CPS CENTER for PRODUCE SAFETY

CPS-WCFS 2013 RFP
FINAL PROJECT REPORT

Project Title

Transfer and survival of organisms to produce from surface irrigation water

Project Period

July 1, 2013 – June 30, 2014

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Objectives

1. Determine recovery of inoculated STEC and generic E. coli in surface water that is representative of what will be used in field trials.

2. Determine transfer of pathogens (STEC) and indicator organisms (E. coli) from irrigation water to the fruit of model crop (cantaloupe) utilizing both overhead and drip irrigation delivery.

3. Determine how sample number impacts overall sensitivity of results by lowering the type II error rate in field trials where natural variation can greatly impact outcomes.

Funding for this project provided by the Center for Produce Safety through:
The Western Center for Food Safety

FINAL REPORT

Abstract

Water used for irrigation is one of the most likely points of pathogen contamination during fruit and vegetable production. While irrigation water is a known point of contamination, there are very few studies that can be used to determine pathogen transfer from contaminated irrigation water to produce and the correlation of water indicator organisms (generic *Escherichia coli*) with pathogen concentration. This study evaluated the transfer of Shiga Toxigenic *E. coli* (STEC) from contaminated surface water to cantaloupe. Cantaloupe plots containing cross-classified combinations of overhead or surface drip irrigation along with bare ground or plastic mulch raised bed preparations were irrigated from a pond naturally harboring STEC. Surface water was sampled weekly for enumeration of STEC, generic *E. coli*, and coliforms using routine enumeration methods. Cantaloupes were harvested and enriched in mTSB with sodium novobiocin (8 ppm), DNA extracted, and tested for the presence/absence of *stx* and *eae* genes using multiplex PCR. Over six weeks, STEC populations in water used for irrigation were found to fluctuate between 0.7 to 2.68 $\log_{10}$ CFU/100 ml. There was no significant correlation between populations of STEC and coliforms or generic *E. coli* in irrigation water, r^2=0.56 and r^2=0.41, respectively. Over a four-week harvest period, 210 cantaloupes were sampled for STEC contamination. All treatment combinations were found to have similar occurrence of STEC-contaminated cantaloupe ($p>0.05$). STEC contamination of bare ground plots with drip irrigation and plastic mulch plots with overhead irrigation was 20.4% and 19.7%, respectively. The percentage of positive samples on overhead-irrigated bare ground plots was 14% and while drip irrigated plots with plastic mulch was 12%. These data suggest that the population of generic *E. coli* or coliforms in irrigation water does not correlate with STEC concentration. Additionally, when high levels of STEC persist in irrigation water, transfer to cantaloupe can occur regardless of irrigation methods and bed preparation.

Background

Surface water is widely used for farming operations in the United States (*40*). Between 2003 and 2008, the use of surface water on farms increased 22% (*40*). Due to the unpredictable nature of surface water contamination, the microbial quality of a surface water source can be highly variable and should be closely monitored (*10*, *20*). In response to the Food Safety Modernization Act, signed into law in January 2011, the United States Food and Drug Administration released its proposed produce safety regulations. The FDA's Produce Safety Rule, issued in January 2013, seeks to establish science-based minimum standards for the growing, harvesting, packaging, and holding of fresh produce on farms (*36*). In the proposed legislation, agricultural water is defined as water that is intended to or is likely to contact produce or food-contact surfaces. The proposed microbial water standards rely on testing for generic *E. coli* as an indicator of pathogen contamination. Irrigation water that directly contacts the edible portion of the crop must have generic *E. coli* counts of less than 235 *E. coli* in a 100 ml single sample or less than 126 *E. coli* per 100 ml in a five-sample rolling geometric mean. However, those using indirect irrigation techniques that do not contact the edible portion of the crop such as drip and furrow irrigation are not required to test irrigation water for microbiological quality.

These proposed standards are based on the assumed relationship between concentration of generic *E. coli* and pathogens in surface waters. This approach is problematic however, since studies have shown that this relationship is weak or non-existent (*12*, *13*, *20*, *26*). While generic *E. coli* may be the most likely indicator of fecal contamination, its correlation

with pathogens in surface water sources needs to be further studied to assess the practicality of these standards. Furthermore, no standards are proposed in the Produce Safety Rule for indirect water applications. Whether or not it is intended to occur or likely to occur, there may be instances where indirect water application leads to direct or indirect contamination of the crop or the growing environment. For instance, pooling from overwatering may contaminate the environment or spray from a compromised drip irrigation line may contact the edible portion of the crop.

In contrast to recreational surface waters, very few studies have focused on the microbiology of surface water used for crop irrigation. The risk associated with using irrigation water that exceeds the proposed water quality standards needs to be characterized. Quantitative data relating to contaminated irrigation water contacting crops is needed to make risk-based assessments that may also aid in developing future standards for microbiological quality of irrigation water.

Modern farming utilizes many different production practices to benefit crop health and maximize yield, but there is little understanding about how these techniques may influence the likelihood of pathogen contamination. Notably, plastic films applied to cover the soil can increase soil temperature, increase soil moisture, maintain soil tilth, and improve crop quality and yield (*27*). These plastic films, commonly called plastic mulch, provide a barrier between the soil layer and the edible portion of the crop, thereby protecting it from contact with soil moisture and pathogens (*29*). Cantaloupes and other produce commodities that are grown in close proximity with the soil could possibly benefit from the use of plastic mulch to help mitigate the risk of contamination. Depending on the specific crop and the method of irrigation, the potential for plastic mulch to reduce crop contamination could vary. The relative contamination risk associated with different production and irrigation methods needs to be better understood by farmers and regulating authorities.

STEC are emerging foodborne pathogens of concern, especially with regard to fresh produce contamination (*24*, *25*). *E. coli* O157:H7 was found to survive for over 60 days in conventional and organic soils (*30*), but has been reported to survive up to 500 days in frozen soil (*4*). Another study documented the survival of *E. coli* O157:H7 on lettuce and parsley leaves for 77 and 177 days, respectively (*14*). Pathogenic *E. coli* strains have been shown to differentially attach to a variety of plant parts, whereas non-pathogenic *E. coli* K12 could not (*16*). Some fruits and vegetables like cantaloupe have rough exterior surfaces allowing for microbial attachment to take place. Additionally, the netted rind of cantaloupes can create microniches that can serve to protect and harbor pathogenic bacteria for extended periods of time (*35*, *37*, *38*). The ability of pathogenic *E. coli* to attach to various fruit and vegetable surfaces coupled with the susceptibility of cantaloupes to microbial attachment and persistence, make these ideal parameters for use in this study.

