Chlorine Disinfection of Tomato Surface Wounds Contaminated with *Salmonella* spp.

K. Felkey¹, D.L. Archer², J.A. Bartz³, R.M. Goodrich², and K.R. Schneider^{2, 4}

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SUMMARY. This study examined the efficacy of chlorine treatments of flume water for eliminating *Salmonella* spp. from inoculated wounds and intact surfaces of tomatoes (*Lycopersicon esculentum*). Water in a scale-model flume was chlorinated to 150 mg·L⁻¹ of free chlorine at pH 6.5 and maintained at a temperature of 25 or 35 °C, depending on the test. Viable *Salmonella* were recovered from all of the inoculation sites (intact fruit surface, punctures, shaves, and stem scars) even after treatment with chlorinated water for up to 120 seconds at either 25 or 35 °C. Generally, the highest *Salmonella* recovery came from puncture wounds and the lowest from the intact surfaces. After 120 seconds at 25 °C, 4.9 to 5.8 log₁₀ units were recovered from the wounds. Populations recovered after the 30-second treatment at 35 °C ranged from 4.1 log₁₀ cfu/mL for intact surfaces to 6.0 log₁₀ cfu/mL in the puncture wounds. At 60- and 120-second treatment times, all wounds had higher mean populations than tomatoes with intact surfaces. Although greater *Salmonella* survival was associated with shorter exposure to the chlorine, water chlorination cannot completely eliminate contamination of tomato fruit by *Salmonella*, even on intact surfaces. Stem scars, in this study, were not readily disinfected with sodium hypochlorite.

A consumer eating raw tomatoes ingests various microorganisms that reside on or in the fruit. Like other fresh fruit and vegetables, tomatoes are not sterile (Samish and Etinger-Tulczynska, 1963). Good agricultural practices (GAPs) dictate that growers take steps to prevent tomato crops from contacting potential sources of harmful microorganisms. For example, most tomato fields begin as transplants set into bedded soil that is covered with plastic mulch (Rushing et al., 1996). As the plants grow, they are tied to stakes. Developing fruit rarely if ever contact the soil. However, some soil can get on fruit from dust or rain splash. Irrigation is typically drip or overhead (Hochmuth, 1998). Although drip irrigation restricts fruit contact with water, Sadovski et al. (1978) observed that tomatoes

drip irrigated with wastewater could become contaminated. By contrast, with overhead irrigation fruit become wetted by water with each application. Thus, use of any source of non-potable water can lead to fruit contamination. Guo et al. (2001) detected *Salmonella* in ripe tomatoes that had been inoculated when the plants were blossoming. Clearly, a single point-source of contamination can lead to the survival of human pathogens on plants for several weeks.

During harvest for the fresh market, tomatoes are hand-picked and placed in large plastic or wooden buckets that hold 40 to 50 lb of fruit. These buckets are then dumped into pallet bins with a capacity of about 1200 lb of fruit (Sargent, 1998). Alternatively, field buckets are emptied into large tractor trailers that can contain several thousand pounds of fruit. The entire field harvest operation is labor intensive. Workers are paid on a piece-rate basis that rewards speed and efficiency. Rapid harvest motions

and bucket unloading promotes fruit injuries. Any damage to the fruit surface facilitates internalization of various forms of microorganisms. Additionally, spoilage organisms such as *Erwinia carotovora*, *Geotrichum candidum*, *Rhizopus stolonifera*, and *Botrytis cinerea* (Sargent, 1998) may grow on the surface of damaged tissues or in unprotected tissues within fruit (Bartz and Wei, 2003). Wells and Butterfield (1997) reported a high incidence of *Salmonella* in association with decayed vegetable samples from supermarket produce displays. Bacterial soft rot appeared to promote the survival of *Salmonella* in co-inoculation tests.

