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Assessment of root uptake and systemic vine-transport of *Salmonella enterica* sv. Typhimurium by melon (*Cucumis melo*) during field production

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ABSTRACT

Among melons, cantaloupes are most frequently implicated in outbreaks and surveillance-based recalls due to *Salmonella enterica*. There is limited but compelling evidence that associates irrigation water quality as a significant risk of preharvest contamination of melons. However, the potential for root uptake from water and soil and subsequent systemic transport of *Salmonella* into melon fruit is uncharacterized. The aim of this work was to determine whether root uptake of *S. enterica* results in systemic transport to fruit at high doses of applied inoculum through sub-surface drip and furrow irrigation during field production of melons. Cantaloupe and honeydew were grown under field conditions, in a silt clay loam soil using standard agronomic practices for California. An attenuated *S. enterica* sv. Typhimurium strain was applied during furrow irrigation and, in separate plots, buried drip-emitter lines delivered the inoculum directly into the established root zone. Contamination of the water resulted in soil contamination within furrows however *Salmonella* was not detected on top of the beds or around melon roots of furrow-irrigated rows demonstrating absence of detectable lateral transfer across the soil profile. In contrast, positive detection of the applied isolate occurred in soil and the rhizosphere in drip injected plots; survival of *Salmonella* was at least 41 days. Despite high populations of the applied bacteria in the rhizosphere, after surface disinfection, internalized *Salmonella* was not detected in mature melon fruit ($n = 485$). Contamination of the applied *Salmonella* was detected on the rind surface of melons if fruit developed in contact with soil on the sides of the inoculated furrows. Following an unusual and heavy rain event during fruit maturation, melons collected from the central area of the beds, were shown to harbor the furrow-applied *Salmonella*. Delivery of *Salmonella* directly into the peduncle, after minor puncture wounding, resulted in detection of applied *Salmonella* in the sub-rind tissue below the fruit abscission zone. Results indicate that *Salmonella* internalization from soil and vascular systemic transport to fruit is unlikely to occur from irrigation water in CA production regions, even if substantially above normal presumptive levels of contamination. Although contaminated irrigation water and subsequently soil in contact with fruit remains a concern for contamination of the external rind, results suggest an acceptable microbial indicator threshold and critical limit for the presence of *Salmonella* in applied water may be possible by defining appropriate microbiological standards for melon irrigation in California and regions with similar climate, soil texture, and crop management practices.

1. Introduction

Consumption of fresh fruits and vegetables is widely recognized as a major factor that contributes to the burden of foodborne illness caused by human pathogens (Brandl, 2006; Scallan et al., 2011) Clearly, there is often a widespread economic impact in the produce industry but more importantly each event has a major impact on productivity, consumer health, and erodes confidence in the food supply (Hoffman, 2011; Opara and Mazaud, 2001). Preharvest components that are involved in produce contamination during crop production have been mostly associated with wildlife, soil amendments and irrigation water (Franz and van Bruggen, 2008; Gagliardi et al., 2003; Suslow, 2010). In the particular case of irrigation water, it often remains unclear how contaminated water acts as vector for the transmission of human pathogens (Pachepsky et al., 2011; Steele et al., 2005; Suslow, 2010). Although the role of irrigation water in various produce related outbreaks is difficult to establish, there is evidence that both ground and surface waters can be contaminated by point and non-point sources such as manure, environmental water runoff and wildlife (FDA, 2008; Greene et al., 2008; Gorski et al., 2011; Pachepsky et al., 2009; Steele et al., 2005; Suslow, 2010). Hence, understanding transport mechanisms of pathogenic microorganisms, their fate in irrigation water and modes of transference to fresh produce during production is of particular importance.

Current water quality standards are primarily based on indicator microorganisms, including total coliforms, *Escherichia coli*, streptococci and enterococci, that ideally could be the result of recent fecal

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contamination (Pachepsky et al., 2011; Suslow, 2010). However, it has long been recognized within the research community that there is limited predictive value of recreational water quality indicator standards for estimating the risk of produce contamination with specific pathogens. Additionally, more recent studies have provided further evidence of the lack of correlation between indicator microorganisms and the presence of pathogenic bacteria in surface water (Duris et al., 2009; Harwood et al., 2005; Shelton et al., 2011; Winfield and Groisman, 2003). Determination of a pathogen threshold dose in water under different modes of irrigation that will likely result in contamination of a crop and the specific marketed edible part, could certainly contribute to the establishment of science-based standards for irrigation water quality (Suslow, 2010).

Melons, including cantaloupe, honeydew, watermelon, and various mixed specialty melons (i.e. casaba, crenshaw, Galia, Juan Canary) are major horticultural crops in the United States. California is responsible for approximately 58% of the domestic production with a national and international distribution. California ranks number one in production acreage of honeydew and cantaloupe (NASS, 2011) and, therefore, preharvest food safety management and inputs, such as irrigation water, are of primary concern. Over the past decade, melons have been implicated in outbreaks of foodborne illness as well as multiple recalls due to positive pathogen detection, most typically due to presumptive or confirmed *Salmonella enterica* and mostly on cantaloupes (Bowen et al, 2006; CDC, 1991, 2002a, 2002b; Mohle-Boetani et al., 1999; Munnoch et al, 2009; Steele et al., 2005; Powell, 2011). As a result, cantaloupes have been classified as a produce item of concern and drawn particular attention of the Food and Drug Administration (FDA) as it relates to microbiological food safety. Commodity Specific Food Safety Guidelines for the Melon Supply Chain (PMA and United Fresh, 2005) and FDA Guide to Minimize Food Safety Hazards of Melons (FDA, 2009) are evolving documents describing the result of hazard analysis and practice-based risk identification upon which both general and specific science-based standards and audit criteria continue to be refined.

One key and consistent area of concern defined in these guidance documents and standards is irrigation water quality. There is limited but strong evidence that associates irrigation water quality as a significant and potentially determinative risk of preharvest contamination of melons (Materon et al., 2007). In addition to contamination of the external rind surfaces, internalization from contaminated water during the production phase has been considered for diverse horticultural food crops. In a recent review, Erickson (2012) thoroughly assessed the scientific basis and evidence for the potential of systemic uptake and internalization of pathogens into food crops, including from both irrigation water and via soil contamination around the root/rhizosphere.

