CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Evaluation of multiple disinfection methods to mitigate the risk of produce contamination by irrigation water

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Objectives
Overall, our objective is to determine the applicability of incorporating disinfection technologies into produce irrigation and frost protection systems in order to mitigate surface-source water that has been potentially contaminated with pathogenic organisms.

Objective 1. Determine inactivation of indicator organisms (E. coli and fecal coliforms) and STEC from a surface-water irrigation source after treatment by sand filtration followed by: 1) UV dosage of 10,000 $\mu W \cdot s/cm^2$, 2) ClO_2 dosage of 20 ppm with 2 minutes of contact time, 3) PAA dosage of 20 ppm with 2 minutes of contact time, or 4) no further treatment (control).

Objective 2. Determine transfer of pathogen (STEC) and indicator organisms (E. coli) from irrigation water to the fruit of model crops (strawberry and cantaloupe) with the three mitigation strategies as compared to no treatment, utilizing both overhead and drip irrigation delivery.

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Abstract
Irrigation is a potential source of pathogen contamination to fresh fruits and vegetables. While the science of drinking-water disinfection is well established, little is known about providing disinfection during crop irrigation. Interferences, such as turbidity, suspended solids, and organic matter, will have to be accounted for when determining the disinfectant dosage provided by the treatment system. The basic goal of this two-year project was to gain knowledge regarding the effectiveness of providing in-line treatment of surface water to mitigate the risk of applying foodborne pathogens during irrigation. Three traditional water disinfection methods were evaluated: ultraviolet light (UV), chlorine (calcium hypochlorite during 2014, and chlorine dioxide during 2015), and peroxyacetic acid (PAA). To address this goal, the project was divided into two objectives: determine the effectiveness of providing disinfection while the water is moving through the irrigation system, and investigate the transfer of pathogens from irrigation water to fresh produce.

A series of raised-bed (plasticulture and bare-ground) research plots were established to grow strawberries (2014 and 2015), tomatoes (2014), and cabbage (2015) using overhead irrigation and drip irrigation. Two ponds known to be contaminated with populations of fecal coliforms, generic *Escherichia coli* as well as Shiga toxigenic *E. coli* (STEC) and *Salmonella* were used as the source water. This water was passed through a sand filter and then divided across four water treatment systems (UV light, chlorine, PAA, and a non-disinfected control). The UV light system was mounted in-line with the irrigation system. Injection pumps were used to transfer concentrated chlorine and PAA into their respective irrigation delivery systems. Each research plot had the ability to be drip and/or overhead irrigated. Water samples were taken before and after treatment to determine the pathogen reduction.

In Tennessee, it is strongly recommended that strawberry producers have the capacity to provide frost protection. This process involves drenching the crop via overhead irrigation, which certainly provides a contamination risk. As such, strawberries were grown and evaluated for contamination before and after frost protection events. Tomatoes and cabbage are also important fresh market crops in Tennessee, and served as late season model crops after the strawberries. Plant tissue samples were taken during peak fruit and/or late harvest of each crop.

Overall, the treatments were successful in reducing pathogen numbers in the irrigation water before crop application. The results indicate that producers can use UV light, chlorine, and PAA to mitigate irrigation water; however, the disinfection system must be closely monitored. At some point during each of the evaluations, a system component failed and the water passed untreated through the irrigation system. Because of other environmental factors, the objective to evaluate pathogen transfer from irrigation water proved inconclusive.

Background
In the U.S., contaminated produce is estimated to cause over 1.1 million illnesses, 7,125 hospitalizations, and 134 deaths annually, with associated costs of $1.4 billion *(3)*. This data speaks to the staggering impact of foodborne pathogen contamination on produce and the resulting impact to public health. Produce continues to be linked to foodborne outbreaks since many fruits and vegetables are consumed raw without a processing step that could inactivate microorganisms, if present *(33)*.

Currently, the safety of produce relies on the implementation of Good Agricultural Practices (GAPs) to prevent microbial contamination during growing, harvesting, and processing.

However, effective mitigation strategies for foodborne pathogens on fresh fruits and vegetables are still lacking. Between 2000 and 2005, there were 60–80 outbreaks annually in the U.S. associated with produce *(25)*. Over a 15-year period beginning in 1990, produce was linked to 713 outbreaks and 34,049 cases of foodborne illnesses *(25)*. Most recently in the U.S., outbreaks related to *Escherichia coli* O157:H7, O145, O26, *Salmonella* serovars, and *Listeria monocytogenes* have been linked to baby spinach, Romaine lettuce, peppers, cantaloupe, mangoes, and sprouts *(7–20)*.

The Food Safety Modernization Act (FSMA) of 2011 has emphasized prevention of foodborne illnesses rather than responding to outbreaks. In response to FSMA, the FDA has recently finalized 21 CFR Part 112: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (aka Produce Safety Rule). Because irrigation water is a likely point of pathogen contamination during fruit and vegetable production, the Produce Safety Rule calls for in-depth initial water quality surveys, which will be amended with annual surveys for generic *E. coli* in any surface-source agricultural water that will contact the edible portion of the plant during growing, unless a water treatment method is used. The testing expenses will greatly impact U.S. fruit and vegetable growers since many utilize surface water as an irrigation source or in agricultural protective sprays, and, more importantly, quantifying generic *E. coli* does not always indicate a food safety risk. If we are to begin reducing the risk of produce contamination, effective mitigation strategies must be utilized in irrigation water application systems.

