APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1995, p. 2127–2131 Vol. 61, No. 6
0099-2240/95/$04.00+0

Fate of *Salmonella montevideo* on and in Raw Tomatoes as Affected by Temperature and Treatment with Chlorine

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Received 31 October 1994/Accepted 9 February 1995

A study was undertaken to determine the survival patterns of *Salmonella montevideo* G4639 on and in tomatoes during storage and the efficacy of chlorine treatment on inactivation of the pathogen. The population of *S. montevideo* on the surfaces of inoculated tomatoes stored at 10°C did not change significantly ($P < 0.05$) throughout an 18-day storage period. Significant increases in population occurred within 7 days and within 1 day when tomatoes were stored at 20 and 30°C, respectively. A significantly higher number of cells was taken up by the core tissue of tomatoes tempered at 25°C when the tomatoes were dipped in a suspension at 10°C compared with the number taken up when the tomatoes were dipped in cell suspensions tempered at 25 or 37°C. Populations remained constant throughout subsequent storage for 8 days at 10°C, regardless of the temperature differential between tomatoes and the dip suspension. Storage of tomatoes at 20°C, however, resulted in significant increases in populations of *S. montevideo*. Populations of the pathogen on the surfaces and in the core tissues of tomatoes were significantly reduced by dipping for 2 min in a solution containing 60 or 110 ppm (60 or 110 μg/ml) chlorine, respectively; however, treatment in solution containing 320 ppm chlorine did not result in complete inactivation. Populations of *S. montevideo* remained unchanged in chopped tomatoes stored at 5°C for 216 h (9 days) but increased significantly after storage for 96 or 22 h at 20 or 30°C, respectively. We recommend that tomato packinghouses maintain their dip tanks at a temperature higher than the temperature of the tomatoes and at a free chlorine concentration of 200 ppm. The temperature of tomatoes should be reduced to 10°C as rapidly as possible after harvesting and should be held at 10°C until they are ripened immediately before consumption.

The presence of pathogens on ready-to-eat salad vegetables is well documented. *Listeria monocytogenes* has been detected on fresh produce (16) and on retail packs of prepared salad vegetables (8, 14) and is capable of growing on the surfaces of ripe tomatoes (7). *Yersinia enterocolitica* (8) and *Aeromonas hydrophila* (9) have been isolated from fresh produce, and human gastroenteritis caused by the consumption of shredded lettuce contaminated with *Shigella sonnei* (10) and epidemiologically linked to the consumption of salad vegetables contaminated by enterotoxigenic *Escherichia coli* (17) has been reported.

Salmonellae are the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States (6). Foods of animal origin, such as poultry and other meat products, eggs, and dairy products, are the most commonly implicated sources of salmonellosis outbreaks (24). Fresh fruits and vegetables are implicated less frequently, although salmonellae have been isolated from several types of salad vegetables (11, 13, 24). An outbreak of infection associated with bean sprouts contaminated with *Salmonella saint-paul* has been reported (18). Four large multistate outbreaks of salmonellosis have been attributed to fresh produce since 1990 (15). Two involved the consumption of tomatoes contaminated with *Salmonella javiana* (26) and *Salmonella montevideo* (15).

A 1993 multistate *S. montevideo* outbreak was associated with eating uncooked tomatoes. The mature green tomatoes were handpicked from staked plants, transported to the packing shed, dumped into a heated, chlorinated water bath, conveyed for hand sorting, and placed in boxes on pallets. The tomatoes were then held for 2 to 4 weeks at temperatures of 13 to 16°C during storage, shipping, and ripening.

In this outbreak, as in previous multistate produce-associated outbreaks, salmonellae were not recovered from this implicated food item; most of the tomatoes were consumed or discarded before the investigation. Although *Salmonella* species have been isolated from numerous types of produce, including tomatoes (11, 13, 24), and diced tomatoes can support the growth of salmonellae, it was not known whether it was feasible for tomatoes to serve as a vehicle for salmonellae under current handling procedures. The purpose of the study described here was to determine the fate of *S. montevideo* on the surfaces and in the stem core tissues of mature green tomatoes under conditions simulating their handling from the packing shed to the retail outlet.

