Leveraging Management Strategies for Seedborne Plant Diseases To Reduce *Salmonella enterica* Serovar Typhimurium Incidence on Tomato Seed and Seedlings

MELANIE L. LEWIS IVEY,[1,2]* XIULAN XU,[1] AND SALLY A. MILLER[1]

[1]*The Ohio State University, Ohio Agriculture Research and Development Center, 1680 Madison Avenue, Wooster, Ohio 44691; and* [2]*Louisiana State University, Louisiana State University Agricultural Center, 101 Efferson Hall, Baton Rouge, Louisiana 70803, USA*

ABSTRACT

Tomatoes have been linked to many outbreaks of salmonellosis over the last decade, but the routes of contamination have yet to be discerned. Many phytopathogens of tomato are seedborne and are effectively managed using seed sanitizers. Seed sanitizers effective against bacterial phytopathogens were evaluated for their efficacy in killing bioluminescent *Salmonella enterica* serovar Typhimurium strain SeT-A14 on tomato seed infested with moderately high and high levels of pathogen. SeT-A14 incidence on seedlings produced from contaminated seed following sanitation was also determined. At a moderately high infestation rate (40%), SeT-A14 was eradicated on seed sanitized with 1.2% sodium hypochlorite (NaClO) mixed with 0.03% surfactant for 2 min, hydrochloric acid (HCl) for 30 min, and trichloromelamine for 2 min. At a higher infestation rate (94%), only NaClO and HCl were effective in eradicating SeT-A14 from the seed. At both infestation rates, 2% Virkon-S for 15 min significantly reduced SeT-A14 incidence compared with the nontreated infested controls but did not eradicate the pathogen. Hot water, a commonly used sanitizer for managing seedborne bacterial plant diseases, significantly reduced SeT-A14 on heavily infested seed, but incidence was still moderate at 17.5%. On seedlings produced from moderately highly infested seed, SeT-A14 was not detected using RapidChek *Salmonella* test strips. Using heavily infested seed, SeT-A14 was detected with the test strips in one of four pooled samples of 14-day-old seedlings produced from nonsanitized seed and from seed sanitized with hot water and trichloromelamine. However, bioluminescence was not observed on 14-day-old seedlings. To our knowledge, this is the first report that provides evidence that *S. enterica* serovar Typhimurium can be seed transmitted and can lead to the contamination of tomato seedlings. In addition to eliminating important bacterial phytopathogens from tomato seed, NaClO or HCl may mitigate the risk of *Salmonella* seedling contamination.

Salmonellosis, caused by *Salmonella enterica,* is the most common foodborne bacterial illness in the United States. Consumption of *Salmonella*-contaminated tomatoes has been linked to many national salmonellosis outbreaks in recent years *(4)*. Although the preharvest and postharvest environments (areas where the tomatoes are grown and packing sheds) are the most likely points of contamination *(3, 28),* other routes of contamination are still in question *(22)*.

Fresh market tomato production is an economically important segment of the U.S. agricultural industry, contributing an average of $1.1 billion annually between 2009 and 2012 to the U.S. economy *(24)*. The inherent risk of tomato contamination by *Salmonella* presents economic and management challenges to the fresh market tomato industry. Since fresh market tomatoes are most often consumed raw, there are currently no kill steps available that would eliminate the pathogen in the event of contamination. For this reason and because contamination

with *Salmonella* and other foodborne bacterial pathogens can occur at any stage in the chain of custody, preventative management strategies at each stage are critical to control efforts and to preventing outbreaks that could damage the fresh market tomato industry. Although little information is available on whether or not tomato seed is a source of human pathogen inoculum, seeds are the major source of inoculum for foodborne illnesses associated with sprout consumption *(27),* and tomato diseases caused by bacteria such as *Xanthomonas* spp., *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), and *Pseudomonas syringae* pv. *tomato* are seedborne.

Seedborne tomato diseases are notoriously difficult to manage once they become established in the field, and they can cause significant crop losses to producers. The most effective means of controlling these diseases is through pathogen exclusion from the production field, which can be achieved through the use of pathogen-free seed. Seed treatments, particularly hot water soaking, sodium hypochlorite (NaClO), and hydrochloric acid (HCl), have long been recommended to kill phytopathogenic bacteria on and within tomato seed *(6, 7, 15–17)*. Although the U.S.

* Author for correspondence. Tel: 225-578-1464; Fax: 330-263-3841; E-mail: mivey@agcenter.lsu.edu.