Pathogen internalization into produce edible portions has been speculatively identified as a major risk as once microorganisms reach internal spaces or tissues, the produce itself becomes a protective barrier against postharvest interventions applicable to fresh product handling, such as a wash-disinfection during packing, fresh processing, or consumer food preparation. Early studies suggested that *S. enterica* and *E. coli* could be transported to edible portions of plants through root systems (Bernstein et al., 2007; Solomon et al., 2002; Klerks et al., 2007) in model systems, however recent studies have demonstrated that pathogen internalization is rather a rare event and highly dose dependent (Erickson et al., 2010a, 2010b; Miles et al., 2009; Zhang et al., 2009). In the particular case of non-foliar contact water, water testing or treatment is recommended as routine practice to reach acceptable standards. However, there are no established critical limits that associate a threshold dose range with the likelihood of pathogenic bacteria to contaminate plants during melon production and the potential for fruit internalization. The objective of this work was to characterize the minimum threshold dose of *S. enterica* in irrigation water applied during field production of melons through furrow or drip irrigation that would minimize the risk of root internalization and systemic transfer to the melon vines and fruit, thus substantially reducing concerns for food-borne illness by consumers.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

S. enterica sv. Typhimurium strain *a*PTVS150 was used in this study. The parental source of *a*PTVS150 was *S. enterica* sv. Typhimurium χ3895, generously made available by R. Curtis (Hassan and Curtiss Iii, 1990). The strain lacks adenylate cyclase and cyclic AMP receptor protein rendering it avirulent yet still immunogenic. This strain has been previously utilized as surrogate in other model systems (Islam et al, 2004). A derivative isolate, *a*PTVS177, is a rifampicin-resistant strain from *a*PTVS150 selected via spontaneous mutation for tolerance to 80 mg/L, which facilitates detection and recovery and minimizes interference from background bacteria during greenhouse trials and field studies. Lab studies verified that *a*PTVS177 had an *in vitro* growth rate in several media indistinguishable from the parent strain and a plating efficiency exceeding 85% following 20 generations without rifampicin amendment in the selective/differential growth media or on plant surfaces (data not shown). The use of *a*PTVS177 in both, greenhouse and field studies was approved by the Office of Environmental Health and Safety (EH&S) and the Institutional Biosafety Committee of the University of California, Davis.

*a*PTVS177 was cultured at 37 °C for 18 h on tryptic soy agar (TSA, BD Diagnostics, Sparks MD, USA), supplemented with 80 mg/L of rifampicin (rif, Fisher Scientific) and 1 g/L of sodium pyruvate {$C_3H_3NaO_3$;(TSARP)}. Approximately five colonies were re-suspended in 5 mL of Butterfield's phosphate buffered saline (BPBS) (Whatman Inc. Piscataway, NJ, USA). A total of 100 μL were spread onto TSARP and incubated for 18 h to allow the formation of a uniform lawn of cells in early stationary phase. Culture preparation on solid media, has been found to produce cells with greater tolerance to acute desiccation death in model and open environment comparisons as encountered in field conditions (Suslow and Schroth, 1982; Wilson and Lindow, 1993; Theofel and Harris, 2009). Cells were harvested by gently scraping the agar surface with a sterile rubber spatula and suspended in BPBS. The resultant bacterial suspension was centrifuged at 1500 ×*g* for 10 min. The pellet was washed twice in BPBS and re-suspended in BPBS to adjust the optical density at 600 nm, approximately 0.750 absorbance, which corresponds to log 9 CFU/mL. The inoculum was then diluted to the desired concentration for field and greenhouse trials (see below). Final inoculum was serially diluted and plated on TSARP to determine the nominal estimated concentration of inoculum.

2.2. Preliminary studies under greenhouse conditions

Three melon cultivars (Cantaloupe “Oro Rico” F1 — OR and “Top Mark” — TM; Honeydew “Summer Dew” HMX 4593 — SD) were planted in UC mix (33% peat, 25% sand, 42% fir bark) watered daily and fertilized as needed with 50% Hoagland's solution following standard practices in research greenhouses of University of California, Davis. A total of 66 plants were established in an effort to produce fruit-bearing vines on a trellis-support system (22 plants per cultivar). At the stage of first male flowers, each vine root-mass was inoculated with 400 mL of log 7 CFU/mL of *a*PTVS177 that were added directly to the soil and root-ball mass. After the first inoculation event, plants were inoculated every week with the same population of *a*PTVS177, until a total of 4 inoculations were completed. After 15 and 49 days from first inoculation a total of 6 vines were excised just above the soil line. Vines were surface sterilized by soaking them into a solution of 1% silver nitrate (Sigma-Aldrich Co. USA) for 1 min and then rinsed in sterile distilled water (SDW) for 1 min (Franz et al., 2007). First and second internode sections of vines were cut transversally with a sterile scalpel and

deposited into a sterile bag. Samples were covered with buffered peptone water (BPW) (BD Diagnostics Sparks MD, USA) supplemented with 80 mg/L of rifampicin and incubated at 37 °C for up to 24 h. A total of 40 μL of the enrichments were spot-plated (20 μL/spot) onto *Salmonella* differential and selective agar Xylose Lactose Tergitol 4 (XLT-4) (BD Diagnostics Sparks MD, USA) supplemented with 80 mg/L of rifampicin (XLT-4/rif) 1 g/L sodium pyruvate then incubated for up to 48 h at 37 °C for colony confirmation.

To determine whether internalization of *Salmonella* occurred, mature melons were first surface sterilized and then analyzed to determine the presence of the bacterium after enrichment. Briefly, 2 sterile paper towels were saturated with 1% silver nitrate and placed on top of the melon rind surface, including the stem-scar area, for 10 min at room temperature. After paper sheets appeared dry these were removed and two new sterile paper towels saturated with 80% ethanol (Sigma-Aldrich Co. USA) were draped over the rind for an additional 10 min. A sharpened cylindrical coring tool (1.5 cm diameter) was disinfected with 70% ethanol and utilized to cut and extract the melon core (stem scar and subtending tissue to approximately 12–15 cm). The melon core sample was divided in two portions by using a sterile scalpel, the stem scar and the sub-rind melon mesocarp flesh, and analyzed separately. Samples were placed in individual sterile bags, covered with BPW/rif and incubated for 24 h of enrichment. A total of 40 μL of enrichment was spotted on XLT-4/rif for colony confirmation, as described above.