MATERIALS AND METHODS

Strain G4639 and preparation of inoculum. *S. montevideo* G4639, isolated from a patient in the 1993 outbreak, was used throughout the study. The strain was obtained from the Centers for Disease Control and Prevention, Atlanta, Ga. A stock culture was maintained on tryptic soy agar (Difco, Detroit, Mich.) at 5°C. The organism was cultured in tryptic soy broth (TSB; pH 7.3; Difco) at 37°C. Loop inocula were transferred to TSB at three consecutive 24-h intervals immediately before their use as inocula for all experiments.

Media for enumeration of *S. montevideo* and aerobic mesophiles. Bismuth sulfite agar (BSA; Difco) was used to enumerate *S. montevideo* in all experiments. In addition, brilliant green agar (BGA; Difco) was also used to enumerate *S. montevideo* in experiments designed to determine its fate on the surfaces of tomatoes during storage and in experiments to determine its uptake by stem tissue. Plate count agar (PCA; Difco) was used to enumerate aerobic mesophiles in chopped, fully ripe tomatoes.

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Source of tomatoes. Mature green tomatoes (Sunny cultivar) were picked by hand from the fields of commercial growers near Homestead, Fla., and were shipped to our laboratory within 48 h after they were removed from the vine. The weights of individual tomatoes used in various experiments ranged from 110 to 140 g. Fully ripened tomatoes (variety not known), purchased from a Griffin, Ga., supermarket, were used in the experiments designed to determine survival and growth characteristics in chopped tomatoes.

Fate of *S. montevideo* on tomato surfaces. Experiments were done to determine the survival and growth characteristics of *S. montevideo* on the surfaces of tomatoes stored at 10, 20, or 30°C for up to 18 days. On the second day after harvesting, stems were removed from the approximately 20% of unwashed, mature green tomatoes on which they were still attached, and the tomatoes were placed in an incubator at 20°C for 22 h. A suspension of *S. montevideo* was prepared by mixing 15 ml of a 24-h TSB culture with 8 liters of sterile 0.1 M potassium phosphate buffer (pH 7.0) tempered at 20°C. Batches of tomatoes (18 to 20) were submerged and constantly agitated in the suspension for 2 min and were then air dried in a laminar flow hood at 22°C for 4 h. Tomatoes were individually placed in plastic bags and were stored at 10, 20, and 30°C for up to 18 days before analyzing for populations ($\log_{10}$ CFU per square centimeter of external surface) of *S. montevideo*. All bags were open during storage to facilitate constant contact of the tomatoes with air. The relative humidity of the storage environment ranged from 45 to 60%.

Tomatoes were analyzed for populations of salmonellae before inoculation and for *S. montevideo* after inoculation and drying (0 day) and after 1, 2, 4, 7, 9, 15, and 18 days of storage at 10, 20, or 30°C. To each bag containing one tomato, 20 ml of a sterile aqueous solution of 0.1% peptone was added. The bag was sealed, and the surface of each tomato was gently rubbed by hand for 1 min to remove *S. montevideo* as well as other microorganisms that were naturally present. The wash fluid was serially (1:10) diluted in 0.1% peptone and surface plated in duplicate onto BSA and BGA. Presumptive colonies of *S. montevideo* that developed after incubation for 24 h at 37°C were counted. Four colonies per plate were randomly selected and confirmed by appropriate biochemical tests (12).

Uptake of *S. montevideo* by core tissue. The extent of uptake of *S. montevideo* by tomato stem scar tissue (core tissue) as influenced by the temperature of the dip suspension and subsequent survival during storage at 10 and 20°C were determined. Mature green tomatoes were tempered at 25°C. Two-liter quantities of 0.1 M phosphate buffer were tempered at 10, 25, or 37°C. Fifteen milliliters of a 24-h TSB culture of *S. montevideo* was added to the buffer, after which 10 tomatoes were submerged and constantly agitated for 2 min, dried, placed in plastic bags, and stored at 10 or 20°C as described above.