Upon reaching the packinghouse, tomatoes are dumped into flume tanks containing 100 to 150 mg·L⁻¹ of free chlorine, typically at a pH of 6.5 to 7, and at a temperature about 5 °C above fruit pulp (Bartz et al., 2001). Chlorination of the water in the tanks is intended to prevent the fruit from becoming cross-contaminated by an accumulation of undesirable microorganisms. The flume water is maintained at about 5 °C above the temperature of the incoming fruit to prevent subsequent flume water and microbial infiltration into the fruit through the stem scar. Upon leaving the flume, tomatoes are conveyed past workers who remove fruit with defects such as excessive color, misshapen physical appearance, decay lesions, or injuries such as cuts or bruises. Small wounds, however, may escape detection. Microorganisms, including *Salmonella* spp., have been reported to survive better in wounded tissues, whereas populations located on the smooth surfaces fail to survive as well (Beuchat and Ryu, 1997). In three studies on tomatoes and chlorine efficacy, *Salmonella* spp. were inoculated on whole tomatoes as well as stem scars and wounds (Wei et al., 1995; Weissinger et al., 2000; Zhuang et al., 1995). Wei et al. (1995) inoculated *Salmonella* Montevideo on whole tomatoes, stem scars, growth cracks, and punctures. The tomatoes were treated with 100 mg·L⁻¹ chlorine

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¹Division of Consolidated Laboratory Services, Commonwealth of Virginia, 600 N. 5th St., Rm. 272, Richmond, VA 23219.

²Food Science and Human Nutrition Department, University of Florida, 359 FHSN Bldg., Newell Dr., Gainesville, FL 32611-0370.

³Plant Pathology Department, University of Florida, PO Box 110680, Gainesville, FL 32611-0680.

⁴To whom reprint requests should be addressed; phone: (352) 392-1991, ext. 313; fax: (352) 392-9467; e-mail: krschneider@ifas.ufl.edu

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	μL	3.3814 × 10 ⁻⁵
29.5735	fl oz	mL	0.0338
25.4000	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
1	ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

for up to 2 min and reductions were only 1.0 to 2.0 log₁₀ units. Weissinger et al. (2000) treated tomatoes inoculated with *Salmonella* Baildon by dipping the whole tomato in 120 and 200 mg·L⁻¹ of chlorine for 40 s. Tomatoes were still positive for *Salmonella* after the 200 mg·L⁻¹ treatment and populations with both treatments were reduced by less than a 1.0 log₁₀ cfu/mL. Zhuang et al. (1995) treated tomatoes with 0, 50, 110, 200, and 320 mg·L⁻¹ of chlorine after inoculating with *Salmonella* Montevideo. Whole tomatoes and core tissues were sampled for *Salmonella* survival. Even at 320 mg·L⁻¹, populations of *Salmonella* were not eliminated (Zhuang et al., 1995).

This study examined the efficacy of chlorine treatment of flume water for eliminating *Salmonella* spp. from inoculated wounds and intact surfaces, using treatment conditions (free chlorine level, pH, and flume water temperatures) currently utilized in Florida fresh tomato packinghouses.

Materials and methods

CULTURES. Bacterial strains associated with fruit- and vegetable-related foodborne illness outbreaks were obtained from University of California, Davis (L. Harris). These *Salmonella* cultures were adapted to be rifampin resistant (rif⁺) to a level of 200 mg·L⁻¹. The serovars used in this study were *Salmonella* Agona, *Salmonella* Gaminara, *Salmonella* Michigan, *Salmonella* Montevideo, and *Salmonella* Poona. All media used in growth studies and experiments were supplemented with 80 mg·L⁻¹ of rifampin (Fisher, Fair Lawn, N.J.) (rif⁺) so that *Salmonella* cells isolated from inoculated tomatoes were relatively free of competition from other bacterial contaminants (Lukasik et al., 2001). The cultures were maintained on tryptic soy agar [TSA (BD Diagnostics, Franklin Lakes, N.J.)] slants. The inoculum was prepared from a successive 3-d transfer in tryptic soy broth [TSB (BD Diagnostics)] supplemented with 80 mg·L⁻¹ of rifampin. The cultures were centrifuged at 3220 g_n for 20 min and washed twice with phosphate buffer saline [PBS (Sigma-Aldrich, St. Louis, Mo.)]. All five strains were combined as a cocktail and diluted in PBS to obtain an approximate inoculum level of 1.0 × 10⁹ cfu/mL of inoculum cocktail.