The routine analysis of surface water sources according to the standards proposed in the FDA's Produce Safety Rule can become expensive and negatively impact many farms using these sources for irrigation. If generic *E. coli* has no correlation to actual pathogen presence, the routine testing of these water sources will be ineffective in promoting food safety. The comment period for the proposed Produce Safety Rule ended in November 2013, but there is still a great amount of research that needs to be done to elucidate the true relationships between pathogens and indicators in irrigation water sources. This study seeks to evaluate the transfer of Shiga Toxigenic *E. coli* (STEC) from contaminated surface water to cantaloupe using common production methods. In addition, the accuracy of using generic *E. coli* to indicate pathogen presence in surface water is investigated.

Research Methods and Results

Cantaloupe production environment

The University of Tennessee Plateau Research and Education Center (Crossville, TN) was selected as the open field farm site for this study. An onsite pond was utilized as a surface water source for irrigating the 20 m x 42.5 m melon plot used to grow and harvest cantaloupes for this study. A general purpose Honda WB30 centrifugal pump was used to transport pond water to the melon plot. Water was pumped through a 150-mesh sand filter and then approximately 323 m of polyvinyl chloride lay-flat water delivery hose to the melon plot. The pump inlet was positioned to accept water from just below the pond surface.

Experimental design

The melon plot was divided into 16 sub-plots that were 6 m in length and 1 m wide in four rows of four sub-plots each. Each subplot contained a combination of irrigation and bed preparation treatments. A cross-classified treatment design was used. Two irrigation treatments, overhead spray and surface drip, were applied to each half of the melon plot. Additional raised-bed preparation treatments, bare ground and black polyethylene plastic mulch, were applied to each subplot. A randomized block design was used, where subplots were randomly assigned as one of four repetitions for cantaloupe growing and harvesting. Blocks were randomly assigned to groups of four subplots that contained the four treatment combinations. A pond frequented by cattle and separated from the plot by approximately 300 m was used as the surface water irrigation source for the study. Cantaloupes (cv. Athena) were direct seeded and managed as described in the 2013 Southeastern U.S. Vegetable Crop Handbook (*17*). Cantaloupe production began at planting in July 2013 and culminated in October 2013 with the last cantaloupe harvest event. Ripe melons were harvested twice per week starting September 10, 2013 and ending October 3, 2013. Irrigation water was sampled weekly at the source and point of application starting August 29, 2013 and ending October 1, 2013.

Collection and transport of water samples

Once per week, samples were collected directly from the overhead sprinklers located at the melon plot and from the water pump outlet hose just before the sand filter. Three samples were collected into sterile 69 oz Whirl-Pak sample bags (Nasco, Fort Atkinson, Wis.) at each sampling location. Sample bags were placed in a cooler with ice for transport to the laboratory for analysis.

Physicochemical analysis of irrigation water

Turbidity, total dissolved solids, total nitrogen, non-purgeable organic carbon, and pH were monitored in irrigation water obtained from the source and point of application. 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, Colo.). 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.

Microbial analysis of irrigation water

Total coliforms, thermotolerant coliforms, and generic *E. coli* were used as hygiene indicators due to their common use as indicators of fecal contamination. Water samples were also analyzed for STEC as pathogens of interest in this study. Sample bags were agitated prior to pipetting the sample amount needed for each analysis. Thermotolerant coliforms were enumerated using Petrifilm Coliform Count Plates (3M, St. Paul, Minn.). Water samples were

diluted 1:10 in 0.1% peptone water, 1 ml was inoculated onto duplicate Petrifilm Coliform Count Plates, and incubated at 44 °C for 24 h. Total coliforms and generic *E. coli* were enumerated by the Colilert Quanti-Tray/2000 procedure (IDEXX Laboratories Inc., Westbrook, Maine). 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 were enumerated using membrane filtration onto a selective and differential chromogenic medium, CHROMagar STEC (CHROMagar, Paris, France). Volumes of 10, 50, and 100 ml were filtered using 0.45-µm S-Pak membrane filters and glass 47 mm filter holders (Millipore Corporation, Bedford, Mass.). The filters were aseptically placed onto CHROMagar STEC and incubated at 37 °C for 24 h. STEC colonies were identified by a mauve (pink/purple) colony color and enumerated.

Collection and transport of cantaloupe samples

Ripe cantaloupes were aseptically harvested twice a week and placed into separate sterile 184 oz Whirl-Pak sample bags. A tan colored rind and the ability of the stem to easily slip from the melon identified ripe cantaloupes. Cantaloupe samples were transported in coolers on ice to the laboratory for analysis.

Cantaloupe sample preparation

Three cantaloupes that were deemed “marketable” from each plot were chosen for sampling. Each cantaloupe was aseptically placed in a new sterile 184 oz Whirl-Pak sample bag and 250 ml of 0.1% peptone with 0.2% Tween 80 was added. The bag was closed and held with an aluminum filter holder clamp (Millipore Corporation; Bedford, Mass.). Each cantaloupe was vigorously rinsed by rubbing the bag against the cantaloupe exterior for 60 s. Cantaloupes were removed from the bags and 10 ml of the resulting liquid rinsate was used for enumeration of STEC and generic *E. coli*. The remaining rinsate in each bag was enriched for PCR detection of STEC.

Microbial enumeration of cantaloupe samples

Each bag containing rinsate was agitated before a 10-ml aliquot was pipetted into a sterile sample cup. A WASP II Spiral Plater (Don Whitley Scientific Ltd., West Yorkshire, United Kingdom) was used to plate 100 µl of the undiluted rinsate sample onto CHROMagar STEC and CHROMagar *E. coli*. The resulting plates were incubated at 37 °C for 24 h. Mauve colonies were identified as STEC on CHROMagar STEC and blue colonies were identified as generic *E. coli* on CHROMagar *E. coli* plates.

Enrichment and DNA extraction of cantaloupe samples

Modified TSB with 8 ppm sodium novobiocin was added to the remaining rinsate in the sample bag at a ratio of 1:4. This was achieved using a BabyGravimat gravimetric dilutor (Interscience, St. Nom La Breteche, France). Each enrichment broth was incubated for 15-22 hours at 42 °C in its respective sample bag. After incubation, the enrichment was agitated in the sample bag and DNA was extracted from the enrichment. For extraction, a boiling lysis procedure was used where 1.4 ml of enrichment was transferred to a sterile 1.5 ml centrifuge tube, centrifuged for 5 minutes at 10,000 g at 25 °C, washed with 500µl 0.85% saline solution, centrifuged for 3 minutes at 10,000 g, washed with 90µl 1X TE buffer, heated at 97 °C for 15 minutes, allowed to cool to room temperature, and centrifuged for 4 minutes at 16,000 g. The resulting supernatant was transferred to a new sterile microcentrifuge tube, and the extracted DNA was stored at -20 °C for detection using multiplex real-time PCR.