Food and Drug Administration and the National Advisory Committee on Microbiological Criteria for Foods recommend seed sanitation practices, including the use of calcium hypochlorite (CaClO), NaClO, and chlorine for decontaminating human pathogens on sprouting seeds (18, 25, 26), there are currently no recommendations for tomato seed or any vegetable seed not intended for direct consumption. Although a study by Trinetta et al. (23) evaluated the efficacy of chlorine dioxide gas, ozone gas, and electron beam (e-beam) irradiation as tomato seed sanitizers against *Salmonella enterica* serovar Poona, the efficacy of these treatments against important bacterial plant pathogens was not determined. Pathogen exclusion strategies, specifically seed sanitation practices, that consider risks associated with both foodborne human pathogens and plant pathogens are both practical and economically desirable for fresh market tomato growers. The objective of this study was to evaluate the efficacy of seed sanitizers with known efficacy against seedborne phytobacterial pathogens in killing *Salmonella enterica* serovar Typhimurium on artificially infested tomato seed.

MATERIALS AND METHODS

***Salmonella enterica* serovar Typhimurium and inoculum preparation.** A bioluminescent strain of *Salmonella enterica* serovar Typhimurium strain SeT-A14 (SeT-A14) constructed by Xenogen Corp. (Alameda, CA) and provided by Dr. Gireesh Rajashekara, Food Animal Health Research Program, The Ohio State University (Wooster) was used in this study. Bioluminescent SeT-A14 was constructed by chromosomal integration of a Tn5 transposon using a suicide vector carrying the entire lux operon from *Photorhabdus luminescens* and a kanamycin-resistant marker gene (2). Inoculum was prepared by growing SeT-A14 in Luria-Bertani broth at 37°C for 18 to 24 h with shaking (200 rpm). The bacterial suspension was diluted in 100 ml of sterile water to give a final concentration of 10^5 CFU/ml for the first experiment and 10^6 CFU/ml for the second experiment.

Seed infestation. Approximately 4 g (880 seeds) of tomato (cv. BHN 961) was artificially infested with SeT-A14 by soaking the seeds in a suspension of SeT-A14 at 10^5 CFU/ml or 10^6 CFU/ml for 30 min with shaking (~150 rpm). Seeds soaked in sterile water served as noninfested controls. Seeds were air dried at room temperature for 18 h prior to sanitation. To determine the inoculum load on the seed, four seeds from the 10^5 CFU/ml bacterial suspension and the 10^6 CFU/ml bacterial suspension were macerated individually in mesh sample bags (Agdia Inc., Elkhart, IN) containing 2 ml of $1 \times$ phosphate-buffered saline (PBS) using a hand-held tissue homogenizer (Agdia Inc.). Each sample suspension was diluted to 10^{-2} in PBS in 10-fold serial dilutions, and 100-μl aliquots of the undiluted, 10^{-1}, and 10^{-2} dilutions were plated on Luria-Bertani medium amended with kanamycin (50 μg/ml). The plates were incubated at 37°C for 18 h prior to enumeration.

Seed sanitation. Infested tomato seeds were sanitized with 25 ml of 10,500 ppm of NaClO (pH = 10.5) for 2 min (16), 1% HCl for 30 min (10), hot water at 37°C for 10 min followed by 50°C for 20 min and a final 5-min cooling step with cold water (16), or 0.2 g/liter trichloromelamine (Beer Clean, Johnson & Son Inc., Racine, WI) for 2 min or a 2% mixture of potassium peroxomonosulfate, sodium dodecylbenzene–sulphonate, and sul-

famic acid (Virkon-S, DuPont Chemical Solutions Enterprise, Wilmington, DE) for 15 min. The seeds were then air dried at room temperature for 18 h. A SeT-A14 infested, nontreated control and a noninfested control were included in each experiment. The experiment was conducted twice. The first experiment included three replications of each treatment and the controls, and the second experiment included four replications. Both experiments included 40 seeds per replication.

Seed germination assay. Infested, sanitized seeds (20 per replication) were planted in 288-cell flats containing Fafard superfine germinating mix (Conrad Fafard Inc., Agawam, MA) and were maintained within a containment Class II growth chamber at 23°C for 14 days. Supplemental lighting (16-h photoperiod) was provided for the last 9 days only. Flats were watered twice daily using a fine misting watering can. The number of germinated seeds (seedlings) was counted 14 days postseeding, and percent germination was determined.

SeT-A14 detection on tomato seed. Infested, sanitized seeds (20 seeds per replication) were placed on Luria-Bertani agar medium in a square polystyrene petri dish (100 by 100 by 15 mm with a 36-13 mm^2 square grid) and incubated at 42°C for 18 h. To visualize viable SeT-A14 on the seed surface, the plates were placed in an in vivo imaging system (IVIS; 100 Series, Xenogen Corp.) set at station D and photographed using a charge-coupled device (CCD) camera (30-s exposure time) and imaging chamber (Xenogen Corp.). Seeds showing bioluminescence due to colonization by SeT-A14 were counted, and incidence was determined for each sanitizer and replication.

