8-2014

Transfer and Survival of Microorganisms to Produce from Surface Irrigation Water

Stuart Jamison Gorman
University of Tennessee - Knoxville, sgorman2@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Stuart Jamison Gorman entitled "Transfer and Survival of Microorganisms to Produce from Surface Irrigation Water." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Faith J. Critzer, Major Professor

We have read this thesis and recommend its acceptance:

John R. Buchanan, Annette L. Wszelaki

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Transfer and Survival of Microorganisms to Produce from Surface Irrigation Water

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Stuart Jamison Gorman
August 2014

ABSTRACT

Surface water irrigation sources are widely used for fruit and vegetable crop production in the United States. Surface water is inherently prone to direct and indirect contamination with animal fecal material. Hence, the microbial quality of surface water sources can be highly variable. Water used for irrigation is considered a common source for produce contamination. In addition to this, fruits and vegetables are commonly consumed fresh or minimally processed, thus emphasizing the need for preventative measures in production of fresh produce. This study will examine transfer from naturally contaminated irrigation water to cantaloupes using drip and overhead spray irrigation methods. Additionally, the use of plots with bare ground or plastic mulch will be evaluated for contamination risk.

Water from a pond naturally contaminated with STEC was passed through a sand filter and used to irrigate cantaloupes. Cantaloupe plots contained cross-classified combinations of overhead or surface drip irrigation in addition to bare ground or plastic mulch raised bed preparation. Surface water was sampled from the source pond and from overhead spray emitters weekly across six consecutive weeks for enumeration of STEC, generic *E. coli*, and coliforms using routine enumeration methods. Cantaloupes were harvested and processed using a rinse technique across four consecutive harvest weeks. Cantaloupe rinsates were enriched and DNA was extracted. Microbial DNA from each cantaloupe was tested for the presence/absence of *stx* and *eae* genes using multiplex PCR.

No significant correlations were observed between STEC and any indicator organism in the irrigation water source. Cantaloupes were contaminated regardless of irrigation method and seedbed preparation with no significant differences between treatments. Contamination rates for bare ground plots with drip irrigation and plastic mulch plots with overhead spray irrigation were

20.4% and 19.7%, respectively. Positive samples were also found for bare ground plots with overhead irrigation (14%) and plastic mulch plots with drip irrigation (12%). Transfer was shown to occur in treatments using drip irrigation. In this study, generic *E. coli* was not found to be a suitable predictor of STEC levels in the pond water used for irrigation.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1. LITERATURE REVIEW

1.1. A GROWING DEMAND FOR FRESH FRUITS AND VEGETABLES

The popularity of fresh fruits and vegetables is on the rise in the United States. Health promotion aspects of fruits and vegetables make them an increasingly popular choice in many diets. Fresh produce can be a significant source of vitamins, minerals, and fiber, which are key components in healthy diets (*8*). The World Health Organization (WHO) recommends consuming a minimum of 400 grams of fruits and vegetables per day as part of a healthy diet that aids in preventing diseases such as heart disease, diabetes, and certain cancers (*138*). Over the past decades, increased consumer awareness and growth in public knowledge related to health and maintaining healthy diets have contributed to the increase in consumption of fresh produce (*31*). From 1976 to 2009, fresh fruit consumption has increased 25% (101.9 to 127.5 lbs/capita) while fresh vegetables have seen a 26% increase (145.3 to 182.9 lbs/capita) (*31*). This trend is expected to increase into the future (*70*). There are many factors driving the increase in consumption of fresh produce that is evident in the United States.

Access to nutritional research and information pertaining to healthy diets and healthy lifestyles has given the general public more nutritional knowledge than ever before. This has spurred interest in health conscience consumers as well as change in government policies in the form of nutritional guidelines. Due to these recent health promotion trends, consumers are demanding quality food and ingredients. This demand often translates into consumption of fresh and minimally processed foods to obtain the maximum nutritional benefit.

Expanding global trade and widening food distribution networks have fed a greater demand for fresh fruits and vegetables in the United States with imported produce from many

countries around the world available for consumption year-round. In addition to this, smaller market niches of organic and local farms have become more popular, adding to the diversity in supply for fresh fruits and vegetables. The increasing demand for fresh produce from local sources is met by the growing trend in farmer's markets and local food marketing. According to self-reported data gathered by the USDA, farmer's markets reported to operate in the United States have increased from 3,706 in 2004 to 8,144 in 2013 (*128*). As new supply options for fresh produce become available, consumers are able to purchase fresh produce from an increasing number of suppliers whose products may be of variable microbial quality. Foodborne illnesses associated with consumption of fresh produce may become more common as an indirect consequence of the increased demand for fresh produce.

1.2. FOODBORNE ILLNESS AND OUTBREAKS LINKED TO FRESH PRODUCE

Data analyzed from the Centers for Disease Control and Prevention (CDC) from 1973 to 1997 shows that the median number of reported foodborne illness outbreaks related to produce increased from two outbreaks per year in the 1970s to seven per year in the 1980s to 16 per year in the 1990s (*107*). According to the CDC, the number of yearly outbreaks associated with produce in the US doubled between 1973-1987 and 1988-1992 (*83*). From 1998 to 2008, produce-related foodborne illnesses accounted for nearly half (46%) of all reported foodborne illnesses (*89*). Furthermore, more illnesses were attributed to leafy greens during this period than any other commodity (*89*). Illness attributed to consumption of fresh produce can be associated with many pathogens, making produce safety an important concern for regulatory agencies. A study by Batz et al. (2012) estimated the disease burden associated with various food commodities and foodborne pathogens. Among twelve food categories, produce was ranked

fourth in cost of illness with approximately 1.4 billion dollars in estimated annual cost of illness (*7*).

This increase in foodborne illness related to produce commodities may be attributed to increased monitoring of foodborne illness and improved epidemiological tracking methods in the United States (*113*). However, reporting of foodborne outbreaks decreased from 2001-2010, while produce outbreak levels were relatively unchanged (*35*). Other factors contributing to produce outbreaks include increased importation of fresh produce into the United States, growing size of the "at-risk" population, widening food distribution networks, and popularity of salad bars and ready-to-eat food items (*36*, *51*, *69*, *107*).

Most fruits and vegetables are produced outdoors in open fields. In this environment, the crop is potentially exposed to enteric pathogens via irrigation water, soil, wildlife, manure, personnel, and other sources (*51*). Additionally, fresh produce is often consumed raw or minimally processed. With very little processing and a lack of inactivation steps, fresh fruits and vegetables can be substantial vectors for foodborne illness. Certain produce commodities that have contributed to more outbreaks are prioritized as foods with greater food safety risk. For foodborne illness that could be traced back to a single produce commodity, leafy vegetables carry higher risk, followed by fruits and nuts, and vine-stalk vegetables (*36*, *49*, *89*, *107*). Moreover, produce commodities are used in many complex foods such as sandwiches, salads, salsas, and mixed vegetable or fruit plates making it difficult to correctly track and estimate foodborne illness associated with consumption of fresh produce.

Various pathogens are responsible for the myriad of produce-associated outbreaks that occur. Among them, Norovirus, *Salmonella,* and *Escherichia coli* O157:H7 are responsible for a large percentage of produce-associated outbreaks (*6*, *36*). These top three enteric pathogens are

of fecal origin, thus underlining the need to reduce fecal contamination and cross contamination in pre-harvest, harvesting, packing, and holding operations. Many recent outbreaks involving *Salmonella* in fresh produce have been linked to the consumption of cantaloupe and alfalfa sprouts (*17*, *18*, *19*, *25*, *28*); while outbreaks involving Shiga-Toxigenic *E. coli* have been mostly associated with leafy greens such as spinach, lettuce, and sprouts (*20*, *21*, *22*, *23*, *26*). Some produce commodities have higher risk of contamination due to unique physical characteristics, growing environments, and chemical characteristics such as pH and presence of natural antimicrobial compounds. For example, the leaves of green leafy vegetables and the rinds of melons may provide microniches to harbor pathogenic bacteria (*32*, *124*). Some commodities like cantaloupes are grown in close proximity to the soil and have a greater chance of contracting pathogens from the soil environment.

Recent publications have focused on ranking the pathogen-food combinations with the greatest burden on public health (*4*, *6*). Using this data, regulating authorities and researchers can prioritize areas that need more focus than others. Among the pathogen-food pairs in the produce category, enterohemorrhagic *E. coli* (EHEC) in leafy greens consistently ranked first, followed by *Salmonella* spp. in tomatoes, and *Salmonella* spp. in leafy greens (*4*). Numerous multi-state outbreaks associated with various produce items have led the produce industry to adopt safer farming practices. Specifically, foodborne illness outbreaks associated with the consumption of contaminated cantaloupes have been more prevalent in the past decade. There have been four multistate outbreaks involving cantaloupe since 2008 (*19*, *24*, *27*, *28*). Two of these outbreaks were traced back to cantaloupes originating from foreign countries, while the other two outbreaks were traced back to farms in the United States. The specific sources of contamination have not yet been identified for any of these outbreaks. Many outbreaks result from pre-harvest

contamination of crops leading governmental regulatory agencies and farmers to focus on more stringent farming methods and record keeping during primary production.

