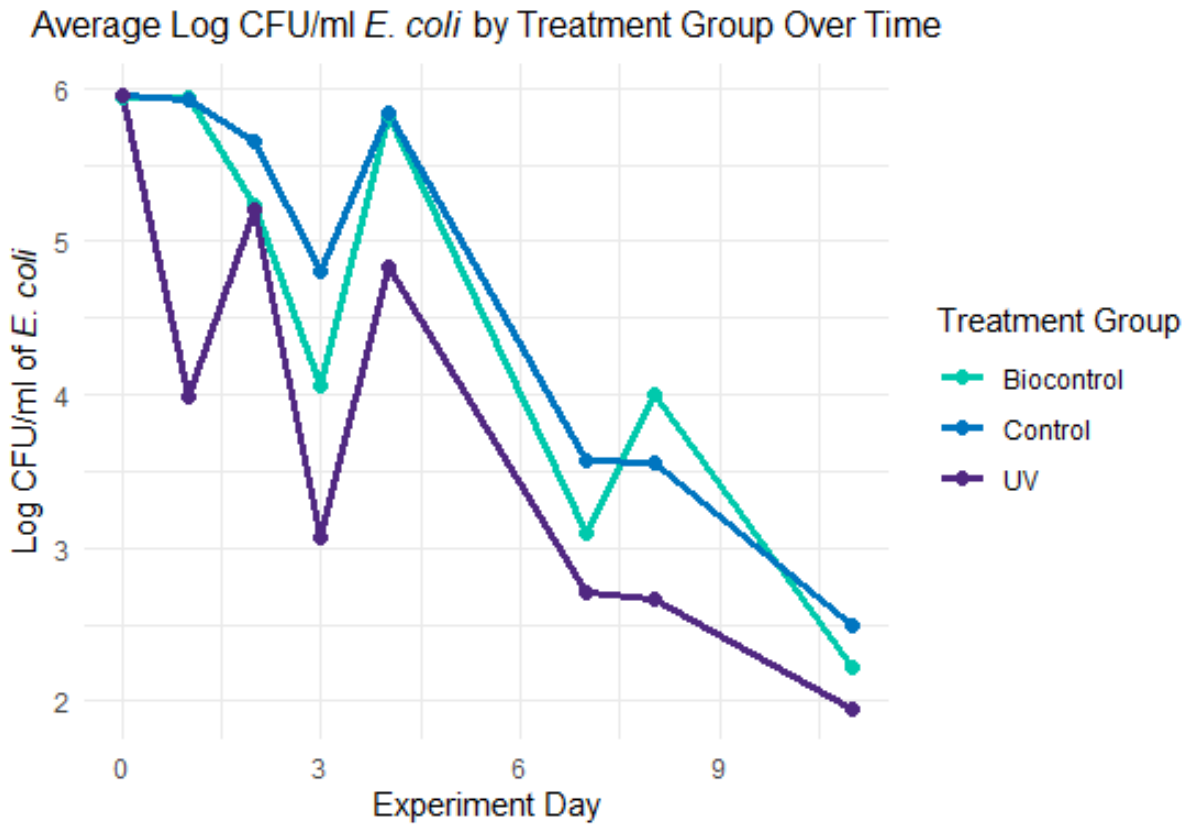

Figure 4.3 Log CFU/ml of *E. coli* declined over the 11 days after propagation for all systems in all treatment groups.

Table 4.2 Average Log CFU/ml of nutrient solution declined over the duration of the experiment for all treatment groups

Experiment Day	Treatment	Average Log CFU/ml
0	Biocontrol	5.94
0	Control	5.95
0	UV	5.95
1	Biocontrol	5.94
1	Control	5.92
1	UV	3.99
2	Biocontrol	5.24
2	Control	5.66
2	UV	5.21

3	Biocontrol	4.06
3	Control	4.81
3	UV	3.07
4	Biocontrol	5.83
4	Control	5.84
4	UV	4.84
7	Biocontrol	3.09
7	Control	3.58
7	UV	2.70
8	Biocontrol	4.00
8	Control	3.56
8	UV	2.66
11	Biocontrol	2.23
11	Control	2.50
11	UV	1.95

Between the first day of the experiment and harvest, the nutrient solution in UV-C systems had the highest reductions for *E. coli* (4.0 log CFU/ml), which was a significantly greater reduction compared to the reduction in the nutrient solution in control systems (3.45 log CFU/ml). Reductions in the biocontrol nutrient solution were neither significantly different from those in the UV-C treated nutrient solution, nor the control systems.

Table 4.3 Total average reductions of E. coli between propagation and harvest in nutrient solution ranged from 4.00 to 3.45 log CFU/ml depending on the treatment group.

Treatment	Day 0 Average Log CFU/ml	Day 11 (Harvest) Average Log CFU	Total Reduction
UV	5.95	1.95	4.00
Biocontrol	5.94	2.23	3.71

Control	5.95	2.50	3.45

Most studies applying bacterial biocontrol products to hydroponic systems are attempting to mitigate plant pathogens rather than human pathogens, so successful reduction is measured in "disease incidence" rates and severity for plants in the systems, rather than direct pathogen enumeration (Ayaz et al., 2023; Siddiqui & Shaukat, 2002; Thongkamngam & Jaenaksorn, 2017). Therefore, the literature did not provide a clear benchmark on what kind of reductions in log CFU could be expected from the biocontrol group in this study.

The legal priority in the U.S. is for growers to completely avoid harvesting and distributing produce contaminated by human pathogens, as outlined by the FDA (FSMA Final Rule on Produce Safety, 2024). There is effectively zero tolerance for human pathogens in fresh produce due to the risk to human lives. Plant pathogens face more leniency, as they pose a risk of economic losses to growers but not an immediate threat to human health, in much the same way as other pests that impact crops. This is one reason that growers may employ more casual and integrated preventative measures against plant pathogens, in comparison to strict food safety practices developed against human pathogens.

While there were fewer colonies of *E. coli* on average in BioStrate samples taken from the biocontrol systems, this difference was not statistically significant.

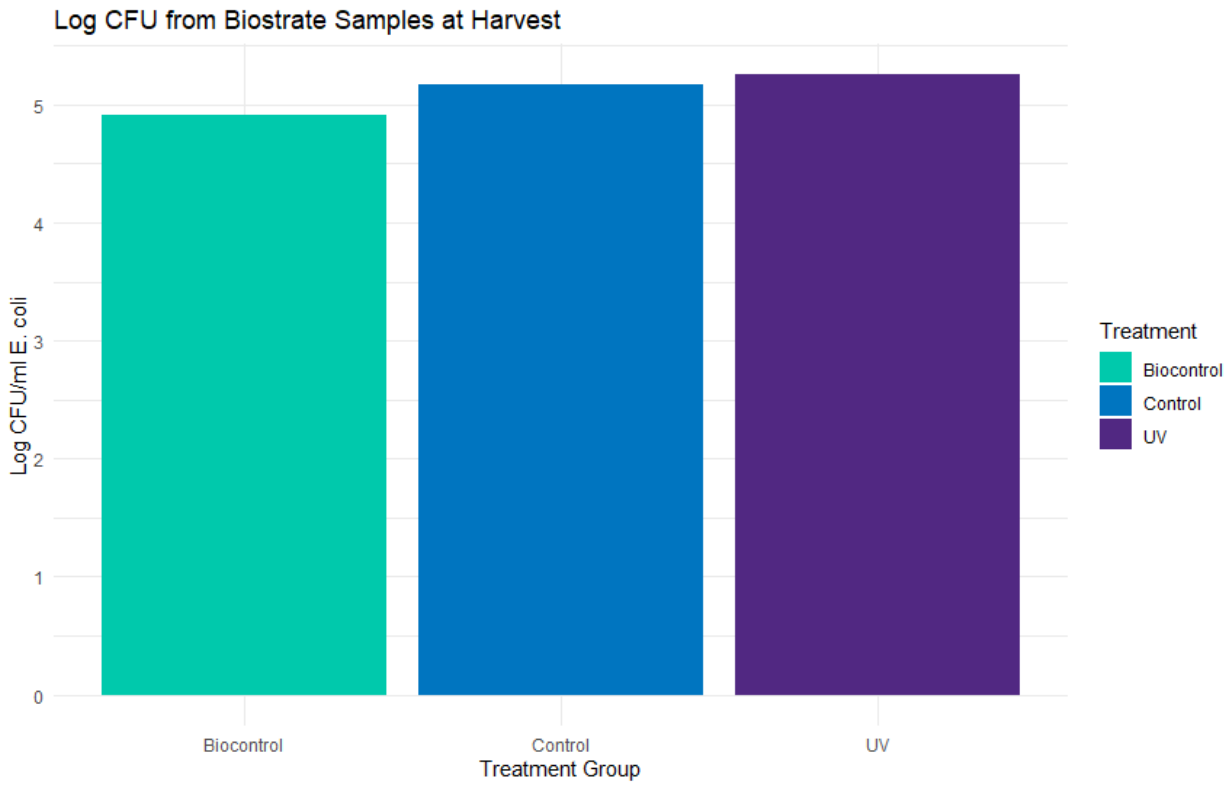

Figure 4.4 Log CFU/ml of E. coli in samples of BioStrate with embedded root tissue collected on the day of harvest were not significantly different between treatment groups.