The stem scar area and tissue (core) of tomatoes were analyzed for populations ($\log_{10}$ CFU per gram) of salmonellae before inoculation with *S. montevideo*, after inoculation and drying (0 day), and after 1, 3, 5, and 8 days of storage at 10°C and 1, 3, 5, 8, and 18 days of storage at 20°C. The cores of three tomatoes from each treatment group were removed with a sterile scalpel for analysis at each storage time. The procedure consisted of cutting around the external stem scar area and excising a conical area of tissue to a depth of 1.5 to 2 cm. Each core was placed in a stomacher bag and was repeatedly struck with a hammer to cause the tissue to become soft and partially homogenized. Sterile 0.1% peptone (10 ml) was added to the macerated core tissue, and the mixture was massaged for 1 min. Samples were serially diluted in 0.1% peptone, and the mixtures were surface plated in duplicate (0.1) or quadruplicate (0.25 ml) onto BSA and BGA. Colonies of *S. montevideo* were counted and confirmed as described above.

Efficacy of chlorine for inactivating *S. montevideo*. Inactivation of *S. montevideo* on the surfaces and in the stem tissues of tomatoes dipped in solutions containing various concentrates of chlorine was determined. Mature green tomatoes were tempered at 25°C. Potassium phosphate buffer (0.1 M; pH 7.0; 3 liters) tempered at 10°C was combined with 30 ml of a 24-h TSB culture of *S. montevideo*. Batches (18 to 22) of tomatoes were submerged in the suspension, constantly agitated for 2 min, air dried for 5 h, and stored at 25°C for 18 h.

Solutions containing free chlorine target concentrations of 0, 50, 100, 200, and 300 ppm (μg/ml) were prepared by adding appropriate amounts of sodium hypochlorite to 0.05 M potassium phosphate buffer (pH 7.1 ± 0.1; 37°C). Batches of six tomatoes (25°C) inoculated with *S. montevideo* were submerged in 1 liter of chlorine solution and were agitated for 2 min. Immediately after removal from solutions, three tomatoes from each treatment group were analyzed for surface populations of *S. montevideo* and three tomatoes from each treatment were analyzed for core populations by the procedures described above. Enumeration was done with BSA but not BGA. The free chlorine content of dip solutions was determined by using Hach chlorine test kits (Hach Co., Ames, Iowa), which are approved by the U.S. Environmental Protection Agency.

Fate of *S. montevideo* in chopped ripe tomatoes. The survival and growth patterns of *S. montevideo* inoculated into chopped ripe tomatoes stored at 5, 20, and 30°C were determined. Fully ripe tomatoes (22°C) were surface disinfected by dipping them in a solution of sodium hypochlorite (0.5%) for 2 min. The cores were aseptically removed, and the tomatoes were cut into approximately 1-cm cubes with a sterile knife. Samples (50 g) in stomacher bags were inoculated with 1 ml of a diluted (10^{-3} in 0.05 potassium phosphate buffer [pH 7.0]) 24-h TSB culture of *S. montevideo* and were thoroughly mixed before incubating at 5, 20, or 30°C.

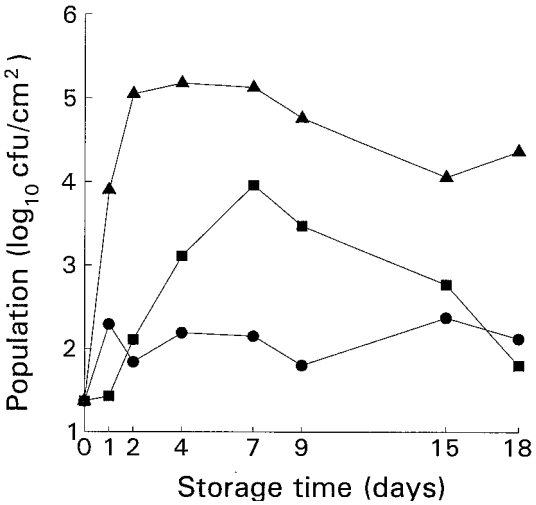

FIG. 1. Effect of storage temperature on populations of *S. montevideo* inoculated onto the surfaces of mature green tomatoes. Storage was at 10°C (●), 20°C (■), and 30°C (▲). Each point represents a mean of values from duplicate samples taken from three replicate experiments ($n = 6$).

Uninoculated, chopped tomatoes and inoculated, chopped tomatoes held for 22, 46, 96, 142, and 216 h at 5°C; 6, 22, 46, 70, 96, and 142 h at 20°C; and 6, 22, 46, and 70 h at 30°C were analyzed for populations ($\log_{10}$ CFU per gram) of *S. montevideo* and aerobic mesophiles. Each sample (50 g) was combined with 50 ml of sterile 0.1% peptone, and the mixture was pummeled in a stomacher for 1 min. Serially diluted samples (0.1 ml) were surface plated in duplicate onto BSA to enumerate the *S. montevideo* population. Serially diluted samples were also surface plated onto PCA to determine the populations of aerobic mesophiles. The colonies appearing on the plates after 3 days of incubation at 20°C were counted.