2.3. Melon field contamination with S. enterica (aPTVS177) through irrigation water

Consecutive field trials were done during summer–fall of 2009, 2010 and 2011 at the University of California Davis Research Farm facility. During trials conducted during June to September 2009 and 2010 melon seedlings (Cantaloupe cultivar Oro Rico F1 and Honeydew cultivar Summer Dew HMX4593) were transplanted in two adjacent field sections (Yolo silt clay loam; class 1 soil). Inoculum was introduced via two different systems, furrow irrigation and sub-surface drip emitters. To mimic contamination of the field using furrow irrigation, 9 beds (150 cm width) were utilized with 18 corresponding furrows. A total of 36 seedlings per bed were transplanted with an in-row spacing of 77 cm. Each half bed corresponded to one block that contained 18 plants of cantaloupe or 18 plants of honeydew, resulting in a total of 18 blocks of replicated-plantings. Honeydew and cantaloupe vines were established on approximately 2 m (center to center) raised beds with flanking furrows. When melon plants were at the initial flowering stage, each furrow was inoculated with 4 porous infusion sachets spaced approximately 7.5 m apart as described by Gutierrez-Rodriguez et al. (2012). Infusion sachets were prepared by mixing 3 kg of sand with 1 L of log 9 CFU/mL of *a*PTVS177 supplemented with 2% non-fat powder milk as an organic carrier to reduce desiccation stress, which resulted in a final concentration of log 7.5 CFU/50 g of sand. After placement of the inoculated infusion sachets, water was immediately applied with a gated pipe into furrows to a uniform depth, without wetting or overflow across the bed surface as is standard industry practice for furrow irrigation in CA (ANR 7218, 2008). Irrigation was managed throughout the trial such that plants during primary fruit set were not in direct contact with contaminated water. Inoculation with porous sachets was repeated after 24 days from the first inoculation event, as previously described. At the peak of irrigation, water samples from each flanking furrow were collected at the mid-point and near the terminal end from the point of irrigation delivery to determine the final estimated concentration of *a*PTVS177 in the water across the field row-length. The population of applied *Salmonella* in water (log CFU/mL) was determined by plating 100 μL of water in triplicate on TSARP and enumerating after 24 h of incubation at 37 °C. In pre-trial tests of the specific research plot areas, no rifampicin resistant *Salmonella* spp. were detected by direct plating or following enrichment of five replicated 100 g samples (data not shown). In parallel, inoculated soils as positive controls were readily enumerated on TSARP.

A second adjacent field plot was utilized to assess the potential for internalization and systemic movement of *Salmonella*, ensuring root zone presence, using a sub-surface drip injection delivery. A total of 5 beds were prepared as described above. A pressurized-tank of 1000 L of water, that fed the main drip manifold lines, was inoculated with 500 mL of log 9 CFU/mL of *a*PTVS177. Water was mixed in the tank by re-circulation and internal agitation prior to release from a discharge-control valve to the drip lay-flat manifold lines. Pumping from the tank continued until the tank was fully discharged. After 2 h, water samples were collected at each bed at the end of the drip line to confirm distribution along the full length and to determine the resultant average concentration of the surrogate pathogen in the water, as described above.

2.4. Fruit collection and analysis

Melons collected during 2009 and 2010 were harvested at "full-slip" maturity stage approximately after 30 and 43 days of furrow and drip line inoculation, respectively. Melons were placed in plastic bags and transferred to laboratory for processing within 4 h. Internalization potential of *a*PTVS177 was determined after melon surface sterilization and colony confirmation as described above for greenhouse trials. Additionally, in order to determine the transfer of *Salmonella* from irrigation water/soil to the rind surface, melons were peeled with a sterile sharp knife. Entire rind tissues, including stem-end and blossom-end cap rind tissue, were placed in a sterile bag, covered with BPW/rif and incubated at 37 °C for 18–24 h for enrichment. Enrichments were spotted onto XLT-4/rif for colony confirmation as described above.

2.5. Soil collection and analysis

Soil samples were collected at approximately 5, 21 and 40 days post-inoculation along each furrow as well as the furrow-bed edge (shoulder) and bed center and directly from the melon root zone, at a depth including 8 to 18 cm below the bed surface. Soil around the drip emitter-tape and melon rhizosphere soil was collected from drip injected beds. Soil was collected from the surface to a depth of 7 cm in 5 random locations per furrow side or bed and composited. An amount of 100 g of soil was taken from the homogenized bulk-composite (approximately 500 g) and added to 200 mL of 0.01 M sodium phosphate supplemented with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The suspension was gently shaken and then allowed to settle for 20 min (Gutierrez-Rodriguez et al., 2012 and supplementary Fig. S1). Aliquots of 100 μL of the supernatant were plated onto TSARP supplemented with 5 mg/L of pentachloronitrobenzene (TSARPP) (Amvac Chemical Co. Newport Beach, CA), added to inhibit the growth of soil fungi, and incubated at 37 °C for 24 h for quantification of *Salmonella*. Additionally, 25 mL of the soil-extraction supernatant was transferred into 75 mL of BPW/rif and incubated for 18–24 h at 37 °C for enrichment. Enriched samples were spotted onto XLT-4/rif for colony confirmation.

2.6. Root uptake of Salmonella on melon vines after soil contamination

During the 2010 field trial, prior to fruit set, 20 vines (10 cv. Oro Rico and 10 cv. Summer Dew) at flowering stage were inoculated within the root zone area in the field with a single dose of 500 mL of log 8 CFU/mL of *a*PTVS177. Plants were located at the tail-water collection end (discharge end) of the field and only within this spatially separated experimental area designated to receive sub-surface inoculation by drip tape delivery. This spatial separation, slope for directional drainage, and raised beds was deemed sufficient to prevent direct inoculum transfer of inoculated soil to areas of non-inoculated plants. Inoculum was deposited at the plant crown area (approximately 14 cm radius) and retained within a shallow crescent trench that was

dug around each plant so as to localize the vertical percolation of contaminated water around the active root zone. Vines were collected after 2 days of inoculation by using a sterile scalpel and the vine ends were covered with Parafilm™ (Pechiney Plastic Packaging Company, Chicago, IL). Samples were transported to the laboratory where they were first rinsed with water to remove adhering soil and then surface sterilized and processed as described above for greenhouse trials. Additionally, to verify that the simulated contaminated irrigation water drenched into the soil was in contact with roots, the root ball from 0 to 20 cm of each plant was collected after the vine excision. Excess adhering soil was shaken off and the roots were rinsed with water to remove major soil particles. Main and lateral roots were cut into approximately 12 cm segments and enriched in 20 mL of BPW/rif for up to 24 h at 37 °C, enrichments were streaked on XLT-4/rif to verify presence of the applied *Salmonella*.

