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

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A R T I C L E   I N F O

Article history:
Received 1 March 2012
Received in revised form 2 July 2012
Accepted 5 July 2012
Available online 13 July 2012

Keywords:
Cantaloupe
Honeydew
Furrow irrigation
Drip irrigation
Salmonella
Internalization

A B S T R A C T

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 factor 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 agricultural 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.

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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 transfer 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
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 Specifications for irrigation water quality (Suslow, 2010). Melons and plant systems (Bernstein et al., 2007; Solomon et al., 2002; Klerks et al., 2004). A derivative isolate, aPTVS177, is a rifampicin-resistant strain from aPTVS150 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 aPTVS177 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 aPTVS177 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.

aPTVS177 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 L of sodium pyruvate {C3H3NaO3{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 aPTVS177 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 aPTVS177, 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 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 aPTVS150 was used in this study. The parental source of aPTVS150 was S. enterica sv. Typhimurium y3895, generously made available by R. Curtis (Hassan and Curtiss lli, 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, aPTVS177, is a rifampicin-resistant strain from aPTVS150 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 aPTVS177 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 aPTVS177 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.

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established on approximately 2 m (center to center) raised beds with blocks of replicated-plantings. Honeydew and cantaloupe vines were spaced at 77 cm. Each half bed corresponded to one block that contained trial such that plants during primary fruit set were not in direct contact

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 HMX4509) 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 sacs spaced approximately 7.5 m apart as described by Gutierrez-Rodriguez et al. (2012). Infusion sacs were prepared by mixing 3 kg of sand with 1 L of log 9 CFU/mL of aPTVS177 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 sacs, 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 sacs 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 aPTVS177 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 aPTVS177. Water was mixed in the tank by recirculation 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 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 aPTVS177 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 (TSARP) (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 aPTVS177. 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 pPTVS177 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 pPTVS177.

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 KH2PO4 and 6.1 mM K2HPO4) 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 with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). The rind was vigorously rubbed to detach bacteria and the suspension was plated on TSARPP with 0.05% Tween 20 (Fisher, Fair Lawn, NJ). 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 pPTVS177 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 pPTVS177.

A second set of random melons (n = 60) from each subplot was tagged and the peduncle of each melon was inoculated with 20 CFU/mL of pPTVS177. After the drop was deposited a days presented refer to time from first inoculation event to 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 pPTVS177 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 (pPTVS177) in soil, fruit and melon vines grown under greenhouse conditions after multiple inoculation events.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Days post inoculationa</th>
<th>Proportion of positive samples after enrichmentb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oro Rico</td>
<td>Top Mark</td>
</tr>
<tr>
<td>Soil</td>
<td>49</td>
<td>3/3</td>
</tr>
<tr>
<td>Vines</td>
<td>15c</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>49d</td>
<td>0/3</td>
</tr>
<tr>
<td>Fruit</td>
<td>32</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>ND</td>
</tr>
</tbody>
</table>

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.

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, pPTVS177 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 pPTVS177, 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 irrigation), 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 pPTVS177 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
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 silt-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).

Table 2

<table>
<thead>
<tr>
<th>Source of water collection</th>
<th>Furrow (log CFU/mL)a</th>
<th>Drip lines (log CFU/mL)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days post inoculation</td>
<td>Year 2009</td>
<td>2010</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.12 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>21b</td>
<td>2.30 ± 0.03</td>
</tr>
</tbody>
</table>

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.

ND, not determined.

Table 3

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Furrow field (log CFU/g of soil)</th>
<th>Drip field (log CFU/g of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Around the drip emitter line a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Furrow a</td>
<td>Bed edge b</td>
</tr>
<tr>
<td>Year 2009</td>
<td>5</td>
<td>3.99 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>21b</td>
<td>2.68 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>41b</td>
<td>6/6a</td>
</tr>
<tr>
<td>Year 2010</td>
<td>15b</td>
<td>1.63 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>37b</td>
<td>1.59 ± 0.27</td>
</tr>
</tbody>
</table>

a Quantiﬁcation was below the limit of detection (1.43 CFU/g of soil).

b 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

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Furrow field Proportion of positive samples for S. enterica/total analyzed samples</th>
<th>Drip field Proportion of positive samples for S. enterica/total analyzed samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit a (C) (HD) (C) (HD)</td>
<td>Fruit a (C) (HD) (C) (HD)</td>
</tr>
<tr>
<td></td>
<td>Year 2009</td>
<td>Year 2010</td>
</tr>
<tr>
<td>35</td>
<td>4/15 ND 3/14 0/15 ND</td>
<td>2/15 ND 0/25 ND</td>
</tr>
<tr>
<td>41</td>
<td>0/15 ND 8/8 ND ND</td>
<td>ND ND 0/6</td>
</tr>
<tr>
<td>46</td>
<td>ND ND 0/15 ND 0/6</td>
<td>ND ND</td>
</tr>
<tr>
<td>31</td>
<td>0/21 ND ND ND</td>
<td>ND ND</td>
</tr>
<tr>
<td>38</td>
<td>0/90 ND ND ND</td>
<td>ND ND</td>
</tr>
<tr>
<td>43</td>
<td>0/90 0/90 15/21 0/75 0/50</td>
<td>ND ND</td>
</tr>
</tbody>
</table>

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.

Table 5

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Proportion of samples positive for detection of S. enterica after enrichment/total samples analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculation dose (CFU/mL)</td>
</tr>
<tr>
<td></td>
<td>4 6 8</td>
</tr>
<tr>
<td>2</td>
<td>4/5 5/7 5/5</td>
</tr>
<tr>
<td>10</td>
<td>0/20 8/20 4/20</td>
</tr>
</tbody>
</table>

a Quantification was intended for these samples, however the population of S. enterica was below the limit of detection (1.43 CFU/melon).
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 irrigant 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
This outcome following controlled contamination events has also been 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 rins 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. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jifoodmicro.2012.07.005.

Acknowledgments

This work was funded by the Center for Produce Safety, the California Melon Research Board, and the California Cantaloupe Advisory Board. We gratefully acknowledge Garry Pearson, Jim Jackson, and Fred Stewart for the transplant production and field trial management and Eduardo Gutierrez-Rodríguez for the trellised melon management. Sharyn Maeda and Francisco Lopez-Galvez for their valuable contribution during the melon sampling and sample processing.

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