WOUNDS (SHAVES, PUNCTURES, AND STEM SCARS). All tomatoes used

in this study were round, unripe, green 'Florida 47' tomatoes. Tomatoes were obtained from a commercial packinghouse prior to any treatment. *Salmonella* recovery was tested on four surface types: intact, punctures, shaves, and stem scars. For puncture tests, three wounds per tomato were made using a sharp, thin strand of sterile metal (approximately 1 mm thick) to a depth of 1 mm. For shaved wounds, a section of the fruit surface was removed to a depth of about 1 mm with a sterile scalpel over an area of approximately 15 mm in diameter. Stem scars represent a common natural wound associated with the removal of stems from the fruit. There were three replicate samples prepared for each wound type. Three replicates of whole, intact tomatoes were utilized as non-wounded surface controls, and a water control (flume water at the same temperature and pH as the chlorinated flume treatment) was performed in order to evaluate the degree of *Salmonella* reduction from the intact fruit surface due to mechanical action only. The water control represents the maximum decontamination effect that could be achieved by the mechanical action of the flume water.

INOCULATION AND *SALMONELLA* RECOVERY. For puncture wounds, a 10-μL aliquot of the *Salmonella* cocktail was placed in each of the three punctures on each tomato. The shave wounds, stem scars, and intact surfaces all received 100-μL aliquots of the inoculum. The inoculum was appropriately diluted to deliver the same titer of *Salmonella* per tomato for each surface type. The inoculated areas were identified with indelible marker and the inoculum was then allowed to air-dry at room temperature for approximately 1 h. As soon as the drying was completed, three replicate samples from each of the four surface types were removed with a sterile knife and placed into a sterile Stomacher bag (Seward, Norfolk, U.K.) containing 100 mL Butterfield's phosphate buffer (BPB) supplemented with 0.1% sodium thiosulfate to determine initial populations (t = 0 s). Counts were recorded as surviving *Salmonella* cfu/mL in recovery buffer.

FLUME TREATMENTS. Water in a scale-model flume was chlorinated to 150 mg·L⁻¹ of free chlorine at pH 6.5 and maintained at a temperature of 25 or 35 °C, depending on the

test. Residual free chlorine levels were measured using a Hach DR-890 (Hach Co., Loveland, Colo.). Temperature, chlorine concentration, and pH measurements were conducted at the beginning and end of each flume treatment to ensure treatment conditions were maintained as stated throughout each run. Inoculated fruit, tempered to room temperature and oriented such that the inoculated areas were fully submerged in the chlorinated water, were floated in the flume for the stated treatment duration. The experiments were also conducted with a water control (no free chlorine) at 25 and 35 °C to represent the pathogen reduction due to water rinsing only. For all treatments, three replicate tomato samples were removed after 30, 60, and 120 s. The inoculated sites were removed and *Salmonella* populations were determined using the same procedures described for the t = 0 samples.

STATISTICS. The means of populations recovered from different inoculation sites (wound types) and chlorination treatment in two separate experiments were compared using analysis of variance (ANOVA)/multivariate analysis of variance (MANOVA) and Duncan's multiple range statistics (Statsoft Statistica, Tulsa, Okla.).

Results

DETERMINATION OF EFFICACY AT 150 MG·L⁻¹ OF FREE CHLORINE, pH 6.5, AND 25 AND 35 °C. Log₁₀ values of 6.5 to 6.8 and 5.8 to 6.5 cfu/mL were recovered from the different inoculation sites prior to the chlorine treatment (t = 0 s) at 25 °C (Table 1) and 35 °C (Table 2), respectively. The t = 0 s values represented the initial inoculum level of untreated fruit and are the values from which log reductions were determined. Viable *Salmonella* were recovered from all of the inoculation sites, including the intact fruit surface, even after treatment with chlorinated water for up to 120 s at either 25 or 35 °C. Water control numbers represent the log₁₀ survivors after exposure to non-chlorinated water for t = 0 to 120 s.

After a 30-s exposure in the chlorine bath, log₁₀ 3.5 to 5.9 cfu/mL were recovered from the different inoculation sites (Table 1). Generally, the highest *Salmonella* recovery came from puncture wounds and the lowest from the intact surfaces. After 120 s at 25 °C, nearly all *Salmonella* had

Table 1. *Salmonella* log₁₀ survivors recovered from tomato inoculated sites after simulated flume treatment of 0, 30, 60, and 120 s at 25 °C (77.0 °F) [free chlorine 150 mg·L⁻¹ (ppm) and pH 6.5] and water control at 25 °C (free chlorine 0 mg·L⁻¹, pH 6.5, intact fruit).

