Salmonella Newport and Typhimurium Colonization of Fruit Differs from Leaves in Various Tomato Cultivars

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ABSTRACT

Several outbreaks of Salmonella enterica infections have been linked to tomatoes. One cost-effective way to complement on-farm preventive Good Agricultural Practices is to identify cultivars with inherent decreased susceptibility to Salmonella colonization. Fruit and leaves of 13 tomato cultivars with distinct phenotypes were screened to evaluate their susceptibility to Salmonella epiphytic colonization. Field-grown fruit or gnotobiotically grown seedling leaves were spot inoculated in replicate with either Salmonella Typhimurium LT2 or a tomato outbreak–associated strain of Salmonella Newport. Initial loads of the Salmonella inocula were 2.5 log CFU per fruit and 3.5 or 7.0 log CFU per seedling. Salmonella cells were retrieved and enumerated using direct plating after 24 h of incubation at room temperature for fruit and 72 h at 26 °C during the day and 18 °C at night for seedling leaves. Epiphytic colonization of fruit by S. enterica was cultivar-dependent and serotype-specific, but did not necessarily correlate with leaf colonization. Fruit of cultivar Heinz-1706 were the least colonized by Salmonella Newport, while the highest populations were retrieved from fruit of Nyagous. By contrast, seedling leaves supporting the lowest populations were Florida 91 VF and the highest were Virginia Sweets for Salmonella Newport. For Salmonella Typhimurium the lowest was Nyagous and the highest was Heinz-1706 and Moneymaker. The tomato outbreak strain of Salmonella Newport attained higher population densities on fruit than did Salmonella Typhimurium, suggesting better adaptation to tomato fruit colonization. Salmonella Newport populations were significantly lower on leaves, but not fruit of the near-isogenic line Movione, compared with the parent cultivar Moneymaker, suggesting the immunity conferring gene Pto could be responding to this outbreak strain. Susceptibility of tomato fruit to Salmonella colonization is highly variable and could be one criterion for cultivar selection for cultivation.

While regular consumption of fruits and vegetables is encouraged owing to their nutritional value and potential in reducing risks associated with chronic diseases (22), the past decades have seen an increase in the number of foodborne illness outbreaks associated with the consumption of fresh produce (7, 8, 15). Outbreaks are not only a risk to public health, but also frequently damage consumer confidence in the safety of the fresh produce supply chain, leading to substantial economic losses to produce growers and associated industries (19).

Salmonella enterica is the most common bacterial etiological agent responsible for produce-related outbreaks in the United States (13). Salmonella on vine-stalk vegetables was the pathogen-commodity pair responsible for the highest number of outbreak-related illnesses in 2008 (7). Among fresh produce commodities, tomatoes have been linked to at least seven multistate outbreaks since 2002 (6, 12). The Salmonella tomato pair is particularly problematic in the Mid-Atlantic region. A variety of Salmonella serotypes have been recovered from tomato production areas (17) and Salmonella Newport isolated from irrigation ponds on the Eastern Shore of Virginia have been matched by pulsed-field gel electrophoresis to the outbreak strain of 2002 and 2005 (9). The latest multistate outbreak caused by Salmonella Newport associated with tomatoes occurred in 2011 and sickened 166 people (5).

Contamination of tomatoes may occur both pre- and postharvest. Although the routes and mechanisms of contamination of fresh produce with Salmonella are still not fully understood, recent food safety efforts have focused on establishing preventive measures. On-farm Good Agricultural Practices and Good Handling Practices have been established, and the Food Safety Modernization Act (23), signed into law in 2011, continues to put the emphasis on prevention. While current Good Agricultural Practices and Good Handling Practices have done much to educate farmers on ways to reduce bacterial contamination of fresh produce, it appears that they are insufficient to completely eliminate tomato contamination since tomato-associated Salmonella illnesses continue to occur (7).

