Innovations in ultraviolet-C technologies for fresh produce growers to reduce human pathogens in agricultural surface water and on fresh produce surfaces

by

Olivia C. Haley

B.S., University of South Carolina, 2015
M.Sc., McGill University, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
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Abstract

Ultraviolet-C (UV-C) irradiation is well-known for its high antimicrobial efficacy and low environmental toxicity compared to its chemical sanitizer counterparts. Although UV-C is well implemented in various food industries, the technology has yet to become popularized in the fresh produce industry. The purpose of this work was to generate knowledge and understanding towards innovations in UV-C technologies for use in the fresh produce industry.

The prevalence of generic *E. coli* in agricultural water samples (N= 426) from Kansas and Missouri was first assessed to determine their potential microbial risk. While there were no statistically significant differences detected between the two states ($P < 0.4023$), the average concentration of generic *E. coli* in surface water sources (158.7 most probable number [MPN]/100 mL; n= 247) was greater than that of ground water sources (20.4 MPN/100 mL, n= 179; $P < 0.0001$). These results indicate a need for safe, environmentally friendly agricultural water treatment options for fresh produce growers in the region.

As agricultural surface waters were shown to carry a significantly higher microbial risk than ground waters, a follow-up study was performed to test the efficacy of two UV-C devices to reduce generic *E. coli* populations in three on-farm agricultural surface water sources. A generalized additive model was used to determine the effect of water characteristics, flow rate, and UV-C device on the antimicrobial efficacy of a low power, low flow (1-9 gallons per minute (GPM), 1.34-gallon capacity) and a high powered, high flow (1-110 GPM, 4.75-gallon capacity) UV-C device at flow rates of 6, 7, and 9 GPM. From this data, an online tool was developed for growers to calculate the predicted efficacy of the devices to treat their agricultural water source.

A survey was then designed to explore the low adoption rate of UV-C devices by the Kansas and Missouri fresh produce growing community. The survey instrument measured grower knowledge of UV-C, their attitudes towards UV-C technology, and included a needs assessment to support the on-farm implementation of UV-C. There was a large variation in grower knowledge of UV-C (N=82) and stepwise regression (n= 62) revealed that overall attitudes were most influenced by grower knowledge of UV-C (P<0.0001), farm size (P=0.0199), farm income (P=0.1047), and state (P=0.1237). Overall, the responses indicate that there is an unmet need for more information regarding the benefits, costs, implementation strategies, and technical skills regarding UV-C devices for agricultural water treatment.

Further on-farm applications were investigated, notably, a UV-C treatment chamber fitted with UV-Light Emitting Diodes (LEDs) on all sides to provide simultaneous treatment of an entire fruit surface. A maximum 0.95-log reduction of generic *E. coli* was detected after 60 s of treatment in blueberries. After optimizing the chamber for improved thermoregulation and UV-C intensity, the chamber achieved a 1.48-log reduction of generic *E. coli* after 60s in fresh strawberries, indicating the technology's suitability for treating diverse fruits. Future study should focus on scaling this technology to fit the needs of the fresh produce industry.

In summary, these studies provide future research directions for on-farm UV-C innovations to improve produce safety. The studies demonstrate that there is a need for safe, effective, non-chemical agricultural water treatment methods – a niche that UV-C devices can be successfully fill. Yet there continues to be a knowledge gap that needs to be addressed to increase the produce grower adoption rate of on-farm UV-C devices. The fresh produce industry could also benefit from innovations in UV-C for whole-fruit treatment, but more research is needed to increase the scale of this technology to treat more fruits simultaneously.

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Dr. Manreet Bhullar

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Dedication

This dissertation is dedicated to my family and friends for their endless support despite my forgetful and neglectful nature throughout these past years. This degree was truly a labor of love. I am forever grateful for their never-ending understanding, undeterred by missed return calls, messages left unanswered, and plans which fell through at the last minute on account of my research. My friends – Enaam, Yeqi, Yvette, Yolanda, Clio – always encourage me and entertain my nonsense, and I look forward to seeing how our lives develop! I love you all.

To my family, thank you for always supporting my curiosity in the world. My earliest memories of science – besides sticking my hand in holes searching for snakes – was a breadboard I played with at Grandma and Grandpa's house (of course in between trying to peak at the 'killer robot' under Uncle's bed)! I'm sure it was difficult to raise a mad scientist, and thank you Mom for tolerating my bug collection, rock collection, leaf collection (and I'm sure many other 'collections'). My dazzling Sunfish, I am *constantly* learning from you, and one day hope to emulate your poise, confidence, and determination. Dad and Mrs. Toni, your advice and guidance in tough situations was invaluable, and thank you for encouraging me to lean on family for support. Jacqueline Louise, you've inspired me to work harder so I can become a scientist that leaves the world even the tiniest bit better for you. Finally, Xavier, you have been my rock, my soundboard, and one of my biggest cheerleaders these last couple of years, and I can't wait for many more.

Enfin, in the words of my Mom, 'I'm glad that bug collection paid off.'

Chapter 1 - The Growing Role of Ultraviolet Light Technologies in the Production and Processing of Fresh Produce: Trends and Challenges to its Implementation for Produce Safety and Quality

ABSTRACT

Ultraviolet (UV) light has been approved for use in the food industry for more than a decade, and there has been much research devoted to its use to improve fresh produce safety, quality, and shelf-life. However, little of this technology has been practically deployed. The purpose of this review is to provide a synopsis of the current state of UV-A, UV-B, and UV-C light technologies with an emphasis on applications during the on-farm production and processing of fresh fruits and vegetables. While there is recent literature reviewing the effects of UV on fresh produce quality, a review on its practical and emerging applications in the fresh produce industry is still missing. Preharvest on-farm UV applications discussed include agricultural water decontamination, with emerging applications begin soil quality improvement, seed decontamination and invigoration, and agrochemical residue removal. Regarding postharvest on-farm UV application, this review highlights the use of UV for whole-fruit produce decontamination, quality, and shelf-life extension, as well as emerging UV applications in fresh cut produce safety and quality and postharvest agricultural water (i.e., flume tank) decontamination. The information in this review is likely to be highly valuable to the academic and industry perspectives.

INTRODUCTION

The global food supply chain is growing, opening the door for the worldwide trade of specialty crops and off-season fresh produce. In fact, more than 1.6 billion metric tons of fresh fruits and vegetables were produced worldwide in 2019 *(195)*, and production is projected to increase due to a growing global population and consumer demand. Even local and regional food supply chains are gaining resiliency, spurred by unprecedented supply chain disruptions (i.e., during the COVID-19 pandemic) and increasing concerns over environmental sustainability *(24)*. Once more, fresh fruits and vegetables are a vital source of vitamins, minerals, and health-promoting phytochemicals (i.e., anthocyanins, glucosinolates) – making fresh produce essential to the human diet *(90)*. Unfortunately, the global food system – including many local and regional food systems – is also facing major challenges in ensuring product safety and quality throughout the entire supply chain *(86)*.

In the fresh produce industry, on-farm production and postharvest operations have stepped into the spotlight as critical control points to prevent the growth of plant and human pathogens and reduce produce quality loss *(193)*. Fresh produce is one of the most common vehicles for human foodborne pathogens, and various outbreaks have been linked to on-farm mismanagement regarding water quality, postharvest handling, soil health practices, etc. Mitigating the spread of plant pathogens (such as fungi) at the pre and postharvest stage is duly of economic concern, as some of these microbes cause billions of dollars in high-value product loss each year *(61)*. Accordingly, on-farm antimicrobial interventions are the first line to protecting the food supply.

Produce safety, quality, and shelf-life are inextricably linked as on-farm antimicrobial interventions used to reduce plant and human pathogens can also impact nutritional value and subsequent produce shelf-life – these interventions will be discussed later in this review. As the consumer demand for safe, fresh, nutritious, minimally processed foods increases, there is a desire for on-farm interventions which potentiate both produce safety and quality *(62, 125)*. Ultraviolet (UV) light technology is one such intervention as it has been used extensively for water and surface decontamination applications and is effective at reducing the microbial load of many produce surfaces and produce-derived products (i.e., fruit juices) while also promoting the maintenance and biosynthesis of health-promoting compounds and extending shelf-life (which will be discussed in detail in this review). The United States Food and Drug Administration (FDA) approved the use of UV light as a germicidal treatment for foods, milk, and juices in 2011 *(216)*, but the technology remains relatively under-utilized in the fresh produce industry.

There have been excellent recent publications providing an overview of the effects of different wavelengths of the UV spectrum on fresh produce quality and safety. For example, Yemmiredddy et al. *(236)* provides a useful overview of UV-C applications of interest to the fresh produce industry that is indispensable for newcomers to the subject. Darré et al. *(60)* also dives into the applications of UV light to fresh produce and details the technology's capacity to induce desirable changes in the phytochemical (nutritional) profile of different commodities. However, a review on the practical and emerging applications of UV light in the fresh produce industry is still lacking. Further discussion including *site-specific* UV light application, *how* the technology can be applied, and *emerging applications* that merit further study is needed. Thus, the purpose of this literature review is to provide a synopsis of the current state of UV light

technologies with an emphasis on its potential applications during the on-farm production and postharvest handling of fresh fruits and vegetables (*Figure 1*). The information in this review is likely to be highly valuable to the academic and industry perspectives.

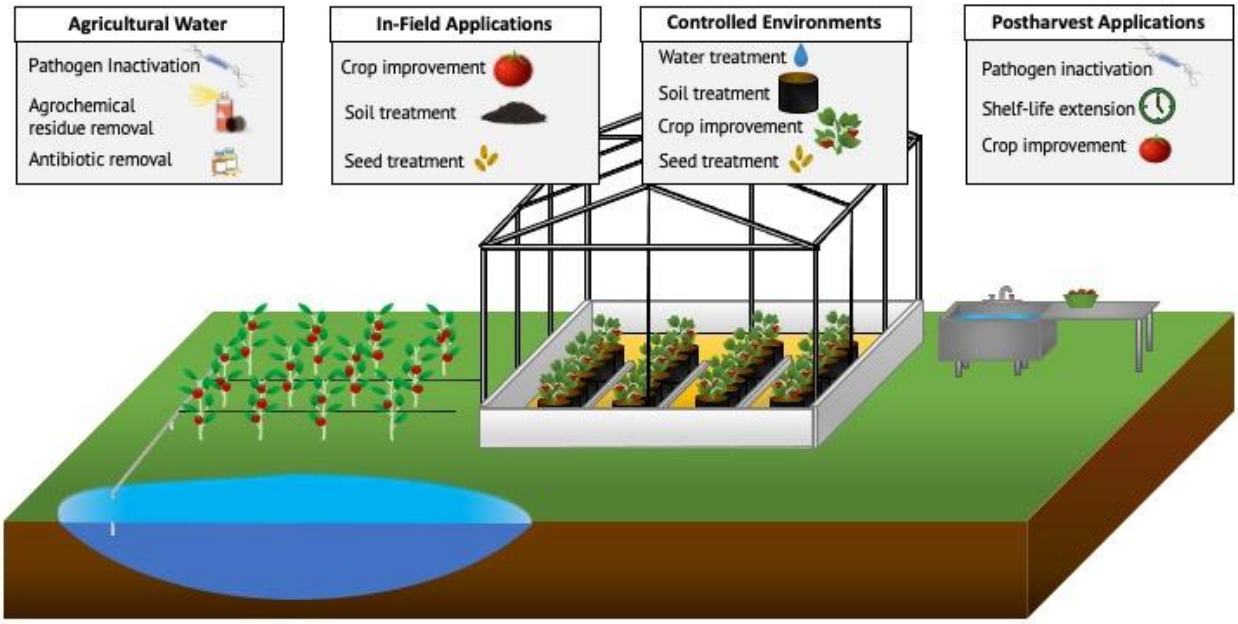


Figure 1 *UV applications during the production and postharvest management of fresh produce*

Ultraviolet Light Technologies: Fundamental Concepts

Ultraviolet light is a form of non-ionizing radiation, carrying sufficient energy to excite atoms and molecules but unable to remove electrons and photons from their atoms, as with x-ray and gamma irradiation *(120)*. The UV spectrum (Figure 2) includes wavelengths from 100 to 400 nm on the electromagnetic spectrum and is further subdivided into: UV-A (315 to 400 nm) which is responsible for skin tanning; UV-B (280 to 315 nm) which is responsible for skin burning and damage leading to skin cancer; UV-C (200 – 280 nm) which is effective in inactivating microorganisms hence called the 'germicidal range', and vacuum UV (100 – 200 nm) which is readily absorbed by all substances and thus only transmitted through a vacuum *(120)*. 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 energy can excite a wide range of biologically relevant biomolecular bonds (*Figure 2*; adapted from Koutchma *(120)*). and induce changes in the thermal, biochemical, structural, and morphological properties of UV-treated objects. These properties will be further discussed in the context of fresh produce in this review.

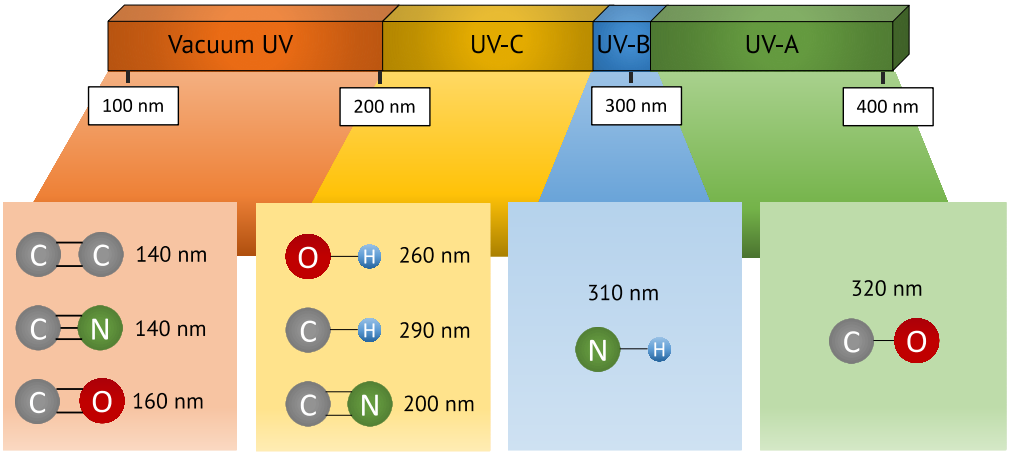


Figure 2 *Biologically relevant bonds excited by UV-A, UV-B, UV-C and vacuum UV*

Furthermore, UV light can be either be absorbed, reflected, refracted, or scattered by different treatment materials. Reflection, refraction, and scattering may have a significant effect on the inactivation efficacy of UV light as once UV irradiation is absorbed, it is no longer free to excite other atoms and thus incapable of decontamination. UV light is also not a highly penetrative form of radiation, having a typical penetration depth of only a few millimeters depending on the surface and optical properties of the target subject *(123, 144)*. For this reason, properties such as turbidity, opacity, and UV transmittance render critical challenges to successfully treat fresh produce and minimally processed fruit products (i.e., cut produce) with UV light.

A variety of bacteria, viruses, fungi, and protozoa can compromise the safety and quality of fresh produce *(112)*.The survival of these microorganisms relies on the replication and translation of their nucleic acids – deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) – which instruct the cell to perform metabolism (e.g., homeostasis), chemical signaling, reproduce, etc. UV light is considered a physical decontamination method as the transfer of photochemical energy from photons to an organism's nucleic acids induces mutagenesis, ultimately leading to cell death *(120)*. 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 *(74, 184)*.

Pyrimidines are more sensitive to UV-induced dimerization, with the thymine-complex and uracil-complex photoproducts requiring the least energy to form *(107)*. Such point mutations, also called DNA lesions, block DNA/RNA replication and transcription, or lead to miscoding, eventually resulting in cell death if it is unable to repair the photodamage. It is also noteworthy that this cycloaddition reaction is energetically inaccessible to thermal reaction pathways *(74)*; i.e., thermal decontamination methods do not carry enough energy to promote this genre of mutagenesis. Moreover, because UV light disrupts cell function – as opposed to chemical sanitizers which typically directly destroy cellular components – it is considered to 'inactivate' rather than 'kill' microorganisms.

There are a variety of extrinsic and intrinsic factors which affect the microbial inactivation efficacy of ultraviolet light *(120)*. Of the extrinsic factors affecting microbial inactivation efficacy, reactor design and matrix characteristics are among the most important and will be

discussed further in the review. The sensitivity of microorganisms to UV radiation, moreover, is heterogenous *(26)* with the extent of DNA/RNA damage dependent on intrinsic factors such as: 1) the degree of UV absorption by cell membrane components, 2) relative concentration of thymine residues in the DNA/RNA backbone, and 3) the expression of DNA repair pathways by the microorganism *(58, 69, 76)*.

Microbial cells employ two primary mechanisms of repairing UV-induced cellular damage: photoreactivation and/or DNA repair pathways *(188)*. Photoreactivation (i.e., light repair) is a light-dependent process in which photolyases absorb and utilize the energy of visible wavelengths of the electromagnetic spectrum to reverse the covalent bonds formed between pyrimidine dimers *(41)*. In light-independent reactions (i.e., "dark repair"), point mutations are enzymatically excised following the UV-induced activation of repair genes *(58)*. Of interest, the SOS response is a major repair pathway initiated by the accumulation of single-stranded DNA during replication as helicase continues unwinding DNA despite DNA polymerase halting at a lesion site *(148)*. This global regulatory network includes the expression of over 50 genes that respond to DNA damage through excision repair, recombination, translesion DNA replication, and the cessation of cell division *(148)*. As excision repair requires a template strand, single-stranded DNA or RNA viruses cannot perform this type of repair mechanism as double-stranded RNA viruses *(97)*. Protozoa such as *Cryptosporidium* spp. possess nucleotide excision repair genes and exhibit the potential to repair UV-induced photodamage *(155, 162)*. However, the UV-treated oocysts are unable to restore infectivity, thus the mechanism of photo repair in protozoal pathogens remains unknown *(186)*.

UV Light Sources. The most common sources of UV light in the food industry are mercury vapor lamps (MVLs) and UV-light emitting diodes (UV-LEDs). MVLs consist of liquid mercury encased in an envelope surrounded by an inert gas; the inert gas serves two purposes: 1) to facilitate the collision of electrons to form energy and 2) to promote stability at high temperatures *(111)*. While there is no risk to human health when the lamp is intact and undamaged, there are increasing human and environmental concerns due to the hazardous nature of mercury if the envelope breaks, and difficulty in disposal after the lamps' lifespan has lapsed *(44)*. For this reason, UV LEDs are considered a far safer and more environmentally friendly alternative. They are also more compact, require little warm-up time, and are more cost-effective than MVLs, owing to recent technological developments *(115, 149, 237)*.

Pulsed xenon flashlamps (PUV) are steadily gaining popularity in the fresh produce literature as they do not contain toxic materials, are more time- and energy-efficient than continuous UV technologies and emit energy which can penetrate plastic packaging *(49)*. PUV employs high-power electrical energy to generate intense, short duration (nanosecond to millisecond) pulses of broad-spectrum light (180 nm to 1,100 nm) *(77)* that inactivates microbes by inducing UV photodamage and the photothermal effect resulting from other non-UV wavelengths *(153, 208, 229)*.

UV Applications in the Production of Fresh Fruits and Vegetables

For fresh fruits and vegetables, the production (preharvest) stage includes agricultural activities from the sowing to harvesting of fresh produce, including activities such as the application of soil amendments and pesticides, crop irrigation, etc. During the production stage there are many

potential routes of pathogen transmission from the environment to fresh produce (*Figure 3*). Of these routes, agricultural water and untreated soil amendments of animal origin (i.e., manure), are the most infamous vectors of pathogen transmission to the soil and plant *(14)*. In fact, various outbreaks have been associated with the use of contaminated irrigation water, and recent studies on full-scale anaerobic digesters indicate that pathogens may persist even in treated soil amendments *(32)*. This review will discuss the main current and developing applications for UV in the production environment: agricultural water, soil, seeds, and fresh produce in the field. Cross-contamination of pathogens from equipment to the growing environment will be included in the context of surface decontamination in the postharvest section.

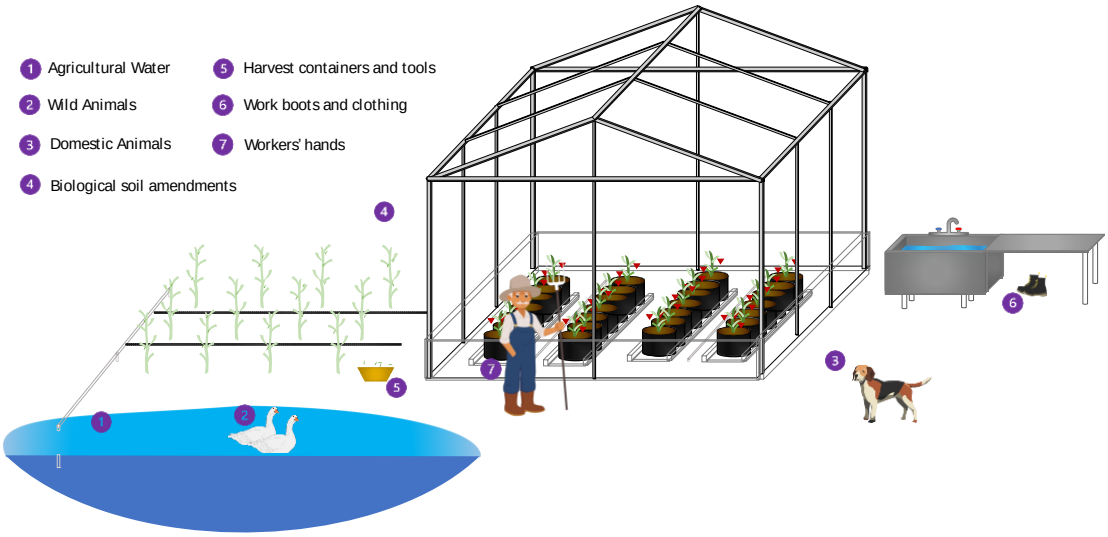


Figure 3 *Examples of potential contamination events during the production and harvest stage of fresh produce*

Agricultural Water Treatment. Agriculture is a major, global consumer of water, and the consumption of agricultural water for crop production is expected to increase due to an

increasingly urbanized population, higher incomes, and shifting in dietary patterns *(63)*. There are three main sources of agricultural water: municipal water, ground water, and surface water. Many farm operations across the United States (and globally) use surface water as a water source for production-related operations (i.e., irrigation, fertigation, crop sprays) because it can be more accessible, economical, and easier to use. However, surface water is more susceptible to environmental contamination (e.g., agricultural run-off, wildlife defecation) as it is typically completely exposed to the environment. In fact, foodborne outbreaks in commodities such as leafy greens *(213, 214)* have been attributed to the use of contaminated surface water in preharvest operations. Even municipal water has been associated with microbial and chemical risk as studies have detected residual chemical pollutants (e.g., pesticides, antibiotics) *(38)* and chlorine-resistant protozoa (i.e., *Cryptosporidium*, *Giardia*) in municipal wastewater effluents even following treatment *(117)*. Although less common, waterborne pathogens have also been reported in ground water sources (namely wells) following events like flooding (Dai et al., 2019) and septic leeching *(150)*. Similarly, rainfall and livestock activity/density have also been attributed to elevated concentrations of *Escherichia coli* in ground water sources *(106)*.

Chemical sanitizers are widely used in fresh produce industries to reduce the microbial population of agricultural waters *(59)*. These sanitizers exert a range of antimicrobial activity from cell protein denaturation (i.e., sodium hypochlorite) to cell wall disruption (i.e., peracetic acid), eventually resulting in microbial cell death *(158)*. Concerns regarding the use of chemical sanitizers are primarily due to the demonstrated resistance by critical produce-related pathogens (i.e., *Cyclospora* spp.) and the formation of dangerous degradation by-products. The efficacy of chemical sanitizers can be somewhat microorganism-specific *(117)*, with differences in

disinfectant sensitivities apparent even between successive generations of the same pathogen species *(192)*. There is also increasing evidence that common sanitizers can induce the viable-but-not-culturable (VNBC) state *(59)*; a state of metabolic dormancy in which the microorganism cannot be propagated but may later regain metabolic activity and infectivity under favorable conditions. Chemical sanitizers may even degrade into harmful byproducts, not only generating safety hazards for workers but also potentially affecting the quality of subsequently irrigated crops and agricultural soils *(135, 161, 163, 225)*. For these reasons, effective alternatives to the sole use of chemical sanitizers are becoming increasingly sought-after.

The produce industry generally employs food-grade disinfectants (e.g., organic acids, sodium hypochlorite, hydrogen peroxide) for the surface decontamination of fresh produce and food contact surfaces. Many of these chemical disinfectants act as oxidizing agents, disrupting the cell membrane of microbes and reacting with a wide variety of biological molecules (e.g., proteins, nucleic acids) *(84)*. However, studies report that common food-grade disinfectants authorized by the FDA and EPA cannot regularly achieve greater than a 3-log reduction of microbial surface populations, and there is a possibility of microbial regrowth during storage. Some pathogens of high public health interest (e.g., *E. coli* O157:H7) can even exhibit resistance to organic acids *(28)*, and recent *Cyclospora* spp. outbreaks demonstrate the inefficacy of chlorine-based sanitizers against 'non-conventional' pathogens such as protozoa *(42, 117)*. While chemical disinfectants can reduce cross-contamination, their use is not sufficient to assure microbial safety, particularly for human pathogens with low infectious doses *(191)* which may regrow after treatment.

Ultraviolet-C (UV-C) technology has shown antimicrobial efficacy against acid-resistant pathogens (i.e., *E. coli* O157:H7) *(200)* and emerging chlorine-resistant pathogens (i.e., *Cyclospora* spp.) *(9)* of high public health interest in drinking water. UV-C light is already used extensively for drinking water decontamination systems; from wastewater treatment facilities to point-of-use water purification devices (i.e., SteriPEN Ultra UV Water Purifier®). The antimicrobial efficacy of UV devices designated for water decontamination largely depends on the lamp power, flow rate, and absorbance of the UV-treated water. In general, lamp power is positively correlated with reactor performance while high flow rates and water absorbencies significantly hinder UV treatments *(128, 231)*. In fact, the relatively high concentration of particulate matter in agricultural surface waters in comparison to drinking water presents the greatest challenge for the application of ultraviolet light to treat production water coming from such water sources. However, there are recent, promising developments in strategies (i.e., thin film UV devices) to improve the performance of UV technologies for the decontamination of highly turbid agricultural surface waters.

Decreasing the impact of high turbidity on decontamination efficacy is at the forefront of UV light-based technologies for agricultural surface water decontamination. Particulate matter as small as 11µm and dilute as <3 NTU can absorb UV-light or shield associated microbes, causing an attenuating effect on microbial inactivation *(10, 39, 40)*. In the past, inexpensive rapid sand filters and polyaluminum chloride coagulation have been used as pre-treatments to improve the antimicrobial efficacy of ultraviolet light-based irradiation of surface waters *(181, 199)*. Moreover, integrating materials with high reflectivity (i.e., aluminum) in comparison to the conventional stainless-steel into the inner surfaces surrounding the lamp has also been shown to

increase reactor performance *(98)*. Fouling of sand filters – for example, the calcification of particulate matter on the sleeve – in this application has also yet to be determined, despite the fact that a high diversity of biofilm-forming genera has been demonstrated to form on rapid sand filters in groundwater, particularly in the summer months during high agricultural productivity *(52)*.

Emerging UV Applications in the Fresh Produce Industry: Preharvest Stage

Agrochemical Residue Removal from Agricultural Water. Ultraviolet light is an emerging technology for the degradation or removal of antibiotic and agricultural pesticide residues in water, an issue drawing concern from the general public and scientific community alike. More than 400,000 metric tons of pesticides were used for agricultural purposes in the United States in 2018 *(82)*, with global pesticide applications projected to increase through 2050 *(140)*. In addition to these agrochemicals, antibiotics – which have been extensively used in animal husbandry – are not typically removed by filters or wastewater treatment plants and thus can persist in the aquatic environment *(171)*. For example, a recent study detected the pesticide glyphosate and its degradation product aminomethylphosphonic acid (AMPA) in 74% and 90% respectively, of sampled streams in the United States *(151)*. Once in this environment, these residues can be transferred to humans through the consumption of drinking water and aquatic animals, and to plants through irrigation. With consumers becoming more conscientious of pesticide residues in food items *(88)*, there is a need for scientifically validated processes to reduce these contaminants in the growing environment and increase consumer confidence in the food system.

The ability of UV to induce the photodegradation of organic contaminants in agricultural water is well supported by the literature, but there has been little traction to bring the idea to fruition in the agricultural sector. Because UV alone exhibits a relatively slow contaminant degradation rate *(233)*, many photocatalytic water systems employ photosensitizers (e.g., TiO_2) or reactive oxygen species (e.g., H_2O_2) which accelerate the degradation of target chemical compounds and potentially exert antimicrobial activity. One of the greatest challenges to marketing UV-assisted pesticide removal is that water matrix components (anions, cations, organic matter, etc.) can have potentiating or detrimental effects on degradation efficiency. For example, while investigating UV/TiO_2 photocatalysis of metronidazole, Tran et al. *(210)* uncovered various processes which were inhibiting the full or efficient degradation. Within the degradation reactions, iron (III) ions competed with the pharmaceutical for reactive organic intermediates, H_2PO_4- reduced the availability of TiO_2, and the presence of humic acid induced the formation of various reaction by-products. Because these chemical reactions are unique to each compound, such water treatment systems would need to be validated for different combinations of water matrix components.

Preharvest Pest Management and Preharvest Quality Enhancement. While appropriate postharvest management is critical to maintaining the quality of fresh produce, a major portion of fresh produce quality is determined by environmental factors encountered in the preharvest field setting. For this reason, the agricultural industry largely relies on pesticides to eliminate pest pressure (e.g., weeds, fungi, and insect damage), thereby promoting high yields, quality, and productivity throughout the growing season. However, there is increasing worldwide concern for the consumption of conventional (non-organic certified) agricultural products as biomonitoring

studies have reported high levels of pesticides in biological samples collected from the general population *(22)*. As many consumers are trending towards food items produced in a pesticide-free environment *(88)*, preharvest UV solutions to promote fresh produce quality and shelf-life stand to play a critical part in the future of UV applications in the agricultural sector.

Regarding promoting produce quality preharvest, UV-B treatments are more commonly associated with improved quality status than UV-A or UV-C applications. For example, Li et al. *(129)* reported that preharvest field applications of UV-B to highbush blueberry (*Vaccinium corymbosum*) increased sugar accumulation, fruit growth, and the production of anthocyanins. A possible exception is in tomatoes wherein preharvest UV-A irradiation increased the content of flavonoids and other phenolic compounds *(146)*, and improved consumer ranking scores relative to tomatoes exposed to UV-B irradiation. These quality-promoting attributes of preharvest UV-A treatments compared to preharvest UV-B treatments were also observed in lettuce *(124)*. Interestingly, the study from Lee et al. *(124)* also emphasized an under-reported mode of quality enhancement: combined UV treatments. In this study, the authors observed that preharvest UV-A/B irradiation was more effective than singular treatment of either UV-A or UV-B in promoting the accumulation of phenolics and other nutritional components (e.g., vitamins, minerals). Of note, these studies also emphasize how much more feasible it is to perform this sort of treatment in the greenhouse or similar controlled environment agricultural system rather than in the field where most horticultural crops are still grown. Hence, clarifying the logistical and economic investment required to perform such UV treatments in the preharvest field setting is a critical future research avenue.

Soil Treatment. Agricultural soils are complex agglomerations of abiotic (i.e., nutrient) and biotic (i.e., soil microbiota) constituents that can either promote or limit the productivity of horticultural commodities. In recent decades, agricultural soil pollutants originating from industrial and agricultural activities *(35)* have garnered significant concern for their adverse impacts on the soil environment and ecosystem. For example, atrazine – a widely used herbicide for weed control – can persist in soils *(8)* and is known to disrupt the soil microbiome *(228)*. Even biochemical substances of animal origin such as 17β-estradiol can be passed from the animal to soil through the application of manure *(119, 164)*. Many soil pollutants can even be absorbed by horticultural crops *(104, 223)*.