2.6.1. Survival of Salmonella on melon surfaces during field production and internalization after direct delivery of the inoculum in the peduncle attached to fruit

During the 2011 field trial, the study was focused on the survival of *S. enterica* on cantaloupe surfaces and on the evaluation of the likelihood of internalization to fruit, assuming that a large population *Salmonella* could reach the peduncle and fruit-adjacent vine tissue. The setup of the field described for furrow-irrigated melons was utilized for this trial, but only 3 beds were planted. The melon field was divided in three sub-plots and randomly selected mature melons (n = 30) from each subplot were tagged and marked with indelible ink with a circumference of about 10 cm in diameter. Marked cantaloupes from each subplot were inoculated with log 4, 6 or 8 CFU/mL of *a*PTVS177 with a spray bottle that was previously calibrated to release 2 mL of inoculum on the marked area. Cantaloupes were harvested after 48 h and 10 days of inoculation to determine the recoverable population of *a*PTVS177.

Collected melons were analyzed by aseptically removing the rind from the inoculated marked area with a sterilized knife. Inoculated melon rind was transferred to a sterile bag containing sterile potassium phosphate buffer (3.9 mM KH_2PO_4 and 6.1 mM K_2HPO_4) supplemented with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP to determine the population of *a*PTVS177. After plating, the remaining cell suspension was enriched with 10 mL of double strength BPW/rif and incubated at 37 °C for up to 24 h. Colony confirmation from enrichments was performed as described above.

A second set of random melons (n = 60) from each subplot was tagged and the peduncle of each melon was inoculated with 20 μL of either log 4, 6 or 8 CFU/mL of *a*PTVS177. After the drop was deposited on the peduncle, the vine tissue was slightly injured with a sterile needle right at the point where the inoculum was deposited to attempt to ensure entrance of the applied *Salmonella* to the plant vascular system with minimal injury. Melons, associated peduncles, and adjacent acropetal and basipetal vines were collected after 9 days of inoculation and transported in individual sterile bags to the laboratory. In the laboratory, adjacent vines and peduncles were detached from mature melons. Melons were surface sterilized prior to fruit and stem scar enrichment as previously described to evaluate the presence of *Salmonella* in internal fruit. Additionally, adjacent vines were disinfected with 95% ethanol and then transversally cut in half for enrichment, peduncle and peduncle basal tissue were also enriched and *Salmonella* confirmation was performed as described above.

3. Results

Preliminary studies to determine the potential for root uptake of *S. enterica* by melon plants and transfer to fruit after soil contamination were performed under greenhouse conditions. A sequence of four soil inoculations was done for a period of one month with a high dose of the pathogen surrogate (400 mL of log 7 CFU/mL). Detection of *Salmonella* was positive only in internodal melon-vine segments immediately above the soil surface for Oro Rico and Summer Dew plants after 15 days of the first inoculation (Table 1). After 49 days of the first inoculation event, *a*PTVS177 *Salmonella* was not detected in any vine tissue assayed from the base of the plant to that immediately attached to the fruit (Table 1). Repetitive contamination of the soil with a high dose did not result in detectable systemic transport into mature fruit for any of the three melon varieties analyzed. However, *Salmonella* was detected in the rhizosphere soil of all pots tested after 49 days of the first inoculation event, indicating that roots were exposed to the pathogen during the flowering and ripening periods (Table 1). These observations suggest that internalization might occur in vines within a short timeframe and distance after the inoculation in a horticultural soil mix, but the applied *Salmonella* isolate did not appear to survive or be transported within the vine after uptake from the soil.

After preliminary experiments, two consecutive field trials were performed during 2009 and 2010 to mimic commercial melon production practices and assessment of the potential for vine internalization following irrigation with contaminated water using both furrow and drip systems. Inoculation of furrows with infusion sachets containing log 9 CFU/50 g of *a*PTVS177, that could mimic animal fecal droppings, achieved a level of contamination of about log 2–3 CFU/mL of *Salmonella* in water across the length of the field (furrow inoculation), while the water samples collected from terminal ends of sub-surface drip lines after tank inoculation delivered a population of log 4–6 CFU/mL (Table 2). Differences in the level of water contamination were observed between the two field trials, which could be mostly associated with technical issues, as it was not practical to precisely control the water flow applied and adsorption of inoculum to the soil in furrows. However, taking into consideration that less than 1000 fecal coliforms per 100 mL is one of the guidelines suggested for irrigation water for crops eaten raw (Steele and Odumeru, 2004), the contamination levels of *Salmonella* surpassed this limit-value during both trials. It is important to point out that, to date, no acceptable quantitative levels of *Salmonella* in surface water sources used for fresh produce production, including melons, in California have been established.

Contamination with *Salmonella* via irrigation water resulted in soil contamination in furrow bottoms and sides of beds and in the rhizosphere of plants inoculated with drip emitters placed within the root zone (Table 3). For furrow-irrigated beds, the population of *Salmonella* declined by approximately 4 logs from the first inoculation event to about 40 days after inoculation. However, *Salmonella* was still detected in soil that was exposed to ambient environmental drying after termination of the experiment. For the 2009 trial, residual *Salmonella* *a*PTVS177 populations in soil were only detected by enrichment, however in the trial in 2010 enumeration of the applied bacterium was still above the quantification limit of detection at the end of the trial (Table 3). In contrast, detection of *Salmonella* from soil collected at

Table 1
Detection of *S. enterica* (*a*PTVS177) in soil, fruit and melon vines grown under greenhouse conditions after multiple inoculation events.

Tissue	Days post inoculation[a]	Proportion of positive samples after enrichment[b]		
		Oro Rico	Top Mark	Summer Dew
Soil	49	3/3	3/3	3/3
Vines	15[c]	2/2	0/2	1/2
	49[d]	0/3	0/5	0/3
	49[c]	0/3	0/3	0/3
Fruit	32	0/3	0/3	ND
	49	ND	0/5	0/3

[a] Days presented refer to time from first inoculation event in all cases; three additional inoculations were performed after 7, 14 and 28 days with 400 mL of log 7 CFU/mL.
[b] Results represent the total of positive detection of *S. enterica* after enrichment/total number of samples analyzed.
[c] Vines collected immediately above soil surface.
[d] Vines (peduncle tissue) attached to fruit.

Table 2
Population densities of *S. enterica* (*a*PTVS177) delivered in irrigation water after contamination of furrows and drip irrigation lines.