1.3. IRRIGATION WATER AS A SOURCE OF PRODUCE CONTAMINATION

1.3.1. Irrigation Water Sources in the United States

Any agricultural input that comes into contact with fresh produce has the potential to cause contamination. For this reason, agricultural water used for irrigation and frost protection must be of suitable microbial quality. Although contamination of produce crops can occur at any time in the production chain, irrigation water and manure are considered the most common sources of contamination (*45*).

In the United States, 70% of commercial farmland is irrigated (*131*). According to the 2008 USDA-NASS National Farm and Ranch Irrigation Survey, total acreage of irrigated land in the United States increased from 52,492,687 acres in 2003 to 54,929,915 acres in 2008 (*130*). Farming operations in the United States utilize various sources for irrigation water. Water sources may likely include treated water or municipal water, groundwater, reclaimed or recycled water, surface water, or a mixture of sources (*38*). Municipal water sources are of the highest microbial quality for irrigation purposes because they have been treated to be safe for drinking.

Groundwater, usually in the form of well water, is generally of suitable microbial quality due to natural filtration through soils (*47*). However, all groundwater sources are potentially susceptible to contamination by a number of ways. The depth, location, and construction of the well can affect the microbiological quality of the well water (*47*). Unprotected wells can allow for runoff contamination during a storm event. Leaching of pathogens from latrines, septic tanks, sewer lines, and unlined landfills can also contaminate groundwater (*47*).

Surface water sources include lakes, rivers, streams, creeks, ponds, and reservoirs. Surface water is generally of questionable microbial quality because surface water is subject to direct contamination by wildlife or indirect contamination by runoff and flooding (*48*). Surface water sources are generally economically feasible solutions for irrigating. In the United States, the use of well water for irrigation increased 12% and use of surface water increased 22% between 2003 and 2008 (*131*).

1.3.2. Pathogen Transfer via Irrigation Water

The transfer of pathogens from contaminated irrigation water to produce surfaces is well documented (*30*, *62*, *111*, *114*). Pathogens such as *E. coli* O157:H7 have been shown to survive in groundwater for 58 days (*96*) and greater than 12 weeks in 8°C municipal water (*133*). Pathogen survival in water depends on water temperature, particle matter, soluble organic matter, and sunlight (*47*). Additionally, materials used in irrigation delivery systems have been shown to influence microbial survival in irrigation water (*104*).

Once contaminated water is distributed for irrigation, or other farm practices involving foliar contact (*e.g.* pesticide and herbicide sprays), contamination can occur by contact with above ground plant surfaces or indirectly by splashing from the soil (*45*). Some studies have even suggested the internalization through uptake of water via the root system (*34*, *111*). However, more recent studies suggest that pathogen internalization may be a rare event that may be dose dependent (*41*, *74*). Once contamination occurs, several factors including environmental conditions, crop type, and strain of bacteria can affect the survival and overall persistence of pathogens on produce. In addition, time elapsed between the most recent irrigation event and harvest can determine the degree of crop contamination for certain pathogens (*113*). Some pathogens have been shown to survive on plant surfaces for the entire growing season (*62*, *110*).

Moreover, recent research suggests that pesticides introduced with source water may promote growth of *Salmonella* and elevate food safety risks of foliar contact water (*73*). In this study, *Salmonella* applied to field grown tomatoes during pesticide application was found to survive up to 15 days on tomato surfaces. To correctly evaluate the risks of using irrigation water of poor microbiological quality, more studies need to be designed to enumerate pathogens on produce surfaces as a function of environmental conditions and time elapsed since irrigation.

1.3.3. Risk Associated with Surface Water Irrigation Sources

The expense of using municipal or lack of availability of well water may encourage many farmers to use surface water sources to irrigate cropland. Using surface water as an irrigation source carries greater risk of contamination. The major sources of pathogens associated with fresh produce are human and animal feces (*11*). Animal and human activity has been shown to directly contaminate surface water sources. For example, river water used for irrigation can be contaminated by upstream human wastewater effluent, wastewater from livestock operations, and use by wildlife such as cattle and deer. Moreover, surface water is susceptible to intermittent nonpoint source runoff resulting from heavy rainfall or flooding (*47*). Agricultural, industrial, and residential waste streams can be point sources that directly contaminate a waterway, while nonpoint source runoff occurs most commonly by rainfall events where pollutants, sewage, or fecal material are drained into bodies of water (*87*, *114*, *118*). Drainage of excess irrigation water can also cause nonpoint source runoff.

In surface waters, the greatest pathogen loads are accumulated after rainfall events (*47*). In the same way, flooding can contaminate surface water sources to a great extent (*14*). Sediments found in surface water are more likely to contain high levels of microorganisms due to attachment to suspended solids and subsequent settling (*47*). Disturbance by heavy rainfall or

flooding can re-suspend sediment that may contain pathogenic microorganisms (*84*, *117*). Fish and other associated wildlife that reside within a surface water source may also contribute to contamination (*117*). Finally, physical properties of surface water sources, such as temperature and pH, may affect the growth and levels of various microbial populations (*57*, *71*, *78*). Due to the unpredictable nature of surface water contamination, microbial quality of a surface water source can be highly variable and should be closely monitored (*48*).

1.3.4. Irrigation Delivery Methods

Water is able to carry many types of microorganisms including enteric pathogens and infectious virus (*78*, *109*, *111*, *122*). As a vehicle for microbial transfer, the quality of water used in agricultural practices directly dictates the potential for microbial contamination (*51*). Furthermore, the method and timing of application can directly affect potential pathogen transfer (*38*, *113*).

Common irrigation methods employed in the United States are furrow or flood irrigation, sprinkler or overhead irrigation, and microirrigation, which include surface drip and subsurface drip irrigation (*47*). Depending on the type of produce, irrigation methods can greatly influence the degree of crop contamination (*48*, *117*). Irrigation methods that do not allow water to contact the edible portion of the plant are generally considered to have less contamination risk (*38*, *84*). For above ground crops, drip irrigation methods can provide less risk of contamination. For lettuce irrigated with water contaminated with *E. coli* O157:H7, drip irrigation methods had significantly less (19%) contamination compared to overhead irrigation (91%) (*110*). Nevertheless, pathogen transfer to produce has been documented when only using furrow and subsurface drip irrigation methods (*112*, *116*). Song et al. (2006) showed that using furrow irrigation methods generally resulted in higher lettuce contamination rates than subsurface drip

irrigation when using water contaminated with *E. coli*, coliphage PRD-1, and *Clostridium perfringens* (*112*). More research using specific irrigation methods is needed to determine the relative risk associated with each.

1.4. SURVIVAL OF ENTERIC PATHOGENS ON FRESH PRODUCE

1.4.1. Survival of Enteric Pathogens in Soils

Recent outbreaks involving fresh vegetables have implicated soil as a vehicle for pathogen transmission (*37*). Modeling pathogen survival in soils can lead to better risk assessment and produce safety standards. The ability of a pathogen to survive in soil may depend on pathogen strain, soil type, crop type, and the environmental conditions that are present (*37*, *85*). Pathogens may survive longer in soils with increased moisture and lower temperatures (*37*). *E. coli* and *Clostridium perfringens* survived longer in subsurface soils compared to surface soils (*112*), which may be due to the increased temperature and lack of moisture in surface soils. *E. coli* O157:H7 was found to survive for over 60 days in conventional and organic soils (*103*), but has been reported to survive up to 500 days in frozen soil (*37*). It has been suggested that some pathogens such as *Salmonella* are able to adapt more readily to the harsh and competitive soil environment (*46*, *134*).

1.4.2. Persistence of Foodborne Pathogens on Produce Surfaces

In the preharvest environment, fruits and vegetables can become contaminated in a variety of ways. However, to become a public health threat the pathogens must be able to persist on or in the crop at the point of consumption (*45*). The surface of fruits and vegetables can be a harsh and inhospitable environment for enteric pathogens (*134*). Survival and subsequent colonization of foodborne pathogens on produce is influenced by the environmental conditions, physiological state of the plant, pathogen, and produce type (*32*, *55*).

Free moisture that is present from rainfall, condensation, or irrigation may promote microbial survival and growth on plant surfaces (*55*). Under conditions of high moisture and warm temperature, growth of *Salmonella enterica* was reported on leaves of cilantro (*15*). Some fruits and vegetables, such as melons, berries, and lettuce, and seed sprouts, have physiological features that are more conducive to attachment and microbial persistence (*55*, *113*). For example, the raised, netted rind of cantaloupes can provide a surface that is readily available for microbial attachment and allows pathogens to be protected from postharvest rinses and sanitizing washes (*124*, *126*, *127*). *E. coli* O157:H7 has been reported to survive in the phyllosphere of lettuce and parsley for up to 77 and 177 days, respectively (*62*).