Height was recorded after seeds had germinated and it was logistically feasible to take measure height, which occurred on average by day four.

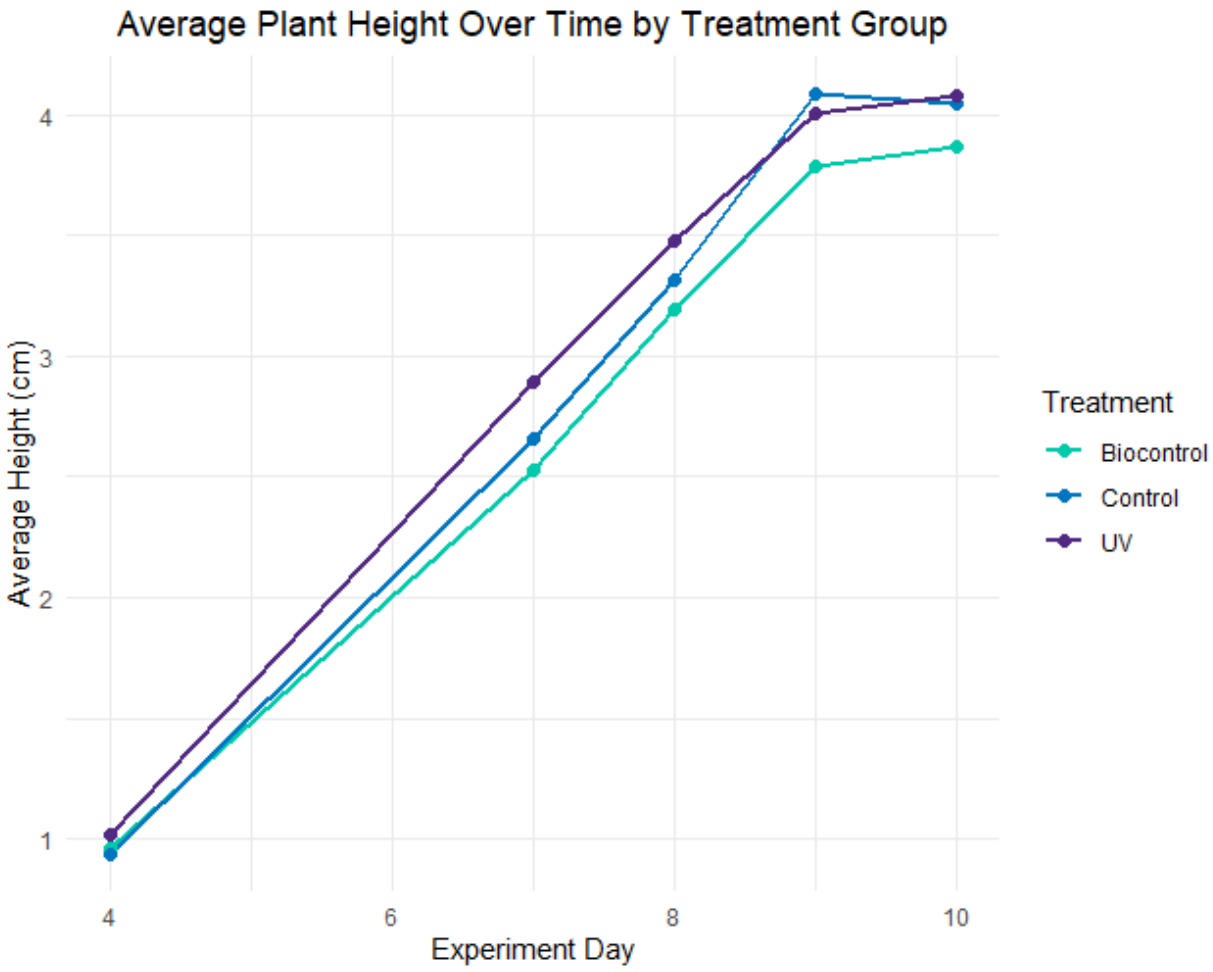

Figure 4.5 Average kale microgreen height increased as expected over time, with no significant differences between treatment groups.

At harvest, the average heights ranged from 3.87 cm in the biocontrol group to 4.08 cm in the UV group as seen in Figure 4.5. Differences between groups were not statistically significant ($P > 0.05$).

The highest average SPAD values were observed in the samples from the biocontrol systems, but differences in SPAD values were not statistically significant between treatment groups.

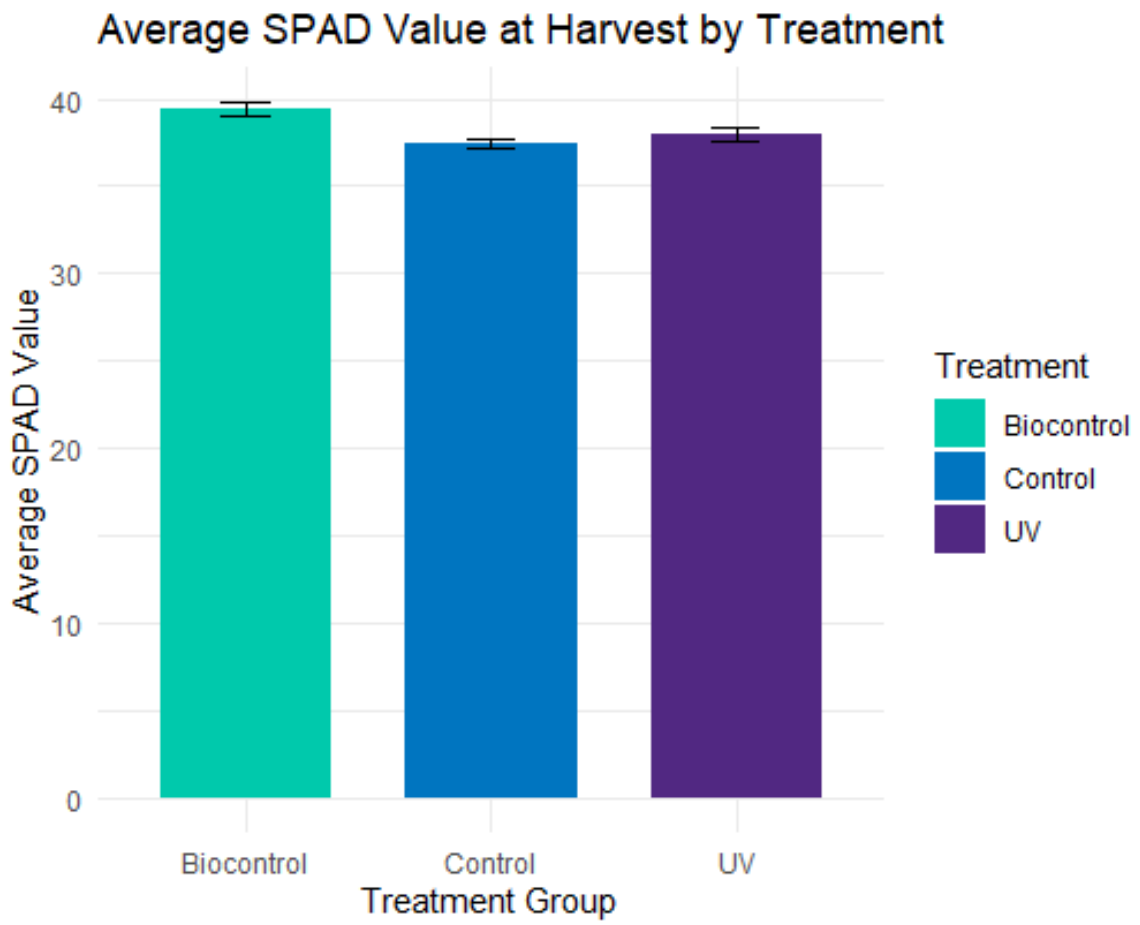

Figure 4.6 Average SPAD value at harvest was not significantly impacted by treatment group.

