AIRBORNE SOIL PARTICULATES AS VEHICLES FOR *SALMONELLA*

CONTAMINATION OF TOMATOES

GOVINDARAJ DEV KUMAR^a, ROBERT C. WILLIAMS^a, HAMZEH ALQUBLAN^b,
NAMMALWAR SRIRANGANATHAN^b, RENEE R. BOYER^a, and JOSEPH D. EIFERT^a

^aDepartment of Food Science and Technology Virginia Polytechnic Institute and State University, Blacksburg, Virginia , USA

^bCenter for Molecular Medicine and Infectious Diseases, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA

Running Head: ***Salmonella*-Tomato contamination by soil particulate**

Key Words: ***Salmonella*, tomato, bioluminescence, soil particulate**

*Author for correspondence: Dr. Robert C. Williams

Department of Food Science and Technology,

Virginia Polytechnic Institute and State University,

Blacksburg, Virginia 24061, USA

Tel: (540) 231-4106; Fax: (540) 231-9293

E-mail: rowilli3@vt.edu¹

¹ Current address: Govindaraj Dev Kumar

Department of Plant Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742

Abstract

The presence of dust is ubiquitous in the produce growing environment and its deposition on edible crops could occur. The potential of wind-distributed soil particulate to serve as a vehicle for *S. Newport* transfer to tomato blossoms and consequently, to fruits, was explored. Blossoms were challenged with previously autoclaved soil containing *S. Newport* (9.39 Log CFU/g) by brushing and airborne transfer. One hundred percent of blossoms brushed with *S. Newport*-contaminated soil tested positive for presence of the pathogen one week after contact ($P < 0.0001$). Compressed air was used to simulate wind currents and direct soil particulates towards blossoms. Airborne soil particulates resulted in contamination of 29% of the blossoms with *S. Newport* one week after contact. Biophotonic imaging of blossoms post-contact with bioluminescent *S. Newport*-contaminated airborne soil particulates revealed transfer of the pathogen on petal, stamen and pedicel structures. Both fruits and calyxes that developed from blossoms contaminated with airborne soil particulates were positive for presence of *S. Newport* in both fruit (66.6%) and calyx (77.7%). Presence of *S. Newport* in surface-sterilized fruit and calyx tissue tested indicated internalization of the pathogen. These results show that airborne soil particulates could serve as a vehicle for *Salmonella*. Hence, *Salmonella* contaminated dust and soil particulate dispersion could contribute to pathogen contamination of fruit, indicating an omnipresent yet relatively unexplored contamination route.

1. Introduction

Salmonella enterica is the leading cause of foodborne illness in the US and tomatoes are the most frequently associated produce item in *Salmonella* outbreaks (CDC, 2008). Tomato-associated *Salmonella enterica* outbreaks have occurred more than 15 times over the last two decades in the United States (Bennett et al., 2015; Berger et al., 2010; Hanning et al., 2009). Approximately 5 billion pounds of fresh market tomatoes are consumed annually in the US (Gupta et al., 2007) and outbreaks associated with tomatoes result in widespread illness and financial burden to both affected individuals and tomato cultivators (Hanning et al., 2009). Salmonellae can survive for extended periods of time in the environment during transition between hosts (Cevallos-Cevallos et al., 2014; Jacobsen and Bech, 2012) and can persist in farm soils and sediments (Greene et al., 2008; Micallef et al., 2012).

Soil particulates can be dispersed in the field due to winds (Ravi et al., 2011) and aerosolized dust particulates have been associated with pathogen transfer in poultry facilities. An assessment of *Salmonella* and *Campylobacter* presence in aerosols within and outside poultry sheds revealed that bacterial numbers in air correlated to their population in poultry litter (Chinivasagam et al., 2009). A study on the microbial composition of a high-throughput chicken slaughtering facility over a four-month period indicated that the highest microbial counts were found in the areas that

had the highest amounts of airborne particulates. Dust was the only environmental factor in the study that had a significant influence on the dispersal of *Salmonella* spp (Lues et al., 2007). While soil, water, and aerosols have been studied for their potential to serve as contamination vehicles of *Salmonella*, dust in the produce growing environment remains lesser explored. Foodborne pathogens could associate themselves with particulate matter and can be dispersed from their source (Berry et al., 2015; Cevallos-Cevallos et al., 2014). Soil, composts and manure are suspected as sources of foodborne pathogens and studies have shown that these can be aerosolized and lead to pathogen spread (Brandl, 2006; Millner, 2009). Agricultural fields are constantly subject to wind based transport of sediments, dusts and aerosols (Ravi et al., 2011). Soil particulates moved by wind range in size up to 1mm in diameter (Zobeck and Van Pelt, 2006) and could serve as a vehicle of Salmonellae.