One cost-effective way to complement primary on-farm preventative interventions to reduce contamination is to identify tomato cultivars with inherent decreased susceptibility to Salmonella contamination. The use of such cultivars could serve as a second tier control measure, by
TABLE 1. *Tomato* (*Solanum lycopersicum*) cultivars used in this study

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Source</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA Red Cherry</td>
<td>Tomato Genetics Resource Center</td>
<td>Cherry variety</td>
</tr>
<tr>
<td>Heinz-1706</td>
<td></td>
<td>Genome sequenced by International Sequencing Project</td>
</tr>
<tr>
<td>Moneymaker</td>
<td></td>
<td>Suitable for Maryland</td>
</tr>
<tr>
<td>Nyagous</td>
<td></td>
<td>Black variety; suitable for Maryland</td>
</tr>
<tr>
<td>LA4013</td>
<td></td>
<td><em>hp</em>-2 (High pigment-2) mutant in Moneymaker background</td>
</tr>
<tr>
<td>Mobox</td>
<td></td>
<td>Near isogenic line in Moneymaker background with <em>R</em> gene immunity to <em>Fusarium oxysporum</em> f. sp. <em>lycopersici</em></td>
</tr>
<tr>
<td>Movione</td>
<td></td>
<td>Near isogenic line in Moneymaker background with <em>R</em> gene immunity to <em>Pseudomonas syringae</em> pv. <em>tomato</em></td>
</tr>
<tr>
<td>Micro-Tom</td>
<td></td>
<td>Miniaturized cultivar</td>
</tr>
<tr>
<td>Florida 91 VFF</td>
<td>Tomato Growers Supply Co.</td>
<td>VFF resistance; recommended for Mid-Atlantic&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rutgers Select</td>
<td></td>
<td>Recommended for Mid-Atlantic&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rutgers VFA</td>
<td></td>
<td>VFA resistance; recommended for Mid-Atlantic&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginia Sweets</td>
<td></td>
<td>Heirloom; Bi-color variety</td>
</tr>
<tr>
<td>Plum Dandy VF</td>
<td>Territorial Seed Co.</td>
<td>Recommended for Mid-Atlantic&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> V, resistance to *Verticillium* wilt; F, resistance to *Fusarium* wilt; A, *Alternaria* resistance. Double letters indicate resistance to two or more strains of the disease.

<sup>b</sup> http://www.mdipm.umd.edu/state_resources/MD%20VEG%20REC%202009.pdf.

<sup>c</sup> http://www.hgic.umd.edu/content/documents/HG70RecommendedVegetableCultivarsrevised2_2010.pdf.

Further minimizing the risk of tomato contamination in the event of on-farm presence of *Salmonella*, or sporadic introduction through random events such as wildlife or rain run-off. A number of studies have shown that leaves of different cultivars vary in their susceptibility to this enteric pathogen (2, 10) and *Salmonella* genes required for colonization were differentially regulated in response to tomato cultivar (18), implying that different plant genotypes impose different selective pressures on this human pathogen.

Although differential epiphytic colonization of tomato leaves with *Salmonella* has been reported, more pertinent data on *Salmonella* colonization of fruit is lacking. Also, the adaptability of *Salmonella* strains isolated from tomato outbreaks has not been assessed against a variety of cultivars. To date, most studies have assessed seedling or leaf colonization with *Salmonella* laboratory strains. To address this data gap, the objective of this study was to screen fruit and seedlings of 13 tomato cultivars with distinct phenotypes. Their susceptibility to epiphytic colonization by *Salmonella enterica* Typhimurium as well as *Salmonella* Newport, a tomato outbreak strain, was investigated.