The ability of pathogens to attach to the plant or fruit surface influences potential pathogen colonization in the plant phyllosphere (*32*). Due to heterogeneous physicochemical conditions on plant surfaces, pathogens may find microniches on the plant surface where survival or growth is favorable (*45*). For example, *E. coli* O157:H7 inoculated onto lettuce leaves was shown to survive longer on the underside of the leaf (*140*).

1.5. EFFECTS OF FARMING PRACTICES ON MITIGATING FRESH PRODUCE CONTAMINATION

1.5.1. Good Agricultural Practices (GAP's)

To minimize the risk of contaminating fresh produce with human pathogens, pre-harvest strategies such as the implementation of GAP's are being emphasized. The term "good agricultural practices" refers to general practices used to minimize microbial food safety hazards during growing, harvesting, sorting, packing, and storage operations (*122*). In 1998, the FDA issued *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* (*122*), which outlines good agricultural and good manufacturing practices

with recommendations and suggestions for worker health and hygiene, use of water, use of manure and municipal biosolids, field sanitation, facilities, and transportation. Many producers and packers have adhered to the guidance from FDA issued guidance documents. However, the FDA's guidance documents do not carry the force of law and being GAP compliant relies on a farm to be audited and GAP certified by an auditing agency. Currently, GAP auditing is voluntary and independently chosen by produce suppliers who want to be GAP certified. Many large retailers require their produce suppliers to be GAP-compliant providing incentive for producers to be audited. Additionally, some organizations for specific commodities have formulated commodity-specific GAP's. For example, all member companies of the Leafy Greens Marketing Agreement (LGMA) are subject to mandatory government audits by the California Department of Food and Agriculture.

A large degree of oversight is needed to audit operations across the United States; consequently, there are several auditing agencies that perform GAP audits. General GAP auditing schemes may differ by auditing agency making it difficult to determine compliance to specific guidance. Auditing agencies certify an operation using a single snapshot of the day the operation was audited in addition to record review. Therefore, it is difficult to enforce GAP compliance after a grower or producer has been GAP certified. Fresh produce from small farms that cannot afford to be audited still continues to be consumed in the United States. Furthermore, the increasing demand for fresh fruits and vegetables, the increasing popularity in local farming and farmers' markets, and consumption of imported produce varieties allows a wide market niche for producers that are not GAP audited.

1.5.2. Growing and Cultivation Methods

While many cultivation methods and soil preparations are employed to benefit crop health and yield, they may also have an impact on crop contamination risk. Plasticulture in fruit and vegetable crop production uses plastic materials to modify the production environment (*101*). Specifically, plastic films applied to cover the soil can increase soil temperature, increase soil moisture, and maintain soil tilth (*93*). Plastic films can create a barrier between the soil layer and the edible portion of the crop protecting the fruit or vegetable from contact with soil moisture and pathogens (*101*). Reflective plastic films have been successfully used to reduce aphid colonization and subsequent transfer of aphid-borne viral disease (*93*, *101*). Greenhouses, row covers, and tunnels can also be an effective means of insect and pest control (*101*).

The use of contaminated livestock wastes, such as manure and manure slurry, is believed to be a major source of crop contamination by pathogens carried by ruminant and non-ruminant livestock (*82*, *85*, *111*). Untreated biological soil amendments like manure should be properly composted or treated before being applied to production of human food or a sufficient time interval should be used from application to harvest to ensure that there are no surviving pathogens. Standard minimum time intervals between application of untreated manure and harvest have been debated. In soils amended with poultry manure compost and dairy manure compost, *Salmonella* Typhimurium survived for up to 231 days (*63*). In conventional and organically managed soils amended with manure, *E. coli* O157:H7 was found to survive for at least 60 days (*103*). Currently, the FDA has proposed standards for untreated biological soil amendments. FDA has proposed a nine-month minimum time interval between application and harvest when amending soil with untreated manure (*121*).

1.6. FOOD SAFETY MODERINZATION ACT (FSMA 2011)

The Food Safety Modernization Act (FSMA) was signed into law on January 4, 2011. This law attempted to connect the gaps in the national food safety network by giving the United States Food and Drug Administration (FDA) additional authority to regulate food facilities, recall contaminated food products, oversee imported foods, and establish food safety standards for produce (*120*). The framework of the FSMA emphasizes prevention of foodborne illness rather than response. Thus, the FSMA seeks to minimize foodborne illness risks by using science-based risk assessment data to implement food safety practices (*6*, *13*).

1.6.1 FDA Proposed Produce Safety Rule (Jan. 2013)

Responding to the FSMA, in January 2013, the FDA released a proposed regulation: Title 21 CFR Part 112: *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption* (*121*). In the proposed legislation, the FDA recommends science-based minimum standards for fruit and vegetable production. With this guidance, the FDA emphasizes issuing guidance for specific commodities, maintaining adequate record-keeping, and increased surveillance (*121*). Additionally, the FDA discusses collaborations and partnerships to research emerging food safety issues and foster GAP compliance.

As a primary source of produce contamination, standards for agricultural water are addressed in Subpart E of the rule (*121*). Those subject to this proposed legislation must test all water that is likely to contact the harvestable portion of produce or food-contact surfaces. All growers will be required to record test results at the beginning of every growing season and every three months during production (*121*).

For irrigation water, no testing is required for growers using treated or municipal water. Furthermore, there is no standard for non-direct water applications such as surface and

subsurface drip systems. 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 milliliter single sample or less than 126 *E. coli* in a five-sample rolling geometric mean. The frequency of irrigation water testing will depend on the water source and the risks of environmental contamination. Farms using untreated surface water prone to runoff must record test results every seven days. Those using untreated surface water that is not subject to runoff must record test results monthly (*121*).

The produce safety rule was designed to help the produce industry by enacting preventative measures to combat foodborne illness, yet adhering to these rules may be cumbersome to farmers and those affected by the proposed rules. More research will be needed to fill the existing gaps in knowledge pertaining to surface water hygienic quality, irrigation methods, and the use of indicator organisms to detect pathogen presence. The first comment period, in which the general public is allowed to comment on provisions of the FDA's proposed produce safety rule, closed November 22, 2013. The FDA has agreed to republish parts of the produce safety rule, including subpart E on agricultural water, for further public comment. The target date for the final publication of this legislation has been extended, however, compliance will not be mandatory at that point.

1.7. MONITORING IRRIGATION WATER QUALITY

1.7.1. Microbial Indicator Organisms

In the absence of risk-based data on irrigation water, a universal measurement for water contamination is needed to determine public health risk associated with water sources (*117*). Routine water examination for the presence of specific enteric pathogens is often an expensive and time-consuming task (*47*). First defined to assess drinking water quality, microbiological analysis of indicator organisms in water has been the method of choice for assessing

microbiological water quality (*52*). Indicator organisms are not human pathogens, but the presence of an indicator organism or group of indicator organisms in a body of water may indicate the presence of fecal contamination (*52*). An ideal indicator organism can be isolated from all water types, occurs alongside pathogens, is found in higher concentrations than pathogens, and is more resistant than pathogens (*52*). Additionally, the density of the indicator organism should correlate to the degree of contamination and relate to the health risk of a water source (*38*, *52*). The most commonly used indicator organisms are enterococci, total coliforms, fecal coliforms, generic *E. coli*, and coliphages (*121*).

The coliform group of bacteria is defined by biochemical properties and growth characteristics. Coliforms are Gram-negative, aerobic or facultative anaerobic, non-sporeforming, rod-shaped bacteria that undergo lactose fermentation to form carbon dioxide gas and acid at 35-37°C within 48 hours (*5*). Coliforms include bacteria from many genera, such as *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* (*47*). Coliform bacteria normally occur in the intestines of warm-blooded mammals and are heavily excreted in animal feces (*47*), making them a logical choice for indicators of fecal contamination. However, there are some coliforms that are naturally present on plants and in soils (*38*, *47*).

Fecal coliforms, a subset of total coliforms, are differentiated in their ability to ferment lactose with the production of acid and gas at 44.5 °C within 24 hours (*52*). Recently, this group has been more accurately termed “thermotolerant” coliforms because they differ from total coliforms by higher optimal growth temperature and not necessarily origin (*38*, *119*). Coliform and fecal coliform bacteria have been reported to frequently occur and survive for extended periods of time in unpolluted tropical waters (*108*). Research has suggested that these organisms

occur naturally in tropical waters emphasizing the need for new indicators in these waters (*56*, *108*).