Previous studies have demonstrated that Salmonellae can be internalized into tomato fruit upon brushing of blossoms with inoculum (Guo et al., 2001; Shi et al., 2007; Zhuang et al., 1995) though natural routes of blossom contamination remain unknown. The objectives of this study were to test the potential of soil particulate to deliver *S. Newport* to tomato blossoms and consequentially to fruit tissue that developed from contaminated blossoms. Biophotonic imaging was employed to visualize spatial distribution and retention of *Salmonella* upon contact of

blossom with airborne soil particulate. The results of this study could help elucidate a ubiquitous but relatively unexplored contamination route of field-grown tomatoes.

2. Materials and Methods

2.1 Bacterial strains

Stock cultures of *Salmonella enterica* subsp. *enterica* serovar Newport were obtained from the culture collection at Virginia Polytechnic Institute and State University. The frozen stock culture was thawed and a loopful of culture was transferred to 25 ml of Brain Heart Infusion broth (BHIB; Becton, Dickinson and Co, Sparks, MD) and incubated for 24 hrs at 37°C. Three subcultures were performed from the initial stock of *S. Newport* at 24 h intervals into 25 ml of BHIB. Incubation of BHIB was performed at 37°C for 24 h. A loopful of culture from the final batch of BHIB was streaked on xylose lysine Tergitol 4 (XLT4); (Becton, Dickinson and Co, Sparks, MD). The plates were incubated for 24 hrs at 37°C. Typical *Salmonella* colonies from the XLT4 plates were confirmed biochemically using 20E API strips (bioMerieux, Hazelwood, MO) and serologically using latex agglutination assay (Oxoid, Ogdensburg, NY). Upon confirmation, colonies were streaked on TSA (Becton, Dickinson and Co, Sparks, MD) slants and incubated for 24 hrs at 37°C, following this, the slants were stored in the refrigerator at 4°C for inoculum preparation.

2.2 Bioluminescent *Salmonella Newport*

Competent *S. Newport* cells were prepared by inoculating 45 ml of LB broth (Becton, Dickinson and Co, Sparks, MD) with 1 ml of an overnight culture of *S. Newport*. Once the optical density (OD_{600}) reached 0.8, the cells were placed in ice for 15 min. The culture was centrifuged at 1400 g for 10 min to pellet the cells and the supernatant was discarded. The pelleted cells were washed three times with 15% ice-cold glycerol and stored at -80°C until use. Competent *S. Newport* was transformed with broad host range plasmid pNSTrc-lux containing the *lux CDABE* operon (Seleem et al., 2008). For electroporation, competent cells were placed in ice and then 40 μl were transferred to a sterile 1.5 ml microcentrifuge tube along with 1 μl ($60 \text{ ng } \mu\text{l}^{-1}$) of plasmid DNA isolated from *E. coli* DH10BTM T1 using a QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA). The cells and plasmid DNA were mixed and spun down. The mixture was then transferred to an ice-chilled 2 mm gap cuvette (Bio-Rad, Hercules, CA). Electrotransformation protocols were adapted from Howe et al. (Howe et al., 2010). For *S. Newport*, the electroporation conditions applied were 2.5 kV, 25 μF and 400Ω using the Gene Pulser II system (Bio-Rad, Hercules, CA). After electroporation, the cells were transferred into 450 μl SOC medium (2% Bacto Tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl_2 , 10 mM MgSO_4 , 20 mM glucose) and incubated at 37°C in a shaking incubator at 100 rpm for 1 hour. For selection of

transformants, 100 µl of the broth was spread plated on TSA plates containing chloramphenicol (30 µg ml⁻¹) and observed for transformants after 24 hours of incubation.