Detection of STEC on cantaloupe samples using multiplex real-time PCR

The protocol described in the USDA/FSIS Microbiology Laboratory Guidebook for detection of Shiga Toxigenic *E. coli* from meat products was followed (*39*).The DNA extractions were screened for presence/absence of STEC using ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, Calif.). Shiga toxin genes (*stx1* and *stx2*) and intimin (*eae*) genes were targeted using specific TaqMan based PrimeTime qPCR primers and probes shown in Table 1 (Integrated DNA Technologies, Coralville, Iowa). This assay detected both Shiga toxin gene sequences under the same fluorescent wavelength, thus differentiation between *stx1* and *stx2* was not possible. Samples that were positive for either *stx* or *eae* genes were confirmed using gel electrophoresis. These samples were used in another set of three serogroup-specific assays to identify genes within the O-antigen gene cluster specific for each serogroup. Shown in Table 2, PrimeTime primers and probes specific to the O157 serogroup or the six most prevalent non-O157 serogroups in the United States (O26, O45, O103, O111, O121, O145) were used.

Cantaloupe quality measurements and grading

Cantaloupes from each plot were weighed and then tested for firmness, soluble solids content, pH, and color to determine marketable quality of the fruit. Four, 1" cubes were cut from the blossom end of each melon and used for pH measurements. The pH was measured using a Calibration Check Portable pH/ORP Meter, HI 9126 (Hanna Instruments, Inc., Woonsocket, R.I.). Fruit color was measured with a MiniScan XE PLUS Spectrophotometer (Hunter Associates Laboratory Inc., Reston, Va.) in L*a*b* mode under CIE Standard Illuminant C. Two readings per fruit were taken on opposite sides of the cantaloupe and averaged for both color and firmness data. Fruit firmness was measured with a Wagner Force Dial-Model FDK 32 (Wagner Instruments, Greenwich, Conn.) with a 10-mm tip. Soluble solids content was measured using a temperature compensating AR200 Automatic Digital Refractometer (Reichert Inc., Depew, N.Y.).

Statistical analysis

The Statistical Analysis Software (SAS) system Version 9.3 (SAS Institute Inc., Cary, N.C.) was used for all analyses. For water samples, all CFU counts were converted to $\log_{10}$ counts per 100 ml before statistical analysis. Analysis of variance was conducted for microbial counts and cantaloupe quality measurements separately 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.

Physicochemical characteristics of irrigation water

For all irrigation water samples taken from the source and point of application, the pH ranged from 6.9 to 9.1 with point of application samples averaging 7.3 and source samples averaging 7.5. Turbidity from all water samples ranged from 4.0 to 27.1 NTU's. Source water directly from the pump (16.9 NTU) was more turbid on average than water collected from the sprinklers at point of application (12.3 NTU). Percent total dissolved solids ranged from 0.004 to 0.042% for all water samples, with source water averaging 0.017% and water at the point of application averaging 0.016%. Total nitrogen ranged from 0.97 to 4.89 mg/l. Averages for source water and sprinkler water were similar at 2.20 and 2.15 mg/l, respectively. Non-purgeable organic carbon (NPOC) ranged from 6.21 to 8.17 mg/l. Averages were similar for both sampling locations with an average of 7.51 mg/l for source water and 7.26 mg/l for water from the point of application.

Microbial indicators and STEC in irrigation water

Over six weeks, STEC populations ranged from 0.7 to 2.68 $\log_{10}$ CFU/100 ml. Figure 1 shows the populations of STEC and hygienic indicators at both sampling points throughout the study. Table 3 describes the lack of a significant correlation between STEC and any of the fecal indicators measured in source irrigation water. Results for irrigation water from the point of application were not statistically analyzed for correlation to STEC concentrations. These concentrations had greater variation and were generally higher due to high microbial loads incurred from the irrigation lines after the sand filter.

Cantaloupe quality measurements

Firmness, color, weight, pH, and soluble solids were not significantly different ($p>0.05$) amongst cantaloupe samples with different treatment combinations. Cantaloupe samples averaged 8.32% soluble solids and weights ranged from 1.011 to 4.880 kg with an average weight of 2.081 kg. Cantaloupe pH ranged from 5.25 to 7.31 and the average cantaloupe pH was 6.38. Average cantaloupe firmness pressure reading was 4.46 grams. Using the CIE L*a*b* system (CIELAB), average cantaloupe lightness was L*=67.86 and the average values for chromaticity were a*=20.04 and b*=27.97.

Enumeration of STEC on cantaloupe samples

Enumeration of STEC on cantaloupe samples was not possible due to the high presence of background microflora recovered from the cantaloupe surface compared to the low concentrations of the target organism, STEC. The amount of background microflora varied greatly for each plate making it difficult to compare accurate colony counts between plates. Consequently, the results from these plates were not utilized.

Molecular detection of STEC on cantaloupe samples

Forty-four of 210 cantaloupe samples were presumed *stx/eae* positive by multiplex PCR. Amongst the presumptive positives, 35 samples were confirmed positive by gel electrophoresis. Table 4 shows the contamination rates among the different plot treatment combinations. Due to the varying sample number for each treatment combination, contamination rates are presented by the ratio of *stx/eae* positive cantaloupe samples to total samples for each treatment combination. There were no significant differences in contamination rates between the four treatment combinations at the $\alpha=0.05$ level of significance. Percentages of *stx/eae* positive cantaloupes ranged from 12 to 20.4%. Table 5 shows the results for the serotyping assays of *stx/eae* positive samples. The majority of positive samples belonged to the O45 serogroup.

Outcomes and Accomplishments

Several studies have assessed the transfer from artificially contaminated irrigation water to field crops (*7*, *9*, *14*, *15*, *21*, *28*, *32*, *33*, *34*). The surface water source used for irrigation in this study was frequented by beef cattle and contained populations of coliforms, generic *E. coli*, and STEC. Consequently, other natural microbial populations associated with this environment were also present in irrigation water and on cantaloupes. Surface water source are commonly used for irrigation in the Southeastern United States. These natural parameters, in addition to using a sand-filter alongside a typical farm irrigation system, helped represent contamination events *in vivo*.