Ultraviolet radiation is postured to be a highly accessible method of photodegradation of pollutants, particularly for those which are distributed in the topsoil *(43)*. For example, Wang et al. *(226)* used a 500W polychromatic mercury lamp to degrade 17β-estradiol in silica gel and natural soil. Similarly, Abd El-Rehim *(4)* found that a combination of UV irradiation and soil burial treatments significantly increased the rate of low-density polyethylene (LDPE)/starch biodegradation. In a more recent study, an electrokinetic soil remediation system utilizing sunlight as the UV source was found to reduce hexavalent chromium – a highly toxic environmental pollutant released from industrial activities – to its more environmentally-friendly trivalent form *(244)*. However, environmental conditions (i.e., humidity) *(226)* and soil physiochemical and biotic components *(211)* can negatively influence the removal rate of pollutants. In addition, as the soil remediation studies were also primarily performed at the benchtop scale, further research into their efficacy at the farm-level is warranted. Accordingly, it has yet to be demonstrated how UV technologies implemented at the soil level could impact the

microbial safety of horticultural commodities grown therein, especially as UV is not a highly penetrative form of radiation.

Seed Treatment. Commercial seeds are well-known disease vectors of human and plant pathogens *(23)*. Sprouted seeds, in particular, have been implicated in various foodborne outbreaks as they are primarily consumed raw *(65)*. Similarly, bacterial, viral, and fungal plant pathogens can colonize the seed surface and be transmitted during storage or delivery, resulting in substantial global crop losses *(159)*. Although physical control measures such as heat or microwave treatment can reduce the pressure from pathogenic contaminants, these methods may result in reduced seed viability *(202)*. There has been relatively little work in the application of UV light to address the microbial safety of seeds, but studies suggest that it can stimulate the stress response, including the production of plant biodefense compounds.

Brown et al. *(31)* attributed the reduced fungal disease incidence of UV-C-treated cabbage seeds (*Brassica oleracea* var. capitata L.) to the induced synthesis of phytoalexins – a group of anti-fungal biodefense molecules. In romaine (*Lactuca sativa* L. 'Romaine') and green bean (*Phaseolus vulgaris* L.), UV-C seed treatments conferred an increased activity of antioxidant scavenging species and tolerance to salinity stress in later stages of development *(7, 169)*. In mung bean (*Vigna radiata*) and cultivars of tomato (*Solanum lycopersicum* L.), UV-A-treated seeds exhibited increased germination rates and a greater increase in shoot and root metrics than UV-C-treated seeds *(95, 145)* . There are fewer studies on UV-B seed treatments likely due to the deleterious effects of this irradiation range on plant metabolism *(121)*. In fact, Shaukat et al. *(197)* observed that UV-B irradiation stimulated the stress response and accelerated germination

rate but reduced the overall germination and subsequent root and shoot growth of mash-bean (*Vigna mungo* (L.) Hepper.). Interestingly, Abbas et al. *(2)* reported a higher content of health-promoting phytochemicals in 21-day old seedlings of *Momordica charantia* L. (bitter melon) in response to UV-B pre-treatment. Accordingly, the priming effect of UV-A, UV-B, and UV-C to support growth, stress resistance, and phytochemical content during development is an avenue for further investigation.

As with its other applications, the low penetration of UV radiation is a potential challenge for the commercialization of the technology as a pre-sown seed treatment to reduce the levels of human pathogens on the seed coat. This limitation means that under most circumstances, the seeds would need to be rotated to ensure whole-surface treatment. However, the research of Phornvillay et al. *(178)* highlights an alternative method integrating hydrogen peroxide (H_2O_2) and micro-nano bubbles with UV-C induced seed surface decontamination. The authors report the efficacy of this advanced oxidative process (AOP) method to reduce total bacteria and *E. coli* on the seed surface while promoting the formation of health-promoting phytochemicals (i.e., flavonoids, phenolic compounds). Interestingly, immersing the seeds during treatment seems critical to reducing the surface microbial concentration as Wang et al. *(221)* found a minimal antimicrobial effect of applying activated rinse water to the surface population of mung beans inoculated with *E. coli* O157:H7, *Salmonella* spp., or *Listeria monocytogenes*. These findings present avenues for future research, including how to integrate such processes with other industry interventions such as artificial seed coating.

UV Applications for Postharvest Produce Quality and Safety Management

Fruits and vegetables are an excellent source of vitamins and minerals, antioxidative compounds (e.g., carotenoids, polyphenols), and bioactive compounds that support human health. In fact, in 2003 the World's Health Organization (WHO) declared increasing the consumption of fruits and vegetables a global priority as a preventative measure against non-communicable diseases. And, in 2015, all members of the United Nations adopted the 2030 Agenda for Sustainable Development Goals (SDGs), of which an objective was to end all forms of hunger and malnutrition in the world by 2030. However, according to the United Nations Food and Agriculture Organization (FAO), approximately one third (or 1.3 billion metric tons) of food produced for human consumption is lost or wasted each year, worldwide *(93)*. As produce is highly perishable, preventing food loss – food that is damaged, spoilt, or suffers a significant reduction in quality before reaching retail – has become a major issue in produce quality. In an increasingly globalized market, moreover, produce safety is coming to the forefront as current widely used interventions (i.e., chemical washes) have proven to be of limited efficacy as the number of outbreaks is rising in concert with the consumption of raw produce commodities *(37)*.

As previously mentioned, food safety and quality are inextricably linked as postharvest treatments which influence microbial safety can have synergistic or antagonistic effects on quality and even shelf-life and maturity. Despite recognition of its potential as a nonthermal method of microbial inactivation and preservation of agricultural commodities, UV light technology remains relatively underdeveloped in the food industry. UV-C is effective in reducing the microbial load of many fruit surfaces *(236)*, and studies suggest low (hormetic) postharvest UV-C doses can induce the production of health-promoting bioactive compounds

and retard the degradation of shelf-life quality attributes *(30, 114, 147, 179, 194)*. The paired germicidal and quality-promoting effect of postharvest UV treatments is beneficial for the entire food chain, offering growers a potentially cost-effective alternative to chemical sanitizers while supporting the production of nutritious, high-quality produce and reducing product loss *(179)*. To this extent, investigating the germicidal and hormetic effects of UV-C light on produce has been the focus of many studies.

As with the case of UV seed treatments, low (hormetic) doses of UV light applied to fresh produce have been shown to stimulate the stress response and provide protection/resistance to microbes throughout development. However, the concept of radiation hormesis – the introduction of low or sublethal dose of UV light, X-rays, and/or gamma rays – is not new and has been studied since as early as the 1980s *(138)*. Though studies generally suggest a UV-C hormetic dose range of 0.5 to 20 kJ m^{-2} *(196)*, this range is produce-dependent with supra-optimal doses resulting in external damage, reduced germination, and increased disease susceptibility *(31, 185, 205)*. Moreover, it appears that some crops are more sensitive, with produce such as papaya developing undesirable sensory characteristics even at lower limits of effective UV treatment. *Table 1* provides a summary of the benefits of postharvest UV applications on produce safety and quality (including shelf-life) by commodity, which will be covered by this review in the following sections. The table is primarily based on findings from the last six years (2018 to 2022) of literature. Fourteen types of benefits from postharvest UV applications were identified (second column) and their subsequent outcomes on fresh produce safety and/or quality were categorized into five major overall effects: promoting shelf-life

extension (green), plant pathogen resistance (purple), nutritional quality (yellow), foodborne pathogen reduction (red), and consumer acceptance (blue).

Table 1 *A summary of the benefits of postharvest UV applications to fresh produce based on literature from 2018 - 2022*

	Benefits of Postharvest UV Applications	Commodity (references)
	Reduced cross-contamination risk in produce wash water	blueberry *(103)*, broccoli *(54, 55)*, carrot *(103)*, lettuce *(103)*, strawberry *(167)*, tomato *(1, 103)*
	Plant biodefense gene or pathogenesis-related enzyme expression	jujube *(109)*, mangosteen *(204)*, pear *(207)*, rice *(130)*, strawberry *(3)*, tomato *(134, 194)*
	Maintenance of cell wall integrity	cherry *(154)*, strawberry *(165, 166)*, jujube *(109)*, nectarine *(242)*
	Increased antioxidant production, capacity, or retention	apple *(19)*, broccoli *(72)*, cherry *(6, 154, 240)*, grape *(198)*, mandarin orange *(177)*, mushroom *(182)*,
	Delayed or reduced pigment degradation	apple *(19)*, bell pepper *(139)*, blueberry *(245)*, broccoli *(13, 73)*, cherry *(6, 154, 240)*, grape *(198)*, lime *(180)*, mushroom *(182)*, nectarine *(242)*
	Plant hormone accumulation	bell pepper *(139)*, mandarin orange *(177)*, strawberry *(3)*, tomato *(194)*
	Increased phytochemical production, accumulation, or retention	apple *(19)*, bell pepper *(139)*, cherry *(6, 154, 240)*, bamboo shoot *(230)*, broccoli *(72, 73)*, grape *(198)*, habanero pepper *(175)*, jujube *(109)*,

		mandarin orange *(177)*, mushroom *(182)*, nectarine *(242)*, peach *(5)*, pear *(207)*, pepino fruit *(243)*, tomato *(134)*
	Reduced population of surface human pathogens (or human pathogen surrogates)	apple *(89)*, blueberry *(34, 94)*, lettuce *(89, 133)*, peach *(232)*, raspberry *(34)*, spinach *(172)*, strawberry *(34)*, tomato *(172)*
	Reduced decay incidence or population of plant pathogens & spoilage microorganisms	blueberry *(108, 245)*, litchi *(239)*, mandarin orange *(177)*, mangosteen *(204)*, mushroom *(227)*, nectarine *(242)*, peach *(5)*, pear *(207)*, persimmon *(50)*, rice *(130)*, strawberry *(3, 83, 165)*
	’Priming’ against physiological damage, plant pathogens, or to improve storage quality	cherry *(147)*, lettuce *(122)*, peach *(246)*, tomato *(194)*
	Water retention, reduced weight loss, and firmness retention	bell pepper *(139)*, blueberry *(108, 245)*, broccoli *(72, 73)*, cherry *(6, 154)*, fig *(201)*, jujube *(109)*, mangosteen *(204)*, peach *(5)*, pepino fruit *(243)*, strawberry *(165, 166)*

	Reduced respiration rate	cherry *(154)*, lime *(180)*, mangosteen *(204)*, pepino fruit *(243)*, strawberry *(165)*
	Improved sensory quality	cherry *(6)*, fig *(201)*, lime *(180)*, peach *(247)*, pepino fruit *(243)*, strawberry *(165)*
	Fresh-cut produce safety and quality	apple *(87)*, bamboo shoot *(230)*, broccoli *(54)*, kiwi *(101)*, lettuce *(96)* , lotus root *(220)*, melon *(103)*, peach *(131)*, pineapple *(103)*, radish *(103)*, strawberry *(21)*, watermelon *(18)*

Color coding indicates the parameter contributes to shelf-life extension (●), plant pathogen resistance (●), nutritional quality (●), foodborne pathogen reduction (●), or consumer acceptance (●)

Whole-Fruit Surface Applications. Many fresh produce are highly perishable as these commodities continue to ripen (and metabolize) even after harvest. Phytohormones such as ethylene are naturally upregulated during ripening to serve as chemical signals for cell wall degradation, glycolysis, sugar accumulation, and water loss. Continuous exposure to endogenous or exogenous ethylene eventually leads to senescence (over-ripening) characterized by wrinkling and tissue browning (amongst other symptoms) *(113)*. The ripening process is accelerated by abiotic and biotic stressors encountered during harvest and processing (i.e., injury, disease), which can also be interconnected. For example, in many fruits mechanical injury often results in micro-fissures within the fruits' tissues *(80, 105)* which promote electrolyte leakage *(105)*, and serve as entry points for bacterial and/or fungal pathogens *(78)*. These microorganisms may also excrete extracellular enzymes (pectinases, hemicellulases), further degrading plant tissues *(78)* and accelerating degradation reactions. In the absence of abiotic stressors, even the natural breakdown of produce tissues (and release of sugars) during ripening increases the fruits' susceptibility to colonization by plant and human pathogenic microorganisms *(75, 99)*. While climate-controlled storage can increase produce shelf-life by slowing the natural degradation processes and reducing microbial colonization/growth, there is still a considerable concern for the safety and quality of commodities that undergo field packing.

Many fruits and vegetables are 'field-packed' – directly packaged after harvesting – including high-value commodities such as berries (i.e., strawberry, blueberry, raspberry), melons (honeydew, watermelon, cantaloupe), some varieties of leafy greens, brassicas (cabbage, broccoli), and herbs *(215)*. As such, these commodities may not undergo a wash step and typically cannot be treated with a postharvest antimicrobial dip or edible wax, which prevents

colonization by human pathogens, plant pathogens, and spoilage microorganisms. Recent studies even suggest that the postharvest, dual application of fungicides and chemical sanitizers may be ineffective due to antagonistic interactions between the active ingredients *(187)*. The inability to apply this genre of postharvest treatments is likely a contributing factor to outbreaks in these commodities and postharvest losses in these field-packed commodities. Thus, more investigation into antimicrobial interventions feasible in the field setting is warranted.

There is expansive literature regarding the UV decontamination of fresh produce, and recent reports provide insight into the underlying UV-induced biochemical reactions which potentiate produce safety and quality. Moreover, there are three genre of microbes which are targeted for UV decontamination of fresh produce surfaces: human pathogens, plant pathogens, and spoilage microorganisms. These points will be discussed in the following sections.

Inactivation of Human Pathogens on Whole-Fruit Surfaces. As previously mentioned, chemical sanitizers are the primary intervention used to reduce the concentration of pathogenic microbes on the surface of fresh produce. However, there is increasing consumer pressure to reduce the amount of chemicals used in agriculture and the food industry, and such antimicrobial interventions may not be available for many produce types (e.g., field packed). One of the greatest challenges to expanding UV decontamination' is identifying the optimal UV dose. This factor is critical to the produce industry as too high of UV doses can result in tissue damage leading to the development of undesirable sensory characteristics like browning or 'off' flavors *(15, 16, 45, 51, 89, 91)*. However, the optimal UV dose for produce is difficult to generalize due to the high variability in produce surface characteristics *(11)*. For example, complex surface

structures like trichomes (i.e., peach) or netting (i.e., cantaloupe) shield microbes from irradiation, requiring higher levels of UV irradiation to achieve microbial reduction *(11)*. Hydrophobic produce surfaces can even enhance this shading effect as bacteria are shown to aggregate on these surfaces, reducing the penetration capability of UV light *(116)*.

Inactivation of Plant Pathogens and Spoilage Microbes on Whole-Fruit Surfaces. Plant pathogens and microbial spoilage are major causes of economic losses in the fruit and vegetable industry and oftentimes limit the distribution of certain products. Globally, the Food and Agricultural Organization of the United Nations (FAO) estimates the burden of plant disease to be about $220 billion per year – approximately 20 to 40 percent of global crop production *(212)*. These economic losses are likely to increase as the abundance of soil-borne and fungal plant pathogens is expected to worsen in the coming years due to climate change *(64)*. Contrarily, it's difficult to measure economic losses due to food spoilage. In fact, spoilage microorganisms are considered part of a commodity's normal microflora, which experiences dynamic shifts favoring such organisms towards the end of the commodity shelf-life *(112)*. Interestingly, recent research indicates that some organic-acid based chemical sanitizers meant to reduce the microbial load of human pathogens on the produce surface may in turn increase the abundance of spoilage microbes – reducing the risk of foodborne outbreaks but increasing the risk of economic losses from pre-mature spoilage *(224)*. Consequently, there is a need for robust antimicrobial interventions in the produce industry which can reduce the burden of human and plant pathogens without promoting the growth of spoilage microorganisms.

The cumulation of previous research indicates that UV-C irradiation can provide broad-range protection against plant and spoilage microorganisms by inducing the synthesis of metabolites and enzymes belonging to the biodefense response. This 'priming' effect confers resistance to plant pathogens, thereby reducing the incidence of microbial decay. For example, the UV-induced synthesis of phytoalexins – compounds that inhibit the growth of parasites and fungi – has been shown to negatively impact the colonization of fungal pathogens on citrus *(27)*, grapevines *(68)*, rice *(118)*, carrots *(152)*, and grapefruit *(66)*. Likewise, UV-C irradiation also induces the expression of pathogenesis-related enzymes (i.e., chitinases, peroxidase, phenylalanine ammonia lyase), which prevent mycorrhizal expansion, disrupt microbial membranes, and signal for the expression of biodefense genes *(48, 204)*. On a physical level, plants simultaneously respond to UV-C irradiation with accelerated levels of suberization, lignin accumulation *(46)*, and delayed pectin degradation *(166)*. These changes in produce surface structure to reinforce the cell wall results in reduced microbial adhesion *(47)*, surface pitting during senescence *(154)*, and improved firmness retention *(166)* in comparison with non-treated produce.

Although UV-C is the most widely investigated spectra due to its 'germicidal effect', UV-A and UV-B applications also display potential (though inconsistent) to augment the plant defense response. For example, Shrouk Abd El Hamid et al. *(3)* demonstrated the use of UV-B irradiation as a priming agent in fresh strawberries to upregulate the fungal cell wall degradation enzymes, jasmonic acid accumulation, and the production of bioactive compounds (i.e., terpenoids) in anticipation of fungal infection. However, the effects of UV-B irradiation are not universal, as Sripong et al. *(204)* reported no advantages of using UV-B over UV-C. In fact, UV-B doses

lower than 40 kJ m-2 were unable to reduce the disease incidence or severity of fruit rot in mangosteen, whereas a UV-C dose of 13 kJ m-2 reduced both experimental parameters. Similarly, Abdipour et al. *(6)* found that UV-C irradiation conferred a greater antioxidative capacity and total phenolic content than UV-B irradiation in sweet cherry (*Prunus avium*), though the best fruit quality was obtained with a combination of UV-B, UV-C, and chitosan coatings.

While there has been much investigation towards the priming activity of UV on the postharvest defense response, recent literature has uncovered there are still challenges towards this avenue of UV application. For example, studies from Zhu et al. *(248)* indicate that processors must account for storage lighting conditions to prevent the reversal of UV-induced photodamage on plant or human pathogens. In fact, the research group found that in white-light or blue-light storage conditions, UV-C treated B. cinerea exhibited an upregulation of UV damage repair-related enzymes, thereby increasing UV-C resistance. Jaramillo Sanchez et al. *(108)* reported trade-offs between UV-C induced cell wall modifications and produce transpiration which could negatively impact UV-treated produce shelf-life. While observing cell wall changes in UV-treated fresh blueberry (var. 'O'Neal'), the group noted a reduction in crystalline wax content indicative of disruptions in epicuticular wax production/formation. These changes in epicuticular wax content appeared to have negatively impacted water retention as the UV-treated fruits exhibited higher rates of water loss than the control fruit. Of interest, studies from Ortiz Araque et al. *(166)* also note improved shelf-life qualities (rate of decay, weight loss, firmness retention) in strawberry fruit treated with multiple, low-dose UV-C cycles instead of the more conventional single UV-C

dose. However, more research on how to implement this irradiation strategy in an industry setting is warranted.

Whole-Fruit Shelf-life Extension and Quality Enhancement. The shelf-life of a food item is determined based on the deterioration of limiting attributes which affect product quality or the consumer intent to purchase *(143)*. In the produce industry, the limiting attributes are not universal and vary based on the commodity and differ even amongst varieties or cultivars *(215)*. These attributes are critical to consumer acceptance, and may be categorized as physical (e.g., visual quality, color, waxiness/shine), organoleptic (e.g., odor, taste), or nutritional (e.g., antioxidant concentration) *(100, 170)*. Postharvest UV treatments have garnered attention in this aspect due to their ability to not only delay the degradation of these attributes, but also support their development when used at commodity-appropriate doses.

The delayed ripening observed in UV-treated produce is likely a consequence of slowed metabolic processes. As previously discussed, senescence in horticultural commodities is accompanied by the release of sugars from cell wall degradation, reduction in fresh weight due to water loss, and pigmentation alterations (e.g., yellowing, browning) due to oxidation. The effects of UV irradiation on gas exchange processes (i.e., ethylene production, respiration) appear to be commodity- and dose-dependent *(209, 235)*. Yet, UV-induced alterations in the degradation patterns of cell wall pectin and accumulation of lignin components *(46, 154, 166)* have been shown to increase water retention *(17)*, and consequently firmness *(57, 206)*, during storage. Additionally, UV-B delays chlorophyll and similar pigment degradation through the inhibition of enzymes like Chl-ase, Chl-POX, MD and MDS *(12, 13, 71, 127, 141, 180, 203)*. Postharvest

UV-C applications have also been shown to increase the antioxidant capacity of blueberries *(176)*, strawberries *(79)*, onions *(238)*, broccoli *(157)*, and peppers *(17)*. Beyond prolonging shelf-life, these metabolic changes are hypothesized to contribute to increased resistance to postharvest physiological damage from chilling injury in pepper *(219)*, banana *(178)*, and peach *(246)*.

Unlike preharvest treatments (discussed in further detail in the developing applications section), UV-A treatments do not appear to confer the same benefits as compared to UV-C *(142, 160)* and the effects of UV-B treatment can be inconsistent. This inconsistency in UV-B irradiation efficacy to promote fruit quality presents an avenue for further study. The effects of UV-B irradiation were likely not initially investigated due to studies reporting the induction of genes related to senescence and cellular decline *(110)*. While postharvest UV-B has since been shown to positively impact pigment degradation, admittedly, further study is still required to elucidate the metabolome changes induced by this range of radiation. For example, while UV-B irradiation resulted in favorable changes in the antioxidant profile of blueberries *(160)* and vitamin D2 content of mushrooms *(182)* after treatment, there were no detectable differences in broccoli *(137)*. A study performed in peach fruit found that UV-B -treated fruit showed both positive and negative accumulation patterns for different phytochemicals – making it difficult to judge the advantageous or disadvantageous nature of such treatments *(189)*. Overall, more study of the metabolome and underlying gene expression patterns is needed to understand the role of UV-B over (or in combination with) UV-C in promoting produce quality.

Emerging UV Applications in the Fresh Produce Industry: Postharvest Stage

Fresh Cut Produce. Fresh-cut fruits and vegetables have steadily gained popularity amongst consumers due to their convenience and compatibility with a healthy lifestyle *(62)*. However, many of these fresh-cut produce items are suitable matrices for the growth of human pathogens *(102, 190)* and spoilage microorganisms *(62)*. Chlorine-based sanitizers are often used to disinfect the surface of fresh-cut produce to improve product shelf-life while reducing the risk of contamination from human pathogens. But similar to the case of the whole-produce industry, such sanitizers present challenges for the reuse of wash water *(241)* and reduction of resistant microorganisms. A discussion on UV applications for fresh-cut produce quality and safety is hence included in this review as these fresh produce are minimally processed, and recent literature has developed the use of UV technologies in this industry.

UV technologies have shown much potential in recent literature to reduce the microbial risk and enhance the quality of fresh-cut produce comparative to the industry status quo. For instance, Santo et al. *(190)* found that the application of UV-C was more effective in reducing the microbial load of *Cronobacter sakazakii* on fresh-cut apple, pear, and melon than the industry standard (100 mg/L free chlorine) or acidified electrolyzed water. For fresh-cut produce with more complex surface structures (e.g., broccoli florets), treating the fruit with UV-C lamps submerged in water or a diluted solvent (i.e., peroxyacetic acid) appear to be more effective in reducing the microbial load than conventional UV applications *(54)*. Although this assertion appears consistent with previous literature in whole-produce UV treatments, more data using consistent UV systems is needed to support this observation. For example, Collazo et al. *(54)* compared the microbial inactivation efficacy between a UV system containing UV-lamps

submerged in a solvent and a non-submerged pulsed-UV system. Such solvent-incorporating UV applications, however, would serve a dual purpose in also reducing the microbial load of wash water *(55)*, thereby reducing the risk for cross contamination of human pathogens between different batches of fresh-cut produce.

The effects UV-C on fresh-cut produce quality are commodity-specific, yet many studies emphasize the positive effect of UV on fresh-cut produce quality. For example, in fresh strawberries, Avalos-Llano et al. *(21)* reported that UV-C treatment significantly enhanced the content of health-promoting phytochemicals including phenols, flavonoids, and anthocyanins by 14%, 23%, and up to 41% (respectively) in comparison with untreated strawberries. Whereas Han et al. *(96)* observed that UV-C treatment reduced the accumulation of phenolic compounds, the treatments also reduced chlorophyll degradation and PAL activity, leading to an increased retention of surface color and improved product marketability. Although UV-B applications to fresh-cut produce are not well-represented in the recent literature, Li et al. *(131)* observed that UV-B-treated fresh-cut peach fruit experienced reduced browning (up to four days in storage) and reduced weight loss throughout storage compared to the untreated fruit. It is critical to note, however, that the quality enhancing properties of UV applications may also depend on the cut intensity (cut size). Through a study performed with fresh-cut watermelon cylinders, researchers found that larger cut intensities best preserved the produce quality (though the antioxidant capacity of the control samples was lower regardless of the experimental cut intensity) *(18)*.

Postharvest Agricultural Water. Postharvest agricultural water is generally defined as water used during or after harvest (including during packing and holding) such as for commodity

rinsing/washing, movement, cooling, ice making, or postharvest fungicide and wax application. After harvest, the debris covering fresh fruits and vegetables is generally removed before proceeding through the packing line by an initial wash using a dump tank– a bin containing water for the batch-style rinsing of fresh produce. However, the batch-style of dump tanks can constitute a high risk for cross-contamination of human/plant pathogens from one affected produce to the rest of the batch; the potential for cross-contamination is of particular concern for human pathogens with a low infectious dose such as shiga-toxigenic *E. coli (164)*.

Chemical sanitizers are often added to the dump (flume) tanks in order to decontaminate the water and reduce the risk of cross contamination. However, the formation of harmful byproducts as the chemical(s) degrade *(161)*, can limit the re-cycling of water with added chemical sanitizers; negatively impacting the operations' environmental footprint. Additionally, changes in the organic content *(218)* and pH *(234)* of the dump-tank water following debris accumulation can negatively affect the efficacy of the chemical sanitizer, requiring time-consuming, ongoing surveillance of these parameters by facility personnel. For example, Murray et al. *(156)* found that the dynamic shifts of antimicrobial byproducts (free chlorine) in chlorinated wash water made maintaining the industry standard challenging in a typical operational setting. This is a potential reason explaining the observed inefficacy of dump tanks to reduce the microbial load of produce in the industry *(29)*. Van Haute et al. *(217)* recently reported that filtering wash water can reduce the concentration of debris and pathogen load of chlorinated wash water, increasing the antimicrobial efficacy and allowing for further water reuse. However, the filtered chlorinated wash water contained a higher concentration of gram-positive and spore-producing genera, potentially contributing to microbial spoilage. Consequently, there is an industry need for

antimicrobial interventions for dump tanks which require less monitoring and are efficacious in reducing the population of human pathogens, plant pathogens and spoilage microorganisms.

UV light is not affected by water characteristics such as pH and does not release dangerous byproducts *(132)*, facilitating the re-cycling of postharvest wash water and increasing the environmental sustainability of the overall operation. Recent literature emphasizes the use of water-assisted UV wherein, unlike in 'dry' applications wherein UV light is applied directly to the produce surface, the UV lamp is submerged into the water with the produce and gentle agitation is applied to facilitate the rotation (and thereby treatment) of the entire produce surface *(1, 56, 103, 168)*. However, many of these studies echo the challenges of using UV light alone to treat postharvest dump tanks due to the potential build-up of organic matter as debris is washed from the produce surface.

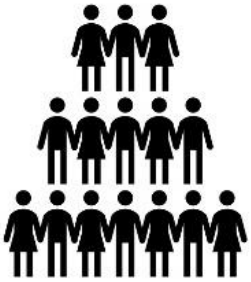
UV light has gained attention in recent studies for increasing the efficacy and reliability of chemical sanitizers applied to dump tanks. For example, in an advanced oxidative process (AOP), UV light accelerates the photodegradation of added hydrogen peroxide (H_2O_2) (typically in the presence of a catalyst) to form free radicals; these species disrupt cellular membranes and eventually lead to cellular death. If used in conjunction with chlorination, the integration of this process can reduce the free chlorine demand and reduce cross-contamination *(222)*. Similarly, Collazo et al. *(56)* observed bacteriostatic effects of UV-C treatments when used in conjunction with peroxyacetic acid (PAA), allowing for the further reuse of wash water (though arguably not reducing the microbial risk) and potentially reducing the risk of biofilm formation. When used in conjunction with LAPEN – a novel antimicrobial solution formulated by Leng et al. *(126)* –

pulsed UV light applications reduced the concentration of *Salmonella* and native surface microbiota of tomatoes; however, the role of this system in preventing microbial spoilage has yet to be investigated.

Major Challenges to the UV Industry

Despite the recent advances in preharvest and postharvest UV applications, there has been little traction in adopting the technology in the food industry. *Figure 4* summarizes the challenges towards implementing UV solutions in the food system using the most recent literature published on the subject (which will be discussed in further detail in this section). This review focuses on five key stakeholders in food system – producers, consumers, the UV industry, and research/extension personnel – their impact or 'role' in the food system, and what is currently the greatest challenge in securing their buy-in of on-farm UV technologies and UV-treated fresh produce.

Buy-in of UV Technologies In the Fresh Produce Industry Depends on Diffusion of Information and Technology



	Producers	Consumers	Food Retailers	UV Industry	Research & Extension
Role	Safe production and postharvest management of fresh produce	Select fresh produce with the highest quality and apparent food safety	Purchase and sale of safe, nutritious, affordable, produce	Manufacture UV innovations for application in many industries	Develop and distribute knowledge of UV to other groups
Challenge	Relatively little awareness of on-farm UV applications *(183)*	Low awareness of irradiated foods negatively affects consumer perceptions *(25)*	Sharing knowledge and spreading consumer awareness on the benefits of UV-treated produce *(70)*	Little marketing of UV technologies to fresh produce growers	Sparse knowledge of UV applications distributed to growers and consumers *(81, 85)*

Figure 4 *The role and challenges of major parties impacting UV applications to fresh produce.*

Increasing Grower Buy-In and Marketing in the Agricultural Sector. There has no doubt been a global rise of UV-focused companies which are producing a wide array of UV innovations. For example, colorimetric UV dosimeters (available in card or sticker form) have recently entered the commercial market as a more accessible and user-friendly method of measuring UV dose *(36)*. Various groups have developed UV-C decontamination robots which roam in pre-programmed paths, treating non-porous surfaces – such as those found in the

produce processing setting – along the way *(20, 92)*. Regarding water treatment, the UV industry has increased versatility, introducing UV systems with dynamic power control which alters the power output according to flow rate *(33)*. While many of these products fit the needs of the agricultural sector, very few of these technologies are marketed towards produce growers, severely impacting their visibility in this sector and subsequent grower adoption.

Communicating novel technologies to the agricultural sector is not a problem unique to the UV industry as the lack of appropriate communication strategies has led to the slow or low adoption of innovations in fields like precision agriculture *(173)* and blockchain *(53)*. There are numerous complex and multi-dimensional factors which impact grower decision-making in the adoption of novel technologies. Yet, grower awareness of the technology and its advantages has a critical role in many adoption models and theories *(173)*. There has yet to be a study that has directly evaluated the awareness and adoption of UV light technologies in the produce industry, but these metrics are assumed to be relatively low. For example, a study investigating attributes affecting grower selection of irrigation water treatment methods found that the average respondent was not aware of the operating cost of UV water treatment, and there was no strong agreement that UV could control human pathogens in irrigation water *(183)*. With the many innovations in UV design and application, there needs to be investigation into the effect of marketing strategies (specifically, the communication of benefits) on grower awareness and intent to purchase. There may also be unrecognized potential in informational campaigns delivering messaging on the impacts of UV-based products to produce growers and Extension personnel. *Figure 6* summarizes the roles and current challenges of the major parties impacting the implementation and acceptance of UV applications in the fresh produce industry.