Co-Principal Investigator Dr. Annette Wszelaki has been sampling water to gather scientific data on irrigation water quality in Tennessee, and contributed to the nationwide Irrigation Water Quality Database for fresh fruit and vegetable production through the National GAPs Program. In 2010, irrigation water was sampled on twelve farms across East and West Tennessee, with a total of 28 irrigation water sources, including twelve surface water sources, fourteen wells, and two municipal water sources. In 2011, water was sampled on thirteen farms across Tennessee, with a total of 30 irrigation water sources, including eleven surface water sources and nineteen wells. In 2012, irrigation samples were taken from fourteen farms across Tennessee, with a total of forty-three water sources, including 32 surface water sources, nine wells, and two municipal sources. Samples were taken three times throughout each production season (April–May, June–July, and late July–August).

Analyses included quantified generic *E. coli*, conductivity, turbidity and pH. In 2010, of 84 samples taken, only two samples were above 235 most probable number (MPN)/100 ml for *E. coli* in water, and one sample was taken from a pond and the other from a river, shortly after a heavy rain event (Wszelaki, unpublished data). In 2011, of 90 samples taken, four samples were above 235 MPN *E. coli* /100 ml, and these samples were taken from ponds, streams and rivers. In 2012, of the 144 samples taken 25 samples were above *E. coli* levels of 235 MPN/100 ml, and these samples were taken from ponds, springs, streams and rivers. Moreover, nine of these samples had *E. coli* levels too numerous to count (TNTC) or >2,420 MPN/100 ml. The samples from ponds increased in *E. coli* counts throughout the season, while flowing water sources did not follow a pattern throughout the season. The weather in 2012 was conducive to increased proliferation of generic *E. coli*, due to both drought conditions and elevated early spring temperatures. While generic *E. coli* is an indicator organism and not an actual count of pathogenic organisms that cause foodborne illnesses, results from 2012 give cause for concern and warrant further investigation on the relationship between weather conditions and foodborne illness outbreaks.

Dr. Wszelaki's irrigation water quality testing results further support the need to validate irrigation water treatment systems that have been evaluated in field applications, and have shown effective inactivation of foodborne pathogens. Ultraviolet (UV) light, chlorine dioxide (ClO_2), and peroxyacetic acid (PAA) have all been used in treating wastewater *(4, 21–23, 27, 34, 39, 48–50, 52)* as well as select applications within the food industry *(26, 29–31, 37, 40, 41, 43, 47)*. These technologies have also been applied to irrigation water, but with the primary goal of inhibiting plant pathogens or nuisance organisms *(2, 5, 6, 24, 28, 32, 38)*.

UV light provides disinfection by the transference of electromagnetic energy from a light source to an organism's genetic material. This physical process damages the DNA and prevents the cell from replicating *(45)*. In clear water, 38,000 $\mu W \cdot s/cm^2$ is the recommended dosage for point-of-use drinking water systems *(35)*. Newman *(36)* reports on the use of UV light in greenhouses and nursery container operations to remove plant pathogens from irrigation water, and found a wide range of UV dosages being used in nurseries, from 80,000 to 250,000 $\mu W \cdot s/cm^2$, with the higher ranges used in turbid, irrigation-return waters. Yoon et al. *(51)* evaluated the use of UV light to disinfect secondary-level effluent in paddy rice culture; their research indicated that a UV dose of 30,000 $\mu W \cdot s/cm^2$ was needed to provide adequate disinfection for safe irrigation of relatively clear water.

Chlorine dioxide is a neutral chlorine compound that disinfects by oxidation. Studies have shown that ClO_2 can be used over wide pH and temperature ranges *(45)*. The limiting factor with the large-scale use of ClO_2 is that is must be generated on site *(42)*. Strong aqueous solutions cannot be stored due to ClO_2 volatility. The gas will accumulate in the headspace of a closed-container and can explode. For small applications of ClO_2, commercial solutions are available with up to 15% ClO_2. When ClO_2 demand is greater than 20 pounds per day, then the economics favor the on-site generation. However, one of the precursors to ClO_2 production is sodium chlorite ($NaClO_2$), which creates salt (NaCl) as a by-product. The osmotic effects of salt may cause crop injury. An alternative chlorine product is calcium hypochlorite ($Ca(ClO)_2$), commonly known as swimming pool chlorine. This sodium-free product can be purchased locally, mixed in the field, and injected into the irrigation system.

Finally, peroxyacetic acid, also known as peracetic acid, is a mixture of the peroxy compound, acetic acid and hydrogen peroxide. PAA disinfects by oxidizing the outer membrane of bacteria and is a frequently used wash water disinfectant and is commercially available under several trade names and in a variety of concentrations.

Mitigation strategies, such as incorporating UV light or chlorine injection into the irrigation system, need to be investigated as potential practices that growers can utilize to reduce the likelihood of a foodborne pathogen contamination event. Water disinfection is not a new technology. UV light, chlorine, and PAA are widely employed disinfectants in the food, water and wastewater industries. However, there is little available information about the design and evaluation of disinfection systems for pathogen control in irrigation water. It is extremely important for growers to understand the benefits of mitigation strategies in addition to limitations. This research provides insight to inactivation of both pathogens (STEC) and indicator organisms (*E. coli* and coliforms) as well as reduction of risk with respect to pathogen transfer.

Research Methods and Results

The field work for this project was conducted at the University of Tennessee Plateau Research and Education Center (Crossville, TN). Laboratory work was conducted at the Food Science Lab and the Biosystems Engineering Water Quality Lab at the University of Tennessee, Institute of Agriculture, in Knoxville, TN.

