UV Light Inactivation of Human and Plant Pathogens in Unfiltered Surface Irrigation Water

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Fruit and vegetable growers continually battle plant diseases and food safety concerns. Surface water is commonly used in the production of fruits and vegetables and can harbor both human- and plant-pathogenic microorganisms that can contaminate crops when used for irrigation or other agricultural purposes. Treatment methods for surface water are currently limited, and there is a need for suitable treatment options. A liquid-processing unit that uses UV light for the decontamination of turbid juices was analyzed for its efficacy in the treatment of surface waters contaminated with bacterial or oomycete pathogens, i.e., Escherichia coli, Salmonella enterica, Listeria monocytogenes, Clavibacter michiganensis subsp. michiganensis, Pseudomonas syringae pv. tomato, and Phytophthora capsici. Five-strain cocktails of each pathogen, containing approximately $10^8$ or $10^9$ CFU/liter for bacteria or $10^4$ or $10^5$ zoospores/liter for Ph. capsici, were inoculated into aliquots of two turbid surface water irrigation sources and processed with the UV unit. Pathogens were enumerated before and after treatment. In general, as the turbidity of the water source increased, the effectiveness of the UV treatment decreased, but in all cases, 99.9% or higher inactivation was achieved. Log reductions ranged from 10.0 to 6.1 and from 5.0 to 4.2 for bacterial pathogens and Ph. capsici, respectively.

For decades, water has been a vector for human- and plant-pathogenic microorganisms (1, 2). Illnesses caused by waterborne microorganisms can occur through the consumption of fruits and vegetables that have come in contact with contaminated water (3, 4). Plant pathogens are also spread through water and can lead to plant disease and yield losses. In the food production environment, agricultural water is defined as any water that is used in the growing, harvesting or packing of produce where water is likely to contact produce directly or to contact surfaces that produce are likely to come in contact with (5). Agricultural water, including that used for irrigation, pesticide or herbicide application, freeze protection, or produce washing, is under increasing scrutiny as a vehicle for food-borne human- and plant-disease-causing microorganisms. Irrigation water is one of the main avenues by which pathogenic microorganisms can reach produce, especially if the irrigation water is obtained from a surface water reservoir. Irrigation water is typically taken from groundwater, surface water, or municipal sources. Water from groundwater and municipal sources is generally free of pathogenic microorganisms, but breaches can still occur. Surface water sources are considered high risk for pathogen contamination because they are open to many routes by which microorganisms causing plant disease or human food-borne illness can enter. Although surface water is a high-risk source, many growers continue to use surface water because it remains the most feasible and economic choice.

Mitigation strategies may be necessary for growers to continue using surface water for agricultural applications, particularly for leafy greens or produce that is intended to be consumed raw. Human-pathogenic bacteria can enter surface water sources through fecal material from wildlife and human activities or contaminated runoff and debris. Three important bacterial species responsible for many food-borne illnesses are pathogenic Escherichia coli, Salmonella enterica, and Listeria monocytogenes, with illness occurring through consumption of contaminated fresh fruits and vegetables. These pathogenic bacteria have been recovered from the environment, including irrigation sources, where produce is grown (6, 7, 8, 9). Illnesses from these pathogens can lead to death and cause significant economic losses for growers if the bacteria are traced back to their farms.

The Food and Drug Administration (FDA) has recently drafted a set of food safety regulations as part of the Food Safety Modernization Act (FSMA), with a principal focus on fresh produce due to its high risk for contamination. Produce consumption accounts for about half of the food-borne illness outbreaks each year, and many of these cases are due to the bacterial pathogens E. coli, S. enterica, and L. monocytogenes (10, 11). Much of the produce focus of FSMA is on the prevention of contamination in the growing environment. These proposed regulations have brought considerable attention to agricultural water and its role in the spread of disease-causing organisms. Testing of irrigation water for generic E. coli, an indicator of fecal contamination, will likely be required for some growers, especially if the water is from a surface water source. If generic E. coli levels are above regulatory thresholds, the irrigation water would not be usable unless a mitigation strategy is applied and further testing reveals generic E. coli levels to be below the threshold.