Increasing Consumer Buy-in of UV-treated Fresh Produce

As previously mentioned, UV light is a form of non-ionizing radiation in contrast to ionizing radiation such as x-ray and gamma irradiation *(120)*. The use of radiation to decontaminate products in the food industry is not new, dating back to as early as 1957 *(67)*. However, similar the case of producers, there appears to be little visibility (and consequently, knowledge) of food irradiation by the average consumer. UV treatment exhibits the most marketable of fresh produce and vegetables in comparison to other forms of radiation (e.g., x-rays, Gamma radiation) *(136)*. However, consumers are still wary of irradiated foods despite the plethora of evidence that these products are safe and fit for human consumption. In fact, irradiation has been used in the food industry for more than 50 years to treat products such as meats, juices, and dry food ingredients, and is approved in more than 60 countries *(174)*. Interestingly, while consumers have become more aware of food safety and more willing to pay a premium to purchase safer food stuffs, consumers rarely hold positive perceptions of irradiated foods.

The lack of available knowledge on food irradiation is largely to blame for uncertain or negative consumer perceptions of irradiated foods. Food irradiation is most common on the North American and European continents, yet consumer studies across different countries in these areas repeatedly find that consumers are mostly unaware of food irradiation *(25, 85)*. In fact, a study by Bearth and Siegrist *(25)* found that consumers reacted more negatively to the term 'irradiation' than 'ionization' – despite the terms being synonymous in the case of x-ray, gamma, and e-beam irradiation. The study also found that produce items labeled with 'treated with irradiation' were perceived as lower quality whereas those labeled with 'treated with ionization' were perceived as having no difference in quality from the unlabeled controls. This low

consumer awareness of food irradiation could negatively impact the overall acceptance of UV-treated produce and deserves equal attention to improving grower acceptance of the technology. Accordingly, this problem requires combined efforts between the food industry, the UV scientific community, and members of the social sciences to overcome. Fortunately, the research has highlighted several methods of increasing consumer acceptance of UV-treated foods.

The future of food irradiation is encouraging from the consumer perspective as research indicates that consumer opinions can be easily improved by labeling strategies and informational campaigns. In fact, Galati et al. *(85)* reported that despite the low consumer awareness of irradiated foods, 89.2% of their survey respondents were interested in receiving more information about the technology. To combat the stigma of food irradiation, D'Souza et al. *(70)* suggest that both food producers and retailers could play a critical role in providing such information to boost consumer attitudes and purchase of UV-treated food items. Regarding labeling practices, although consumers experience aversion to statements like 'treated with irradiation', consumer acceptance increased in fresh packed blueberries labeled with this statement when combined with the technologies' benefits (e.g., 'treated with irradiation for food safety'). In a study to gauge the perceptions of irradiated spinach, providing information on food irradiation dispelled misleading food irradiation myths (e.g., that treated food was harmful to human health) and increased consumer acceptance by 90% *(81)*. These findings show that the onus is not only on the consumer to accept irradiated foods, but also lies with the food industry and scientific community to ensure that scientifically validated information which a consumer can use to form an informed opinion is available.

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Chapter 2 - Comparative Assessment of the Microbial Quality of Agricultural Water on Kansas and Missouri Fresh Produce Farms

ABSTRACT

In 2015, the United States Food and Drug Administration (FDA) published the Produce Safety Rule (PSR) providing guidance for growers to minimize food safety risks associated with growing, harvesting, packing, and holding fresh produce. To mitigate foodborne outbreaks attributed to contaminated agricultural water, the PSR requires growers to test their water for microbial contamination. The increased production of fruits and vegetables in Kansas and Missouri necessitates the investigation of agricultural water quality in these states. This study assessed and compared the prevalence of generic *E. coli* in agricultural water sources in both states. A total of 426 agricultural water samples were analyzed using the IDEXX Colilert with Quanti-tray/2000 method. While there were no statistically significant differences in the prevalence of *E. coli* in agricultural waters detected between the two states ($P < 0.4023$), the average number of *E. coli* in surface water sources (158.7 MPN/100mL ($n = 247$) was statistically greater than that of groundwater sources (20.4 MPN/100mL ($n = 179$), $P < 0.0001$) and seasonal effects were detected ($P < 0.0001$). These results demonstrate the higher microbial risk of surface water compared to groundwater in both states and the need for continued grower education on safe water management practices.

INTRODUCTION

Fresh produce is a major vehicle for foodborne pathogens *(10)* as such food commodities are often consumed raw and there is no 'kill step' *(29, 33)*. From 2009 to 2018, there were 753 foodborne outbreaks associated with leafy greens alone in the United States, resulting in 15,603 illnesses, 1,604 hospitalizations, and 151 deaths *(4)*. In 1998, the United States Food and Drug Administration (FDA) published a general guide of voluntary on-farm practices to minimize microbial safety hazards associated with fresh produce *(38)*. However, following numerous foodborne outbreaks attributed to contaminated agricultural water in commodities such as leafy greens *(19, 26)*, tomatoes *(1)*, and melons *(42)*, water management practices became a critical theme in produce safety *(20)*. In 2015, the FDA finalized the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) outlining science-based practices to reduce the prevalence and transmission of pathogens to produce, including through agricultural water *(9)*.

The FSMA PSR highlights information on contamination routes and best practices to minimize risks of contamination. One of the routes focuses on agricultural water quality and highlights the needs for water testing and establishing a microbial water quality profile (MWQP) *(37)*. This profile is developed using generic *Escherichia coli* (*E. coli*) as an indicator organism for fecal contamination with a testing frequency dependent on the inherent risks associated with the agricultural water source. There are three primary water source types associated with produce operations: public water supplies, ground water, and surface water. Public (municipal) water supplies assume the 'lowest risk' of microbial contamination since the water is treated and its microbial quality is regularly monitored by a utility entity; this source, however, may be cost-prohibitive for larger operations that consume a greater amount of water *(45)*. Ground water

(e.g., well water) is considered a 'moderate risk' with the potential for microbial contamination events due to poor aquifer quality and/or well structural integrity. Although more convenient to access and use *(11)*, surface water (e.g., ponds, rivers, creeks, rainwater catchment systems) are considered the 'highest risk' since growers are generally unable to completely isolate surface water sources from external sources of microbial pollution (i.e., contaminated soil, wild animal and/or livestock feces). Besides the water source, factors such as proximal livestock density *(14, 18)*, climate *(21, 27)*, and frequency of water treatments (e.g., chlorine shock, filtration) *(3, 40)* can affect microbial water quality.

The PSR states that all agricultural water must be "safe and of adequate sanitary quality for its intended use" (21 CFR 112.43). Based on a rolling 4-year dataset of *E. coli* test results, an agricultural water source used for pre-harvest operations (i.e., irrigation, fertigation) must have a geometric mean (GM) of less than 126 Colony Forming Units (CFU) of *E. coli* per 100 mL of water, and a statistical threshold value (STV) of 410 or less CFU generic *E. coli* per 100 mL of water. Of note, both mathematical values are important when building a MWQP as the GM provides information on the average amount of generic *E. coli* in the water source, and the STV captures the variation in *E. coli* levels during the year – potentially as a result of adverse events (i.e., rainfall). Water sources used for postharvest purposes (i.e., rinsing produce or washing hands) must contain no detectable *E. coli* per 100 mL of water *(37)*. Moreover, untreated surface water is not permitted for postharvest use. Although the FDA extended the compliance dates of the PSR agricultural water testing requirements to facilitate grower adherence to the rule, studies indicate that agricultural water remains one of the least understood topics of the FSMA PSR in Midwestern states *(5, 24, 25, 28)*. Interestingly, to the authors' knowledge, the microbial safety

of agricultural waters in these states has rarely been studied, with the exception of Iowa *(2)*. Bhullar et al. *(2)* reported few contamination events for ground water sources in Iowa; however, contamination of agricultural surface waters with generic *E. coli* was prevalent in Iowa, with some surface water samples exceeding the FDA's maximum allowed GM thresholds.

Each year, Kansas and Missouri growers produce approximately $26 million *(16)* and $81 million *(6)* of fruits and vegetables, respectively, with recent notable increases in the production of specialty crops. For example, berry production increased dramatically in Kansas, with the number of blueberry farms increasing by 269 percent, blueberry acres by 250 percent, blackberry farms by 112 percent, and blackberry acres by 260 percent 2007 to 2017 *(22)*. The number of Kansas farms producing tree fruit also increased by 46 percent from 2007 to 2017 *(22)*. To support the outputs of this growing industry, the purpose of this study is to understand the microbial quality of agricultural waters used by growers in Kansas and Missouri and identify opportunities for Extension education and outreach. The objectives of this study are thus to 1) evaluate and 2) compare the prevalence of microbial contamination in agricultural water sources on Kansas and Missouri farms.

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MATERIALS AND METHODS

Sample collection and submission. From 2018 to 2020, individual growers, Extension educators, or trained laboratory personnel collected water samples from agricultural water sources on Kansas and Missouri produce-growing operations (*Figure 7*) according to shared instructions prepared by the Kansas State University (KSU) / University of Missouri Extension produce safety team *(available at: https://www.ksre.k-state.edu/foodsafety/produce/testing.html*). The water samples were collected in 100 mL sample containers with added sodium thiosulfate (IDEXX Laboratories, Westbrook, ME) to reduce the effect of residual chlorine on *E. coli* stability during transit, and then mailed overnight or submitted in-person in a refrigerated cooler box with ice to a microbial water quality testing laboratory for analysis according to their state of residence. Kansas growers submitted water samples to either the Food Safety Laboratory at KSU in Olathe or Manhattan, whereas Missouri growers submitted the samples to the KSU lab in Olathe or the Missouri State Public Health Laboratory (MSPHL). The samples were accompanied by a submission form specific to the laboratory to provide information on water sources (*available at: https://www.ksre.k-state.edu/foodsafety/produce/testing.html*). Water samples were received from various locations in eastern Kansas and throughout Missouri, as shown in *Figure 5*. Because these labs provide microbial water testing services free of charge to produce growers through grant funding, multiple entries from the same grower may have occurred throughout each year. Also, note that it was unknown if the operations which submitted samples for analysis treated their agricultural water before use in production or postharvest activities.

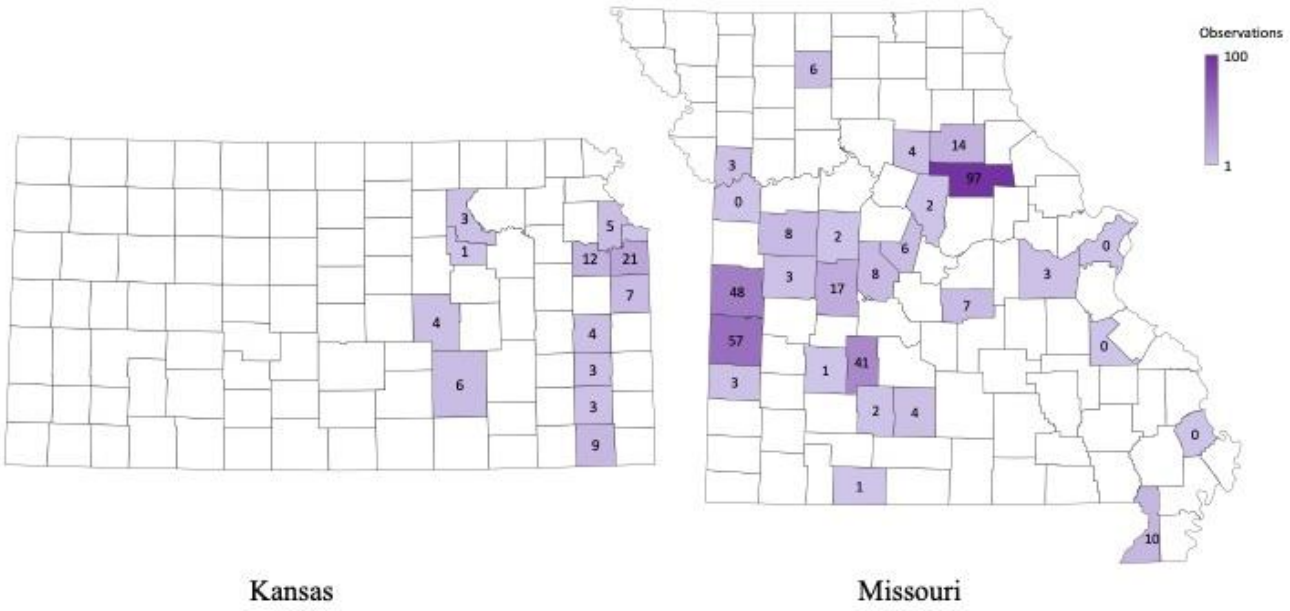

Figure 5 *Distribution of agricultural water source samples from counties in Kansas and Missouri. The number in each county indicates the number of samples included in the dataset from that county.*

Sample testing methodology. The three labs used the IDEXX Colilert with Quanti-Tray/2000 test method (U.S. Food & Drug Administration, 2014) (IDEXX Laboratories, Westbrook, ME) to obtain the Most Probable Number (MPN) of generic *E. coli* per 100 mL of water. Briefly, one snap-pack of Colilert reagent was added to each 100mL water sample and completely dissolved through vigorous shaking before being sealed in a Quanti-Tray and incubated for 24 hours at 35ºC ± 0.5ºC. The Colilert reagent is a proprietary Defined Substrate Technology (DST) wherein two nutrient indicators – ONPG and MUG – act as the major source of carbon for coliforms and *E. coli* (respectively), promoting their selective growth in the sample during incubation (Fricker et al., 1997). β-glucuronidase, an enzyme conserved in 94 to 96% of *E. coli (12)* metabolizes

MUG, cleaving the MUG-bound fluorescent reporter 4-methyl-umbelliferone. The MPN of generic *E. coli* was determined based on the number of large and small wells which fluoresced under ultraviolet light (Spectroline, Melville, NY); an MPN table was provided by the manufacturer (IDEXX Laboratories, Westbrook, ME) for the MPN calculations. The lower limit of detection was <1.0 MPN/100 mL and the upper limit of detection was >2419.6 MPN/100mL. Note that the FDA determined this method as scientifically valid and at least equivalent in accuracy, precision, and sensitivity to the conventional EPA 1603 method for quantifying generic *E. coli* in water which utilizes the CFU per 100mL unit *(35)*.

Data preparation. The data from the KSU Food Safety Laboratories and the Missouri State Public Health Laboratory were shared with the project team and reviewed prior to analysis. To prepare the dataset for analysis, the sample source was first classified as ground water or surface water according to the sample description. Entries in which the samples exceeded a 24-hour holding time were not used in subsequent analyses. Personal identifiers (e.g., grower name, address) were deleted from the working datasets to protect the privacy of the grower.

Statistical analysis. The study was considered an incomplete block design with STATE as a fixed blocking factor. All generic *E. coli* counts were documented as most probable number (MPN)/100 mL values and transformed to fit a logarithmic distribution for statistical analysis. For samples which exceeded the microbial testing threshold value (>2419.6 MPN/100mL), 2419.6 MPN/100mL was used for further analyses; 1 MPN/100mL was used for values below the testing threshold value (<1 MPN/100mL). To investigate the variation in generic *E. coli* concentrations attributed to season; the seasons were divided into winter (December to

February), spring (March to May), summer (June to August) and fall (September to November). Differences between the means were computed using the GLIMMIX procedure (SAS 9.4, Cary, NC) with the Tukey-Kramer adjustment for multiple comparisons of unequal sample sizes. Statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Source type distribution. Previous literature has primarily emphasized the study and surveyance of surface water microbial quality *(17, 30, 32)*; the microbial quality of other types of agricultural water sources (ground, municipal, etc.) within a growing region had rarely been directly compared. The findings of this study illustrate the considerable use of ground water sources within produce-growing operations in midwestern states. In fact, 41.8% (33/79) of the water samples from Kansas and 42.1% (146/347) of the water samples from Missouri were from ground water sources (*Table 2*). Bhullar et al. *(2)* reported similar findings in Iowa wherein 67% (69/101) of the agricultural water samples for microbial testing were collected from ground water sources. Future studies would benefit from examining the microbial quality of agricultural water source types according to their usage in the region (pre-harvest or post-harvest use) to facilitate more directed agricultural Extension and outreach activities. In the Midwest and regions of similar ground water usage trends, this directive would include to also investigate the microbial water quality of ground water sources.

Table 2 *Source type distribution of water samples tested by source in Kansas and Missouri*

Source Type	Kansas		Missouri	
	***n*= 79**	**%**	***n*= 347**	**%**
Ground water	33	41.8	146	42.1
Surface water	46	58.2	201	57.9

**Note: Table 2 shows the number of ground and surface water samples submitted from produce growers in Kansas and Missouri in this study.*

Prevalence of generic *E. coli* in agricultural water sources. Generic *E. coli* was detected in 77.7% (192/247) of surface water and 29% (34/179) of the ground water samples collected in this study (*Table 3*). The widespread presence of generic *E. coli* in surface water sources is well-documented, particularly because this source type is exposed to contamination events from contact with domestic or wild animal fecal matter *(39)*. In fact, previous studies of surface water microbial quality from the midwestern states of Iowa *(2)* and Ohio *(44)* reported the presence of generic *E. coli* in 31% (32/101) and 96.9% (219/226) of the samples collected, respectively. Moreover, in Iowa, Bhullar *(2)* noted 5.8% (4/69) of the ground water samples contained generic *E. coli*; of note, many of these ground water samples were collected from shallow wells (<60 ft). Other studies have also shown *E. coli* contamination of ground water sources *(8, 13, 15, 23)*, with one study *(8)* indicating that such contamination can be exacerbated by certain types of well construction. Further investigation is needed to determine the cause of the *E. coli* contamination of ground water sources in Kansas and Missouri and ways to mitigate these risks.

Table 3 *Generic E. coli prevalence data in ground and surface water sources in Kansas and Missouri*

MPN/100mL	Ground water		Surface water	
	***n*= 179**	**%**	***n*= 247**	**%**
<1	145	81.0	55	22.3
1-126	31	17.3	152	61.5
126-2419.6	2	1.1	35	14.2
>2419.6	1	0.6	5	2.0

**Note: Table 2 shows the relative prevalence of generic E. coli (CFU/100mL) in ground and surface water sources based on the provided data (N=426).*

Overall, the agricultural water source type was a significant source of variation in the concentration of generic *E. coli* ($P < 0.0001$), though there were no statistically significant differences detected between the states ($P < 0.4023$). This study not only reported higher microbial risk in surface waters but also concurs with the PSR's classification of surface water as 'higher risk' and ground waters as "lower risk" for microbial contamination *(36)*. The average *E. coli* concentration for agricultural water samples tested in Kansas and Missouri was 158.7 MPN/100mL (*n*= 247) for surface water and 20.4 MPN/100mL (*n*= 179) for ground water source samples (*Table 4*). Accordingly, the concentration of generic *E. coli* in surface water sources was significantly greater than that of ground water ($P < 0.0001$) sources.

Table 4 *Average generic E. coli concentration (CFU/100mL) of ground and surface water sources in Kansas and Missouri*

Source Type	**Kansas** ***n*= 79**	**Missouri** ***n*= 347**	**Overall** ***n*= 426**
Ground water	2.8 (*n*= 56)	24.3 (*n*= 123)	20.4 (*n*= 179)
Surface water	31.4 (*n*= 29)	188.3 (*n*= 218)	158.7 (*n*= 247)

**Note: Table 4 shows the average generic E. coli concentration (CFU/100mL) in ground and surface water sources based on the provided data.*

Microbial risk of Kansas and Missouri source waters in relation to the PSR. Per the regulations outlined in the PSR, the results of this study indicate that surface waters in Kansas and Missouri are more likely to be unfit for use in production-related activities than ground source waters (*Figure 6*). However, growers need to develop a water quality profile to determine the compliance with the PSR rule. More information on microbial water quality profile can be found in Subpart E of the Produce Safety Rule.

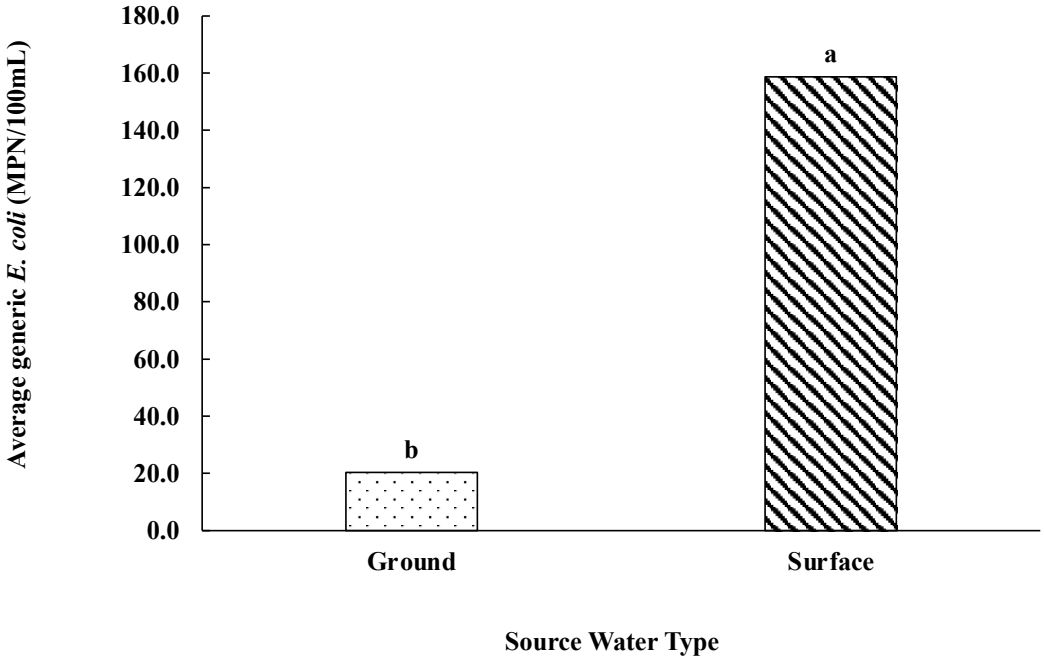

Figure 6 *Average E. coli concentration for agricultural water sources in Kansas and Missouri (n= 426).*

**Note: Statistically significant differences between the log-transformed means of the source water types are denoted with letters (α = 0.05).*

The seasonality of generic *E. coli* concentrations in surface water sources are well recorded in the literature with the concentration of generic *E. coli* typically increasing with rising air temperatures *(31, 43)*. In this study, season was a statistically significant source of variation ($P < 0.0001$). From further analysis performed post-hoc, surface water sources contained a statistically higher concentration of generic *E. coli* than ground water sources in the spring ($P < 0.0001$) and summer ($P < 0.0001$) months; no statistically significant differences were detected between the sources during the fall ($P = 0.0541$) or winter ($P < 0.2100$). Notably, the 2.0% ($n = 5$) of surface water samples and 0.6% ($n = 1$) of ground water samples which exceeded the threshold

value of the testing method (> 2419.6 MPN/100mL) were recorded during the spring and summer months (April – August).

Potential challenges to PSR compliance. The findings of this study indicate that some produce growers in Kansas and Missouri may still face barriers to accessing microbial water quality testing services despite lowered costs, based on the fact that many samples submitted could not be tested due to exceeding the 24h hold time. The National Water Summit facilitated by the Produce Safety Alliance in 2018 emphasized the cost of agricultural water testing being a major challenge to growers. During the summit, groups expressed concern that the estimate of $1,058 in testing costs per year per farm calculated by the FDA may not cover additional costs to the grower such as time to collect and transport the sample or testing costs if the operation uses water from multiple sources *(41)*. Offsetting the cost of agricultural water testing for Kansas and Missouri growers was a critical motivator of requesting this grant funding, particularly as a study of food safety practices by Perry *(24)* in the North Central Region (encompassing Kansas and Missouri) revealed that up to 43% of the surveyed growers (N=253) may not be testing their agricultural water. However, this study suggests sample transit/holding times as another potential obstacle for growers in the Midwest, in addition to the cost. The database originally contained a total of 760 observations, but only 507 (66.71%) of the observations could be used for this analysis. The remaining samples were excluded from the statistical analysis since they exceeded the 24-hour sample transit/holding time limit specified by the Environmental Protection Agency (EPA) Revised Total Coliform Rule to prevent microbial decline *(2, 34)*. Potential actions to address these challenges are included in the subsequent Conclusions/ Recommendations section of this manuscript.

Study Limitations. Accessing the three laboratories' databases presented an excellent way to leverage existing data and capture a snapshot of regional water quality, but this methodology inherently carries a few limitations. Firstly, the sampling region in Kansas is heavily East-biased whereas the sampling region in Missouri is more evenly distributed. Although the geographical distribution is likely a result of the sampling sites proximity to the testing labs, this aspect introduces regional bias to the dataset. Further, there is a much larger number of produce growers in eastern Kansas than in western Kansas, based on the contacts that the project team receives from both parts of the state and the fact that membership in organizations such as the Kansas Specialty Crop Growers Association is much more heavily based in eastern Kansas. Effectively, the findings of this study may not reflect the reality of agricultural water quality used for produce in Western Kansas, and further investigation to expand the dataset and include more sampling sites in Western Kansas is needed. The databases also did not always specify from which source the samples were taken on each farm (i.e., Pond A, Pond B), so it was impossible to ascertain if there were resamples or if each data entry was a unique water source. As a consequence, only the average was reported in this manuscript, as it would be inappropriate to calculate the GM and STV for different water sources.

CONCLUSIONS/RECOMMENDATIONS

To the authors' knowledge, this is the first study, in Kansas and Missouri, to provide an individualized and comparative analysis of the microbial quality of agricultural water sources used in produce-growing operations. Agricultural water regulations and management practices outlined in the FSMA PSR are among the least understood topics by growers in Mid-western states *(5, 24, 25, 28)*. As the production of fresh fruits and vegetables increases in Kansas and

Missouri, ensuring produce safety is critical for consumer health and for supporting the local and state-wide economy. This study was designed to determine the prevalence of *E. coli* in agricultural water sources from Kansas and Missouri produce-growing operations. The study findings highlighting the contamination of surface and ground waters with generic *E. coli* reinforces the need for effective, accessible water quality control and treatment methods to minimize the microbial risks on fresh produce farms.

The results of this study largely coincide with the conclusions of previous studies in the Midwest *(2, 5, 24, 25)* that continued Extension education and outreach are needed to improve grower knowledge on water testing requirements and treatment methods. Kansas State Research and Extension and University of Missouri Extension personnel are fulfilling this need by providing digital materials for growers regarding produce safety and water safety at (https://www.ksre.k-state.edu/foodsafety/produce/index.html) and (https://extension.missouri.edu/programs/food-safety) respectively. Continued efforts are also being made to provide printed materials for Plain community growers (Amish and Mennonite) as knowledge gaps become increasingly clarified *(7, 24)*.

Agricultural water treatments are another factor affecting microbial risk which was not explored in this study. Identifying the common chemical and/or physical agricultural water treatments (if used) within Kansas and Missouri produce-growing operations will aid in guiding Extension education on agricultural water treatment methods and may help improve produce safety. As a recommendation for future agricultural water surveys, it would be beneficial to continue to investigate the microbial quality of varying agricultural water source types (as opposed to solely

surface water) and information on environmental factors contributing to an increased *E. coli* prevalence (i.e., temperature, precipitation, animal grazing, farming practices), to establish a water quality profile reflective of the sources commonly used in the region and facilitate more directed produce safety Extension and outreach activities. These approaches will help with drawing science-based conclusion on minimizing food safety risks.

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Chapter 3 - Developing a decision-making tool for agricultural surface water decontamination using ultraviolet-C light

ABSTRACT

Ultraviolet (UV) light-assisted water treatment systems are an increasingly investigated alternative to chemical sanitizers for agricultural surface water decontamination. However, the relatively high concentration of particulate matter in surface water is a major challenge to expanding its application in the production of fresh produce. The objective of this project was to test the efficacy of two commercial UV-C devices to reduce the microbial risk of agricultural water in order to develop a web application to assist growers in decision-making related to the on-farm implementation of UV technologies for agricultural water treatment. An on-farm study using three agricultural water sources was performed to determine the microbial reduction efficacy of a low power, low flow (LP/LF; 1-9 gallons per minute (GPM), 1.34-gallon capacity) and a high powered, high flow (HP/HF; 1-110 GPM, 4.75-gallon capacity) device at flow rates of 6, 7, and 9 GPM. A threshold of 30% UVT for the HP/HF device was observed, wherein lower water transmissibility significantly impacted microbial inactivation. Although less effective at lower %UVT, the LP/LF device costs less to install, maintain, and operate. The observations were used to design an online tool for growers to calculate the predicted reduction of generic *Escherichia coli* using either device based on the %UVT of their water source. These results of this study demonstrate the utility of UV light in reducing the microbial risk of agricultural water, and further studies are needed using different UV devices and higher flow rates to expand the use of the decision-making tool.

INTRODUCTION

Surface waters (i.e., ponds, rivers, streams) are commonly used in produce-growing operations as they are typically cost-effective and convenient to access and utilize in large- to small-scale growing operations *(9)*. Unfortunately, surface waters also carry a high risk of microbial contamination as they are typically exposed to external sources of fecal contamination (i.e., agricultural run-off, birds, wild/domestic animals) *(32)*. Pathogenic bacteria (e.g., *E. coli* O157:H7), viruses (e.g., Norovirus), and parasites (e.g., *Cyclospora* cayetanensis) are readily transmitted to fresh produce through contact with contaminated water *(7)* and such events can easily result in foodborne illness, particularly if the produce is consumed raw. Currently, chemical sanitizers are the most commonly used intervention in agricultural operations to reduce the microbial populations of their agricultural water *(7)*. However, these chemicals may have negative consequences for plant and human health *(22, 27)*, and their efficacy can be limited by the water body's physiochemical characteristics (i.e., pH, organic matter content) *(8, 21)*. With new information that these sanitizers may induce the viable-but-not-culturable (VNBC) state of potentially pathogenic microorganisms *(7)*, there is increasing interest in non-chemical antimicrobial alternatives within the fresh produce industry.

Ultraviolet-C (UV-C) light is increasingly viewed as an alternative for agricultural surface water decontamination since it is highly effective, user-friendly, and does not produce toxic by-products *(2, 3, 26)*. UV-C technologies are effective against acid-resistant pathogens (i.e., *E. coli* O157:H7) and emerging chlorine-resistant pathogens (i.e., *Cyclospora* spp.) of high public health interest in water *(1, 16, 29)*. Furthermore, there have been no reports in the literature of UV-C causing a VBNC state, although this is clearly a topic that needs more research. However, the

relatively high abundance of UV-absorbing and UV-scattering matter in surface waters presents the greatest challenge for the application of UV-assisted agricultural water treatment. The abundance of such particulate matter has been historically represented in the literature by measures of turbidity – the amount of light scattered by matter suspended or dissolved in the water (soil, clay, organic matter, etc.). Understanding how turbidity influences treatment efficacy is critical because UV-C is not a highly penetrative form of radiation; thus, even particulate matter as small as 11µm can 'shield' microbes from treatment and reduce the amount of irradiation available for decontamination *(4)*. However, turbidity is not the sole determining factor for UV efficacy. There are also certain solutes in agricultural waters that may not significantly impact turbidity but can absorb or scatter UV light at specific wavelengths *(39)*, further reducing the efficacy of UV-assisted agricultural water treatment systems. For this reason, both turbidity, as well as the percentage of UV-C light transmitted through agricultural water at a certain wavelength (percent UV transmittance; %UVT) are key measures in UV studies. Beyond these metrics, water flow rate, UV-C intensity, and the type of pathogen present in the water source could impact the treatment efficacy *(14, 40)*.

Despite the growing applicability of UV-C technologies for agricultural water decontamination (as discussed in this section), there has been relatively little traction amongst growers toward the adoption of the technology by growers. The lack of documentation available regarding the resource and capital inputs of this technology is likely a major deterrent to its implementation. This factor has been documented in other fields such as precision agriculture, where access to information regarding ongoing cost, labor intensity, and efficacy was a major barrier to the deployment of novel agricultural technologies *(25, 31)*. Furthermore, the initial price tag of UV-

C technologies is high compared to its chemical sanitizer counterparts (e.g., chlorine), which presents another prohibitive factor if the ongoing costs are not sufficiently low to justify the high initial investment *(18, 30)*. Yet, Tak and Kumar *(30)* examined the use of UV-C decontamination in drinking water treatment plants and argued that due to the low ongoing costs of UV-C decontamination, this technology could reduce the overall costs incurred by using chlorination by at least 63%. However, there are few studies that have explored the initial and ongoing costs associated with UV-C water treatment systems in the fresh produce production setting.

