Agricultural Water

Center for Produce Safety
Five Year Research Review

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CENTER for PRODUCE SAFETY

PRODUCE MARKETING ASSOCIATION

WESTERN GROWERS

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Disclaimer

This report has been prepared to provide a summary of scientific and technical information related to factors that affect the microbial safety of agricultural water and is derived from CPS-funded research reports and other public research sources. The intent of drafting this document is to provide users with currently available information regarding factors that affect the microbial safety of agricultural water, and the information contained within is intended to be used in a manner consistent with existing applicable regulations, standards and guidelines. The information provided herein is offered in good faith and believed to be reliable, but is made without warranty, expressed or implied, as to merchantability, fitness for a particular purpose, or any other matter. The information contained within this report is not designed to apply to any specific operation. It is the responsibility of the user of this document to verify that any information contained within this document is accurate and applicable for its operation. The publishing trade associations, their members and contributors do not assume any responsibility for compliance with applicable laws and regulations, and recommend that users consult with their own legal and technical advisors to be sure that their own procedures meet with applicable requirements.

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1.0 Background

The Center for Produce Safety (CPS) is a leader in the delivery of science-based research for the produce industry. CPS' research has made and continues to make valuable contributions to the industry's food safety knowledge. Recognizing those contributions, the Produce Marketing Association and Western Growers Association, in collaboration with CPS, are in the process of assessing CPS research findings for 2008 through 2013. The review is intended as a produce industry resource to help individuals and companies better understand the state of agricultural water knowledge in regard to food safety. This report, which focuses on agricultural water, is intended as the first in a series.

2.0 Agricultural Water

Agricultural water is a major risk factor in the contamination of fresh produce (Beuchat, 1997; Steele, 2004; Suslow, 2003). Epidemiological evidence that contaminated irrigation water can increase the risk of human disease was demonstrated in a large study in Mexico where higher incidence of disease was reported in households that consumed food irrigated with untreated wastewater than in households that consumed non-irrigated food or food irrigated with water from treated wastewater effluent reservoirs (Cifuentes, 1998). Additionally, an *E. coli* O157:H7 outbreak associated with consumption of shredded lettuce was linked back to the unintentional cross-contamination of well water intended for irrigation with water from a dairy manure lagoon (U.S. Food and Drug Administration and California Food Emergency Response Team, 2008). Irrigation water was also identified as a likely contributing factor in *Cyclospora cayetanensis* infections from raspberries (Herwaldt, 2000). Finally, the same strain of *Salmonella* Saintpaul was identified in irrigation water and on serrano peppers implicated in a salmonellosis outbreak in 2008 (Centers for Disease Control and Prevention, 2008). These incidents are a sampling of reported contamination events providing evidence that water is a risk factor in conjunction with the production and harvesting of fresh produce.

Water use is extensive in produce growing (e.g., irrigation, frost protection, direct application of pesticides), harvesting (e.g., hydration, rinsing) and cooling (e.g., hydrocooling, hydrovac, and ice) operations. Water can be a carrier of many different human pathogens including pathogenic strains of *E. coli, Salmonella, Shigella, Cyclospora cayetanensis, Cryptosporidium parvum* and *Giardia lamblia* and viruses, such as hepatitis A virus. If present, pathogens can potentially enter a water system anywhere from its source through distribution and use (U.S. Food and Drug Administration, 2013a and U.S. Food and Drug Administration, 2013b). Ensuring agricultural water does not become a means of produce contamination and subsequent illness has several challenges. The first relates to the microbial quality of the water itself – which pathogens may be present in agricultural water supplies; how does one determine what microbial quality of water is acceptable for various agricultural water uses and how do agricultural practices and crop type and condition affect the microbial quality of agricultural water needed? Second, how does one know when agricultural water poses a risk to consumers? And when agricultural water microbial quality issues exist, is corrective action necessary based solely on the potential for transference of human pathogens from the agricultural water to the harvestable portion of the crop? If it is necessary, is it even possible (i.e., are there cost-effective technologies available for remediation)? Finally, can tools such as quantitative microbial risk assessment (QMRA) be used to predict potential contamination outcomes, identify prevention strategies and/or prioritize risk management efforts?

Research related to agricultural water microbial quality is limited. Most research regarding water microbial quality (e.g., pathogen prevalence, indicator organisms) has been conducted for objectives related to reclaimed water, drinking and recreational water supplies and the effects of agriculture on the environment. However, much of this research is applicable to and has benefited agriculture.

The microbial quality of and the user's control over source waters as well as both the method and timing of application are key determinants in assessing relative likelihood of produce contamination attributable to agricultural water use practices. To date, agricultural water microbial quality research has focused on pathogen or indicator organism occurrence, transfer of pathogens to crops and management strategies to reduce the likelihood of produce contamination and consumer risk.

This report on the quality of agricultural water reviews and synthesizes much of the existing body of research including past CPS-funded research, and current CPS-funded research that addresses the issues of:

1) Human Pathogen Prevalence, Quantity and Persistence in Agricultural Water,
2) Transfer and Persistence of Human Pathogens from Contaminated Agricultural Water to the Agricultural Environment and Produce,
3) Managing Agricultural Water Safety, and
4) Tools to Assess the Risk Posed by Agricultural Water Use and Practices.

Included in each of this reports' four sections addressing these issues are key findings from completed CPS-funded research and summaries of ongoing research. Final reports for completed CPS-funded research are available on CPS' website: https://cps.ucdavis.edu/grant_opportunities_awards.php. Additionally, select CPS studies that have been published in peer-reviewed journals have been noted along with the study's key findings. In addition to findings from research funded by CPS grant funds, this report includes studies from the scientific body of knowledge that also address the CPS or industry research questions. Relevant studies from the scientific literature were identified in searches using PubMed and Google Internet search engines. The report concludes with a list of data gaps or identifiable agricultural water research needs.

2.1 Human Pathogen Prevalence, Quantity and Persistence in Agricultural Water

The CPS has asked the following research questions related to pathogen prevalence, quantity and persistence in agricultural water in their 2009-2013 requests for proposals:

- What is the frequency of generic *E. coli* detection by water source and production location? Are some water sources more prone to generic *E. coli* contamination? Is there a seasonality component to detection of generic *E. coli* when it is found?
- Irrigation water is a potential source for contamination of tomatoes. What levels of *Salmonella* and indicator organisms are normally found in the water and soils that are used to grow tomatoes in Florida?
- If water used for tree fruit irrigation or overhead cooling is contaminated with microorganisms, what is the transference to the fruit?

2.1.1 What do we know? – Studies from the scientific body of knowledge

Prevalence: Some watersheds that have been extensively sampled demonstrate a consistent pathogen presence, and surveys of waterbodies in various parts of the United States and abroad have reported human pathogen occurrence (Thurston, 2002). A study conducted in south central Georgia reported *Salmonella* prevalence as high as 79% in rural surface water (Haley, 2009). *Campylobacter* was found in 43% and *Salmonella* was found in 62% of samples taken in a rural watershed (Vereen, 2013). Edge et al. (2012) collected 902 water samples from 27 sites in four intensive agricultural watersheds across Canada and found waterborne pathogens in 80% of water samples. These samples also had low generic *E. coli* concentrations (<100 CFU/100 ml).

Weather: Susceptibility to runoff significantly increases the variability of surface water quality. Precipitation relative to fecal deposition is associated with increased levels of microbial populations in runoff from agricultural lands. Lewis et al. (2010) demonstrated that fecal coliform bacteria levels in storm runoff were positively affected by the timing of manure application to a storm event and inversely affected by the presence of vegetative buffers or filter strips. Meals' and Braun's work (2006) also showed a

positive association between rainfall and timing of manure application in concentrations of generic *E. coli* in field runoff. In a study analyzing the association between land-use and environmental variables and isolations of *E. coli* O157:H7, *Salmonella* spp., and *Campylobacter* spp. from an agricultural watershed in southern Alberta, an increase in the presence of these three pathogens was predicted by total rainfall in the days prior to sampling, sampling during the summer months, and sampling at sites downstream from high-density livestock operations, respectively. Increased levels of *E. coli* O157:H7 and fecal coliforms in irrigation ponds on produce farms were positively associated with precipitation and runoff (Gu, 2013). The incidence of *E. coli* O157:H7 increased significantly when heavy rain increased the flow rates of the rivers in a major produce production region in California (Cooley, 2007). Elevated temperatures and rainfall have been demonstrated to be associated with increased numbers of fecal coliform and enterococci concentrations, but not generic *E. coli* in a Florida freshwater lake (Staley, 2012a).

Sediments: A number of publications have shown that sediments may act as an *E. coli* reservoir and re-suspension of sediment, rather than runoff from surrounding lands, can create elevated generic *E. coli* concentrations in water (Pachepsky, 2011a). Riverbed sediments have been found to represent a possible reservoir of human pathogens. As sediment compartments (suspended and bed), riverbeds have been found to typically have higher prevalence and levels of human pathogens than water alone (Droppo, 2009). Generic *E. coli* has been demonstrated to survive in sediments much longer than in the overlying water and was inactivated at slower rates when organic carbon contents were elevated (Garzio-Hadzick, 2010). In a study by Czajkowska et al. (2005), *E. coli* O157:H7 survived for extended periods in sediment becoming undetectable only after 60 days at 24°C.

Biofilms: Little has been published about the role if any that biofilms may play as human pathogen reservoirs in irrigation water delivery systems. This may be important as biofilms have been found to play a role in microbiological contamination of drinking water distributing systems (Juhna, 2007). Additionally, elevated generic *E. coli* levels in agricultural water irrigation pipes between irrigation events indicate that *E. coli* growth can occur and most of the increase in *E. coli* numbers was associated with the biofilm on the irrigation pipe walls (Pachepsky, 2012). Pathogenic *Salmonella* have been isolated from aquatic biofilms that persist and keep the *Salmonella* viable, which provides for the potential for release in irrigation systems (Sha, 2013).

Persistence: Human pathogens commonly involved in foodborne illness persist in water for varying lengths of time (Maule, 2000). The presence of fecal material may affect the survival of pathogens (Gu, 2013; McGee, 2002). Most human pathogens can survive in water for varying lengths of time depending on factors such as temperature, salinity, dissolved oxygen, pH, the amount of pathogen present, predation, exposure to ultra-violet (UV) light and nutrient availability (Metge, 2002; Steele, 2004; Wilkes, 2011; Wanjugi, 2013). Though the viability of most pathogenic bacteria in water decreases over time, bacterial endospores survive for an undetermined amount of time while fecal coliforms, *Salmonella* spp., and *Shigella* spp. generally survive less than 30 days at 20-30˚C (Steele, 2004). *Salmonella* spp. has been demonstrated to remain viable for longer than many other enteric bacteria in freshwaters suggesting that the aquatic environment may represent a relatively stable environment for these bacteria (Chao, 1987). *Salmonella* serovars DT104, O78, and ML14 survived for 45 days in autoclaved river water at approximately 105 CFU/ml (from an initial population of approximately 108 CFU/ml) whereas plate counts of untreated or filtered river water supported fewer *Salmonella* (Santo, 2000). Wang and Doyle (1998) measured the survival of *E. coli* O157:H7 in autoclaved municipal water, reservoir water and water from two recreational lakes at 8, 15 or 25°C. The bacterium

survived longer in all three water types at 8°C, and the least amount of time at 25°C. At 8°C, populations declined from 10 to 100 CFU/ml in 91 days. At 25°C, populations decreased below the detection limit between day 49 and 84 in all but the autoclaved municipal water. *E. coli* O157:H7 has been demonstrated to survive up to 109 days in water, and *E. coli* O157:H7 collected from inoculated cattle were detected up to 10 weeks longer than the laboratory-prepared cultures suggesting that pathogen survival in low-nutrient conditions may be enhanced by passage through the gastrointestinal tract of cattle (Scott, 2006).

Quantity: Microbial populations may differ depending on the time of year water is sampled (Fonseca, 2011; Gu, 2013; Pahl, 2013; Wilkes, 2011). *E. coli* O157 has been demonstrated to be able to grow in sterile freshwater at low carbon concentrations (Vital, 2008).