Transfer of STEC from irrigation water to cantaloupe

A goal of this study was to characterize the amount of contamination occurring from contaminated irrigation water used for growing cantaloupes. Due to insect predation and delayed ripening for most plots, ripe cantaloupes were picked opportunistically. This allowed the

choice of the highest quality melons from each plot for sampling, but resulted in varying numbers of samples from each plot. The high rate of recovery of background microflora from cantaloupe samples made enumeration of STEC and generic *E. coli* on cantaloupes impossible. Any dilution of the rinsate risked diluting the target organisms to below detectable levels. To guarantee the highest sensitivity for the detection of pathogen contamination on melons, an enrichment step was used prior to DNA extraction and PCR analysis. As a consequence of the enrichment step, a complete quantitative analysis of the contamination was not possible. However, attachment and persistence of STEC on cantaloupe surfaces can be confirmed by detection.

After selective enrichment, presence/absence of STEC on cantaloupes via multiplex PCR analysis was used to determine which cantaloupe samples and corresponding treatments were contaminated. A similar study by Holvoet et al. (2014) successfully analyzed samples of lettuce irrigated with naturally contaminated water by using multiplex PCR for detection of *Salmonella* and STEC (*12*). The presence of STEC detected on cantaloupe samples indicates the ability of these organisms to attach and persist on melon surfaces. Furthermore, the ability of these organisms to grow in enrichment indicates a potential food safety risk associated with the contaminated melons.

Comparing production methods

Overhead irrigation methods are generally regarded as a higher risk for contamination, as water is distributed onto the edible portion of above-ground crops (*11*, *14*, *34*), compared to non-direct applications such as surface and subsurface drip irrigation (*6*, *8*, *21*, *22*, *32*). However, in the current study, cantaloupe rinds were contaminated with STEC regardless of irrigation treatment or raised bed preparation with no significant difference among treatments. Although not significantly different from other cantaloupe production treatments, drip irrigation plots with plastic mulch contained the least contaminated samples. Many studies have demonstrated the reduction of contamination by using drip irrigation (*8*, *21*, *32*). Accordingly, a previous study by Sadovski et al. (1978) found that certain manipulations to drip irrigation systems, such as emitter depth and the addition of plastic mulch could reduce contamination risk associated with using poor quality irrigation water (*28*). The widespread occurrence of *stx/eae* positive results with all treatment combinations suggested that contamination might have resulted from a variety of vectors including water, soil, and insects. These results suggest that high levels of STEC in irrigation water result in a heavily contaminated environment.

The effect of using plastic mulch in this experiment was most likely negated due to cantaloupes growing off of the raised bed onto the bare ground between plots and by soil blown onto the plastic mulch by the wind. Rainfall events and overhead irrigation sprays cause splashing of contaminated water and soil particles onto the crop exterior (*2*, *19*). Moreover, plastic mulch has been found to have an increased splashing effect from simulated rainwater compared to bare ground plots (*2*). Therefore, the use of plastic mulch with overhead irrigation in this experiment may have increased the contamination of cantaloupes..

Crops such as melons, that are in close proximity to the soil or directly contact the soil, may also become contaminated by non-direct water application through contaminated soils (*9*, *18*, *28*). Drip irrigation increases the moisture content of the soil surface. A study by Song et al. (2006) links the occurrence of increased soil moisture beneath cantaloupes to greater microbial recovery (*34*). Regardless of the type of crop being produced, indirect water applications such as drip or furrow irrigation should be included in the Food Safety Modernization Act's definition of agricultural water due to their ability to influence the overall contamination of the production environment. The obstacles in this experiment can be improved upon, allowing the enumeration of pathogens surviving on the produce crop in relation to concentrations associated with contaminating vectors. The fates of pathogens distributed onto produce needs to be further studied to determine safe pathogen levels in irrigation water.

Irrigation water quality from a surface water source

The prevalence of STEC in surface waters around the world is well documented (*1*, *3*, *5*, *12*, *31*). However, few studies have focused on crop contamination via naturally contaminated surface water (*12*, *23*). All irrigation water counts from the point of application were increased and highly variable possibly due to biofilms and leftover organic sediment in the irrigation lines and the sand filter. Back-flushing of the irrigation lines and sand filter was performed during the study to mitigate these risks. Therefore, microbial counts taken after the sand filter at the plot were subject to extreme variation. This may be an important issue with monitoring irrigation water quality, because test results from water sources may not reflect the microbial populations present in irrigation systems, as growers do not generally back-flush irrigation lines after use.

The lack of significant linear correlations between pathogens and indicator organisms in water is well documented (*12*, *13*, *20*, *26*, *31*, *42*). Accordingly, source water data (Figure 1) from the current study shows erratic relationships between populations of indicator organisms and STEC. Results from a study by Won et al. (2013) depict the variable nature of surface water sources and their spatial and temporal variations suggesting that single sample standards, such as less than 235 *E. coli* (CFU/100 ml), may only provide brief and insufficient detail of surface water source quality (*41*). Moreover, the results from the current study suggest that generic *E. coli* cannot be used to accurately predict STEC levels in surface water when low to moderate linear relationships are present. The lack of correlation between the concentration of STEC and any fecal indicator tested, suggests that these organisms may indicate but not accurately represent pathogen concentration. The weak correlation between generic *E. coli*, STEC, and other fecal indicators in this study, in addition to similar results from other studies, frames the need for revision in the FSMA's standards for agricultural water used for crop irrigation.

Summary of Findings and Recommendations

Rather high rates of STEC contamination were observed regardless of cultivation method in this study. With airborne and vector transmission of microorganisms likely causes of contamination in addition to irrigation water there is an increased need to understand complex dynamics of pathogen contamination of produce in the field when multiple routes of contamination are at play. Once these factors are better understood, science-based guidance may be given to growers with animal production or other sources of contamination on land adjacent to produce production.

In this study, no significant correlations between indicator organisms (generic *E. coli*, coliforms, or thermotolerant coliforms) and STEC were found in irrigation water. As a result, monitoring for generic *E.coli*, as proposed in the produce safety rule, may not achieve the goal of reducing the burden of foodborne illness associated with fresh produce. Research should continue to focus on discovering better candidate indicator organisms that are better correlated with a food safety risk in irrigation water.