Source of water collection		Furrow (log CFU/mL)[a]		Drip lines (log CFU/mL)[a]	
Days post inoculation	Year	2009	2010	2009	2010
0		2.12 ± 0.44	3.01 ± 0.41	4.17 ± 0.07	5.89 ± 0.01
21[b]		2.30 ± 0.03	2.41 ± 0.39	ND[c]	ND

[a] Results represent the mean and standard deviation of n = 9 and n = 5 water samples in furrow and drip irrigation collected water, respectively. Samples from furrow water correspond to a composite of two samples collected from left and right sides of each bed.
[b] A second application of infusion sachets was applied at day 21 from the first inoculation.
[c] ND, not determined.

bed edges was positive only for one sample in each trial year, while soil collected from the surface and subsurface, around the main crown and root zone, at the bed center tested negative for the presence of the pathogen (Table 3). This indicates that the contamination in the water did not transfer laterally in the soil profile and it was not in direct contact with the fruit and vegetative tissues. Thus, if internalization could occur, it appears unlikely to be associated with uptake of water through the root system under these conditions with a slit-clay loam soil texture. Soil collected from the rhizosphere around the drip line after contamination with water, achieved a population of about log 4 CFU/g of soil, measured after 5 days of inoculation. As observed in the furrows, the *Salmonella* population tended to decline, however it was still quantifiable during the growing season and several weeks after irrigation cut-off and soil drying (data not shown).

Internalization potential of *Salmonella* in melons grown in both furrow irrigated and drip injected beds was evaluated within 30 to 45 days after the first inoculation event during a consecutive 2 year period. Harvesting times differed each year because melons reached full-slip maturity at different points, as a consequence of variable environmental conditions. During the trial of 2009, in general, limited mature fruit yields were achieved due to low vine vigor, and thus the sample size had to be restricted to the analysis of 75 melons while during the 2010 trial a total of 416 fruit were analyzed. From both cultivars over two years, *Salmonella* was not detected inside the fruit tissue (Table 4). In contrast, evaluation of surface contamination during the 2009 trial indicated that a low percentage of the melons that developed in contact with soil within the furrow were contaminated (Table 4). Detection of *Salmonella* on melon surfaces during 2010 was carried out on melons collected from the center of the furrow-irrigated

Table 3
Presence and population densities of *S. enterica* (*a*PTVS177) in soil after contamination with inoculated irrigation water.

Days post inoculation	Furrow field (log CFU/g of soil)			Drip field (log CFU/g of soil)
	Furrow[a]	Bed edge[b]	Bed center[b]	Around the drip emitter line[a]
Year 2009				
5	3.99 ± 0.20	ND	ND	4.10 ± 0.31
21	2.68 ± 0.25	1/30	0/6	2.57 ± 0.22
41	6/6[b]	0/9	0/9	ND
Year 2010				
15	1.63 ± 0.25	1/9	0/9	3.44 ± 0.44
37	1.59 ± 0.27	0/9	0/9[c]	1.94 ± 0.43

ND, not determined for that sample point.
[a] Values represent the mean and standard deviation (n = 9 and 6 for furrow and drip irrigated fields, respectively) of 5 composited soil samples collected along the furrows or beds.
[b] Samples are represented as the total number of samples that tested positive for *S. enterica*/total number of analyzed samples.
[c] Samples collected from the bed center and edge were processed by enrichment as quantification was below the limit of detection (1.43 CFU/g of soil).

Table 4
Detection of *S. enterica* (*a*PTVS177) in melon fruits and on rind surfaces after exposure to contaminated water through furrow and drip irrigation.

Days post inoculation	Furrow field				Drip field	
	Proportion of positive samples for *S. enterica*/total analyzed samples					
	Fruit[a]		Rind surface[b]		Fruit[a]	
	(C)	(HD)	(C)	(HD)	(C)	(HD)
Year 2009						
35	0/14	0/15	3/14	2/15	0/25	ND
41	0/15	ND[c]	8/8	ND	ND	
46	ND		ND	0/15	ND	0/6
Year 2010						
31	0/21	ND	ND		ND	
38	0/90	ND	ND		ND	
43	0/90	0/90	15/21[d]	ND	0/75	0/50

[a] Melon fruits were collected from the bed and were not in contact with soil in the furrow.
[b] Melon fruits collected from the furrow.
[c] ND, not determined for that time point.
[d] Samples were collected from the bed after two days of a heavy rain event (C), (HD) cantaloupe and honeydew respectively.

beds after an unseasonal heavy rain event. In this case, approximately 70% of the analyzed rinds were contaminated with *Salmonella* (Table 4), likely the result of splash transference of contaminated soil onto the fruit surface (Cevallos-Cevallos et al., 2012). Nevertheless, as occurred with melons in contact with furrow soil, this event did not result in detectable internalization of the pathogen into edible portions at harvest.

Cantaloupe vines that were inoculated in the field with a single high dose of *Salmonella* around the root area, similar to the greenhouse pot studies, had predictably high populations within the rhizosphere soil of all samples. Excised cantaloupe vines were negative for *Salmonella* after 48 h of inoculation, while 1 out of 10 vines of honeydew was positive for internalization.

After lack of observed internalization in melon fruits, a third trial was done during 2011, to further examine the survival of the *Salmonella* on cantaloupe surfaces in the field once contaminated. Melons were inoculated with three doses of the attenuated surrogate, which resulted in positive detection of the bacterium in all samples after 48 h, regardless of the inoculation dose (Table 5). It is important to point out that quantification was below the limit of detection after 2 days of exposure to the prevailing environmental stresses for all doses, suggesting a rapid drop in viability or recovery of the applied isolate. Detection of *Salmonella* 10 days after inoculation was negative for melons inoculated with log 4 CFU/mL, while 40 and 20% of the analyzed melons inoculated with log 6 and log 8 CFU/mL respectively were still positive for the applied *Salmonella* (Table 5).

In addition to survival of *Salmonella* on fruit surfaces, internalization into fruits once the inoculum was delivered through a peduncle injury was evaluated. *Salmonella* was detected in the inoculated peduncle and also in adjacent acropetal and basipetal tissues including the fruit

Table 5
Detection of *S. enterica* (*a*PTVS177) on cantaloupe rinds after inoculation of their surfaces with different doses of inoculum.

Days post inoculation	Proportion of samples positive for detection of *S. enterica* after enrichment/total samples analyzed[a]		
	Inoculation dose (CFU/mL)		
	4	6	8
2	4/5	5/5	5/5
10	0/20	8/20	4/20

[a] Quantification was intended for these samples, however the population of *S. enterica* was below the limit of detection (1.43 CFU/melon).

Table 6
Internalization of *S. enterica* (*a*PTVS177) into melon fruit and associated tissues after 9 days of inoculation in melon peduncle during field production.