Experimental Design – 2014 Strawberry Trial

Four separate irrigation blocks were established—UV, chlorine, PAA, and a non-disinfected control. Each block consisted of eight raised-bed plots: four of the plots were covered in plastic mulch (plasticulture) and four were bare soil. These eight plots were further divided between overhead irrigation and drip irrigation. All plots were installed with both overhead irrigation (for frost protection) and drip irrigation (for fertigation). A typical plot was 1 m (3 ft) wide by 6 m (20 ft) long. A "blank" row with a tarpaulin curtain was placed between irrigation blocks to minimize water drift between treatments. The water source was pond-1, a cattle-watering pond with high concentrations of generic *E. coli* and STEC organisms.

Experimental Design – 2014 Tomato, 2015 Strawberry, and 2015 Cabbage Trials

Because of the extreme environmental contamination found during the 2014 Strawberry trial, it was decided to move the research plots 850 m (2,800 ft) further away from the beef herd. The same irrigation blocks (UV, chlorine, PAA and non-disinfected control plots) were re-established with both overhead and drip irrigation. In addition, a municipal water irrigation block was created to help minimize potential contaminant sources and serve as a negative control. The municipal block only had drip irrigation, and the water was supplied in a trailer-mounted 950-L (250-gallon) tank. Pond-2 served as the water source for the remainder of this project. Cattle did not have direct access to pond-2 but were in the same drainage area. Tomatoes and cabbage are generally not overhead irrigated, thus overhead irrigation plots were not used during these trials.

Design of Water Treatment Systems

A general purpose centrifugal pump was used to move pond water from the contaminated ponds to the irrigation plots. A sand filter (150-mesh equivalent) was located adjacent to the disinfection system and provided filtered water to the irrigation systems. After filtration, the water was divided across the four treatments.

UV disinfection: A Sterilight Silver model SSM-37 (Viqua, Guelph, Ontario, Canada) closed-vessel UV light module was incorporated into the hydraulic network system serving the UV block. This device was operated at a UV dosage of approximately 35,000 $\mu W \cdot s/cm^2$ during frost protection and 47,000 $\mu W \cdot s/cm^2$ during drip irrigation. This configuration was used in both year one and year two.

Chlorine: For year one, calcium hypochlorite ($Ca(ClO)_2$) was used as the chlorine source. Chlorine was metered into the irrigation system using a diaphragm injection pump. A 12% available-chlorine concentrate was produced and used to supply the metering pump. This concentrate was injected at a rate that created irrigation water with 20 ppm available chlorine. The hydraulic network was constructed to provide at least 2 minutes of water-chlorine contact time before the water was applied to the crop. Chlorine concentrations were monitored with test strips (pHydrion, Micro Essential Laboratory, Brooklyn, NY). During year two, chlorine dioxide was the chlorine source. A stock concentrate of 2% chlorine dioxide was produced by dissolving a proprietary powdered-mixture containing sodium chlorite (ICA TriNova, Newnan, GA). This mixture was injected into the irrigation stream to achieve a final chlorine dioxide concentration of 10 ppm. Chlorine dioxide concentrations were monitored with test strips (Insta-Test ClO_2, LaMotte Company, Chestertown, MD).

Peroxyacetic acid (PAA): During year one, a 12% commercially-prepared PAA concentrate (SaniDate 12, BioSafe Systems, East Hartford, CT) was used as the PAA source. During year two, this concentrate was diluted by 50%, and the injection rate was doubled. For each year, the final PAA concentration in the irrigation water was 20 ppm, with a 2-minute contact prior to

exiting the system. PAA concentrations were monitored with test strips (Insta-Test PAA, LaMotte Company).

Irrigation Water Sample Collection

Irrigation was scheduled on the basis of 50 mm (2 inches) of water per week. Nutrients were applied by fertigation and followed the recommendations in the 2013 Southeastern U.S. Vegetable Crop Handbook (Vance Publishing, Lincolnshire, IL).

Once per week during irrigation/fertigation, three sets of water samples were taken from each treatment plus the source water. Sterile, 100-ml plastic bottles, pre-loaded with 10 mg of sodium thiosulfate, were used to collect water for *E. coli* and coliform analysis, and 500 mL Nalgene bottles (U.S. Plastic, Lima, OH) were used to collect water samples for physical-chemical analysis. For STEC analysis, 69-oz (2-L), sterile Whirl-Pak bags (Fort Atkinson, WI) with 110 mg of added sodium thiosulfate were used to collect water samples. All collected water samples were packed on ice in a cooler for transport to the laboratory.

Irrigation Water Analysis

Following the protocols in Standard Methods for the Examination of Water and Wastewater *(1)*, the following water quality parameters were determined: turbidity, total dissolved solids, total nitrogen, non-purgeable organic carbon, and pH. Wet and dry weights of water samples were measured using an analytical balance to determine total dissolved solids. Turbidity was measured using a Hach 2100P Portable Turbidimeter (Hach Company, Loveland, CO). Total nitrogen was measured using a Shimadzu TNM-1 measuring unit (Shimadzu Co., Kyoto, Japan). Non-purgeable organic carbon was measured using a Shimadzu TOC-V CPH unit, and pH was measured using a Hach HQ40d multimeter.

Total coliforms and generic *E. coli* were enumerated by the Quanti-Tray/2000 procedure (IDEXX Laboratories Inc., Westbrook, ME). For this modified MPN method, a 100-ml water sample was mixed with Colilert reagent, poured into an open Quanti-Tray/2000, sealed using the automated IDEXX Quanti-Tray Sealer, and incubated at 37°C for 24 h. The number of positive wells was converted to MPN.

STEC was enumerated using membrane filtration onto a selective and differential chromogenic medium, CHROMagar STEC (CHROMagar, Paris, France). A 100-ml volume was filtered using 0.45-µm S-Pak membrane filters and glass 47-mm filter holders (Millipore Corporation, Bedford, MA). The filters were aseptically placed onto CHROMagar STEC and incubated for 24 h at 37°C prior to enumeration.