Much emphasis is being placed on human pathogen contamination of produce, but growers are also concerned about plant-pathogenic organisms, which can lead to large yield and economic losses. A major mode of dispersal for many plant pathogens is through water which can become contaminated through infested debris, soil, or runoff. All major groups of plant pathogens, in-
cluding bacteria, viruses, fungi, nematodes, and oomycetes, have been found in irrigation water (2). Several plant-pathogenic isolates of the Gram-negative bacterium *Pseudomonas syringae* have been isolated from many different surface water sources (32, 33). In New York surface water, the pathogens that we have recovered include the oomycete *Phytophthora capsici* and the Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (L. A. Jones and C. D. Smart, unpublished data).

Given that irrigation water is a major carrier for human and plant pathogens, a mitigation strategy that could deal with both groups would be of great benefit to any grower. There are currently several methods available for the treatment of water, such as chlorine, ozone, UV, and filtration, but not all methods are suitable for surface water sources due to its complexity and variability. Water quality parameters such as pH, turbidity, color, dissolved solids, and microbial load can adversely affect treatment efficacies and can change seasonally or even hourly in surface water with weather events or human activities.

UV light has been used successfully for treating human bacterial and protist pathogens in drinking water (12). It has also been used to successfully disinfect water contaminated with plant-pathogenic oomycetes and bacteria in nursery settings where recycling is a common method of water and nutrient conservation (13, 14). UV light treatment of drinking and nursery water can be effective, since these waters are high quality, with low turbidity (<1.0 nephelometric turbidity unit [NTU]) and microbial loads. Previously, UV light was not considered suitable for the treatment of surface water due to high turbidity levels (>1.0 NTU), which can block or absorb UV light, shielding pathogens from treatment. One UV treatment system, UV CiderSure (FPE, Inc., Rochester, NY), however, has been designed to overcome this problem and is capable of consistently achieving a minimum 5-log reduction of *E. coli* O157:H7 in unfiltered apple cider. Apple cider is a liquid with a varying high content of solids and with high turbidity in the range of 1,000 to 2,400 NTU (15). The UV processing unit is designed to deliver the same UV dose to all pathogens by using computational fluid dynamics and adjustable flow rates. In this study, the UV processing unit designed to treat turbid liquids with high solid contents was evaluated for efficacy in decontaminating surface waters contaminated with bacterial and oomycete pathogens, including *E. coli* O157:H7, *S. enterica, L. monocytogenes, Pseudomonas syringae* pv. tomato, *Clavibacter michiganensis* subsp. *michiganensis*, and *Phytophthora capsici*.

### MATERIALS AND METHODS

#### Water sources

For this experiment, the UV inactivation of each pathogen was tested in three water sources. Two of the water sources were from actively used surface water irrigation sources, a creek (Tompkins Co., NY) and a pond (Ontario Co., NY). Phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4) and reverse osmosis (RO) water were used as low-turbidity water sources for all experiments with bacterial pathogens and *Ph. capsici*, respectively. Surface water was collected in the fall of 2012 and stored in 55-gallon drums (food-grade plastic). Surface water was stirred thoroughly, and then pH (HI 2211 pH/ORP meter; Hanna, Woonsocket, RI) and turbidity (2100P portable turbidimeter; Hach, Loveland, CO) measurements were recorded before use. All turbidity values were recorded in nephelometric turbidity units. RO water was produced as needed (Barnstead Nanopure II; Thermo Fisher Scientific, Waltham, MA).