Inoculum dose (log CFU/mL)	Percentage of samples that tested positive for *S. enterica* after enrichment[a]				
	Sub-rind abscission flesh	Stem scar	Peduncle base	Peduncle	Adjacent vine
4	5	5	55	75	25
6	75	80	75	75	40
8	55	60	80	95	90

[a] n = 20 for each inoculum dose.

mesocarp flesh (Table 6). This suggests that if *Salmonella* could reach vascular tissue via a wound event, cells could be disseminated or transferred through the vine for at least limited distances. In this artificial contamination scenario *Salmonella* was detected in a greater percentage of peduncles and adjacent vines than in the fruit. This indicates that movement of the bacterium is limited or survival/recovery is highly transient. The results of this trial further suggest that the ability of *Salmonella* to reach the fruit internal tissues is strongly dose dependent (Table 6).

4. Discussion

This study aimed to determine a threshold contamination dose of *S. enterica* in irrigation water to evaluate the potential for root uptake and systemic transfer to melon fruit. Evaluation of two irrigation methods, furrow and sub-surface drip irrigation, were utilized to deliver an avirulent strain of *S. enterica* during the field production of cantaloupe and honeydew. Furrow and sub-surface drip irrigation are widely used for melon production and both are non-foliar water application methods. In addition to a reduced risk of food contamination (Suslow et al., 2003), the attraction of using drip systems resides in minimizing water use, particularly in a location where water is scarce, as in semi-arid regions, or may be restricted by regulatory statutes (Sensoy et al., 2007; Song et al., 2006). Additionally, sub-surface drip irrigation can minimize water contact with edible portions of a crop and exposure to contamination with plant and human pathogens (Alum et al., 2000; Oron et al., 1992). During melon production fruit contact with irrigation-wetted soil is mostly minimized by careful crop management which has significant advantages for fruit quality (ANR 7218, 2008). However in the broad context of global melon production, there is inadequate information regarding the suitability of different irrigation sources and pathways involved in melon contamination by irrigation water, including the potential for fruit internalization under diverse conditions to develop sound science-based guidance and policy.

In this study water in furrow and in drip lines was contaminated with a dose of *Salmonella* greater than 1000 CFU/mL (Table 2). This population largely surpasses any documented levels suggested for irrigation water for produce consumed raw, even if based on the population of indicator *E. coli* (Steele et al., 2005). Thus under worst-case scenario conditions there appears to be a low plausibility of *Salmonella* internalization into melon fruits from typical water sources and modes of application. Even under worst-case scenario conditions, we were unable to detect *Salmonella* internalized in melon fruits that were irrigated through furrows and sub-surface drip irrigation, in two-year consecutive trials. This result is supported by similar studies performed for various commodities, showing that internalization of human pathogens into plants during produce production in a mineral field soil, is unlikely to occur (Erickson et al., 2010b; Gu et al., 2011; Miles et al., 2009; Zhang et al., 2009).

It was expected that if internalization occurred, this event would be most likely in a sub-surface drip irrigation field, as a soil with higher clay content (typical for CA melon production), would limit movement of bacteria from irrigation furrows to the root zone during plant growth. In this study, this hypothesis appears to be supported, as *Salmonella* was not detected in soil collected from the interior horizontal and vertical profiles of the bed. In contrast, inoculation delivered through drip lines, in which bacteria would be in direct contact with rhizosphere and newly emerging roots (Berg et al., 2005; Ongeng et al., 2011). The potential for uptake and internalization to occur would be greater in accordance with the extended survival of *Salmonella* in the bulk soil into which new roots develop (Semenov et al., 2009). It was noticed that the survival or culture-based recovery of *Salmonella* in soil associated with the furrow was lower, potentially due to more extreme surface exposure stress, and thus it was necessary to inoculate the soil twice during each field trial to maintain exposure through the period of fruit set to maturity. For drip inoculations, a single event allowed bacteria to establish in the root zone and rhizosphere soil throughout the growing season and beyond the date of termination if irrigation events (Table 3). However, our findings also suggest, that internalization occurs at a limited frequency and minimal transport-distance in the plant vascular system. When internalization does occur the applied *Salmonella* appears to be unable to reach the fruit peduncle. However, long-distance systemic transfer of *Salmonella* has been demonstrated for tomato plants (Gu et al., 2011). It was not determined whether absence of recovery following enrichment signifies cell death or a viable but not culturable physiological condition which has been reported for serovar Typhimurium (Panutdaporn et al., 2006; Passerat et al., 2009).

In this study, detection of *Salmonella* on melon plants was mostly associated with rind surfaces, particularly from melons that were in direct contact with the soil from the furrow or from the top of the bed between furrow-contaminated rows after an event of heavy rainfall (Table 4). This understandably indicates that soil transference is a key risk factor for surface contamination. In open field production, dissemination of bacteria from soil or other sources can be the result of multiple factors, including wildlife, aerosol or soil transference or splash dispersal as occurred in this study. Surface contamination is a recognized risk, as once bacteria are in contact with melon rind and firmly attached, postharvest washing operations cannot ensure their removal (Ukuku and Fett, 2002b, 2006). In addition, several studies have demonstrated that pathogen transfer from rind to melon fruit during processing or home preparation can occur (Ukuku, 2004; Ukuku and Fett, 2002a; Selma et al., 2008).

Internalization to fruit was only positive after intentional peduncle damage and inoculation with different doses of *Salmonella* in the vine tissue adjacent to the fruit. Damage to the peduncle and co-incident inoculation with *Salmonella*, has not been demonstrated to occur in a natural field production situation, however this inoculation method demonstrated that transport from vine to fruit across the abscission zone (slip) is possible if the bacterium does reach the vascular system immediately adjacent to the fruit. The fact that we observed the differences in internalization to fruit and adjacent vines in a dose dependent manner, suggests that the vascular system is sufficiently accessible to allow a low number of *Salmonella* cells to move over a short distance. Current results do not support the probability of long-distance systemic transfer and survival that would extend the distance from the root to the fruit during commercial melon production. Studies characterizing the physiological status of *S. enterica* in the vascular system and its ability to behave as a metabolically active endophyte on in a viable but non culturable state would need to be carried out.

Artificial inoculation of the surface with different doses of *Salmonella* showed that after application, there is a rapid drop of the quantifiable *Salmonella* population as detection of the bacterium was only possible after sample enrichment. Specifically, a greater proportional survival was observed on samples inoculated with log 6 and log 8 CFU/mL compared to those inoculated with log 4 CFU/mL (Table 5). Likely, after inoculation, exposure to the open environment at the test location, with extended daytime temperatures often above 35 °C, low humidity and high solar UV radiation, cause *Salmonella* cells to die off (Panutdaporn

et al., 2006). This outcome following controlled contamination events has been also observed in various field trials with leafy greens inoculated with *E. coli* O157:H7 (Gutierrez-Rodriguez et al., 2012; Moyne et al., 2011). It would be important to consider, that if contamination of melon surface occurs, the presence of soil, organic matter and vine cover, could enhance the survival as those can offer additional protection to the bacterium.