Plant Tissue Sampling

Strawberry Evaluation Ripe strawberries (cv. Chandler) were picked from each plant using sterile gloves and placed into sterile Whirl-Pak bags. Strawberries undergoing the same experimental treatment were placed into the same Whirl-Pak bags. Undamaged, marketable strawberries were chosen for sampling. Each strawberry sample was prepared by weighing out 25 ± 2.5 g of strawberries in a stomacher bag diluted 1:5 with buffered peptone water (BPW) containing 0.2% Tween 80. A maximum of five samples per plot were weighed out. Sample bags were manually rubbed for 60 seconds. Then 10 ml of rinsate from each sample was placed in a sample cup and spiral plated onto Chromagar STEC and XLT4 media in duplicate. An additional 20 ml from each sample was filtered onto 0.45-µm membrane filters, and transferred to Chromagar STEC and XLT4.

The remaining rinsate from each sample bag was pipetted into two sterile 150-ml sample bottles for selective enrichment of STEC. Modified tryptic soy broth (TSB) with 0.8 mg/L sodium

novobiocin was added to a separate bottle at a 2:1 dilution with the bottled rinsate. These selective enrichments were incubated at 37°C for 24 h and streaked onto Chromagar STEC to confirm presence or absence of STEC. Aliquots (1.4 ml) of these selective enrichments were kept in sterile 1.5-ml microcentrifuge tubes for further quantitative real-time PCR assays.

Tomato Evaluation Tomatoes (cv. Florida 47) were picked using sterile gloves and placed into sterile Whirl-Pak bags; tomatoes undergoing the same experimental treatment were placed into the same Whirl-Pak bags. Undamaged tomatoes larger than 3.5 cm were chosen for sampling. Each sample consisted of five tomatoes placed into a new sterile sampling bag. A maximum of six samples were chosen for each tomato plot, and some plots contained as few as four samples. A total of 250 ml of BPW with 0.2% Tween 80 was added to each sample bag containing five tomatoes. Sample bags were manually rubbed and rinsed with the rinsate solution for 60 seconds, and then 10 ml was allotted for spiral plating onto Chromagar STEC and XLT4 media.

The remaining rinsate was pipetted into separate sterile Whirl-Pak bags each containing 115 ml of rinsate. These two bags for each sample were incubated at 37°C for 18 h. Then the bags were diluted with modified TSB with 0.8 mg/L sodium novobiocin at a 1:4 dilution rate for selective enrichment of STEC and incubated at 37°C for 24 h and streaked onto Chromagar STEC to confirm presence or absence of STEC in the sample. Aliquots (1.4 ml) of these selective enrichments were kept in sterile 1.5-ml microcentrifuge tubes for further quantitative real-time PCR assays.

Cabbage Evaluation Nine cabbage (cv. Grand Vantage) heads per plot were sampled. Outer leaves were removed to reveal the inner head, from which two exterior leaves were removed. Using a gravimetric dilutor, leaves were diluted (1:5) in BPW with 0.2% Tween 80 in a sterile sample bag. Samples were hand massaged for 15 seconds. Diluent from each sample bag was spiral plated (0.1 ml) in duplicate and filtered (10 mL) onto CHROMagar STEC plates and incubated at 37°C for 48 h.

Remaining diluent in sample bags was divided in half for use in selective enrichments of target bacteria. Four parts modified TSB with 8 mg/L novobiocin was mixed with one part diluent and incubated at 42°C to selectively enrich for STEC. Enrichments were streaked for isolation onto CHROMagar STEC and incubated for 48 h. Aliquots (1.4 ml) of these selective enrichments were kept in sterile 1.5-ml microcentrifuge tubes for further quantitative real-time PCR assays.

Statistical Analysis

The Statistical Analysis Software (SAS) system Version 9.3 (SAS Institute Inc., Cary, NC) was used for all analyses. For microbial samples, all CFU or MPN counts were converted to $\log_{10}$ counts per 100 ml before statistical analysis. Analysis of variance was conducted for microbial counts using mixed models and least squares means separated with LSD ($p < 0.05$) to analyze the effect of each cross-classified treatment combination of irrigation method and bed preparation. A Pearson's partial correlation test was used to determine the strength of relationship between the concentration of fecal indicators and the concentration of STEC in irrigation water from the surface water source.

Outcomes and Accomplishments

During the summer of 2013, funding from the U.S. FDA Western Center for Food Safety (secured by Dr. Critzer) was used to establish the strawberry plots that served as our model crop for frost protection and for irrigation. This crop overwintered well and began new growth in late March 2014. The night of April 15-16, 2014, was the only frost protection event required that season; this night had greater than 12 hours of below freezing temperatures, with a low

temperature of 27°F. For the remainder of the growing season, liquid nutrients were applied once per week, providing the equivalent of one-half inch of water. Supplemental water was added to maintain a weekly water application of approximately two inches. A routine was established such that one-half of the ripe berries were harvested on Monday mornings, overhead irrigation was provided on Monday afternoons, and the remaining ripe berries were harvested on Tuesday mornings. This procedure was an attempt to provide before and after information about pathogen transfer. The third portion of the weekly harvest was on Thursdays. The last harvest was taken on May 27, 2014.