Treatment	<i>Salmonella</i> survivors (log cfu/mL) recovered from inoculation site ^a				
	Stem scar	Scrape	Puncture	Intact	Water control ^b
0 s	6.77 ± 0.17 a	6.52 ± 0.16 a	6.56 ± 0.34 a	6.52 ± 0.48 a	5.99 ± 0.29 d
30 s	5.80 ± 0.38 a,b	4.82 ± 0.79 b	5.92 ± 0.20 a	3.49 ± 0.64 c	4.94 ± 0.39 e
60 s	5.16 ± 0.66 a	5.34 ± 0.64 a	5.85 ± 0.50 a	3.29 ± 0.71 b	5.06 ± 0.20 e
120 s	4.91 ± 0.17 a	5.10 ± 1.04 a	5.83 ± 0.13 a	0.16 ± 0.28 b	4.57 ± 0.27 e

^aMean ± SD for triplicate determinations. For chlorination results, means with the same letter in the same row are not significantly different (*P* > 0.05).

^bFor water control only, means with the same letter in the same column are not significantly different (*P* > 0.05).

Table 2. *Salmonella* log₁₀ survivors recovered from tomato inoculated sites after simulated flume treatment of 0, 30, 60 and 120 s at 35 °C (95.0 °F) [free chlorine 150 mg·L⁻¹ (ppm) and pH 6.5] and water control at 35 °C (free chlorine 0 mg·L⁻¹, pH 6.5, intact fruit).

Treatment	<i>Salmonella</i> survivors (log cfu/mL) recovered from inoculation site ^a				
	Stem scar	Scrape	Puncture	Intact	Water control ^b
0 s	6.48 ± 0.66 a	5.77 ± 0.28 a	6.49 ± 0.09 a	6.34 ± 0.28 a	5.60 ± 0.54 d
30 s	5.59 ± 0.17 a	5.65 ± 0.31 a	6.00 ± 0.11 a	4.06 ± 2.03 a	4.84 ± 0.13 e
60 s	5.76 ± 0.30 a	5.43 ± 0.19 a	5.67 ± 0.14 a	2.81 ± 2.58 b	4.74 ± 0.13 e
120 s	5.48 ± 0.18 a	5.21 ± 0.06 a	5.78 ± 0.15 a	1.49 ± 2.58 b	4.24 ± 0.65 e

^aMean ± SD for triplicate determinations. For chlorination results, means with the same letter in the same row are not significantly different (*P* > 0.05).

^bFor water control only, means with the same letter in the same column are not significantly different (*P* > 0.05).

been eliminated from intact surfaces, whereas 4.9 to 5.8 log₁₀ units were recovered from the various wounds. The largest populations were recovered from the puncture wound sites and the smallest from the stem scar area; however, these values were not different from each other. Populations recovered from the scrape wounds were similar to those extracted from the stem scar sites. Exposure of intact fruit to non-chlorinated water at the various treatment times did not result in significant differences in recovered *Salmonella* populations.

Populations recovered after the 30-s chlorination treatment at 35 °C ranged from log₁₀ 4.1 for intact surfaces to 6.0 cfu/mL in the puncture wounds (Table 2). At the 60- and 120-s treatment times, all wounds had higher mean populations than tomatoes with intact surfaces. After the 120-s treatment, mean populations recovered from wounds ranged from log₁₀ 5.2 to 5.8 cfu/mL, whereas the intact surface had a mean recovered value of log₁₀ 1.5 cfu/mL. Flume treatment with the non-chlorinated water resulted in a significant decrease in initial populations for all exposure times, although there were no significant differences among the exposure times themselves.

The 25 °C log₁₀ reductions over 120 s ranged from 0.7 for punctures to 6.4 for intact tomatoes (Fig. 1). The

largest reduction of *Salmonella* was observed with intact tomatoes. Similar reductions in *Salmonella* counts were observed in the 35 °C trials.