Escherichia coli, a thermotolerant coliform, is more consistently associated with fecal contamination than other indicators (*119*, *121*). *Escherichia coli* is a member of the family *Enterobacteriaceae* that includes genera of known pathogens such as *Salmonella*, *Yersinia*, and *Shigella* (*42*). The absence of urease and the presence of β-glucuronidase allow *E. coli* to be easily distinguished from other fecal coliforms (*47*). Due to a number of commercial products, generic *E. coli* can be rapidly detected and enumerated at a relatively low cost compared to detection of pathogenic microorganisms (*121*) and is emerging as the choice indicator for fecal contamination. However, due to frequent water testing requirements, the expense of these detection methods still may be taxing for many fruit and vegetable producers. In contrast to other thermotolerant coliforms, generic *E. coli* occurs in the environment less frequently in the absence of fecal contamination (*119*). Several proposed standards for microbial water quality focus on the use of generic *E. coli* as an indicator for fecal contamination of a water source (*68*, *81*). Other indicators of fecal contamination such as enterococci, *Clostridium perfringens*, and *Enterococcus faecalis* are being used mainly for their ability to persist in water in certain regions of the world (*3*, *52*).

1.7.2. Physicochemical Water Parameters

Physicochemical water measurements can be performed more rapidly than conducting microbial analyses of water. These physicochemical water parameters typically include, conductance, turbidity, pH, temperature, and oxidation-reduction potential (ORP) (*78*). These parameters are characteristics of water that may describe the environment in which a pathogen might be present. Although these measurements have been used to monitor the microbiological

quality of drinking and recreational water, they have been reported to be poor indicators for pathogen presence in surface waters (*78*). Weak correlations among pathogens and measurements such as ORP, turbidity, and conductivity suggest that other physicochemical factors may be involved (*78*). The relationships among pathogens, indicator organisms, and water characteristics are complex and may be influenced by a variety of factors. Due to spatial and temporal variations in water chemistry, it may be difficult to accurately assess the true physicochemical conditions of a water source without high sampling frequency (*106*, *136*).

1.7.3. Methods for Microbial Enumeration in Water Samples

While many indicator organisms are being utilized for various purposes, the test methods to detect them must be reliable and readily available. By exploiting physiological and biochemical attributes of target organisms, test methods can be developed to specifically detect and identify organisms of interest. The American Public Health Association has developed standard methods for the microbial analysis of water. These widely used methods can be found in The Standard Methods for Water and Wastewater Analysis (*5*).

MPN or most probable number is a statistical method for enumerating microorganisms in a sample. The presence or absence of organisms in serial dilutions is used to estimate the concentration of bacteria in a sample (*38*). Most probable number methods do not directly measure the bacterial population in a sample. Due to this, results tend to be more variable than direct plating methods (*97*). Novel modified MPN methods such as Colilert® and Colisure® have been developed specifically for the detection of *E. coli* and coliforms (*61*). In these procedures, a water sample is mixed with Colilert® or Colisure® powder creating a color change for coliforms and ultraviolet fluorescence for *E. coli* within 24 hours. These methods can be used in conjunction with Quanti-Tray® and Quanti-Tray 2000® to statistically quantify *E. coli* and

coliforms in a water sample detecting down to one organism per 100 milliliters (*61*). For quantification, a water sample containing either Colilert® or Colisure® is distributed across wells in a plastic tray by an automated sealer and incubated. Counting the number of wells with positive color change or fluorescence and consulting an MPN table allows the user to estimate the concentration of coliforms or *E. coli* in a water sample. The Colilert® and Colisure® methods have been validated by the FDA and EPA for water testing claiming to have 95% confidence intervals comparable to membrane filtration techniques (*61*). Another study suggests that while these methods may be similar in sensitivity to membrane filter and direct plating techniques, specificity is slightly lower (*59*).

Direct plating methods provide an indirect count of bacteria in a water sample by cultivation on a solid agar medium containing specific nutrients for growth, selection, or differentiation of microorganisms. Direct plating techniques may involve spreading a small volume of liquid on the surface of solid agar or mixing a volume of liquid sample into liquid agar and allowing solidification to occur resulting in a more anaerobic environment. In addition to the substances that comprise the agar medium, growth conditions such as temperature, humidity, and oxygen availability will direct the growth of certain organisms. Single cells or groups of cells form visible colonies in the agar medium and are counted as colony forming units (CFU's). The number of CFU's counted in the agar medium is used to estimate the number of microorganisms in the original sample by accounting for dilution factors used prior to plating. These concentrations are expressed as CFU's per milliliter of liquid sample or per gram of solid sample.

Membrane filtration is another standard method that can be used to enumerate bacteria in a water sample. The most widely used method for testing drinking water (*97*, *102*); membrane

filtration can concentrate bacteria in a sample with low initial bacteria levels. Using a membrane filtration method, water is vacuum-filtered through a porous membrane filter while microorganisms are trapped on the filter. The filter is then placed on a solid agar medium and incubated allowing the entrapped bacteria to grow utilizing the nutrients on the agar surface. When using membrane filtration, the ability to examine of larger volumes of water is a significant advantage, which can increase sensitivity and reliability of the method (*77*, *97*). Although, membrane filtration techniques are widely used, the filtering process along with preparation of selective and differential media for use with membrane filters can be expensive and time consuming (*102*). Moreover, organic matter and sediments entrapped on a filter can alter results by providing nutrient sources not normally present in a particular selective or differential growth medium (*77*). Finally, water samples with high background microflora can overcrowd a filter making it difficult to enumerate target organisms and allowing unwanted bacteria to outcompete organisms of interest; thus reducing plate counts (*77*).

3M™ Petrifilm™, a sample-ready culture medium, was developed for the detection of *E. coli* and coliforms in the food and dairy industries (*33*, *98*). Petrifilm™ plates use a thin layer of cold-water-soluble gelling agent with nutrients and biochemical indicators to provide a simple, low cost culture medium (*77*). The relatively low cost, which can be as low as $1.04 USD per plate (*77*), allows for reduced operating costs compared to traditional methods (*1*).

Results from Petrifilm™ products have been compared to standard methods such as membrane filtration, conventional agar plating, and modified MPN methods such as Colilert® and Colisure® (*59*, *77*, *102*, *132*). A study comparing Difco mFC agar with Petrifilm™ EC plates found that typical fecal coliform colonies on Petrifilm™ EC plates were confirmed fecal coliforms more often (87.1%) than colonies of mFC plates (68.5%) (*102*). When comparing

Petrifilm™ EC plates to membrane filtration onto m-Endo agar, Colilert®, and Colisure®, Petrifilm™ EC plates were found to have the highest specificity (90.9%) and also the lowest sensitivity (39.5%) due to its low sample volume of 1 milliliter (*59*). Although Petrifilm™ has significantly high correlations with the results from other test methods for water sampling, the small amount of inoculum may lower overall test sensitivity when dealing with samples of low initial levels of bacteria (*59*, *102*, *132*). Currently, Petrifilm™ products are widely accepted and validated for microbiological analysis of food and beverages (*1*). Petrifilm™ methods are simple, inexpensive, and can be performed reliably with limited equipment and training making them a suitable choice for water sampling purposes (*77*, *102*, *115*, *132*).

Alternative methods that rely on enzymatic activities of *E. coli* and coliforms, such as Colilert® and Petrifilm™, provide specificity and slightly reduced incubation time. However, these methods can be more expensive than traditional methods and incubation time is still too long for same-day results (*97*). Molecular methods that allow for specific and rapid detection of indicator organisms without the need for cultivation have been proposed. Immunological methods such as enzyme-linked immunosorbent assays (ELISA) and immunofluorescent assays (IFA) can provide specific and sensitive detection along with rapid quantification, but are limited by the amount of cross-reactivity between commercial antibodies and non-targeted cells (*97*). Nucleic acid-based methods such as fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) can be performed rapidly and provide even greater detection specificity. These methods, however, require dedicated laboratory equipment and reagents, skilled personnel, and can be expensive for routine use making them less suitable for use as a standard method for testing water quality (*97*). Alongside the need for frequent and persistent water

monitoring remains the need for economically feasible test methods that can be easily performed and interpreted allowing a quicker response for health related issues.

1.8. MICROBIOLOGICAL RECOVERY AND ENUMERATION FROM FRESH FRUITS AND VEGETABLES

1.8.1. Methods for Sampling and Recovery

The method of microbiological recovery can have significant effects on subsequent microbial enumeration. Most food matrices are complex and recovery methods can vary depending on the food type. For sampling the entirety of a food item, portions or whole food items can be processed into a homogeneous mixture with buffered medium to obtain a representative sample. With the exception of microbial internalization in fruits and vegetables, contamination most commonly occurs on the surface or exterior of the fruit or plant part. When sampling whole raw fruits and vegetables such as tomatoes and herbs, recovery may be decreased by release of acids or antimicrobial compounds during sample processing (*9*, *16*). Similarly, nutrients and carbon sources within fruits and vegetables may likely be released into the homogenate, possibly promoting favorable conditions for certain microorganisms. For these reasons, methods for processing samples may depend on the type of produce and suspected location of contamination (*58*). One particular study used a cork borer and a sterile cutting knife to create circles of only cantaloupe rind for sample processing (*125*). When sampling food surfaces, carriers like rinses, swabs, sponges, and adhesive tape can also be used to obtain surface samples (*54*). In addition to this, impression techniques can be used to directly contact the food surface with the surface of a growth medium. However, impression techniques should not be used when sampling surfaces with high microbial loads, as dilutions are not possible with impression techniques (*54*).