**MATERIALS AND METHODS**

**Tomato cultivars and bacterial strains.** Thirteen tomato (*Solanum lycopersicum*) cultivars were selected based on a range of distinct fruit phenotypes, including morphology (California Red Cherry, Heinz-1706, and Micro-Tom), pigment formation (LA4013, Nyagous, and Virginia Sweets), resistance to phytopathogens (Florida 91 VFF, Mobox, Movione, and Rutgers VFA), and suitability of cultivation in the Mid-Atlantic region of the United States (Moneymaker, Rutgers Select, and Plum Dandy VF). Mobox and Movione are near-isogenic lines, bred from the parent cultivar Moneymaker for resistance to phytopathogens. These two cultivars were included in this study with a specific purpose of answering a question regarding effects of phytopathogen resistance on the outbreak strain *Salmonella* Newport. The 13 cultivars used in this study are listed in Table 1. Two *S. enterica* subsp. *enterica* serotypes were selected, *Salmonella* Typhimurium LT2 (ATCC 700720), a frequently used laboratory strain in food safety research, and *Salmonella* Newport, an isolate recovered from a salmonellosis outbreak associated with tomato consumption (9), both adapted for rifampin resistance. Rifampin-adapted strains were used in all inoculations except for experiments with tomato seedlings grown under sterile conditions in culture plates, in which *Salmonella* Typhimurium LT2 lacking rifampin resistance was used. These *Salmonella* strains were maintained at −80°C in Brucella broth (BD, Sparks, MD) containing 15% glycerol, and plated on Trypticase soy agar (TSA; BD) plates incubated at 35°C overnight, prior to experiments. For growth of rifampin-resistant *Salmonella* strains, archiving and culture media were supplemented with 50 µl/ml rifampin (Tokyo Chemical Industry Co. LTD., Japan).

**In vitro tomato seedling growth.** Tomato seeds were surface sterilized by soaking in 2.7% sodium hypochlorite for 30 min, followed by six to seven rinses in sterile water, as recommended by the Tomato Genetics Resource Center (UC Davis, Sacramento, CA). Seeds were germinated in the dark on Murashige and Skoog medium (MP Biomedicals LLC, Solon, OH) supplemented with 2% sucrose and 1.2% agar. Germinated tomato seedlings were grown gnotobiotically in an upright position in culture plates measuring 13 by 13 mm² at a 16L:8D photoperiod and at 26°C during the day and 18°C at night.

**Tomato fruit harvesting and surface sterilization.** To evaluate the epiphytic colonization on tomato fruit, 13 cultivars were grown at experimental field plots at the Wye Research and Education Center, University of Maryland (Queenstown). Tomato transplants were started at the Research Greenhouse Complex, University of Maryland, and transplanted into the Wye Research and Education Center field plots 3 weeks after germination. Plants were grown to fruit maturity under recommended irrigation and fertilization regimes. Pesticide application was discontinued 1 month prior to tomato harvesting. Ripe fruit were picked into sterile sampling bags avoiding direct contact with gloved hands, and the bags were transported in coolers on ice to a cold chamber at
4°C. Within 24 h of sampling, tomato fruit were submerged in 2% household bleach for 10 min to sterilize the surface of fruit and then rinsed adequately with deionized water twice. Surface-sterilized fruit was dried in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) with the bag open for 1.5 days prior to Salmonella inoculation.

**Preparation of Salmonella inocula.** Overnight cultures of Salmonella grown on TSA at 35°C were suspended in sterile phosphate-buffered saline (PBS) at an OD<sub>600</sub> of 0.5, which yields approximately 10<sup>8</sup> CFU/ml. Further dilutions were made in sterile PBS. Actual cell concentrations of Salmonella suspension were enumerated on TSA plates. TSA culture plates containing 50 µl/ml rifampin were used to prepare and enumerate rifampin-resistant Salmonella strains.

**Tomato seedling and fruit inoculation.** At 3 weeks postgermination, multiple locations on leaves were spotted with 100 µl of either 3.2 × 10<sup>4</sup> or 10<sup>8</sup> CFU/ml Salmonella Typhimurium LT2, or 3.2 × 10<sup>4</sup> CFU/ml Salmonella Newport, or sterile PBS. Square culture plates holding the inoculated seedlings were resealed with micropore tape (3M, St. Paul, MN) maintaining high relative humidity inside the plates but allowing aeration, and reincubated. For surface-sterilized fruit, 50 µl of 6.4 × 10<sup>3</sup> CFU/ml rifampin-adapted Salmonella Typhimurium LT2 or Salmonella Newport, or sterile PBS were spot inoculated on intact areas of the fruit surface forming five droplets of 10 µl, spotted as tightly within a minimum diameter as possible. The inoculated fruit were incubated in sterile Whirl-Pak bags at room temperature. The bags were closed to maintain humid conditions, and care taken to avoid Salmonella inocula from contacting the sides of the bags during incubation. For fruit, inoculations were done in replicates of five, except for Rutgers Select and Rutgers VFA with replicates of three to four for Salmonella Newport, due to low fruit yields. For seedlings, inoculations were performed in replicates of three to five. Data were pooled from separate experiments up to a total of 10 replicates.