Seed and seedling colonization by SeT-A14. Six nonsanitized infested seeds (both inoculum levels) were incubated in square polystyrene petri dishes containing water agar (1.5% agar) for 6 days. Four replications per inoculum level were tested. The seeds were allowed to germinate in the dark for the first 3 days and with a 10-h photoperiod for the last 3 days. The germination temperature was maintained at 29°C for the entire period. Seed and seedling colonization by SeT-A14 was observed daily using IVIS (Station D) and was photographed using a CCD camera (3-min exposure time) and imaging chamber (Xenogen Corp.). On days 4, 5, and 6, bioluminescent images were taken after quenching plant phosphorescence by incubating the seedlings in the dark in the imaging chamber for 10 min (34). On day 6, one seedling from each replication was dissected into three sections: cotyledon with seed coat attached, hypocotyl, and radicle. Each section was placed in a mesh sample bag (Agdia Inc.) containing PBS and was macerated. The extract was diluted, and dilutions were plated on Luria-Bertani medium amended with kanamycin (50 μg/ml). Cultures were incubated, and bioluminescent colonies were enumerated as described above.

SeT-A14 detection on tomato seedlings. Tomato seedlings (14 days old) were placed in the IVIS imaging chamber in the dark for 10 min to quench plant phosphorescence (station D), and bioluminescent images were taken with a 3-min exposure time using a CCD camera to visualize viable SeT-A14. Following imaging, all the viable seedlings from each treatment and replicate were cut off at the soil line, pooled into Whirl-Pak sampling bags (Nasco, Ft. Atkinson, WI), and tested for the presence of SeT-A14 using the RapidChek *Salmonella* test kit (SDIX, Newark, DE). Pooled seedlings were suspended in RapidChek *Salmonella* medium (1:10, wt/vol) prewarmed to 42°C, pulsed for 30 s (~1,400 pulses) using a PUL 100 pulsifier (Microbiology

International, Frederick, MD), and incubated for 24 h at 42°C. Following enrichment, three 1-ml aliquots from each sample were transferred to a 14-ml polypropylene tube containing 10 ml of sterile prewarmed (42°C) Hajna tetrathionate broth base (BD, Sparks, MD) and enriched a second time at 42°C for 24 h. Approximately 500 μl of the enriched broth from each replicate was transferred to manufacturer-supplied tubes and tested for SeT-A14 using a RapidChek *Salmonella* test strip. After 10 min, each sample was scored for the presence (+) or absence (−) of SeT-A14.

Statistical analysis. Data were tested using the Kruskal-Wallis or one-way analysis of variance (ANOVA) tests. Medians were separated using Dunn's test with a Minitab macro *(19)* in combination with the Kruskal-Wallis test, and means were separated using Tukey's honestly significant difference test. Data were considered to be statistically significant at a 95% confidence level (α = 0.05). All statistical tests were conducted with Minitab 15.1.20.0 (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Seed sanitizers are essential to any integrated tomato disease management program and are also recommended for sprouting seeds produced for human consumption. Without the use of seed sanitizers, it is a challenge for tomato producers to successfully control tomato diseases caused by seedborne bacterial pathogens. Although tomato seed has not yet been implicated as a route of contamination for an outbreak of *S. enterica,* recent studies have demonstrated that some serovars of *S. enterica* can be transported inside tomato plants through hydathodes *(12),* stomata, flowers, and roots *(8).* Furthermore, a recent study by Gu et al. *(11)* demonstrated that, following leaf inoculation, *S. enterica* serovar Typhimurium can be detected inside tomato fruit and on seed harvested from inoculated plants. Although the likelihood of seed contamination is low, management practices that further reduce the risk of seed contamination without adding economic burden to the producer can go a long way to enhance producer and consumer confidence in the food supply.

Four of the sanitizers (NaClO, HCl, hot water, and Virkon-S) used in this study have proven efficacy against one or both of the phytopathogens Cmm and *Xanthomonas* spp. on tomato seed, without compromising seed germination or seedling vigor *(9, 10, 15, 20, 32, 33).* Although trichloromelamine has not been tested for direct application on tomato seed, it is effective against Cmm on contaminated tools used for tomato grafting and crop maintenance *(1).* The efficacy of these same sanitizers in reducing the incidence of bioluminescent SeT-A14 on tomato seeds and seedlings is summarized in Table 1. SeT-A14 exhibited strong bioluminescence on infested nonsanitized seed (Fig. 1A), and a 40.0 and 93.8% infestation rate was achieved for nonsanitized seed infested with a SeT-A14 suspension of 10^5 and 10^6 CFU/ml, respectively. At the moderately high infestation rate (40%), SeT-A14 was eradicated on seed sanitized by NaClO for 2 min, HCl for 30 min, and a slurry of trichloromelamine for 2 min (Table 1). However, when pathogen inoculum was increased to 10^6 CFU/ml, only the NaClO and HCl treatments