References

1. Ahmed, W., S. Sawant, F. Huygens, A. Goonetilleke, and T. Gardner. 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. *Water Res.* 43:4918–4928.
2. Cevallos-Cevallos, J. M., M. D. Danyluk, G. Gu, G. E. Vallad, and A. H. C. van Bruggen. 2012. Dispersal of Salmonella Typhimurium by rain splash onto tomato plants. *J. Food Prot.* 75:472–479.
3. Chigor, V. N., V. J. Umoh, and S. I. Smith. 2010. Occurrence of Escherichia coli O157 in a river used for fresh produce irrigation in Nigeria. *Afr. J. Biotechnol.* 9:178–182.

4. Diez-Gonzalez, F., and A. Mukherjee. 2009. Produce safety in organic vs. conventional crops, p. 83–100. *In* Microbial safety of fresh produce. Wiley-Blackwell, Ames, Iowa.
5. Duris, J. W., S. K. Haack, and L. R. Fogarty. 2009. Gene and antigen markers of Shiga-toxin producing E. coli from Michigan and Indiana river water: cccurrence and relation to recreational water quality criteria. *J. Environ. Qual.* 38:1878–1886.
6. Enriquez, C., A. Alum, E. Suarez-Rey, C. Choi, G. Oron, and C. Gerba. 2003. Bacteriophages MS2 and PRD1 in turfgrass by subsurface drip irrigation. *J. Environ. Eng.* 129:852–857.
7. Erickson, M., C. Webb, J. Diaz-Perez, S. Phatak, J. Silvoy, L. Davey, A. Payton, J. Liao, L. Ma, and M. Doyle. 2010. Infrequent internalization of Escherichia coli O157:H7 into field-grown leafy greens. *J. Food Prot.* 73:500–506.
8. Forslund, A., J. H. J. Ensink, B. Markussen, A. Battilani, G. Psarras, S. Gola, L. Sandei, T. Fletcher, and A. Dalsgaard. 2012. Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater. *Water Res.* 46:5917–5934.
9. Forslund, A., F. Plauborg, M. N. Andersen, B. Markussen, and A. Dalsgaard. 2011. Leaching of human pathogens in repacked soil lysimeters and contamination of potato tubers under subsurface drip irrigation in Denmark. *Water Res.* 45:4367–4380.
10. Gil, M. I., M. V. Selma, T. Suslow, L. Jacxsens, M. Uyttendaele, and A. Allende. 2013. Pre- and post-harvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Crit. Rev. Food Sci. Nutr.* (Ahead of Print).
11. Hamilton, A. J., F. Stagnitti, R. Premier, A.-M. Boland, and G. Hale. 2006. Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Appl. Environ. Microbiol.* 72:3284–3290.
12. Holvoet, K., I. Sampers, M. Seynnaeve, and M. Uyttendaele. 2014. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. *Int. J. Food Microbiol.* 171:21–31.
13. Hörman, A., R. Rimhanen-Finne, L. Maunula, C.-H. von Bonsdorff, N. Torvela, A. Heikinheimo, and M.-L. Hänninen. 2004. Campylobacter spp., Giardia spp., Cryptosporidium spp., Noroviruses, and indicator organisms in surface water in southwestern Finland, 2000-2001. *Appl. Environ. Microbiol.* 70:87–95.
14. Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Persistence of enterohemorrhagic Escherichia coli O157: H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J. Food Prot.* 67:1365–1370.
15. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Fate of Salmonella enterica serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Appl. Environ. Microbiol.* 70:2497–2502.
16. Jeter, C., and A. G. Matthysse. 2005. Characterization of the binding of diarrheagenic strains of E. coli to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. *Mol. Plant. Microbe Interact.* 18:1235–1242.
17. Kemble, J. M., K. Ivors, F. J. Louws, K. M. Jennings, and J. F. Walgenbach. 2013. Southeastern U.S. 2013 vegetable crop handbook. Vance Publishing, Lincolnshire, IL.
18. Lapidotand, A., and S. Yaron. 2009. Transfer of Salmonella enterica serovar Typhimurium from contaminated irrigation water to parsley is dependent on curli and cellulose, the biofilm matrix components. *J. Food Prot.* 72:618–623.
19. Liu, C., N. Hofstra, and E. Franz. 2013. Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by Escherichia coli O157 and Salmonella spp. *Int. J. Food Microbiol.* 163:119–128.

20. McEgan, R., G. Mootian, L. D. Goodridge, D. W. Schaffner, and M. D. Danyluk. 2013. Predicting Salmonella populations from biological, chemical, and physical indicators in Florida surface waters. *Appl. Environ. Microbiol.* 79:4094–4105.
21. Oliveira, M., I. Viñas, J. Usall, M. Anguera, and M. Abadias. 2012. Presence and survival of Escherichia coli O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *Int. J. Food Microbiol.* 156:133–140.
22. Oron, G., Y. DeMalach, Z. Hoffman, and Y. Manor. 1992. Effect of effluent quality and application method on agricultural productivity and environmental control. *Water Sci. Technol.* Vol. 26:1593–1601.
23. Pahl, D. M., A. Telias, M. Newell, A. R. Ottesen, and C. S. Walsh. 2013. Comparing source of agricultural contact water and the presence of fecal indicator organisms on the surface of “Juliet” grape tomatoes. *J. Food Prot.* 76:967–974.
24. Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* 19:407–415.
25. Park, S., R. W. Worobo, and R. A. Durst. 1999. Escherichia coli O157: H7 as an emerging foodborne pathogen: a literature review. *Crit. Rev. Food Sci. Nutr.* 39:481–502.
26. Payment, P., and A. Locas. 2011. Pathogens in water: value and limits of correlation with microbial indicators. *Ground Water* 49:4–11.
27. Pennsylvania State University Extension. 2011. Use of plasticulture for the production of melons — vegetable, small fruit, and mushroom production (Penn State Extension). Available at: (http://extension.psu.edu/plants/vegetable-fruit/news/2011/use-of-plasticulture-for-the-production-of-melons).
28. Sadovski, A. Y., B. Fattal, D. Goldberg, E. Katzenelson, and H. I. Shuval. 1978. High levels of microbial contamination of vegetables irrigated with wastewater by the drip method. *Appl. Environ. Microbiol.* 36:824–830.
29. Schrader, W. L. 2000. Plasticulture in California vegetable production. UCANR Publications, Oakland, CA.
30. Semenov, A. V., E. Franz, L. Van Overbeek, A. J. Termorshuizen, and A. H. Van Bruggen. 2008. Estimating the stability of Escherichia coli O157: H7 survival in manure-amended soils with different management histories. *Environ. Microbiol.* 10:1450–1459.
31. Shelton, D. R., J. S. Karns, C. Coppock, J. Patel, M. Sharma, and Y. A. Pachepsky. 2011. Relationship between eae and stx virulence genes and Escherichia coli in an agricultural watershed: implications for irrigation water standards and leafy green commodities. *J. Food Prot.* 74:18–23.
32. Solomon, E. B., C. J. Potenski, and K. R. Matthews. 2002. Effect of irrigation method on transmission to and persistence of Escherichia coli O157: H7 on lettuce. *J. Food Prot.* 65:673–676.
33. Solomon, E. B., S. Yaron, and K. R. Matthews. 2002. Transmission of Escherichia coli O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68:397–400.
34. Song, I., S. Stine, C. Choi, and C. Gerba. 2006. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. *J. Environ. Eng.* 132:1243–1248.
35. Suslow, T. 2003. Key points of control and management for microbial food safety: melons. UCANR Publications, Oakland, CA.
36. U.S. FDA. 2013. Food Safety Modernization Act (FSMA) - FSMA proposed rule for produce safety. Available at: (http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334552.htm#E).