#### Human and plant pathogen strains

Six pathogen species, i.e., *E. coli, S. enterica, L. monocytogenes, P. syringae, C. michiganensis*, and *Ph. capsici*, were used in this study. Five strains of each pathogen were used to prepare a five-strain cocktail for UV inactivation experiments (Table 1). The *E. coli, S. enterica, and L. monocytogenes* strains are clinical or food isolates obtained from M. Wiedmann’s food safety laboratory at Cornell University. After storage at ~80°C, human bacterial pathogens were passed once through tryptic soy broth (TSB) (Hardy Diagnostics, Santa Maria, CA). All *Ps. syringae, C. michiganensis*, and *Ph. capsici* strains were obtained from the Smart lab and were isolated from field samples collected in New York. All *Ps. syringae* and *C. michiganensis* strains were isolated from tomato. *Ph. capsici* strains were isolated from pepper (strain 0664-1), pumpkin (strains 0759-8 and MMZ-4A), zucchini (strain 0752-15), and butternut squash (strain 06180-4).

#### UV radiation and inactivation

UV radiation (254 nm) was delivered for each pathogen species was tested separately in three water treatments with bacterial and oomycetes pathogens, including *E. coli* O157:H7, *S. enterica, L. monocytogenes, Pseudomonas syringae* pv. tomato, *Clavibacter michiganensis* subsp. *michiganensis*, and *Phytophthora capsici*.

<table>
<thead>
<tr>
<th>Species or subspecies</th>
<th>Strains or serovars</th>
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</thead>
<tbody>
<tr>
<td>Human pathogens</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>933, 2722, ATCC 43895, ATCC 35150, ATCC 4389</td>
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<tr>
<td><em>Salmonella enterica</em> subs. <em>enterica</em></td>
<td>Hartford, Montevideo, Rubislaw, Gaminara, Cuban</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2812, 2289, L99, 104025, F2586-V1</td>
</tr>
<tr>
<td>Plant pathogens</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas syringae</em> pv. tomato</td>
<td>09150, 09110, 09084, 0761, 0578</td>
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<tr>
<td><em>Clavibacter michiganensis</em> subsp. <em>michiganensis</em></td>
<td>11015, 10-4, 0767, 09085, 0690</td>
</tr>
<tr>
<td><em>Phytophthora capsici</em></td>
<td>0664-1, 06180-4, 0759-8, 0752-15, MMZ-4A</td>
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### Media and culture conditions

*E. coli, S. enterica, and L. monocytogenes* were maintained on tryptic soy agar (TSA) (Hardy Diagnostics, Santa Maria, CA), while *Ps. syringae, C. michiganensis*, and *Ph. capsici* were maintained on King’s B (KB) agar (16), D_ANX agar (17), and PARP agar (18), respectively. For preparation of the five-strain cocktails, *E. coli* and *S. enterica* were grown for 18 h at 37°C; *L. monocytogenes* was grown for 24 h at 37°C in TSB, and *Ps. syringae* was grown at 28°C for 18 h in KB broth. *Clavibacter michiganensis* was grown for 48 h at 37°C in Luria-Bertani (LB) broth (19). All bacteria were incubated on a rotary platform shaker at 250 rpm. *Phytophthora capsici* was cultured on 15% V8 agar for 7 days at room temperature (25 to 28°C) under continuous fluorescent light for sufficient sporangium production. Zoospore suspensions were produced according to the protocol used by Dunn et al. (20).

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Pseudomonas enumerated on the Oxford antimicrobial supplement (Difco, Franklin Lakes, NJ); samples were incubated at 37°C for 18 h.

Pathogens were enumerated immediately after UV irradiation, and treated samples were incubated in sterile 1-litre bottles and filtered for enumeration. Noninoculated samples from each water source were also processed for each pathogen. All UV inactivation experiments were repeated once, for a total of six 1-litre samples for each pathogen concentration and water source pairing.