Average fresh weight was highest in UV systems, but this difference was not statistically significant.

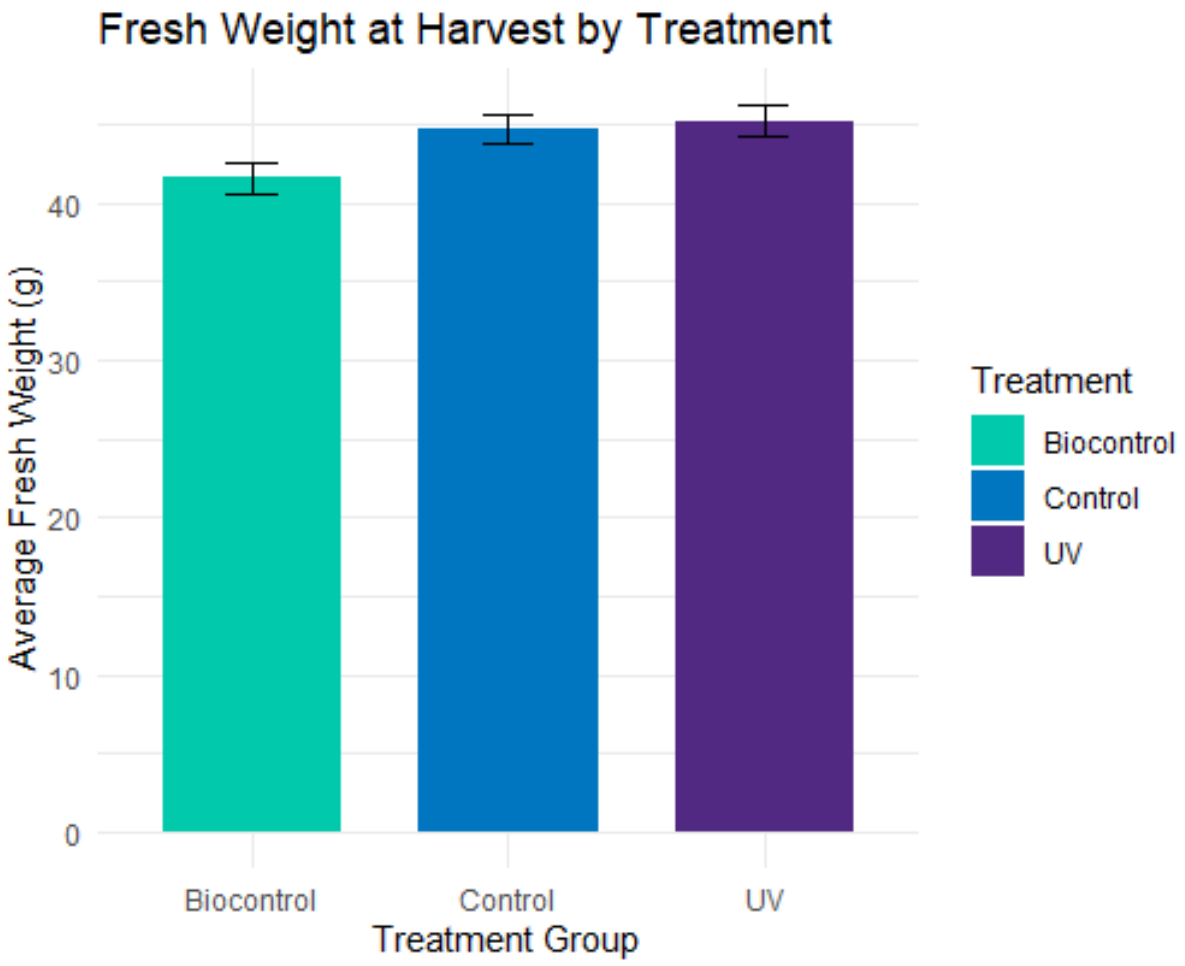

Figure 4.7 There was no significant difference in the average fresh weight per NFT channel between the treatment and control groups.

No significant differences were observed in crop growth parameters between any of the groups, indicating that this particular PGPB product did not have measurable benefits on the height, SPAD value, or fresh weight of the kale microgreens used in this study. While no comparable studies on microgreens could be found, this contrasts with the findings of a report growing hydroponic lettuce with the addition of PGPB, in which the addition of PGPB was significantly associated with increased plant length, chlorophyll content, and biomass (Aini et al., 2024). It is possible that the longer growth period of lettuce allowed for the impact of the

biocontrol product to become clearer. Similarly, the limited timeline for the growth of microgreens may serve to obscure differences that could otherwise be observed over a longer time period.

CONCLUSION

Future research could compare the efficacy in human pathogen reduction by biocontrol products that are specifically designed to mitigate plant pathogens (rather than PGPB products which also happen to have some biocontrol functions) in hydroponic environments to UV interventions. Biocontrol products could also be evaluated against PGPB products in terms of impacts on plant health and growth, to see if there is a measurable benefit to plant physiology outside of the potential biocontrol functions of any biorational products. There is also a research gap pertaining to the specific functions of numerous bacterial strains found in soil and hydroponic rhizospheres, which could be evaluated for their potential impacts on pathogen reduction and plant growth.

Overall, this study has shown that when used as directed, a common PGPB inoculant product did not reduce a surrogate human pathogen in hydroponic nutrient solution as effectively as a UV-C treatment did. Other variations of this study that may be worth future exploration include higher dosages of PGPB products, examining impacts on crops with longer growth cycles, and testing an integrated approach combining both UV and a PGPB or biocontrol product. In addition, nutritional quality and phytochemical composition should be included as important parameters in future evaluations to better understand the overall impact on crop health and value. As the UV intervention provided immediate reductions after seeds were sown, it could potentially be applied first as a strong mitigation against any contamination introduced at the start of the growth cycle; the PGPB or biocontrol product could then be applied as a preventative

measure to keep pathogens under control throughout the rest of the crop growth period. Ultimately there are still many questions to be answered in order to find the best way to utilize biorational products to improve crop physiology and food safety.

Chapter 5 - Impact of Commercial Plant Growth Promoting Bacteria Products on Growth, Chlorophyll, Carotenoids, and Ascorbic Acid Content of Hydroponic Kale Microgreens

ABSTRACT

Efficient, intensive production of crops in the United States is heavily dependent on the use of fertilizers, which are often synthetically produced. While cultivation methods like hydroponics offer significant advantages in the efficient use of water, hydroponics are even more reliant on fertilizer inputs than traditional agriculture, due to inherent the lack of mineral material to provide essential plant micronutrients. Much like the essential plant nutrients provided by soil, the many microorganisms found in soil which aid in various aspects of plant growth are another factor that can be utilized in soil-based agriculture but may need to be deliberately included in hydroponic cultivation in order to optimize yields. Plant growth-promoting bacteria (PGPB) can be added to hydroponic nutrient solution, and different PGPB products claim to improve plant health through approaches including increasing the mobilization and efficient uptake of nutrients (Baas et al., 2016) or through less direct methods like competition with plant pathogens which could otherwise hinder plant health and performance.

This study compares the effects of two commercially available PGPB products on the growth and nutritional quality of hydroponic kale microgreens. Nutrient Film Technique hydroponic systems were used to grow kale microgreens for 11 days while evaluating the effects of two PGPB products on plants in NFT desktop systems. Parameters measured include height (which was recorded three times during the growth period), chlorophylls a and b, carotenoids, and ascorbic acid content (which were all determined upon harvest). While the microgreens

treated with the *Pseudomonas putida*-based PGPB product had a greater average height, chlorophyll a, and chlorophyll b, these values were not significantly different from the *Lactobacillus casei*-based PGPB product or the control group. Although the PGPB products investigated in this report show potential for improved crop performance, further studies using additional products, bacterial strains, and hydroponic crops will be needed to better understand the broader impacts of PGPB on parameters of crop growth.