Agricultural Water Sources and Distribution Systems: Sources of irrigation water can be generally ranked by the microbial contamination hazard. In order of increasing risk, these are: potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater (Leifert, 2008; Pachepsky, 2011b). A comprehensive survey of human pathogen contamination levels in agricultural water sources has not yet been compiled for the U.S. or for any other country.

Ground Water: Ground water sources that are properly designed, located and constructed generally provide high-quality agricultural water with little variability in microbial quality (Close, 2008; Gerba, 2009). Microbial quality of well water can be affected by the design of wells, nature of the substrata, and the depth to groundwater and rainfall (Gerba, 2009). Long distance transport of pathogens is possible in fractured limestone and clay soils, and gravel sandy soils (Gerba, 2009). A study of well water from 268 households in southeast Nebraska showed 37% of samples contained fecal coliforms as high as 950 cells per 100 ml water. Only 10% of the wells met Nebraska's criteria for private well construction and 30% of these wells contained one or more coliform bacteria per 100 ml. The highest incidence of coliform occurred in dug or augered wells with open-jointed casing (Exner, 1985). Domestic wells at 1,292 farmsteads in Ontario were sampled in 1991 and 1992 and tested for coliform bacteria as well as other contaminants. Thirty-four percent of wells had more than the maximum acceptable number of coliform bacteria. The percentage of wells contaminated by coliform bacteria decreased significantly with increasing separation of the well from the feedlot or exercise yard on livestock farms (Goss, 1998).

Surface Water: Surface water poses the highest potential for contamination and the greatest variability in microbial quality among commonly used agricultural water sources. Water microbial quality can be quickly degraded in storage ponds, due to wildlife and other factors (Higgins, 2009; McLain, 2008). In general surface water presents a higher risk of pathogen contamination than do groundwater sources as demonstrated by numerous studies that have shown there is a significant likelihood that surface waters will contain human pathogens (Betancourt, 2005; Pahl, 2013; Furtula, 2013). Telias et al. (2011) investigated the effect of water on the microbial population of tomatoes and showed that despite the major differences observed in the bacterial composition of ground and surface water used in their study, the season-long use of these very different water sources did not have a significant impact on the bacterial composition of the tomato fruit surface.

Treated Waste Water: In the U.S., the use of treated municipal wastewater as a source of agricultural water for produce crop irrigation occurs only on a very limited scale. Nineteen U.S. states regulate the use of wastewater in crop production with varying degrees of regulation. Some states require very stringent treatment

of effluents to reduce the concentration of human pathogens to acceptable levels prior to irrigation, while other states utilize site limitations and restrictions on crop utilization to allow time for pathogen die off (National Research Council, 1996). An extensive review of California's water recycling criteria for irrigation water use and recommendations from a panel of independent advisors was compiled by the National Water Research Institute in 2012 (NWRI, 2012).

Reclaimed Tail Water: The authors are currently unaware of any research that addresses the risk or mitigation strategies for using reclaimed tail water for irrigation.

2.1.2 What have we learned? – CPS-funded ongoing and completed research

Gillor (2009), University of California, Davis & Ben-Gurion University, Science-based monitoring for produce safety: Comparing indicators and pathogens in water, soil and crops.

- There was no statistically significant difference in fecal indicator bacteria levels on tomatoes drip irrigated with treated waste water as compared to tomatoes drip irrigated with potable water. Thus microbial contamination on the surface of tomatoes did not appear to be associated with the irrigation water source when comparing these two agricultural water sources.
- Indicator bacteria testing did not predict the presence of pathogens in any of the matrices (soil, water, crop) tested. High concentrations of fecal indicator bacteria were detected in agricultural water and on tomato surfaces from all irrigation treatment schemes, while human pathogen contamination on tomato surfaces (*Cryptosporidium* and *Salmonella*) was only detected on crops irrigated with treated waste water.
- Publication: Orlofsky et al., 2011.

Marco (2009), University of California, Davis, Contribution of phyllosphere microbiota to the persistence of *Escherichia coli* O157:H7 ATCC 700728 on field-grown lettuce.

- Total bacterial phyllosphere populations on romaine lettuce differed over time during the four-week field trials and season of planting (spring and fall 2009). During the spring 2009, bacterial population amounts also differed significantly depending on method of irrigation and exposure to attenuated *E. coli* O157:H7.
- Zero to seven days after inoculation with attenuated *E. coli* O157:H7, overhead irrigated romaine lettuce plants typically contained two- to five-fold more total bacteria than drip irrigated plants.
- Substantial plant-to-plant variation exists in microbial diversity patterns on lettuce.
- Field grown romaine lettuce has been found to harbor indigenous bacteria that are antagonistic towards the growth of virulent *E. coli* O157:H7 and a total of 28 *E. coli* O157:H7 inhibitory bacterial isolates were identified in this project.
- Publication: Williams et al., 2013.

Koike (2009), University of California Cooperative Extension, Survival of *E. coli* on soil amendments and irrigation water in leafy green field environments.

- Attenuated *E. coli* O157:H7 and generic *E. coli*, when applied to soil, failed to move significantly into irrigation water runoff.

Wright (2010), University of Florida, Science-based evaluation of regional risks for *Salmonella* contamination of irrigation water at mixed produce farms in the Suwannee River watershed.

- *Salmonella* was detected in all irrigation ponds tested in the Upper Suwannee River watershed, but these *Salmonella* populations were very low (at or near the most probable number [MPN] methodology detection limit). *Salmonella* persisted at low population densities in this watershed.
- The highest prevalence and population of *Salmonella* for water samples was found in August-October, while *Salmonella* prevalence and populations in sediment was much more sporadic with peaks occurring in April, June and September.
- Ponds within this watershed that had consistently higher levels of *Salmonella* detected, had no distinguishable pond characteristics or agricultural practices that differentiated these ponds from those with lower levels.

Atwill (2009), University of California, Davis, Epidemiologic analysis and risk management practices for reducing *E. coli* in irrigation source water supplies and distribution systems.

- The majority of water samples tested (79% or 35,093/44,249) contained no detectable generic *E. coli* and 0.86% (380/44,249) exceeded >235 *E. coli* / 100 ml.
- Approximately 8% of well samples had detectable generic *E. coli* compared to 86% of canal and 48% of reservoir samples.
- On-farm reservoir samples have much higher concentrations of generic *E. coli* than well water samples in the central coast of California.
- In California and Arizona, seasonality is a factor in test results with exceedances (>235 generic *E. coli* / 100 ml) more common in summer and fall. There was a positive association between mean air temperature and the probability of an exceedance for canal sources (from mostly California/Arizona desert growing region).
- As the sample volume increases from 100 ml to 1 L or more, the probability of detection of generic *E. coli* increases. Therefore, companies should consider amending current sampling volume practices by increasing the sampling volume to ensure more accurate assessment of generic *E. coli* levels in irrigation water sources.

2.1.3 What is being funded? – CPS-funded new research

Gibson (2013), University of Arkansas, Evaluation of pathogen survival in fresh water sediments and potential impact on irrigation water quality sampling programs.

The objective of this study is to evaluate the relationship between pathogens and fecal indicator bacteria in fresh water sources over time and the role that fresh water sediments may play in the harboring and distributing pathogens in water sources used for irrigation.

2.2 Transfer and Persistence of Human Pathogens from Contaminated Agricultural Water to the Agricultural Environment and Produce.

Although research has shown that pathogens can survive in agricultural water, questions remain as to the risk this poses to the agricultural environment, produce crops and ultimately the consumer. In assessing relative likelihood of contamination from agricultural water use practices, key determinants are microbial quality of source waters, method and timing of application, pathogen species and concentration, commodity characteristics, and climatic conditions. In their Draft Qualitative Assessment of Risk to Public Health from On Farm Contamination of Produce, the U.S. Food and Drug Administration (FDA) composed the following table to present a relative comparison of the likelihood of produce contamination attributable to practices related to agricultural water.

Table 7. Relative Likelihood of Produce Becoming Contaminated with Pathogens of Public Health Concern from Agricultural Water				
	Least → Most			
Source	Public Drinking Water	Ground water	Surface water protected from runoff	Surface water unprotected from runoff
And where contamination is known to exist, the likelihood of contamination is a function of the following factors:				
Contact with commodity	Indirect contact		Direct contact	
Commodity effects	Unlikely infiltration		Susceptible to infiltration	
	Surface not conducive to adhesion		Surface conducive to adhesion	
Application timing	Early in crop growth	Late in crop growth	During harvest	Postharvest

From: FDA, 2013 - Draft Qualitative Assessment of Risk to Public Health from On-Farm Contamination of Produce.

In CPS' 2009-2013 request for proposals, research questions addressing transfer and persistence of pathogens to the agricultural environment and produce include the following:

- If contaminated irrigation water is applied to crops, how long will human pathogens (e.g., *Salmonella*, Shiga toxin-producing *E. coli* [STECs] or *Listeria monocytogenes* [*L. monocytogenes*]) persist on the edible portion of the crop? What factors enhance or diminish survival?
- What effects does irrigation delivery method (e.g., drip, flood, sprinkle or furrow irrigation) have on pathogen transference?
- Is water used for irrigation or application of agricultural chemical sprays a risk for contamination in pistachio and walnut crops?
- Drought conditions and seasonal irrigation water shortages can lead to growers using alternative pre-harvest water sources (typically for irrigation and spraying) where the microbial quality of the water may be less than desirable. Therefore, how long do human pathogens (or surrogates/indicators) survive on the surface of leafy greens, tomatoes, peppers, tree fruits or strawberries if transferred via irrigation or spray water? What environmental factors affect survivability? Are there alternative irrigation water delivery systems that minimize transference of pathogens? What water source treatments might be available to minimize transference of pathogens to the crop?

2.2.1 What do we know? – Studies from the scientific body of knowledge

Agricultural Water Practices – Use and Application Methods: While dependent on environmental conditions, research has demonstrated human pathogens persist for various lengths of time in the agricultural environment and on produce when introduced by contaminated irrigation water (Brandl, 2006; Delaquis, 2007; Fan, 2009; Hanning, 2009; Sapers, 2006; Teplitski, 2009). Research has also demonstrated the ability of nonpathogenic *E. coli* to persist for up to 28 days, whereas *E. coli* O157:H7 did not survive for more than 14 days in inoculated spinach plants (Patel, 2010).

Irrigation: Numerous studies have demonstrated that pathogens in contaminated irrigation water may be transferred to irrigated crops (Geldreich, 1971; Hillborn, 1999; Ruiz, 1987; Sadovski, 1978; Wheeler, 2005). In a

study investigating *E. coli* contamination risk in lettuce using three different irrigation systems, investigators injected attenuated nonpathogenic *E. coli* into the water stream of overhead, subsurface drip, and surface furrow. Sprinkler irrigated lettuce tested positive for the nonpathogenic *E. coli* for up to seven days, where subsurface drip and furrow methods produced only one positive sample (Fonseca, 2011). Solomon et al. (2002a) investigated transmission of *E. coli* O157:H7 from spray and surface irrigation water to lettuce plants and found that spray irrigation resulted in more plants testing positive.