**Enumeration** Pathogen populations in both inoculated and noninoculated samples were enumerated before and after UV inactivation on selective or semiselective media. Before UV exposure, bacteria (CFU/liter) were enumerated by serial dilution plating, and *Ph. capsici* zoospores were enumerated with a hemacytometer (Hausser Scientific, Horsham, PA). Equal numbers of zoospores from each strain were combined to produce a five-strain cocktail for inoculation of 1-litre water samples at 10⁵ and 10⁶ zoospores/litre. The higher inoculum levels were included to ensure the assessment of the upper inactivation rates. Three 1-litre samples were prepared from individual cocktail preparations for each pathogen concentration and water source pairing. Subsamples (1 ml) were taken immediately from inoculated 1-litre water samples for enumeration before UV inactivation. Samples were gently mixed prior to subsampling, UV inactivation, and filtering. The 1-litre water samples were processed immediately after UV irradiation, and treated samples were collected in sterile 1-litre bottles and filtered for enumeration. Noninoculated samples from each water source were also processed for each pathogen. All UV inactivation experiments were repeated once, for a total of six 1-litre samples for each pathogen concentration and water source pairing.

**Calculations and statistics** The percent inactivation ([N₀ − N]/N₀) was calculated for each pathogen concentration by water source pairing. Pathogen counts were also converted into logarithmic units, and log reduction was calculated as log(N/N₀), where N corresponds to the after-treatment count and N₀ to the initial count. Data were analyzed by analysis of variance using R statistical software (24). Tukey’s honestly significant difference (HSD) was used to determine significant differences (α = 0.05) between log reduction means or percent inactivation means of all pathogen concentration and water source pairings.

**RESULTS**

**UV inactivation of pathogens by water source** The efficacy of UV inactivation of each of the six pathogen species was analyzed in the three water sources, which varied in pH and turbidity. The pH and turbidity of the water sources were monitored throughout the UV inactivation experiments, and the average and range of both parameters are presented for each water source in Table 2. The RO water had an average neutral pH (6.96), while the PBS, creek, and pond water exhibited alkaline pH levels (8.01, 8.32, and 8.21, respectively). The pH did not vary more than 0.19 pH units in any water source. The turbidity of the RO water and PBS were consistent at 0.1 NTU. The turbidity in the creek water was higher and more variable and ranged from 3.0 to 4.4 NTU. Turbidity was by far the highest and most variable in the pond water, which ranged from 15.8 to 22.7 NTU.

The average percent inactivation and log reduction values for all pathogens by water source can be found in Tables S1 to S6 in the supplemental material. Percent inactivation for all pathogens by water source was 99.9% or greater. The log reductions ranged from 10.0 to 6.1 and from 5.0 to 4.2 for bacterial pathogens and *Ph. capsici*, respectively (Tables S1 to S6). In all water sources, *C. michiganensis* consistently had higher total numbers of CFU/liter after UV treatment than the other bacterial pathogens (Tables S1, S3, and S5). *Escherichia coli* and *S. enterica* had similar numbers of CFU/liter after UV treatment in all water sources and were consistently lowest of the bacterial pathogens (Tables S1, S3, and S5). *Pseudomonas syringae* and *L. monocytogenes* had intermediate numbers of CFU/liter after UV treatment among the bacterial pathogens; they performed similarly to each other in PBS (Table S1), but in the creek and pond water, *L. monocytogenes* had more surviving CFU/liter than *P. syringae* (Tables S3 and S5).

**UV inactivation of pathogens by species.**

(i) **E. coli O157:H7** One hundred percent inactivation was achieved for the lower concentrations and 99.9% inactivation was achieved for higher concentrations in each water source (see Tables S1, S3, and S5 in the supplemental material). No significant difference in log reduction was found among water sources at the lower concentrations (Fig. 1). For the higher concentrations, the log reduction was not different between PBS (10.0) and creek water (9.5); however, the log reduction was significantly less (7.3) in pond water (Fig. 2). No CFU were recovered in uninoculated controls before or after UV inactivation.

(ii) **S. enterica** At the lower concentrations in PBS and creek water, 100% inactivation was achieved, while 99.9% inactivation was achieved for the lower concentration in pond water and for the higher concentrations in each water source (see Tables S1, S3, and S5 in the supplemental material). At the lower concentrations, there were no log reduction differences between PBS (8.8) and creek (8.6) water or between creek and pond (8.3) water. A significant difference was found between PBS and pond water (Fig. 1). For the higher concentrations, there were significant differences in log reduction between each pair of water sources (Fig. 2). No *S. enterica* CFU were recovered in uninoculated controls before or after UV inactivation.