INTRODUCTION

Crops have traditionally been grown in soil that is home to robust microbial communities, and in many cases these microbes are drawn to plants and function symbiotically with them. Root exudates provide a nutrient source that attracts microorganisms ranging from fungi and bacteria to protozoa and algae (Vlasselaer et al., 2024). Free living rhizobacteria are present in the root zone outside of the plant tissue, while symbiotic bacteria are endophytes which are present within plant tissue (Turan et al., 2016).

Some of these microbes can enhance plant growth directly by releasing bioactive compounds that help plants better endure abiotic and biotic stressors or help plants obtain key nutrients (Ayaz et al., 2023; Fanai et al., 2024). To assist plants in overcoming abiotic stress, PGPB can encourage the production of phytohormones, the expression of heat shock protein, the modification of root morphology, or the regulation of osmosis (Fanai et al., 2024).

However, plant pathogens can also indirectly influence the microbial community, because infected plants mounting an immune response will alter the composition of root exudates, which in turn precipitates changes in the microbial community (Vlasselaer et al., 2024). Certain oomycete pathogens have demonstrated an enhanced capacity for host colonization, aided by their release of proteins which repress beneficial bacteria (Gómez-Pérez et

al., 2023). This suggests an evolutionary incentive exists for plant pathogens to neutralize microbial competitors. For example, studies have shown that healthy hydroponic tomato plants has been observed to have significantly higher quantities of *Paenibacillus* spp., which is a group of beneficial bacteria antagonistic towards rhizogenic *Agrobacterium*, compared to infected plants (Vargas et al., 2021). This highlights the potential role of beneficial microbes in suppressing plant pathogens.

While PGPB are often recognized for their ability to directly stimulate plant growth, their protective role against pathogens may be equally important. These beneficial microbes can improve plant health not only by enhancing growth and quality but also by reducing disease incidence through microbial antagonism (Aini et al., 2024). This could be especially important in hydroponics, where plant pathogens like those causing hairy root disease can spread rapidly through shared, recirculating nutrient water and devastate crops of multiple species (Vargas et al., 2021). Suppressing these harmful plant pathogens is an indirect way in which PGPB can enhance plant growth outcomes.

PGPB primarily suppress harmful microbes by producing antimicrobial compounds. For instance, the production of protease can serve as a defense system against plant pathogens, and this variety of PGPB is thought to aid in plant growth indirectly via pathogen suppression (Fanai et al., 2024). Protease-producing bacteria include *B. subtilis* subsp. *natto*, *B. clausei*, *B. licheniformis*, *B. lentus*, *A. salinivibrio*, and *Cryptococcus aureus* (Kalaiarasi & Sunitha, 2009). Similarly, alpha-amylases antagonize plant pathogens by hydrolyzing the cell wall of the pathogen; they are produced by endophytes including *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus strearothermophilus,* and *Geobacilus bacterium* (Far et al., 2020). Several varieties of the *Pseudomonas* and *Bacillus* species also produce hydrogen cyanide, which can

inhibit a range of plant pathogens and pests by disrupting cellular respiration (Alemu, 2016; Sehrawat et al., 2022).

Bacteria that produce catalase are helpful to plants in mitigating the effects of oxidative stress. PGPB species with notable catalase activity include *B. marinus, B. insolitus, B. sphaericus, B. pasteurii, B. laterosporus,* and *B. badiu* (Talaiekhozani, 2013). These could help protect plants from potential negative impacts of lingering residue from hydrogen peroxide-based sanitizers used in cleaning hydroponic system components.

One of the direct mechanisms through which PGPB can encourage plant growth is the biosynthesis of plant hormones such as cytokines, auxins, and gibberellic acid. Bacterial species that encourage cytokine biosytnthesis include *Paenibacillus polymyxa, Rhizobium leguminosarum* and *Pseudomonas fluorescens* (Fanai et al., 2024). Cytokines are involved in regulating plant growth and in protection from drought stress (Mekureyaw et al., 2022). Gibberellic acid, which has been suggested to improve stress tolerance and induce growth in plants, is produced by *B. pumilus* and *Bacillus licheniformis* (Gutiérrez-Mañero, 2001). Production of physiologically active IAA (Indole-3-acetic acid) has been linked to rhizospheric bacteria including *Arthrobacter spp. Bradyrhizobium, Bacillus, Pantoea, Rhanella, Burkholderia, Arthrobacter, Herbaspirillum, Pseudomonas, Enterobacter, Mesorhizobium,* and *Brevundimonas* (Prasad et al., 2019). IAA is the main auxin which helps with leaf formation, root development, phototropism, and fruit development (Chandra et al., 2019) among other critical elements of plant growth, underscoring the beneficial role these plant hormones synthesized by bacteria could have on plant growth.

In addition to the production of plant hormones, PGPB can also assist with the availability and uptake of key plant nutrients. Nitrogen, as a key component of chlorophyll and

driver of plant growth, must be fixed from the atmosphere in order to be used by plants. Some of the bacteria which can fix nitrogen through symbiotic relationships with plant roots include *Bradyrhizobium, Mesorhizobium, Sinorhizobium, Azhorhizobium, Pararhizobium, Neorhizobium*, and *Pseudomonas* (Nascimento et al., 2019). Other species which contribute to nitrogen fixation without the symbiotic relationships include *Achromobacter, Herbespirillum, Azoarcus* (Turan et al., 2016), *Glucanoacetobacter, Azoarcus, Azotobacter, Azospirillum, Acetobacter, Enterobacter, Burkholderia, Pseudomonas, Cyanobacteria* and *Diazotrophicus* (Basile & Lepek, 2021).

The other two plant macronutrients, phosphorus and potassium, are often naturally found in insoluble forms that are unavailable to plants. Phosphorus-solubilizing bacteria which enable this key nutrient to be taken up by plants include *Pseudomonas putida*, *Azospirillum lipoferum, Bacillus firmus* and *Bacillus polymyxa* (Mohamed et al., 2019), *P. chlororaphis, Serratia marcescens, B. subtilis, B. megaterium, Arthrobacter aureofaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis* (Kaymak, 2011)*, Burkholderia, flavobacterium, Rhizobium, Erwinia, Acetobacter, Micrococcus, Agrobacterium* and *Achromobacterium* (Youssef & Eissa, 2014). The bacterial species associated with potassium solubilization include *Bacillus megaterium, Arthrobacter* (Keshavarz Zarjani et al., 2013), *Bacillus mucilaginosus,*and *Paenibacillus mucilaginosus* (Hu et al., 2006). These bacteria produce organic acids which work on the insoluble potassium usually found in soil, and potassium itself activates enzymes involved in plant growth, and influences the quality of fruits and vegetables (Prajapati & Modi, 2012).

While many species have been identified to contribute to plant growth through enhanced nutrient uptake, only a select few of those species has become widely used in commercial settings (Fanai et al., 2024). Formulations of PGPB products for commercial use face pressure to

achieve the maximum overall gains in crop yield with the most efficient use of bacterial strains. One approach to this challenge is to combine multiple bacteria into a consortium with a variety of benefits. Combinations of *B. subtilis, A. brasilense,* and *P. fluorescens* coupled with phosphorus pentoxide have been found to increase sugarcane yields (Fernandes et al., 2023). Additionally, consortia of nitrogen-fixing *A. subtilis* and *A. brasilense* improved parameters including root and shoot development, leaf chlorophyll, transpiration, and carbon dioxide uptake (Galindo et al., 2024). Most commercially available PGPB take advantage of this strategy, and thus it becomes more useful to examine the efficacy of such products in terms of whole-plant health and growth outcomes rather than trying to isolate the impact of any one bacterial species.

The PGPB products selected for this study met specific criteria: they were formulated for hydroponic use, clearly listed all bacterial strains included, and contained minimal additional ingredients. In contrast, mycorrhizal fungi products are typically sold as a powder or inoculant intended for direct application to plant roots during seedling transplantation, with claimed benefits such as enhanced root development and nutrient uptake (*Vital Roots*, 2025). However, because their effectiveness relies on more established root systems, they are not well-suited for hydroponic systems or microgreen cultivation in shallow substrate mats. Therefore, mycorrhizal products were excluded from this study.