It was determined that 30% of the strawberry samples were contaminated with STEC, independent of irrigation treatment (Table 7; $p > 0.05$). We anticipate that other routes of contamination resulted in similar contamination levels amongst all produce, although significantly lower populations of STEC were present in treated water (UV, PAA, chlorine) used for irrigation and frost protection than the untreated positive control (Table 1; $p < 0.05$). In retrospect, while the cattle were 100 m (300 ft) from the plots, it seems that the plots were too close to the STEC source. Flies, bioaerosols, and other vectors could have transferred pathogens to the research plots. In hope of controlling the natural contaminant sources, this field location was abandoned and the experiment was relocated further away from the cattle, and a negative control (municipal water) irrigation block was added as a treatment. With the plots reestablished, a fall tomato crop was transplanted on August 14, 2014. Each treatment had four plots for a total of 20 plots. These plots were drip irrigated only, with no overhead irrigation. After an early frost, green tomatoes were harvested on October 6, 2014.

On September 18, 2014, 1,600 strawberry plugs were transplanted across 40 plots adjacent to the tomato plots. There were 8 plots per treatment and five treatments: chlorine dioxide, PAA, UV, municipal water (negative control), and untreated surface water (positive control). During January 2015, row covers were applied to the strawberry plots. During the spring of 2015, the strawberry crop was cultivated and harvested. There was no need to frost protect this season. Irrigation water (both overhead and drip) samples were collected throughout the growing season. Once ripening began, the plots were harvested using the following routine. On Mondays, half the ripe berries were gathered from all plots during the mornings; during those same afternoons, half of the plots received overhead irrigation and half of the plots received drip irrigation. On the next mornings (Tuesdays), the remaining ripe berries were picked. On Thursdays a complete picking was conducted to prevent overripe berries from causing spoilage.

The strawberry crop was terminated June 5, and cantaloupes were seeded back into the same plots on June 10. This location received 7.7 inches of rain in June and 11.75 inches of rain in July; in total, this was 11 inches over average. By August 8, 2015 crop failure was obvious and the project team decided to switch to cabbage as a late-season model crop. The cabbage was transplanted into newly formed beds on August 21, 2015. On November 3, 2015, the cabbage was harvested.

Summary of Findings and Recommendations

Objective 1. Determine inactivation of indicator organisms (E. coli and fecal coliforms) and STEC from a surface-water irrigation source after treatment by sand filtration followed by: 1) UV dosage of 10,000 $\mu W \cdot s/cm^2$, 2) ClO_2 dosage of 20 ppm with 2 minutes of contact time, 3) PAA dosage of 20 ppm with 2 minutes of contact time, or 4) no further treatment (control).

Calcium Hypochlorite - $Ca(ClO)_2$

Calcium hypochlorite was the chlorine source used during 2014. Stock concentrates were produced using 454 g (1 lb) packs of 68% calcium hypochlorite (i.e., swimming pool shock) to

get a stock solution of 12% available chlorine. At this concentration, not all of the inert ingredients within the packs are soluble and thus form a significant precipitant. The precipitant was removed to prevent clogging of the metering pumps. For this project, a 12% concentration was chosen because the PAA solution was also 12%; this allowed both metering pumps to be operated at the same setting. Contact time was provided by adding sufficient pipe volume such that two minutes elapsed before the water was applied to the crop.

Overall, calcium hypochlorite performed very well. This product significantly inactivated generic *E. coli* and STEC organisms as compared with the non-treated control (Table 1 and 2). Generic *E. coli* and STEC were not detected in 2014 strawberry and tomato crops irrigated with water treated with calcium hypochlorite. The target available chlorine concentration was 20 ppm to ensure the satisfaction of the chlorine demand created by the organic matter in pond-1 and the short contact time; this dosage was higher than needed. It is recommended that producers have an injection system that can provide 10 to 20 ppm of available chlorine, and then the dosage can be lowered until 3 to 5 ppm chlorine residual remains in the water that is applied to plant surfaces.

Chlorine Dioxide - ClO_2

Chlorine dioxide was the chlorine source during 2015 and was injected at a rate to produce a 10 ppm concentration in the irrigation water. As shown in Tables 3 and 4, this product performed similarly to calcium hypochlorite, and inactivated STEC below detection limits. Generic *E. coli* was detected twice, but populations were at or below 11 MPN/100 ml. Some plant damage on the chlorine dioxide plots was attributed to the sodium content of the disinfectant solution. This product must be manufactured on site; however, there are now vendors that will provide the chlorine dioxide precursors in smaller packets (as opposed to a shipping container) that produce final product volumes that are reasonable for producers to use for disinfection. It is recommended that producers have the capacity to inject chlorine dioxide at a rate that can produce a 5 to 10 ppm chlorine concentration in the irrigation water.

Peroxyacetic Acid - PAA

PAA performed very well as a disinfectant of raw surface water, even with a short contact time. PAA seems to have a slightly greater affinity for oxidizing microbes than for the dissolved organic matter, which reduces the potential for dissolved organic matter to interfere with disinfection. PAA also readily decomposes to carbon dioxide and water in the environment. This product is commercially available in several concentrations. It is somewhat difficult to compare the various PAA formulations; this product is a mixture of peracetic acid, hydrogen peroxide, acetic acid and water. Peracetic acid is the primary active ingredient; however, hydrogen peroxide and acetic acid also have disinfectant properties. The solution used for this project was 12% peracetic acid, 18.5% hydrogen peroxide, and 20% acetic acid.

The disinfectant performance was very good, but care must be taken when using this product. Initially, the 12% concentrate was used as the stock solution. However, the concentration produced sufficient volatilization that the metering pumps would frequently vapor-lock, allowing large water volumes to pass without treatment. As shown in Table 1, when injection was properly controlled, the compound performed moderately well. Tables 2, 3, and 4 show the improvement with PAA performance achieved with changes in management. This problem was alleviated by diluting this concentrate by 50% (thus doubling the injection rate), and by replacing the diaphragm metering pump with a peristaltic metering pump. A second potential issue with using PAA is the change in water pH; after treatment, the irrigation water dropped from approximately 6.8 to approximately 4.5. The pH change has the potential to acidify the soil and

change the availability of nutrients. The issue of pH change needs further research to determine whether this concern is warranted.