2.3 Plant growth conditions

Bonnie “Sweet 100” sweet cherry tomato plants were purchased from a local store during the summer of 2011. Plants had not reached flowering stage when procured and were *Salmonella* free. The plants were transferred to alcohol-sanitized pots containing Miracle-Gro potting mix (Scotts Miracle-Gro Products, Port Washington, N.Y.), which tested negative for the presence of *Salmonella* on XLT4 agar. The plants were irrigated with tap water on a daily basis and Miracle-gro Liquid All-Purpose Plant Food (Scotts Miracle-Gro Products, Port Washington, N.Y.) was used weekly as per manufacturer’s instructions. Once the plants reached flowering stage (2 weeks), they were transferred to a Precision incubator (Thermo Scientific, Waltham, MA). Blossoms (n=3) from each of the plants used during the study were negative for the presence of *Salmonella* before the start of the treatments. Plants were grown at 25°C and 70% relative humidity, with 12 hour light and dark intervals. They were irrigated on a daily basis with tap water. A drip tray was placed at the bottom shelf of the incubator to collect plant debris and water droplets after watering. Once blossom formation occurred, each peduncle of tomato blossoms was tagged for identification with a labeled tape at the base stem. A plastic sampling

cup with its base cut was placed around the flowers to prevent dispersal of inoculum to other plant tissues.

2.4 Preparation of soil

Soil was obtained from a tomato farm on the eastern shore of Virginia (United States). The sandy soil was passed through a No. 20 sieve (U.S.A Standard Testing Sieve) to obtain uniform grain size. The soil (in 10 g portions) was autoclaved (121°C for 12 min) to eliminate populations of existing microorganisms. Autoclaved soil was tested for sterility by performing an aerobic plate count. The soil sample was also screened for the presence of *Salmonella* spp. by plating on XLT4 agar. The 10 g soil aliquots were stored in a sterile 50 ml centrifuge tube and stored at 4°C until use.

2.5 Preparation of Inoculum

A loopful of *S. Newport* was inoculated into 45 ml TSB (Tryptic soy broth) and incubated in a shaking incubator at 200 rpm for 24 hours, 37°C. The broth was then centrifuged at 1400 g for 10 min to pellet the cells. The supernatant was discarded and the cells were washed twice with sterile deionized distilled water to rid them of remaining media. The washed cells were pelleted by spinning in a centrifuge at 1400 g for 10 min and the supernatant was discarded. Ten grams of sterile soil (a_w 0.92) was added to the pellet and the mixture was vigorously mixed using a sterile

spatula by adding 1 g aliquots of soil and mixing with the pellet. The container with its lid open was placed in a desiccator containing CaSO₄ (DrieriteTM Xenia, OH) for 48 hours to facilitate the drying process. The soil was then mixed using a vortexer to create equal distribution of *S. Newport* in soil.

2.6 Brush inoculation

An ethanol (70%) sanitized paintbrush moistened with sterile distilled water was used to apply dust particle-*S. Newport* mixture to healthy tomato blossoms (n=6; 2 each from three different plants). Care was taken not to dislodge blossoms during dust application. Blossoms that were dislodged were discarded. Petals, stamens and pedicel of tomato blossoms from each peduncle were brushed with approximately 100 mg of *S. Newport* inoculated soil (9.39 log CFU/g) to ensure contact with the entire surface area of the blossom. For the control treatment, autoclaved soil particles (121°C for 12 min) were brushed on to blossoms.

2.7 Air inoculation

Fourteen blossoms from three different plants (4, 6, and 4 blossoms from plants 1, 2, and 3, respectively) were treated with inoculated dust. Nine blossoms (3 each from 3 different plants) were used as controls and treated with non-inoculated dust. For the inoculation of blossoms, 100 hundred mg of the dried *S. Newport*-soil mixture (9.39 log CFU/g) was placed on the edge of a

sterile spatula. A Nalgene aerosol spray bottle (#2430-0200, Fisher Scientific, Pittsburgh, PA, USA) was pressurized with ambient air. The nozzle of the sprayer and the dust were placed 6 inches near the flower and pressurized air was released by pressing nozzle once to deliver the soil onto the blossom surface. Care was taken to prevent errant distribution of dust by using a collection cup around each blossom bunch. Inoculation of blossoms was performed in a sterile cabinet. For the control treatment, autoclaved soil particulates (121°C for 12 min) were air inoculated into a blossom bunch from the same plant and the flowers were tested.