Overhead Sprinkler: Oliveira et al. (2012) found that after sprinkler irrigating lettuce with water containing *E. coli* O157:H7 three to eight weeks after seedling transplant, initial high levels (103 – 106 CFU/g) on lettuce leaves were reduced to undetectable levels in two to three weeks under field conditions. Markland et al. (2013) inoculated basil, lettuce, and spinach plants with *E. coli* O157:H7, *E. coli* O104:H4, and avian pathogenic *E. coli* (APEC; two strains were used – *stx*+ and *stx*-) via overhead irrigation in a laboratory growth chamber. At 10 days post inoculation, *E. coli* O104:H4, APEC*stx*+ and APEC*stx*- populations were present on basil plants at concentrations of 2.3, 3.1, and 3.6 $\log_{10}$ CFU/g, respectively. *E. coli* O157:H7 was no longer detected on basil four days after inoculation. On spinach and lettuce, *E. coli* O157:H7 populations declined from 105.7 CFU/g, to undetectable three days post-inoculation. At seven days post-inoculation, APEC populations were still measureable at 0.6 to 1.6 log10 CFU/g – reduced 103–105 CFU/g on day 0. The study authors noted that the APEC and *E. coli* O104:H4 strains may be more adapted to environmental conditions than *E. coli* O157:H7. A field study by Islam et al. (2004a) showed transfer of *E. coli* O157:H7 (105 CFU/ml) during spray irrigation to lettuce and parsley with levels reaching non-detection at day 77 and 175 of sampling, respectively. Using the same experimental design, the researchers repeated their experiments with *Salmonella* Typhimurium resulting in non-detectable levels on lettuce and parsley at sampling day 84 and 231, respectively (Islam, 2004b). Following an initial 3-5 log reduction in the first 72 hours, secondary-growth spinach was shown to have culturable *E. coli* for up to six days after spray irrigation with water containing a non-pathogenic strain of *E. coli* O157:H7 at concentrations of 104-107 CFU/100 ml (Wood, 2010). However, Moyne et al. (2011) reported that neither drip nor overhead sprinkler irrigation consistently influenced the survival of *E. coli* O157:H7 on lettuce.

Drip Irrigation: Subsurface drip irrigation has been demonstrated to decrease the risk of produce contamination in some crops (Song, 2006). However, parsley plants drip-irrigated with *Salmonella* contaminated water were found to have *Salmonella* in their stems and leaves (Lapidot, 2009).

Furrow Irrigation: Both 12- and 30-day-old lettuce plants grown in a greenhouse were positive for *E. coli* O157:H7 after application of irrigation water containing low levels (101, 102, 103, 104 CFU/ml) of the pathogen (Mootian, 2009).

Crop Protection / Nutrients / Growth Regulator Sprays: If contaminated water is used in solution preparation, agricultural chemical application may serve as a human pathogen source for fresh produce. Numerous studies have investigated the survival of human pathogens in agricultural chemical solutions. *Salmonella* Newport and Montevideo were able to survive and were not significantly reduced in 11 out of 12 pesticide formulations tested by Mahovic et al. (2013). Guan et al. (2005) tested survival and growth of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *Shigella* in seven different pesticide solutions and found that most formulations were somewhat inhibitory and, with the exception of *Salmonella*, did not allow growth. Verhaelen et al. (2013) investigated the ability of eight

pesticide solutions to reduce virus contamination in water, and found that murine norovirus was found to remain infectious in seven solutions at the highest concentration applied in practice. The pesticides, atrazine, malathion, and chlorothalonil, and inorganic fertilizer, did not affect the survival of generic *E. coli*, *Enterococcus faecalis*, *Salmonella enterica*, human polyomaviruses and adenovirus in water (Staley, 2012b). There is also evidence that pesticides may support the growth of *Salmonella*, if introduced with source water, and may elevate risk during foliar contact application beyond that of the water source alone (Lopez-Velasco, 2013).

Frost Protection: The authors are unaware of any published literature regarding the relative risk posed by use of contaminated irrigation water for either bloom or fruit frost protection.

Root Uptake: Greenhouse studies by Solomon et al. (2002b) and Bernstein et al. (2007) suggested that *E. coli* O157:H7 could be transported into the edible part of lettuce from soil and *Salmonella* Newport could be transported to romaine lettuce seedling leaves through root system. However, other studies, some of which were conducted in outdoor field conditions, have not demonstrated root uptake, internalization and translocation of pathogenic *E. coli* and *Salmonella* from plant root to the edible portions of spinach and other crops (Sharma, 2009; Jablasone, 2004; Miles, 2009; Zhang, 2009; Erickson, 2010a). However Zheng et al. (2013) reported that transplanted tomato plants (within three days) were more susceptible to having bacteria internalized by root uptake as evidenced by increased incidence of internalized bacteria after transplant. These researchers also found that *Salmonella* internalization through the root system was influenced by *Salmonella* serovar as well as plant growth stage.

Other Factors: Further research has shown that numerous factors such as temporality, seasonality and plant characteristics may also play a role in human pathogen transference and persistence on produce.

Pathogen Concentrations: Currently it is inconclusive whether or not the initial concentration of pathogens in agricultural water used for irrigation affects the propensity for produce contamination as the scientific evidence is varied. Human pathogen concentrations in irrigation water may not be the most important factor determining pathogen colonization or internalization in growing produce. This is because human pathogens may not be able to compete well with natural microbiota found in or on the produce item (Pachepsky, 2011b). Some studies have shown a positive relationship with *E. coli* O157:H7 concentrations and spinach contamination while other studies have demonstrated that irrigation water containing 10 to 100 CFU/ml of *E. coli* O157:H7 can lead to contamination for up to 15 days in 30% of the crop (Erickson 2010b; Mootian, 2009).

Serotypes: Spinach irrigated with water containing *Salmonella* strains isolated from either poultry or produce at 6 CFU/100 ml was found to have higher concentrations from produce-isolated *Salmonella* over 35 days (Patel, 2013). Transfer of *Salmonella* to parsley leaves via irrigation water has been demonstrated to be dependent on serotype specific curli-forming abilities of the *Salmonella* strains (Lapidot, 2009). Additionally, significantly higher attachment of *E. coli* O157:H7 occurs on iceberg lettuce and cabbage when attachment strains express curli – a thin fiber on the bacterium's surface that mediates adhesion and entry to the host cell (Patel, 2011).

Plant Injury: Human pathogens may be internalized into produce via contaminated agricultural water through plant stoma, stem scars or wounds and the conditions may sometimes allow for pathogen growth (Aruscavage, 2008; Gomes, 2009; Kroupitski, 2009; Materon, 2007; Mitra, 2009). Two days following overhead irrigation with water containing 107 CFU/ml nonpathogenic *E. coli*, injured iceberg lettuce

was found to sustain significantly higher microbial persistence than uninjured lettuce or lettuce with injuries that occurred greater than two days prior to irrigation (Barker-Reid, 2009). It has also been observed that *E. coli* O157:H7 cells preferentially attached to coarse, porous, or injured surfaces than to uninjured surfaces of green peppers (Han, 2000). Liao and Sapers (2000) observed that *Salmonella* Chester preferentially attaches to injured apple tissue than to unbroken skin. This may be due to the differences in topographical structures and specific physicochemical properties (Liao, 2000).

Temporal/Seasonal Effects: Parsley spray irrigated by water contaminated with *Salmonella* Typhimurium was found to have a higher concentration of the pathogen on leaves when irrigated at night or in the winter (Kisluk, 2012). Nonpathogenic *E. coli* persistence increased from five days during the summer to 17 days during winter months in the soil of furrow irrigated lettuce fields (Fonseca, 2011). Oliveira et al. (2012) found higher levels in the fall than in the spring after sprinkler irrigating lettuce with water containing *E. coli* O157:H7.

Crops: Produce crops may also differ in their propensity to become contaminated with human pathogens via contaminated agricultural water. Song et al. (2006) found that when agricultural water was intentionally contaminated with generic *E. coli* and *Clostridium perfringens* and used to furrow or drip irrigate produce crops the microorganisms of interest were only recoverable on the surfaces of cantaloupe and lettuce, but not on bell peppers. Additionally, crops with harvestable portion that develop on or near the ground (e.g., lettuce and parsley) were more likely to be contaminated with *Salmonella* than produce items grown off the ground (e.g., tomatoes and pimento) (Melloul, 2001). The surface topology of fruits, vegetables and food contact surfaces has been found to influence the bacterial attachment to and removal from a surface (Wang, 2012).

Cultivars: Cultivars may influence the susceptibility of some produce commodities (spinach and tomatoes) to contamination with human pathogen via contaminated irrigation water; however, more research is needed (Barak, 2008; Mitra, 2009).

2.2.2 What have we learned? – CPS-funded ongoing and completed research

Suslow (2011), University of California, Davis, Comparative assessment of field survival of *Salmonella enterica* and *Escherichia coli* O157:H7 on cilantro (*Coriandrum sativum*) in relation to sequential cutting and re-growth.

- Cilantro cultivar had no significant effect on attachment and survival of avirulent *Salmonella* or attenuated *E. coli* O157:H7 on cilantro. However, in general avirulent *Salmonella* persistence was greater than attenuated *E. coli* O157:H7 during the field production and postharvest washing and storage.
- Populations of avirulent *Salmonella* and attenuated *E. coli* O157:H7 declined after inoculation onto cilantro, to below the limit of quantitative detection, but were still detected after 12 days post inoculation by selective enrichment.
- Postharvest washing of cilantro with 50 mg/L sodium hypochlorite did not disinfect the inoculated cilantro (log 6) prior to refrigerated storage.

- Viable attenuated *E. coli* O157:H7 populations were confirmed throughout storage, including the final time point 14 days postharvest and 26 days post inoculation.
- In relation to the potential for re-growth on field cultivated cilantro, no culturable bacteria were detected 22 days after the first cut.
- Avirulent *Salmonella* and attenuated *E. coli* O157:H7 inoculated onto cilantro before cutting, could not be detected after a 22 days re-growth period. Hence commercial field contamination events may be more likely from agricultural inputs, environmental sources or practices that may contaminate cilantro close to harvest.

Suslow (2010), University of California, Davis, Risk assessment of *Salmonella* preharvest internalization in relation to irrigation water quality standards for melons and other cucurbits.

- Root uptake and systemic transfer of *Salmonella enterica* delivered through irrigation water is highly limited and systemic transfer to the edible portion of cucurbits is of low risk concern.
- Optimized irrigation strategies and hardening-off of transplants for young established vines prior to in-season cultivation results in plant-based limitations on vascular mobility of *Salmonella* in the vine.
- Publication: Lopez-Velasco et al., 2012.

Suslow (2009), University of California, Davis, Comparison of surrogate *E. coli* survival and epidemiology in the phyllosphere of diverse leafy green crops.

- Attenuated *E. coli* O157:H7 inoculated onto spring mix could be detected by enrichment eight and 14 days after inoculation.
- Survival of attenuated *E. coli* O157:H7 inoculated onto spring mix varied by variety and greater survival occurred when intermittent rainy weather occurred after inoculation versus when warm, windy weather was observed.
- Uniform contamination of spinach leaves did not result in uniform survival, which indicates that this variability may require consideration of increasing leafy green sample sizes to increase the probability of detection.
- Publication: Tomás-Callejas et al., 2011.

Teplitski (2009), University of Florida, Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest.

- *Salmonella* proliferated less within green tomatoes versus more mature tomatoes.
- Tomato varieties differed by 10- to 1,000-fold in their susceptibility to *Salmonella* proliferation.
- Publication: Noel et al., 2010.

Koike (2008), University of California Cooperative Extension, Examination of the survival and internalization of *E. coli* on spinach under field production environments.

- When attenuated *E. coli* O157:H7 and generic *E. coli* was delivered to spinach roots via sub-surface drip irrigation, it could not be recovered from spinach foliage; however, inoculated bacteria were readily recovered from soil adjacent to the drip lines.
- Attenuated *E. coli* O157:H7 and generic *E. coli* sprayed on soil did not survive for long periods of time under commercial growing conditions in the Salinas Valley. In general a 102 and 105 reduction was observed eight and 15 days post inoculation for attenuated *E. coli* O157:H7 and generic *E. coli*, respectively.
- Spinach plants inoculated with attenuated *E. coli* O157:H7 and generic *E. coli* at various stages of development (first true leaf, first true leaf +7 days and first true leaf +14 day) had no detectable applied bacteria after two weeks.
- Attenuated *E. coli* O157:H7 and generic *E. coli* sprayed on whole plants that were then turned under the soil survived up to 85 days.

Koike (2009), University of California Cooperative Extension, Survival of *E. coli* on soil amendments and irrigation water in leafy green field environments.