Research is limited on the applications of PGPB in hydroponics, particularly with regards to broad, whole-plant physiological outcomes. It is even more limited with regards to microgreen production, with most studies focusing on crops with a much longer growth period. Past studies have usually focused on things like attempting to identify the composition of hydroponic microbiomes through genetic sequencing (Vargas et al., 2021), assessing the impact of PGPB strains on crops grown in soil in combination with specific fertilizer treatments (Fernandes et al.,

2023; Lima et al., 2024), determining the mechanism through which a bacterial species is able to benefit a plant as described above, or culturing a specific strain of bacteria in a laboratory setting for use in experiments (Aini et al., 2024; Yedidia et al., 2001). By contrast, this study explores which available strains or other characteristics might make a commercially produced PGPB product effective and useful for hydroponic growers who are interested in improving yield. Additionally, this study seeks to determine which kind of PGPB products may be associated with higher concentrations of desirable phytochemicals, including carotenoids and vitamin C, in hydroponic microgreens.

MATERIALS AND METHODS

Three replicate NFT hydroponic systems were assigned to each of the three treatment groups (control, PGPB 1, and PGPB 2) in a structured non-randomized manner, resulting in a total of nine systems used in each of the three repetitions of the 11-day experiment. The nine systems were placed within five hydroponic towers (Farm 48 and 24+ models, AeroGrow International, Inc., Boulder, Colorado) consisting of stackable frames with built-in full-spectrum LED light panels. The layout of the systems can be seen in Figure 5.1. All systems were housed indoors, in the Fresh Produce Safety Laboratory at Kansas State University Olathe, and the experiment was repeated three times.

Nine NFT desktop systems (CropKing Inc., Lodi, Ohio) were filled with eight L of DI water. Adjustable flow rate pumps (Vivosun, Ontario, California) were added to each system, and set to ensure they were at the minimum flow rate possible, which was on average 823 ml/min. The pumps were controlled by timers (iPower, China), which were set to run for one minute every two hours, during a 12-hour period per day. The NFTs were positioned perpendicularly inside AeroGarden Farm 24+ towers (AeroGrow International, Inc., Boulder,

Colorado), with lights set to 14-hour photoperiods to achieve a target DLI of 12 mol/m^2/d based on an observed average PAR value of 357.59 µmol/m^2/s using an Apogee DLI-600.

BioStrate growing media (CropKing Inc., Lodi, Ohio) was cut into strips that filled one channel evenly, submerged in the reservoir of the system in which they were to be placed, and shaped with two small parallel folds down the center to act as a channel to reduce the water flow displacing seeds. Mesh screens were installed at the draining end of each channel to prevent seeds from washing into the reservoir. 1.7g (+/- 0.09) of kale (*Brassica napus*, 'Red Russian') microgreen seeds (Johnny's Selected Seeds, Fairfield, Maine) were scattered across the mat uniformly.

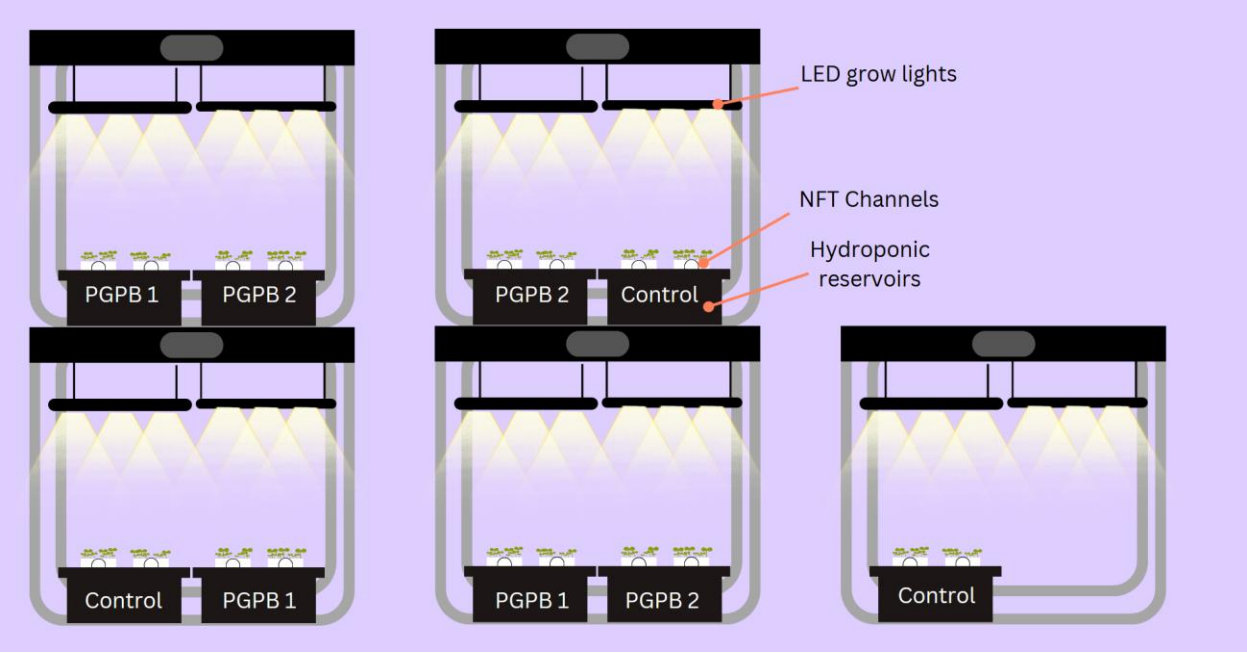

Figure 5.1 Schematic layout of the hydroponic systems and vertical towers used in the study. Treatment groups (PGPB 1, PGPB 2, and Control) were assigned in a structured, non-randomized design in order to ensure even distribution within the towers.

Hydro-Gro Leafy fertilizer with 4.30% N, 9.30% P205, 35% K20, 3.9% Mg, 200 mg/kg B, 105 mg/kg Cu, 2100 mg/kg Fe, 190 mg/kg Mn, 42 mg/kg Mo, and 210 mg/kg Zn (CropKing

Inc., Lodi, Ohio) and calcium nitrate fertilizer with 15.5% N and 19.0% Ca were mixed with distilled water to create stock solutions according to manufacturer recommendations, which were added to the reservoirs to adjust EC within the range of 1.2 - 1.6. The pH was adjusted to the range of 5.5 - 6.0 by using 1% KOH to raise the pH and 1% H_2SO_4 to lower the pH. These levels were maintained throughout the growth period with monitoring and adjustment being conducted three times per week. Systems were assigned to treatment groups – either PGPB 1, PGPB 2, or control – in order to ensure equal distribution of all groups between lower and upper systems. Three systems were assigned to each group.

The first PGPB product used in this study (assigned the identifier "PGPB 1") contains four beneficial bacterial strains: *Citrobacter freundii, Enterobacter cloacae, Pseudomonas putida, and Comamonas testosterone*. This microbial consortium is reported to be particularly effective in solubilizing mineral phosphorus and increasing the phosphorus available to plants (Baas et al., 2016). Notably, *Pseudomonas putida*, the dominant strain by proportion, has demonstrated strong antagonistic activity against a variety of plant-associated bacteria, including the ability to disrupt and invade biofilms (Purtschert-Montenegro et al., 2022).

The other product tested in this study (PGPB 2) consists of water, organic molasses, and allegedly a blend of yeast and photosynthetic bacteria which are not listed on the product label. It also includes a labeled concentration of 1x106 CFU/ml of *lactobacillus casei*, a probiotic commonly used in fermented dairy products. *Lactobacillus casei* can function as a protective culture in raw fresh vegetable products like pre-washed salad mixtures, by competing with pathogens and producing antimicrobial compounds like organic acids and bacteriocins (Gobbetti & Minervini, 2014). Some strains have also shown protective effects against multi-drug resistant *Salmonella* (Gobbetti & Minervini, 2014), and various *Lactobacillus* strains have demonstrated

biocontrol potential against plant pathogenic fungi (Kharazian et al., 2017). Although no literature was found specifically evaluating *Lactobacillus casei* for biocontrol or plant growth promotion in hydroponic systems, the manufacturer claims that the proprietary effective microbials consortium has beneficial effects on various crop physiological parameters .

