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# Airborne soil particulates as vehicles for *Salmonella* contamination of tomatoes

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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 over >15 times during the last two decades in the United States (Bennett et al., 2015; Berger et al., 2010; Hanning et al., 2009). Approximately 5 billion lb 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 1 mm in diameter (Zobeck and Van Pelt, 2006) and could serve as a vehicle of Salmonellae.

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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 h 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 h 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, Ogdensberg, NY). Upon confirmation, colonies were streaked on TSA (Becton, Dickinson and Co, Sparks, MD) slants and incubated for 24 h 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 ng $\mu l^{-1}$) of plasmid DNA isolated from *E. coli* DH10B™ 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 400 Ω 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 h. 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 h 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 h 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 h, 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 $CaSO_4$ (Drierite™ Xenia, OH) for 48 h 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,