2.8 Microbiological testing of blossoms

Inoculated and control blossoms were tested one week after inoculation for the presence of *S. Newport*. The inoculated blossom bunches were excised and each individual blossom was placed in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 10 ml of sterile peptone diluent (0.1% peptone). The bag was massaged to facilitate particulate dislodging and the wash water was streaked on the XLT4 agar. Plates were incubated at 37°C for 48 hours.

2.9 Microbiological testing of fruit and calyxes

Fruits without calyx were tested from three different plants (n=6 fruits per plant), fruits with calyx attached was tested from one plant (n=25) and calyxes from one plant (n=11) were tested for the presence of *Salmonella* three weeks after blossom inoculation by dust particulate. Three

control fruits, from three plants each (n=9) were tested for the presence of *Salmonella*. Fruits and calyxes tested were not from the same fruit set, to uphold randomness in sample selection. To test for surface contamination of *S. Newport*, the excised tissue was placed in labeled Whirl-Pak bags and massaged in 10 ml sterile peptone water to dislodge surface-attached cells. One hundred microliters of the peptone water was plated on XLT4 and incubated at 37°C for 24 h. To account for internalized *S. Newport*, test samples were surface sterilized by immersing in 10% commercial bleach, air dried, and then immersing in 70% ethanol for a duration of 15 min each. The ethanol-washed tissue was dried under UV radiation for one hour. During UV exposure, the test tissue was turned every 15 min to ensure uniform exposure. The surface-sterilized tissue was then placed in a whirlpak bag and macerated with a mortar and pestle to ensure exposure to the enrichment medium. The macerated tissue was incubated in 10 ml of buffered peptone water for 24 hours at 37°C. After incubation of the tissue in buffered peptone water (BPW), 100 µl of the BPW was plated on XLT4 and incubated at 37°C for 24 hours to account for pathogen presence. Following this, 1 ml of the BPW in which the tissue had been incubated was transferred to 10 ml of Tetrathionate broth. After 24 hours incubation at 37°C, 100µl of the tetrathionate broth was spread-plated on XLT4. The XLT4 plates were incubated at 37°C for 24 hours. Black colonies indicative of *Salmonella* on XLT4 plates were considered positive. These were confirmed as *Salmonella* through latex agglutination test (*Salmonella*: DR1108, Oxoid, Basingstoke).

2.10 Preparation of inoculum for bioluminescent imaging

Dispersal of *Salmonella* cells on flowers was also studied using bioluminescent *S. Newport* transformed with the broad range pNSTrc-lux plasmid containing the *lux CDABE* operon. Control blossoms were inoculated with autoclaved soil free of bacteria. Inoculum was prepared by the same procedure described previously for blossom inoculation except that the media used to grow the *S. Newport* cells and the distilled deionized water used for washing the pelleted cells contained chloramphenicol ($30 \mu\text{g mL}^{-1}$) to aid in plasmid retention by *S. Newport* cells.

2.11 Inoculation and imaging

Inoculation was performed using pressurized air from a Nalgene aerosol spray bottle as described earlier. Once the blossoms were inoculated, they were excised after 30 min and placed in a sterile petri dish for imaging. Imaging was performed using an electron-multiplying charge-coupled device camera (EMCCD, Andor, Ixon) 4 hours after inoculation.

2.12 Statistical analysis

The experiment of flower inoculation studies was a randomized complete blocked design. Only data from experiments conducted in triplicates was statistically analyzed. The effects of brushing and air delivery of inoculated dust on tomato blossoms were analyzed by Chi-square analysis and

Fisher's exact test, using the FREQ procedure of SAS 9.1 (SAS Inst., Inc., Cary, NC, USA). Air inoculation of blossoms and resulting contamination of fruit was also analyzed by Chi-square analysis and Fisher's exact test, using the FREQ procedure. A probability value of less than 0.05 indicated a statistically significant.