The PGPB products were added to the reservoirs at the time of sowing seeds and re-added every seven days in amounts corresponding to the manufacturer recommendations. For PGPB 1, Mammoth P (Growcentia, Inc., Loveland, Colorado), this was 1.27 ml per 8L of nutrient water. For PGPB 2, EM1 (TeraGainX, Tucson, Arizona), it was 10.42 ml per 8L of nutrient water. Height measurements were taken at three locations within each NFT channel 3 times throughout the growth period beginning four days after propagation. Six measurements were taken in each NFT system on each sampling day. The locations were approximately at each end and the middle of the growth channel, with a representative plant of average size being chosen to approximate the height for the location.

Harvest and postharvest methodology. Plants were harvested 11 days after seeds were added to systems. Harvesting was performed by cutting stems of plants with scissors, as near to the BioStrate mat as possible. At this stage, the majority of plants still only had cotyledons, but a few had started to sprout true leaves. For ascorbic acid determination, samples of 2g (+/- 0.01 g) of the harvested plants (comprised of cotyledons, stems, and in some cases early true leaves) were homogenized in 50 ml tubes with 20ml of an acid mixture containing 6% phosphoric acid and 2N acetic acid. Samples were frozen at -20C for later determination of ascorbic acid content in accordance with the recommended protocol (Pliakoni, Eleni & Kent, Tori, 2025).

Samples of 0.3g (+/- 0.02 g) collected for determination of chlorophylls a and b and carotenoids were homogenized in 30 ml centrifuge tubes containing 10ml of concentrated

methanol. These were incubated for 24-48hrs in darkness at 4°C. Following this, samples were adjusted to the same weight within 0.05g by removing volume as needed, and centrifuged at 1000 x g at 4°C for 10 minutes. After centrifuging, approximately 6.5 ml of supernatant was carefully pipetted from the tubes and transferred to a clear 10 ml glass test tube without disturbing the cell pellet at the bottom. The 10 ml tubes were then placed in a DR 3900 spectrophotometer (Hach) and readings were taken at 666, 653, and 470 nm. These values were used to calculate chlorophylls a and b, as well as total carotenoids, according to established convention from prior literature (Wellburn, 1994). Calculations were performed with the following equations from Wellburn 1994, based on the use of methanol as the solvent and the spectrophotometric resolution of 1nm, and yielding results in µg/ml:

$$\text{Chlorophyll A: } C_a = 15.65A_{666} - 7.34A_{653}$$

$$\text{Chlorophyll B: } C_b = 27.05A_{653} - 11.21A_{666}$$

$$\text{Carotenoids: } C_{x+c} = \frac{(1000A_{470} - 2.86C_a - 129.2C_b)}{221}$$

Results were then standardized by the extract volume (6.5 ml) and converted to µg/g of fresh weight by dividing the total µg by the fresh weight.

Data analysis. Observations of chlorophylls and carotenoids in the second of three trials were excluded due to highly unusual spectrophotometric readings, including some beyond the equipment's range which resulted in negative carotenoids values. Height values were excluded from analysis in systems where plants died due to seeds clogging pump components, determined by filtering out observations where all height observations in the system were less than 0.25 cm. A MANOVA model was used to assess the impact of treatment group upon concentrations of chlorophylls a and b, as well as carotenoids. A Shapiro-Wilks test was used to verify the normality of residuals after fitting the model, and a Levene test was used to confirm sufficient

homogeneity of variance. The impact of treatment on plant height and vitamin C content was assessed using a Kruskal-Wallis test. Data was analyzed using R version 4.3 (R Foundation for Statistical Computing, Vienna, Austria.)

RESULTS AND DISCUSSION

This study sought to examine the impacts of two different kinds of PGPB products on the physiological parameters of kale microgreens grown in NFT hydroponic systems, including height throughout growth, and levels of chlorophyll a & b, carotenoids, and ascorbic acid upon harvest. Height was measured starting from four days after propagation in each trial, and recorded on six sampling days in total by the time microgreens were harvested on day 11. PGPB 1 systems reached an average height of 4.41 ± 1.31 cm by harvest, compared to 4.17 ± 1.21 cm for control systems and 3.57 ± 1.14 cm for PGPB 2 systems. These differences were not statistically significant ($p = 0.08$).

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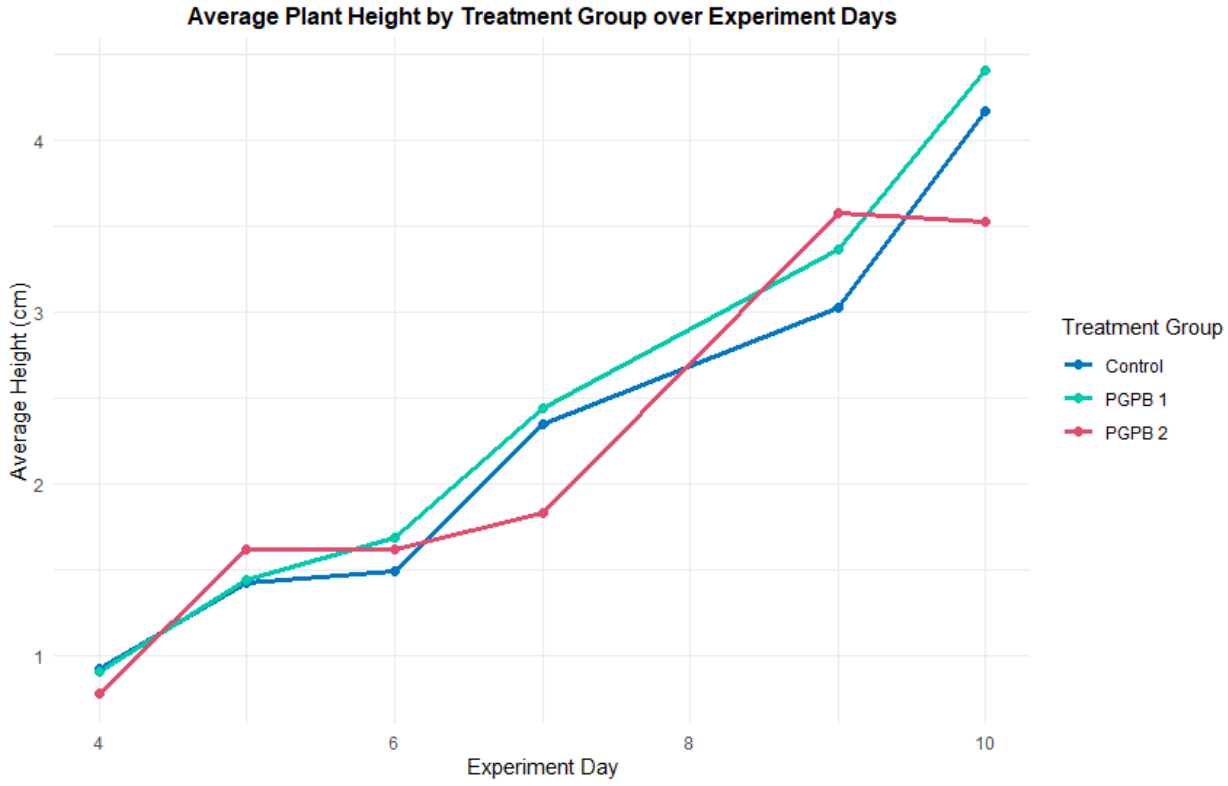

Figure 5.2 Average height (cm) of kale microgreens measured on Days 4, 5, 6, 7, 9, and 10 after propagation under control, PGPB 1 and PGPB 2 treatments in a vertical hydroponic tower during the study.

This study sought to quantify their actual impact of PGPB products on plant performance by measuring photosynthetic pigments and Vitamin C content in microgreen tissue. Chlorophyll, a key indicator of plant productivity, plays a central role in light harvesting during photosynthesis (Rabinowitch & Govindjee, 1965) Its concentrations can be influenced by various environmental stressors, such as temperature fluctuations, water availability, and nitrogen levels

(Zhang et al., 2021). Carotenoids not only extend the range of light absorption but also serve a protective role by mitigating photooxidative damage (Swapnil et al., 2021).