Ultraviolet Light - UV

The results of using UV light are shown in Tables 1, 2, 3, and 4. A particular advantage of using UV light is that a module can be installed on the irrigation pipeline to treat all the water. However, this is also a disadvantage because the pathogen kill-zone is limited to the volume within the module – there is no downstream residual treatment. UV systems are designated by water flow rate and UV intensity. For point-of-use drinking water treatment, the U.S. EPA recommends a UV dosage of 40,000 $\mu W \cdot s/cm^2$. This value has a two-fold safety factor. Further, a NSF-certified UV system must be able to provide this exposure when about 50% of the transmitted radiation is blocked by a dirty quartz sleeve or by turbid water. Because the UV module has a fixed volume, as the flow rate changes, so does the UV dosage. As such, the user must size the UV device based on the greatest flow rate expected to be treated. For this project, the greater flow rate was during overhead irrigation, for which the water received a 35,000 $\mu W \cdot s/cm^2$ dosage. During drip irrigation, the dosage was 47,000 $\mu W \cdot s/cm^2$.

As mentioned, UV is very sensitive to turbidity. This project was able to successfully remove pathogens from surface water with turbidities as high as 35 NTUs. There is a notable difference in UV disinfection success between Table 1 and Tables 3, 5, and 7. While UV provided successful treatment in all cases, when the source water was switch to pond-2 (less turbidity, see Table 5), the pathogen reduction was more complete.

Based on these findings, it is recommended that a UV device should be selected that can provide a minimum dosage 40,000 $\mu W \cdot s/cm^2$ at the required flow and that the maximum turbidity should be limited to approximately 30 NTUs. The UV module should be placed for easy maintenance and include an intensity monitor let the operator know when the UV transmission is cannot provide the required dosage.

Overall, all disinfection treatments performed better than the untreated positive control and were found to be similar to municipal water (Table 6). This demonstrates that these disinfection methods are promising mitigation strategies that can be applied by growers to reduce risk. They should especially be considered when water will contact the edible portion of the crop.

Operator Observations

It is important to consider irrigation system start-up. At start-up, the pipelines are not under pressure and the pump will transfer water at a greater rate than during steady-state conditions. As discussed in this report, injection rates and UV dosages have been based on steady-state conditions. Either the start-up water can be diverted until the water flow rate reaches steady-state, or additional disinfection capacity can be added to account for the increased flow rate. If fertigation is used, then the fertigation system needs to be disinfected. Water used to dissolve the nutrients must be from a sanitary source and the injection equipment must be sanitized before use. An alternative is to place the fertigation system before the disinfection system.

Objective 2. Determine transfer of pathogen (STEC) and indicator organisms (E. coli) from irrigation water to the fruit of model crops (strawberry and cantaloupe) with the three mitigation strategies as compared to no treatment, utilizing both overhead and drip irrigation delivery.

The original intent of this objective was to evaluate the movement of pathogens from the irrigation water onto the model crops, with the anticipation that the plots irrigated with treated

water would demonstrate less contamination as compared to the non-treated control plots. To minimize cross-contamination, the plots were separated by curtains and there were blank rows between treatments to increase the separation. As seen in Table 7, the STEC contamination was fairly well distributed across all treatments in the 2014 strawberry trial (24–40%). The untreated control has slightly more contaminated samples (40%) than the treated irrigation blocks, but it is certainly not significant ($p > 0.05$). These plots were located 100 m (300 ft) downwind of a pasture containing beef cattle (stocking density approximately 1 cow per acre). It was assumed that other environmental pressures (insects, small mammals, birds, and bioaerosols) played a significant role in crop contamination such that any irrigation treatment effects were eliminated. No conclusions could be drawn from this trial. The research plots were reestablished 850 m (2,800 ft) away from this pasture, but still downwind for subsequent trials.

Tomatoes were transplanted into the new plots. The tomato trial resulted in only one positive sample, isolated from the control plot, but no significant difference was found between treatment blocks (Table 8; $p > 0.05$). This crop was only drip irrigated, and the results indicate that drip irrigating tomatoes is a good agricultural practice.

Strawberries were transplanted the fall of 2014 and grown out in 2015. As shown in Table 9, the 2015 strawberries were as contaminated as the 2014 crop. This crop did not receive frost protection, thus the drip irrigated plots were not overhead frost protected (a confounding factor from 2014), and still there was equal contamination across all treatments (43–32%). The project team is attempting to find more information about the contaminant sources beyond the irrigation water. Isolates collected from plant samples are being submitted for molecular genetic analysis to hopefully provide more information as to the pathogen reservoir.

The final model crop was cabbage. As shown in Table 10, there was minimum contamination (4–7%), but it was found among all treatments, with the exception of municipal water. These plots only received drip irrigation, but were significantly closer to the soil than the tomato crop.

On the basis of the above results, we cannot draw specific conclusions about pathogen transfer from irrigation water. Other environmental factors are apparently a greater cause of contamination. While the specific vectors are unknown at this time, it appears that their effect overwhelmed any potential treatment effect provided by disinfecting the irrigation water.

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APPENDICES

Publications and Presentations

Chang, T. 2015. Evaluation of multiple disinfection methods to mitigate contaminated irrigation water. An unpublished thesis presented for the Master of Science degree, The University of Tennessee, Knoxville, May 2015.