A variety of sample preparation methods for enrichment or direct plating of raw fruit and vegetable samples are being utilized by researchers, regulatory agencies, and analytical testing services. Common sample processing methods include homogenizing, blending, stomaching, macerating, shaking, and rubbing (*16*). Many factors are involved in raw fruit and vegetable sample preparation and a single method may not be able to achieve maximum recovery from all fresh fruits and vegetables. When sampling cantaloupe rinds for recovery of *Salmonella* and native microflora, homogenization with a blender rather than a stomacher resulted in greater recovery (*125*). However, a study sampling the surfaces of 26 different fresh produce items found no significant difference in microbial recovery between washing, stomaching, and homogenizing (*16*). Further research comparing sampling methods for fresh produce is needed to determine which methods are suitable for certain produce commodities.

A wide variety of structural and surface morphologies are exhibited in fruits and vegetables. Some fruits and vegetables, particularly melons such as cantaloupe, have surfaces that are more conducive to microbial attachment and growth than others (*80*, *124*). The ability of wash solutions to recover microorganisms can be reduced by surface irregularities of cantaloupes, such as crevices, roughness, and pits, which favor microbial attachment (*123*, *124*). Using surfactants, which reduce surface tension, has shown to increase sanitizer efficacy on fresh fruits and vegetables (*10*, *94*). On the other hand, a separate study reported that surfactants at 0.1% concentration were no more effective in removing microorganisms from produce surfaces than water (*95*). Using surfactants to improve wettability of hydrophobic crevices present on fruit and vegetable surfaces may be promising for sampling methods as well as for sanitizing.

1.8.2. Common Methods for Microbial Enumeration

Once fruit and vegetables samples have been collected and processed, there are a number of ways to detect and enumerate target microorganisms. Food producers and public health laboratories commonly focus on detection instead of enumeration where presence/absence results are sufficient. However, quantitative data gained from enumeration assays can be useful for microbial risk assessment and provide beneficial epidemiological data (*58*). Many rapid and improved methods for detection and enumeration are available, but have not yet been evaluated thoroughly for reliability (*12*, *97*, *72*, *50*).

The use of colony counting with selective and differential solid agar media is a common microbiological enumeration method. To determine the number of target bacteria in a product, suspensions from a sample are diluted, inoculated onto a growth medium, and the resulting colonies are counted after incubation (*64*). Sensitivity and specificity vary greatly among commercial media products depending on the organism being cultured. Stressed and wounded organisms can be difficult to recover without the use of an agar overlay technique or enrichment steps (*77*, *97*). Incorporating chromogenic and fluorogenic enzyme substrates into media can help selectively isolate and differentiate foodborne pathogens of interest eliminating the need for further biochemical testing to establish identity (*76*). These substrates are acted on by specific enzymes produced by microorganisms and change color or fluoresce upon enzymatic cleavage of the substrate (*97*, *76*). The possibility of high background microflora and the occurrence of false positives are the principle disadvantages associated with chromogenic and fluorogenic media (*58*). Organisms such as Shiga Toxigenic *E. coli* (STEC) are present at much lower levels than natural microflora in the environment and dilutions to reduce background may also dilute the target organism to below enumerable levels (*58*, *77*). These media may be supplemented with

additional antibiotics or other selective agents to further increase specificity (*50*), however, these additions may reduce sensitivity by impeding growth of stressed target organisms. Although traditional agar plate methods are low cost and easy to use, they lack the sensitivity and rapidity of alternative molecular methods (*50*).

PCR or polymerase chain reaction allows a gene sequence from a sample to be amplified and subsequently detected for strain specific confirmation. Quantitative real-time PCR (qPCR) reactions can amplify a genetic sequence and detect the quantity of gene product in real time using labeled DNA probes that produce a fluorescent signal upon each round of amplification (*79*). DNA quantification is based on the exponential increase of initial DNA amount along with the amount of cycles performed (*75*). Using genetic characteristics allows for higher sensitivity and specificity than conventional culture-based methods where even slow growing or viable but non-culturable cells are detected. On the other hand, one of the major drawbacks of enumerating with PCR assays is the detection of non-viable or noninfectious cells where the presence of a genetic sequence is not indicative of gene expression or viability (*58*). Reverse transcriptase PCR can be used to monitor gene expression in RNA extracted from produce samples, however, it requires extensive methods for extraction and purification due to the vulnerability of RNA to degradation. Also, free DNA and DNA from dead cells can be inactivated with propidium monoazide and ethidium monoazide prior to quantitative real-time PCR for quantification of solely viable cell DNA (*90*). With regard to STEC, multiple virulence genes must be detected to determine virulence, but most extractions do not occur from a pure culture so the genetic sequences could have originated from different cells (*58*).

Finally, PCR reactions are inhibited by a wide variety of substances found in food items (*64*, *139*), and some PCR inhibitors are known to exist in fresh produce (*58*). For example,

polyphenolic compounds found in many fruits and vegetables have been shown to decrease PCR sensitivity (*135*). Additionally, unripe fruits and some vegetables contain tannic acids, which are known PCR inhibitors (*100*). Currently, quantitative real-time PCR methods for enumeration are limited to samples with higher microbial loads of at least 10^3- 10^4 cells per gram (*64*). Bacterial concentration methods, such as the use of immunomagnetic beads and metal hydroxides for isolation, can enhance enumeration sensitivity of subsequent qPCR assays (*64*). Since an enrichment step is prohibited for enumeration purposes, more focus needs to be placed on sample preparation for greater cell recovery and highly purified template DNA. With the continued development of sample preparation and sample concentration techniques, qPCR methods are becoming more reliable to use for bacterial enumeration (*79*, *90*).

Quantifying bacteria present on fruit and vegetable surfaces can help to set industry standards and limits regarding acceptable levels of microorganisms in water used for irrigation, frost protection, and application of herbicides and pesticides. To effectively evaluate transfer and assess the risk from a contaminating vector to a produce commodity, reliable quantitative data should be collected from a sufficient number of samples to distinguish acceptable levels from unacceptable levels of pathogens in the farm environment. Due to the wide range of pathogens, natural microflora, and heterogeneous components associated with various fruits and vegetables, specific methods for sampling, detection, and enumeration must be chosen with regard to the produce commodity and organism of interest.

CHAPTER 2. TRANSFER AND SURVIVAL OF MICROORGANISMS TO PRODUCE FROM SURFACE IRRIGATION WATER

2.1. 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.

2.2. INTRODUCTION

Surface water is widely used for farming operations in the United States (*131*). Between 2003 and 2008, the use of surface water on farms increased 22% (*131*). 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 (*48*, *78*). 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 (*121*). 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 (*57*, *60*, *78*, *92*). 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 (*93*). 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 (*101*). 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 (*89*, *91*). *E. coli* O157:H7 was found to survive for over 60 days in conventional and organic soils (*103*), but has been reported to survive up to 500 days in frozen soil (*37*). Another study documented the survival of *E. coli* O157:H7 on lettuce and parsley leaves for 77 and 177 days, respectively (*62*). Pathogenic *E. coli* strains have been shown to differentially attach to a variety of plant parts, whereas non-pathogenic *E. coli* K12 could not (*65*). 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 (*118*, *124*, *127*). 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* 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.

2.3. MATERIALS AND METHODS

2.3.1. 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.

2.3.2. 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 (*66*). 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.

2.3.3. 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.

2.3.4. 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.

2.3.5. 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.

2.3.6. 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.

2.3.7. 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.

2.3.8. 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.

2.3.9. 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.

2.3.10. 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 (*129*).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.

2.3.11. 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.).

2.3.12. 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.

2.4. RESULTS

2.4.1. 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.

2.4.2. 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.

2.4.3. 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.

2.4.4. 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.

2.4.5. 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 α=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.

2.5. DISCUSSION

Several studies have assessed the transfer from artificially contaminated irrigation water to field crops (*41*, *44*, *62*, *63*, *82*, *99*, *110*, *111*, *112*). 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*.

2.5.1. 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 regardless of organism viability.

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 (*57*). 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 zero-tolerance organisms to grow in enrichment indicates the food safety risk associated with the contaminated melons.

2.5.2. Comparing production methods

Overhead irrigation methods are generally regarded as a higher risk for contamination, as water is distributed onto the edible portion of aboveground crops (*53*, *62*, *112*), compared to non-direct applications such as surface and subsurface drip irrigation (*40*, *43*, *82*, *86*, *110*). 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 (*43*, *82*, *110*). 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 (*99*). 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 (*29*, *71*). Moreover, plastic mulch has been found to have an increased splashing effect from simulated rainwater compared to bare ground plots (*29*). 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 (*44*, *67*, *99,116*). 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 (*112*). 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.