5. Conclusions

The outcomes of this study strongly indicate that root uptake and systemic transport of *Salmonella* from soil, as a consequence of contaminated irrigation water, is highly unlikely to occur, particularly if it is considered that conditions in this study corresponded to an exaggerated worst-case scenario. Preventing these conditions is expected from the industry in collaboration with public health organizations during operation under Good Agricultural Practices management. Clearly any crop management input, or other sources of contamination that could result in transfer of pathogens, such as *Salmonella*, into the growing area has the potential to result in fruit-surface contamination at low levels, and certainly viable *Salmonella* cells on the production soil surface, could be transferred to fruit by various vectors and by human and equipment movement or direct contact during harvest operations. While contamination of the external rind from irrigation sources remains a concern in melon production, results suggest acceptable pathogen criteria may be defined to establish critical limits for melon irrigation in California and regions with similar arid and semi-arid climate, soil texture, and crop management practices.

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References

Alum, A.E., Oron, C.E., Gerba, C.P., 2000. Control of viral contamination of reclaimed irrigated vegetables by drip irrigation. Water Use Conf J. Am Wat. Wks. Assoc. Conf J. Am Wat. Wks. Assoc.

ANR 7218, 2008. Cantaloupe Production in CA(Accessed at) http://anrcatalog.ucdavis.edu/.

Berg, G., Eberl, L., Hartmann, A., 2005. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environmental Microbiology 7, 1673–1685.

Bernstein, N., Sela, S., Pinto, R., Ioffe, M., 2007. Evidence for internalization of *Escherichia coli* into the aerial parts of maize via the root system. Journal of Food Protection 70, 471–475.

Bowen, A., Fry, A., Richards, G., Beuchat, L., 2006. Infections associated with cantaloupe consumption: a public health concern. Epidemiology and Infection 134, 675–685.

Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annual Review of Phytopathology 44, 367–392.

Centers for Disease Control and Prevention, 1991. Multistate outbreak of *Salmonella* poona infections—United States and Canada, 1991. Morbidity and Mortality Weekly Report 40, 549–552.

Centers for Disease Control and Prevention, 2002a. From the Centers for Disease Control and Prevention. Multistate outbreaks of *Salmonella* serotype poona infections associated with eating cantaloupe from Mexico–United States and Canada, 2000–2002. JAMA: The Journal of the American Medical Association 288, 2967–2969.

Centers for Disease Control and Prevention, 2002b. Multistate outbreaks of *Salmonella* serotype poona infections associated with eating cantaloupe from Mexico–United States and Canada, 2000–2002. MMWR. Morbidity and Mortality Weekly Report 51, 1044–1047.

Cevallos-Cevallos, J.M., Danyluk, M.D., Gu, G., Vallad, G.E., van Bruggen, A.H., 2012. Dispersal of *Salmonella* Typhimurium by rain splash onto tomato plants. Journal of Food Protection 75, 472–479.

Duris, J.W., Haack, S.K., Fogarty, L.R., 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, 1878–1886.

Erickson, M.C., 2012. Internalization of fresh produce by foodborne pathogens. Annuual Review of Food Science and Technology 3, 283–310.

Erickson, M.C., Liao, J., Payton, A.S., Riley, D.G., Webb, C.C., Davey, L.E., Kimbrel, S., Ma, L., Zhang, G., Flitcroft, I., Doyle, M.P., Beuchat, L.R., 2010a. Preharvest internalization of *Escherichia coli* O157:H7 into lettuce leaves, as affected by insect and physical damage. Journal of Food Protection 73, 1809–1816.

Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J., Ma, L., Doyle, M.P., 2010b. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. Journal of Food Protection 73, 500–506.

FDA, 2009. Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards of Melons; Draft Guidance. CFSAN. updated version 08/24/2011. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm174171.htm/.

Franz, E., van Bruggen, A.H.C., 2008. Ecology of *E. coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. Critical Reviews in Microbiology 34, 143–161.

Franz, E., Visser, A.A., van Diepeningen, A.D., Klerks, M.M., Termorshuizen, A.J., van Bruggen, A.H.C., 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. Food Microbiology 24, 106–112.

Gagliardi, J.V., Millner, P.D., Lester, G., Ingram, D., 2003. On-farm and postharvest processing sources of bacterial contamination to melon rinds. Journal of Food Protection 66, 82–87.

Gorski, L., Parker, C.T., Liang, A., Cooley, M.B., Jay-Russell, M.T., Gordus, A.G., Atwill, E.R., Mandrell, R.E., 2011. Prevalence, distribution and diversity of *Salmonella enterica* in a major produce region of California. Applied and Environmental Microbiology. http://dx.doi.org/10.1128/AEM.02321-10.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F., Painter, J.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiology and Infection 136, 157–165.

Gu, G., Hu, J., Cevallos-Cevallos, J.M., Richardson, S.M., Bartz, J.A., van Bruggen, A.H.C., 2011. Internal colonization of *Salmonella enterica* serovar Typhimurium in tomato plants. PLos One 6, e27340.

Gutierrez-Rodriguez, E., Gundersen, A., Sbodio, A.O., Suslow, T.V., 2012. Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of *Escherichia coli* on spinach. Journal of Applied Microbiology 112, 109–118.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R., Rose, J.B., 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. Applied and Environmental Microbiology 71, 3163–3170.

Hassan, J.O., Curtiss Iii, R., 1990. Control of colonization by virulent *Salmonella* typhimurium by oral immunization of chickens with avirulent Δcya Δcrp *S.* typhimurium. Research in Microbiology 141, 839–850.

Hoffman, S., 2011. U.S. Food Safety Enters a New Era. USDA Economic Research Service. Amber Waves. (December 2011. 6 pp.).

Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P., Jian, X., 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Applied and Environmental Microbiology 70, 2495–24502.

Klerks, M.M., van Gent-Pelzer, M., Franz, E., Zijlstra, C., van Bruggen, H.C., 2007. Physiological and molecular responses of *Lactuca sativa* to colonization by *Salmonella enterica* serovar Dublin. Applied and Environmental Microbiology 73, 4905–4914.

Materon, L., 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.

Miles, J.M., Sumner, S.S., Boyer, R.R., Williams, R.C., Latimer, J.G., McKinney, J.M., 2009. Internalization of *Salmonella enterica* serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. Journal of Food Protection 72, 849–852.