Presentations:

Buchanan, J. R. 2016. Beyond chlorination: Other ways to keep your irrigation water safe. Pick TN Conference, Knoxville, TN, February 11-13.

Critzer, F. J. 2016. Produce Safety: Don't kill your customer. Pick TN Conference, Knoxville, TN, February 11-13.

Wszelaki, A. L., F. J. Critzer, J. R. Buchanan, and D. Lockwood. 2016. Central region fruit, vegetable, and food safety update. Tennessee Extension Agent In-Service, Murfreesboro, TN, February 2-3.

Wszelaki, A. L., F. J. Critzer, J. R. Buchanan, and D. Lockwood. 2016. Western region fruit, vegetable, and food safety update. Tennessee Extension Agent In-Service, Lexington, TN, January 26-27.

Buchanan, J. R., F. Critzer, A. Wszelaki and D. Lockwood. 2015. Evaluation of multiple disinfection methods to mitigate the risk of produce contamination by irrigation water - progress report. Center for Produce Safety Symposium, Atlanta, Georgia, June 24.

Gorman, S., L. Gann, A. L. Wszelaki, F. Critzer, and J. R. Buchanan. 2015. Disinfection Methods to Mitigate Food Safety Risks Associated with Contaminated Irrigation Water on Drip-irrigated Tomatoes. Journal of Food Protection, Supplement A, 78:280, Portland, OR, July 25-28.

Buchanan, J. R., F. Critzer, A. L. Wszelaki, and D. W. Lockwood. 2014. Irrigation water disinfection strategies. Center for Produce Safety Symposium, Long Beach, CA, June 24.

Buchanan, J. R. and A. L. Wszelaki. 2014. Mitigation of agricultural water. Plateau Research and Education Center, Steak and Potatoes Field Day, Crossville, TN, August 6.

Buchanan, J. R. and F. J. Critzer. 2013. New applications for old tools: Strategies for mitigating risks in agricultural irrigation. Steak & Potatoes Field Day, Plateau Research and Education Center, Crossville, TN, August 8.

Budget Summary

Approximately $17,000 of the original $280,483 was not spent. Not all of the money allocated for supplies was needed, and not all of the money allocated for graduate student tuition was used. Both of these costs are considered direct costs, so lower indirect costs were charged.

Tables and Figures

Table 1. Range of pathogen concentrations measured in irrigation water before and after treatment during the spring of 2014 strawberry trial. (Source water was pond-1.)

Date	Organism	Before Treatment	Treated Water		
			$Ca(ClO)_2$	PAA[2]	UV
		CFU 100 mL^{-1}	CFU 100 mL^{-1}		
8 May 2014	*E. coli*[1]	579.4	<1	<1	<1
	STEC	50	0	12	0
12 May 2014	*E. coli*	1553.1	<1	4.1	15.8
	STEC	100	0	5	2
13 May 2014	*E. coli*	866.4	<1	39.9	<1
	STEC	-	-	-	-
15 May 2014	*E. coli*	47.1	<1	488.4	7.4
	STEC	-	-	-	-
19 May 2014	*E. coli*	-	<1	<1	2
	STEC	150	0	0	11
23 May 2014	*E. coli*	93	<1	36.4	6.3
	STEC	-	-	-	-
27 May 2014	*E. coli*	325.5	<1	<1	3.1
	STEC	-	-	-	-
2 Jun 2014	*E. coli*	-	-	-	-
	STEC	42	0	72	34

[1] *E. coli* values are given as MPN 100 mL^{-1}
[2] PAA performance as a disinfectant is represented poorly by this table. The primary issue is that hydrogen peroxide gas evolves under vacuum and care must be taken to prevent an air-lock from developing during water treatment.

Table 2. Range of pathogen concentrations measured in irrigation water before and after treatment during the fall of 2014 tomato trial. (Source water was pond-2.)

Date	Organism	Before Treatment	Treated Water		
			$Ca(ClO)_2$	PAA[2]	UV
		CFU 100 mL^{-1}	CFU 100 mL^{-1}		
14 Aug 2014	*E. coli*[1]	2	<1	<1	<1
	STEC	96	0	0	0
21 Aug 2014	*E. coli*	<1	<1	<1	<1
	STEC	3	0	0	0
28 Aug 2014	*E. coli*	457	<1	<1	<1
	STEC	200	0	0	0
4 Sept 2014	*E. coli*	10	<1	<1	<1
	STEC	14	0	0	0
11 Sept 2014	*E. coli*	3	<1	3.1	<1
	STEC	82	0	26	0
18 Sept 2014	*E. coli*	301	<1	<1	<1
	STEC	20	0	0	0
25 Sept 2014	*E. coli*	2	<1	<1	<1
	STEC	1	0	0	0
2 Oct 2014	*E. coli*	25	<1	<1	<1
	STEC	11	0	0	0

[1]*E. coli* values are given as MPN 100 mL^{-1}
[2]PAA values on September 11, 2014 represent an injection pump failure

Table 3. Range of *E. coli* and STEC concentrations measured in irrigation water before and after treatment during the spring of 2015 strawberry trial. (Source water was pond-2.)