**Salmonella retrieval from inoculated tomato seedlings and fruit, and Salmonella enumeration.** Three days after Salmonella inoculation, seedling leaves were aseptically cut and transferred to sterile 50-ml conical tubes containing 20 ml of PBS. The tubes were sonicated in Branson Ultrasonic Cleaner (Branson Ultrasonics Corporation, Danbury, CT) for 2 min and vortexed briefly at maximum speed in order to dislodge attached Salmonella cells from the plant surface. For fruit 24 h postinoculation, the fruit skin where the Salmonella inocula had been mounted was cut off using a sterile scalpel and transferred into sterile 1.5-ml microcentrifuge tubes containing 1 ml of PBS. Tubes were vortexed at maximum speed for 10 min. Serial dilutions were prepared from the rinsates, and plated on TSA for Salmonella Typhimurium LT2 or TSA with 50 µl/ml rifampin culture plates for rifampin-adapted Salmonella Typhimurium LT2 and Salmonella Newport quantification.

**Statistical analysis.** Enumeration data in CFU per unit of sample were log transformed to satisfy the assumptions on normality of residuals and homogeneity of variances. Differences in log CFU per unit of sample detected between levels of treatments were tested for significance using one-way analysis of the variance and Tukey’s honestly significant difference (HSD) test. Student’s t test was performed when a comparison between only two levels of treatments was necessary. Specific interest comparing Mivione or Mobox with its parent cultivar Money-maker was tested by a preplanned comparison procedure, called contrasts. Statistical analyses were carried out using JMP Pro 10 (SAS Institute Inc., Cary, NC).

**RESULTS**

Epiphytic colonization of tomato fruit with Salmonella is cultivar-dependent and serotype-specific. Fruit of different cultivars, field harvested and then surface sterilized, were inoculated with either Salmonella Newport or Salmonella Typhimurium. Growth of Salmonella populations was observed for both Salmonella serotypes on all cultivars screened but was generally higher for the former (Fig. 1). When each of the cultivars was initially loaded with 2.5 log CFU Salmonella Newport per fruit, 1.4 to 3.1 log CFU increases in population density were observed 1 day postinoculation, and was cultivar-dependent. Heinz-1706 was significantly less colonized per fruit than Nyagouz, 3.9 versus 5.6 log CFU, respectively (P = 0.0139; Fig. 1a). Salmonella Newport populations on Micro-Tom and Virginia Sweets, at 3.9 log CFU per fruit for both, were also less than those on Nyagous (P = 0.0930 and 0.0797, respectively), but not significant at the P < 0.05 level. The highest log CFU of Salmonella Newport was retrieved from Nyagous, followed by LA4013 and Florida 91 VFF (5.5 and 5.4 log CFU per fruit, respectively). For Salmonella Typhimurium, 0.7 to 2.2 log CFU increases in population density were observed on fruit (Fig. 1b). The largest population of Salmonella Typhimurium was recovered from LA4013 (4.7 log CFU per fruit), which was followed by Rutgers VFA and Florida 91 VFF (4.5 and 4.3 log CFU per fruit, respectively), while the smallest was from Mobox, Heinz-1706, and Rutgers Select (3.2, 3.4, and 3.4 log CFU per fruit, respectively).