were effective in eradicating SeT-A14 on the seeds (Table 1 and Fig. 1C and 1D). At both infestation rates, sanitation of seed with Virkon-S significantly reduced SeT-A14 incidence compared to the nontreated infested controls but did not eradicate the pathogen (Table 1 and Fig. 1E). Although soaking in hot water, which is commonly used to eradicate Cmm and *Xanthomonas* phytopathogens from seed, significantly reduced SeT-A14 on heavily infested seed, only an 81.3% pathogen reduction was achieved. The hot water treatment was ineffective in reducing SeT-A14 on seed infested with a moderately high level of the pathogen. Hot water–treated seed infested with a moderately high level of pathogen germinated prematurely due to inadequate seed drying conditions created by a microenvironment with high moisture (data not shown). Since *S. enterica* and other foodborne bacterial pathogens grow exponentially when conditions are favorable (high moisture, 21 to 25°C) for sprouting *(21, 26),* it is probable that high moisture levels during the drying process mimicked sprouting conditions and favored rapid growth of SeT-A14. In addition, the hot water conditions (37°C for 10 min, 50°C for 20 min) required to kill Cmm and *Xanthomonas* spp. on and in the seed without affecting germination or seedling vigor are optimal growth conditions for *S. enterica.* Depending on exposure time, *S. enterica* can be killed at temperatures ranging from 60 to 101°C. Jaquette et al. *(14)* demonstrated that hot water soaking at 57 to 60°C for 5 min could eliminate *S. enterica* serovar Stanley from alfalfa seeds without negatively impacting germination. Because moisture favors the growth of *S. enterica* and bacterial phytopathogens, dry heat may be an effective alternative to hot water in killing both types of pathogens on tomato seed.

Identifying seed sanitizers and sanitation conditions that can eradicate pathogens on seeds without adversely affecting germination, seedling vigor, or yield is challenging. Although many sanitizers, if used properly, can eradicate plant pathogens on vegetable seeds *(13),* there are no treatments currently in use that can eliminate human pathogens on seeds or sprouts without affecting germination or yield *(21, 26).* Whereas none of the seed sanitizers evaluated in this study significantly reduced germination 14 days postplanting compared with the nontreated infested controls, only NaClO (10,500 ppm, pH 10.5, 2 min) and HCl eliminated SeT-A14 from tomato seeds at both levels of pathogen inoculum (Table 1). In this study, the rate of NaClO required to eradicate *Salmonella* from tomato seed was much lower than the recommended standard (20,000 ppm) for killing *Salmonella* on seeds used for sprouting *(26).* SeT-A14 was not detected on seedlings produced from NaClO- or HCl-treated infested seed using the RapidChek *Salmonella* test strips (Table 1), and bioluminescence was also not observed on the seedlings (data not shown). Further, under moderately high pathogen inoculum load, SeT-A14 was neither observed by bioluminescence (data not shown) nor detected using the Rapid-Chek *Salmonella* test strips (Table 1) on seedlings produced from seed sanitized with trichloromelamine or hot water. However, when the pathogen inoculum concentration on

TABLE 1. *Efficacy of seed treatments in eradicating bioluminescent* Salmonella enterica *serovar Typhimurium strain SeT-A14 from artificially infested BHN 961 tomato seed and treatment effect on seed germination*

Sanitizer, treatment conditions	Moderately high *Salmonella* inoculum (10^5 CFU/ml)			High *Salmonella* inoculum (10^6 CFU/ml)		
	Incidence on seed (%)[a]	Incidence on seedlings[b]	Germination (%)[c]	Incidence on seed (%)[a]	Incidence on seedlings[b]	Germination (%)[c]
Nontreated infested control	40.0 A	– – – –	91.7 A	93.8 A	+ – – –	86.3 A
Hot water, 37°C for 10 min and 50°C for 20 min, and cold water for 5 min	43.3 A	– – – –	98.3 A	17.5 B	+ – – –	92.5 A
Sodium hypochlorite, 1.2% mixed with Silwet (0.03%) for 2 min	0.0 B	– – – –	100.0 A	0.0 C	– – – –	91.3 A
Hydrochloric acid, 1% for 30 min	0.0 B	– – – –	91.6 A	0.0 C	– – – –	91.3 A
Virkon-S, 2% product for 15 min[d]	1.7 B	– – – –	96.7 A	1.3 C	– – – –	90.0 A
Trichloromelamine, 0.2 g/liter for 2 min	0.0 B	– – – –	91.6 A	6.3 BC	+ – – –	93.8 A
P value	0.006	NT[e]	0.505	0.001	NT	0.873

[a] Incidence of *Salmonella enterica* serovar Typhimurium strain SeT-A14 on seed following sanitation was calculated using the following formula: number of seeds exhibiting bioluminescence per total number of seeds tested × 100. Bioluminescence was observed using the in vivo imaging system (IVIS) 100 and photographed using a CCD camera and imaging chamber. Data were analyzed using the Kruskal-Wallis test, and medians were separated using Dunn's test. Values are expressed as medians; medians followed by the same letter are not significantly different at $P \leq 0.05$.