- Both generic *E. coli* and attenuated pathogenic *E. coli* persisted in soil six days following drip-irrigation inoculation, but only generic *E. coli* strains were recovered from soil sampled near the root zone 20 days post-inoculation.
- Attenuated *E. coli* O157:H7 and generic *E. coli* did not move significantly into irrigation water runoff or in the soil. Generic and attenuated O157:H7 *E. coli* strains were not detected by iso-grid membrane filtration for all runoff samplings.

Teplitski (2010), University of Florida, Irrigation regime, fruit water congestion and produce safety: parameter optimization to reduce susceptibility of tomatoes and peppers to post-harvest contamination, pathogen transfer and proliferation of *Salmonella*.

- Once contamination occurs, tomato maturity affects the growth of *Salmonella* in tomatoes. Ripe tomatoes (stage 6) are significantly more susceptible to *Salmonella* than younger tomatoes.
- Peppers were generally more susceptible to infections with *Salmonella* than tomatoes.
- Soft rot or lesions on tomatoes caused by *Xanthomonas* and/or *Pseudomonas* spp. significantly promoted growth of *Salmonella* in fruit. Additionally, tomato fruit with no soft rot or lesions but with signs of phytopathology elsewhere on the plant were not susceptible to *Salmonella*.
- Irrigation within two weeks prior to harvest did not significantly affect the ability of *Salmonella* to proliferate in the fruit.
- *Salmonella* growth is promoted by water moving into the tissue in green or pink tomatoes but not red tomatoes.
- Publication: Marvasi et al., 2013.

Rock (2011), University of Arizona, Assessment of *E. coli* as an indicator of microbial quality of irrigation waters use for produce.

- Public health risk is a function of source-water quality and irrigation-delivery system used with drip irrigation presenting the lowest risk of illness followed by furrow and sprinkler irrigation, respectively.

2.2.3 What is being funded? – CPS-funded new research

Critzer (2013), University of Tennessee, Transfer and survival of organisms to produce from surface irrigation water.

- The study purpose is to understand the transfer and survival of foodborne pathogens as well as generic *E. coli* and fecal coliforms from naturally contaminated surface water that will be applied to cantaloupes using drip and spray irrigation methods on bare-ground and plasticulture systems.

Vellidis (2013), University of Georgia, Does *Salmonella* move through the irrigation systems of mixed produce farms of the southeastern United States?

- The expected outcome of the proposed project is information on whether *Salmonella* moves through the irrigation systems of mixed produce farms of the southeastern United States and if so, does it persist on the crop until harvest. The ability of chlorine dioxide treatment to eliminate *Salmonella* from the irrigation water after it is withdrawn from the pond will be explored. Finally, the validity of measuring generic *E. coli* as an indicator for *Salmonella* serovar will be assessed.

Vellidis (2013), University of Georgia, Does splash from overhead sprinkler irrigation systems contaminate produce with *Salmonella* in the southeastern United States?

- The overall goal of this proposal is to develop knowledge that will allow vegetable producers who rely on untreated surface sources of irrigation water coupled with overhead sprinkler irrigation to effectively address recently proposed U.S. Food and Drug Administration rules.

Waite-Cusic (2013), Oregon State University, Survival of generic *E. coli* and *Salmonella* during the growth, curing, and storage of dry bulb onions produced with contaminated irrigation water.

- The primary aim of the proposed research is to quantify the survival of generic *E. coli* and *Salmonella* associated with dry bulb onions through the late stages of growth, water cessation, curing, and storage when inoculated through contaminated irrigation water at realistically high levels (5,000; 10,000 CFU/100 ml).

2.3 Managing Agricultural Water Safety

The following research questions related to managing agricultural water safety to prevent crop contamination were included in the 2009-2013 requests for proposals:

- If generic *E. coli* is detected, why was it found? Were there any structural or operational issues that may have led to contamination (e.g. broken well head, a rain event, etc.)? What mitigation steps were used by growers that had positive test results? How effective were these measures?
- What mitigation step(s) can be applied to various agricultural water sources that would diminish the risk of pathogen contamination to the crop?
- What preventative controls can be applied to agricultural water and how effective are these preventive controls at reducing, controlling or eliminating microbial hazards that may lead to adulteration of produce at the time of harvest?
- How do water-sampling protocols affect the outcome of water-testing programs?
- What are the most common methods used to measure generic *E. coli*?
- Can a sampling model be constructed so that higher risk irrigation water sources are sampled more frequently and lower risk sources less frequently, i.e. can the industry use its water testing resources more efficiently?

2.3.1 What do we know? – Studies from the scientific body of knowledge

Monitoring: Assessing and managing the microbial quality of agricultural water as a farm input is not easy. Agricultural water monitoring is often used to manage agricultural water microbial quality so as to minimize produce contamination; however, it is limited in its ability to monitor risks due to limited sampling frequency, the dynamic nature of agricultural water microbial quality and the time lag between obtaining agricultural water testing results and use of agricultural water (Maki, 2002; Wang, 1998; Winfield, 2003; Gerba 2009; Won, 2013b). Additionally, current agricultural water sampling strategies are based on an assumption that bacteria are floating as single cells in water and do not account for the potentially significant concentration of bacteria associated with suspended and bed sediments (Droppo, 2009).

Indicator Organisms: Because there are many pathogens that can potentially contaminate agricultural water and cause illness if consumed, it is not practical to test for any one pathogen to assess microbial quality. Generic *E. coli*, commonly used as an indicator organism for fecal contamination, is currently used in both recreational water quality standards and drinking water standards as one of several indicators that water is suitable for human contact and consumption. However, despite its use as an indicator of fecal contamination, studies have demonstrated that generic *E. coli* does not consistently correlate with pathogen presence (Benjamin, 2013; Duris, 2009; Edge, 2012; McEgan, 2013; Nieminski, 2010; Vereen, 2013; Wilkes, 2009; Won, 2013a). *L. monocytogenes* was found to have an inverse relationship with fecal indicators (Wilkes, 2009). Benjamin et al. (2013) did not find *Salmonella* or *E. coli* O157:H7 to be correlated with generic *E. coli* concentrations. Studies by Forslund et al. (2012) and Pahl et al. (2013) also found limited association between fecal indicator organisms in irrigation water and the populations on tomatoes. *E. coli* O157:H7 has been found to persist longer in pond water than generic *E. coli* and fecal enterococci most likely due to *E. coli* O157:H7 being less susceptible to environmental stressors like exposure to solar radiation and predation (Jenkins, 2011). *E. coli* has also demonstrated to have the ability to multiply in soil. Hence *E. coli* concentrations can be artificially elevated above that expected from fecal impacts alone and thus challenges the use of generic *E. coli* as a suitable indicator of water quality in tropical and subtropical environments (Solo-Gabriele, 2000). Regrowth of fecal indicator bacteria in river sediments may also lead to a decoupling of the association between fecal indicator bacteria and human pathogens concentrations in water and thus limit the ability of fecal indicator bacteria as indicator for human illnesses (Litton, 2010). The use of bifidobacteria species has been proposed by numerous studies; however, recent research has shown that their use as potential markers to monitor human fecal pollution in natural waters is questionable (Lamendella, 2008).

Biomarkers: Attempts at other methods to identify fecal contamination have been made, though none are as widely accepted as generic *E. coli* (Busta, 2003). Fremaux et al. (2009) used genetic markers to detect the presence of human and ruminant fecal matter, though none of the fecal markers used were able to predict the presence of *Campylobacter* spp. and Shiga toxin producing-*E. coli*. Stelma and Wymer (2012) recommend a number of techniques to increase the likelihood of detection of present pathogens including increasing monitoring frequency, and using a conservative polymerase chain reaction (PCR) method, and a suite of indicators. Litton et al. (2010) found that HF183 *Bacteriodes* may be a good candidate marker for fecal contamination for inland waters. *E. coli eae* and *stx* virulence genes were found not to correlate with generic *E. coli* concentrations when studied in an agricultural watershed (Shelton, 2011).

Agricultural Watershed and Delivery System Management: Protecting surface water from runoff from fecal sources and eliminating or avoiding environmental human pathogen reservoirs at the agricultural water intake have been identified as means to reduce the likelihood of agricultural water contamination (Pachepsky, 2011b). The benefits of vegetative areas have been explored for their ability to protect the microbial quality of agricultural water. In their study investigating runoff from 0.7, 1.7, and 2.7 meter buffer strips, Stout et al. (2005), showed that peak concentrations of fecal coliforms decreased as buffer length increased. Microbial concentrations are also affected by the amount of dry vegetation matter and land slope (Tate, 2006; Atwill, 2006). A study by the U.S. Department of Agriculture (USDA) revealed the importance of the specific soil surface hydrology characteristics (e.g., soil storage capacity and proximity to surface water table) in the vegetative buffer's ability to effectively retain manure-borne bacteria (Cardoso, 2012).

Work by Atwill et al. (2002) and Fox et al. (2011) demonstrated the influence of soil type and flow concentration, respectively, on effective retention of bacteria. Results of a study by Knox et al. (2008), demonstrated the importance of maintaining vegetative areas (e.g., wetlands) surrounding agricultural lands to benefit from contaminant filtration. Cahn et al. (2009) demonstrated that neither vegetated ditches nor the addition of polyacrylamide reduced the concentration of coliforms or generic *E. coli* in run-off from sprinkler irrigated lettuce grown in the Salinas Valley. In addition to protection from runoff – suspended sediments with which fecal indicators and generic *E. coli* are associated, aquatic biota, bank soils, and biofilms in pipe-based irrigation systems have been demonstrated to affect human pathogens in water systems used as agricultural water sources (Pachepsky, 2011a).

Treatments: Various forms of physical and chemical mechanisms have been explored as methods to remove human pathogens from agricultural water sources. Most of these methods have been studied in relation to use of reclaimed wastewater in agricultural applications or for treatment of wastewater effluent before release into the environment.

UV Light: Use of ultra-violet (UV) light, filters containing sand and/or materials with reactive components such as metal ions has been explored as a potential treatment of wastewater with some degree of success in other countries that have a more limited water supply (Khamkure, 2013; Rajala, 2003). The ability of these technologies to disinfect water varies with microbial species. For example, a study on wastewater from municipal wastewater treatment facilities in the U.S. showed that UV irradiation was effective in killing viruses, but the bacterial community, after an initial decline, was able to recover under laboratory conditions that mimicked a receiving stream (Blatchley, 2007). Wastewater treatment plant effluent containing levels of generic *E. coli* greater than 103 CFU/ml was treated by solar disinfection processes and then used to irrigate lettuce; just 26/28 lettuce samples tested positive for the presence of generic *E. coli* versus all lettuce samples irrigated with raw wastewater effluent (Bichai, 2012).

Other: Use of polyacrylamide and biopolymer preparations to protect surface and ground waters from agricultural runoff contaminants including enteric microorganisms has been demonstrated to be a cost-effective method that typically eliminates 70-90% of contaminants from irrigation water (Entry, 2002; Sojka, 2005).

2.3.2 What have we learned? – CPS-funded ongoing and completed research

Wright (2010), University of Florida, Science-based evaluation of regional risks for *Salmonella* contamination of irrigation water at mixed produce farms in the Suwannee River watershed.

- Fecal indicator bacteria (generic *E. coli* and fecal coliforms) testing was of little or no predictive value for *Salmonella*. There was some correlation with fecal coliforms that may have been from a common source.

Kniel (2009), University of Delaware, Mitigation of irrigation water using zero-valent iron (ZVI) treatment.

- ZVI is a useful addition to a sand filtration system to reduce *E. coli* O157:H7 and *Salmonella* contamination in irrigation water. Efficiency of removal was >102 over three months, and ranged from 102-104 removal.

- ZVI looks to be a relatively simple and inexpensive tool that can be added to existing sand filtration units to reduce pathogen contamination risks in agricultural water.
- Publication: Ingram et al., 2012.

2.3.3 What is being funded? – CPS-funded new research

Kniel (2013), University of Delaware, Use of zero valent iron (ZVI) in irrigation of tomatoes with manure-contaminated water at varying *E. coli* levels.