3. Results

3.1 *S. Newport transfer to tomato blossoms from soil through brush inoculation*

Blossoms brushed with a *S. Newport* – soil mixture were analyzed one-week post contact for the presence of *Salmonella*, to determine both pathogen transfer and retention on blossoms. All six blossoms tested were positive for the presence of *S. Newport* one week after brushing with the *Salmonella* - soil mixture ($P < 0.001$), indicating both transfer of pathogen from soil to blossom surface and retention of *S. Newport* on blossom surface was possible (Table 1). None of the control blossoms that were brushed with autoclaved soil tested positive for the presence of *S. Newport* (Table 1). Brushing of petals, stamens and pedicels resulted in dislodging of eleven blossoms. Dislodged blossoms were discarded and not used to test for the presence of *S. Newport*.

3.2 *S. Newport transfer to tomato blossoms from soil through air inoculation*

Compressed air was used to mimic wind-based dispersal of soil. Soil- *S. Newport* mixture came in contact with petal, stamens and pedicel. Post treatment, 7 blossoms were dislodged and these were discarded and not tested for the presence of *S. Newport*. Four out of the 14 blossoms (28.6%) tested (28.6%) were positive for the presence of *S. Newport* one week after inoculation, indicating airborne soil particulates can serve as a contamination vehicle for *Salmonella* and that

pathogen retention was possible on blossoms one week after contact with air dispersed soil particulate (Table 1).

3.3 Bioluminescent imaging of compressed air inoculated blossoms to visualize spatial distribution of pathogen on blossom tissue.

Biophotonic imaging of the blossoms post contact with contaminated soil particulates revealed that the bioluminescent *S. Newport* cells from soil particulates were transferred onto blossom tissue such as petals, stamen and pedicel (Figure 1 B, C). Location of bioluminescent bacteria was determined by the presence of luminescence, denoted by color in the image (Figure 1B). Dust particle adhesion or deposition was not observable during visual examination of blossom tissue. Soil particulate dislodged off the blossom after contact. Control blossoms did not exhibit bioluminescence. It was observed from the image (Figure 1), that contaminated soil served as a vehicle of *S. Newport* transfer to blossoms and that blossom tissues could retain pathogen after contact with airborne soil particulate.

3.4 Fruit contamination

Blossoms that were inoculated with *Salmonella* soil particulate using pressurized air developed into fruits within 2-3 weeks. Tomatoes at the immature green stage were tested for the presence of *S. Newport* to determine flower to fruit transmission. Out of the 25 fruit with calyx attached

tested from one plant, 10 were positive for the presence of *S. Newport* in surface sterilized tissue. To determine if presence of *S. Newport* was restricted to calyx or fruit, separate fruit and calyx tissue samples were tested (Table 2). *S. Newport* was isolated from the surface of 2 fruits (n=25) and from 1 calyx (n=9). Both surfaced sterilized fruits (66.66%) and calyxes (77.77%) were positive for the presence of *S. Newport* (Table 2). None of the control fruits that were formed from flowers inoculated with autoclaved soil particulate tested positive for presence of *S. Newport*.

4. Discussion

Dust is defined as fine particulate matter removed from land surface due to winds (Ravi et al., 2011) and its presence is ubiquitous in the crop growing environment. Presence of compost piles, animal feces, farms and poultry houses might contribute to dispersal of airborne enteric pathogens. *Salmonella* can internalize into tomato fruit and calyxes developing from blossoms that have been inoculated with the pathogen (Zheng et al., 2013). Previous studies have demonstrated *Salmonella* internalization into tomato fruit through brushing of blossoms with inoculum, vacuum cycles and stem injection (Guo et al., 2002; Shi et al., 2007; Zheng et al., 2013) but very limited information is available about potential vehicles of blossom contamination in the field. The results suggest that dust particles contaminated with high

populations of *Salmonella* under conditions, used in this study, could serve as a vehicle for *Salmonella* transfer to tomato blossoms leading to fruit contamination (Table 2), but the conditions tested might not be representative of the farm environment.

The study used a high inoculum (8.39 CFU/100mg of dust) of *S. Newport* cells for the contamination of blossoms to facilitate biophotonic imaging. Similar concentrations have been used in previous blossom inoculation studies (Guo et al., 2002; Zheng et al., 2013). The high inoculum levels are not characteristic of *Salmonella* populations detected in environmental matrices, since environmental detection of pathogens often requires enrichment to exceed the detection limit of culture based media (Micallef et al., 2012). While the inoculum levels selected were required to facilitate bioluminescent imaging for spatial distribution, more studies are required to evaluate the risk of dust based contamination of blossoms with naturally occurring levels of inoculum. Conclusions about the risks of dust contact with blossoms cannot be made based solely on the results of this study. The study also used autoclaved soil that was free of competing bacteria to improve chances of survival and detection of *Salmonella* from tomatoes. Presence of competing microorganisms could affect pathogen survival in dust and hence sterile dust was used as a vehicle for *Salmonella*. Studies performed with non-autoclaved dust would require robust biomarkers such as chromosomally acquired, stable and distinct antibiotic resistance profiles for traceability and selection of *Salmonella* from tomatoes.