Cultivar-dependent and serotype-specific differential colonization by Salmonella was also observed on leaves of tomato seedlings, but the patterns differed from the fruit colonization data. Seeding leaves of seven different cultivars grown sterilely in culture plates for 3 weeks were inoculated with either Salmonella Newport or Salmonella Typhimurium. Consistent with fruit colonization, overall population growth for both serotypes was observed on all the cultivars (Fig. 2). For Salmonella Newport, seedlings were initially loaded with 3.5 log CFU. Three days postinoculation, 5.9 to 7.6 log CFU per seedling were recovered (Fig. 2a). Florida 91 VFF and Mivione were the cultivars least susceptible to Salmonella Newport colonization (5.9 and 6.4 log CFU per seedling, respectively), compared with the most colonized Virginia Sweets (7.6 log CFU per seedling) (P < 0.05). When inoculated with Salmonella Typhimurium, Nyagous and again Mivione and Florida 91 VFF exhibited reduced susceptibility to Salmonella colonization (6.7, 6.7, and 6.8 log CFU per seedling, respectively) and were significantly different from Money-maker and Heinz-1706 (7.8 and 7.6 log CFU per seedling, respectively; P < 0.05; Fig. 2b). These data contrast with counts obtained from fruit colonization experiments, where Heinz-1706 was the least colonized and Nyagous the most colonized (Fig. 1a). Cultivar-dependent differential coloni-
Salmonella Newport, a tomato outbreak strain, colonizes tomato fruit more efficiently than Salmonella Typhimurium. To determine whether Salmonella Newport, a tomato outbreak strain, is better adapted to colonize and persist on tomato plants than Salmonella Typhimurium, Salmonella colonization data on seedling leaves and fruit were combined by Salmonella strain and compared. The population of Salmonella Newport was significantly higher than that of Salmonella Typhimurium on tomato fruit (Fig. 4a). By contrast, Salmonella Newport was less able to colonize seedling leaves than Salmonella Typhimurium (Fig. 4b). These differences were statistically supported ($P < 0.05$). In pairwise comparisons between cultivars inoculated with the two Salmonella serotypes, significant differences in cell counts were observed on fruit of Florida 91 VFF, LA4013, Mobox, Nyagous, and Plum Dandy VF, with higher counts recovered for Salmonella Newport compared with Salmonella Typhimurium (Fig. 1). On leaves, higher cell counts were recovered from Florida 91 VFF and Heinz-1706 for Salmonella Typhimurium compared with Salmonella Newport (Fig. 2). Cell counts from the other five cultivars were not statistically different between the serotypes.

Potential role of plant innate immunity to Salmonella Newport colonization. To analyze the effects of plant innate phytopathogen resistance on colonization by Salmonella, the population levels of Salmonella Newport obtained from Movione and Mobox fruit and Movione seedling leaves were compared against Moneymaker. Both Movione and Mobox are near-isogenic lines bred from their parent cultivar Moneymaker, selected for resistance to Pseudomonas syringae pv. tomato or Fusarium oxysporum f. sp. lycopersici as a result of harboring the Pto or the I-2 gene, respectively. Salmonella counts obtained from leaves of Movione were significantly lower for Salmonella Newport ($P = 0.0124$) than the counts from Moneymaker (Fig. 2a). By contrast, there were no differences in the population levels of Salmonella Newport on tomato fruit between Movione and Moneymaker ($P = 0.8131$; Fig. 1a). Similarly, no significant difference in Salmonella cell
counts on tomato fruit between Mobox and Moneymaker was observed for *Salmonella* Newport ($P = 0.6450$). The same patterns were observed with the laboratory strain *Salmonella* Typhimurium LT2.

**DISCUSSION**

While interest in understanding biological factors involved in *Salmonella*–fresh produce crop plant interactions is growing (3, 14, 18), the role of plant genotypes or enteric pathogen serotype remains less investigated. Adaptability of *Salmonella* strains isolated from tomato outbreaks has also not been well addressed. In this study, associations between 13 tomato genotypes and two *Salmonella* serotypes yielded differential levels of *Salmonella* populations colonizing tomato fruit and seedling leaves. Fruit and leaves of the same cultivar differed in their ability to suppress or support *Salmonella* growth. The tomato outbreak strain of *Salmonella* Newport was a better colonizer of fruit than *Salmonella* Typhimurium. Susceptibilities for fruit and leaves for individual cultivars did not always follow the same trend. The near-isogenic lines of Moneymaker, cultivar Movione, was less susceptible to *Salmonella* Newport leaf colonization, compared with the background genotype, although no expression data to support this observation were obtained.