[b] The presence of *Salmonella enterica* serovar Typhimurium strain SeT-A14 was tested using the RapidChek *Salmonella* test kit. The presence of *Salmonella enterica* serovar Typhimurium strain SeT-A14 on 14-day-old seedlings (four replications) following enrichment with the RapidChek *Salmonella* test kit is presented as ''−'' for negative and ''+'' for positive.

[c] Data were analyzed using ANOVA, and means were separated using Tukey's honestly significant difference test. Values are expressed as means; means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

[d] Potassium peroxomonosulfate, sodium dodecylbenzene–sulphonate, and sulfamic acid.

[e] NT, not tested.

the seed was increased, SeT-A14 was detected using the RapidChek *Salmonella* test strips in one of four of the pooled seedling samples produced from the same two treatments (Table 1). Interestingly, Virkon-S did not eradicate SeT-A14 from infested tomato seed, but the pathogen could not be detected with the RapidChek *Salmonella* test strips on tomato seedlings produced from treated seed. This discrepancy may be due to sublethal injury of SeT-A14 following exposure to Virkon-S. Many chemical and physical strategies used to control bacterial
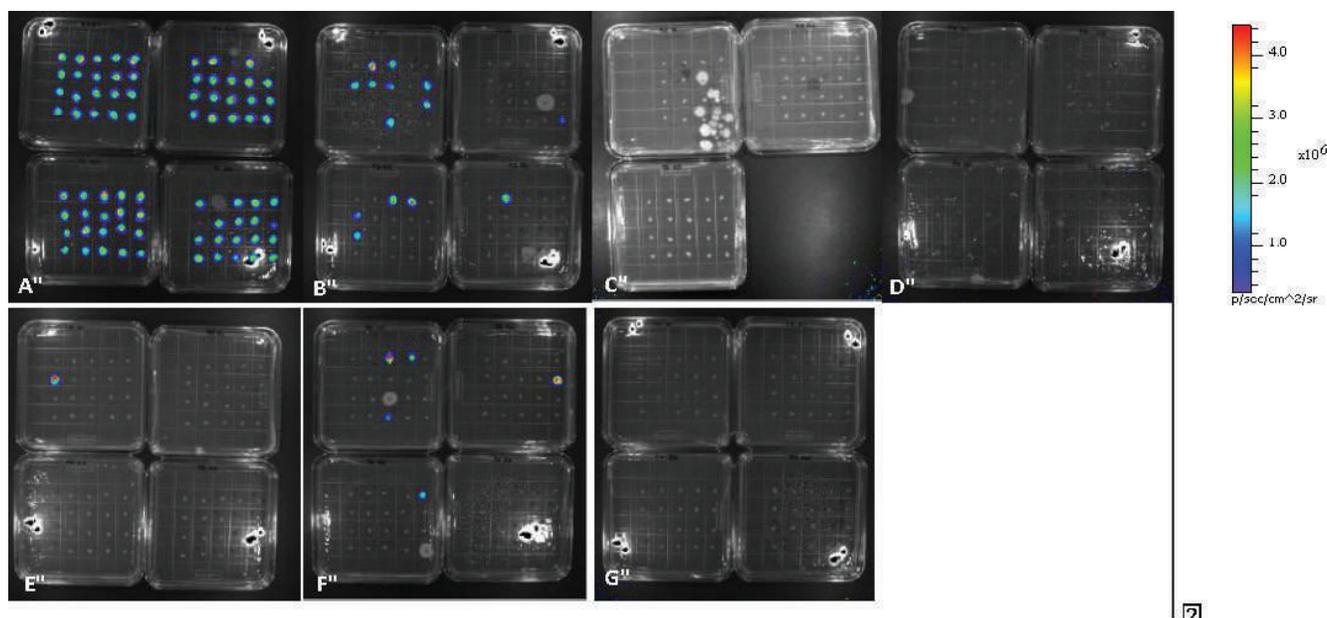