Dust exposure of blossom was performed once under high levels of containment. The contact with dust particle was simulated using a pressurized gust of wind and hence there was only one contamination event per blossom. The dust samples used in this study were dried for 48h prior to airborne transfer. Previous work on pathogen contaminated dust and soils have indicated that *Salmonella* can be stressed when inoculated into dry soils, hence a high population were used to compensate for viability loss of the pathogen (Ravishankar and Dev Kumar, 2014).

Internalization was defined as the presence of *S. Newport* in surface-sterilized fruit tissue. The combination of bleach (approximately 6000 ppm) followed by immersion in 70% ethanol was carried out to compensate for loss of free chlorine due to presence of soil and organic matter on tomato surface. The use of these treatments were based on previous studies on tomato internalization (Guo et al., 2002; Zheng et al., 2013). The presence of *S. Newport* on sanitizer-treated fruit tissues highlights the ability of dust transferred *Salmonella* to persist from blossom to fruit set (Table 2) when high inoculum loads are present.

Biophotonic imaging of the blossoms after dust contact demonstrated the spatial distribution of *Salmonella* and proved that transfer of pathogens to produce tissue could happen through dust transfer (Figure 1). A plasmid based system was used in *S. Newport* due to requirement of higher bioluminescence to image bacteria on plant surfaces. Plasmid based luminescence systems have

a copy number of about 30 per cell resulting in higher luminescence compared to chromosomally integrated systems (Howe et al., 2010) which result in significantly lesser luminescence but more stability. Expression of luminescence by *Salmonella* in nutritionally challenged environmental matrices such as dust deteriorates over time and hence higher concentrations of inoculum was employed.

The presence of aerosolized bacteria has been demonstrated in poultry processing facilities and farms. Soil in the farm environment could serve as the most obvious source of dust (Ravi et al., 2011, Millner, 2009, Cevallos-Cevallos et al., 2014). Smaller soil particles could be dispersed due the wind, rain and changes in humidity. *Salmonella* has been isolated from soils in agricultural areas and adhesion to soil particles might be related to cell surface hydrophobicity (Stenstrom, 1989). Soil and sediment could act as a reservoir of organic molecules for bacterial nutrition or a substratum for attachment and hence serve as a niche for pathogenic bacteria (Chao, 1987; Thomason et al., 1977; Winfield and Groisman, 2003).

The high percentages of *Salmonella* contaminated fruits and calyxes (Tables 1 and 2) indicate that the environment and weather conditions could play an important role in produce quality and safety (Cevallos-Cevallos et al., 2012a; Cevallos-Cevallos et al., 2012b; Winfield and Groisman, 2003). The experiments were performed in a plant growth incubator for containment of dispersed

dust. The lower yield of blossoms and fruit in a contained environment resulted in limited availability of blossoms and fruit that were tested. Enumerating the population of cells in fruit contaminated through the blossom route and determining survival and increase in populations during ripening and storage of fruit is required to develop a better understanding of the fate of *Salmonella* in tomatoes.

The results from this study indicate that contact of tomato blossoms with *Salmonella* contaminated dust vehicle can result in pathogen transfer to the petal, sepal, stamen and pedicel. A high percentage of fruit and calyxes that developed from these blossoms harbored *S. Newport*. While the conditions in the tomato growing farm environment may vary from the parameters used in this study, limiting the use soil amendments during blossom formation in tomatoes could reduce the risk of tomato blossom contamination.

Acknowledgements

The authors would like to thank Dr. Haiou Shen for technical assistance with imaging. Funding for this work was provided by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Figure 1. Image of blossoms (A), air-inoculated with bioluminescent *S. Newport*-Soil Particulate mixture. Biophotonic image of blossoms (B) indicate spatial distribution of bioluminescent *S. Newport* transferred by air-particulate contact. Biophotonic image (C) indicates bioluminescence intensity of *S. Newport* using a color scale from red to blue indicating highest to lowest luminescence respectively.