While chlorophyll concentrations can provide insight into plant performance and health, carotenoids and vitamin C are measured in this study primarily due to human nutrition and consumer appeal. As humans cannot synthesize vitamin C, it is an essential dietary component involved in neurotransmitters biosynthesis, connective tissue formation, iron absorption, and antioxidant defense (*Vitamin C - Health Professional Fact Sheet*, n.d.). Similarly, carotenoids are valued in the human for their role as precursors to vitamin A, which assists in growth, vision, and immune functions within the human body, as well as their antioxidants properties (Almodaifer et al., n.d.).

The potential impact of PGPB on photosynthetic pigments is not entirely consistent based on findings in existing literature. One study found that a seed treatment with an extract from the unicellular alga *Chlorella vulgaris* increased the concentration of chlorophyll b in *Eruca sativa* microgreens, but at lower treatment levels the effect was smaller or even negative on concentrations of chlorophyll a and carotenoids (Del Prete et al., 2025). Another study applying *Bacillus thuringiensis* as a biostimulant on pea microgreens found the treatment resulted in a significant increase in chlorophyll b and carotenoids, but did not impact chlorophyll a levels (Pérez-Leal et al., 2025). No such differences were observed in the research conducted in this study due to the PGPB treatments.

The PGPB 1 systems had the highest mean concentrations of chlorophyll a (461 ± 21.78 µg/g) and chlorophyll b (294.64 ± 35.99 µg/g. The PGPB 2 systems had the highest mean

carotenoid concentrations (69.67 ± 31.77 µg/). None of these were statistically significant in their differences from the control group ($P = 0.18$).

This study sought to quantify their actual impact of PGPB products on plant performance by measuring photosynthetic pigments and Vitamin C content in microgreen tissue. Chlorophyll, a key indicator of plant productivity, plays a central role in light harvesting during photosynthesis (Rabinowitch & Govindjee, 1965) Its concentrations can be influenced by various environmental stressors, such as temperature fluctuations, water availability, and nitrogen levels (Zhang et al., 2021). Carotenoids not only extend the range of light absorption but also serve a protective role by mitigating photooxidative damage (Swapnil et al., 2021).

While chlorophyll concentrations can provide insight into plant performance and health, carotenoids and vitamin C are measured in this study primarily due to human nutrition and consumer appeal. As humans cannot synthesize vitamin C, it is an essential dietary component involved in neurotransmitters biosynthesis, connective tissue formation, iron absorption, and antioxidant defense (*Vitamin C - Health Professional Fact Sheet*, n.d.). Similarly, carotenoids are valued in the human for their role as precursors to vitamin A, which assists in growth, vision, and immune functions within the human body, as well as their antioxidants properties (Almodaifer et al., n.d.).

The potential impact of PGPB on photosynthetic pigments is not entirely consistent based on findings in existing literature. One study found that a seed treatment with an extract from the unicellular alga *Chlorella vulgaris* increased the concentration of chlorophyll b in *Eruca sativa* microgreens, but at lower treatment levels the effect was smaller or even negative on concentrations of chlorophyll a and carotenoids (Del Prete et al., 2025). Another study applying *Bacillus thuringiensis* as a biostimulant on pea microgreens found the treatment resulted in a

significant increase in chlorophyll b and carotenoids, but did not impact chlorophyll a levels (Pérez-Leal et al., 2025). No such differences were observed in the research conducted in this study due to the PGPB treatments.

The PGPB 1 systems had the highest mean concentrations of chlorophyll a (461 ± 21.78 µg/g) and chlorophyll b (294.64 ± 35.99 µg/g. The PGPB 2 systems had the highest mean carotenoid concentrations (69.67 ± 31.77 µg/). None of these were statistically significant in their differences from the control group ($P = 0.18$).

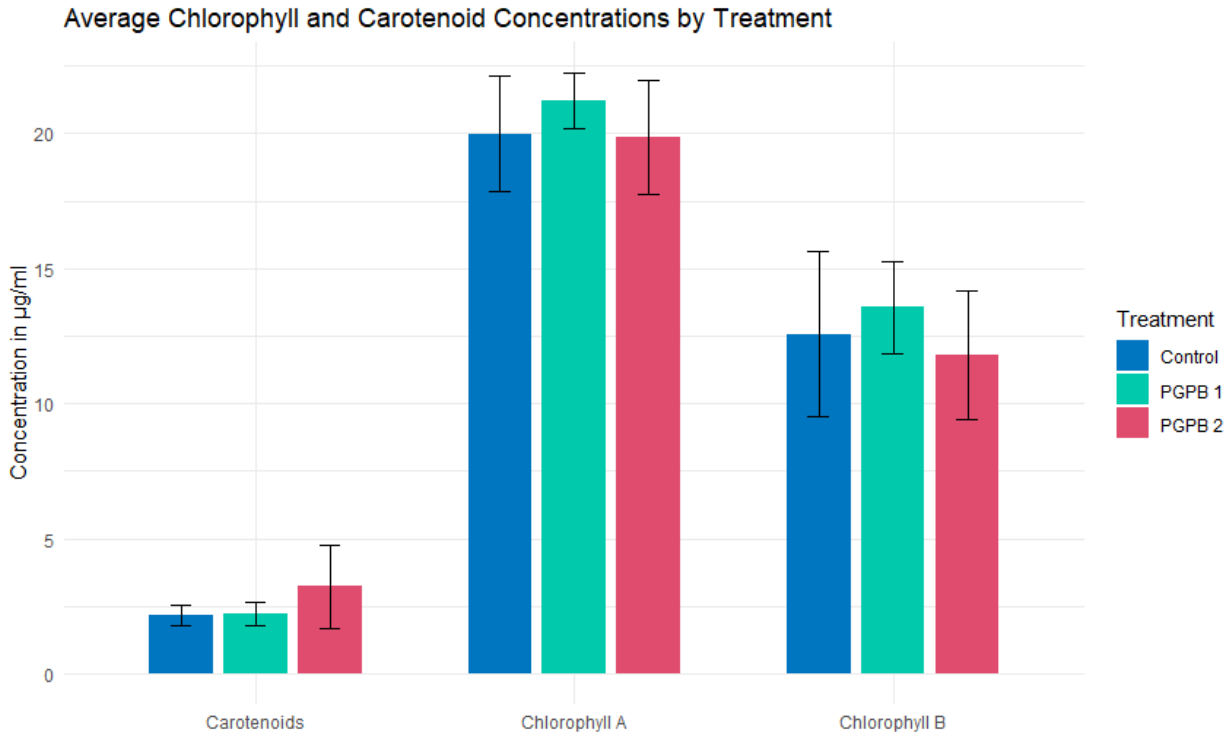

Figure 5.3 Average levels of chlorophyll a & b and carotenoids in µg/ml in kale microgreens from the treatment groups control, PGPB 1, and PGPB 2 upon harvest 11 days after propagation in NFT hydroponic systems.

Levels of carotenoids across all treatment groups in this study (on average about 55 µg/g of fresh weight) were much lower than the 222 µg/g found in another study examining the impact of photoperiods on kale microgreens (Liu et al., 2022). Chlorophyll a concentrations were

on average 580 µg/g for the 14 hour photoperiod in that study (Liu et al., 2022), compared to 442 µg/g on average in this study. Chlorophyll b levels were higher however, being 220 µg/g in that study (Liu et al., 2022) and 275 µg/g in this one. Differences could possibly be attributed to different cultivars used in the respective studies, since that study used Chinese kale instead of Red Russian, because microgreen species and cultivar are considered the major source of variation in mineral and phytochemical content in microgreens (Li et al., 2023).

There were no significant effects observed for vitamin C content among treatments (P = 0.98). The control group had a mean Vitamin C concentration of 17.6 ± 3.03 mg per 100 g of fresh weight, identical to that of PGPB 1 treatment. The PGPB 2 treatment showed the highest mean value at 18.5 ± 3.77 mg per 100 g fresh weight. Another study found that Chinese kale microgreens receiving a similar 14-hour photoperiod contained approximately 100 mg of vitamin C per 100 g of fresh weight (Liu et al., 2022).