FIGURE 1. *Efficacy of sanitizing treatments in reducing or eradicating bioluminescent* Salmonella enterica *serovar Typhimurium strain SeT-A14 on artificially infested (10^6 CFU/ml) BHN 961 tomato seed. Images show replicated plates of nonsanitized SeT-A14 infested seed (A), SeT-A14 infested seed after exposure to hot water (B), 1.2% sodium hypochlorite (C), 1% hydrochloric acid (D), 2% mixture of potassium peroxymonosulfate, sodium dodecylbenzene–sulphonate, and sulfamic acid (Virkon-S) (E), or 0.2 g/liter trichloromelamine, and noninoculated control seed (F). The rainbow scale represents photon counts (photons/s/cm^2/sr).*
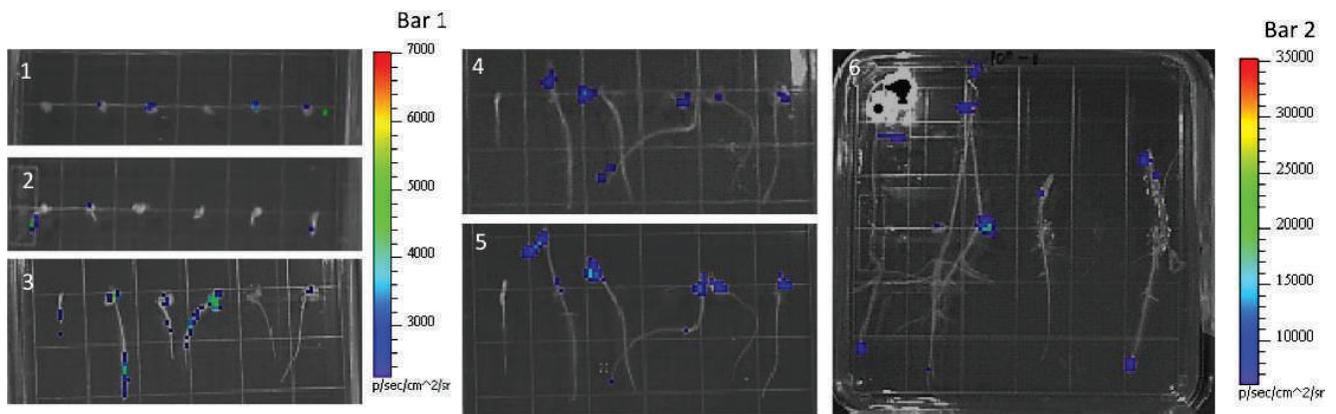
FIGURE 2. Salmonella enterica *serovar Typhimurium strain SeT-A14 (10^6 CFU/ml) seed and cotyledon colonization. Images were acquired daily using an in vitro imaging system (IVIS). Bacteria were observed on seed coats throughout the germination period, and they colonized other seedling components as they emerged. The number in the upper left corner of each image indicates the day after seed plating. The rainbow scales (bar 1 for days 1, 2, and 3 and bar 2 for days 4, 5, and 6) represent photon counts (photons/s/cm^2/sr).*

foodborne pathogens cause sublethal injuries, resulting in damaged but viable cells *(31)*. Although viable cells were observed on the seed following exposure to Virkon-S, these cells may have been injured and unable to sufficiently reproduce or colonize the seedlings to detectable levels with the RapidChek *Salmonella* test strips.

Following artificial seed infestation, SeT-A14 colonized the seed, cotyledons, and radicles over a 6-day period (Fig. 2). On day 1, bioluminescence was observed on the seed coat of 33.3% ($n = 24$) and 16.7% ($n = 24$) of the seed inoculated with 10^6 and 10^5 CFU/ml, respectively. By day 3, bioluminescence was observed on the seed coats (66.7% of seeds infested with 10^6 CFU/ml and 54.2% of seeds infested with 10^5 CFU/ml) and radicles. Beginning on day 4 and at both inoculum levels, luminescence was consistently observed on the seed coats, cotyledons, and root tips. Although bioluminescence was not observed on any of the hypocotyls, SeT-A14 was recovered from the hypocotyls (6.0 Log CFU per hypocotyl, Fig. 3). SeT-A14 was also recovered from the cotyledons (6.3 Log CFU per cotyledon) and radicles (6.4 Log CFU per radicle). Total cell numbers were higher on the radicles than on the
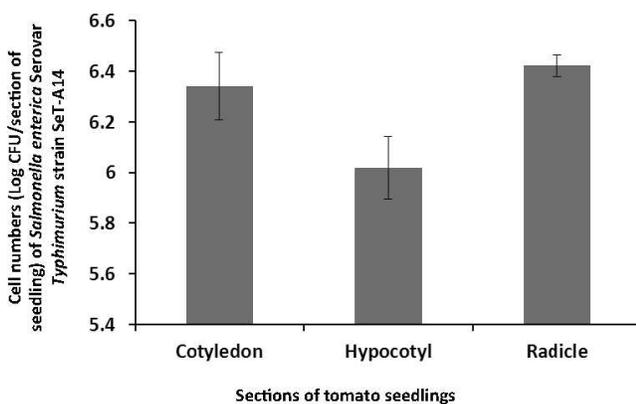
hypocotyls ($P = 0.05$). Cell numbers on the cotyledons, hypocotyls, or radicles (day 6) did not differ between these two inoculum levels ($P = 0.655$). A similar colonization pattern by bioluminescent *Salmonella* Montevideo has been reported on sprouting mung beans *(30)*. Interestingly, however, the colonization pattern by the plant bacterial pathogen Cmm on tomato seedlings differs in that Cmm recovery is highest from the hypocotyl *(33)*.