Date	Organism	Before Treatment	Treated Water		
			PAA	ClO_2	UV
		CFU 100 mL^{-1}	CFU 100 mL^{-1}		
17 Apr 2015	*E. coli*[1]	172 – 344	<1 – 41	1 – 11	<1
	STEC	14 – 22	0	0	0
23 Apr 2015	*E. coli*	4 – 17	<1 – 20	<1	<1
	STEC	20 – 60	0	0	0
28 Apr 2015	*E. coli*	3 – 13	<1 – 7	<1 – 1	<1
	STEC	10 – 38	0	0	0
7 May 2015	*E. coli*	5 – 15	<1 – 2	<1	<1
	STEC	30 – 62	0	0	0
11 May 2015	*E. coli*	7 – 387	<1	<1	<1
	STEC	56 – 146	0	0	0
18 May 2015	*E. coli*	6 – 17	<1	<1 – 2	<1
	STEC	40	0	0	0
28 May 2015	*E. coli*	2 – 15	<1	<1	<1
	STEC	3 – 4	0	0	1 – 3

[1]*E. coli* values are given as MPN 100 mL^{-1}

Table 4. Range of pathogen concentrations measured in irrigation water before and after treatment during the fall of 2015 cabbage trial. (Source water was pond-2.)

Date	Organism	Before Treatment	Treated Water		
			PAA	ClO_2	UV
		CFU 100 mL^{-1}	CFU 100 mL^{-1}		
30 Aug 2015	*E. coli*[1]	5.2 – 10.9	<1	<1	<1
	STEC	760	2	0	0
4 Sept 2015	*E. coli*	4.1 – 11	<1	<1	<1
	STEC	0	0	2	1
10 Sept 2015	*E. coli*	5.2 – 6.3	<1	<1	<1
	STEC	150 – 470	0	0	0
17 Sept 2015	*E. coli*	4.1 – 7.4	<1	<1	<1
	STEC	60 – 140	0	0	0
24 Sept 2015	*E. coli*	1 – 4.2	<1	<1	<1
	STEC	20 – 60	0	0	0
1 Oct 2015	*E. coli*	1 – 2	<1	<1	<1
	STEC	-	0	0	0
8 Oct 2015	*E. coli*	3.1 – 4.2	<1	<1	<1
	STEC	0 – 20	0	0	0
15 Oct 2015	*E. coli*	1 – 4.1	<1	<1	<1
	STEC	0 – 40	0	0	0
23 Oct 2015	*E. coli*	10.4 – 22.3	<1	<1	<1
	STEC	60 – 80	0	0	0
28 Oct 2015	*E. coli*	24.9 – 36.9	<1	<1	<1
	STEC	10 – 30	0	0	0

[1]*E. coli* values are given as MPN 100 mL^{-1}

Table 5. Comparison of water quality parameters for the water sources.

Parameter	Pond-1	Pond-2	Municipal
Total Suspended Solids (mg/L)	0.01 – 0.02	0.008 – 0.02	0.001 – 0.003
Total Dissolved Solids (mg/L)	0.1 – 0.2	0.1 – 0.2	0.08 – 0.1
pH	6.9	6.9	7.1 – 8.1
Total Nitrogen (mg/L)	1 – 3	0.5 – 0.8	0.1 – 0.4
Total Carbon (mg/L)	20 – 30	16 – 19	4 - 8
Turbidity (NTU)	26 – 35	3 – 9	0.1 – 0.3
E. coli MPN per 100 mL	100 – 800	13 – 400	<1 – 1

Table 6. Overall performance of disinfection treatments to reduce populations of STEC in irrigation water.

Treatment	Mean Population of STEC (CFU/100 ml)[1]	Standard Error
Control	20.18[A]	1.38
ClO_2	1.34[B]	1.44
$Ca(ClO)_2$	0.97(ND[2])[B]	0
PAA	1.30[B]	1.39
UV	1.26[B]	1.38
Municipal	0.97(ND)[B]	0

[1]Means followed by different letters are significantly different ($p < 0.05$).
[2]ND, for not detected per 100 ml.

Table 7. STEC detection in strawberry samples by treatment collected during the 2014 strawberry trial[1].

Treatment	STEC Not Detected	STEC Detected	Total No. Samples	% No Detect
Control	148	100	248	60
$Ca(ClO)_2$	168	83	251	67
PAA	195	61	256	76
UV	163	97	260	63

[1]This data includes all samples of berries picked before/after irrigation and on Thursdays.

Table 8. STEC detection in tomato samples by treatment collected during the 2014 tomato trial.

Treatment	STEC Not Detected	STEC Detected	Total No. Samples	% No Detect
Control	29	1	30	97
$Ca(ClO)_2$	26	0	26	100
PAA	30	0	30	100
UV	32	0	32	100
Municipal	29	0	29	100

Table 9. STEC detection in strawberry samples by treatment collected during the 2015 strawberry trial[1].

Treatment	STEC Not Detected	STEC Detected	Total No. Samples	% No Detect
Control	64	49	113	57
ClO_2	65	33	98	66
PAA	73	51	124	59
UV	72	52	124	58
Municipal	68	32	100	68

[1]This data includes all samples of berries picked before/after irrigation and on Thursdays.

Table 10. STEC detection in cabbage samples by treatment collected during the 2015 cabbage trial.

Treatment	STEC Not Detected	STEC Detected	Total No. Samples	% No Detect
Control	42	3	45	93
ClO_2	34	2	36	94
PAA	34	2	36	94
UV	26	1	27	96
Municipal	36	0	36	100

Suggestions to CPS

STEC is generally associated with beef cattle, and the project team knew that the location selected for this investigation had STEC in a pond used for cattle watering. What we did not know was the extent to which STEC would be in the environment surrounding the beef pasture. Beef and fresh produce are commonly grown in the same watersheds, and are often only separated by a strong fence. This project team feels strongly that new knowledge must be gained about the risk of having cattle proximate to fresh produce. Our data indicates that the beef operation does not have to be a concentrated feed lot but rather that pastured-fed beef operations have the potential to serve as STEC reservoirs.