2.5.3. Irrigation water quality from a surface water source

The prevalence of STEC in surface waters around the world is well documented (*2*, *30*, *39*, *57*, *105*). However, few studies have focused on crop contamination via naturally contaminated surface water (*57*, *88*). 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 (*57*, *60*, *78*, *92*, *105*, *137*). 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 (*136*). 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.

2.6. ACKNOWLEDGEMENTS

We would like to thank John Walter Hitch and Ann Moore from the University of Tennessee Plateau Research and Education Center for assistance in water sampling and in the growing and harvesting of cantaloupes for this project. This research was funded by the Western Center for Food Safety project "Transfer and Survival of Organisms to Produce from Surface Irrigation Water".

LIST OF REFERENCES

1. 3M. 2014. Indicator organism testing. Available at: (http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/product-applications/one/).

2. 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.

3. Anderson, K. L., J. E. Whitlock, and V. J. Harwood. 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.* 71:3041–3048.

4. Anderson, M., L.A. Jaykus, S. Beaulieu, and S. Dennis. 2011. Pathogen-produce pair attribution risk ranking tool to prioritize fresh produce commodity and pathogen combinations for further evaluation. *Food Control* 22:1865–1872.

5. American Public Health Association. 2005. Microbiological examination, p. 9–1 – 9–140. *In* A.D. Eaton, E.W. Rice, and R.B. Baird (eds.), Standard methods for the examination of water and wastewater. American Public Health Association, Washington D.C.

6. Batz, M. B., S. Hoffmann, and J. G. J. Morris. 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute at University of Florida. Available at: (http://www.rwjf.org/en/research-publications/find-rwjf-research/2011/01/ranking-the-risks.html).

7. Batz, M. B., S. Hoffmann, and J., J. Glenn Morris. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot.* 75:1278–1291.

8. Bazzano, L. A. 2005. Dietary intake of fruit and vegetables and risk of diabetes mellitus and cardiovascular diseases. World Health Organization. Available at: (http://www.who.int/entity/dietphysicalactivity/publications/f&v_cvd_diabetes.pdf).

9. Beuchat, L. R. 2006. Sampling, detection, and enumeration of pathogenic and spoilage microorganisms, p. 543–564. *In* G.M. Sapers, J.R. Gorny, and A.E. Yousef (eds.), Microbiology of fruits and vegetables. CRC Press, Boca Raton, FL.

10. Beuchat, L. R., and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg. Infect. Dis.* 3:459–465.

11. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204–216.

12. Bhunia, A. K. 2008. Biosensors and bio-based methods for the separation and detection of foodborne pathogens, p. 1–44. *In* Steve L. Taylor (ed.), Advances in food and nutrition research. Academic Press.

13. Bihn, E. A. 2011. Survey of current water use practices on fresh fruit and vegetable farms and evaluation of microbiological quality of surface waters intended for fresh produce production. Dissertation, Cornell University. Available at: (http://ecommons.library.cornell.edu/handle/1813/30644).

14. Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biol. Technol.* 15:305–311.

15. Brandl, M. T., and R. E. Mandrell. 2002. Fitness of *Salmonella* enterica serovar Thompson in the cilantro phyllosphere. *Appl. Environ. Microbiol.* 68:3614–3621.

16. Burnett, A. B., and L. R. Beuchat. 2001. Comparison of sample preparation methods for recovering Salmonella from raw fruits, vegetables, and herbs. *J. Food Prot.* 64:1459–1465.

17. Centers for Disease Control and Prevention. 2009. Investigation of an outbreak of *Salmonella* Saintpaul infections linked to raw alfalfa sprouts. Available at: (http://www.cdc.gov/salmonella/saintpaul/alfalfa/).

18. Centers for Disease Control and Prevention. 2011. July 6, 2011 - *Salmonella* Enteritidis infections linked to sprouts. Available at: (http://www.cdc.gov/salmonella/sprouts-enteritidis0611/070611/index.html).

19. Centers for Disease Control and Prevention. 2011. June 23, 2011 - *Salmonella* Panama infections linked to cantaloupe. Available at: (http://www.cdc.gov/salmonella/panama0311/062311/index.html).

20. Centers for Disease Control and Prevention. 2012. Multistate outbreak linked to organic spinach and spring mix blend. Available at: (http://www.cdc.gov/ecoli/2012/O157H7-11-12/index.html).

21. Centers for Disease Control and Prevention. 2011. Multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce. Available at: (http://www.cdc.gov/ecoli/2011/ecoliO157/romainelettuce/120711/index.html).

22. Centers for Disease Control and Prevention. 2012. Multistate outbreak of *E. coli* O26 infections linked to raw clover sprouts at Jimmy John's restaurants. Available at: (http://www.cdc.gov/ecoli/2012/O26-02-12/index.html).

23. Centers for Disease Control and Prevention. 2010. Multistate outbreak of human *E. coli* O145 infections linked to shredded romaine lettuce from a single processing facility. Available at: (http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html).

24. Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado. Available at: (http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html).

25. Centers for Disease Control and Prevention. 2010. Multistate outbreak of *Salmonella* Newport - June 29, 2010. Available at: (http://www.cdc.gov/salmonella/newport/index.html).

26. Centers for Disease Control and Prevention. 2014. Outbreaks involving *E. coli*. Available at: (http://www.cdc.gov/ecoli/outbreaks.html).

27. Centers for Disease Control and Prevention. 2008. *Salmonella* Litchfield infections. Available at: (http://www.cdc.gov/salmonella/litchfield/).

28. Centers for Disease Control and Prevention. 2012. *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe. Available at: (http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html).

29. 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.

30. 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.

31. Cook, R. 2011. Tracking demographics and US fruit and vegetable consumption patterns. *Dep. Agric. Resour. Econ. Univ. Calif. Davis*. Available at: (http://agecon.ucdavis.edu/people/faculty/roberta-cook/docs/Articles/BlueprintsEoEConsumptionCookFinalJan2012Figures.pdf).

32. Critzer, F. J., and M. P. Doyle. 2010. Microbial ecology of foodborne pathogens associated with produce. *Curr. Opin. Biotechnol.* 21:125–130.

33. Curiale, M. S., T. Sons, D. McIver, J. McAllister, B. Halsey, D. Roblee, and T. Fox. 1991. Dry rehydratable film for enumeration of total coliforms and *Escherichia coli* in foods: collaborative study. *J. Assoc. Off. Anal. Chem.* 74:635–648.

34. Deering, A. J., L. J. Mauer, and R. E. Pruitt. 2012. Internalization of *E. coli* O157: H7 and *Salmonella* spp. in plants: a review. *Food Res. Int.* 45:567–575.

35. Dewaal, C. S., and M. Glassman. 2013. Outbreak alert! 2001-2010: a review of foodborne illness in America. Available at: (http://cspinet.org/new/pdf/outbreak_alert_2013_final.pdf).

36. Dewaal, C. S., G. Hicks, K. Barlow, L. Alderton, and L. Vegosen. 2006. Foods associated with foodborne illness outbreaks from 1990 through 2003. *Food Prot. Trends* 26:466–473.

37. 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.

38. Draper, A. H. 2012. Microbial survey of Pennsylvania surface water used for specialty crop irrigation and development of sampling, handling, and shipping procedures for surface water testing. Thesis, Pennsylvania State University. Available at: (https://etda.libraries.psu.edu/paper/16017/).

39. 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: occurrence and relation to recreational water quality criteria. *J. Environ. Qual.* 38:1878–1886.

40. 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.

41. 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.

42. Feng, P., S. D. Weagant, M. A. Grant, and W. Burkhardt. 2002. Enumeration of *Escherichia coli* and the coliform bacteria. *In* U.S. Food and Drug Administration (ed.), Bacteriological analytical manual online, 8th ed. Available at: (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm).

43. 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.

44. 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.

45. Franz, E., and A. H. van Bruggen. 2008. Ecology of *E. coli* O157: H7 and *Salmonella enterica* in the primary vegetable production chain. *Crit. Rev. Microbiol.* 34:143–161.

46. Garcia, A. V., and H. Hirt. 2014. *Salmonella enterica* induces and subverts the plant immune system. *Plant-Microbe Interact.* 5:141.

47. Gerba, C. P. 2009. The role of water and water testing in produce safety, p. 129–142. *In* Microbial safety of fresh produce. Wiley-Blackwell, Ames, Iowa.

48. 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).

49. Gould, H. L. 2013. Surveillance for foodborne disease outbreaks — United States, 1998–2008. *MMWR.* 62:1–34.

50. Gracias, K. S., and J. L. McKillip. 2004. A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Can. J. Microbiol.* 50:883–890.

51. Gravani, R. B. 2009. The role of good agricultural practices in produce safety, p. 101–117. *In* Microbial safety of fresh produce. Wiley-Blackwell, Ames, Iowa.