Although seed contamination and transmission of pathogenic bacteria from infested seed to plant are sporadic and rare events, the consequences of such events can be devastating to a grower and the industry alike. For example, one to five seeds infested with Cmm per 10,000 seeds, under favorable environmental conditions, can initiate a bacterial canker epidemic *(5)* resulting in complete crop losses. Sprouting seed contamination by *S. enterica* also occurs sporadically and at low levels *(25, 29)*; but, because the consequences of foodborne illnesses are so great, there is zero tolerance for *S. enterica* in sprouting seed *(25, 26)*. Because *S. enterica* has not previously been shown to be seed transmitted to tomato and because contaminated tomato seed has never been linked to a salmonellosis outbreak, no tolerance levels on tomato seed have been established.

To our knowledge, this is the first report to provide evidence that *S. enterica* can be seed transmitted and persist at levels detectable using enrichment procedures for up to 14 days on tomato seedlings in vitro. Tomato seeds typically are germinated under warm (21 to 27°C), moist conditions in humid chambers for 5 to 7 days after seeding, conditions favorable for *Salmonella* growth and persistence. Additional studies are needed to determine how long *S. enterica* can persist on seedlings following seed transmission under typical seedling production conditions and whether subsequent fruit internalization and fruit contamination can occur.

FIGURE 3. *Mean populations (Log CFU per section of seedling) of* Salmonella enterica *serovar Typhimurium strain SeT-A14 recovered from the cotyledons, hypocotyls, and radicles of 6-day-old tomato seedlings produced from infested seed. Vertical bars represent the standard error where* n = 8.

REFERENCES

1. Baysal-Gurel, F., K. Tifft, and S. A. Miller. 2009. Sensitivity of *Clavibacter michiganensis* subsp. *michiganensis* to different disinfectants. *Phytopathology* 99:S9.

2. Burns-Guydish, S. M., I. N. Olomu, H. Zhao, R. J. Wong, D. K. Stevenson, and C. H. Contag. 2005. Monitoring age-related susceptibility of young mice to oral *Salmonella enterica* serovar Typhimurium infection using in vivo murine model. *Pediatr. Res.* 58: 153–158.

3. Centers for Disease Control and Prevention. 2007. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants—United States, 2005–2006. *Morb. Mortal. Wkly. Rep.* 56:909–911.

4. Centers for Disease Control and Prevention. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted through food—10 states, 2009. *Morb. Mortal. Wkly. Rep.* 59:418–422.

5. Chang, R. J., S. M. Ries, and J. K. Pataky. 1991. Dissemination of *Clavibacter michiganensis* subsp. *michiganensis* by practices used to produce tomatoes. *Phytopathology* 81:1276–1281.

6. Dhanvantari, B. N., and R. J. Brown. 1993. Improved seed treatments for the control of bacterial canker of tomato. *Can. J. Plant Pathol.* 15: 201–205.

7. Egel, D., R. Foster, E. Maynard, R. Weinzierl, M. Babadoost, P. O'Malley, A. Nair, R. Cloyd, C. Rivard, M. Kennelly, B. Hutchison, S. Gu, R. J. Precheur, C. Welty, D. Doohan, and S. A. Miller. 2013. Midwest vegetable production guide for commercial growers 2013. Purdue University. Available at: www.btny.purdue.edu/Pubs/ID/ID-56. Accessed 15 March 2013.

8. Erickson, M. C. 2012. Internalization of fresh produce by foodborne pathogens. *Annu. Rev. Food Sci. Technol.* 3:283–310.

9. Fatmi, M., and N. W. Schaad. 1991. Seed treatments for eradicating *Clavibacter michiganensis* subsp. *michiganensis* from naturally infested tomato seed. *Plant Dis.* 75:383–335.

10. Gilbertson, R. L. 2006. Combining traditional and molecular approaches for improved understanding of the nature and importance of seed transmission in bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis. Phytopathology* 96:S132.

11. Gu, G., J. M. Cevallos-Cevallos, G. Vallad, and A. H. C. van Bruggen. 2013. Organically managed soils reduce internal colonization of tomato plants by *Salmonella enterica* serovar Typhimurium. *Phytopathology* 103:381–388.

12. Gu, G., J. M. Cevallos-Cevallos, and A. H. C. van Bruggen. 2013. Ingress of *Salmonella enterica* Typhimurium into tomato leaves through hydathodes. *PLoS ONE* 8:e53470. doi:10.1371/journal.pone.0053470.

13. Guo, X., J. Chen, R. E. Brackett, and L. R. Beuchat. 2001. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through ripening. *Appl. Environ. Microbiol.* 67:4760–4764.

14. Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seed and growth and survival of the pathogen during sprouting and storage. *Appl. Environ. Microbiol.* 62:2212–2215.