A few studies have been conducted to evaluate cultivar effects on the colonization of tomato plants with *Salmonella*, testing tomato leaves to investigate cultivar effects, although differences in *Salmonella* population levels on tomato seedling leaves were more obvious between tomato (*Solanum lycopersicum*) and its closely related species (*Solanum pimpinelifolium*) than between different tomato cultivars (1). Recently, Gu et al. (10) also reported cultivar effects on the internalization and survival of *Salmonella* Typhimurium in tomato leaves. Barak et al. (2) reported that *Salmonella* contamination incidence rates of soil-germinated tomato seedlings varied depending on the cultivar they screened, with the cultivars Nyagous and Yellow Pear being less frequently contaminated. In the present study, seedling leaves of Nyagous were also the least colonized by *Salmonella* Typhimurium among the seven cultivars, but by contrast Nyagous fruit supported the largest *Salmonella* Newport populations and among the largest *Salmonella* Typhimurium populations. This suggests that the cultivar-mediated effects on *Salmonella* populations in tomato leaves and fruit were highly dependent on the interaction between the host and the pathogen.

**FIGURE 3.** Association between population increases in log CFU per fruit or log CFU per seedling of (a) *Salmonella* Newport and (b) *Salmonella* Typhimurium, for each cultivar.

**FIGURE 4.** *Salmonella* Newport and *Salmonella* Typhimurium colonization of tomato (a) fruit in log CFU per fruit and (b) seedling leaves in log CFU per seedling, for all cultivars. Asterisks denote the significance by one-way analysis of the variance at $P < 0.05$. 
Salmonella colonization observed on tomato seedlings is not necessarily correlated with that of tomato fruit, a significant finding, since only fruit are consumed. This discrepancy between leaves and fruit is best observed with the cultivars Florida 91 VFF and Heinz-1706. Young leaves of Florida 91 VFF were among the least colonized by either Salmonella Newport or Salmonella Typhimurium compared with the other cultivars, whereas fruit of Florida 91 VFF were among the most favored by both Salmonella serotypes. The opposite pattern, with higher population levels on young leaves but lower on fruit, was recorded for Heinz-1706. Interestingly, increases in Salmonella population levels were higher in leaves compared with fruit, revealing complex and tissue-specific interactions and responses between this pathogen-crop pair.

Although tomato leaves are not edible, data on susceptibility to leaf colonization are relevant since Salmonella residing on leaves can be transmitted to fruit (1, 11). However, since only fruit is eaten, data on Salmonella colonization on or in tomato fruit of various cultivars also need to be considered for establishment of food safety recommendations. Beuchat and Mann (4) concluded that survival and growth of Salmonella was unaffected by tomato variety when Salmonella grew in stem scar and pulp tissues using store-bought tomatoes sorted by shape and size (round, Roma, and grape). In contrast, Xia et al. (25) reported that tomato fruit of the cultivar Mountain Spring were less susceptible to Salmonella Thompson internalization than the cultivars Applause and BHN961. Knowledge about the susceptibility of field-grown fruit of various cultivars to surface attachment and colonization by Salmonella could be an important criterion in cultivar selection by growers, particularly in geographical areas where Salmonella appears to be endemic. Such is the case on the Delmarva Peninsula, east of the Chesapeake Bay in Virginia, Maryland, and Delaware, an area supporting intensive tomato cultivation. Multiple serotypes, including Salmonella Newport, Salmonella Javiana, and Salmonella Thompson, have been isolated from tomato farms in this area (17), and even linked to outbreaks (9).

To our knowledge, this is the largest screen of field-grown tomato fruit of different cultivars assessing susceptibility to Salmonella fruit colonization, including those that farmers in the Mid-Atlantic region can select for cultivation. Under consistent field conditions, several cultivar-specific differences were observed. For instance, compared with the dark pigmented cultivar Nyagous, fruit of cultivar Heinz-1706 supported significantly lower concentrations of both Salmonella Newport (P = 0.0002) and Salmonella Typhimurium (P = 0.0582). Recognizing that Salmonella responses to green and mature fruit differ (18), only mature fruit were used across all the cultivars examined throughout the experimental protocols. The cultivar-dependent differences in levels of Salmonella population on tomato fruit, therefore, can be attributed to genetic variation among cultivars because all cultivars were grown simultaneously and harvested at equivalent ripeness stage. Recently, tomato maturity and genotype were also found to be factors for Salmonella proliferation for cultivars Florida-47, Solar Fire, and Bonny Best (16). Yet differences in fruit surface morphology or chemistry between cultivars, on which phyllospheric microbes rely for their food and protection from abiotic stresses, have not been examined. Further research in this area is needed to begin to unravel the mechanisms regulating these differences.