37. Ukuku, D. O., and W. F. Fett. 2006. Effects of cell surface charge and hydrophobicity on attachment of 16 Salmonella serovars to cantaloupe rind and decontamination with sanitizers. *J. Food Prot.* 69:1835–1843.
38. Ukuku, D. O., and G. M. Sapers. 2001. Effect of sanitizer treatments on Salmonella Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *J. Food Prot.* 64:1286–1291.
39. USDA-FSIS. 2013. Detection and isolation of non-O157 Shiga toxin-producing Escherichia coli (STEC) from meat products and carcass and environmental sponges. Available at: (http://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES).
40. USDA-NASS. 2009. Specialty crops2007 US census of agriculture. US Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.
41. Won, G., T. R. Kline, and J. T. LeJeune. 2013. Spatial-temporal variations of microbial water quality in surface reservoirs and canals used for irrigation. *Agric. Water Manag.* 116:73–78.
42. Won, G., P. J. Schlegel, J. M. Schrock, and J. T. LeJeune. 2013. Absence of direct association between coliforms and Escherichia coli in irrigation water and on produce. *J. Food Prot.* 76:959–966.

APPENDICES

Publications and Presentations (required)

Critzer, F.J., S.J. Gorman, J. Buchanan, A.L. Wszelaki, and D. Lockwood. 2014. Transfer and Survival of Organisms to Produce from Surface Irrigation Water. Center for Produce Safety Produce Research Symposium, Newport Beach, CA, June 24-25.

Gorman, S.J., J. Buchanan, A.L. Wszelaki, D. Lockwood, F.J. Critzer. 2014. Transfer and Survival of STEC to Cantaloupe from Surface Irrigation Water. Annual Meeting, International Association for Food Protection, Indianapolis, IN, Aug. 2-6.

Budget Summary (required)

	Food Sci & Tech	Biosystems Engineering	Plant Sciences	PREC	All Entities
Salaries	17,271.01	5,045.93	19,337.44	7,115.18	$48,769.56
Fringe Benefits	7,916.60	208.09	2,387.69	3,362.16	$13,874.54
Travel	1,431.52				$1,431.52
Materials and Supplies	31,372.07	1,836.05		14,098.66	$47,306.78
				F&A	$39,382.95
				TOTAL	$150,765.35

Tables and Figures (optional)

Table 1. *Degenerate primers and probes used for amplification and detection of stx1, stx2, and eae genes in 5' nuclease PCR assays*

Target gene	Forward primer, reverse primer, and probe sequences[a]
stx1	TTTGTYACTGTSACAGCWGAAGCYTTACG
	CCCCAGTTCARWGTRAGRTCMACDTC
	FAM-CTGGATGAT-ZEN-CTCAGTGGGCGTTCTTATGTAA-IABk
stx2	TTTGTYACTGTSACAGCWGAAGCYTTACG
	CCCCAGTTCARWGTRAGRTCMACDTC
	FAM-TCGTCAGGC-ZEN-ACTGTCTGAAACTGCTCC-IABk
eae	CATTGATCAGGATTTTTCTGGTGATA
	CTCATGCGGAAATAGCCGTTM
	MAX-ATAGTCTCG-ZEN-CCAGTATTCGCCACCAATACC-IABk

[a] In the sequence Y is (C,T), W is (A,T), R is (A,G), M is (A,C), D is (A,G,T).

Table 2. *Primers and probes used for amplification and detection of O antigen specific genes in 5' nuclease PCR assays*

Target gene (serogroup)	Forward primer, reverse primer, and probe sequences
***wzx* (O26)**	GTATCGCTGAAATTAGAAGCGC
	AGTTGAAACACCCGTAATGGC
	FAM-TGGTTCGGTTGGATTGTCCATAAGAGGG-BHQ1
***wzx* (O45)**	CGTTGTGCATGGTGGCAT
	TGGCCAAACCAACTATGAACTG
	FAM-ATTTTTTGCTGCAAGTGGGCTGTCCA-BHQ1
***wzx* (O103)**	TTGGAGCGTTAACTGGACCT
	ATATTCGCTATATCTTCTTGCGGC
	MAX-AGGCTTATC-ZEN-TGGCTGTTCTTACTACGGC-IABk
***wbdI* (O111)**	TGTTCCAGGTGGTAGGATTCG
	TCACGATGTTGATCATCTGGG
	MAX-TGAAGGCGA-ZEN-GGCAACACATTATATAGTGC-IABk
***wzx* (O121)**	AGGCGCTGTTTGGTCTCTTAGA
	GAACCGAAATGATGGGTGCT
	MAX-CGCTATCAT-ZEN-GGCGGGACAATGACAGTGC-IABk

Table 2. *Continued.*

Target gene (serogroup)	Forward primer, reverse primer, and probe sequences
***wzx* (O145)**	AAACTGGGATTGGACGTGG
	CCCAAAACTTCTAGGCCCG
	FAM-TGCTAATTGCAGCCCTTGCACTACGAGGC-BHQ1
***wzy* (O157)**	CCTGTCAAAGGATAACCGTAATCC
	TTGTTCTCCGTCTTGTCCTAAACT
	FAM-AAAACAACGAGCATACAACCCCTACCAAT-BHQ1

Table 3. *Pearson correlation coefficients between fecal indicators and STEC in irrigation water*[a]

	Pearson Correlation Coefficients			
	E. coli	Fecal Coliform	Coliform	STEC
E. coli	1.00000	--------	--------	--------
Fecal Coliform	0.66236[b]	1.00000	--------	--------
Coliform	0.23237[b]	0.07717[b]	1.00000	--------
STEC	0.41177[b]	0.43852[b]	0.56424[b]	1.00000

[a] Irrigation water source samples taken before the sand filter.