- This study will determine the efficacy of zero-valent iron for use in reducing microbial indicator populations in surface water containing bovine manure with a high organic load and known amount of generic *E. coli*.

Buchanan (2013), University of Tennessee, Evaluation of multiple disinfection methods to mitigate the risk of produce contamination by irrigation water.

- The project deliverables will include inactivation rates of STEC, generic *E. coli*, and fecal coliforms for each irrigation water disinfection system (UV light, peroxyacetic acid, chlorine dioxide) as well as information regarding transfer of these organisms to produce and the effect on produce yield and quality when utilizing indirect and direct irrigation methods and plasticulture and bare-ground cultivation techniques.

Rock (2013), University of Arizona, Evaluation of risk-based water quality sampling strategies for the fresh produce industry.

- The goals of this research are to assess and quantify factors that 1) determine variability of generic *E. coli*, pathogenic *E. coli*, and *Salmonella* occurrence in irrigation water over time, based on historic data and data collected as part of this study, at specific locations in Arizona and Southern California. This data will be used to assess the impact of risk events such as rainfall, water quality factors including temperature and turbidity, canal size, and watershed characteristics (potential sources of fecal contamination), on the occurrence of these organisms; 2) assess the impact of occurrence, duration and intensity of rainfall events on generic and pathogenic *E. coli*/*Salmonella* in irrigation waters with the goal of determining how long after a specific rainfall event the irrigation water quality will be affected; 3) Use an exposure scenario risk-based model for generic and pathogenic *E. coli*/*Salmonella* in irrigation waters to quantify the risks of infection with different sampling frequencies of irrigation waters based on environmental factors (e.g., rainfall), irrigation methods, and type of produce; and 4) develop a cell phone/computer application that can be used for guidance for frequency of sampling after high-risk events.

2.4 Tools to Assess the Risk Posed by Agricultural Water Use and Practices

To investigate tools to assess risk posed by agricultural water use and practices, the CPS has asked the following research questions in their 2009-2013 requests for proposals:

- Growers use a variety of water sources for field operations and irrigation (e.g., wells, on-farm reservoirs supplied by wells, municipal reservoirs, canals, natural ponds, water reclamation projects, lakes, rivers and springs). What are the risk factors associated with each source of water by source and use? What are the transfer coefficients for pathogens by source, concentration and use? Can these transfer coefficients be used to model pathogen risk profiles for each type of water source?
- What factors (source of water, use, and delivery method) affect the risk for contamination of harvested product by agricultural water? Can these risks be quantified?
- Can quantitative risk factors be associated with specific irrigation water sources?
- Research on the survival of foodborne pathogens is usually limited to biosafety level 2 or higher facilities. Selection of appropriate surrogate organisms is complicated by the limited scientific data that validates their use. What phenotypic traits are most important for validating the use of nonpathogenic surrogates that would mimic survival of STECs, *Salmonella* and *L. monocytogenes* on preharvest plants (greenhouse, field trials) contaminated by a water source?
- How does water quality influence the specific risks of contamination of tree fruit and survival of pathogens on fruit surfaces when water is applied preharvest (e.g., overhead irrigation, evaporative cooling, pesticide application) or post-harvest (e.g., hydro cooling)?

2.4.1 What do we know? – Studies from the scientific body of knowledge

Agricultural water contaminated with human pathogens may contaminate produce or by consumption of contaminated agricultural water cause adverse health consequences. Risk assessment characterizes and estimates potential adverse health effects associated with exposure to hazards like human pathogens. Quantitative microbial risk assessment (QMRA), can establish a relationship between the concentrations of pathogenic microorganisms in agricultural water and the probability of illness using statistical exposure and infectivity models. In 2006, the World Health Organization (WHO) narrowly addressed the safe use of wastewater in agriculture in its comprehensive assessment of risk posed by agricultural water use (WHO, 2006). Additionally there are other studies that have used QMRA to assess risk of illness from consuming produce irrigated with contaminated agricultural water. Stine et al. (2005) estimated a 1:10,000 risk of infection when consuming a crop that has been irrigated the day before harvest with water containing 2.5 CFU/100 ml *Salmonella* and 2.5 x 10-5 MPN/100 ml hepatitis A virus. Mota et al. (2009) calculated an annual risk of infection of 9 x 10-6 – 1.04 x 10-4 when consuming bell peppers grown in Mexico irrigated with water contaminated by *Cryptosporidium* at concentrations ranging from 17 – 1,633 oocysts/100 L. Seidu et al. (2013) found that current QMRA models underestimate the number of days between the harvest and irrigation of lettuce, in order to achieve an acceptable annual risk of infection by *E. coli* O157:H7. However, the current state of knowledge does not allow for accurate predictions of microbial reservoirs in agricultural water or the specific pathogens' survival patterns in specific agricultural water used for irrigation, even though factors affecting pathogen survival and patterns of population changes in time are generally known (Pachepsky, 2011b).

2.4.2 What have we learned? – CPS-funded ongoing and completed research

Pleus (2011), Intertox, Inc., Apple growing and packing microbial risk factors and their potential to lead to foodborne disease outbreaks.

- According to the QMRA, if growers apply evaporative cooling water containing 2400 MPN/100 ml generic *E. coli* to the orchard 10 to 12 hours prior to harvest, the probability of gastrointestinal illness for the elderly and adult population consuming contaminated unwaxed Washington fresh-pack apples (worst case scenario) is 1 case in 77 million and 1 case in 67 million, respectively.
- The time interval between evaporative cooling water application and harvest and washing apples with commercial cleaners during packing had the most effect on reducing apple contamination as indicated by the sensitivity analysis.
- The QMRA supports current practices such as those related to evaporative cooling and exclusion of bruised and dropped apples are protective of human health.

Rock (2011), University of Arizona, Assessment of *E. coli* as an indicator of microbial quality of irrigation waters use for produce.

- A QMRA model is only as useful as the quality of the data and the assumptions made to build it.
- According to the QMRA, if irrigation water has a generic *E. coli* density of 126 per 100 ml (or 12.6 generic *E. coli* per 10 ml), and based on Stine et al. (2005), 1.1 x 10-4 of the 126 generic *E. coli* per 100 ml (0.00008%) will be transferred to lettuce for furrow irrigation system and 8.8 x 10-7 of the 126 generic *E. coli* per 100 ml (0.0000007%) will be transferred to lettuce for subsurface drip irrigation system. That corresponds to a risk of

gastrointestinal illness of 1.1 cases in 100,000 for furrows and 9 cases in 100 million for subsurface irrigation system.

- For a sprinkler irrigation system and based on Stine et al. (2011), 0.011 of the 126 generic *E. coli* per 100 ml (0.009%) will be transferred to lettuce resulting in a risk of gastrointestinal illness of 1.1 cases in 1,000.
- Irrigation water containing 126 generic *E. coli* per 100 ml for lettuce would appear to present a minimal risk for furrow and subsurface drip. However, further research on contamination of lettuce by spray irrigation appears warranted to reduce uncertainty in the risk estimate.

2.4.3 What is being funded? – CPS-funded new research

Rock (2013), University of Arizona, Evaluation of risk-based water quality sampling strategies for the fresh produce industry.

- One of the goals of this research is to assess and quantify factors that use an exposure scenario risk-based model for generic and pathogenic *E. coli*/*Salmonella* in irrigation waters to quantify the risks of infection with different sampling frequencies of irrigation waters based on environmental factors (e.g., rainfall), irrigation methods, and type of produce.

2.5 Data gaps: What still needs to be done

In order to provide research that adds value to the fresh produce industry, it is important to identify areas that require more research.

2.5.1 Sampling strategies that provide an estimate of the true underlying distribution of bacteria in a water system

A monitoring protocol is needed that is based on the spatial and temporal variability of human pathogen prevalence, persistence, and concentrations in agricultural water. Most ranch- or farm-level water sampling is conducted at particular locations (i.e., close to the point of use) in the system to meet a food safety requirement and does not capture the bacterial distribution of a water system. Because of these focused sampling plans, it is difficult to assess the true spatial and temporal distribution of human pathogens in the water system. Sampling is typically conducted for the purpose of establishing microbial population occurrence/prevalence levels (i.e., is it present or not?). However, the spatial and temporal distribution and variability of human pathogens in production areas is not fully understood. Questions that remain unanswered include: Where does most growth occur? Are there consistent reservoirs of bacterial communities, and if so, do they correlate with pathogens? How do microbial populations vary temporally? Further exploring the spatial and temporal distribution and variability of human pathogens and indicator organisms will lead to a better understanding of resident and transient bacterial populations, and could lead to a better indicator of pathogen presence as well as technology and practices to mitigate or reduce the risk in these areas.

2.5.2 Correlation of field and water system management practices with pathogen positive/negative agricultural water samples

Many best practices and mitigation measures in use today, though based on known risk factors, have not been evaluated for positive or negative correlation with actual pathogen occurrence. A better understanding of the

relationship between pathogen occurrence and field and water management practices would provide growers with practical information to improve the efficiency of their food safety programs. Although management practices may vary across production areas, this type of data collection would be streamlined by the development of a standardized methodology that could be adapted for use by specific commodity groups, a particular growing region, etc.

2.5.3 Development of low-cost, large-scale water treatment for agricultural water disinfection

Surface water sources used for agricultural water in the United States have varying microbial quality. If this water does not meet existing or forthcoming microbial standards for agricultural water, efficient, low-cost, large-scale water treatment is one of the options in maintaining current fresh produce production in these areas. Although water treatment technologies are being used for drinking water systems, these technologies as currently designed may compromise soil and crop quality and may not be practical for use in agriculture. With water quality issues on the forefront in agriculture, adapting existing technologies or designing new technologies is critical for the continuance of production in growing regions with inadequate water quality. Simple and inexpensive methods for improving the microbial quality of marginal agricultural water at the farm level need to be developed, tested, and demonstrated.

2.5.4 A better understanding of risk factors leading to survival and/or growth of pathogens on fresh produce following application of contaminated water used in chemical/ nutrient sprays, irrigation, evaporative cooling.

Factors that influence the survival of human pathogens on crops are not completely understood. Some fresh produce crops repeatedly are linked to pathogen occurrence, and in some cases, to foodborne illness. Other crops have never been associated with foodborne illness and may have limited or no data on pathogen occurrence. Are there commodity-specific characteristics associated with pathogen occurrence, survival, and growth? When human pathogens are present, what environmental factors or conditions contribute to pathogen die-off, survival, growth?

2.5.5 Quantitative Microbial Risk Assessment (QMRA)

What level of pathogens in agricultural water applied to fresh produce constitutes a risk to human health? Calculating this risk estimate requires knowledge about a pathogen — e.g., prevalence, survival, growth, infective dose, etc., in addition to commodity-specific information regarding water use during cultivation and handling processes. Most fresh produce commodities lack sufficient data on pathogen occurrence throughout the production and packing process to conduct a QMRA. However, the strategic coordination of research awards from grant authorities such as CPS may assist in standardizing, collecting and assembling the necessary information to conduct a QMRA.

3.0 GLOSSARY

agricultural water – Water used in growing, harvesting, packing, and holding activities on produce where water is intended to, or is likely to, contact produce or food contact surfaces (U.S. Food and Drug Administration, 2013a).

attenuated (bacteria) – To reduce or eliminate the virulence (disease causing ability) of a pathogenic microorganism (U.S. Environmental Protection Agency, 2014).

avirulent – Not virulent; such microorganisms have lost the capacity to infect a host and cause disease (U.S. Environmental Protection Agency, 2014).