The purpose of this study was to investigate the efficacy and assess the costs of two commercially available UV-based water treatment systems used for agricultural water decontamination purposes. To achieve this goal, the objectives of this study were threefold: (1) characterize and compare the antimicrobial efficacy of two commercially- available UV-C devices in artificially-inoculated and natural surface waters, (2) conduct an economic study to compare the costs associated with the UV-C devices, and (3) develop an online decision-making tool for fresh produce growers in Kansas and Missouri seeking to integrate commercially-available UV-C devices into their growing operations.

MATERIALS AND METHODS

General characteristics of the experimental UV-C devices. Two UV-C devices exhibiting a peak emission wavelength of 254 nm were tested in this study. The devices were selected based on their different characteristics (e.g., shell size, lamp number; *Table* 5), company reputation, and availability of the device for testing. The research group did not receive any financial support from either company for this research. The first device, hereafter referred to as high

powered, high flow (HP/HF; model no. UV-25T80; SARIN Energy Solutions, Overland Park, KS, USA), and exhibited an optical power output of 320W at a voltage of 240V. The second device, hereafter referred to as low powered, low flow (LP/LF; model no. Minipure MIN-9; Atlantic Ultraviolet Corp., Hauppauge, NY, USA) exhibited an optical power output of 34W at a voltage of 120V. The device specifications (flow rate capacity, shell size, etc.) are reported below as per the manufacturers in *Table 5*. It is important to note that because this study takes a proof-of-concept approach wherein direct comparisons between the two devices are desired, the selected experimental flow rates for both devices were limited to 1-9 gallons per minute (GPM; 3.8 – 34.1 liters per minute) to reflect the lower capacity of the smaller unit.

Table 5 *Specifications of the experimental UV-C devices as provided by the manufacturers*

Specification	**HF/HP**	**LF/LP**
Manufacturer	SARIN Energy Solutions	Atlantic Ultraviolet Co.
Model	UV-25T80	Minipure MIN-9
Flow Rate Capacity	1 – 110 GPM (3.8 – 416.4 LPM)	1 – 9 GPM (3.8 – 34.1 LPM)
Power	320 W	34 W
No. of UV Lamps	4	1
Shell Size (l x w x h)	906mm x 159mm x 360mm	750mm x 108mm x 146mm
Body Material	Stainless Steel	Stainless Steel

Note. Abbreviations: GPM = gallons per minute, LPM = liters per minute

Characterizing the devices' antimicrobial efficacy in artificially inoculated surface water

Culturing and enumeration of microorganisms. Validation studies were initially completed under controlled laboratory conditions because there was no manufacturer information available to determine the potential efficacy of the devices in natural source waters with high concentrations of UV-absorbing particulate matter. *Escherichia coli* (ATCC #25922), *Salmonella enterica* ser. Typhimurium (ATCC #14028), and *Listeria innocua* (ATCC #33039) were used as test microorganisms for the laboratory validation studies. The strains were acquired from the Bhullar Food Safety Laboratory (Kansas State University, Olathe, KS, USA), and stored in 25 to 50% glycerol (Fisher Scientific, Waltham, MA, USA) in cryovials at −80°C. The strains were initially propagated by transferring a loopful of the frozen culture to Tryptic Soy Agar (TSA, Remel Inc., San Diego, CA, USA) and incubation at 35 ± 2 ºC for 24 ± 2 hours. To generate 48-hour subcultures, one colony from the master plate was then transferred to a culture tube containing 10 mL of Tryptic Soy Broth (TSB, Remel Inc., San Diego, CA, USA) or Brain-Heart Infusion broth (BHI, Remel Inc., San Diego, CA, USA) according to the organism, and the tube was incubated at 35 ± 2 ºC for 24 ± 2 hours. Then, 100µL of the incubated culture was transferred to 30mL of TSB or BHI and incubated at 35 ± 2 ºC for another 24 ± 2 hours. The cells were prepared for experimentation by centrifugation (Allegra X-30 R, Beckman Coulter, Brea, CA, USA; 10,000 rpm, 10 min, and 20ºC) and washed twice with 0.1% (w/v) phosphate buffer saline (PBS, Thermo Fisher Scientific, Waltham, MA, USA). The final volume was adjusted to 40 mL to achieve a concentration of at least 6 log10 CFU/mL.

Experimental setup. Because the HP/HF device required larger volumes to operate, only the LP/LF could be feasibly used for laboratory validation. *Figure 7* depicts the setup and workflow

for the laboratory validation experiments. The materials to construct the system can be readily purchased from common home improvement retailers and are detailed in the supplementary materials (*Appendix A Table A.1*). Briefly, the laboratory water treatment system was established using a 30-gallon (113.6 L) inlet vessel (ULINE, Pleasant Prairie, WI, USA) containing a pump (TotalPond, West Palm Beach, FL, USA) connected with vinyl tubing to the inlet of the UV-C device; the device outlet was then connected via vinyl tubing to a 30-gallon (113.6 L) outlet vessel (ULINE, Pleasant Prairie, WI, USA) to collect the wastewater. The inoculated surrogate surface water was continuously stirred using an electric drill (Model P215, Ryobi, Fuchu, Hiroshima, JP) equipped with a mixer attachment purchased from a local hardware supplier. An in-line digital flow meter (Fill-Rite Model TT10A, Tuthill Corporation, Ft. Wayne, IN, USA) and a PVC flow regulator purchased from a local hardware supplier were used to measure and modulate the flow of the inoculated simulated surface water throughout the system.

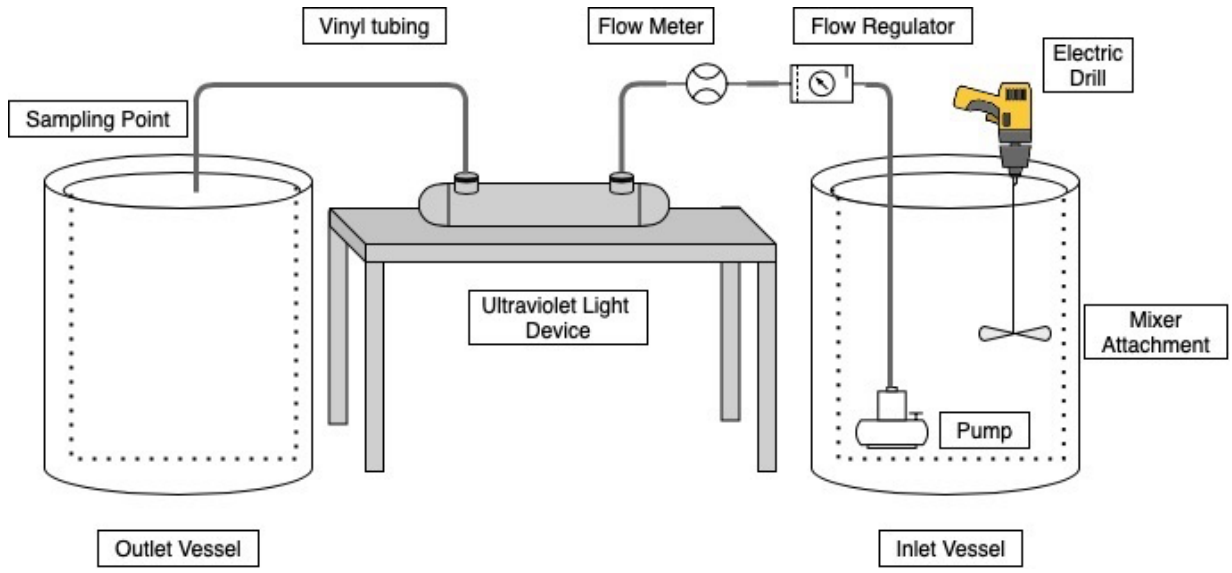

Figure 7 *The experimental setup for the laboratory validation trials*

Inactivation experiments. The inlet vessel was filled with 20 gallons (75.7 L) of distilled water and the percent UV-C transmission at 254nm (%UVT) was adjusted to either 20%, 30% or 40% using humic acid (GS Plant Foods, Sanford, FL, USA). The simulated agricultural surface water was then mixed for 5 minutes, and the %UVT and absorbance at a wavelength of 254 nm (A254) were measured using a spectrophotometer (Genesys 10UV; Thermofisher Scientific, Waltham, MA, USA). The simulated agricultural surface water was then inoculated with a washed cell suspension of either *Escherichia coli*, *Salmonella* Typhimurium, or *Listeria innocua*. The device was then turned on, and the inoculated simulated agricultural water was mixed for 5 minutes. Three control samples were extracted from the inlet tank before the experimentation began. At each of the experimental flow rates – 9, 7, and 6 GPM (34, 26.5, 22.7 liters per minute) – three 10mL samples were collected in 10 mL centrifuge tubes (Fisher Scientific, Waltham, MA, USA) from the outlet tube before reaching the wastewater collection vessel. After each experiment, the entire system was sanitized via a sodium hypochlorite rinse with a contact time of 30 minutes. Afterward, the sodium hypochlorite was rinsed from the system with distilled water. The wastewater in the outlet tank was pumped into 5-gallon (18.9 L) vessels (Fisher Scientific, Waltham, MA, USA), autoclaved, and disposed of appropriately. Each experiment had three replicates and was repeated three times on different days for each microorganism.

Microbial enumeration. The surviving microbial population was enumerated within one hour of each experiment. Serial dilutions were prepared as needed with 0.1% buffered peptone water (BPW, Remel Inc., San Diego, CA, USA), and 100 µL of the sample was plated onto TSA via the spread plate method. The plates were then incubated at 35 ± 2 ºC for 24 ± 2 hours. Plates yielding 25 to 250 colony-forming units (CFU) were counted, and log reductions were

calculated. All the experiments were conducted in triplicate, with three repeats conducted on three different days for three months (e.g., n= 27 for each treatment).

Characterizing the devices' antimicrobial efficacy in natural surface water

Description of field sampling points. The microbial inactivation efficacy of both devices was tested using three different surface water sources used for irrigation at the Olathe Horticulture Research and Extension Center in Olathe, KS, USA. The surface water sources were selected due to their varying levels of turbidity and %UVT, and included unfiltered stream water, stream water filtered via sand-filtration, and pond water. The unfiltered stream water was pumped from the stream (Honda GX Series, American Honda Motor Co., Inc., Torrance, CA, USA) and either filtered with a sand filter or diverted into the pond. The pond was stocked with fish by the facility and aerated with a windmill aerator. A photo of the sampling points is included (*Figure 8*). Weather conditions on the sample collection days (temperature, humidity, wind speed, precipitation) were retrieved from a local weather station located at the research center (http://mesonet.k-state.edu).

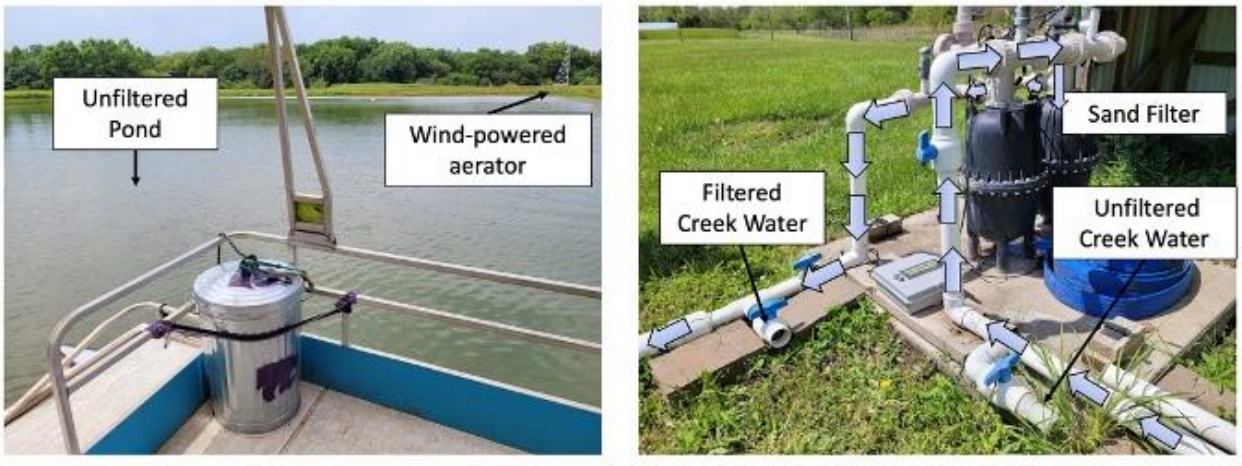

Figure 8 *The sampling sites for the field testing of the ultraviolet light (UV-C) water treatment devices included an unfiltered pond (left) as well as an unfiltered and filtered stream (right).*

UV-C treatments. For the unfiltered and filtered stream water sampling points, the UV-C devices were installed in-line with the system. A valve purchased from a local hardware supplier and a flow meter (Fill-Rite Model TT10A, Tuthill Corporation, Ft. Wayne, IN, USA) were installed to monitor and modulate the flow of water through the devices. The flow rate was monitored in units of gallons per minute (GPM). The microbial population of surface waters is dynamic, differing according to the influence of weather and environmental factors *(19, 37)*. Thus, two 100mL water samples were collected in 100mL sample bottles with added sodium thiosulfate (IDEXX Laboratories, Westbrook, ME, USA) prior to illuminating the UV-C light to serve as the control; a third control was collected at the end of the experiment. The added sodium thiosulfate prevents continued microbial reduction during transport due to the presence of chlorine free radicals and improves the accuracy of test results *(20)*.

After a 5-minute warm-up period for the UV-C devices, 100mL samples were collected in triplicate 10 seconds apart at each flow rate; a 30-second interval was applied between flow rates to flush the device. For the pond water sampling point, a pump (TotalPond, West Palm Beach, FL, USA) was also installed to establish the flow rates of 6 GPM, 7 GPM, and 8.5 GPM. At the unfiltered and filtered stream water sampling sites, flow rates of 6 GPM, 7 GPM, and 9 GPM were established. An error of ± 0.1 GPM was applied to each flow rate.

Surface water microbial enumeration. The generic *E. coli* per 100mL water sample was quantified using the Colilert with Quanti-Tray/2000 test method (IDEXX Laboratories, Westbrook, ME, USA) as per the manufacturer's instructions. This method was selected because it is commonly used by growers to determine the microbial water quality status and has been

used in prior studies with similar objectives *(13)*. Briefly, one snap-pack of Colilert reagent was added to each 100mL sample, dissolved completely via vigorous shaking, and sealed in a Quanti-Tray before incubation for 24 hours ± 2 hours at 35ºC ± 0.5ºC. The most probable number (MPN) of total coliforms was determined based on the number of large and small wells which developed yellow coloration; the MPN of generic *E. coli* was determined based on the number of large and small wells which fluoresced under ultraviolet light (Spectroline, Melville, NY, USA). An MPN table provided by the manufacturer (IDEXX Laboratories, Westbrook, ME, USA) was used as a reference for the final MPN calculations. Note that the lower limit of detection for this methodology was <1.0 MPN/100 mL and the upper limit of detection was >2419.6 MPN/100mL.

For statistical analysis, the surviving *E. coli* counts were converted into logarithmic units [log(N)] and represented as log abundance following treatment. Because the initial *E. coli* concentration of the agricultural surface waters was not uniform, the percent inactivation (*Equation 1*) was also calculated as per Jones et al. *(12)*:

Equation 1 *The percent inactivation of E. coli in agricultural water following UV-C treatment*

$$\left[\frac{N_o - N}{N_o}\right] \quad [1]$$

where N_o is the initial microbial load calculated from samples collected before treatment and N is the microbial load calculated from samples collected after treatment.

Surface water physiochemical analyses. On each sampling day, a 500mL water sample was collected from each of the sources to determine their physiochemical characteristics. The 500mL samples were stored in darkness at 4ºC until analysis and allowed to acclimate to room temperature (25ºC ± 2ºC) on the laboratory counter prior to beginning the physiochemical analyses. After reaching room temperature, the samples were homogenized with a 0.5 inch stir bar for 5 minutes at 700 rpm on a magnetic stir plate. After 5 minutes (but while still homogenizing), three 100mL sub-samples were extracted from the sample and placed into three separate sample containers; this formed three separate sub-samples for analysis. The sub-samples were homogenized with a 0.5 inch stir bar for 30 seconds at 350 rpm on a magnetic stir plate. While still homogenizing, the pH (accumet AE150, Fisher Scientific, Waltham, MA, USA), turbidity (2100Q Portable Turbidimeter, Hach Company, Loveland, CO, USA), conductivity (sensION EC5 DL, Hach Company, Loveland, CO, USA), absorbance, and transmittance at 254nm (Genesys 10S UV-Vis, Thermo Fisher Scientific, Waltham, MA, USA) were measured. The absorbance and transmittance were measured using a quartz cuvette with a 1-cm path length.

Developing a predictive model and web-based application

For the laboratory validation trials, the data were analyzed using a linear model (i.e., multiple linear regression; *(6)*). The linear model included log abundance as the response variable, and the predictor variables included pathogens (i.e., *E. coli*, *S.* Typhimurium, and *L. innocua*), flow rate, the %UVT, and if the sample was a control. The main effects, 2-way and 3-way interactions of the pathogen, flow rate, and %UVT were also included in the linear model. The predictor variable control was treated as a binary predictor variable (i.e., control = 0, treatment =1). This predictor variable was used as an interaction term to remove the effect of the flow rate and

the %UVT from the model for control samples since neither term would influence the log abundance for these samples. As a result, the linear model could be used to make unique predictions of the log abundance for all three pathogen types at a pre-specified flow rate and %UVT if a treatment is applied. If a treatment is not applied (e.g., the sample is a control sample), then the model will predict the log abundance for untreated samples. To estimate log reduction, the predicted log abundance of a treated sample (according to flow rate and observed percent transmission) was subtracted from the predictions for untreated water (negating flow rate and %UVT).

The data generated from the field validation component of the study was analyzed using generalized additive models (GAMS; *(38)*). Generalized additive models are similar to the well-known linear model, but enable important modifications that include the specification (assumption) of the distribution of the response variable is not normal. In addition, GAMs enable the predictor variables to have a more flexible effect (known as a spline) on the response variable when compared to the linear model. The first analysis of the field data used a GAM that included log abundance of generic *E. coli* as the response variable. Flow rate, %UVT, the device, and if the water was treated or not were included as predictor variables. All main effects, 2-way and 3-way interactions of flow rate, %UVT, and device were included in the GAM. For the response variable (surviving *E. coli* population), the model assumed a Tweedie distribution since these data included many zeros (i.e., treated samples wherein *E. coli* was not detected); hence the normal distributional assumption of the linear model was not tenable.

Previous studies report turbidity being a significant factor for UV-C treatment efficacy *(28)* Thus, a post-hoc test to determine the suitability of turbidity as a surrogate predictor variable for %UVT was performed; the implications of this analysis will be discussed in further context in the discussion section. For this analysis of the field data, the same modeling technique was used and %UVT as a predictor variable was replaced with turbidity. As previously mentioned, both analyses included the predictor variable "treatment" which was encoded as a binary predictor variable (i.e., untreated= 0, treated=1). The predictor variable treatment is similar to the predictor control in the model for the laboratory validation data and was used as an interaction term to remove the effect of the device, flow rate, and %UVT (or turbidity) from the model for untreated samples since these predictors will not influence the log abundance in untreated water. When predictions of percent reduction were of interest, the GAMs were used to predict the log abundance for treated and untreated water and to calculate the percent reduction (%).

To show how observed percent transmission and turbidity changed over time (i.e., between May and August) in the field, a GAM was used with observed percent transmission (or turbidity) as the response variable (assuming a beta distribution) and thin-plate splines were used for the predictor variable (month).

Of note, the statistical analyses and modeling in this study follows the American Statistics Association's statement on the use of P-values *(36)*. In brief, the statistical analysis in this study relies on the use of statistical models to enable inference and prediction. Regarding inference, the goal was to quantify the effects of variables that can be manipulated to control microbial reduction (e.g., flow rate) of the UV-C devices or that have an impact on the effectiveness of the

UV-C devices (e.g., %UVT). As such, the variables of interest were not tested to determine if they have an effect or not (i.e., for statistical significance), rather, the analysis estimates quantities of interest and quantifies the uncertainty. For example, the microbial reduction of the UV-C devices at a flow rate of 6, 7, and 9 GPM was tested. Following this example, the goal was to understand how flow rate influenced log abundance and make predictions at untested values (e.g., at a flow rate of 6.5 GPM). This type of inference, which differs from statistical testing, requires reporting point estimates and predictions with uncertainty quantification using confidence intervals and prediction intervals rather than *P*-values. In addition, we report values that assess model fit and adequacy, such as the coefficient of determination (i.e., R^2).

Following the analyses, a web-based application was developed to display and communicate the results of the analyses using data from the field validation component of the study and the GAMs. All statistical analyses were conducted in program R *(24)*, and the web-based application was developed using R Shiny *(6)*. The functionality of the application will be discussed further in the following sections.

Economic analysis to determine initial and ongoing costs of the devices

The costs of installing, operating, and maintaining each UV-C device were calculated in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The costs were reported over a 10-year operational period and according to the volume of agricultural water treated (in gallons). Gallons was selected (rather than liters) since growers most relate economic benefits by comparing costs associated with treating agricultural water by gallons.

Installation costs. The installation costs were calculated based on the initial cost of each device, their corresponding fittings, and the hours dedicated to installation and training farm workers on its operation and maintenance (e.g., weekly inspections and changing the bulbs). The cost of the fittings for each device (e.g., PVC pipe adapter, hose barb, flow meter) are included as supplementary material in *Appendix A Table A.1* and *Appendix A Table A.2*. The hours dedicated to device installation and training farm operators were calculated using the wage estimator for farming, fishing, and forestry occupations from the United States Bureau of Labor Statistics – 14.27 USD per hour at the time of calculation. (https://www.bls.gov/ooh/farming-fishing-and-forestry/agricultural-workers.html).

Maintenance costs. Regarding the maintenance costs, the devices themselves do not need to be replaced, but the efficacy of the UV-C lamp decreases with time and needs to be replaced before the operational life spans have lapsed (as specified by the manufacturer). According to the manufacturers, the UV-C lamps for the HP/HF and LP/LF units have an operational life span of 8,000 and 10,000 hours (respectively). For simplicity, it was assumed that the lamp would be replaced yearly in the economic analysis. The replacement lamps were quoted at $24.00 per unit ($24 x 4 = $96.00 to replace the complete set) for the HP/HF device and $72.00 per unit for the LP/LF device. Each manufacturer provides instructions for the operator in English regarding how to change the lamp. The model accounts for half an hour of labor to install the new lamps and a quarter of an hour (15 minutes) for a weekly inspection of the system every year for 10 years. This estimate is based on the authors' experience working with UV-C devices. Of note, neither lamp records operational hours; thus, the operator would need to keep a log(s) detailing operational use, repairs, and maintenance; this cost of implementing practices monitoring the

operating time is not accounted for as the practice can differ from operation to operation (i.e., labor, installing a flow meter).

Operating costs. The operating costs were calculated based on the energy inputs required to operate each device – 0.320 kWh and 0.034 kWh for the HP/HF device and the LP/LF device, respectively – and the average retail price of electricity in Kansas at the time of calculation (2021; 10.38 cents/kWh) as reported by the United States Energy Information Administration (US EIA; https://www.eia.gov/electricity/state/kansas/). After consultation with extension professionals, the operational costs were also calculated based on the energy required per 10,000 gallons of treated water to facilitate a more direct comparison with other water treatment strategies.

RESULTS

Laboratory Validation Results. Laboratory validation was performed to understand the potential efficacy of the selected devices prior to implementation in the field setting. The predicted change in log pathogen abundance of *E. coli*, *S.* Typhimurium, and *L. innocua* due to the UV-C transmission at 254nm (%UVT) and flow rate of the surrogate agricultural water through the tested UV-C water system was depicted as color images (heatmaps) (*Figure 9A*). For a given flow rate and %UVT, the color displayed in the images is a prediction from the linear model about the expected log pathogen abundance shown in the sidebar. Note that the color coding in the top row of panels corresponds to the log pathogen abundance as per the key to the right of the heat maps. The microbial responses are also displayed in a more conventional format using a linear format in 20%, 30%, and 40% UVT conditions in *Figure 9B*, *9C*, and *9D*

(respectively). The laboratory validation data was modeled with a linear correlation between the flow rate and log reduction (log CFU/mL) compared to the control at each experimental %UVT by the pathogen. The high values of the coefficient of determination (R2 = 0.95) demonstrated that the explanatory variables in the model (pathogen type, flow rate, etc.) accounted for a large proportion of the variability observed in the log pathogen abundance. At 20% UVT, the LP/LF device achieved a maximum 1.70-log, 1.66-log, and 1.51-log reduction of *E. coli*, *S.* Typhimurium, and *L. innocua* (respectively). At 30% UVT, the LP/LF device achieved a maximum 2.37-log, 2.13-log, and 2.22-log reduction of *E. coli*, *S.* Typhimurium, and *L. innocua* (respectively). At 40% UVT, the LP/LF device achieved a maximum 3.32-log, 3.57-log, and 3.73-log reduction of *E. coli*, *S.* Typhimurium, and *L. innocua* (respectively).

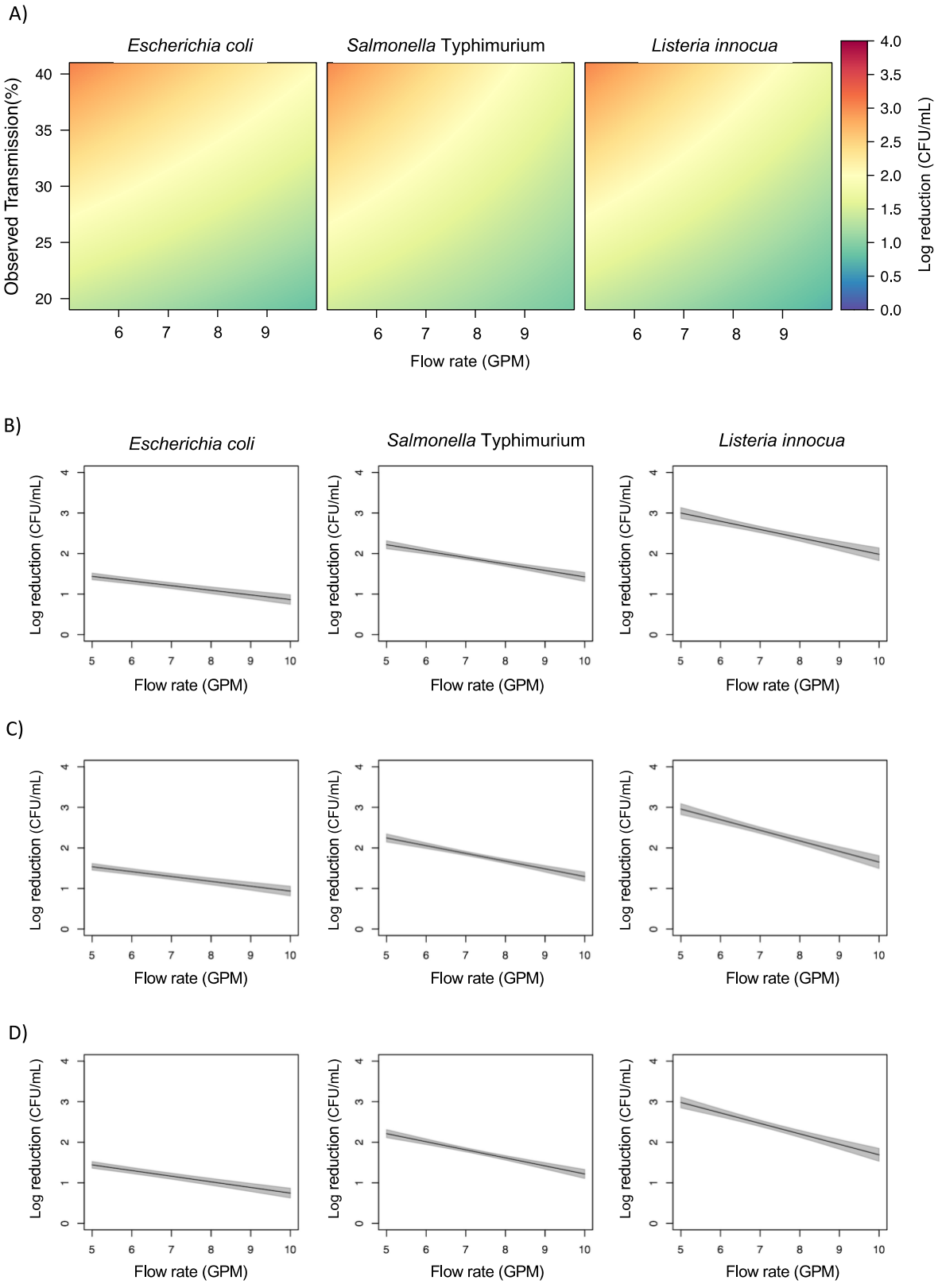
A)
Escherichia coli
Salmonella Typhimurium
Listeria innocua
Observed Transmission(%)
Flow rate (GPM)
Log reduction (CFU/mL)
B)
C)
D)

Figure 9 *The log reduction of E. coli, S. Typhimurium, and L. innocua with the LP/LF device according to %UVT and flow rate depicted in dynamic (A) and linear (B-D) formats.*

Field Validation Results. The predicted log abundance and percent reduction of *E. coli* in natural agricultural water after UV-C treatment according to the water sources' %UVT is shown in *Figure 10*. The high values of the coefficient of determination (R2 = 0.843) demonstrated that the explanatory variables in the constructed model accounted for a large proportion of the variability observed in the log pathogen abundance. At the lowest observed % UVT (8.4%), the HP/HF device achieved a maximum 2.01-log reduction of *E. coli* (*data not shown*). At the highest observed % UVT (79.4%), the HP/HF device achieved a complete log reduction of *E. coli*. In contrast, the LP/LF device at 8.4% UVT did not achieve any reduction of *E. coli*. At 79.4% UVT, a complete reduction of *E. coli* was observed.

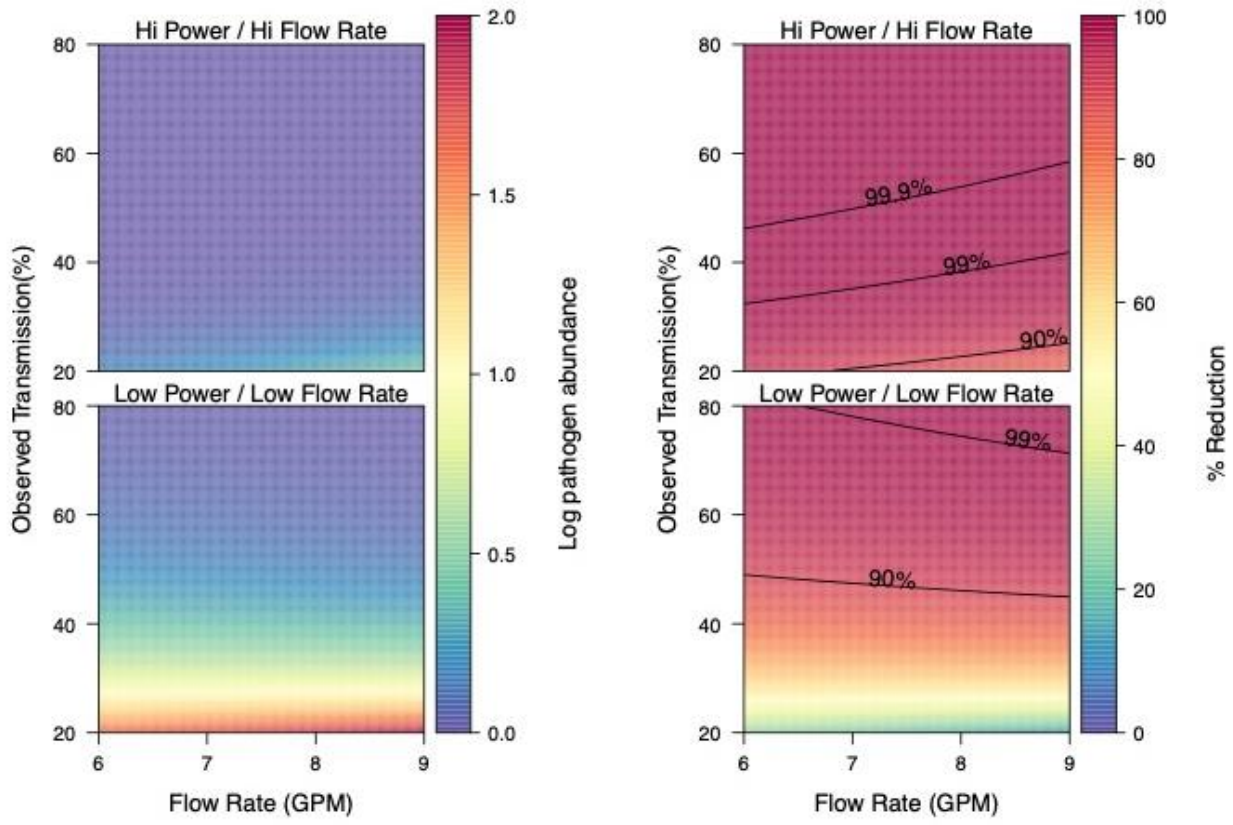

Figure 10 *The predicted log abundance (left) and percent reduction (right) of E. coli in agricultural water based on percent UV-C transmission (%UVT) after treatment with either device at various flow rates.*

Figure 11 shows the effect of turbidity and flow rate on the log abundance and percent reduction of *E. coli* in natural agricultural water after UV-C treatment. Once again, the color coding corresponds to the log pathogen abundance as per the key to the right of the heat map. The high values of the coefficient of determination ($R^2 = 0.659$) demonstrated that this model accounted for a large portion of the variation within the treatments. At the lowest observed turbidity (5.35 NTU), both devices achieved a complete inactivation of *E. coli*, whereas almost no inactivation was observed with either device at the highest turbidity reading (185.67 NTU).