[b] These correlations were not significant at $p<0.05$.

Table 4. *STEC contamination rates among plot treatment combinations*

Plot Treatment Combination	% Contaminated[a]	*Stx/eae* positives/total samples
Drip-Bare Ground	20.4%	10/49
Overhead-Plastic Mulch	19.7%	12/61
Overhead-Bare Ground	14%	7/50
Drip-Plastic Mulch	12%	6/50

[a] Treatment combinations were not significantly different at $p<0.05$.

Table 5. *Serogroup identifications of stx/eae positive samples*

Serogroup (gene)	Number of positive samples / total samples
O26 (*wzx*)	0 / 35
O45 (*wzx*)	33 / 35
O103 (*wzx*)	0 / 35
O111 (*wbdI*)	0 / 35
O121 (*wzx*)	0 / 35
O145 (*wzx*)	1 / 35
O157 (*wzy*)	0 / 35

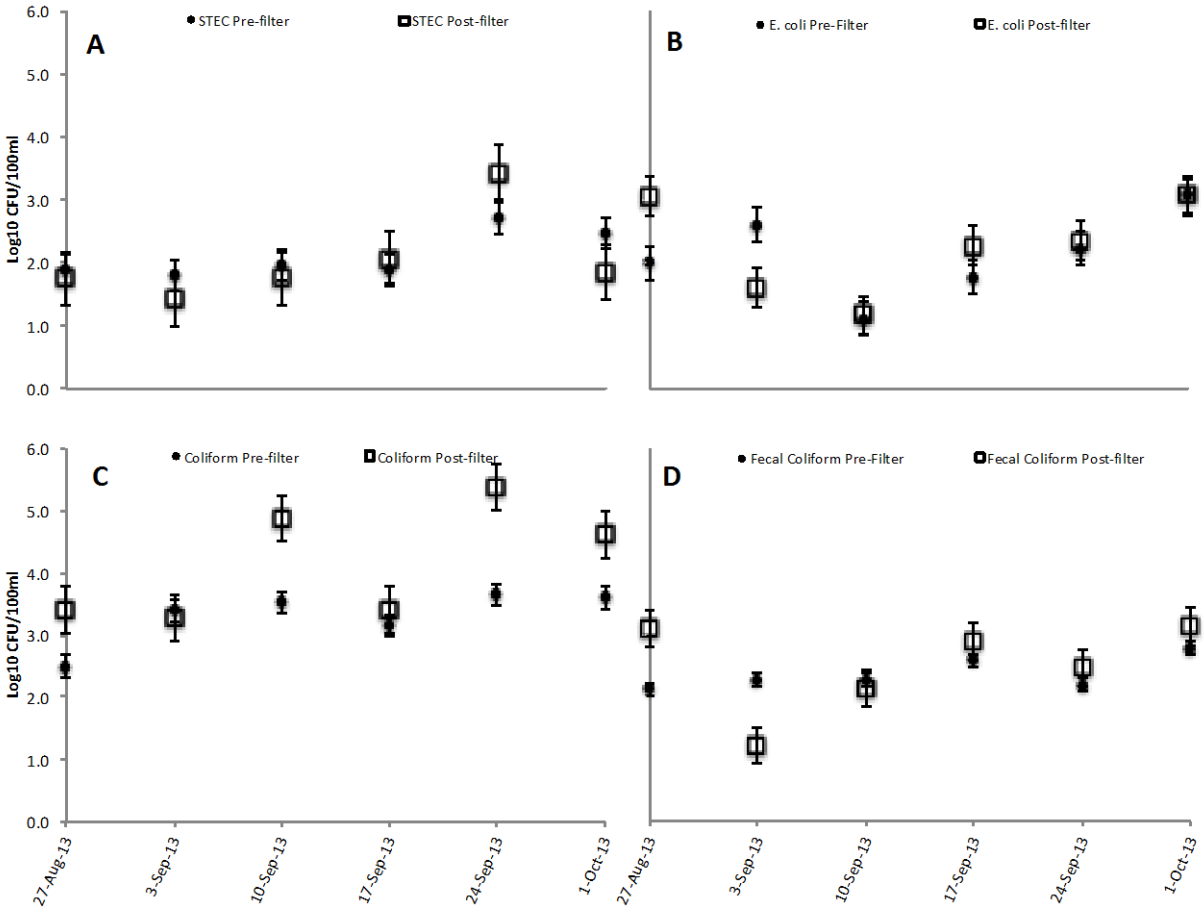

Figure 1. Concentrations in $\log_{10}$ CFU/100 ml from both sampling points across six weeks of water sampling for A: STEC, B: generic *E. coli*, C: Total Coliforms, D: Fecal Coliforms.

Data: STEC concentrations for all irrigation water samples

Week	Treatment	Sample	Log10 CFU/100ml
27-Aug-13	unfiltered	1	1.944482672
27-Aug-13	unfiltered	2	1.913813852
27-Aug-13	unfiltered	3	1.73239376
27-Aug-13	filtered	1	1.698970004
27-Aug-13	filtered	2	1.698970004
27-Aug-13	filtered	3	1.84509804
3-Sep-13	unfiltered	1	1.414973348
3-Sep-13	unfiltered	2	2.017033339
3-Sep-13	unfiltered	3	1.73239376
3-Sep-13	filtered	1	1.612783857
3-Sep-13	filtered	2	0.77815125
3-Sep-13	filtered	3	1.505149978
10-Sep-13	unfiltered	1	1.954242509
10-Sep-13	unfiltered	2	2.113943352
10-Sep-13	unfiltered	3	1.698970004
10-Sep-13	filtered	1	1.698970004
10-Sep-13	filtered	2	1.301029996
10-Sep-13	filtered	3	2
17-Sep-13	unfiltered	1	1.146128036
17-Sep-13	unfiltered	2	2.10720997
17-Sep-13	unfiltered	3	1.973127854
17-Sep-13	filtered	1	2.146128036
17-Sep-13	filtered	2	2
17-Sep-13	filtered	3	2.041392685
24-Sep-13	unfiltered	1	2.544068044
24-Sep-13	unfiltered	2	2.826074803
24-Sep-13	unfiltered	3	2.681241237
24-Sep-13	filtered	1	3.439332694
24-Sep-13	filtered	2	3.426511261
24-Sep-13	filtered	3	3.404833717
1-Oct-13	unfiltered	1	2.491361694
1-Oct-13	unfiltered	2	2.428134794
1-Oct-13	unfiltered	3	2.480006943
1-Oct-13	filtered	1	1.857332496
1-Oct-13	filtered	2	1.832508913
1-Oct-13	filtered	3	1.86923172