Bacteriodes – The most prominent anaerobic bacterial species in the human gut; also bile-resistant, non-spore-forming, Gram-negative rod-shaped.

bifidobacteria – Bacteria that are common inhabitants of the gastrointestinal tracts of mammals, birds, and certain cold-blooded animals (Turroni, 2011).

cell – The smallest unit of living matter capable of functioning independently (National Academy of Sciences, 2014).

coliforms – Gram-negative, non-sporeforming, rod-shaped bacteria that ferment lactose to gas. They are frequently used as indicators of process control, but exist broadly in nature (Western Growers Association, 2013).

colony forming units (CFU) – Viable microorganisms (bacteria, yeasts, and mold) capable of growth under the prescribed conditions (medium, atmosphere, time, and temperature) develop into visible colonies (colony forming units) on agar which are counted (Western Growers Association, 2013).

curli – Thin, aggregative surface fibers on the surface of many pathogenic *E. coli* and *Salmonella* strains that mediate entry into host (e.g., human, animals) cells (Gophna, 2001).

enteric – Of, relating to, or affecting the intestinal tract.

fecal coliforms – Coliform bacteria that grow at elevated temperatures and may or may not be of fecal origin. Useful to monitor effectiveness of composting processes. Also called "thermotolerant coliforms" (Western Growers Association, 2013).

fecal indicator – A microbiological organism (e.g., *E. coli*), or group of organisms (e.g., thermotolerant coliforms), that may be used in certain circumstances to indicate an association with fecal material and hence the potential for illness risk (U.S. Environmental Protection Agency, 2014).

human pathogen – A disease causing agent such as a virus, parasite, or bacteria (Western Growers Association, 2013).

inoculate – The act of introducing microorganism or suspension of microorganisms (e.g., bacteria) into a culture medium (Biology online, 2014).

microbiota – The community of various microorganisms that occur on a given substrate such as on or in plant surfaces, soil, produce, etc.

most probable number (MPN) – A statistical method used to estimate and enumerate microbes in samples, particularly when present in small numbers (Western Growers Association, 2013).

phyllosphere – The above-ground portions of a plant (Lindow, 2003).

phytopathology – The study of plant diseases (American Phytopathological Society, 2014).

potable water – Water that meets quality standards of drinking water such as described in the U.S. Environmental Protection Agency Clean Water Act and World Health Organization's Guidelines for Drinking Water Quality (National Cantaloupe Guidance, 2013).

reclaimed tail water – Water running off the lower end of a field as part of normal irrigation practices that is collected, treated, and reused (Schwankl, 2007).

reservoir (pathogen) – An organism in which a parasite that is a pathogen for some other organism lives and reproduces without harming its host (National Academy of Sciences, 2014).

serotype – Groups within a single species of microorganisms, such as bacteria or viruses, which share distinctive surface structures (Centers for Disease Control and Prevention, 2014).

species – One of the most basic units of biological classification, ranking just below the genus and comprising individuals or populations capable of interbreeding (National Academy of Sciences, 2014).

strain – A genetic variant or specific subtype of microorganism or virus (National Academy of Sciences, 2014).

wastewater effluent – The final product of all earlier treatment processes that can be discharged to a stream, river, bay, lagoon, or wetland (Davis, 2004).

4.0 REFERENCES

American Phytopathological Society (APS). 2014. What is phytopathology or plant pathology? The American Phytopathological Society.

Aruscavage D, Miller SA, Ivey ML, Lee K, LeLeune JT. 2008. Survival and dissemination of *Escherichia coli* O157:H7 on physically and biologically damaged lettuce plants. *Journal of Food Protection*, 71(12):2384–2388.

Atwill ER, Hou L, Karle BM, Harter T, Tate KW, Dahlgren RA. 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. *Applied and Environmental Microbiology,* 68(11):5517-27.

Atwill ER, Tate KW, Pereira MD, Bartolome J, Nader G. 2006. Efficacy of natural grassland buffers for removal of *Cryptosporidium parvum* in rangeland runoff. *Journal of Food Protection*, 69(1):177-84.

Atwill R, Tate K, Jay-Russell M, Harris J. 2011. Epidemiologic analysis and risk management practices for reducing *E. coli* in irrigation source water supplies and distribution systems. CPS Final Report.

Barak JD, Liang A, Narm K-E. 2008. Differential attachment and subsequent contamination of agricultural crops by *Salmonella enterica*. *Applied and Environmental Microbiology,* 74:5568–5570.

Barker-Reid F, Harapas D, Engleitner S, Kreidl S, Holmes R, Faggian R. 2009. Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. *Journal of Food Protection*, 72(3):458-64.

Benjamin L, Atwill ER, Jay-Russell M, Cooley M, Carychao D, Gorski L, Mandrell RE. 2013. Occurrence of generic *E. coli*, *E. coli* O157 and *Salmonella* spp. in water and sediment from leafy green produce farms and streams on the Central California coast. *International Journal of Food Microbiology*, 165:65-76.

Bernstein N, Sela S, Neder-Lavon S. 2007. Assessment of contamination potential of lettuce by *Salmonella enterica* serovar Newport added to the plant growing medium. *Journal of Food Protection*, 70:1717–1722.

Betancourt WQ, Rose JB. 2005. Microbiological assessment of ambient waters and proposed water sources for restoration of a Florida wetland. *Journal of Water and Health,* 3(2):89-100.

Beuchat LR, Ryu JH. 1997. Produce handling and processing practices. *Emerging Infectious Diseases,* 3(4):459-465.

Bichai F, Polo-López MI, Fernández Ibañez P. 2012. Solar disinfection of wastewater to reduce contamination of lettuce crops by *Escherichia coli* in reclaimed water irrigation. *Water Research*, 46(18):6040-50.

Blatchley ER, Gong WL, Alleman JE, Rose JB, Huffman DE, Otaki M, Lisle JT. 2007. Effects of wastewater disinfection on waterborne bacteria and viruses. *Water Environment Research,* 79(1):81-92.

Brandl MT. 2006. Fitness of human enteric pathogens on plants and implications for food safety. *Annual Reviews of Phytopathology,* 44:367-392.

Busta FF, Suslow TV, Parish ME, Beuchat LR, Farber JN, Garrett EH, Harris LJ. 2003. The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety,* 2(supplement):179-185.

Cahn M, Suslow TV, Sbodio AO, Kamal SL. 2009. Using vegetation and polymers to control sediments, nutrients and bacteria in irrigation run-off from vegetable fields. *Horticultural Sciences,* 44:1068-1069.

Cardoso F, Shelton D, Sadeghi A, Shirmohammadi A, Pachepsky Y, Dulaney W. 2012. Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. *Journal of Environmental Management,* 110:1-7.

Centers for Disease Control and Prevention (CDC). 2008. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. MMWR 57:929–934.

Chao W, Ding R, Chen R. 1987. Survival of pathogenic bacteria in environmental microcosms. *Chinese Journal of Microbiology and Immunology,* 20:339-348.

Cifuentes E. 1998. The epidemiology of enteric infections in agricultural communities exposed to wastewater irrigation: perspectives for risk control. *International Journal of Environmental Health Research,* 8:203–213.

Close M, Dann R, Ball A, Pirie R, Savill M, Smith Z. 2008. Microbial groundwater quality and its health implications for a border strip irrigated dairy farm catchment, South Island, New Zealand. *Journal of Water and Health,* 6(1):83-98.

Cooley M, Carychao D, Crawford-Miksza L, Jay MT, Myers C, Rose C, Keys C, Farrar J, Mandrell RE. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PLoS One,* 11:e1159.

Czajkowska D, Witkowska-Gwiazdowska A, Sikorska I, Boszczyk-Maleszak H, Horoch M. 2005. Survival of *Escherichia coli* serotype O157:H7 in water and in bottom-shore sediments. *Polish Journal of Environmental Studies,* 14:423-430.

Davis ML, Masten SJ. Principles of Environmental Engineering and Science. New York: McGraw-Hill, 2004.

Delaquis P, Bach S, Dinu L-D. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. *Journal of Food Protection*, 70(8):1966–1974.

Droppo IG, Liss SN, Williams D, Nelson T, Jaskot C, Trapp B. 2009. Dynamic existence of waterborne pathogens within river sediment compartments. Implications for water quality regulatory affairs. *Environmental Science and Technology,* 43:1737-1743.

Duris JW, Haack SK, Fogarty LR. 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. *Journal of Environmental Quality*, 38(5):1878-86.

Edge TA, El-Shaarawi A, Gannon V, Jokinen C, Kent R, Khan IU, Koning W, Lapen D, Miller J, Neumann N, Phillips R, Robertson W, Schreier H, Scott A, Shtepani I, Topp E, Wilkes G, van Bochove E. 2012. Investigation of an *Escherichia coli* environmental benchmark for waterborne pathogens in agricultural watersheds in Canada. *Journal of Environmental Quality*, 41(1):21-30.

Entry JA, Sojka RE, Watwood M, Ross C. 2002. Polyacrylamide preparations for protection of water quality threatened by agricultural runoff contaminants. *Environmental Pollution,* 120(2):191-200.

Erickson MC, Webb CC, Diaz-Perez JC, Phatak SC, Silvoy JJ, Davey L, Payton AS, Liao J, Ma L, Doyle MP. 2010a. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. *Journal of Food Protection*, 73(3):500–506.

Erickson MC, Webb CC, Diaz-Perez JC, Phatak SC, Silvoy JJ, Davey L, Payton AS, Liao J, Ma L, Doyle MP. 2010b. Surface and internalized *Escherichia coli* O157: H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *Journal of Food Protection*, 73(6):1023-1029.

Exner ME, Spalding RF. 1985. Ground-water contamination and well construction in southeast Nebraska. *Groundwater,* 23(1):26-34.

Fan X, Niemira BA, Doona CJ, Feeherry FE, Gravani RB. 2009. Microbial Safety of Fresh Produce. Eds. Blackwell-Wiley and IFT Press.

Fonseca JM, Fallon SD, Sanchez CA, Nolte KD. 2011. *Escherichia coli* survival in lettuce fields following its introduction through different irrigation systems. *Journal of Applied Microbiology,* 110(4):893-902.

Forslund A, Ensink JH, Markussen B, Battilani A, Psarras G, Gola S, Sandei L, Fletcher T, Dalsgaard A. 2012. *Escherichia coli* contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater. *Water Research*, 46(18):5917-34.

Fox GA, Matlock EM, Guzman JA, Sahoo D, Stunkel KB. 2011. *Escherichia coli* load reduction from runoff by vegetative filter strips: a laboratory-scale study. *Journal of Environmental Quality*, 40(3):980-8.

Fremaux B, Gritzfeld J, Boa T, Yost CK. 2009. Evaluation of host-specific Bacteroidales 16S rRNA gene markers as a complementary tool for detecting fecal pollution in a prairie watershed. *Water Research*, 43(19):4838-49.

Furtula V, Jackson CR, Farrell EG, Barrett JB, Hiott LM, Chambers PA. 2013. Antimicrobial resistance in Enterococcus spp. isolated from environmental samples in an area of intensive poultry production. *International Journal of Environmental Research and Public Health,* 10(3):1020-36.

Garzio-Hadzick A, Shelton DR, Hill RL, Pachepsky YA, Guber AK, Rowland R. 2010. Survival of manure-borne *E. coli* in streambed sediment: Effects of temperature and sediment properties. *Water Research*, 44:2753-2762

Geldreich EE, Bordner RH. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market - a review. *Journal of Milk and Food Technology,* 34:184-195.

Gerba CP. 2009. The role of water and water testing in produce safety. Chapter 7 in Microbial Safety of Fresh Produce X. Fan et al. Eds. Blackwell-Wiley and IFT Press.

Gillor O, Wuertz S. 2013. Science-based monitoring for produce safety: Comparing indicators and pathogens in water, soil and crops. CPS Final Report.

Gomes C, Da Silva P, Moreira R. G, Castell-Perez E, Ellis A, Pendleton M. 2009. Understanding *E. coli* internalization in lettuce leaves for optimization of irradiation treatment. *International Journal of Food Microbiology*, 135:238–247.

Gophna U, Barlev M, Seijffers R, Oelschlager TA, Hacker J, Ron EZ. 2001. Curli fibers mediate internalization of *Escherichia coli* by eukaryotic cells. *Infection and Immunity,* 69(4):2659-65.

Goss MJ, Barry DAJ, Rudolph DL. 1998. Contamination in Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. *Journal of Contaminant Hydrology,* 32(3-4):267-293.