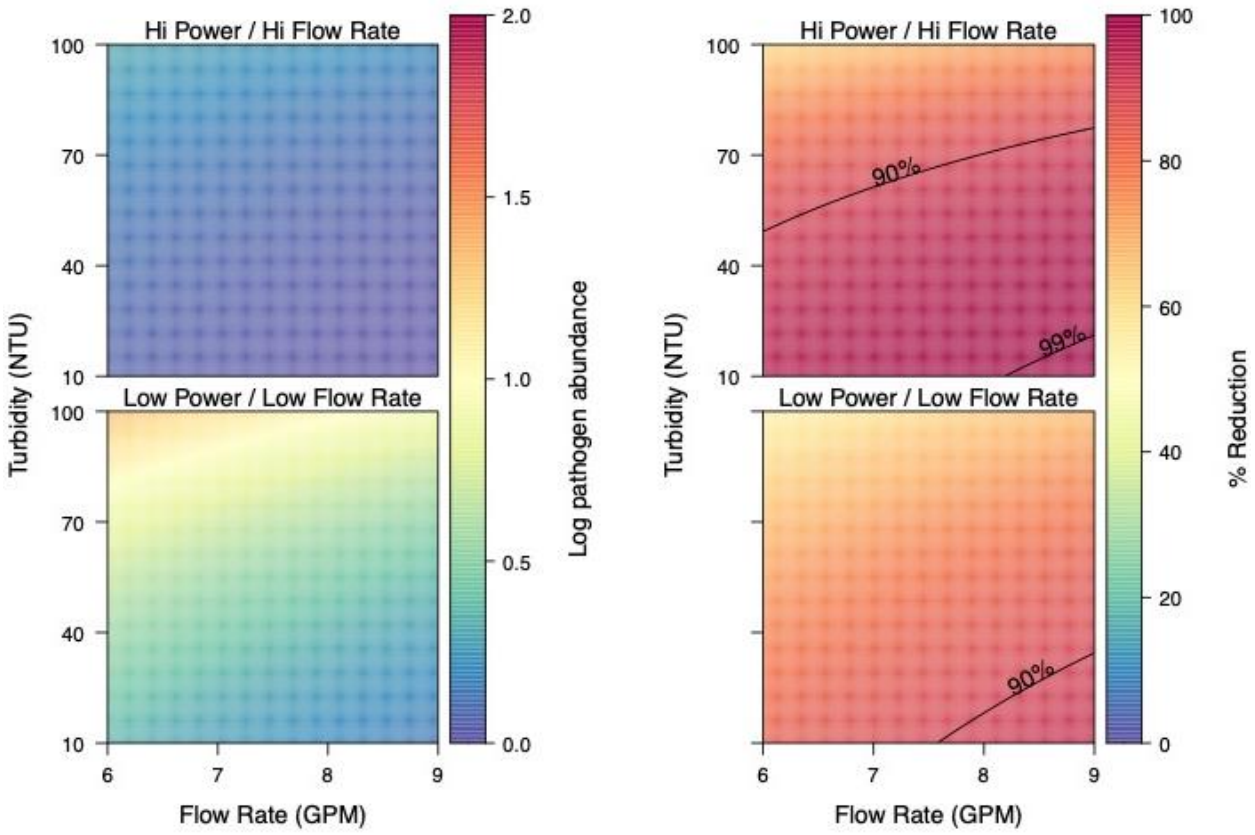

Figure 11 *The predicted log abundance (left) and percent reduction (right) of E. coli in agricultural water based on turbidity (NTU) after treatment with either device at various flow rates.*

Due to its statistical and practical relevance to predicting the microbial inactivation of each UV-C device, the temporal variation in the %UVT of each water source was also explored (*Figure 12*). Note that the shading indicates the 95% confidence interval. Over the sampling season (May–August) the observed transmission of the unfiltered stream water ranged between 8.3% to 79.0%, filtered stream water between 8.4% and 78.0%, and unfiltered pond water between 61.8% to 79.7%. Amongst the sources, the lowest %UVT values were observed during May and were roughly the same later during the months of June and July/August. By month, the observed

transmission of the unfiltered stream was 15.2% (SD = 7.4%) in May, 75.3% (SD = 1.2%) in June, and 73.0% (SD = 4.2%) in July/August. The observed transmission of the sand-filtered stream was 16.0% (SD = 7.0%) in May, 75.7% (SD = 0.6%) in June, and 73.6% (SD = 4.1%) in July/August. The observed transmission of the unfiltered pond was 63.4% (SD = 1.1%) in May, 74.5% (SD = 1.6%) in June, and 76.2% (SD = 2.4%) in July/August.

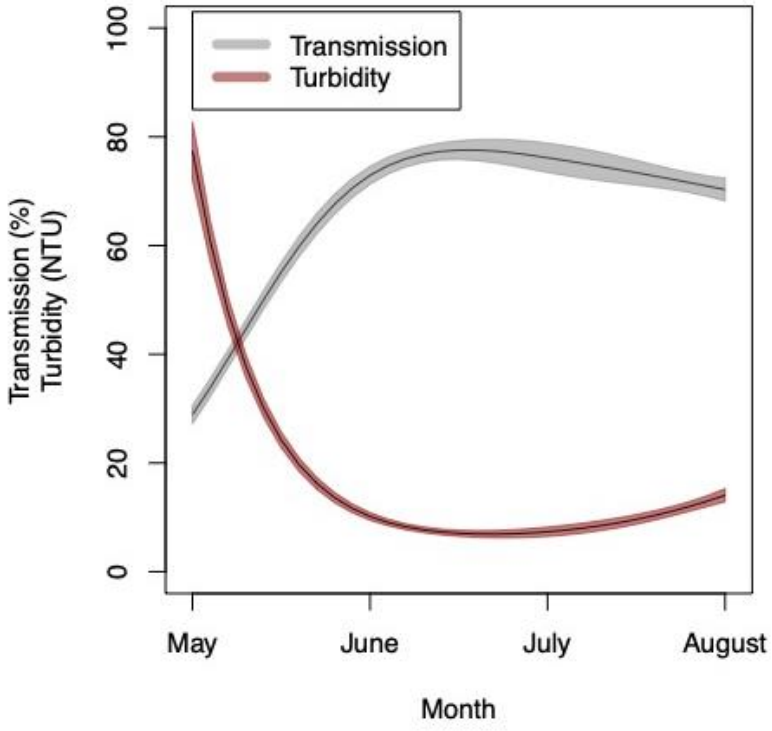

Figure 12 *The observed transmission (%UVT at 254nm) of the experimental surface water sources at each testing interval.*

Over the sampling season (May–August), the turbidity of the unfiltered stream ranged between 7.2 NTU and 189.0 NTU, the filtered stream between 6.9 NTU and 182.0 NTU, and the unfiltered pond between 5.2 NTU and 18.6 NTU (*Figure 12*). Like the values for percent

transmission, the largest turbidity values were observed early in the growing season during the month of May. The average turbidity of the unfiltered stream was 109.5 NTU (SD = 64.0 NTU) in May, 9.8 NTU (SD = 3.0 NTU) in June, and 13.5 NTU (SD = 4.5 NTU) in July/August. For the sand-filtered stream, the average turbidity was 107.9 NTU (SD = 62.0 NTU) in May, 8.9 NTU (SD = 1.7 NTU) in June, and 13.4 NTU (SD = 3.7 NTU) in July/August. The average turbidity of the unfiltered pond was 16.2 NTU (SD = 2.3 NTU) in May, 11.6 NTU (SD = 1.9 NTU) in June, and 8.7 NTU (SD = 2.6 NTU) in July/August.

Economic analysis. Assuming the UV-C lamps were replaced every year for 10 years, the total costs for installing and maintaining the devices in-line with the irrigation water system was approximately 6,256.19 USD for the HP/HF device and 4,020.63 USD for the LP/LF device (Table 5). Of note, these costs are based on the 2021 U.S. dollar and account for inflation. Also note that though the HF/HP device contained more lamps than the LF/LP device, the cost of each individual lamp was lower; the labor to change the lamps is also not likely underestimated as the accessible design of the devices makes changing the lamp equivalent to the ease of changing a lightbulb.

Table 6 *The installation, maintenance, and operating cost of the HP/HF and LP/LF UV-C devices over a 10-year period and according to usage rates*

Device	Installation Costs⁺ (USD)	Maintenance Costs⁺ (USD)	Flow Rate (GPM)	Run time (hours)	Operating Costs* (USD)
HP/HF	$ 2,891.92	$ 3,364.27	6	27.8	$ 0.9227
	$ 2,891.92	$ 3,364.27	7	23.8	$ 0.7909
	$ 2,891.92	$ 3,364.27	8	20.8	$ 0.6920
	$ 2,891.92	$ 3,364.27	9	18.5	$ 0.6151
LP/LF	$ 889.43	$ 3,131.20	6	27.8	$ 0.0980
	$ 889.43	$ 3,131.20	7	23.8	$ 0.0840
	$ 889.43	$ 3,131.20	8	20.8	$ 0.0735
	$ 889.43	$ 3,131.20	9	18.5	$ 0.0654

*Note: * indicates the costs computed per 10,000 gallons of water treated by the device; ⁺ indicates computed over a 10-year usage interval*

During installation, there is one hour dedicated to training and installation of the device; the rest of the cost is for the device and the fittings. For maintenance of the HP/HF device, 33.7% (1132.92 USD) of the cost is incurred by purchasing the lamps yearly for 10 years, 1.3% (42.10 USD) by the 15 minutes of labor to install the lamps each year for 10 years, and 65.1% (2,189.25 USD) encompasses the labor cost of performing weekly, 15-minute system inspections for 10 years. For maintenance of the LP/LF device, 28.7% (899.85 USD) of the cost is incurred by purchasing the lamps yearly for 10 years, 1.3% (42.10 USD) by the 15 minutes of labor to install

the lamps each year for 10 years, and 69.9% (2,189.25 USD) encompasses the labor cost of performing weekly, 15-minute system inspections for 10 years.

To compute the operating costs, the total operating time required to treat 10,000 gal. of agricultural water was calculated based on the flow rate (6 to 9 GPM). Accordingly, the operating time required to treat 10,000 gal. of water at 6, 7, 8, and 9 GPM is 27.8, 23.8, 20.8, and 18.5 hours (respectfully). The operating costs of the HP/HF device ranged between 0.9227 USD and 0.6151 USD per 10,000 gallons of water at the experimental flow rates. Using the LP/LF device, the operating costs ranged between 0.0980 to 0.0654 USD per 10,000 gallons of water treated at the experimental flow rates.

DISCUSSION

Laboratory validation yielded conservative estimates in natural agricultural water

Neither of the two UV devices evaluated were commercialized or marketed with the intention of treating agricultural water – rather, they were intended for home-use to treat drinking water. Thus there was no basis to compare the laboratory data against the manufacturer's claims of efficacy in the agricultural water medium. The predictive models resulting from the laboratory validation studies showed similar trends to the field study but were more conservative in predicting the reduction of generic *E. coli*. In both the laboratory and field studies, the flow rate was positively correlated with the log reduction (regardless of strain), whereas %UVT was negatively correlated. This agrees with previously reported literature that increasing flow rate and reducing %UVT reduces the efficacy of UV-C water treatment systems (which will be discussed in further detail in the following sections).

These results also highlight the importance of not relying on laboratory or benchtop scale data to estimate the efficacy of a UV-assisted water decontamination system. Within the simulated laboratory conditions, there are likely very few (if any) additional factors that can be accounted for to explain variability in treatment efficacy. This concept was demonstrated in the data obtained from the laboratory testing in this study, as the R^2 value of 0.95 indicated the strong fit of the data to the linear model (*Figure 9B-C*). Accordingly, these results indicate that the factors already in the model are sufficient, and there are a few other factors that could be incorporated to improve the fit.

Field data shows a threshold for device efficacy critical to grower decision-making

In the field setting, there was a threshold under which the UV-C devices exhibited differences in their ability to disinfect the agricultural surface water, which then disappeared under high %UVT conditions. For water bodies with %UVT greater than 25 to 30% (*Figure 10B*), the HP/HF device was more effective in reducing the population of *E. coli* in the agricultural water, far outperforming the LP/LF device. However, once the %UVT increased above a 60% threshold (*Figure 10*), there were few differences of applied importance between the performance of the two devices. In practice, these findings indicate that below the 60% UVT threshold, growers are recommended to use the HP/HF device. Yet, if the agricultural water surpasses the %UVT threshold, the choice of the device is inconsequential regarding antimicrobial performance. For water sources surpassing the 60% UVT threshold, the choice should be based on the growers' required flow rates, as the LP/LF device is not capable of rates exceeding 9 GPM. Interestingly, the results also indicate the need to expand the flow rate treatments for the first UV-C device as

complete inactivation of *E. coli* was observed at most of the flow-%UVT combinations and a lower threshold (25% to 30%) than the second device.

Logistical concerns in needing to monitor the %UVT over the production months

Despite %UVT being the most impactful water characteristic on device efficacy, the dramatic change in the water sources' %UVT presents logistical concerns for growers seeking to implement UV-C devices into their agricultural water treatment regimen. The most extreme changes in %UVT were observed in samples from the unfiltered stream and filtered stream water where, interestingly, the on-farm sand filtration unit did not significantly alter the %UVT or turbidity of the source stream. In practical terms, this finding could indicate that growers will need to monitor key water physiochemical characteristics (%UVT) over the growing season to ensure that the water source can be effectively treated with UV-C. This monitoring would be in addition to the microbial water quality analyses required by water management practices outlined in the current Subpart E of the Food Safety Modernization Act (FSMA) Water Rule, and those recommended by the proposed changes to the rule from December 2021. Further discussion on the implications of these results within the regulatory landscape will be addressed in the following sections of this manuscript.

In addition to monitoring the water source's physiochemical characteristics over the growing season, growers also need to understand how environmental changes (e.g., rainfall, flooding, agricultural run-off) impact these characteristics. Within agricultural surface waters, residues from agrochemicals (e.g., pesticides) can inhibit the transmission of UV-C and, thus, the availability of UV-C radiation for microbial inactivation *(39)*. In an urban farm environment,

Hata et al. *(10)* reported significant increases in enteric viral load and suspended solids in surface waters following their exposure to sewer overflow due to rainfall. While turbidity can be measured with relatively cost-effective and accessible instrumentation, measuring %UVT can also require highly specialized and costly equipment (i.e., spectrophotometry); the purchasing and maintenance (e.g., calibration, cleaning) of this equipment presents further costs for growers.

The data provides insight into the potential for turbidity as a surrogate predictor for efficacy
Due to the aforementioned logistical limitations for growers towards monitoring their water sources' %UVT over time, this study also investigated the use of turbidity as a surrogate predictor for %UVT. Turbidity and %UVT are somewhat interconnected; increasing turbidity can lead to decreasing %UVT, though their correlation may not be as strong in the presence of UV-blocking chemical species which do not obscure visible light (e.g., agrochemicals) *(3, 4, 5, 39)*. Surrogate predictors are often used when data collection of the primary predictor is inaccessible or unavailable. Because %UVT monitoring is not accessible to most growers, we decided to explore the use of turbidity as a surrogate predictor estimating the efficacy of the UV-assisted water treatment systems.

While the trends were similar, the substitution of turbidity for %UVT in the statistical model led to less accurate predictions of the efficacy of the devices, which is particularly apparent for the LP/LF device. For water sources with turbidity measurements of less than 50 to 60 NTU, the HP/HF device leads to the complete inactivation of *E. coli* regardless of flow rate (*Figure 11*). For the LP/LF device, however, there was no obvious threshold, and the figure (*Figure 11*) depicts that the treatment was more effective at higher flow rates, which is contrary to prior

knowledge as higher flow rates decrease the exposure time of the microorganism to the UV treatment. This observation could be explained by turbulent flow – the degree of turbulence (mixing) of fluid within the device. Sherman-Wood et al. *(28)*, for example, reported that the limited mixing of the fluid layers in surface water sources with high turbidity significantly reduced the uniformity of UV-C treatment. Similar to the results of this study, Sherman-Wood et al. *(28)* also reported a threshold of 50 NTU wherein afterward the decontamination efficacy significantly decreased. Usaga et al. *(34)* overcame these limitations and improved reduction efficacy by using a UV-C device (marketed for the treatment of beverages) with an incorporated turbulent flow regime. However, it is not clear the initial or ongoing costs associated with using such equipment for agricultural water.

What is safe water worth? A discussion on the costs of each UV-C device and grower challenges

A major limitation of previous studies is the lack of information on the costs for growers that are interested in integrating UV-C devices into their operations. Yet, such analysis is critical to facilitate grower buy-in into UV-C technology and answer the key question, "but how much does it [the system] cost?". This study is one of the few that directly reports the cost of implementing a UV-C device into an on-farm water system within a small-scale growing operation. The two systems had varying initial and ongoing investments, which can complicate the grower's decision-making process. For example, the cost of installing the LP/LF device into the existing water distribution system was 30.8% lower than the cost of installing the HP/HF device, discouraging the purchase of the higher capacity device for growers concerned about the high initial investment. But while the HP/HF device required higher initial capital, the maintenance

costs over the ten-year period were very similar (3,364.27 USD versus 3,131.20 USD per ten years for the HP/HF device and LP/LF device, respectively). The similarity in maintenance cost was largely due to the lower cost per UV-C lamp of the HP/HF device, despite the device requiring the maintenance of four lamps for manufacturer-recommended operation conditions.

The reduced labor costs of UV-C-based agricultural water treatment systems could provide an advantage for growers implementing this technology over conventional chlorine-based sanitizer treatments. It would also be allowable in organic systems or for growers that do not wish to add chlorine to their irrigation water. There are a variety of agricultural water physiochemical characteristics that can affect the degradation kinetics of chlorine sanitizers (organic matter content, free chlorine content, temperature, etc.) *(17)* and much research has been dedicated to reducing the labor associated with monitoring each of these metrics. For example, Qin et al. *(23)* reported the development of an accessible water quality monitoring system equipped with various sensors to facilitate the real-time measurement of water pH, free chlorine content, and temperature. However, such systems are typically still costly, and disproportionately so for smaller growers. The ongoing labor costs associated with the UV-C devices include 15 minutes per week of operation for weekly inspection and 15 minutes yearly for replacing the UV-C lamps. The yearly 15-minute maintenance is appropriate as replacing the UV-C lamp is a simple process wherein the spent lamp is simply unscrewed from the unit, and the new lamp is screwed in. During the weekly 15-minute inspection, the grower needs to only check the lamp for sleeve fouling, verify that the lamp is functioning through the UV-blocking viewing window, and check that there aren't any cracks in the lamp.

How do the UV-C devices fit into the Produce Safety Rule regulatory framework?

As a relatively unknown intervention to Midwestern growers, it is critical to discuss the results of this study in the context of how it impacts compliance with the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)*(32)*. In December 2021, the United States Food and Drug Administration (FDA) released proposed revisions to Subpart E of the PSR outlining standards related to agricultural water *(33)*. Although they have yet to be finalized, the proposed revisions move from testing as the primary metric for water quality to growers performing a holistic, preharvest Agricultural Water Assessment (AgWA) evaluating key factors and conditions "reasonably likely to introduce known or reasonably foreseeable hazards onto produce or food contact surfaces.". One way for covered farms to be exempt from conducting a pre-harvest AgWA is if their water source is treated in accordance with standards outlined in the FSMA PSR (which is supported by records). While the PSR recognizes UV-C light as an approved treatment for agricultural water since it is classified by the United States Environmental Protection Agency as a pesticide device *(32)*, it is unclear what documentation would be needed to fulfill the requirement for "supporting records." As federal food safety regulation evolves in the US, growers that proactively utilized interventions such as the UV-C light may benefit compared to those that only utilize reactive approaches.

There are various aspects of using UV-C-based water treatment systems which provide an opportunity for collecting "supporting records." The findings of this study indicate growers may need to monitor %UVT (or turbidity) over the growing season and after extreme weather events to ensure that the water source can be effectively treated with UV-C. The operators should also monitor the system for factors that can reduce its efficacy or introduce contamination, such as

sleeve fouling, build-up of particulate matter at the bottom of the system, cracks in the connectors, etc. Additionally, some growers may choose to collect water samples downstream of the UV-C device to generate quantitative data on how the intervention affects the population of generic *E. coli* from their water source. Such data could be stored in the form of a log(s) detailing operational use, maintenance, and microbial water quality testing. Labor for collecting water samples was not included in the cost estimates because the frequency of microbial water testing can depend on the operator, how often extreme weather events occur, and the prevalence of other factors that can impact the water sources' physiochemical characteristics (e.g., animal activity that disturbs sediment near the collection site).

An overview of the developed web application to facilitate grower usage of UV-C devices

The results from this study were used to design a web application to facilitate grower decision-making in determining if either UV-C device is suitable for their operation. The application (https://trevorhefley.shinyapps.io/uv-1/) is free, and the functionality of the application using the internet browsers Safari© (Version 15.6, Apple Inc., Cupertino, CA, USA) and Google Chrome© (Version 108.0.5359.94, Google LLC., Mountain View, CA, USA) was verified. The home screen of the application (*Figure 13*) requests growers input two parameters: 1) Initial generic *E. coli* load in the water source and 2) the measured %UVT of the water source they intend to treat with UV-C. The possible input values for the Initial *E. coli* load are restricted to between 0 to 2,419 CFU/100mL; for building the application, this mostly reflects the detection limits of the Colilert with Quanti-Tray/2000 test method. The possible input values for the %UVT (percent UV transmission) are restricted to the tested values, 8% to 79%.

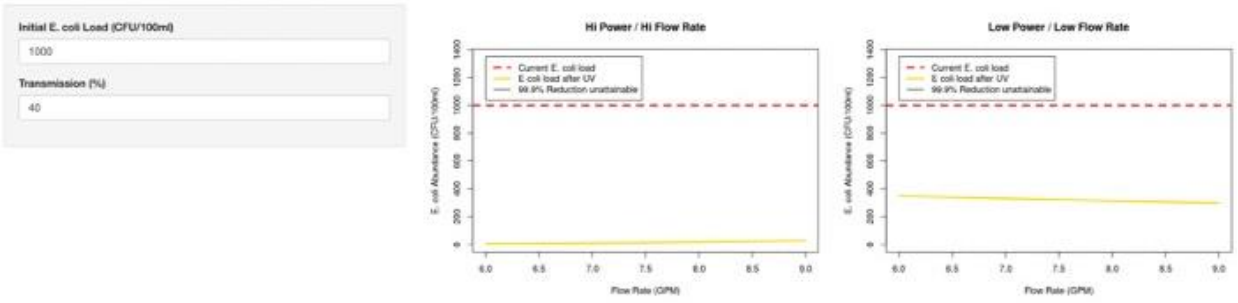

Figure 13 *The home screen of the developed web application*

The application then provides two graphs depicting the expected post-treatment *E. coli* load based on the UV-C device. The graphs provide a visual representation of the current *E. coli* load of the water source (red dotted line) and expected *E. coli* load after UV-C treatment (yellow line) based on the flow rate (in GPM). Because there were instances where the LP/LF system did not ensure a 3-log (99.9%) reduction of *E. coli*, this limitation is depicted via a vertical black line.

Study Limitations and Future Considerations

As previously mentioned, because this study utilized an exploratory and proof-of-concept approach, the experimental flow rates were limited to reflect the capacities of the smaller unit. Based on the manufacturer's recommendations, the LP/LF device was only tested between the range of 1 to 9 GPM. To make direct comparisons between the two devices, the experimental flow rates for the HP/HF device mirrored this range. There is limited publicly available information regarding actual on-farm flow rates and water usage for produce-growing operations. But based on the feedback from extension specialists and growers, these experimental flow rates are assumed to be on the lower level, potentially reflecting water usage on urban farms. Thus, further exploration of the microbial inactivation capacity of the HP/HF

device at different flow rates within its' recommended usage range (1 to 110 GPM) is warranted. Such results could provide insight into the device's suitability for farms with larger water flow rate requirements.

Another topic that merits further characterization is the likelihood of sleeve fouling. Sleeve fouling occurs when foulants (e.g., minerals) form sediments on the lamp sleeve surface, absorbing UV-C radiation that would otherwise be available for water treatment *(35)*. In fact, Li et al. *(15)* reported an average 72.9% decrease in the UV-C fluence of their experimental UV-C device over a 6-month usage period due to lamp output attenuation and sleeve fouling. For this study, the agricultural water was removed from the UV-C devices by tipping the devices until little to no water was left in the cylinder. However, growers are not likely to regularly disconnect and empty the cylinders, either due to time constraints or low accessibility of the device after installation. Future experimentation to monitor lamp sleeve fouling from different agricultural water sources throughout the year could be beneficial in adding more information to the decision-making process for growers.

CONCLUSIONS/RECOMMENDATIONS

Various recent studies have reported the successful implementation of UV-C light in small-scale and large-scale operations on surface water *(2, 11, 12, 28)*. However, to the authors' knowledge, this is the first study that has performed an accompanying cost analysis of the UV-C devices used for agricultural water treatment. The outcome of this study also presents the first web application accessible to growers, which provides guidance on the use of UV-C devices and facilitates their decision-making process in adopting UV-C technologies for on-farm agricultural

water treatment. Future studies expanding the number of devices, experimental flow rates, and agricultural water sources with a wider range of turbidity levels can improve the accuracy and applicability of the web application.

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Chapter 4 - Knowledge, Attitudes, and Perceptions of Ultraviolet-C Light Technologies for Agricultural Surface Water Decontamination by Produce Growers in Kansas and Missouri

ABSTRACT

Ultraviolet-C light (UV-C) technology is used extensively for drinking water treatment but has yet to become popularized in fresh produce production in the central United States. Thus, it is imperative to investigate the major driver(s) for UV-C adoption (or lack thereof) by produce growers. A survey instrument was designed to determine factors that most impact the attitudes of fresh produce growers (N=82) in Kansas and Missouri towards the adoption of UV-C technology for agricultural water treatment. Grower knowledge of UV-C light was measured using five close-ended constructs evaluated on a binary scale (1=correct, 0=incorrect). An overall attitude score was calculated from eight constructs using a 5-point Likert scale (Strongly Agree/Strongly Disagree). The data indicated a large variation in grower knowledge of UV-C (M=2.61, SD=1.32). Stepwise regression (n=62) revealed that the overall attitudes were most influenced by grower knowledge of UV-C ($P<0.0001$), farm size ($P=0.0199$), farm income ($P=0.1047$), and state ($P=0.1237$). Growers perceived cost (33.3%; 27/81) and technical skills (30.9%; 25/81) as major barriers to UV-C light implementation and 34.6% (28/81) felt the technology wasn't appropriate for their operation. This data improves the current understanding of different factors that could impact produce grower adoption of UV-C technologies for agricultural water decontamination.

INTRODUCTION

Agricultural water is a critical factor for microbial contamination due to the history of water-related foodborne outbreaks in horticultural commodities such as leafy greens *(17)*. Such outbreaks disproportionately occur from operations using surface waters, which carry a higher microbial risk in fresh produce operations as it is usually not feasible to isolate these water sources from environmental contamination (i.e., wildlife or other animals) *(36)*. Chemical sanitizers (i.e., sodium hypochlorite) are the most common intervention in the fresh produce industry to reduce pathogens in agricultural surface water *(7)*. However, degradation by-products of chemical sanitizers can have negative consequences on the aquatic environment, human health, and crop growth *(20, 28)*. Further, chemicals such as chlorine have reduced efficacy in waters containing high levels of organic matter, which can commonly occur in surface waters. As sustainability is a key consideration in agricultural practices, more growers are seeking to reduce the use of chemicals in their operation. Ultraviolet-C (UV-C) light is increasingly recognized by the UV industry as a viable alternative to chemical sanitizers for agricultural surface water decontamination. However, there has been relatively little deployment of UV-C technologies by fresh produce growers.

UV-C is a popular technology for wastewater and drinking water decontamination since it is highly effective against human pathogens of high public health interest *(16, 32)* and is generally considered user-friendly. Unlike some chemical sanitizers *(38)*, there is also scarce evidence that UV-C may produce toxic byproducts. UV-C is considered a physical decontamination method wherein microorganisms are inactivated following UV-C-induced photodamage to DNA. Without DNA repair mechanisms, the cell ceases to replicate, perform metabolic processes, or

reproduce, and eventually dies *(22, 27)*. Of the entire UV spectrum, wavelengths within the UV-C range (200-280nm) are the most effective to inactivate microorganisms, hence this range is denoted the 'germicidal range'. Various studies report strategies for the successful implementation of UV-C to treat agricultural surface water in small-scale and large-scale operations *(1, 4, 25, 29, 37)*. However, the low adoption of UV-C technologies indicates a disconnect between the UV-C industry, academia, and fresh produce growers.

Produce grower adoption of UV-C technologies for agricultural water decontamination purposes has not been thoroughly studied, though previous studies indicate it could be very low due to barriers such as low grower awareness of UV-C and low access to guidance on UV-C technologies. For example, Lamm et al. *(15)* reported that the majority (66.5%) of surveyed nursery and greenhouse growers in the United States did not perceive themselves as knowledgeable of chemical agricultural water treatment methods; of note, those researchers chose to include ultraviolet light as a chemical, rather than a physical treatment method. Although the study's category 'chemical agricultural water treatment methods' represented multiple decontamination strategies, it could be indicative of the knowledge level of nursery and greenhouse growers towards the use of UV-C technologies for agricultural water treatment. Raudales et al. *(26)* also identified an unmet need for more information on UV-C light tailored towards growers such as the level of maintenance and supervision required during operation of UV-C technology. Because there is little information on actual produce grower adoption of UV-C for agricultural water decontamination, it is necessary to identify the factors affecting grower attitudes towards such technology.

The purpose of this study was to determine growers' level of knowledge, attitudes, and barriers to integrating UV-C technology for agricultural water decontamination in their produce operations. Kansas and Missouri growers were selected for this study because these states have a rapidly growing specialty crop industry, and previous reports *(12, 39)* indicate that many growers in those states are in need of more information on agricultural water treatment strategies. The objectives of this study are thus:

1) to identify agricultural water treatment practices of Kansas and Missouri produce growers,
2) to evaluate growers' level of knowledge of UV-C for agricultural water decontamination,
3) to determine produce grower's attitudes toward UV-C water treatment systems, and
4) to determine growers' perceived barriers to investing in and/or integrating UV-C water treatment systems into their growing operations.