52. Griffin, D. W., E. K. Lipp, M. R. Mclaughlin, and J. B. Rose. 2001. Marine recreation and public health microbiology: quest for the ideal indicator. *BioScience* 51:817–825.

53. 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.

54. Harrigan, W. F. 1998. Methods of sampling, p. 148–152. *In* Laboratory methods in food microbiology. Academic Press.

55. Harris, L. J., J. N. Farber, L. R. Beuchat, M. E. Parish, T. V. Suslow, E. H. Garrett, and F. F. Busta. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* 2:78–141.

56. Hazen, T. C., and G. A. Toranzos. 1990. Tropical source water, p. 32–53. *In* G.A. McFeters (ed.), Drinking Water Microbiology. Springer New York.

57. 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.

58. Hoorfar, J., P. Feng, G. Duffy, B. Malorny, and R. Binet. 2014. Bottlenecks and limitations in testing for pathogens in fresh produce, p. 274–291. *In* J. Hoorfar (ed.), Global Safety of Fresh Produce. Woodhead Publishing.

59. Hörman, A., and M.-L. Hänninen. 2006. Evaluation of the lactose Tergitol-7, m-Endo LES, Colilert 18, Readycult Coliforms 100, Water-Check-100, 3M Petrifilm EC and DryCult Coliform test methods for detection of total coliforms and Escherichia coli in water samples. *Water Res.* 40:3249–3256.

60. 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.

61. IDEXX Laboratories. 2014. A comparison of IDEXX coliform/*E. coli* tests. Available at: (https://www.idexx.com/water/comparison.html).

62. 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.

63. 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.

64. Jasson, V., L. Jacxsens, P. Luning, A. Rajkovic, and M. Uyttendaele. 2010. Alternative microbial methods: an overview and selection criteria. *Food Microbiol.* 27:710–730.

65. 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.

66. 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.

67. 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.

68. Leafy Greens Marketing Agreement. 2012. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. Available at: (http://www.caleafygreens.ca.gov/food-safety-practices).

69. León, J. S., L.-A. Jaykus, and C. L. Moe. 2009. Food safety issues and the microbiology of fruits and vegetables, p. 255–290. *In* N. Heredia, I. Wesley, and S. García (eds.), Microbiologically Safe Foods. John Wiley & Sons, Inc.

70. Lin, B.-H. 2004. Fruit and vegetable consumption looking ahead to 2020. U.S. Dept. of Agriculture, Economic Research Service, Washington, DC. Available at: (http://purl.fdlp.gov/GPO/gpo28196).

71. 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.

72. Lopez-Roldan, R., P. Tusell, S. Courtois, and J. L. Cortina. 2013. On-line bacteriological detection in water. *Trends Anal. Chem.* 44:46–57.

73. Lopez-Velasco, G., A. Tomas-Callejas, D. Diribsa, P. Wei, and T. V. Suslow. 2013. Growth of *Salmonella enterica* in foliar pesticide solutions and its survival during field production and postharvest handling of fresh market tomato. *J. Appl. Microbiol.* 114:1547–1558.

74. Lopez-Velasco, G., A. Sbodio, A. Tomás-Callejas, P. Wei, K. H. Tan, and T. V. Suslow. 2012. Assessment of root uptake and systemic vine-transport of *Salmonella enterica* sv. Typhimurium by melon (*Cucumis melo*) during field production. *Int. J. Food Microbiol.* 158:65–72.

75. Mackay, I. M. 2004. Real-time PCR in the microbiology laboratory. *Clin. Microbiol. Infect.* 10:190–212.

76. Manafi, M. 2000. New developments in chromogenic and fluorogenic culture media. *Int. J. Food Microbiol.* 60:205–218.

77. Mattelet, C. 2006. Household ceramic water filter evaluation using three simple low-cost methods : membrane filtration, 3M Petrifilm and hydrogen sulfide bacteria in northern region, Ghana. Thesis, Massachusetts Institute of Technology.

78. 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.

79. Nadkarni, M. A., F. E. Martin, N. A. Jacques, and N. Hunter. 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* 148:257–266.

80. National Food Safety Guidelines. National commodity specific food safety guidelines for cantaloupes and netted melons. Available at: (http://www.cantaloupe-guidance.org/docs/national-commodity-specific-food-safety-guidelines-cantaloupes-and-netted-melons).

81. North American Tomato Trade Working Group. 2008. Commodity specific food safety guidelines for the fresh tomato supply chain. Available at: (http://www.unitedfresh.org/assets/files/Tomato%20Guidelines%20July08%20FINAL.pdf).

82. 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.

83. Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker. 2000. Surveillance for foodborne-disease outbreaks—United States, 1993–1997. *MMWR CDC Surveill Summ* 49:1–62.

84. OMAFRA. 2010. Improving on-farm food safety. Available at: (http://www.omafra.gov.on.ca/english/crops/facts/10-037.htm#1).

85. Ongeng, D., L. U. Haberbeck, G. Mauriello, J. Ryckeboer, D. Springael, and A. H. Geeraerd. 2014. Modeling the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* in the agricultural environment: current perspective. *J. Food Sci.* 79:R421–R427.

86. 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.

87. Pachepsky, Y., D. R. Shelton, J. E. McLain, J. Patel, and R. E. Mandrell. 2011. Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Adv. Agron.* 113:73.

88. 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.

89. 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.

90. Pan, Y., and F. Breidt. 2007. Enumeration of viable *Listeria monocytogenes* cells by real-time PCR with propidium monoazide and ethidium monoazide in the presence of dead cells. *Appl. Environ. Microbiol.* 73:8028–8031.

91. 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.

92. Payment, P., and A. Locas. 2011. Pathogens in water: value and limits of correlation with microbial indicators. *Ground Water* 49:4–11.

93. 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).

94. Predmore, A., and J. Li. 2011. Enhanced removal of a human Norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Appl. Environ. Microbiol.* 77:4829–4838.

95. Raiden, R. M., S. S. Sumner, J. D. Eifert, and M. D. Pierson. 2003. Efficacy of detergents in removing *Salmonella* and *Shigella* spp. from the surface of fresh produce. *J. Food Prot.* 66:2210–2215.

96. Ritchie, J. M., G. R. Campbell, J. Shepherd, Y. Beaton, D. Jones, K. Killham, and R. R. E. Artz. 2003. A stable bioluminescent construct of *Escherichia coli* O157:H7 for hazard assessments of long-term survival in the environment. *Appl. Environ. Microbiol.* 69:3359–3367.

97. Rompré, A., P. Servais, J. Baudart, M.-R. de-Roubin, and P. Laurent. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *J. Microbiol. Methods* 49:31–54.

98. Russell, S. M. 2000. Comparison of the traditional three-tube most probable number method with the Petrifilm, SimPlate, BioSys optical, and Bactometer conductance methods for enumerating *Escherichia coli* from chicken carcasses and ground beef. *J. Food Prot.* 63:1179–1183.

99. 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.

100. Schrader, C., A. Schielke, L. Ellerbroek, and R. Johne. 2012. PCR inhibitors – occurrence, properties and removal. *J. Appl. Microbiol.* 113:1014–1026.

101. Schrader, W. L. 2000. Plasticulture in California vegetable production. UCANR Publications, Oakland, CA. Available at: (http://anrcatalog.ucdavis.edu/pdf/8016.pdf).

102. Schraft, H., and L. A. Watterworth. 2005. Enumeration of heterotrophs, fecal coliforms and *Escherichia coli* in water: comparison of 3M Petrifilm plates with standard plating procedures. *J. Microbiol. Methods* 60:335–342.

103. 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.

104. Shelton, D. R., L. A. Kiefer, Y. A. Pachepsky, G. Martinez, G. W. McCarty, and T. H. Dao. 2013. Comparison of microbial quality of irrigation water delivered in aluminum and PVC pipes. *Agric. Water Manag.* 129:145–151.

105. 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.

106. Simeonov, V., J. A. Stratis, C. Samara, G. Zachariadis, D. Voutsa, A. Anthemidis, M. Sofoniou, and T. Kouimtzis. 2003. Assessment of the surface water quality in northern Greece. *Water Res.* 37:4119–4124.

107. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67:2342–2353.

108. Solo-Gabriele, H. M., M. A. Wolfert, T. R. Desmarais, and C. J. Palmer. 2000. Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. Environ. Microbiol.* 66:230–237.

109. Solomon, E. B., H.-J. Pang, and K. R. Matthews. 2003. Persistence of *Escherichia coli* O157: H7 on lettuce plants following spray irrigation with contaminated water. *J. Food Prot.* 66:2198–2202.

110. 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.

111. 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.

112. 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.

113. Soon, J. M., L. Manning, W. P. Davies, and R. Baines. 2012. Fresh produce-associated outbreaks: a call for HACCP on farms? *Br. Food J.* 114:553–597.