Discussion

Areas on or in tomatoes that enable bacteria to become embedded in the fruit tissues could provide protection for *Salmonella* in an aqueous chlorinated environment. Wells and Butterfield (1999) found that the incidence of *Salmonella* on produce was higher on items that had decay caused by bacteria or fungi or had physical

injury than on those that were injury and decay free. The wounds offer not only protection against desiccation and other stresses, but also an environment conducive to multiplication (Wells and Butterfield, 1999). Zhuang et al. (1995) demonstrated chlorine dip treatments were less effective in reducing *Salmonella* populations in core tissues than on those on tomato surfaces. Wei et al. (1995) reported the failure of water chlorination to remove *Salmonella* from stem scars and growth cracks.

Under certain conditions, the

Fig. 1. Log₁₀ reductions of *Salmonella* populations after a 120-s treatment of 150 mg·L⁻¹ (ppm) free chlorine at pH 6.5. Trials were performed at 25 and 35 °C (77.0 and 95.0 °F). Each bar represents the mean of three replicate samples.

stem scar and wounds on tomato can offer routes by which aqueous suspensions of bacteria can become internalized. Bartz and Showalter (1981) reported that if fruit were immersed in an environment, such as a flume tank, that was cooler than the fruit, bacterial suspensions could infiltrate the stem scars on tomatoes as the fruit cooled. Bartz et al. (2001) noted that water-soluble dyes rapidly penetrated into freshly wounded tissues of tomato fruit where sap from damaged cells flooded nearby intercellular spaces. Johnson (1945) reported that bacteria internalized in tobacco leaf tissues if the surface openings were water-congested and further, suggested that capillary forces in the intercellular spaces were involved. When bacterial suspensions are in intercellular spaces, liquid disinfectants appear to displace the suspensions further into the tissues (Bartz et al., 2001).

In the 25 °C study, with tomatoes that were inoculated with an aqueous cell suspension of *Salmonella* that was allowed to dry on the unbroken surface, populations were reduced up to 6.4 log units by a 2-min immersion treatment with chlorinated water at 150 mg·L⁻¹ (pH 6.5 at 25 °C). By contrast, populations placed in wounds were reduced by only 0.6 to 1.9 log units, depending on the test and the type of wound. Greater survival with each type of inoculation site, including stem scar, unbroken surface, puncture, or scrape, was associated with shorter exposure to the chlorine.

In the second study, tomatoes were placed in flume water at a temperature of 35 °C to establish a +10 °C temperature differential between the flume water and the fruit. This simulates the optimal commercial practice of maintaining the flume water at a warmer temperature than the incoming fruit in order to prevent possible bacterial infiltration. Theoretically, use of warmer chlorine solutions should lead to increased activity of the hypochlorous acid (White, 1999). One would expect improved wound sanitization at 35 °C, as compared to results at 25 °C, due to this increased sanitization activity. However, this study did not demonstrate any increase in efficacy at 35 °C (Fig. 1). This could be due to the reduced tendency of the sanitizing solution to infiltrate the wounded areas due to the temperature differential. After a 60-s treatment, significantly

fewer *Salmonella* were recovered from the unbroken surfaces as compared with the wounds (Tables 1–2). In the 35 °C study, the 120-s-treated, intact tomatoes demonstrated a 4.9 log₁₀ reduction, whereas the log₁₀ reduction with the stem scar, puncture, and scrape wounds averaged 1.0, 0.7, and 0.6, respectively.

Clearly, water chlorination cannot be relied upon to eliminate contamination of tomato fruit by *Salmonella*, particularly if these bacteria have gained entry into the fruit through wounds prior to sanitation. Any tomatoes that have wounds, such as punctures, cuts, or scrapes, must be culled at the packinghouse to minimize the chance of *Salmonella* contamination. Stem scars are a naturally occurring consequence of harvesting tomatoes and therefore cannot be avoided. However, this study suggests stem scars, as with the wounds examined in this study, cannot be readily disinfected through the use of the industry standard sanitizer, sodium hypochlorite. Thus, it is imperative for tomato growers to minimize or prevent contamination through the use of good agricultural practices prior to and during harvesting. Additionally, the equipment used during the harvest must be kept sanitized and field crews must be properly trained and use good hygienic practices. Proper and continuous water chlorination is required at the packinghouse, as recommended by U.S. Food and Drug Administration (1998). This study shows *Salmonella* populations were reduced when fruit surfaces were intact. Finally, the packing equipment, fruit boxes, and transportation equipment must be kept clean, new, and/or sanitized to prevent cross-contamination.

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