MATERIALS AND METHODS

Conceptual framework of the study. The conceptual framework of this study is largely drawn from the technology acceptance model (TAM) wherein the ease of use and grower/employee knowledge of the technology are major drivers affecting behavioral attitudes *(8, 9)*. Attitudes (rather than adoption) are emphasized in this study's framework because consultation with state extension personnel indicated a low adoption rate of UV-C technologies amongst produce growers in Kansas and Missouri. As such, demographic explanatory variables –farm size, farm annual income from fresh produce sales, state, training certifications held, highest level of education, age, years of farming experience, and grower knowledge of UV-C – were used to explore internal and external factors affecting grower attitudes towards UV-C technology. Farm

size (acreage) was found to positively impact the adoption of novel agricultural technologies like precision agriculture due to the economy of scale *(24)*. In this study, physical size (acreage) and income are both included to represent urban agriculture operations that produce large quantities of horticultural crops in small spaces. State is included as a factor because grower attitudes may be influenced by the perceived profitability *(33)* due to different market sizes for fresh produce within Kansas and Missouri. Training certifications, education, age, and years of farming experience were included as possible explanatory variables indicating the likelihood of exposure to the basic concepts of UV-C light and its uses for agricultural water decontamination.

The grower attitudes towards UV-C for agricultural water treatment were further divided into perceived usefulness, perceived ease of use, and perceived resource availability. Perceived usefulness and perceived ease of use are pre-defined in the TAM *(8)* whereas perceived resource availability was added in accordance with relevant literature *(18)*. In this study, perceived usefulness is the belief that the use of UV-C technology will eventually enhance the grower's agricultural water quality management. perceived ease of use is the belief that the use of UV-C technology will require minimal management (physical and mental effort). perceived resource availability is the belief the individual grower has access to the required resources (i.e., money, information) to use the technology. This factor was added to the framework since it was previously identified as highly influential to the adoption of novel agricultural technology *(3, 18)*.

Target Population. The target population was defined as fresh produce growers in Kansas and Missouri. Because there is no publicly available complete registry of produce growers in either

state, it was not possible to calculate the required number of responses based on population size and nonresponse rate. However, a target response rate of around 100 survey responses was followed, based on the methodology of similar survey studies in the region *(39)*.

Survey Design. The survey instrument was developed using the Qualtrics platform (Qualtrics, Provo, UT) for data collection and designed to measure produce growers' water quality management behavior, knowledge of and attitudes towards ultraviolet light technology, and assess their needs to be able to implement UV technology in their operation. A modified Delphi approach similar to that of Perry et al. *(23)* was used to develop the survey constructs. Briefly, five extension professionals and two other food safety experts were recruited to evaluate the preliminary survey questions for content and face validity. The survey was then pilot tested with produce growers from the target population. The survey instrument was amended using the feedback from the growers and finalized after expert re-review. The Kansas State University Human Research Institutional Review Board approved the use of the survey.

There was a total of 20 questions in the final survey (*Supplemental File 1*). The grower's current water management practices were assessed through six (6) close-ended questions. The grower's level of knowledge of UV light and its applications was measured using a series of five (5) close-ended constructs with a 3-point scale (Agree/Don't Know/Disagree). The grower's attitudes toward UV-based water decontamination systems for on-farm operations was evaluated using eight (8) constructs related to the measuring variable with a 5-point Likert scale (Strongly Agree/Mostly Agree/ I don't know/Mostly Disagree/ Strongly Disagree). The barriers to on-farm implementation of UV-C technology for agricultural water decontamination and the needs to

overcome these barriers were assessed using two (2) close-ended, check-all-that-apply (CATA) questions. Lastly, the survey instrument collected demographic data regarding the grower's state of operation, gender, age, years of farming experience, highest level of education, farm/operation size, farm income, and current certifications. The possible options for the demographic information were determined according to the format of the U.S. Department of Agriculture Census. This format was selected based on grower familiarity with the census, in anticipation that this familiarity would prompt growers to be more likely to complete the demographic questions.

Survey Distribution. The survey was available between February and October 2022 and administered to Kansas and Missouri growers in either electronic- or paper-based format. Organizations with a significant proportion of the target population (i.e., specialty crop growers' groups, farmers' market associations) assisted in distributing the survey electronically through email listservs or in paper format at in-person events. To incentivize survey participation, growers could choose to be entered into a drawing to win one of fifteen 25-dollar visa gift cards. Of note, it was not possible to calculate the (non)response rate as this study used a convenience sampling approach *(10)*. Convenience sampling (or, 'accidental' sampling) is a quick, inexpensive sampling method based on gathering responses from members of the target population who are conveniently available *(31)*. This method has been previously used to study the agricultural water treatment behaviors of growers in the region by sampling at events where growers are likely to be present (e.g., grower trainings, field days) *(39)*.

Data Analysis. Similar to the methodology of Chen et al. *(5)*, the grower's knowledge of UV-C light and its applications was expressed as a knowledge index (KI) calculated as the sum of correct answers (assigned 1 point each). Incorrect and "I don't know" responses received 0 points. The respondent's attitudes towards UV-C water treatment systems for on-farm operations was codified (Strongly Agree = 5, Mostly Agree = 4, I don't know = 3, Mostly Disagree = 2, Strongly Disagree = 1). The sum of all the attitude constructs is hereafter referred to as the attitude score (AS). The AS was then divided into scores for perceived ease of use, perceived usefulness, and perceived resource availability to test for factors which affect each aspect of the attitudes. To divide the AS, the perceived ease of use was calculated from the sum of scores for the first to third construct, the perceived usefulness from the sum of fourth, sixth, seventh, and eight construct, and the perceived resource availability from the score of the fifth construct. The constructs are a modified form of the constructs from the TAM survey instrument of Davis *(8)*.

Descriptive analysis of the data was performed in Microsoft Excel (Microsoft Corporation, Redmond, WA) and statistical analysis was performed in SAS (Version 9.4, SAS Institute, Cary, NC). Stepwise regression was applied to determine the factors affecting the total attitude score, perceived ease of use, perceived usefulness, and perceived resource availability scores. Responses were not used for stepwise regression if the participants did not answer a significant portion of the demographic questions. Due to the relatively low number of valid, completed surveys received (n = 66), the demographics were restructured to facilitate analysis. Notably, the explanatory variables included the knowledge index (KI), state (STATE; Kansas or Missouri/Other), age (AGE; ≤ 54 or > 54 years old), farming experience (FARM_EXP; ≤ 9 years or > 9 years), education (EDUCATION; (Some high school/High school diploma/Some college

or master's degree/doctoral degree), farm size in acres (FARM_SIZE; <1 acres or ≥ 1 acres), farm income (FARM_INCOME; <1,000 USD/year or ≥ 1,000 USD), and training certifications held (GAP/FSMA/HACCP or USDA organic/Other).

RESULTS

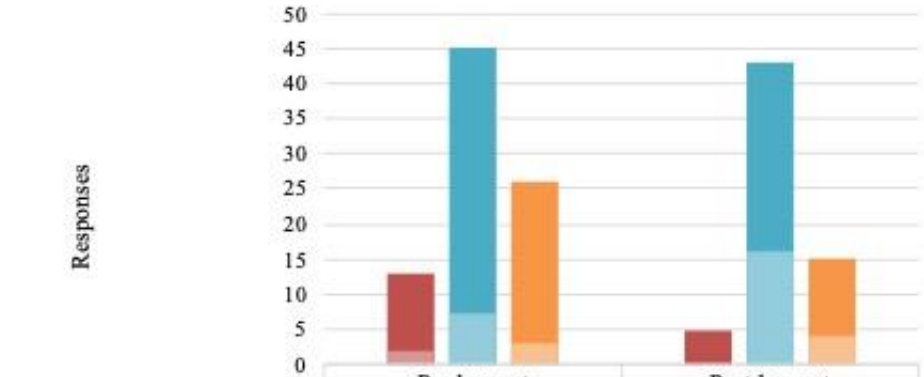
Demographic characteristics of the respondents. There were 91 total responses received, 82 of which were valid (from respondents currently growing fresh produce). The demographics are shown in *Table 7*. Because participants could elect not to answer the question (i.e., selected Prefer not to say), different questions may have a different number of responses. As previously mentioned, due to the relatively low number of surveys with complete demographic information received, the demographics were restructured to facilitate analysis. For state, the demographics were re-categorized into growers who indicated they lived in Kansas, and growers who indicated they lived in Missouri or another state. For age, the categories were either ≤ 54 or > 54 years old. Farming experience was either ≤ 9 years or > 9 years, farm size in acres as either <1 acres or ≥ 1 acres, and farm income as either <1,000 USD/year or ≥ 1,000 USD). For education, the responses Some high school/High school diploma/Some college were combined to form one category, and master's degree/doctoral degree were combined to form a second category. The categories for training certifications held were either responses of GAP/FSMA/HACCP or USDA organic/Other.

Table 7 *Demographic information of the survey participants*

Construct	**Response**	**% (*n*)**
State the farm is located (*n*= 79)	Kansas	60.8% (48)
	Missouri	26.6% (21)
	Other	12.7% (10)
Age (*n*= 78)	Under 25	2.6% (2)
	25 to 34	16.7% (13)
	35 to 44	20.5% (16)
	45 to 54	12.8% (10)
	55 to 65	23.1% (18)
	65 to 74	20.5% (16)
	75 and older	3.8% (3)
Years of farming experience (*n*= 78)	Less than 4	34.6% (27)
	5 to 9	17.9% (14)
	10 to 14	14.1% (11)
	15 to 19	10.3% (8)
	20 to 24	3.8% (3)
	25 to 29	3.8% (3)
	30 years and above	15.4% (12)
Highest level of education (*n*= 78)	High school diploma/GED	3.8% (3)
	Some college	20.5% (16)
	College/University degree	44.9% (35)
	Master's degree	24.4% (19)

	Doctoral degree	6.4% (5)
Farm Size (*n*= 79)	<1 acre	39.2% (31)
	1 to 9 acres	43.0% (34)
	10 to 49	11.4% (9)
	70 to 99	1.3% (1)
	100 to 139	1.3% (1)
	140 to 179	1.3% (1)
	500 or more	2.5% (2)
Annual Farm Income (*n*= 63)	Less than 1,000 USD	31.7% (20)
	1,000 USD to 2,499 USD	12.7% (8)
	2,500 USD to 4,999 USD	6.3% (4)
	5,000 USD to 9,999 USD	17.5% (11)
	10,000 USD to 24,999 USD	12.7% (8)
	25,000 USD to 49,999 USD	7.9% (5)
	50,000 USD to 99,999 USD	6.3% (4)
	100,000 USD to 499,999	1.6% (1)
	500,000 USD or more	3.2% (2)
Training certifications held (*n*= 80)	GAP	15.0% (12)
	FSMA	42.5% (34)
	HACCP	1.2% (1)
	USDA organic	2.5% (2)
	Other	3.8% (3)
	None	45.0% (36)

Agricultural water treatment practices. The preharvest and postharvest agricultural water treatment practices of the surveyed growers are shown in *Figure 14*. For most growers responding to the survey, at least one of the water sources used for preharvest operations was a municipal water source (64.2%, 52/81). Ground water was the next most common source (35.8%, 29/81) followed by surface water (18.5%, 15/81). Growers were also using municipal (72.8%, 59/81), ground (23.5%, 19/81), and surface (6.2%, 5/81) water sources at a similar frequency for postharvest operations. Interestingly, most of the water sources used for preharvest operations (87.5%, 84/96) and postharvest operations (75.9%, 63/83) were untreated. Growers treating their preharvest agricultural water reported using chlorine-based sanitizers (*n*= 4), UV-light (*n*= 2), organic acids (*n*= 1), or other treatments (*n*= 5). Growers treating their postharvest agricultural water reported using chlorine-based sanitizers (*n*= 6), hydrogen peroxide (*n*= 3), organic acids (*n*= 1), UV-light (*n*= 1), or other treatments (*n*= 7).



	Pre harvest	Post harvest
Surface Water (Untreated)	13	5
Municipal Water (Untreated)	45	43
Ground Water (Untreated)	26	15
Surface Water (Treated)	2	0
Municipal Water (Treated)	7	16
Ground Water (Treated)	3	4

Figure 14 *The source distribution for agricultural water sources used in preharvest and postharvest operations of surveyed fresh produce growers.*

Knowledge index. Grower knowledge of UV-C light (knowledge index; KI) was measured using five close-ended constructs evaluated on a binary scale (1=correct, 0=incorrect). Accordingly, the maximum possible score for KI was 5 and the minimum possible score was 0. The mean knowledge index (KI) was 2.61 with a standard deviation of 1.32 (*Table 8*). The third construct was the most difficult as participants most often provided an incorrect answer or selected 'I don't know'. In contrast, the first and fifth construct appeared to be the least difficult and had the highest percentage of correct answers.

Table 8 *The perceived barriers and needs of growers to facilitate the adoption of UV-C technology for agricultural water source treatment (n= 81).*

Survey construct	**Correct % (*n*)**	**Incorrect % (*n*)**	**I don't know % (*n*)**
1. UV light can kill disease-causing germs	74.4% (61)	1.2% (1)	24.4% (20)
2. UV light is invisible to the naked eye	48.8% (40)	17.1% (14)	34.1% (28)
3. UV light cannot penetrate clothing	12.2% (10)	34.1% (28)	53.7% (44)
4. UV lamps have an infinite lifespan	53.7% (44)	3.6% (3)	42.7% (35)
5. UV light effectiveness depends on time and intensity	72.0% (59)	0.0% (0)	28.0% (23)

Grower attitudes towards UV-C for agricultural water decontamination. There was a total of 82 responses received for the attitude and perceptions assessment portion of the survey. *Table 9* summarizes the results of asking growers to "Please answer with the extent you agree or disagree with the following statements about ultraviolet (UV) light-based water treatment

systems", by construct. Growers mostly answered 'I don't know' to each of the survey constructs, and there were few who answered 'Strongly Disagree' or 'Mostly Disagree' to any of the constructs. Almost 33% (27/82) of the growers agreed to some extent that UV water treatment systems are easy to install, 39% (32/82) expressed some extent of agreement that the systems are easy to maintain, and 46.3% (38/82) agreed to various degrees that such systems were easy to operate

Table 9 *The mean and standard deviation of grower responses to constructs evaluating attitudes and perceptions towards UV-C for agricultural water treatment (n = 82).*

Survey construct:	Strongly Disagree % (*n*)	Mostly Disagree % (*n*)	I don't know % (*n*)	Mostly Agree % (*n*)	Strongly Agree % (*n*)
1. They are easy to install	0% (0)	1.2% (1)	65.9% (54)	25.6% (21)	7.3% (6)
2. They are easy to maintain	0% (0)	0% (0)	61.0% (50)	30.5% (25)	8.5% (7)
3. They are easy to use	0% (0)	1.2% (1)	52.4% (43)	34.1% (28)	12.2% (10)
4. They are safe for humans to use	0% (0)	2.4% (2)	36.6% (30)	35.4% (29)	25.6% (21)
5. They are affordable	1.2% (1)	13.4% (11)	69.5% (57)	13.4% (11)	2.4% (2)
6. They can reduce the number of microorganisms in agricultural water	0% (0)	0% (0)	30.5% (25)	31.7% (26)	37.8% (31)
7. They can reduce the number of microorganisms on the surface of irrigated crops	0% (0)	2.4% (2)	58.5% (48)	14.6% (12)	24.4% (20)
8. They are suitable for treating agricultural water(s) in my operation	1.2% (1)	3.6% (3)	59.8% (49)	19.5% (16)	15.8% (13)

(*Table 10*) shows how the constructs translated into quantitative scores for overall attitudes, perceived ease of use, perceived resource availability, and perceived usefulness of UV-C for agricultural water treatment. The mean total attitude score (AS) calculated from the sum of all the constructs was 29.02 (SD = 4.16) out of a total possible score of 40; scores closer to 40 indicate a more positive view of UV-C technologies whereas scores closer to 0 indicate more negative views. The mean score for perceived ease of use, calculated from the sum of the first, second, and third constructs, was 14.38 (SD = 2.29) out of a possible score of 15.00. Scores closer to 15.00 indicate that UV-C technology was perceived as relatively easy to use whereas scores closer to 0 indicate that the technology was perceived as more difficult to use. The mean score for perceived resource availability as indicated by the score of the fifth construct was 3.34 (SD = 0.55) out of a possible score of 5.00. Scores closer to 5 indicate that grower perceive the resources needed to implement UV-C technology (e.g., capital, guidance) as more available than scores closer to 0. The mean score for perceived usefulness as calculated from the sum of the fourth, sixth, seventh, and eighth constructs was 11.30 (SD = 2.12) out of a possible 15.00 with scores closer to 15.00 indicating that UV-C technology was perceived as useful to the grower's operation.

Table 10 *The mean and standard deviation of grower overall attitudes, perceived ease of use, perceived resource availability, and perceived usefulness of UV-C for agricultural water treatment.*

Survey Constructs:	***M***	***SD***
Overall Attitudes	29.02	4.16
Perceived Ease of Use	14.38	2.29
Perceived Resource Availability	3.34	0.55
Perceived Usefulness	11.30	2.12

For the stepwise regression models, Knowledge Index (KI), state (STATE), grower age (AGE), years of farming experience (FARM_EXP), education (EDUCATION), farm size in acres (FARM_SIZE), annual farm income from fresh produce sales in USD (FARM_INCOME), and trainings completed, or certificates held (TRAIN_CERT) were considered. The best fit (R2 = 32.1%) to the Attitude Score (AS) data was achieved with a model incorporating KI ($P < 0.0001$), FARM_SIZE ($P = 0.0199$), FARM_INCOME ($P = 1.047$), and STATE ($P = 0.1237$). The best fit (R2 = 19.2%) to the Perceived ease of use scores was achieved with a model incorporating KI ($P < 0.0015$) and FARM_SIZE ($P = 0.0197$). The best fit (R2 = 7.4%) to the Perceived resource availability scores was achieved with a model incorporating KI ($P = 0.146$) and FARM_SIZE ($P = 0.032$). Lastly, the best fit (R2 = 33.5%) to the Perceived usefulness score was achieved with a model incorporating KI ($P < 0.0001$), FARM_INCOME ($P = 0.0368$), and STATE ($P = 0.0549$).

Grower barriers and resources needed to adopt UV-C technology. *Table 11* shows the responses of growers to constructs assessing the barriers and needs of growers to facilitate the adoption UV-C for agricultural water decontamination. Note that because growers could select more than one response, the total number of responses may exceed the number of respondents (n= 81). Growers indicated that technical skills (30.9%) and cost (33.3%) were major barriers to UV-C adoption whereas 34.6% reported that UV-C was not appropriate for their operation. Several growers (29.6%) indicated that there were 'Other' barriers preventing UV-C adoption, warranting further investigation. Growers (n= 81) generally perceived needing more information about the benefits of using UV-C (63.0%), money (61.7%), and technical training (55.6%) to overcome these barriers (*Table 11*).

Table 11 *The perceived barriers and needs of growers to facilitate the adoption of UV-C technology for agricultural water source treatment (n= 81).*

Survey construct		Responses % (n)
Barriers	Technical Skills	30.9% (25)
	Cost	33.3% (27)
	Complicated	16.0% (13)
	Maintenance	11.1% (9)
	Not appropriate for my operation	34.6% (28)
	Other	29.6% (24)
Needs	Money	61.7% (50)
	Technical Training	55.6% (45)
	Time	28.4% (23)
	More information about its benefits	63.0% (51)
	Other	9.9% (8)

DISCUSSION

Water trends and a potential niche for UV-C treatment for municipal water. Zhao et al. *(39)* previously reported that many fresh produce growers in Kansas and Missouri were not testing their water according to the standards of the Produce Safety Rule at the time. Most growers in this survey used municipal water for preharvest and postharvest activities, but 28.4% (23/81) of the growers used untreated ground or surface water for preharvest and postharvest activities. The FSMA PSR requires that to use surface water postharvest, it must be treated. Thus, these results are potentially concerning as a recent study found that many surface and

ground water sources in the region may not be suitable for postharvest use without some sort of treatment *(12)*. Overall, these data could indicate there is still a critical need for grower education and engagement on proper agricultural water management practices.

Interestingly, the reported water treatment practices from this study combined with other recent studies also indicate a potential niche for UV-C technologies. Although 64.2% (52/81) of growers in this study reported using municipal water in preharvest activities, McGehee et al. *(19)* recently reported that the residual chlorination of municipal water may be phytotoxic, even if the chlorine levels meet industry standards. Accordingly, even though growers using municipal water are using a microbiologically safe water source, they could also be inadvertently decreasing their crop productivity. In this study, 14.8% (12/81) of the growers reported using a mix of municipal water and either ground or surface water for preharvest use (*data not shown*). In the absence of events that would logistically restrict ground or surface water use (e.g., drought, aquifer depletion, or not having access to ground or surface water), these growers could potentially save money by using UV-C to treat these sources for preharvest activity (if there were concerns about the microbial quality of the water source) rather than paying for a municipal water supply. To perform a cost comparison between UV-C treated water and municipal water, however, growers would need more information regarding the initial and ongoing costs of UV-C water decontamination systems, which is currently lacking in the literature.

Growers show mixed attitudes towards UV-C. The attitudes of produce growers in this study both conform and contradict with previous literature. The Likert scale used to assess grower attitudes in this study translated into high scores indicating positive attitudes, low scores

indicating negative attitudes, and scores closer to the middle indicating neither positive nor negative attitudes (neutral or the grower doesn't know). Overall, growers had a more positive attitude towards the capacity of UV-C as an antimicrobial intervention for agricultural water (as indicated by the mean attitude score). When separated into perceived ease of use, perceived usefulness, and perceived resource availability, growers had highly positive attitudes towards the perceived ease of use of UV-C technology. Of note, the perceived ease of use included constructs indicating the extent to which users agreed that UV light-based water treatment systems were easy to install, use, and maintain. This finding is in contradiction with Raudales et al. *(26)* that reported growers perceiving UV-C as one of the most difficult water treatment methods to monitor and maintain. However, this difference could be attributed to the different audience (Northeast versus Central United States), survey design, and sample sizes between the two studies.

Although the mean score for perceived resource availability (3.34 (SD = 0.55) out of a possible score of 5) indicated a more positive attitude towards resource availability, it is also very close to a neutral score of 3. This score indicates that many growers either didn't know or disagreed with the notion that they have access to the resources required to implement UV-C in their operation (notably, capital). Not only is this finding consistent with the literature *(26)*, but it is also consistent with barriers later identified by the growers in this study. More than 50% of the growers reported needing more knowledge resources, monetary resources, and training resources to feel confident in the choice to implement UV-C in their operation. Although addressing monetary concerns may be more difficult, the UV-C industry and academia have a vested

interest in providing more opportunities to address the lack of technical training and guidance for growers on UV-C devices, though this point will be elaborated on in the following section.

Grower buy-in is a major factor in the deployment of UV-C in fresh produce operations *(11)*, and may be highly impacted by understanding of the benefits of the technology to their current operation *(6, 30, 34, 35)*. Although growers had positive attitudes towards the usefulness of UV-C, they also identified the lack of information about its benefits as a barrier. The mean score for Perceived Usefulness (11.30 (SD = 2.12) out of a possible 15) indicated that many of the participants perceived UV-C water decontamination as useful to their operation. This finding agrees with previous studies that show growers tend to believe that UV-C has some efficacy against plant pathogens and human pathogens *(26)*. However, this also could appear contradictory as the barrier most identified by growers was also access to more information on the benefits of UV-C to their operation. To clarify the information of most interest to growers, future studies in this domain should leverage open-ended questions or focus group discussion which would allow for more idea development than close-ended questions.

Growers need more technical guidance on UV-C. Previous studies on grower adoption of novel agricultural technologies suggests that growers must have confidence that the technology is effective *(2, 34)*. However, almost a quarter of the growers in this survey either didn't know or disagreed with the knowledge index construct that UV light can kill disease-causing germs, a statement that is well supported in the literature *(13, 21, 37)*. Moreover, 39% of the surveyed growers did not know that UV-C couldn't penetrate clothing, indicating that the human safety element of using UV-C is not widely known by growers. While the constructs such as 'UV light

is invisible to the naked eye' and 'UV lamps have an infinite lifespan' may not be consequential to how growers perceive UV-C, it could still be important to know for growers to safely operate the UV-C devices. Once more, growers recognized the lack of technical guidance as a barrier to UV-C implementation. In fact, 55.6% of growers responded that they need more technical training before implementing UV-C in their operation. This point is reinforced by the contribution of grower knowledge of UV-C to the total and individual attitudes in the stepwise regression models for the attitude score, perceived ease of use, perceived usefulness, and perceived resource availability.

More information is needed on factors impacting grower adoption of UV-C. Stepwise regression was able to identify which information collected in this study most impacted the attitudes and perceptions of growers towards UV-C. Knowledge Index appeared in all four regression models, which could provide further evidence that knowledge of UV-C is critical in the grower decision-making process on whether to adopt UV-C for water treatment. However, understanding the effects of the other demographic factors warrants further study. For example, stepwise regression indicated that overall attitudes were affected by farm size, farm income, and state. Typically, the inclusion of farm size and farm income would not be surprising, as a previous study on the adoption of precision agriculture demonstrated that larger (in acreage) farms are more likely to invest in technologies which could be perceived as too costly for smaller operations *(14)*. However, growers in this survey largely did not know if UV-C devices for water treatment were affordable (*Table 9*), which complicates the interpretation of two factors. More investigation on the effect of state is also justified, particularly to understand if there are

differences in perceived profitability (driven by market size) between Kansas and Missouri growers is indeed what is affecting overall attitudes and perceived usefulness.

The low R^2 values also indicate that there are likely other variables impacting grower attitudes which were not measured in this study. The models had R^2 values between 7.5% to 33.5%, indicating there is still a large portion of the variation which is not accounted for. Future studies could measure growers' prior exposure to information about UV-C technologies by having growers self-evaluate their knowledge (e.g., I perceive myself as extremely knowledgeable/ extremely unknowledgeable on UV-C concepts) as in previous studies *(23, 26)* Instead, in this study factors such as age, experience, and education were used as surrogate predictors of grower knowledge of UV-C. To this point, there was not definitive information regarding if the respondents were covered (or not) by the FSMA PSR, which could potentially impact their exposure to agricultural water treatment methods. Moreover, grower perceptions of the cost-effectiveness of UV-C could also be assessed, which was elaborated by Adrian et al. *(2)* as perceived net benefit. Lastly, survey respondents can be asked about the crops they grow which could impact the investment they are willing to make in agricultural water treatment strategies. For instance, growers who produce commodities not commonly consumed raw (i.e., potatoes) may not feel the same pressure to implement agricultural water management strategies as those who grow leafy greens which are more often consumed raw. Similarly, grower who do not apply irrigation to the edible portions of the PSR-covered produce during growing, and growers who do not wash their PSR-covered produce prior to sale (as is the case for strawberries) or may also not feel this pressure.

CONCLUSION/RECOMMENDATIONS

UV-C has been shown to be an effective and environmentally friendly technology for agricultural water treatment, but has not been popularized in the fresh produce industry of the central United States. The present study indicates that the lack of grower adoption may be largely due to the absence of technical and general knowledge resources tailored towards growers. The level of grower knowledge of UV-C was shown to be a major predictor of grower attitudes towards UV-C for agricultural water treatment. Considering the identified barriers, growers held slightly positive attitudes towards UV-C, and showed a degree of neutrality/uncertainty towards the extent that they perceived having sufficient resources to implement UV-C in their operations. More investigation is required to determine other significant factors as the statistical models had a larger proportion of variation which was not accounted for. Clearly more work is needed to provide resources and education to growers about the potential benefits of utilizing UV-C for water treatment for growing produce and other specialty crops.

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Chapter 5 - The attenuation of microbial reduction in blueberry fruit following UV-LED treatment

ABSTRACT

Ultraviolet-C (UV-C) irradiation is a well-recognized technology for improving blueberry postharvest quality and previous literature indicates that it has the potential for dual-use as an antimicrobial intervention for this industry. However, the practicality and feasibility of deploying this technology in fresh blueberry fruit are significantly hindered by the shadowing effect occurring at the blossom-end scar of the fruit. The purpose of this study was to determine if treating the blueberry fruit within a chamber fitted with UV-Light Emitting Diodes (LEDs) emitting a peak UV-C at 275nm could minimize this shadowing and result in improved treatment efficacy. Ten blueberry fruits were dip-inoculated with *E. coli* at a concentration of 105 CFU/mL and irradiated within the system at doses of 0, 1.617, 3.234, 9.702, and 16.17 mJ/cm^2 (0, 30, 60, 180, and 300 seconds). Statistical analysis was performed to characterize the extent of microbial survival as well as the UV-C inactivation kinetics. A maximum 0.91 – 0.95 log reduction was observed, which attenuated after 60 seconds of treatment. The microbial inactivation and survival were thus modeled using the Geeraerd-tail model in Microsoft Excel with the GInaFIt add-in (RMSE = 0.2862). Temperatures fluctuated between 23 ± 0.5º C and 39.5 ºC ± 0.5º C during treatment but did not statistically impact the treatment efficacy (P = 0.0823). The data indicates that the design of a UV-LED system may improve the antimicrobial efficacy of UV-C technology for the surface decontamination of irregularly shaped fruits, and that further optimization could facilitate its use in the industry.

INTRODUCTION

Blueberries (*Vaccinium corymbosum*) are a high-value horticultural commodity with ever-increasing popularity among consumers due to the fruit's flavor, health benefits, and versatility. According to Protzman et al. *(28)*, more than 1 million metric tons of blueberries were produced globally in 2019, which was more than double the production in 2010. However, blueberries (like most fresh produce) are highly susceptible to microbial contaminants. A microbial surveillance study of berry fruits in Norway, for example, reported the detection of *Toxoplasma gondii*, *Cyclospora* cayetanensis, and *Cryptosporidium* spp. in imported blueberries destined for sale in the Norwegian market *(31)*. In the United States – a top producer of blueberry fruit (Food and Agriculture Organization, 2020) – both fresh and frozen blueberries have been implicated in foodborne disease outbreaks *(21)*. As the global production and trade of blueberries expands, the industry is facing increasing pressure to assure the fruits' postharvest quality and microbial safety throughout the global supply chain. Ultraviolet-C (UV-C) irradiation is a well-recognized tool for improving blueberry quality during postharvest storage, but more studies are needed to improve its capacity as an antimicrobial intervention.

Despite being an abiotic stressor, the literature consistently reports the benefits of dry UV-C applications to blueberry fruit shelf-life, including delayed softening, sugar catabolism, and lipid peroxidation *(23, 32, 33)*. Such applications were also found to induce the accumulation of antioxidants (e.g., anthocyanins), antioxidative enzymes, and other enzymes in the plant defense response to biotic stress, improving the fruit's nutritional content and resistance to plant pathogens *(13, 23, 27, 33, 34)*. Unlike 'wet' or water-assisted UV-C technologies, dry UV-C applications do not incorporate water or aqueous solutions during the treatment process. Yet, dry

UV-C technologies have historically had a lackluster or highly variable effect on the populations of human pathogens on the surface of fresh blueberry fruit (*Table 12*), likely due to the shadowing effect occurring at the blossom-end scar. As demonstrated in previous studies, such complex surface structures can harbor pathogens and protect them from the conventional one-dimensional UV-C treatment design *(1, 30)*. Prior studies attempted to overcome this morphological challenge by manually or mechanically rotating the fruit during treatment or by extending the treatment time, but these methods have a minimal impact as there is still an observed attenuation in microbial reduction despite increasing the UV-C dose delivered to the fruit *(19)*. On a more practical note, the rotation method is not feasible for deployment in the produce processing environment and higher than optimal UV-C doses may negatively impact blueberry fruit quality such as excessive water loss *(15)*. Studies from Liu et al. *(19)* also demonstrated the impact of the inoculation technique; while spot inoculation ensures a more consistent starting microbial load, the drip inoculation method better accounts for the realities of the shading effect.