Table 1. Effect of brush application and pressurized air dispersal of *S. Newport*-soil mixture on the persistence of *S. Newport* on tomato blossoms one week after inoculation.

Method of Inoculation	Pathogen positive blossoms from Plant 1	Pathogen positive blossoms Plant2	Pathogen positive blossoms Plant 3	Total positive
Brush (n ^a =6)	2	2	2	6
Air (n=14)	1	1	2	4
Control ^b -Brush (n=9)	0	0	0	0
Control ^c - Air (n=9)	0	0	0	0

^a n represents number of blossoms tested for presence of *S. Newport*

^b Control blossoms from three plants were inoculated with sterile uninoculated soil using brushing

^c Control blossoms from three plants were inoculated with sterile uninoculated soil using compressed air delivery

Table 2. *S. Newport* presence on surface and internal tomato fruit tissue developed from *S. Newport*- Soil Particulate inoculated blossoms

Plant	Surface presence of <i>S. Newport</i> in tissue	Presence of <i>S. Newport</i> after 24 hour incubation in buffered peptone water	Presence of <i>S. Newport</i> after 24 hour enrichment in TT broth^b	Total number of positive samples <i>S. Newport</i>
Fruit (n ^a =18)	0	1	12	12
Calyx (n=11)	1	1	7	7
Control (n=9)	0/9	0/9	0/9	0/9

^a n represents total number of fruits tested, obtained from three different plants

^b Tetrathionate broth

References

1. 2008. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2007. *MMWR Morb Mortal Wkly Rep* 57, 366-370.
2. Bennett, S., Littrell, K., Hill, T., Mahovic, M., Behraves, C.B., 2015. Multistate foodborne disease outbreaks associated with raw tomatoes, United States, 1990–2010: a recurring public health problem. *Epidemiol. Infect.* 143, 1352-1359.
3. Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ. Microbiol.* 12, 2385-2397.
4. Berry, E.D., Wells, J.E., Bono, J.L., Woodbury, B.L., Kalchayanand, N., Norman, K.N., Suslow, T.V., López-Velasco, G., Millner, P.D., 2015. Effect of Proximity to a Cattle Feedlot on *Escherichia coli* O157: H7 Contamination of Leafy Greens and Evaluation of the Potential for Airborne Transmission. *Appl. Environ. Microbiol.* 81, 1101-1110.
5. Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implications for food safety. *Annu Rev Phytopathol.* 44, 367-392.
6. Cevallos-Cevallos, J.M., Danyluk, M.D., Gu, G., Vallad, G.E., van Bruggen, A.H., 2012a. Dispersal of *Salmonella* Typhimurium by rain splash onto tomato plants. *Journal of Food Protection*® 75, 472-479.

7. Cevallos-Cevallos, J.M., Gu, G., Danyluk, M.D., Dufault, N.S., van Bruggen, A.H., 2012b. *Salmonella* can reach tomato fruits on plants exposed to aerosols formed by rain. International Journal of Food Microbiology 158, 140-146.
8. Cevallos-Cevallos, J.M., Gu, G., Richardson, S.M., Hu, J., Van Bruggen, A.H., 2014. Survival of *Salmonella enterica* Typhimurium in water amended with manure. Journal of Food Protection® 77, 2035-2042.
9. Chao, W., R. Ding, and R. Chen, 1987. Survival of pathogenic bacteria in environmental microcosms. Chinese J. Microbial Immunol. (Taipei) 20, 339-348.
10. Chinivasagam, H.N., Tran, T., Maddock, L., Gale, A., Blackall, P.J., 2009. Mechanically Ventilated Broiler Sheds: a Possible Source of Aerosolized *Salmonella*, *Campylobacter*, and *Escherichia coli*. Appl. Environ. Microbiol. 75, 7417-7425.
11. Greene, S., Daly, E., Talbot, E., Demma, L., Holzbauer, S., Patel, N., Hill, T., Walderhaug, M., Hoekstra, R., Lynch, M., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiol Infect. 136, 157-165.
12. Guo, X., Chen, J., Brackett, R.E., Beuchat, L.R., 2001. Survival of Salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit

- development through fruit ripening. *Applied and environmental microbiology* 67, 4760-4764.
13. Guo, X., Chen, J., Brackett, R.E., Beuchat, L.R., 2002. Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. *J. Food. Protect.* 65, 274-279.
14. Gupta, S.K., Nalluswami, K., Snider, C., Perch, M., Balasegaram, M., Burmeister, D., Lockett, J., Sandt, C., Hoekstra, R.M., Montgomery, S., 2007. Outbreak of *Salmonella* Braenderup infections associated with Roma tomatoes, northeastern United States, 2004: a useful method for subtyping exposures in field investigations. *Epidemiol. Infect.* 135, 1165-1173.
15. Hanning, I.B., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathog Dis.* 6, 635-648.
16. Howe, K., Karsi, A., Germon, P., Wills, R., Lawrence, M., Bailey, R., 2010. Development of stable reporter system cloning luxCDABE genes into chromosome of *Salmonella enterica* serotypes using Tn7 transposon. *BMC Microbiol.* 10, 197.
17. Jacobsen, C.S., Bech, T.B., 2012. Soil survival of *Salmonella* and transfer to freshwater and fresh produce. *Food. Res. Inter.* 45, 557-566.

18. Lues, J.F.R., Theron, M.M., Venter, P., Rasephei, M.H.R., 2007. Microbial Composition in Bioaerosols of a High-Throughput Chicken-Slaughtering Facility. *Poult. Sci.* 86, 142-149.
19. Micallef, S.A., Goldstein, R.E.R., George, A., Kleinfelter, L., Boyer, M.S., McLaughlin, C.R., Estrin, A., Ewing, L., Beaubrun, J.J.-G., Hanes, D.E., 2012. Occurrence and antibiotic resistance of multiple *Salmonella* serotypes recovered from water, sediment and soil on mid-Atlantic tomato farms. *Environ Res.* 114, 31-39.
20. Millner, P.D., 2009. Bioaerosols associated with animal production operations. *Biores. Tech.* 100, 5379-5385.
21. Ravi, S., D'Odorico, P., Breshears, D.D., Field, J.P., Goudie, A.S., Huxman, T.E., Li, J., Okin, G.S., Swap, R.J., Thomas, A.D., Van Pelt, S., Whicker, J.J., Zobeck, T.M., 2011. Aeolian processes and the biosphere. *Rev. Geophys.* 49, RG3001.
22. Ravishankar, S., Dev Kumar, G., 2014. Assessing Contamination Risk of Dust, Soil, Compost, Compost Amended Soil and Irrigation Water as Vehicles of Pathogen Contamination on Iceberg Lettuce Surfaces. Arizona Iceberg Lettuce Council. <https://agriculture.az.gov/sites/default/files/Final%2013-09.pdf>.

23. Seleem, M.N., Ali, M., Boyle, S.M., Sriranganathan, N., 2008. Reporter genes for real-time in vivo monitoring of *Ochrobactrum anthropi* infection. FEMS Microbiol Lett. 286, 124-129.
24. Shi, X., Namvar, A., Kostrzynska, M., Hora, R., Warriner, K., 2007. Persistence and Growth of Different *Salmonella* Serovars on Pre- and Postharvest Tomatoes. J. Food Protect. 70, 2725-2731.
25. Stenstrom, T.A., 1989. Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. Appl. Environ. Microbiol. 55, 142-147.
26. Thomason, B.M., Dodd, D.J., Cherry, W.B., 1977. Increased recovery of salmonellae from environmental samples enriched with buffered peptone water. Appl. Environ. Microbiol. 34, 270-273.
27. Winfield, M.D., Groisman, E.A., 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl. Environ. Microbiol. 69, 3687-3694.
28. Zheng, J., Allard, S., Reynolds, S., Millner, P., Arce, G., Blodgett, R.J., Brown, E.W., 2013. Colonization and internalization of *Salmonella enterica* in tomato plants. Appl. Environ. Microbiol. 79, 2494-2502.

29. Zhuang, R., Beuchat, L., Angulo, F., 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61, 2127-2131.
30. Zobeck, T.M., Van Pelt, R.S., 2006. Wind-induced dust generation and transport mechanics on a bare agricultural field. J. Hazard Mat. 132, 26-38.