(iii) **L. monocytogenes** For all concentrations and water source pairings, 99.9% inactivation was achieved (see Tables S1, S3, and S5 in the supplemental material). Significant differences in log reduction were observed among all water sources at both the

<p>| Table 2 pH and turbidity values for water sources used in UV inactivation experiments |
|----------------------------------------|-----------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Water source</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
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<tr>
<td></td>
<td>Avg</td>
<td>Range</td>
</tr>
<tr>
<td>PBS</td>
<td>8.01</td>
<td>7.88–8.10</td>
</tr>
<tr>
<td>RO</td>
<td>6.96</td>
<td>6.90–7.00</td>
</tr>
<tr>
<td>Creek</td>
<td>8.32</td>
<td>8.26–8.37</td>
</tr>
<tr>
<td>Pond</td>
<td>8.21</td>
<td>8.17–8.36</td>
</tr>
</tbody>
</table>
lower and higher pathogen concentrations (Fig. 1 and 2). No *L. monocytogenes* CFU were recovered in uninoculated controls before or after UV inactivation.

(iv) *Ps. syringae*. For all concentrations and water source pairings, 99.9% inactivation was achieved (see Tables S1, S3, and S5 in the supplemental material). Log reductions at the lower concentrations were different in pond water (6.9) than in PBS (8.1) or creek water (7.7), but not between PBS and creek water (Fig. 1). Log reductions at higher concentrations were different for each water source (Fig. 2). Background *Pseudomonas* organisms were present in uninoculated pond water (55 CFU/liter before UV inactivation and 0 CFU/liter after UV inactivation). For inoculated samples, background *Pseudomonas* organisms were included in the values for before and after UV inactivation.

(v) *C. michiganensis*. For all concentrations and water source pairings, 99.9% inactivation was achieved (see Tables S1, S3, and S5 in the supplemental material). Differences in log reductions at the lower concentrations were found between PBS (7.5) and creek (6.9) or pond (6.8) water but not between creek and pond water (Fig. 1). Differences in log reductions were found between each water source at the higher concentrations (Fig. 2). No *C. michiganensis* CFU were recovered in uninoculated controls before or after UV inactivation.

(vi) *Ph. capsici*. One hundred percent inactivation was achieved at the level of 10⁴ zoospores/liter in RO and creek water, and 99.9% inactivation was achieved in pond water (see Tables S2, S4, and S6 in the supplemental material). At 10⁵ zoospores/liter, 99.9% inactivation was achieved in the three water sources.

FIG 1 Average log reductions for the lower inoculum levels of each pathogen in each water source. (A) *E. coli* O157:H7; (B) *S. enterica*; (C) *L. monocytogenes*; (D) *Ps. syringae* pv. tomato; (E) *C. michiganensis* subsp. *michiganensis*; (F) *Ph. capsici* (no error bars are shown because all samples resulted in 100% inactivation). Different letters indicate significantly different groups ($\alpha = 0.05$).

FIG 2 Average log reductions for the higher inoculum levels of each pathogen in each water source. (A) *E. coli* O157:H7; (B) *S. enterica*; (C) *L. monocytogenes*; (D) *Ps. syringae* pv. tomato; (E) *C. michiganensis* subsp. *michiganensis*; (F) *Ph. capsici*. Different letters indicate significantly different groups ($\alpha = 0.05$).
S2, S4, and S6). No significant difference in log reduction was found between water sources at 10^4 zoospores/liter (Fig. 1). At 10^5 zoospores/liter differences in log reductions were found for each water source (Fig. 2). No _Ph. capsici_ isolates were recovered in uninoculated controls before or after UV inactivation.