Table 12 *A selection of studies exploring the use of dry UV applications to reduce microbial contamination on the surface of fresh blueberry fruit.*

Study	UV Source	Wavelength	Dose	Pathogen	Inoculation	Log_{10} Reduction
Cao et. al *(6)*	PUV	180–1100 nm	6 J/cm^2	*Salmonella enterica* ser. Newport, Montevideo, St. Paul, Stanley	Dip	0.6 – 0.9 log
Kim and Hung, *(17)*	UV lamp	200 – 280 nm	1.2 – 12 kJ/m^2	*Escherichia coli* O157:H7	Spot	2.14 – 4.05 log
Bialka and Demirci, *(4)*	PUV	100 to 1100 nm	1.1 – 32.4 J/cm^2	*Escherichia coli* O157:H7	Spot	1.3 – 4.9 log
				Salmonella enterica ser. Agona, Baildon, Gaminara, Michigan, Montevideo	Spot	1.1 – 3.8 log

Liu et al. *(19)*	UV lamp	254 nm	0.95 – 4.74 J/cm^2	*Escherichia coli* O157:H7	Dip	1.0 – 1.6 log
				Escherichia coli O157:H7	Spot	0.8 – 4.0 log

**Note: PUV = pulsed UV*

The purpose of this research was to redefine this challenge as an engineering barrier (rather than a fruit morphological barrier) to increase the efficacy of dry UV-C surface treatments for fresh produce with irregular surface morphologies. Liu et al. *(19)* began to address this challenge by installing four UV-C mercury lamps within an enclosed reactor to reduce the shadowing effect. However, the authors still only observed a 1.0 – 1.6 log reduction of *E. coli* O157:H7 following dip inoculation after 2 minutes of treatment which did not change despite increasing the dose.

Therefore, there is a need to continue to improve the UV-C application method to determine if UV-C light can effectively reduce the microbial load in fresh blueberries. The objective of this research was to determine if a series of UV-LED microarrays installed on all six major planes (top, bottom, and four sides) of a UV-reflective chamber (*Figure 15*) could provide simultaneous exposure of the entire fruit, and then explore if this coverage could in turn improve the antimicrobial efficacy of UV-C light and facilitate its use in the industry. This chapter was published in the Journal of Food Protection, Vol 86, Olivia C. Haley, Eleni D. Pliakoni, Cary Rivard, Londa Nwadike, Manreet Bhullar, *The Attenuation of Microbial Reduction in Blueberry Fruit Following UV-LED Treatment*, Copyright the International Association for Food Protection (2023), https://doi.org/10.1016/j.jfp.2023.100056.

MATERIALS AND METHODS

UV-C Chamber Fluence Characterization. A custom-made chamber fitted with UV-LED modules exhibiting an optical power output of 290 mW per module and a peak emission wavelength of 275 nm was used for this study (*Figure 15*). The UV-LED modules (WOB_16A, Seoul Viosys Co., Ansan, Republic of Korea) were selected because previous reports indicated

that this wavelength is highly effective in repressing photoreactivation and is more energy efficient (thus potentially more cost-effective) *(25)*. The outer layer and inner walls of the UV-C chamber were composed of stainless steel for reflectivity.

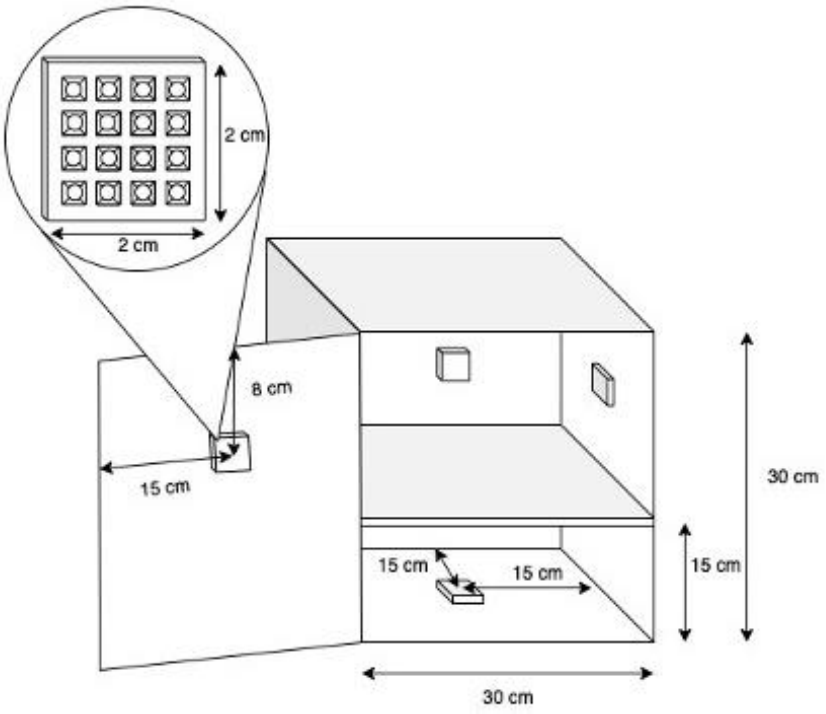

Figure 15 *The dimensions of the enclosed UV-C treatment system (UV-C Chamber) used in this study*

The UV-C fluence of the system was characterized spatially and temporally, along with the changes in temperature, in a separate trial from the blueberry experiment. To measure the spatial distribution, the shelf within the system was divided into a 5 x 5 matrix and the UV-C fluence measured with an ILT960-UV Spectroradiometer (International Light Technologies, Peabody, MA, USA) in each unit at 11.0 cm, 12.5 cm and 13.5 cm from the top panel. Measurements were recorded after a 2-minute warmup period. The average fluence was calculated based on the average fluence within the units designated as the testing area. The change in temperature with system operation time was recorded with a data logger (EasyLog EL-USB-2, Lascar Electronics,

Whiteparish, UK) according to the time intervals of each block of treatments in the sequence: 120 seconds (warm-up), 570 seconds (first block), 570 seconds (second block), 570 seconds (third block). This approach was taken to characterize the potential effects of the changing microclimate of the chamber on the observed microbial reduction.

Culturing and enumeration of microorganisms. To avoid overhandling, the blueberries were not pre-treated prior to beginning experimentation. Rather, rifampicin-resistant *Escherichia coli* (ATCC #25922) was used as the test organism in the described trials to reduce the impact of the blueberry fruits' background microbiota. Specifically, the antimicrobial rifampicin limits the growth of background flora on the rifampicin-supplemented agar plates such that only the inoculated rifampicin-resistant *E. coli* are enumerated. The strain was acquired from the Bhullar lab (Kansas State University, Olathe, KS, USA), and stored in 25 to 50% glycerol (Fisher Scientific, Waltham, MA, USA) in cryovials at −80°C. The strain was initially propagated by transferring a loopful of the frozen culture to Tryptic Soy Agar (TSA, Remel Inc., San Diego, CA, USA) and incubation at 35 ± 2 ºC for 24 ± 2 hours. To propagate 48-hour subcultures, one colony from the master plate was then transferred to a culture tube containing 10 mL of Tryptic Soy Broth (TSB, Remel Inc., San Diego, CA, USA), and the tube was incubated at 35 ± 2 ºC for 24 ± 2 hours.

Then, 100µL of the incubated subculture was then transferred to 30mL of TSB and incubated at 35 ± 2 ºC for another 24 ± 2 hours. The cells were prepared for experimentation by centrifugation (Allegra X-30 R, Beckman Coulter, Brea, CA, USA; 10,000 rpm, 10 min, and 20ºC) and washed twice with 0.1% (w/v) phosphate buffer saline (PBS, Thermo Fisher

Scientific, Waltham, MA, USA). The volume was then adjusted to 30mL to achieve a concentration of at least 8 log CFU/mL. To prepare the inoculum for the trials, 10mL of the washed subculture was added to a sterile bottle containing 990mL of 0.1% buffered peptone water (BPW; Thermo Fisher Scientific, Waltham, MA, USA) for a final concentration of at least 6 log CFU/mL.

Blueberry inoculation. Fresh blueberries were acquired from a local grocery store on the morning of each trial and stored at 4ºC until experimentation. The blueberries were initially sorted to exclude fruits which had an irregular shape, size, or excessive postharvest damage (i.e., lacerations, scarring, or visible bruising). Ten blueberries were then randomly assigned to a treatment. The number of blueberries per replicate was determined based on preliminary trials (data not shown) and to avoid overcrowding the unit's shelf. Each treatment included three replicates. The fresh weight of each replicate was measured using a balance (Scout Pro SP-202, Ohaus Corporation, Parsippany, NJ, USA) and recorded prior to inoculation.

The 10 blueberries of each replicate were placed into an 8 oz. Whirlpak bag (Nasco Sampling LLC, Fort Atkinson, WI, USA) labeled with the appropriate treatment time using flame-sterilized tweezers, and 50mL of inoculated diluent was added to completely submerge the blueberries. The fruit were rested in the inoculated diluent in the biosafety cabinet (BSC) to allow for adherence of the *E. coli* to the fruit surface. After 30 minutes, the bags were cut open with flame-sterilized scissors to facilitate the removal of the berries without damaging the skin. The fruit were removed from the bag with flame-sterilized tweezers and placed on weigh boats, which were sterilized via a 30-minute soak in 10% sodium hypochlorite followed by rinsing with

distilled water. The spent diluent was autoclaved before disposal. The blueberry fruit were dried in the BSC for 20 minutes; after 10 minutes, the fruit were transferred to another sterile weigh boat and dried on the other side for another 10-15 minutes. After drying, the fruit were transported to the UV275 chamber for experimentation.

Inactivation Experiments and Enumeration. UV-C treatment intervals of 0, 30, 60, 180, and 300 seconds were selected for experimentation based on preliminary data (not shown). Ten blueberries were inoculated per replicate and treated simultaneously. There were three replicates per time interval and the experiments were repeated three times on different days. To reduce the impact of temperature increase in the treatment chamber, the replicates were arranged in a randomized complete block design wherein each 'block' represents a series containing one replicate for each of the experimental treatments. The inoculation, treatment, and enumeration procedure used in this study are summarized in *Figure 16*.

The 10 fruit were placed onto designated markers on the shelf within the UV-C chamber using flame-sterilized tweezers and treated for their respective time interval (*Figure 16*). After the UV-C treatment, the blueberries were transferred to labeled 8oz. Whirlpak bags containing 25mL of 0.1% BPW. The surviving microbial population was plated within one hour of each experiment. Serial dilutions were prepared as needed with 0.1% buffered peptone water (BPW, Remel Inc., San Diego, CA, USA), and 100 µL of the sample was plated onto TSA containing 80 µg/mL rifampicin via the spread plate method to enumerate the surviving *E. coli* on the surface of the fruit. The plates were then incubated at 35 ± 2 ºC for 24 ± 2 hours. Plates yielding 25 to 250 colony forming units were counted, and log reductions were calculated.

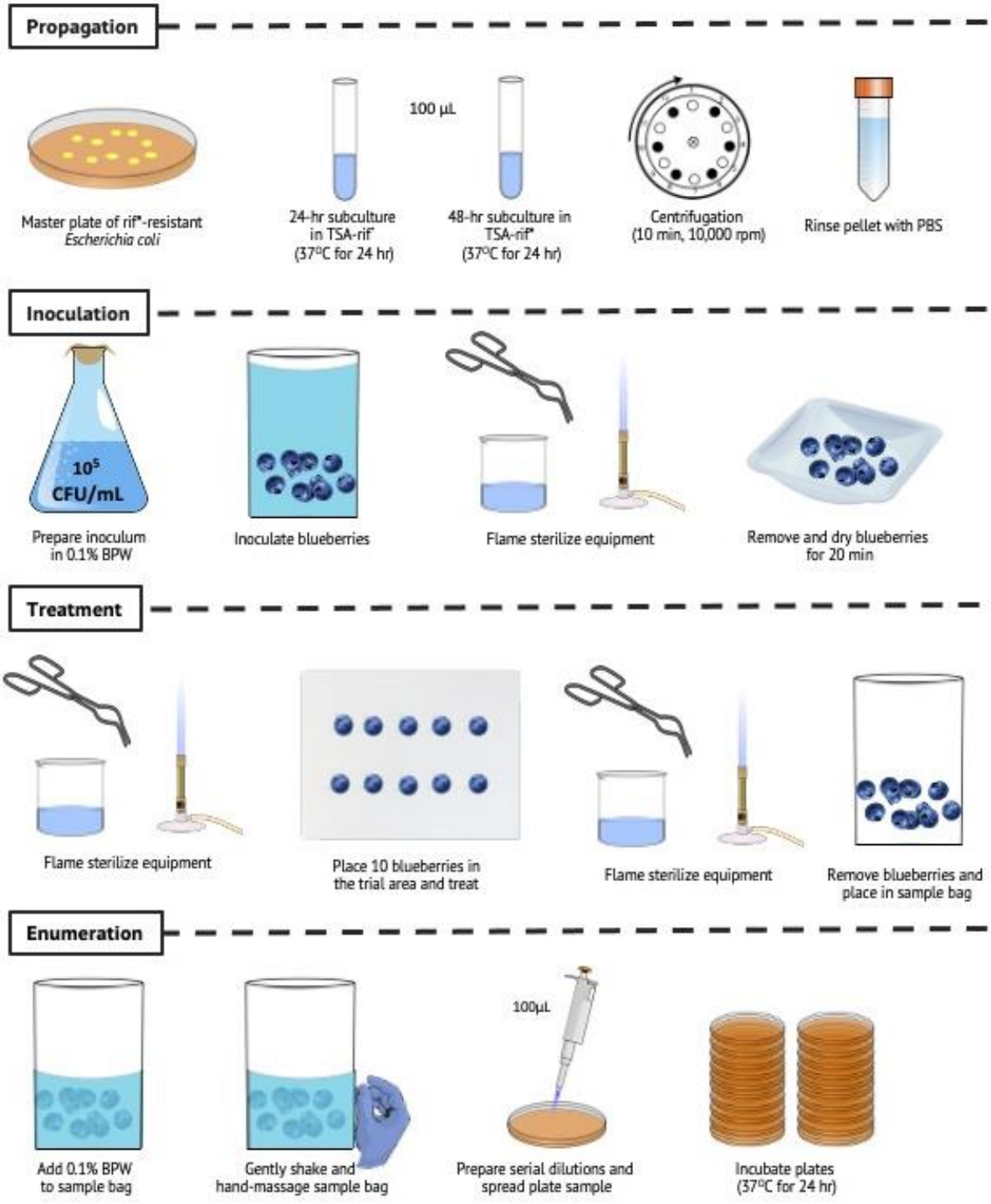

Figure 16 *A pictorial representation of the overall procedure used for microbial propagation, blueberry inoculation and treatment and the enumeration of the surviving population*

Data Analysis. The study was considered as having a randomized complete block design with REPLICATE as a random blocking factor, and UV_DOSE as a fixed factor. The surviving generic *E. coli* counts were transformed to fit a logarithmic distribution prior to statistical analysis and expressed as log colony forming unit per gram of sample (log CFU/g). Differences between the means of the treatments were computed using the GLM procedure in SAS (version 9.4, Cary, NC, USA) with the Tukey-Kramer adjustment for multiple comparisons. Statistical significance was established at $p < 0.05$.

Modeling of UV-C Inactivation Kinetics. The microbial inactivation data were analyzed in the Microsoft® Excel for Mac desktop application (Version 16.63.1, Microsoft, California, USA) using the GInaFiT version 1.8 add-in *(12)*. The goodness of fit was evaluated for the log-linear regression *(5)*, biphasic *(7)*, log-linear with tail (Geeraerd-tail model; *(11)*), Weibull *(20)*, Weibull with tail *(2)*, double Weibull *(9)*, and biphasic with shoulder *(12)* models. Initially, plotting the inactivation data revealed a nonlinear relationship between the UV-C dose and the inactivation of *E. coli* in this study. Accordingly, the most appropriate model to describe the data was selected based on the root mean square error (RSME) since previous literature indicated the low performance of the R^2 and adj-R^2 parameters in evaluating nonlinear model validity and performance *(29)*.

RESULTS AND DISCUSSION

Characterization of UV-C fluence and distribution within the system. Due to the shelf's material (acrylic) there was no significant penetration of UV-C from the bottom module. Moreover, the wide variation in the UV-C fluence of the system at different positions indicated a

non-uniformity outside of the testing area (*Figure 17*); note that 'Front' and 'Back' indicates the direction of the system. Directly atop the system's shelf (13.5 cm from the top), the average fluence of the UV-C system at 275 nm on the shelf within the testing area – the area excluding the units on the outer edges of the system – was 0.0539 mJ/cm^2 ± 0.0062 mJ/cm^2. However, the fluence ranged from 0.1071 mJ/cm^2 to 0.01553 mJ/cm^2. At 12.5 cm, the average fluence was 0.0531 mJ/cm^2 ± 0.0050 mJ/cm^2 and ranged from 0.1094 mJ/cm^2 to 0.0180 mJ/cm^2 across the plane. At 11.0 cm, the average fluence was 0.0482 mJ/cm^2 ± 0.0053 mJ/cm^2 and ranged from 0.2040 mJ/cm^2 to 0.0124 mJ/cm^2.

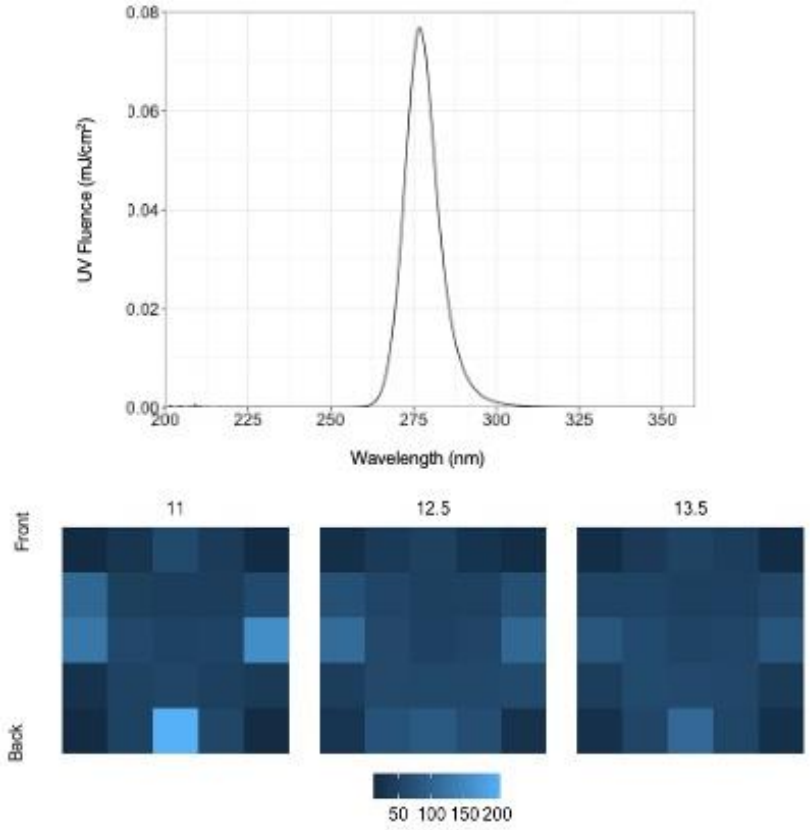

Figure 17 *The emission spectra (top) and the spatial distribution (bottom) of UV-C fluence (mJ/cm2) at a distance of 11, 12.5, and 13.5 cm from the top of the UV-C system.*

The lower fluence observed at the corners of the UV-C system is most likely due to the viewing angle (degree of light spreading) of the UV-LEDs. The viewing angle of UV-LEDs is typically 140º *(8)*, and with the rapid decrease in fluence of UV-LED sources with increasing distance, the corners of the system will naturally experience a lower UV-C fluence. The larger fluence measurements, on the other hand, coincided with areas directly next to a module. Interestingly, the average fluence in the testing area decreased as the distance between the sensor and the UV-LED array on the top of the system narrowed. This finding likely results from the measuring instrument as it is not able to capture light from all angles. As the sensor is placed closer to the top of the system, there is less incident light captured from the sensor's periphery.

The low fluence at the front of the system indicated a malfunction in the front UV-LED module; this hypothesis was corroborated by visual inspection of the system, wherein two of the sixteen LEDs in the microarray were not functioning by the end of the study. As the temperature in the chamber increased significantly over time (discussed in further detail in the sections below), it is likely that excess heat generated by the module surpassed the thermal resistance capacity of the aluminum heat sinks, resulting in LED burnout. Because the timing of the LED malfunction is unknown, the effect of burnout on the determinations was accounted for by looking at the REPLICATE effect. As this effect is nonsignificant ($P < 0.05$), it is not likely that burnout contributed to affecting the determinations. However, this malfunction emphasizes a need for improved heat dissipation for future study (which is addressed in more detail in the section discussing the impact of system temperature and UV_{275} fluence fluctuation). Another suggestion for future studies is to monitor the fluence across the different repeats to detect the occurrence of LED burnout.

Overall system microbial inactivation efficacy. According to the analyses, the survival of rifampicin-resistant *E. coli* was significantly reduced by UV-C application regardless of the experimental dose ($P < 0.0001$). At a cumulative dose of 1.62, 3.23, 9.70, 16.17 mJ/cm^2 (30, 60, 180, 300 seconds), the mean and standard deviation of the microbial reduction compared to the control was 0.54 (SD = 0.23), 0.93 (SD = 0.34), 0.91 (SD = 0.35), and 0.95 (SD = 0.36) log, respectively. After 60 seconds of treatment, the population of *E. coli* on the surface of the treated blueberry fruit did not significantly decrease despite increasing the dose.

When comparing these results with that of similar studies, these findings indicate that approaching the problem from an engineering perspective and altering the design of the chamber was somewhat effective in improving the application of UV-C technologies for whole-fruit surface decontamination. For example, the results of this study were similar to that of Cao et al. *(6)* who reported a reduction of 0.6 log on the surface of dip-inoculated fresh blueberries directly following 30 seconds of UV-C application. However, the system used in this study was able to garner a similar log reduction without the use of a shaker to turn the fruit on all sides. In practice, this finding indicates that this system could eliminate the need for manual or mechanical rotation of fruit during treatment, reducing labor needs, as well as reducing the likelihood of negative impacts on shelf-life due to mechanical damage and excess handling encountered by the blueberry fruit during dry UV-C applications. Although Liu et al. *(19)* reported a slightly higher inactivation efficacy in dip-inoculated blueberry fruit after 2 minutes, the system in this study was able to achieve a reduction of >0.9 log before attenuation after 60 seconds. Reducing the distance of the target produce from the UV-LED sources could reduce the dissipation of UV-C

energy, increasing the quantity of irradiation available for decontamination and increasing the microbial inactivation efficacy. However, the fruit quality could also be impacted as noted in later sections.

Capacity of the UV-C system to address the shadowing effect. As previously mentioned, the model fit was assessed using the root mean square error (RSME) since initially plotting the inactivation data revealed a nonlinear relationship between the UV-C dose and the inactivation of *E. coli*. Although the effect of the replicate (REPLICATE) was nonsignificant ($P > 0.05$), the surviving population of each replicate (rather than the mean of each treatment) was used to evaluate the model due to the low sample size ($n = 9$). The Geeraerd-tail model was selected to describe the inactivation kinetics of *E. coli* by UV-C treatment based on the low RSME (*Table 13*). This model describes the relationship between the UV-C dose and predicted microbial response (*Equation 1*) where N is the population (CFU/g) at time t (s), No is the initial population (CFU/g), Nres is the surviving ('UV-C resistant') population (CFU/g), and kmax is the maximum inactivation rate per second.

Table 13 *The goodness-of-fit parameters for the models estimating the inactivation of E. coli on the surface of fresh blueberries after treatment with UV275 light*

Model	RMSE	R^2	Adj-R^2
Log Linear	0.3881	0.3105	0.2944
Log Linear + Tail	0.2862	0.6337	0.6163
Weibull	0.3011	0.5944	0.5751
Weibull + Tail	0.3045	0.5951	0.5655
Double Weibull	0.3043	0.5957	0.5662
Biphasic	0.2896	0.6337	0.6069
Biphasic + Shoulder	0.4023	0.3105	0.2415

This model describes an inactivation curve having both a log-linear portion and a tailing effect, and has been used to describe microbial survival in food items following treatment by ultraviolet radiation *(14)*, sonication *(22)*, etc. The fit of this model to the experimental data is portrayed graphically in *Figure 18*.

Equation 2 *The Geeraerd-tail model for microbial inactivation*

$$N = (N_0 - N_{res}) * e^{(-kmax*t)} + N_{res} \quad (2)$$

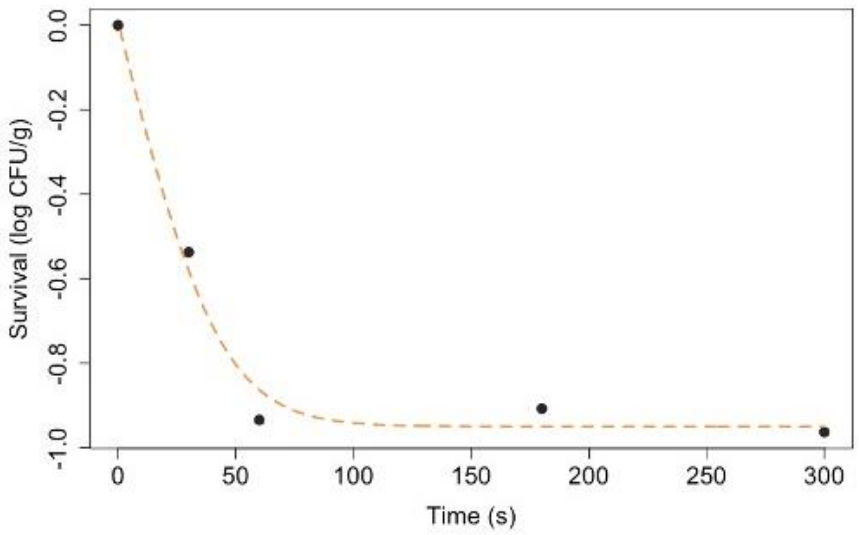

Figure 18 *The inactivation curve of E. coli on the surface of fresh blueberry fruit after treatment with UV-C according to the Geeraerd-tail model*

Based on the findings of Geeraerd et al. *(11)*, the tailing of microbial survival following UV-C treatment represents the survival of a microbial subpopulation due to (1) a greater UV-C resistance of the subpopulation, (2) differential population exposure to the same lethal dose, or (3) incomplete inactivation. In apricot fruit, Hakguder and Unluturk *(14)*, attributed the observed tailing to the heterogenous mixture of natural microbiota on the fruits' surface. By contrast, in this study the sorted blueberry fruit were inoculated with rifampicin-resistant *E. coli* and the surviving microorganisms were enumerated on agar supplemented with rifampicin. Therefore, the heterogeneity of the blueberry surface microbiota was not a likely contributor to the tailing. It is possible that the dissipation of UV-C energy rendered the system incapable of fully minimizing the shadowing effect at the blossom-end scar; a potential solution is to reduce the size of the system as previously suggested. However, it could also be that the formation of biofilms or multiple microbial cell layers on the surface of the fresh blueberry fruit during inoculation meant that the system was not able to fully penetrate the layers of microbiota for

effective UV-C treatment. Biofilm formation has been previously observed on the surface of fresh fruits and vegetables *(3)* and such matrices can physically shield subpopulations from UV-C radiation *(10)*.

Impact of system temperature and UV_{275} fluence fluctuation. The starting temperature of the UV-C system was 22º C ± 0.5º C. Based on the temperature data, (*Figure 19*), the temperature of the chamber during the first, second, and third replication were as high as 23 ± 0.5º C to 26.5 ºC ± 0.5º C, 26.5ºC ± 0.5º C to 32.5 ºC ± 0.5º C, and 32.5 ºC ± 0.5º C to 39.5 ºC ± 0.5º C, respectively.

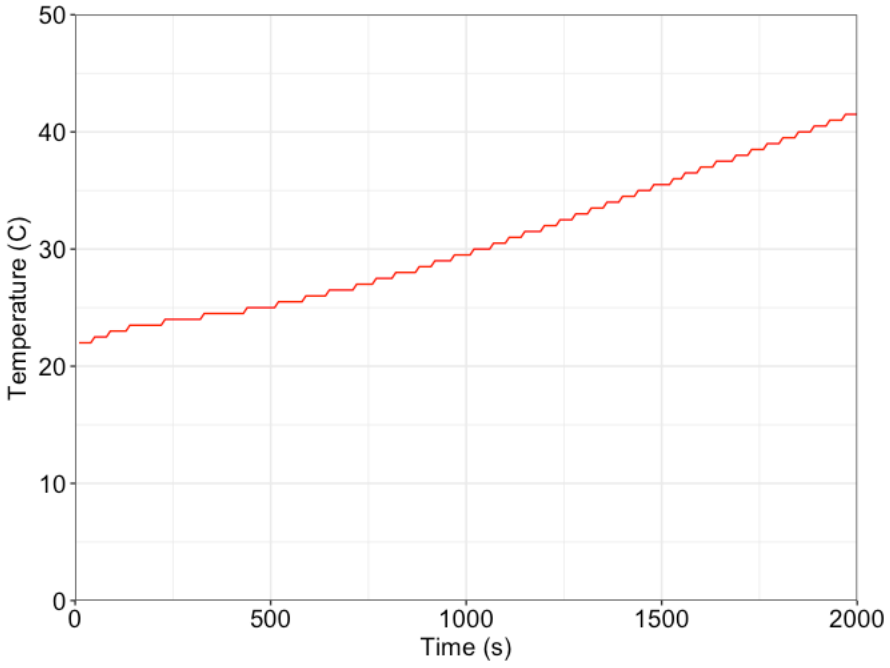

Figure 19 *The change in the internal temperature (ºC) of the UV-C system as a function of time*

In the statistical analyses, the blocking effect (REPLICATE) was used as a proxy to evaluate the impact of the fluctuations in temperature during treatment since the replicates in each block were

exposed to roughly the same temperatures. Moreover, the blocking effect could provide information regarding the impact of the exponential decay in UV-C intensity on the microbial reduction efficacy of the system.

The lack of statistical significance of the blocking effect (REPLICATE) (P= 0.0823) indicates that there is likely not an effect on the survival of generic *E. coli* attributable to the temperature or the UV-C fluence decay within the chamber. However, it is important to note several limitations of this approach. Because it is not possible to separate the two effects (temperature and fluence) using this approach, it is possible that the individual effects of either factor on the systems' efficacy is underestimated. Moreover, this does not address the treatments' effects on the blueberry quality.

Like most fresh produce, blueberry fruit are highly susceptible to supra-optimal temperatures *(18)* which accelerate biochemical reactions leading to water loss and spoilage. Of note, prior research has also highlighted the impact of supra-optimal UV-C doses on blueberry fruit physiology (i.e., ethylene production, transpiration rate). For example, Xu et al. *(33)* reported the beneficial effect of UV-C irradiation on reducing the rate of water (fresh weight) loss at a dose of 4 J/m^2 whereas Cao et al. *(6)* found that pulsed ultraviolet (PUV) applications of 6 J/m^2 accelerated fruit fresh weight loss. For this reason, it is also critical from the industry perspective to understand the repercussions of the temperature and fluence fluctuations during operation on blueberry quality and shelf-life. While the UV-C system included aluminum heat sinks to dissipate the excess heat generated by the LEDs, additional modifications (e.g., installation of a fan and venting) are necessary before conceptualization in a production environment.

Scope of Future Research. While heat dissipation and UV-C fluence present physical limitations, the fact that the blueberry cultivar is unknown presents another limitation of this study and other such UV-C studies. As reported by Perkins-Veazie et al. *(27)*, the physiological response of blueberry fruit to UV-C irradiation can be highly variable, even at the 'optimal' doses for postharvest quality potentiation. The blueberries from this study were acquired from a local retailer whose supplier purchased the fruit from operations in various regions, each using cultivars fit for their operation. This is standard practice, and the overall implication is that it is very difficult, if not impossible, to identify the cultivar used in the study to account for cultivar-specific responses.

Another consideration for future development is coupling the UV-LED system with and another antimicrobial intervention (e.g., chemical sanitizers) as a hurdle technology – the combination of two (or more) decontamination techniques wherein the treatment promotes a greater microbial reduction as compared to when the techniques are applied alone *(16)*. For example, Park et al. *(26)* observed that treating spinach leaves and tomato fruit with combined UV-C and chlorine dioxide gas (ClO_2) was more effective in reducing the pathogen load of fresh produce than either treatment alone. A similar synergistic effect has also been reported between water-assisted UV-C and peracetic acid *(24)* wherein the treatment was also effective in reducing the microbial populations within wash water. Accordingly, it would be beneficial to determine if the efficacy of the UV-LED system can be improved with the addition of another antimicrobial component.