114. Steele, M., and J. Odumeru. 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. *J. Food Prot.* 67:2839–2849.

115. Stepenuck, K. F., L. G. Wolfson, B. W. Liukkonen, J. M. Iles, and T. S. Grant. 2011. Volunteer monitoring of *E. coli* in streams of the upper midwestern United States: a comparison of methods. *Environ. Monit. Assess.* 174:625–633.

116. Stine, S. W., I. Song, C. Y. Choi, and C. P. Gerba. 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *J. Food Prot.* 68:913–918.

117. Suslow, T. V. 2010. Produce safety project issue brief: standards for irrigation and foliar contact water. The Pew Charitable Trusts at Georgetown University. Available at: (http://www.pewtrusts.org/~/media/Assets/2009/PSPWaterSuslow1pdf.pdf?la=en).

118. Suslow, T. 2003. Key points of control and management for microbial food safety: melons. UCANR Publications, Oakland, CA. Available at: (http://anrcatalog.ucdavis.edu/pdf/8103.pdf).

119. Tallon, P., B. Magajna, C. Lofranco, and K. T. Leung. 2005. Microbial indicators of faecal contamination in water: a current perspective. *Water. Air. Soil Pollut.* 166:139–166.

120. U.S. FDA. 2011. Food Safety Modernization Act (FSMA). Available at: (http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm247548.htm).

121. 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).

122. U.S. FDA. 1998. Guidance & regulation - guidance for industry: guide to minimize microbial food safety hazards for fresh fruits and vegetables. Available at: (http://www.fda.gov/Food/GuidanceRegulation/ucm064574.htm).

123. Ukuku, D. O., and W. F. Fett. 2002. Effectiveness of chlorine and nisin-edta treatments of whole melons and fresh-cut pieces for reducing native microflora and extending shelf-life. *J. Food Saf.* 22:231–253.

124. 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.

125. Ukuku, D. O., and W. F. Fett. 2004. Method of applying sanitizers and sample preparation affects recovery of native microflora and *Salmonella* on whole cantaloupe surfaces. *J. Food Prot.* 67:999–1004.

126. Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2001. Influence of washing treatment on native microflora and *Escherichia coli* population of inoculated cantaloupes. *J. Food Saf.* 21:31–47.

127. 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.

128. USDA-AMS. 2013. Farmers market growth. *Agric. Mark. Serv. - Farmers Mark. Growth*. Available at: (http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateS&leftNav=WholesaleandFarmersMarkets&page=WFMFarmersMarketGrowth&description=Farmers%20Market%20Growth). Accessed 29 July 2014.

129. 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). Accessed 29 July 2014.

130. USDA-NASS. 2008. Farm and ranch irrigation survey-2007 US census of agriculture. US Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. Available at: (http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Farm_and_Ranch_Irrigation_Survey/index.php). Accessed 29 July 2014.

131. USDA-NASS. 2009. Specialty crops-2007 US census of agriculture. US Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. Available at: (http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Specialty_Crops/speccrop.pdf). Accessed 29 July 2014.

132. Vail, J. H., R. Morgan, C. R. Merino, F. Gonzales, R. Miller, and J. L. Ram. 2003. Enumeration of waterborne *Escherichia coli* with petrifilm plates: comparison to standard methods. *J. Environ. Qual.* 32:368–373.

133. Wang, G., and M. P. Doyle. 1998. Survival of enterohemorrhagic *Escherichia coli* O157: H7 in water. *J. Food Prot.* 61:662–667.

134. Warriner, K., and W. Jongen. 2005. Pathogens in vegetables, p. 1–43. *In* Improving the safety of fresh fruit and vegetables. Woodhead Publishing.

135. Wei, T., G. Lu, and G. Clover. 2008. Novel approaches to mitigate primer interaction and eliminate inhibitors in multiplex PCR, demonstrated using an assay for detection of three strawberry viruses. *J. Virol. Methods* 151:132–139.

136. 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.

137. 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.

138. World Health Organization. Promoting fruit and vegetable consumption around the world. *WHO*. Available at: (http://www.who.int/dietphysicalactivity/fruit/en/). Accessed 29 July 2014.

139. Yeni, F., S. Acar, Ö. G. Polat, Y. Soyer, and H. Alpas. 2014. Rapid and standardized methods for detection of foodborne pathogens and mycotoxins on fresh produce. *Food Control* 40:359–367.

140. Zhang, G., L. Ma, L. R. Beuchat, M. C. Erickson, V. H. Phelan, and M. P. Doyle. 2009. Lack of internalization of *Escherichia coli* O157:H7 in lettuce (*Lactuca sativa* L.) after leaf surface and soil inoculation. *J. Food Prot.* 72:2028–2037.

APPENDIX

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

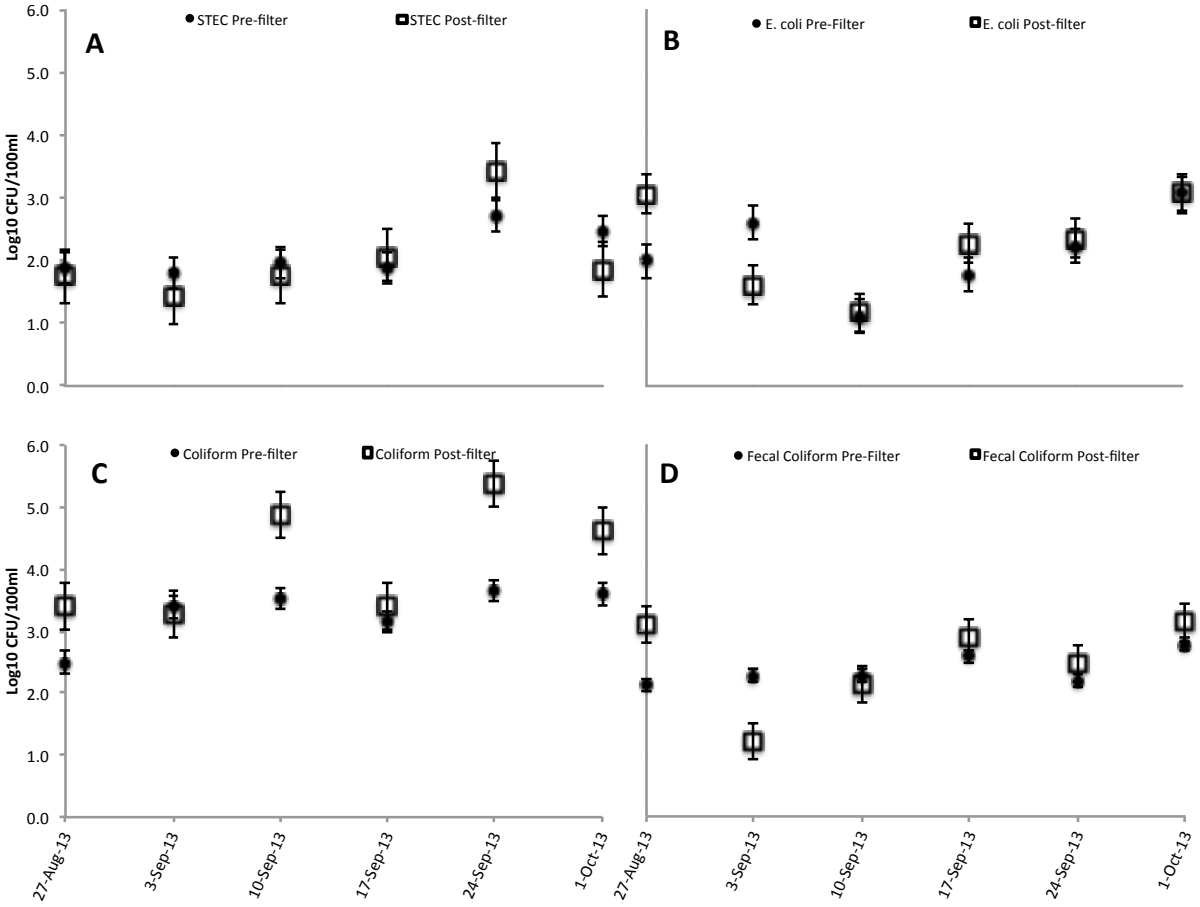

Figure 1. Concentrations in $\log_{10}$ CFU/100 ml from both sampling points across six weeks of water sampling for A: STEC, B: generic *E. coli*, C: Total Coliforms, D: Fecal Coliforms.

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

VITA

Stuart Gorman was born in Kingsport, Tennessee on July 21, 1989 to parents James and Diana Gorman. Stuart grew up in Blountville, Tennessee and graduated from Dobyns-Bennett High School in 2007. He then moved to Knoxville, Tennessee to attend the University of Tennessee where he received his Bachelor of Science degree in Microbiology in 2011. In 2012, Stuart volunteered in the Department of Microbiology at East Tennessee State University before obtaining an internship at Eastman Chemical Company as a Chemical Analyst. After completing his internship, Stuart entered into the Food Science and Technology graduate program at the University of Tennessee where he studies food microbiology.