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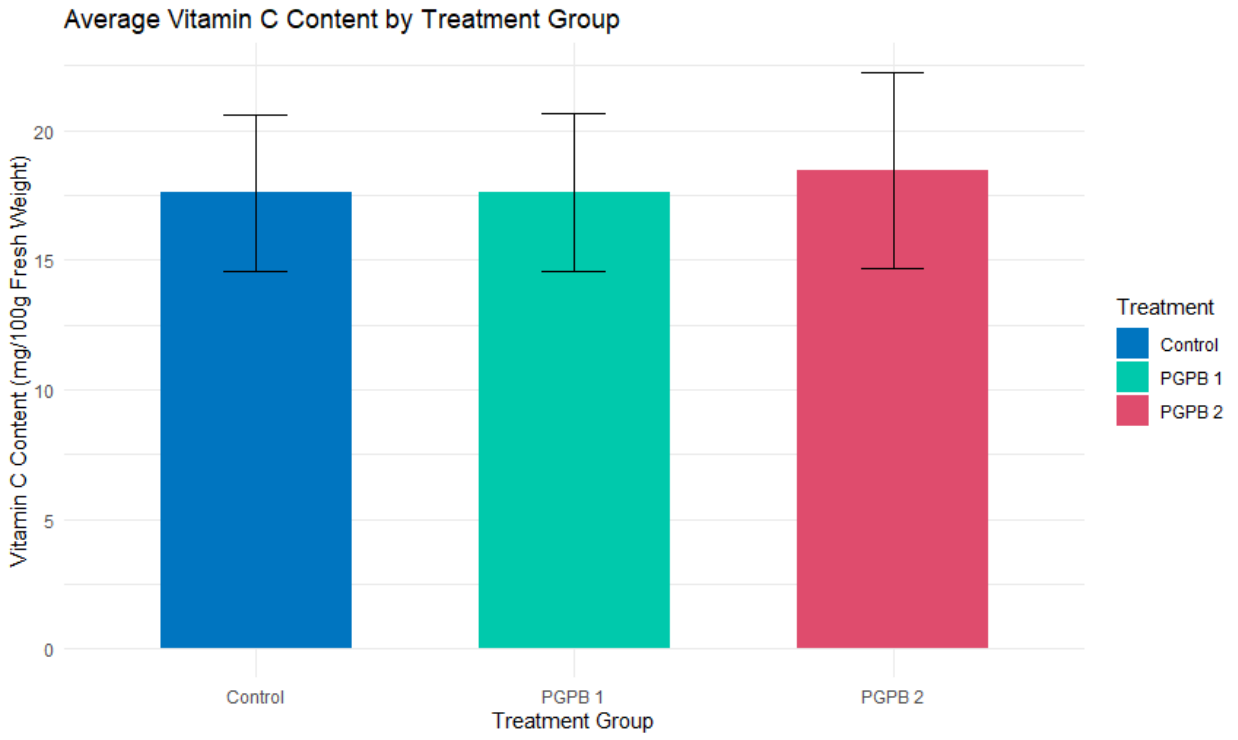

Figure 5.4. The average vitamin C content in mg per 100 g of fresh weight for kale microgreens grown for 11 days in NFT hydroponic systems in the control, PGPB1, and PGBP 2 treatment groups.

Most available sources claim that hydroponic kale microgreens contain an extremely high level of vitamin C, usually above 90 mg (Liu et al., 2022; Neves & Andrew, 2023). Vitamin C content observed in this study did not exceed 25 mg. This may be in part caused by different varieties of kale, or by different maturity standards for harvesting. Kale microgreens, like the microgreens of most plants, contain significantly higher concentrations of nutrients during the microgreen stage compared to later stages of plant development. Studies have shown significant

reductions in the nutrient content of Red Russian Kale microgreens harvested upon the emergence of two true leaves compared to the same microgreens harvested with only cotyledons (Waterland et al., 2017). While microgreens are not strictly defined by the presence or absence of true leaves, harvesting tends to occur at a time that maximizes the size of seedlings with cotyledons, which often overlaps the emergence of the first true leaves due to individual plant-level variation in germination and growth rates. This study chose a harvest date seeking to maximize height while minimizing the proportion of plants with true leaves, yet actual harvested plants did in many cases contain early true leaves. This later stage of maturity may contribute to lower concentrations of many nutrients and may partially explain the lower vitamin C levels.

Another possibility is that the nutrient solution may have contributed to the lower vitamin C content. Fertigation of microgreens typically results in significant increases in yield variables like height and fresh weight (Li et al., 2023), with the impact depending on the fertigation rate and the specific species in question. However, another study found that increasing the frequency of general all-purpose fertilizer applications beyond half of the days during cultivation could decrease the levels of vitamin C and minerals in spinach microgreens (Petropoulos et al., 2021). It is possible that the constant supply of nutrient solution in this study could have impacted the vitamin C levels in a similar way.

CONCLUSION

Other studies have found PGPB strains to have a significant positive impact on the accumulation of chlorophylls a and b and carotenoids in hydroponic microgreens. This study did not find any significant impact of either PGPB product on the chlorophyll and carotenoids of the kale microgreens. In fact, levels of the carotenoids and ascorbic acid were unexpectedly low for

the treatment and control groups in this study, which could possibly be due to genetic factors or a high concentration of nutrients in the hydroponic solution.

Future studies could test different concentrations of nutrient solution in this experimental design to determine if high nutrient availability may be linked to the lower levels of vitamin C recorded in this study. Future studies could also work to overcome the challenge of maintaining equally distributed seeds within channels; despite uniform flow rates being used throughout all systems, several systems regularly washed seeds to one end of the channel, leading to a denser grouping of seedlings. Other routes of expanding on this research could include testing more varieties of PGPB and comparing the effects on different kinds of hydroponic crops, including those with a longer time to maturity. Testing other crops or microgreen varieties may also help rule out any genetic explanations for the lower phytochemical concentrations observed in this study.

Chapter 6 - Conclusion

The objectives of this research included testing the microbial reduction efficacy of UV and biocontrol interventions on *E. coli* in the nutrient solution of recirculating hydroponic systems. Treatments were evaluated on the significance of reductions, as well as their impacts on plant growth parameters. UV treatments were found to significantly reduce *E. coli* levels in nutrient solution by 1.36-1.46 log CFU/ml. In romaine lettuce, the same treatment had no detectable impact on measured plant growth parameters, indicating that the treatment is safe to use during crop growth. PGPB products were associated with slightly greater declines in *E. coli* over time in hydroponic systems growing kale microgreens compared to control systems without PGPB, but those reductions were not as high as UV systems and were not significant. Additionally, no clear benefit to photosynthetic pigments or antioxidant content was observed in PGPB-treated microgreens.

A key limitation of the UV treatment performed in this research was its reliance on pumping nutrient solution out of the reservoirs, into a secondary container, and then back into the reservoirs. This would be a cumbersome process not likely to be adapted to commercial applications, and it also does not address any microbial colonization of surfaces such as substrate and water lines within the system. Additional limitations include the measurement of total nitrogen rather than its individual components, and the lack of plant tissue nutrient analyses to complement and elucidate photosynthetic pigment analyses for the PGPB studies.

Future research could examine more efficient ways to incorporate UV treatments into the hydroponic system without the necessity of pumping nutrient solution out of the reservoirs, or find ways to introduce antimicrobials into materials likely to support bacterial colonization, such as the rockwool growing media. Studies should also explore in more detail the specific

components of nitrogen present in nutrient solution before and after UV treatment, and verify if nitrate is being oxidized. It may also be worth confirming if iron levels in nutrient solution are being significantly impacted by UV treatments, and if this actually impacts iron availability or uptake for the plants. Studies with PGPB may apply the same treatment to crops other than microgreens to see if findings are consistent with this study, or if the nutrient solution may have any effect on vitamin C levels. Further investigation into the possibility of lower chlorophyll a, carotenoids, and vitamin C content should also be conducted, for different crops as well as alongside plant tissue nutrient analysis and assessment of photosynthetic performance.

Ultimately, food safety interventions should be proactive in nature and seek to prevent the introduction of contamination during cultivation, rather than relying solely on postharvest decontamination. This is especially true in the hydroponic production of leafy greens, which will be consumed fresh in most cases and could pose a severe food safety risk if contamination is introduced and not adequately addressed by postharvest treatments. This research has offered several approaches to integrate sustainable microbial interventions addressing bacterial human pathogens in the recirculating nutrient solution of hydroponic systems, without negatively impacting crop growth. Findings provide a baseline for future research to refine the application of UV and biocontrol interventions in hydroponic nutrient solution.

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