**DISCUSSION**

The surface water sources for this study were chosen because they were actively used irrigation sources that are representative of the pH and turbidity of surface waters in New York State as determined by an irrigation water survey conducted by Jones and Smart (unpublished data). For UV inactivation experiments, water sources were monitored for pH and turbidity to ensure uniformity of water sources during experimentation. Additionally, the values of these two water quality parameters are important when considering a water treatment option. Chlorination is one of the most effective and economical water disinfection methods but is not recommended for water with a pH above 7.5 or water with high levels of particulates, due to low levels of hypochlorous acid formation and binding to organic matter, respectively. The pH values of the surface waters in this study were on average 8.32 and 8.21 for the creek and the pond, respectively, making these sources poor choices for chlorine disinfection. Studies have found that pH is not a significant factor in UV treatment efficacy (25, 26).

Turbidity, on the other hand, has been found to adversely affect UV treatment, but the relationship between turbidity level and UV efficacy is not consistent. Water components that influence turbidity have variable UV-blocking and -absorbing qualities, and therefore turbidity can be used only as a general guideline to determine UV transmittance. In general, as turbidity increases, UV transmittance and bactericidal efficacy decrease (27, 28). The average log reduction results from this study support this trend, as tests conducted with more turbid water were generally less effective at inactivating pertinent challenge pathogens than those with a less turbid source. In some cases, for example, with _E. coli_ and _Ph. capsici_, there was no significant log reduction difference between the 0.1-NTU (PBS or RO) water source and the 3.9-NTU (creek) source. This may be due to the fact that complete or almost complete inactivation occurred in the less turbid water, making the log reduction equal to the initial pathogen concentrations and not wholly representative of the full measure of UV efficacy. In New York State, the turbidity of surface water used for irrigation is commonly between 1 NTU and 20 NTU, but it can vary considerably throughout the year (Jones and Smart, unpublished data). The creek water had an average turbidity of 3.9 NTU and the pond of 19.6 NTU; these values are representative of the range of turbidities of the majority of surface water sources in New York.

The percent inactivation for all pathogens and water source pairings was found to be 99.9% or greater. These data show that UV light as a mitigation strategy can be effective against a broad spectrum of pathogens in complex surface water sources. When analyzing percent inactivation data, differences in UV efficacy among water sources are not apparent, but with examination of the average log reduction data, we can begin to discern how UV efficacy is affected by the different water sources. For the bacterial pathogens, log reductions ranged from 10.0 (_E. coli_ in PBS) to 6.1 (_C. michiganensis_ in pond water). No validation standards have been developed for the treatment of bacterial or oomycete pathogens in surface water that is intended for irrigation. To be recognized as a valid method for treatment of juices by the FDA, the treatment must obtain a 5-log reduction of a pertinent pathogen in juice (29). The log reduction results for bacterial pathogens from this experiment would meet the FDA’s requirements for juice. No such standard exists for oomycetes. Log reduction values for _Ph. capsici_ zoospores were less than those for all bacterial pathogens, because the highest concentration of zoospores that we could obtain was 5 × 10^5/liter. Thus, it would be impossible to have a log reduction of 6 or greater as was observed with the bacterial pathogens. The zoospores were clearly highly susceptible to UV treatment, as there was 100% inactivation in pond water at the lower pathogen concentration (5 × 10^4/liter). The only other pathogen with 100% inactivation in pond water was _E. coli_ at the lower concentration. _Clavibacter michiganensis_, in all water sources, was the least susceptible to UV treatment, followed by _L. monocytogenes_ in creek and pond water, among the bacterial pathogens, with the highest CFU/liter after treatment. Typically, Gram-positive bacteria are more recalcitrant to UV radiation than Gram-negative bacteria (30).

UV may not be applicable to all irrigation situations, particularly those applications requiring very high volumes. The path length through which UV can penetrate water is very small and limits the volume of water that can be treated at one time. Highly turbid waters may not be good candidates for UV treatment without pretreatment. In addition, UV light treatment systems have the potential to significantly lower the risk of both plant and human pathogen contamination of crops from surface water through irrigation or other agricultural applications and could be integrated into effective food safety and plant health programs.

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