Studies that have used cocktail inocula consisting of multiple S. enterica serovars preclude the distinction of serovar-specific responses to various tomato cultivars (1, 2, 4). Salmonella Newport exhibited a higher survival rate on tomato cultivar Micro-Tom leaves than Salmonella Typhimurium following a Salmonella cocktail inoculation (26). Shi et al. (21) inoculated red tomato fruit of cultivar Abigail VPET with different Salmonella serovars individually and found that Salmonella Enteritidis, Salmonella Typhimurium, and Salmonella Dublin were less adapted to grow on or in tomato fruit than Salmonella Hadar, Salmonella Montevideo, and Salmonella Newport. In this study, serotype-specific, cultivar-dependent, and plant part-specific Salmonella colonization for the outbreak strain of Salmonella Newport was revealed, providing support to the idea of selecting cultivars on the basis of their resistance to enteric pathogen colonization and the endemic pathogens of a given geographical area of cultivation, although this has not been validated in the field. Leaves provided a more favorable niche for Salmonella Typhimurium, while Salmonella Newport grew best on tomato fruit, suggesting that the tomato outbreak strain is better equipped to colonize and persist on tomato fruit. This could be one explanation for the frequency of Salmonella Newport infections associated with tomato consumption in the mid-Atlantic, compared with other serotypes, in spite of a diversity of serotypes being prevalent in that region (17). A comparison of Mid-Atlantic serotypes could validate this.

Tomato cultivar Movione contains the Pto bacterial resistance locus in cultivar Moneymaker background. The Pto gene encodes a kinase that confers resistance in tomato to Pseudomonas syringae pv. tomato expressing the avirulence gene avrPto by directly interacting with type III secretion system effector proteins AvrPto and AvrPtoB from P. syringae pv. tomato (20). Although no expression data for Pto were obtained in this study, when comparing the cultivar Moneymaker with Movione, Movione was significantly less susceptible to colonization of seedling leaves by Salmonella Newport. Further studies could investigate whether the Pto gene in tomato leaves responds to type III secretion system effector proteins in Salmonella, and whether a different response is elicited in fruit, as suggested in this study. Cultivar Mobox is another near-isogenic line bred from Moneymaker harboring the I2 gene conferring resistance to Fusarium oxysporum f. sp. lycopersici, a wilt-inducing fungus in tomato, probably through recognition of effector proteins (24). No difference in fruit colonization was observed between these two cultivars.

In a repeated experiment with Salmonella Typhimurium colonizing tomato seedlings, it is interesting to note that equivalent Salmonella Typhimurium populations, 7 to 8 log CFU per seedlings, were recovered in 3 days, irrespective of
initial levels loaded per seedling, 3.5 versus 7 log CFU. Additionally, leaves supported higher Salmonella population densities compared with fruit. These findings suggest that there are spatial and/or nutritional limiting factors impacting Salmonella growth on the tomato phyllosphere that differ with different plant parts. Assessing the role that plants play in influencing their associated microbiota is of interest from a food safety standpoint and requires further research.

In conclusion, these findings reveal that tomato plant genetics play a crucial role in determining the success of Salmonella establishment, colonization, and persistence on various plant parts. The highly variable predisposition of tomato fruit to Salmonella colonization offers the opportunity to use this heterogeneity to a food safety advantage. More research is required to better elucidate what other factors might interplay with this plant–enteric pathogen interaction to determine pathogen colonization success. Ultimately, a cultivars inherent susceptibility to Salmonella colonization could be one important criterion for cultivar selection for cultivation.

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