Gu G, Luo Z, Cevallos-Cevallos JM, Adams P, Vellidis G, Wright A, van Bruggen AH. 2013. Factors affecting the occurrence of *Escherichia coli* O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology,* 59(3):175-82.

Guan TT, Blank G, Holley RA. 2005. Survival of pathogenic bacteria in pesticide solutions and on treated tomato plants. *Journal of Food Protection*, 68(2):296-304.

Haley BJ, Cole DJ, Lipp EK. 2009. Distribution, diversity, and seasonality of waterborne *Salmonella*e in a rural watershed. *Applied and Environmental Microbiology,* 75(5):1248-55.

Han Y, Sherman DM, Linton RH, Nielsen SS, Nelson PE. 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. *Food Microbiology,* 17(5):521–533.

Hanning I, Nutt JD, SC Ricke. 2009. Salmonellosis outbreaks in the United States due to fresh produce: Sources and potential intervention measures. *Foodborne Pathogens and Disease,* 69:635-648.

Herwaldt BL. 2000. *Cyclospora cayetanensis*: A review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clinical Infectious Disease,* 31(4):1040–1057.

Higgins J, Warnken J, Teasdale PR, Arthur JM. 2009. Decline in recycled water quality during short-term storage in open ponds. *Journal of Water and Health,* 7(4):597–608.

Hillborn ED, Mermin JH, Mshar PA, Hadler JL, Voetsch A, Wojtkunski C, Swartz M, Mshar R, Lamber-Fair MA, Farrar JA, Glynn MK, Slutsker L. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Archives Internal Medicine,* 159:1758–1764.

Ingram DT, Callahan MT, Ferguson S, Hoover DG, Chiu PC, Shelton DR, Millner PD, Camp MJ, Patel JR, Kniel KE, Sharma M. 2012. Use of zero-valent iron biosand filters to reduce *Escherichia coli* O157: H12 in irrigation water applied to spinach plants in a field setting. *Journal of Applied Microbiology,* 112(3):551-560.

Islam M, Doyle MP, Phatak SC, Millner P, Jiang X. 2004a. 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. *Journal of Food Protection*, 67(7):1365-70.

Islam M, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X. 2004b. Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease,* 1(1):27-35.

Jablasone, J., Brovko, L. Y., and Griffiiths, M. W. 2004. The potential for transfer of *Salmonella* from irrigation water to tomatoes. Journal of the Science of Food and Agriculture, 84(3):287–289.

Jenkins MB, Fisher DS, Endale DM, Adams P. 2011. Comparative die-off of *Escherichia coli* O157:H7 and fecal indicator bacteria in pond water. Environmental Science and Technology, 45(5):1853–1858

Juhna T, Birzniece D, Larsson S, Zulenkovs D, Sharipo A, Azevedo NF, Ménard-Szczebara F, Castagnet S, Féliers C, Keevil CW. 2007. Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks. *Applied and Environmental Microbiology,* 73:7456-7464.

Khamkure S, Cervantes EP, Zermeño González A, Cervantes RL, Melo PG, Ramírez H. 2013. Number of residual thermotolerant coliforms on plants and in soil when using reclaimed domestic wastewater for irrigation. *Water Science and Technology,* 67(2):380-6.

Kisluk G, Yaron S. 2012. Presence and persistence of *Salmonella enterica* serotype Typhimurium in the phyllosphere and rhizosphere of spray-irrigated parsley. *Applied and Environmental Microbiology,* 78(11):4030-6.

Kniel K, Hoover D. 2011. Mitigation of irrigation water using zero-valent iron treatment. CPS Final Report.

Knox AK, Dahlgren RA, Tate KW, Atwill ER. 2008. Efficacy of natural wetlands to retain nutrient, sediment and microbial pollutants. *Journal of Environmental Quality*, 37(5):1837-46.

Koike S, Cahn M, Smith R, Suslow T. 2010. Examination of the survival and internalization of *E. coli* on spinach under field production environments. CPS Final Report.

Koike S, Cahn M. 2011. Survival of *E. coli* on soil amendments and irrigation water in leafy green field environments. CPS Final Report.

Kroupitski Y, Pinto R, Brandl MT, Belausov E, and Sela S. 2009. Interactions of *Salmonella enterica* with lettuce leaves. *Journal of Applied Microbiology,* 106:1876–1885.

Lamendella R, Santo Domingo JW, Kelty C, Oerther DB. 2008. Bifidobacteria in feces and environmental waters. *Applied and Environmental Microbiology,* 74:575-584.

Lapidot A, Yaron S. 2009. Transfer of *Salmonella enterica* serovar Typhimurium from contaminated irrigation water to parsley is dependent on curli and cellulose, the biofilm matrix components. *Journal of Food Protection*, 72(3):618-23.

Leifert C, Ball K, Volakakis N, Cooper JM. 2008. Control of enteric pathogens in ready-to-eat vegetable crops in organic and 'low input' production systems: a HACCP-based approach. *Journal of Applied Microbiology,* 105(4):931-950.

Lewis DJ, Atwill ER, Lennox MS, Pereira MD, Miller WA, Conrad PA, Tate KW. 2010. Management of microbial contamination in storm runoff from California coastal dairy pastures. *Journal of Environmental Management,* 39(5):1782-9.

Liao CH, Sapers GM. 2000. Attachment and growth of *Salmonella* Chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments. *Journal of Food Protection*, 63:876–883.

Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Applied and Environmental Microbiology,* 69(4):1875-1883.

Litton RM, Ahn JH, Sercu B, Holden PA, Sedlak DL, Grant SB. 2010. Evaluation of chemical, molecular and traditional markers of fecal contamination in an effluent dominated urban stream. *Environmental Science and Technology,* 44:7369-7375.

Lopez-Velasco G, Sbodio A, Tomás-Callejas A, Wei P, Tan KH, Suslow TV. 2012. Assessment of root uptake and systemic vine-transport of *Salmonella enterica* sv. Typhimurium by melon (Cucumis melo) during field production. *International Journal of Food Microbiology*, 158(1):65-72.

Lopez-Velasco G, Tomas-Callejas A, Diribsa D, Wei P, Suslow TV. 2013. Growth of *Salmonella enterica* in foliar pesticide solutions and its survival during field production and postharvest handling of fresh market tomato. *Journal of Applied Microbiology,* 114(5):1547-1558.

Mahovic M, Gu G, Rideout S. 2013. Effects of pesticides on the reduction of plant and human pathogenic bacteria in application water. *Journal of Food Protection*, 76(4):719-22.

Maki RP, Hicks RE. 2002. *Salmonella* Typhimurium survival and viability is unaltered by suspended particles in freshwater. *Journal of Environmental Quality*, 31:1702-1709.

Markland SM, Shortlidge KL, Hoover DG, Yaron S, Patel J, Singh A, Sharma M, Kniel KE. 2013. Survival of pathogenic *Escherichia coli* on basil, lettuce, and spinach. *Zoonoses Public Health,* 60(8):563-71.

Marvasi M, Hochmuth GJ, Giurcanu MC, George AS, Noel JT, Bartz J, Teplitski M. 2013. Factors that affect proliferation of *Salmonella* in tomatoes post-harvest: the roles of seasonal effects, irrigation regime, crop and pathogen genotype. *PLoS One,* 8(12):e80871.

Materon LA, Martinez-Garcia M, McDonald V. 2007. Identification of sources of microbial pathogens on cantaloupe rinds from pre-harvest to post-harvest operations. *World Journal of Microbiology and Biotechnology,* 23:1281–1287.

Maule A. 2000. Survival of verocytotoxigenic *Escherichia coli* O157 in soil, water and on surfaces. *Journal of Applied Microbiology Symposium Supplement,* (29):71S-78S.

McEgan R, Mootian G, Goodridge LD, Schaffner DW, Danyluk MD. 2013. Predicting *Salmonella* populations from biological, chemical, and physical indicators in Florida surface waters. *Applied and Environmental Microbiology,* 79(13):4094-105.

McGee P, Bolton DJ, Sheridan JJ, Earley B, Kelly G, Leonard N. 2009. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. *Journal of Applied Microbiology,* 93(4):706-13.

McLain JET, Williams CF. 2008. Seasonal variation in accurate identification of *Escherichia coli* within a constructed wetland receiving tertiary-treated municipal effluent. *Water Research*, 42:4041–4048.

Meals DW, Braun DC. 2006. Demonstration of methods to reduce *E. coli* runoff from dairy manure application sites. *Journal of Environmental Quality*, 35(4):1088-100.

Melloul AA, Hassani L, Rajouk L. 2001. *Salmonella* contamination of vegetables irrigated with untreated wastewater. *World Journal of Microbiology and Biotechnology,* 17:207–209.

Metge D. 2002. "Fate and transport of bacterial, viral, and protozoan pathogens during ASR operations – What microorganisms do we need to worry about and why?" US Geological Survey Artificial Recharge Workshop Proceedings, Sacramento, CA.

Miles JM, Sumner SS, Boyer RR, Williams RC, Latimer JG, McKinney JM. 2009. Internalization of *Salmonella enterica* serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. *Journal of Food Protection*, 72(4):849–852.

Mitra R, Cuesta-Alonso E, Wayadande A, Talley J, Gilliland S, Fletcher J. 2009. Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen *Escherichia coli* O157:H7 in spinach. *Journal of Food Protection*, 72(7):1521–1530.

Mootian G, Wu WH, Matthews KR. 2009. Transfer of *Escherichia coli* O157:H7 from soil, water, and manure contaminated with low numbers of the pathogen to lettuce plants. *Journal of Food Protection*, 72(11):2308-12.

Mota A, Mena KD, Soto-Beltran M, Tarwater PM, Cháidez C. 2009. Risk assessment of *Cryptosporidium* and *Giardia* in water irrigating fresh produce in Mexico. *Journal of Food Protection*, 72(10):2184-8.

Moyne AL, Sudarshana MR, Blessington T, Koike ST, Cahn MD, Harris LJ. 2011. Fate of *Escherichia coli* O157: H7 in field-inoculated lettuce. *Food Microbiology,* 28(8):1417-1425.

National Cantaloupe Guidance. 2013. National commodity specific food safety guidelines for the cantaloupes and netted melons.

National Research Council (NRC). 1996. Use of Reclaimed Water and Sludge in Food Crop Irrigation, National Research Council. National Academy Press, Washington, DC.

National Water Research Institute. 2012. Review of California's Water Recycling Criteria for Agricultural Irrigation.

Nieminski E, Durrant GC, Hoyt MB, Owens ME, Peterson L, Peterson S, Tanner WD, Rosen J, Clancy JL. 2010. Is *E. coli* an appropriate surrogate for Crysptosporidium occurrence in water? *Journal of the American Water Works Association,* 102:65-78.

Noel JT, Arrach N, Alagely A, McClelland M, Teplitski M. 2010. Specific responses of *Salmonella enterica* to tomato varieties and fruit ripeness identified by in vivo expression technology. *PLoS One,* 5(8):e12406.

Oliveira M, Viñas I, Usall J, Anguera M, Abadias M. 2012. Presence and survival of *Escherichia coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, 156(2):133-40.

Orlofsky EM, Gillor O, Bernstein N, Shapiro K, Miller W, Wuertz S. 2011. Science based monitoring for produce safety: The correlation between fecal indicator bacteria and pathogens in effluent irrigated tomatoes. Proceedings of the 11th World Wide Workshop for Young Environmental Scientists, Arcueil, France. Urban waters: Resource or Risk, 61.

Pachepsky Y, Morrow J, Guber A, Shelton D, Rowland R, Davies G. 2012. Effect of biofilm in irrigation pipes on microbial quality of irrigation water. *Letters of Applied Microbiology,* 54:217-224.

Pachepsky YA, Shelton DR. 2011a. *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. *Critical Reviews in Environmental Science and Technology,* 41(12):1067-1110.

Pachepsky Y, Shelton DR, McLain J, Patel J, Mandrell R. 2011b. Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy,* 113:73-138.