Data: Fecal coliform concentrations for all irrigation water samples

Week	Treatment	Sample	Log10 CFU/100ml
27-Aug-13	unfiltered	1	2.161368002
27-Aug-13	unfiltered	2	2.301029996
27-Aug-13	unfiltered	3	2.161368002
27-Aug-13	filtered	1	2.929418926
27-Aug-13	filtered	2	3
27-Aug-13	filtered	3	3.278753601
3-Sep-13	unfiltered	1	2.161368002
3-Sep-13	unfiltered	2	2.397940009
3-Sep-13	unfiltered	3	2.301029996
3-Sep-13	filtered	1	1.977723605
3-Sep-13	filtered	2	1.954242509
3-Sep-13	filtered	3	1.954242509
10-Sep-13	unfiltered	1	1.954242509
10-Sep-13	unfiltered	2	1.954242509
10-Sep-13	unfiltered	3	1.954242509
10-Sep-13	filtered	1	1.977723605
10-Sep-13	filtered	2	2
10-Sep-13	filtered	3	2.397940009
17-Sep-13	unfiltered	1	2.544068044
17-Sep-13	unfiltered	2	2.544068044
17-Sep-13	unfiltered	3	2.653212514
17-Sep-13	filtered	1	2.929418926
17-Sep-13	filtered	2	2.929418926
17-Sep-13	filtered	3	2.77815125
24-Sep-13	unfiltered	1	2.176091259
24-Sep-13	unfiltered	2	2
24-Sep-13	unfiltered	3	2.301029996
24-Sep-13	filtered	1	2.389166084
24-Sep-13	filtered	2	2.397940009
24-Sep-13	filtered	3	2.602059991
1-Oct-13	unfiltered	1	2.602059991
1-Oct-13	unfiltered	2	2.544068044
1-Oct-13	unfiltered	3	3.021189299
1-Oct-13	filtered	1	3.113943352
1-Oct-13	filtered	2	3.079181246
1-Oct-13	filtered	3	3.161368002

Data: Total coliform concentrations for all irrigation water samples

Week	Treatment	Sample	Log10 MPN/100ml
27-Aug-13	unfiltered	1	2.495544338
27-Aug-13	unfiltered	2	2.440121603
27-Aug-13	unfiltered	3	2.537567257
27-Aug-13	filtered	1	3.383815366
27-Aug-13	filtered	2	3.383815366
27-Aug-13	filtered	3	3.383815366
3-Sep-13	unfiltered	1	3.383815366
3-Sep-13	unfiltered	2	3.383815366
3-Sep-13	unfiltered	3	3.383815366
3-Sep-13	filtered	1	3.383815366
3-Sep-13	filtered	2	2.861534411
3-Sep-13	filtered	3	3.383743576
10-Sep-13	unfiltered	1	3.354108439
10-Sep-13	unfiltered	2	3.773786445
10-Sep-13	unfiltered	3	3.271841607
10-Sep-13	filtered	1	4.991403303
10-Sep-13	filtered	2	4.812110841
10-Sep-13	filtered	3	4.812110841
17-Sep-13	unfiltered	1	3.019614716
17-Sep-13	unfiltered	2	3.298044843
17-Sep-13	unfiltered	3	3.113843119
17-Sep-13	filtered	1	3.383815366
17-Sep-13	filtered	2	3.383815366
17-Sep-13	filtered	3	3.383815366
24-Sep-13	unfiltered	1	3.561101384
24-Sep-13	unfiltered	2	3.745074792
24-Sep-13	unfiltered	3	3.631443769
24-Sep-13	filtered	1	5.383815366
24-Sep-13	filtered	2	5.383815366
24-Sep-13	filtered	3	5.383815366
1-Oct-13	unfiltered	1	3.651278014
1-Oct-13	unfiltered	2	3.57863921
1-Oct-13	unfiltered	3	3.537819095
1-Oct-13	filtered	1	4.613418945
1-Oct-13	filtered	2	4.588047497
1-Oct-13	filtered	3	4.613418945

Data: Generic *E. coli* concentrations for all irrigation water samples

Week	Treatment	Sample	Log10 MPN/100ml
27-Aug-13	unfiltered	1	1.947433722
27-Aug-13	unfiltered	2	1.995635195
27-Aug-13	unfiltered	3	2.033825694
27-Aug-13	filtered	1	3.150326536
27-Aug-13	filtered	2	3.049179245
27-Aug-13	filtered	3	2.937718444
3-Sep-13	unfiltered	1	2.536684673
3-Sep-13	unfiltered	2	2.738384124
3-Sep-13	unfiltered	3	2.487986331
3-Sep-13	filtered	1	1.887054378
3-Sep-13	filtered	2	0.491361694
3-Sep-13	filtered	3	1.57863921
10-Sep-13	unfiltered	1	1.037426498
10-Sep-13	unfiltered	2	1.086359831
10-Sep-13	unfiltered	3	1.190331698
10-Sep-13	filtered	1	1.227886705
10-Sep-13	filtered	2	1.164352856
10-Sep-13	filtered	3	1.130333768
17-Sep-13	unfiltered	1	1.688419822
17-Sep-13	unfiltered	2	1.877371346
17-Sep-13	unfiltered	3	1.702430536
17-Sep-13	filtered	1	2.25163822
17-Sep-13	filtered	2	2.176091259
17-Sep-13	filtered	3	2.321805484
24-Sep-13	unfiltered	1	2.171141151
24-Sep-13	unfiltered	2	2.162862993
24-Sep-13	unfiltered	3	2.331022171
24-Sep-13	filtered	1	2.454997217
24-Sep-13	filtered	2	2.269512944
24-Sep-13	filtered	3	2.267171728
1-Oct-13	unfiltered	1	3.080373917
1-Oct-13	unfiltered	2	2.937718444
1-Oct-13	unfiltered	3	3.150326536
1-Oct-13	filtered	1	3.19119942
1-Oct-13	filtered	2	2.964165311
1-Oct-13	filtered	3	2.991403303

Suggestions to CPS (optional)
None at this time