15. Lewis Ivey, M. L., and S. A. Miller. 2005. Evaluation of hot water seed treatment for the control of bacterial leaf spot and bacterial canker on fresh market and processing tomatoes. *Acta Hortic. (ISHS)* 695:197–204.

16. Miller, S. A., and M. L. Lewis Ivey. 2005. Hot water treatment of vegetable seeds to eradicate bacterial plant pathogens in organic production systems. HYG-3086-05. The Ohio State University Extension, Columbus.

17. Mtui, H. D., M. A. Bennett, A. P. Maerere, S. A. Miller, M. D. Kleinhenz, and K. P. Sibuga. 2010. Effect of seed treatments and mulch on seedborne bacterial pathogens and yield of tomato (*Solanum lycopersicum* Mill.) in Tanzania. *J. Anim. Plant Sci.* 3: 1006–1015.

18. National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *Int. J. Food Microbiol.* 52:123–153.

19. Orlich, S. 2000. Multiple comparisons with a MINITAB Macro: Dunn's test. Minitab, Inc., State College, PA.

20. Pradhanang, P. M., and G. Collier. 2009. How effective is hydrochloric acid treatment to control *Clavibacter michiganensis* subsp. *michiganensis* contamination in tomato seed? *Acta Hortic. (ISHS)* 808:81–86.

21. Taormina, P. J., L. R. Beuchat, and L. Slutsker. 1999. Infections associated with eating seed spouts: an international concern. *Emerg. Infect. Dis.* 5:626–634.

22. Teplitski, M., J. D. Barak, and K. R. Schneider. 2009. Human enteric pathogens in produce: un-answered ecological questions with direct implications for food safety. *Curr. Opin. Biotechnol.* 20:166–171.

23. Trinetta, V., N. Vaidya, R. Linton, and M. Morgan. 2011. A comparative study on the effectiveness of chlorine dioxide, ozone gas and e-beam irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds. *Int. J. Food Microbiol.* 146:203–206.

24. U.S. Department of Agriculture, Economic Research Service. 2013. North American fresh-tomato market. Available at: http://www.ers.usda.gov/topics/in-the-news/north-american-fresh-tomato-market.aspx#backgroundstatistics. Accessed 12 March 2013.

25. U.S. Food and Drug Administration. 1999. Guidance for industry: reducing microbial food safety hazards for sprouted seeds. Available at: http://www.foodsafety.gov/~dms/prodguid.html. Accessed 30 August 2011.

26. U.S. Food and Drug Administration. 1999. Microbiological safety evaluations and recommendations on sprouted seed. Available at: http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/ucm078789.htm#summary. Accessed 20 May 2013.

27. U.S. Food and Drug Administration. 2004. Produce safety from production to consumption: 2004 action plan to minimize foodborne illness associated with fresh produce consumption. Available at: http://www.fda.gov/Food/FoodborneIllnessContaminants/BuyStoreServeSafeFood/ucm2006739.htm. Accessed 26 March 2013.

28. U.S. Food and Drug Administration. 2007. Tomato safety initiative. Available at: http://www.fda.gov/Food/FoodborneIllnessContaminants/BuyStoreServeSafeFood/ucm115334.htm. Accessed 14 May 2013.

29. Van Beneden, C. A., W. E. Keene, R. A. Strang, D. H. Werker, A. S. King, B. Mahon, K. Hedberg, A. Bell, M. T. Kelly, V. K. Balan, W. R. MacKenzie, and D. Fleming. 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. *JAMA (J. Am. Med. Assoc.)* 281: 158–162.

30. Warriner, K., S. Spaniolas, M. Dickinson, C. Wright, and W. M. Waites. 2003. Internalization of bioluminescent *Escherichia coli* and *Salmonella* Montevideo in growing bean sprouts. *J. Appl. Microbiol.* 95:719–727.

31. Wesche, A. M., J. B. Gurtler, B. P. Marks, and E. T. Ryser. 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *J. Food Prot.* 72:1121–1138.

32. Xu, X., M. Bennett, and S. A. Miller. 2009. Evaluation of seed treatments to prevent bacterial canker in greenhouse tomatoes. *Phytopathology* 99:S145.

33. Xu, X., S. A. Miller, F. Baysal-Gurel, K.-H. Garteman, R. Eichenlaub, and G. Rajashekara. 2010. Bioluminescence imaging of *Clavibacter michiganensis* subsp. *michiganensis* infection in tomato seeds and plants. *Appl. Environ. Microbiol.* 76:3978–3988.

34. Xu, X., G. Rajashekara, P. A. Paul, and S. A. Miller. 2012. Colonization of tomato seedlings by bioluminescent *Clavibacter michiganensis* subsp. *michiganensis* under different humidity regimes. *Phytopathology* 102:177–184.