Pahl DM, Telias A, Newell M, Ottesen AR, Walsh CS. 2013. Comparing source of agricultural contact water and the presence of fecal indicator organisms on the surface of 'juliet' grape tomatoes. *Journal of Food Protection*, 76(6):967-74.

Patel J, Sharma M, Ravishakar S. 2011. Effect of curli expression and hydrophobicity of *Escherichia coli* O157:H7 on attachment to fresh produce surfaces. *Journal of Applied Microbiology,* 110:737–745.

Patel J, Singh M, Macarisin D, Sharma M, Shelton D. 2013. Differences in biofilm formation of produce and poultry *Salmonella enterica* isolates and their persistence on spinach plants. *Food Microbiology,* 36(2):388-94.

Patel JR, Millner PD, Nou XW, Sharma M. 2010. Persistence of Enterohemorrhagic and non-pathogenic *Escherichia coli* O157:H7 on spinach leaves and in rhizosphere soil. *Journal of Applied Microbiology,* 108:1789–1796.

Pleus R. Apple growing and packing microbial risk factors and their potential to lead to foodborne disease outbreaks. CPS Final Report.

Rajala RL, Pulkkanen M, Pessi M, Heinonen-Tanski H. 2003. Removal of microbes from municipal wastewater effluent by rapid sand filtration and subsequent UV irradiation. Water Science and Technology, 47(3):157-62.

Rock C, Gerba C, Bright K. 2012. Assessment of *E. coli* as an indicator of microbial quality or irrigation water use for produce. CPS Final Report.

Ruiz, B. G. V., R. G. Vargas, and R. Garcia-Villanova. 1987. Contamination of fresh vegetables during cultivation and marketing. *International Journal of Food Microbiology*, 4:285-291.

Sadovski AY, Fattal B, Goldberg D, Katzenelson E, Shuval HI. 1978. High levels of microbial contamination of vegetables irrigated with wastewater by the drip method. *Applied and Environmental Microbiology,* 36(6):824-830.

Santo Domingo JW, Harmon S, Bennett J.. 2000. Survival of *Salmonella* species in river water. *Current Microbiology,* 40:409-417.

Sapers GM, Gorny JR, Yousef AE, eds. 2006. Microbiology of Fruits and Vegetables. CRC Taylor & Francis Group, Boca Raton.

Schwankl LJ, Prichard TL, Hanson BR. 2007. Reducing runoff from irrigated lands: Tailwater return systems. University of California, Division of Agriculture and Natural Resources, Publication 8225.

Scott L, McGee P, Sheridan JJ, Earley B, Leonard N. 2006. A comparison of the survival in feces and water of *Escherichia coli* O157:H7 grown under laboratory conditions or obtained from cattle feces. *Journal of Food Protection*, 69:6-11.

Seidu R, Sjølander I, Abubakari A, Amoah D, Larbi JA, Stenström TA. 2013. Modeling the die-off of *E. coli* and Ascaris in wastewater-irrigated vegetables: implications for microbial health risk reduction associated with irrigation cessation. *Water Science and Technology,* 68(5):1013-21.

Sha Q, Vattem DA, Forstner MRJ, Hahn D. 2013. Quantifying *Salmonella* population dynamics in water and biofilms. *Microbiology Ecology,* 65(1):60-67.

Sharma M, Ingram DT, Patel J R, Millner PD, Wan X, Hull AE, Donnenberg MS. 2009. A novel approach to investigate the uptake and internalization of *Escherichia coli* O157:H7 in spinach cultivated in soil and hydroponic medium. *Journal of Food Protection*, 72:1513–1520.

Shelton DR, Karns JS, Coppock C, Patel J, Sharma M, Pachepsky YA. 2011. Relationship between *eae* and *stx* virulence genes and *Escherichia coli* in an agricultural watershed: Implications for irrigation water standards and leafy green commodities. *Journal of Food Protection*, 74:18-23.

Sojka RE, Entry JA, Orts WJ, Morishita DW, Ross CW, Horne DJ. 2005. Synthetic- and bio-polymer use for runoff water quality management in irrigated agriculture. *Water Science and Technology,* 51(3-4):107-115.

Solo-Gabriele HM, Wolfert MA, Desmarais TR, Palmer CJ. 2000. Sources of *Escherichia coli* in a coastal subtropical environment. *Applied and Environmental Microbiology,* 66(1):230-237.

Solomon EB, Potenski CJ, Matthews KR. 2002a. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. *Journal of Food Protection*, 65(4):673-6.

Solomon EB, Yaron S, Matthews KR. 2002b. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology,* 68:397–400.

Song I, Stine SW, Choi CY, Gerba CP. 2006. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. *Journal of Environmental Engineering,* 132(10):1243–1248.

Staley C, Reckhow KH, Lukasik J, Harwood VJ. 2012a. Assessment of sources of human pathogens and fecal contamination in a Florida freshwater lake. *Water Research*, 46:5799-5812.

Staley ZR, Senkbeil JK, Rohr JR, Harwood VJ. 2012b. Lack of direct effects of agrochemicals on zoonotic pathogens and fecal indicator bacteria. *Applied and Environmental Microbiology,* 78:8146-8150.

Steele M, Odumeru J. 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection*, 67(12):2839-2849.

Stelma GN Jr, Wymer LJ. 2012. Research considerations for more effective groundwater monitoring. *Journal of Water and Health,* 10(4):511-21.

Stine SW, Song I, Choi CY, Gerba CP. 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection*, 68(5):913-8.

Stout WL, Pachepsky YA, Shelton DR, Sadeghi AM, Saporito LS, Sharpley AN. 2005. Runoff transport of fecal coliforms and phosphorus released from manure in grass buffer conditions. *Letters in Applied Microbiology,* 41(3):230-4.

Suslow T, Lopez-Velasco G, Callejas A, Sbodio A, Wei P, Diribsa D, Pham T, Camacho A. 2012. Comparative assessment of field survival of *Salmonella enterica* and *Escherichia coli* O157:H7 on cilantro (*Coriandrum sativum*) in relation to sequential cutting and re-growth. CPS Final Report.

Suslow TV, Oria MP, Beuchat LR, Garrett EH, Parish ME, Harris LJ, Farber JN, Busta FF. 2003. Production practices as risk factors in microbial food safety of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety,* 2(s1):38-77.

Tate KW, Atwill ER, Bartolome JW, Nader G. 2006. Significant *Escherichia coli* attenuation by vegetative buffers on annual grasslands. *Journal of Environmental Quality*, 35(3):795-805.

Telias A, White JR, Pahl DM, Ottesen AR, Walsh CS. 2011. Bacterial community diversity and variation in spray water sources and the tomato fruit surface. *BMC Microbiology,* 11:81.

Teplitski M, Barak JD, Schneider KR. 2009. Human enteric pathogens in produce: un-answered ecological questions with direct implications for food safety. *Current Opinion in Biotechnology,* 20:166–171.

Teplitski M, Noel J. 2010. Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest. CPS Final Report.

Thurston-Enriquez JA, Watt P, Dowd SE, Enriquez R, Pepper IL, Gerba CP. 2002. Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. *Journal of Food Protection*, 65(2):378-82.

Tomás-Callejas A, López-Velasco G, Camacho AB, Artés F, Artés-Hernández F, Suslow TV. 2011. Survival and distribution of *Escherichia coli* on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions. *International Journal of Food Microbiology*, 151(2):216-22.

U.S. Environmental Protection Agency. 2014. Thesaurus of terms used in microbial risk assessment: 5.3 Pathogen characterization or infectious disease hazard characterization terms. Accessed May 30, 2014.

U.S. Food and Drug Administration and California Food Emergency Response Team. 2008. Investigation of the Taco John's *Escherichia coli* O157:H7 outbreak associated with iceberg lettuce, Department of Health, Sacramento, CA.

U.S. Food and Drug Administration, 2013a. Standards for the growing, harvesting, packing, and holding of produce for human consumption.

U.S. Food and Drug Administration, 2013b. Draft qualitative assessment of risk to public health from on farm contamination of produce.

Vereen E Jr, Lowrance RR, Jenkins MB, Adams P, Rajeev S, Lipp EK. 2013. Landscape and seasonal factors influence *Salmonella* and *Campylobacter* prevalence in a rural mixed use watershed. *Water Research*, 47(16):6075-6085.

Verhaelen K, Bouwknegt M, Rutjes SA, de Roda Husman AM. 2013. Persistence of human norovirus in reconstituted pesticides — pesticide application as a possible source of viruses in fresh produce chains. *International Journal of Food Microbiology*, 160(3):323-8.

Vital M, Hammes F, Egli T. 2008. *Escherichia coli* O157 can grow in natural freshwater at low carbon concentrations. *Environmental Microbiology,* 10(9):2387-96.

Wang G, Doyle MP. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection*, 61(6):662-667.

Wang H, Zhou B, Feng H. 2012. Surface characteristics of fresh produce and their impact on attachment and removal of human pathogens on produce surfaces. In: Decontamination of Fresh and Minimally Processed Produce. Vicente M. Gomez-Lopez, Editor, Wiley-Blackwell, Oxford, UK.

Wanjugi P, Harwood VJ. 2013. The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. *Environmental Microbiology,* 15(2):517-526.

Western Growers Association. 2013. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens.

Wheeler C, Vogt TM, Armstrong GL, Vaughan G, Weltman A, Nainan OV, Dato V, Xia G, Waller K, Amon J, Lee TM, Highbaugh-Battle A, Hembree C, Evenson S, Ruta MA, Williams IT, Fiore AE, Bell BP. 2005. An outbreak of hepatitis A associated with green onions. *New England Journal of Medicine,* 353:890-897.

Wilkes G, Edge T, Gannon V, Jokinen C, Lyautey E, Medeiros D, Neumann N, Ruecker N, Topp E, Lapen DR. 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research*, 43(8):2209-23.

Wilkes G, Edge TA, Gannon VPJ, Jokinen C, Lyautey E, Neumann NF, Ruecker N, Scott A, Sunohara M, Topp E, Lapen DR. 2011. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. *Water Research*, 45(18):5807-5825.

Williams TR, Moyne AL, Harris LJ, Marco ML. 2013. Season, irrigation, leaf age, and *Escherichia coli* inoculation influence the bacterial diversity in the lettuce phyllosphere. *PLoS One,* 8(7):e68642.

Winfield MD, Groisman EA. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology,* 69(7):3687-3694.

Won G, Kline TR, LeJeune JT. 2013b. Spatial-temporal variations of microbial water quality in surface reservoirs and canals used for irrigation. *Agricultural Water Management,* 116:73-78.

Won G, Schlegel PJ, Schrock JM, LeJeune JT. 2013a. Absence of direct association between coliforms and *Escherichia coli* in irrigation water and on produce. *Journal of Food Protection*, 76(6):959-966.

Wood JD, Bezanson GS, Gordon RJ, Jamieson R. 2010. Population dynamics of *Escherichia coli* inoculated by irrigation into the phyllosphere of spinach grown under commercial production conditions. *International Journal of Food Microbiology*, 143(3):198-204.

World Health Organization (WHO). 2006. WHO Guidelines for the safe use of wastewater, excreta and greywater (Vol. 2), Wastewater use in agriculture. World Health Organization (Ed.).

Wright A, van Bruggen A, Vellidis G, Danyluk M. 2013. Science-based evaluation of regional risks for *Salmonella* contamination of irrigation water at mixed produce farms in the Suwannee River watershed. CPS Final Report.

Zhang G, Ma L, Beuchat LR, Erickson MC, Phelan VH, Doyle MP. 2009. Lack of internalization of *Escherichia coli* O157:H7 in lettuce (Lactuca sativa L.) after leaf surface and soil inoculation. *Journal of Food Protection,* 72(10):2028–2037.

Zheng J, Allard S, Reynolds S, Millner P, Arce G, Blodgett RJ, Brown EW. 2013. Colonization and Internalization of *Salmonella enterica* in tomato plants. *Applied and Environmental Microbiology,* 79(8):2494-2502.