CONCLUSIONS/RECOMMENDATIONS

This study demonstrated the utility and limitations of an enclosed UV-LED system as an antimicrobial intervention for fresh produce surfaces. The system was able to reduce the population of generic *E. coli* on inoculated blueberry surfaces by 0.91 – 0.95 log within one minute, without needing to rotate the fruit midway through treatment. While the reduction efficacy could potentially be further increased by reducing the distance between the fruit and the LED modules, this approach requires addressing the system's poor heat dissipation which caused temperatures within the chamber to rise by more than 15 ºC. Effectively, improving the system's thermoregulation and optimizing the distance between the UV-LED modules and the fresh produce to minimize the dissipation of LED intensity are the most immediate next steps to improve the systems' microbial reduction capacity. Although the impact of the UV-C system on the blueberry fruit quality parameters over time was not measured in this study, this aspect is also critical to characterize for this system to be accepted as a two-pronged approach toward improving both blueberry quality and safety. Though the UV-C system needs to undergo further conceptualization before deployment in the industry, this is still a promising technology for fresh produce surface decontamination. Once more, in processing environments seeking to reduce the use of chemical sanitizers to rinse the surface of fresh produce, the UV-LED system stands to save energy costs (compared to UV-C systems containing conventional low-pressure mercury lamps) and water resource costs.

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Chapter 6 - The reduction of *Escherichia coli* on the surface of fresh strawberries by UV-LEDs is limited by complex surface structures.

ABSTRACT

Recent studies have investigated the use of ultraviolet-C (UV-C) irradiation as a dual-purpose technology for improving strawberry (*Fragaria* × *ananassa*) postharvest quality and food safety. However, UV-C is not a highly penetrative form of irradiation, thus its antimicrobial efficacy is significantly reduced by shadowing from the fruits' complex surface structures (sepals and achenes). A table-top treatment chamber fitted with six, UV-Light Emitting Diodes (LEDs; 275nm) modules was designed to overcome this shadowing effect and provide a simultaneous, high-powered 360° UV-C treatment. The purpose of this study was to determine if the UV-C chamber could minimize the shadowing effect from the strawberry sepals and confer greater treatment efficacy. Strawberry fruits were dip-inoculated with rifampicin-resistant generic *Escherichia coli* to achieve a surface concentration of at least 4-log CFU/g. To determine the contribution of the sepals to microbial survival, the sepals of select fruit were then either left intact or removed. The fruit were then treated individually on a UV-transparent shelf at a dose of 0, 9.1, 18.3, or 27.4 mJ/cm^2 (0, 30, 60, or 90 seconds). Following treatment, the fruit were surface washed, and the surviving *E. coli* were enumerated on rifampicin-supplemented tryptic soy agar. There was a statistically significant effect of time ($P < 0.0001$), with a highly suggestive effect of leaf presence ($P = 0.0568$). After 90 seconds, an average log reduction of 1.48 ($SD = 0.31$) was observed in fruit with sepals, and 1.53-log ($SD = 0.30$) in fruit without sepals. Both inactivation curves exhibited non-linearity, providing further evidence that the sepals were not significantly inhibiting UV-C efficacy. The data improves the current

understanding of UV-C limitations in the fresh strawberry industry. Particularly, the strawberry surface achene structures instead of the sepals may pose the greatest challenge to UV-C technology.

INTRODUCTION

The United States is a major producer of strawberry fruit (*Fragaria x ananassa*) with approximately 1.2 million pounds (~$2.2 billion dollars) harvested from more than 43,000 acres of agricultural lands in 2020 *(19)*. Since its introduction in the 19th century, many American cultivars have been developed and the commodity's economic importance and consumption in the United States has steadily increased *(12, 15, 22)*. Once more, strawberry are an excellent source of vitamins and health-promoting phytochemicals (e.g., anthocyanins) beneficial to the human diet. However, strawberry fruit are prone to colonization by human pathogens, particularly foodborne enteric viruses like norovirus and Hepatitis A *(5)*. Because strawberry fruit are often packaged directly in the field following harvest (e.g., field-packaged) to prevent mechanical damage (i.e., bruising, cuts) *(26)*, there is limited opportunity to implement post-harvest treatments in this commodity.

Ultraviolet-C (UV-C) light is a promising technology for the treatment of strawberry fruit during harvest. UV-C light is a physical decontamination method wherein the transfer of photochemical energy to a microorganism's nucleic acids induces mutagenesis, ultimately leading to cell death if the microorganism is not able to repair the photodamage *(7, 24)*. Accordingly, UV-C highly effective at reducing the load of human pathogens on the surface of fresh strawberries *(1, 21)*. UV-C treatments also show the potential to improve strawberry quality and shelf-life by increasing resistance to gray mold (*Botrytis cinerea*) *(14)* and powdery mildew (*Podosphaera aphanis*) *(13)*, reducing the rate of cell wall degradation *(20, 23)*, and promoting the accumulation of antioxidants *(3, 17)*. As UV-C treatments also do not require water, this method

does not contribute to the formation of humid environments which promote the growth of molds and mildew *(9)*.

However, more information is needed to address three major areas before UV-C can be implemented for strawberry decontamination at harvest. First, UV-C light is not a highly penetrative form of radiation, having a typical penetration depth of only a few millimeters in depending on the food's surface characteristics *(16, 18)*. Thus, surface structures such as achenes (seeds) and sepals (green, leaf-like structures at the top of the fruit) provides a suitable habitat for microbes to be shielded from treatment *(1)*.; this phenomena is called the shadowing effect. Studies typically overcome this shadowing by manually rotating the fruit during treatment, leading to longer treatment times and a higher UV-C dose. Specifically, UV-C doses in strawberries typically range from 60 mJ/cm^2 to almost 800 mJ/cm^2 *(1, 2, 21)*. However, strawberries are susceptible to UV-C overdoses which can induce tissue damage *(6)* and abnormal sepal shriveling or browning *(2)*. On a fundamental level, the contribution of achenes to the shadowing effect are well-documented *(1, 25)*, but there is little evidence available on how the sepals impact UV-C efficacy, and its implications in the fresh produce industry. For example, if the sepals are a major driver of the shadowing effect, UV-C technology could be an excellent technology to preserve the quality and safety of fresh cut strawberry since the sepals are removed in this application.

Recent studies in apricots and blueberries have demonstrated the ability of UV-C chambers to improve the antimicrobial effectiveness of UV-C by reducing the shadowing that occurs due to complex surface structures *(10, 11)*. Haley et al. *(11)* reported using a UV-C chamber fitted with

six UV-LEDs to reduce the UV-C dose required for microbial reduction in blueberries. If this UV-C treatment style conferred a similar reduction in UV-C dose requirements to treat strawberries, UV-C overdose would be less likely to occur (including its accompanying negative effects on produce quality).The objective of this study was thus to determine if this design would 1) reduce the UV-C dose required to treat fresh strawberries, and 2) determine the contribution of the sepals to the shadowing effect. Because this study takes a proof-of-concept approach, its results will be used to inform future research directions on incorporating UV-C technology into the field-packing operations of strawberry and similar fruit.

MATERIALS & METHODS

UV-C Chamber Fluence Characterization. A stainless-steel UV-C chamber was fitted with six UV-LED modules (model no. WOB_16A, Seoul Viosys Co., Ansan, Republic of Korea) on the interior of all six sides. The UV-LED modules were equidistant from the center of the treatment area on UV-C transmitting quartz shelving. Each module had an optical power output of 290 mW and a peak emission wavelength at 275 nm. This design was selected based on previous studies in blueberries (Haley et al., 2023). A fan and aluminum heat sinks were installed for thermoregulation. The UV-C fluence within the testing area was measured using an ILT960-UV Spectroradiometer (International Light Technologies, Peabody, MA, USA) after a 2-minute warmup period. To account for the fluence variations of UV-LEDs, the average fluence was calculated from three readings taken 30 seconds apart. The change in temperature during operation of the UV-C chamber was recorded over 10 minutes with a data logger (EasyLog EL-USB-2, Lascar Electronics, Whiteparish, UK).

Culturing and enumeration of microorganisms. Due to the strawberry's delicate epicarp (outermost layer), the fruit were not initially treated to remove the pre-existing surface microbiota. Instead, the strawberries were inoculated with rifampicin-resistant *Escherichia coli* (ATCC #25922) and enumerated using tryptic soy agar (TSA; Remel Inc., San Diego, CA) supplemented with rifampicin (rif; Thermo Fisher Scientific, Waltham, MA) at a concentration of 80 µg/mL. The strain was initially acquired from the Bhullar Food Safety Laboratory at Kansas State University and had been cryogenically preserved with 25 to 50% glycerol (Fisher Scientific, Waltham, MA) at −80°C. To prepare the inoculum, a 48-hour subculture was propagated in 30 mL of tryptic soy broth (Remel Inc., San Diego, CA) supplemented with rifampicin (80 µg/mL). The cells were harvested via centrifugation at 10,000 rpm for 10 min (Allegra X-30 R; Beckman Coulter, Brea, CA) and washed thrice with 0.1% (w/v) phosphate buffer saline (Thermo Fisher Scientific, Waltham, MA). The final volume was then adjusted in 0.1% buffered peptone water (BPW; Thermo Fisher Scientific, Waltham, MA) to achieve a 6-log CFU/mL concentration. Separate 48-hour cultures were propagated for each day of experimentation.

Strawberry fruit inoculation. Fresh strawberries were acquired from a local supermarket on the morning of each trial. The strawberries were sorted to exclude fruits which had signs of damage (i.e., scarring, visible bruising), and then weighed to achieve further uniformity. Each strawberry fruit was then randomly assigned to a treatment, and individually placed into an 8 oz. Whirlpak bag (Nasco Sampling LLC, Fort Atkinson, WI) containing 50mL of the inoculum. The fruit were submerged in the inoculum for 30 minutes before being removed from the sample bags with flame-sterilized tweezers, placed onto sterile petri dishes, and allowed to dry for one hour in a

biosafety cabinet. After drying, the sepals were either left intact or removed at the base of the sepal (but without damaging the flesh) using flame-sterilized scissors, and the fruit were transported to the UV-C chamber for treatment.

Inactivation experiments and microbial enumeration. The UV-C treatments followed a randomized complete block design wherein each block contained a single replicate of the treatments: 0, 30, 60, and 90-second UV-C treatment intervals for both sepal-having (SH) and sepal-less (SL) strawberries. The UV-C treatment intervals were selected based on preliminary data (*data not shown*). There were five blocks (thus five replicates per treatment) and the experiments were repeated three times on different days. Each replicate consisted of a single fruit.

For UV-C treatment, the fruit were placed onto the center of the quartz shelf using flame-sterilized tweezers and treated for the appropriate time interval. Immediately thereafter, the strawberries were transferred into sterile 8oz. Whirlpak bags and rinsed with 25mL of 0.1% BPW to harvest the surviving surface rifampicin-resistant generic *E. coli* populations. The quartz shelf was sterilized via 100% ethanol (Thermo Fisher Scientific, Waltham, MA) and allowed to dry between uses. Serial dilutions were prepared as needed in 0.1% BPW, and 100 µL of the diluent was plated onto TSA-rif (80 µg/mL) via the spread plate method. The plates were incubated at 35 ± 2 ºC for 24 ± 2 hours and plates with 25 to 250 colony forming units (CFU) were counted. The surviving generic *E. coli* counts were log transformed prior to statistical analysis and expressed as log CFU per gram of sample (log CFU/g).

Statistical analysis. Statistical analysis was performed using the GLM procedure in SAS (version 9.4, Cary, NC, USA). The linear mixed model included the individual and interaction effects of block (BLOCK; random), UV-C treatment interval (UVC_DOSE; fixed) and sepal presence (SEPAL; fixed) to explain variation in the surviving log population of *E. coli* (LOG_POP). As initial observations indicated the presence of two distinct subgroups in the data, an F-test for the treatment effect under the two conditions (slice = SEPAL) was performed. The differences between the means of the treatments were computed with the Holm-Bonferroni method for multiple comparisons. Statistical significance was established at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The UV-C chamber reduces the dose required for microbial reduction. The findings of this study indicate that the UV-C chamber could reduce the negative impacts of conventional UV-C doses on postharvest quality without compromising microbial reduction. With previous UV-C applications, the reduction of *E. coli* ranged between 0.8 to approximately 4-log, depending on factors such as the inoculation method (e.g., dip versus spot inoculation) and UV-C dose. Yet, the doses delivered to the fruit were often 10-fold to 100-fold greater than the doses used in this study. For example, the UV-C fluence at 275 nm within the testing area of the chamber (0.30 mJ cm^{-2}) conferred a UV-C dose of 0, 9.1, 18.3, and 27.4 mJ cm^{-2} for 0, 30, 60, and 90 seconds of treatment. Yet despite the substantially lower dose, the strawberries with and without sepals still achieved an average 1.53-log and 1.48-log reduction (respectively) after 90 seconds, which is comparable to previous studies. Although the postharvest benefits of these lower doses were not further explored, it is evident that the chamber has the potential to reduce the symptoms of UV-C overdose (i.e., sepal browning) characteristic of the conventional UV-C applications.

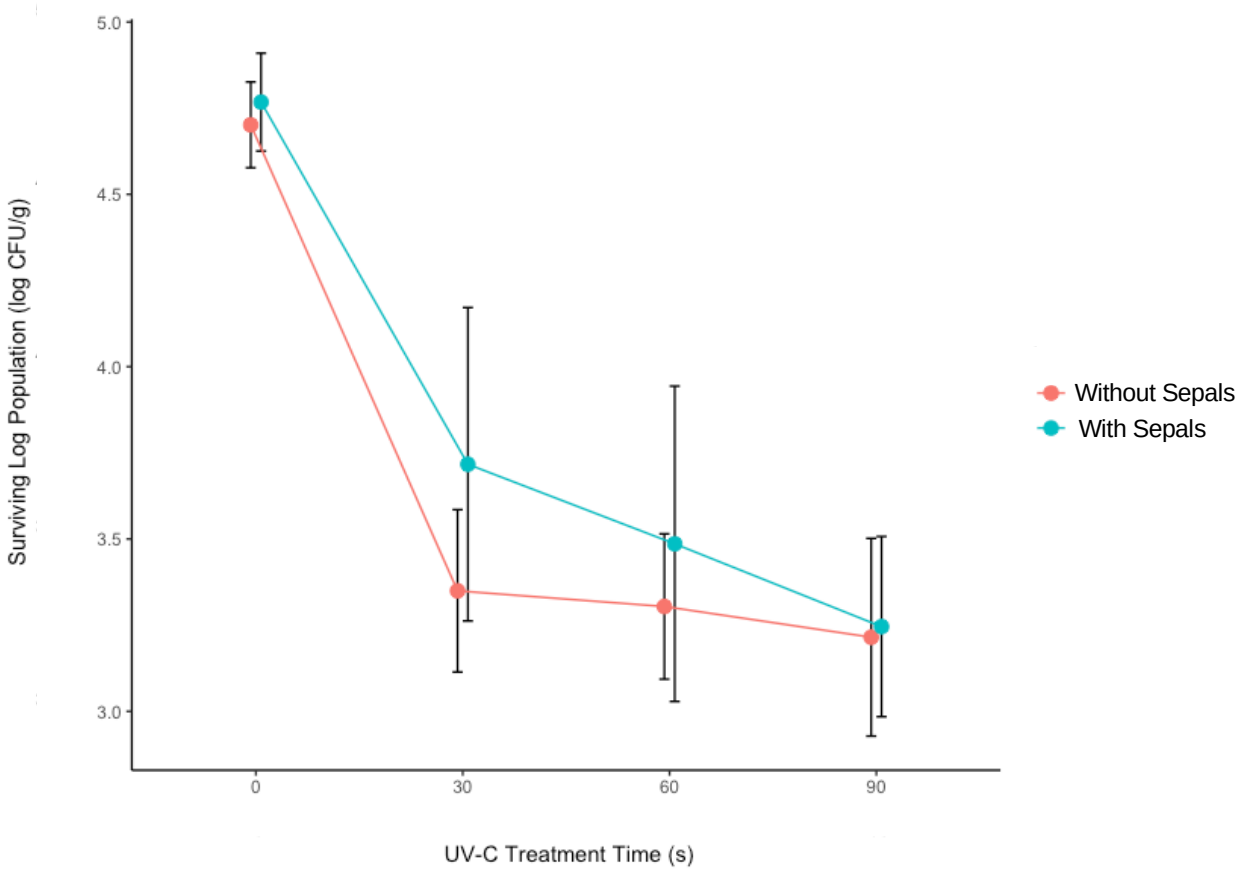

Figure 20 *The reduction of E. coli on inoculated strawberry fruit following UV-C treatment.*

**Note: statistically significant ($P \leq 0.05$) within-group differences are denoted with different letters*

Although the UV-C treatment is effective in reducing the surface population of rifampicin-resistant *E. coli*, the tailing indicates there are still barriers to minimizing the shadowing effect from the strawberries' surface structures. The interaction effect between UV-C dose and sepal presence were non-significant ($P = 0.4683$), but the difference in the group means by UV-C dose were statistically significant ($P < 0.0001$); the effect of sepals will be discussed in the next section. For strawberries with sepals, there was a surviving *E. coli* population of 4.77, 3.72, 3.49, and 3.24 log CUF/g after 0, 30, 60, and 90 seconds of UV-C treatment, respectively (*Figure 20*).

There were statistically significant differences detected between the untreated and treated strawberries with sepals ($P < 0.001$). Amongst the treatments, there were statistically significant differences only between the groups of strawberries with sepals treated for 30 and 90 seconds ($P = 0.0072$). For the SL strawberry group, 4.70, 3.35, 3.30, and 3.22-log CUF/g of *E. coli* was recovered from strawberries treated for 0 (control), 30, 60, and 90 seconds, respectively. There were statistically significant differences detected between the untreated and treated strawberries without sepals ($P < 0.001$), however, there were no statistically significant differences detected between the groups of strawberries with sepals treated for 30, 60, or 90 seconds ($P > 0.05$).

In the absence of a shadowing effect, the survival curve often appears more linear *(25)*, and such is the case for fruits with smoother surfaces. However, increasing the UV-C dose did not continue to (statistically) significantly reduce the microbial populations on the surface of the strawberries, indicating that shadowing is still occurring. Due to the low penetration of UV-C, future studies could also investigate potential shadowing due to the formation of a dead cell layer above viable cells. As suggested by previous publications, the formation of biofilms or multiple microbial cell layers on the surface of dip-inoculated produce could lead to the system not being able to fully penetrate the layers of microbes for effective UV-C treatment. Biofilm formation has been previously observed on the surface of fresh fruits and vegetables *(4)* and such matrices can physically shield subpopulations from UV-C radiation *(8)*. More investigation on strategies to overcome the shadowing effect in UV-C would be beneficial to improving the microbial safety of field-packed fruits and vegetables.

Strawberry sepals provide minimal contribution to shadowing. The data also provides evidence for a minor effect of the sepals on the microbial reduction of UV-C. Although there was a highly suggestive effect of leaf presence ($P = 0.0568$), the generic *E. coli* populations of the SH and SL strawberries followed similar trends and were nearly identical at the lowest (0s) and highest (90s) UV-C doses. As previously discussed, this finding demonstrates that despite the absence of the sepals, similar shadowing still occurs. However, there was a remarked difference in the microbial reduction rate between the SH and SL strawberries. The Holm's multiple comparison test detected statistically significant differences between SH strawberries treated for 30 and 90 seconds ($P = 0.0216$), indicating a more gradual decline to the plateau (maximum reduction). In contrast, the SL strawberries experienced an initial sharp decline in microbial population wherein afterwards there were no statistically significant differences detected amongst the mean surviving microbial populations of the UV-C treated strawberries. The more rapid rate of microbial reduction in the SL strawberries could mean that removing the sepals reduces the initial effects of shadowing but provides further evidence that the sepals are not the major limiting structures.

Improved outlook for industry implementation. This study was successful in addressing the design flaws of previous iterations of UV-C treatment chambers. For example, Haley et al. previously identified the major obstacles to the deployment of the UV-C chamber design in the industry as uneven fluence distribution and excessive heat generation. To improve the fluence distribution, the size of the testing area was reduced, and a quartz shelving unit was installed to transmit a higher UV-C fluence underneath the fruit. Although the shelf was costlier than an acrylic shelf, the more even fluence distribution with the quartz shelf is likely a contributing

factor to the microbial reduction efficacy. In fact, the quartz shelf had a measured UV-C transparency of 71.6% for an average fluence of 0.06 mJ cm^{-2} s^{-1} from the bottom module in the testing area. As exposure to uncontrolled temperatures can have negative consequences on the postharvest quality, implementing additional heat sinks and a fan vastly improved the thermoregulation within the unit. The temperature within the UV-C chamber did not increase more than 1.5 ºC within the 10 minutes of operation (*Figure 21*) in comparison with the almost 4ºC increase from previous studies *(11)*.

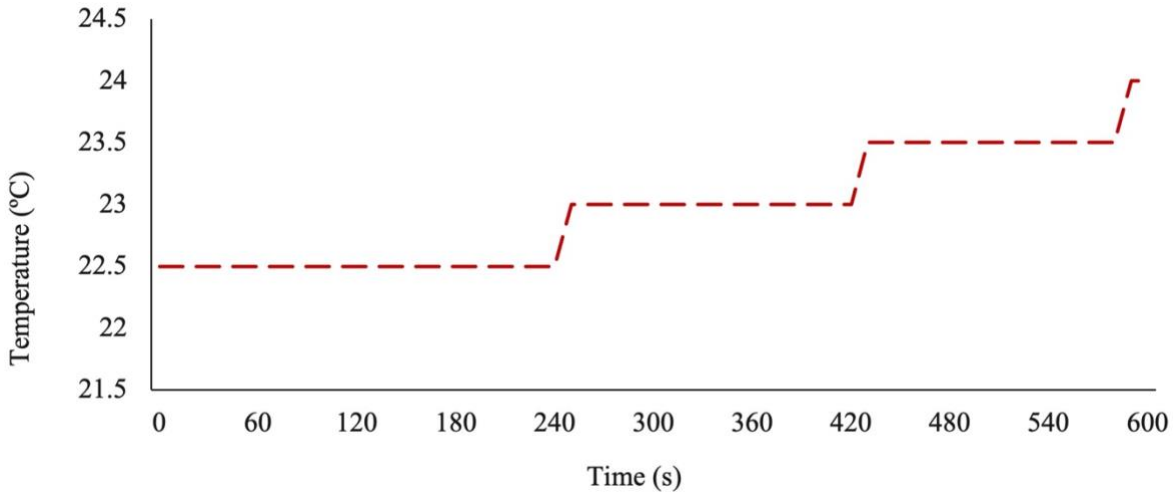

Figure 21 *The change in temperature within the UV-C chamber according to operation time.*

CONCLUSION/RECOMMENDATIONS

The whole-surface treatment approach of the UV-C chamber has the potential to addresses many problems limiting the use of UV-C in the strawberry industry. For example, the sepal browning following UV-C treatment in the literature could be indicative of applying too high a dose. In this study, a comparable reduction of *E. coli* was achieved using a 10- to 100-fold lower UV-C dose than what is previously reported. With the reduced energy inputs of UV-LEDs, the

improved thermoregulation and dose distribution are also highly advantageous from the industry perspective. In effect, the findings of this study indicate the whole-coverage approach of the UV C chamber is more effective to treat fruit with complex surface structures, with less potential to cause postharvest damage. Further research is warranted to identify the causation of the shadowing effect that limits the efficacy of UV-C treatment. Overall, the results of this proof-of-concept study indicate that the UV-C technology has the potential to be deployed in the fresh strawberry industry as a treatment at harvest for improving microbial safety and preserving post-harvest quality. The next steps to deploying this technology in the produce industry is its scaling-up and automation.

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Chapter 7 - Conclusions

Ultraviolet-C (UV-C) irradiation is well-known for its high antimicrobial efficacy and low environmental toxicity compared to its chemical sanitizer counterparts. Although UV-C is well implemented in diverse food industries, the technology has yet to become popularized in the fresh produce industry. The purpose of this work was to research innovations in UV-C technologies to facilitate their integration into the fresh produce value chain.

Agricultural water is a common point of entry for microbial contaminants into the fresh produce value chain. The contents of this dissertation provide insight into the challenges of Kansas and Missouri fresh produce growers in accessing microbiologically safe agricultural water. Analyses of generic *E. coli* concentration in water samples from produce-growing operations in these states indicate that surface water sources may be generally unsafe to use in production activities. More concerning, the average surface and ground water source exceeded the limit for safe use for covered postharvest activities. Further, the FSMA PSR requires that surface water used post-harvest be treated before use. These findings indicated that growers need more information on safe agricultural water quality management practices, and effective technologies to reduce the microbial risks of their growing operations during and after harvest.

This dissertation reports on innovative studies of ultraviolet-C (UV-C) based devices to address the need for agricultural water decontamination methods during production-related activities. Two commercially available UV-C devices intended for household water decontamination were validated against surrogate agricultural water in the laboratory, and natural agricultural water in the field. The capacity of the devices to decontaminate generic *E. coli* in agricultural water

according to changes in flow rate, percent UV-C transmission, and turbidity was characterized and used to build a predictive model. The predictive model formed the basis of a web-based application developed for use by growers to determine the estimated efficacy of either device based on their agricultural water characteristics. In a follow-up study performed to investigate the low adoption rate of UV-C agricultural water decontamination by Kansas and Missouri produce growers, growers were asked a series of questions to determine their attitudes, perceptions, attitudes, and challenges towards adopting the technology for their operation. Ultimately, many growers expressed the need for more information regarding the benefits of UV-C technologies to their operation, and there was a remarked wide range of levels of UV-C knowledge. The results indicate that while the UV-C devices may be effective to treat agricultural water, they will not likely be deployed in the fresh produce industry without targeted campaigns to promote their use amongst growers.

A series of studies directly applying UV-C to the surface of fresh produce was performed which could improve the application of this technology for postharvest produce safety management. UV-C was applied to two different types of fruits – fresh blueberries and fresh strawberries – within a UV-C chamber providing simultaneous UV-C treatment to multiple sides of the fruit. In general, this approach was able to reduce the treatment time and UV-C dose required to achieve microbial inactivation. Further optimization of the UV-C chamber improved the thermoregulation and UV-C dose distribution of the system, making the technology highly advantageous from the industry perspective. Effectively, the findings of this series of studies indicate that the whole-coverage approach of the UV-C chamber could be more effective to treat fruit with complex surface structures, with less potential to cause postharvest damage than

conventional UV-C applications. Overall, the technology has the potential to be deployed in the fresh produce industry as a post-harvest treatment for improving microbial safety and preserving post-harvest quality, pending its scaling and automation.

In summary, these studies provide future research directions for on-farm UV-C innovations to improve produce safety. The studies demonstrate that there is a need for safe, effective, non-chemical agricultural water treatment methods – a niche that UV-C devices can be successfully fill. Yet there continues to be a knowledge gap that needs to be addressed to increase the grower adoption rate of on-farm UV-C devices. The fresh produce industry could also benefit from innovations in UV-C for whole-fruit treatment, but more research is needed to increase the scale of this technology.

Appendix A – Supplementary Tables

Appendix A Table A. 1 *The installation costs of the LP/LF UV-C device per the methodology of this study*

Item	Supplier	Order Quantity	Unit Price	Total
Minipure MIN-9 (UV-C device); 120V, 34W	Atlantic Ultraviolet Corp.	1	$ 677.00	$ 677.00
3/4 in. MHT x 1/2 in. MIP Brass Adapter Fitting	Home Depot	2	$ 5.18	$ 10.36
1/2 in. to 1-1/4 in. Stainless Steel Hose Clamp (10-Pack)	Home Depot	1	$ 8.74	$ 8.74
Fill-Rite TT10AN, 2-35 GPM Digital In-line Turbine Meter	Amazon	1	$ 133.68	$ 133.68
3/4 in. MHT x 1/2 in. MIP Brass Adapter Fitting	Home Depot	2	$ 4.98	$ 9.96
3/4 in. PVC Schedule 40 FIP x FIP Ball Valve	Home Depot	1	$ 2.27	$ 2.27
3/4 in. Barb x 3/4 in. MIP Nylon Adapter Fitting	Home Depot	2	$ 3.53	$ 7.06
1/2 in. x 520 in. White PTFE Tape	Home Depot	1	$ 1.94	$ 1.94
3/4 in. vinyl tubing, 10 ft.	Home Depot	1	$ 24.15	$ 24.15
Hours of Installation	N/A	0.5	$ 14.27	$ 7.14

Hours of Training	N/A	0.5	$ 14.27	$ 7.14

Note: The total installation cost is $ 889.43; Abbreviations: inch (in.; 1 in. = 2.54 cm.), gallons per minute (GPM; 1 GPM = 3.8 lpm), foot (ft.; 1ft. = 30.5cm.)

Appendix A Table A. 2 *The installation costs of the HP/HF UV-C device per the methodology of this study*

Item	Supplier	Order Quantity	Unit Price	Total
SARIN UV System (UV-C device); 240V, 320W	SARIN Energy Solutions	1	$ 2,500	$ 2,500.00
SS Clamp, 1/2 in. to 1-1/4 in.	Home Depot	1	$ 8.94	$ 8.94
PVC Pipe Adapter, Flanged 2-1/2 in. Socket-Connect Adapter	McMaster-Carr	2	$ 16.66	$ 33.32
Bushing Adapter, 2-1/2 in. Socket Male x 1 in. NPT Female	McMaster-Carr	2	$ 19.23	$ 38.46
Barbed Hose Fitting Adapter, 3/4 in. Hose ID, 3/4 in. NPT male, 125 PSI	McMaster-Carr	2	$ 1.80	$ 3.60
Gasket with Bolt Hose for 2-1/2 in. Pipe, ANSI 150, 1/8 in. thick	McMaster-Carr	2	$ 14.50	$ 29.00
Barbed Hose Fitting Adapter, 1 in. Hose ID, 1 in. NPT male, 125 PSI	McMaster-Carr	2	$ 2.14	$ 4.28
Thread Sealant, 16 oz.	McMaster-Carr	1	$ 31.65	$ 31.65
Hex Head Screw, Grade 8 Steel, 5/8 in.-11 Thread Size, 3 in. long, (5-Pack)	McMaster-Carr	2	$ 10.01	$ 20.02
Steel Nylon-Insert Locknut, Grade 8, 5/8 in.-11 Thread Size, (5-Pack)	McMaster-Carr	2	$ 4.80	$ 9.60

Stainless steel washer for 5/8 in. Screw Size, 0.688 ID, 1.5 OD, (10-Pack)	McMaster-Carr	2	$ 6.33	$ 12.66
3/4 in. Barb x 3/4 in. MIP Nylon Adapter Fitting	Home Depot	2	$ 3.53	$ 7.06
Fill-Rite TT10AN, 2-35 GPM Digital In-line Turbine Meter	Amazon	1	$ 133.68	$ 133.68
3/4 in. MHT x 1/2 in. MIP Brass Adapter Fitting	Home Depot	2	$ 4.98	$ 9.96
3/4 in. PVC Schedule 40 FIP x FIP Ball Valve	Home Depot	1	$ 2.27	$ 2.27
3/4 in. Barb x 3/4 in. MIP Nylon Adapter Fitting	Home Depot	2	$ 3.53	$ 7.06
3/4 in. vinyl tubing, 10 ft.	Home Depot	1	$ 24.15	$ 24.15
1/2 in. x 520 in. White PTFE Tape	Home Depot	1	$ 1.94	$ 1.94
Hours of Installation	N/A	0.5	$ 14.27	$ 7.14
Hours of Training	N/A	0.5	$ 14.27	$ 7.14

Note: The total installation cost is $ 2,891.92.

Abbreviations: inch (in.; 1 in. = 2.54 cm.), Pound per square inch (PSI; 1 PSI = 6.9 kPa), ounce (oz.; 1 oz. = 29.6 mL), gallons per minute (GPM; 1 GPM = 3.8 lpm), foot (ft.; 1ft. = 30.5cm)

Supplemental Material 1 *The grower survey used to evaluate the current practices, knowledge, attitudes, and perceptions of fresh produce growers in Kansas and Missouri towards ultraviolet light for agricultural water treatment*

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