Mohle-Boetani, J.C., Reporter, R., Werner, S.B., Abbott, S., Farrar, J., Waterman, S.H., Vugia, D.J., 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. The Journal of Infectious Diseases 180, 1361–1364.

Moyne, A.L., Sudarshana, M.R., Blessington, T., Koike, S.T., Cahn, M.D., Harris, L.J., 2011. Fate of *Escherichia coli* O157:H7 in field-inoculated lettuce. Food Microbiology 28, 1417–1425.

Munnoch, S.A., Ward, K., Sheridan, S., Fitzsimmons, G.J., Shadbolt, C.T., Piispanen, J.P., Wang, Q., Ward, T.J., Worgan, T.L., Oxenford, C., Musto, J.A., McAnulty, J., Durrheim, D.N., 2009. A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. Epidemiology and Infection 137, 367–374.

National Agricultural Statistics Service (NASS), 2011. Vegetables Annual Summary. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1183.

Ongeng, D., Muyanja, C., Ryckeboer, J., Geeraerd, A.H., Springael, D., 2011. Rhizosphere effect on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in manure-amended soil during cabbage (*Brassica oleracea*) cultivation under tropical field conditions in Sub-Saharan Africa. International Journal of Food Microbiology 149, 133–142.

Opara, L.U., Mazaud, F., 2001. Food traceability from field to plate. Agriculture 30, 239–247.

Oron, G., DeMalach, Y., Hoffman, Z., 1992. Effect of effluent quality and application method on agricultural productivity and environmental control. Water Science Technology 267, 1593–1601.

Pachepsky, Y.A., Guber, A.K., Shelton, D.R., McCarty, G.W., 2009. Size distributions of manure particles released under simulated rainfall. Journal of Environmental Management 90, 1365–1369.
Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J., Mandrell, R.E., 2011. Irrigation waters as a source of pathogenic microorganisms in produce: a review. Advances in agronomy. Academic Press, Amsterdam, pp. 74–121.
Panutdaporn, N., Kawamoto, K., Asakura, H., Makino, S.-I., 2006. Resuscitation of the viable but non-culturable state of *Salmonella enterica* serovar Oranienburg by recombinant resuscitation-promoting factor derived from *Salmonella* Typhimurium strain LT2. International Journal of Food Microbiology 106, 241–247.
Passerat, J., Got, P., Dunkan, S., Monfort, P., 2009. Respective roles of culturable and viable-but-nonculturable cells in the heterogeneity of *Salmonella enterica* serovar Typhimurium invasiveness. Applied and Environmental Microbiology 75, 5179–5185.
Powell, D., 2011. Cantaloupe related outbreaks and recalls(Accessed at) http://bites.ksu.edu/cantaloupe-related-outbreaks12 Feb. 2012.
Produce Marketing Association (PMA), United Fresh and Vegetable Association, 2005. Commodity specific food safety guidelines for the melon supply chain. First edition. http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatoryInformation/UCM168625.pdf.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., et al., 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Disease. http://dx.doi.org/10.3201/eid1701.P11101.
Selma, M.V., Ibanez, A.M., Allende, A., Cantwell, M., Suslow, T.V., 2008. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. Food Microbiology 25, 162–168.
Semenov, A.V., van Overbeek, L., van Bruggen, A.H.C., 2009. Percolation and survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in soil amended with contaminated dairy manure or slurry. Applied and Environmental Microbiology 75, 3206–3215.
Sensoy, S., Ertek, A., Gedik, I., Kucukymuk, C., 2007. Irrigation frequency and amount affect yield and quality of field-grown melon (*Cucumis melo* L.). Agricultural Water Management 88, 269–274.
Shelton, D.R., Karns, J.S., Coppock, C., Patel, J., Sharma, M., Pachepsky, Y.A., 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.
Solomon, E.B., Yaron, S., Matthews, K.R., 2002. 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, S.W., Choi, C.Y., Gerba, C.P., 2006. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. Journal of Environmental Engineering 132, 1243–1248.
Steele, M., Odumeru, J., 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. Journal of Food Protection 67, 2839–2849.
Steele, M., Mahdi, A., Odumeru, J., 2005. Microbial assessment of irrigation water used for production of fruit and vegetables in Ontario, Canada. Journal of Food Protection 68, 1388–1392.
Suslow, T.V., 2010. Standards for irrigation and foliar contact water, produce safety project issue brief. Available at: http://www.producesafetyproject.org/admin/assets/files/Water-Suslow-1.pdf. Accessed December 18 2012.
Suslow, T., Schroth, M.N., 1982. Rhizobacteria of sugar beets: effects of seed application and root colonization on yield. Phytopathology 72, 199–206.
Suslow, T.V., Oria, M.P., Beuchat, L.R., Garrett, E.H., Parish, M.E., Harris, L.J., Farber, J.N., Busta, F.F., 2003. Chapter II: production practices as risk factors in microbial food safety of fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety 2S, 38–77.
Theofel, C.G., Harris, L., 2009. Impact of preinoculation culture conditions on the behavior of *Escherichia coli* O157:H7 inoculated onto Romaine lettuce (*Lactuca sativa*) plants and cut leaf surfaces. Journal of Food Protection 72, 1553–1559.
U.S. Food and Drug Administration, 2008. Center for Food Safety and Applied Nutrition. Guide to Minimize Microbial Food Safety Hazards of Fresh-Cut Fruits and Vegetables. U.S. Food and Drug Administration. Center for Food Safety and Applied Nutrition, Washington, D.C.
Ukuku, D.O., 2004. Effect of hydrogen peroxide treatment on microbial quality and appearance of whole and fresh-cut melons contaminated with *Salmonella* spp. International Journal of Food Microbiology 95, 137–146.
Ukuku, D.O., Fett, W., 2002a. Behavior of *Listeria monocytoge*nes inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. Journal of Food Protection 65, 924–930.
Ukuku, D.O., Fett, W.F., 2002b. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. Journal of Food Protection 65, 1093–1099.
Ukuku, D.O., Fett, W.F., 2006. Effects of cell surface charge and hydrophobicity on attachment of 16 *Salmonella* serovars to cantaloupe rind and decontamination with sanitizers. Journal of Food Protection 69, 1835–1843.
Wilson, M., Lindow, S., 1993. Effect of phenotypic plasticity on epiphytic survival and colonization by *Pseudomonas syrinage*. Applied and Environmental Microbiology 59, 410–416.
Winfield, M.D., Groisman, E.A., 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Applied and Environmental Microbiology 69, 3687–3694.
Zhang, G., Ma, L., Beuchat, L.R., Erickson, M.C., Phelan, V.H., Doyle, M.P., 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, 2028–2037.