Statistical analysis. All experiments were done in triplicate, and duplicate samples from each treatment were analyzed at each sampling time. Mean populations of *S. montevideo* and aerobic mesophiles detected on and in the tomatoes were subjected to analysis of variance and Duncan's multiple range test (SAS Institute, Cary, N.C.) to determine significant differences ($P < 0.05$) between treatments.

RESULTS

The use of BSA and BGA in initial experiments to enumerate *S. montevideo* on the surfaces and in the core tissues of tomatoes revealed that neither medium was superior to the other. For simplicity, the data obtained with BSA as an enumeration medium are presented here. BSA was chosen for enumerating *S. montevideo* in subsequent experiments because of the ease of visually detecting colonies. Populations of *S. montevideo* in the dip suspension used for whole tomatoes ranged from 7.13 to 7.59 $\log_{10}$ CFU/ml. The population of *S. montevideo* in the suspension used to inoculate chopped tomatoes was 6.08 $\log_{10}$ CFU/ml.

Shown in Fig. 1 are the results from studies designed to determine the fate of *S. montevideo* on the surfaces of inoculated mature green tomatoes stored at 10, 20, and 30°C. The population of the pathogen did not change significantly ($P < 0.05$) on tomatoes stored at 10°C throughout the 18-day storage period. However, significant increases in the population of *S. montevideo* occurred within 7 days and within 1 day when tomatoes were stored at 20 and 30°C, respectively. Between 7 and 9 days, the number of *S. montevideo* detected on tomatoes stored at 20°C decreased and remained at levels that were not significantly different from those detected during the first 4 days throughout the remainder of the storage period. At 30°C, populations were significantly higher from day 1 to day 18 compared with the initial population (day 0). A comparison of

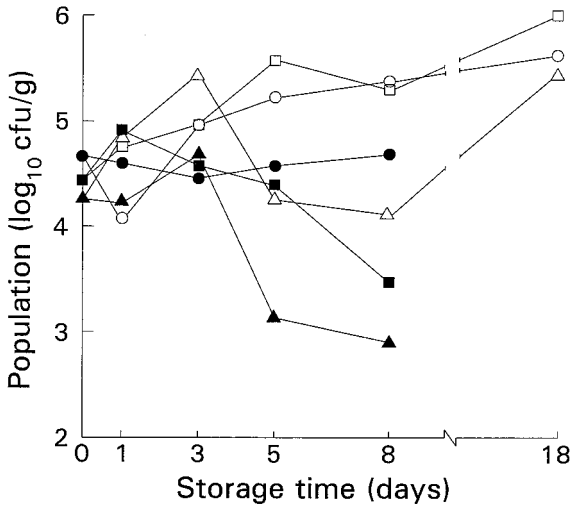

FIG. 2. Effect of temperature differential between tomatoes (25°C) and dip suspension (10, 25, or 37°C) on uptake of *S. montevideo* by core tissue and effect of subsequent storage temperature (10 or 20°C) on survival. The temperatures of the dip suspension were 10°C with storage of tomatoes at 10°C (●) and 20°C (○), 25°C with storage at 10°C (■) and 20°C (□), and 37°C with storage at 10°C (▲) and 20°C (△). Each point represents a mean of values from duplicate samples taken from three replicate experiments ($n = 6$).

the effect of temperature on the number of *S. montevideo* detected on tomatoes at any given storage time revealed that significantly higher populations occurred between 1 and 7 days and at 18 days on tomatoes stored at 30°C compared with the populations on tomatoes stored at 10 or 20°C. At 9 and 15 days, the storage temperature did not have a significant effect on the population of *S. montevideo*.

Results from studies to determine the effect of temperature differential between tomatoes and dip suspension on the uptake of *S. montevideo* by core tissue and the effect of subsequent storage temperature on changes in populations on *S. montevideo* in tissue are illustrated in Fig. 2. Some of the *S. montevideo* isolates detected in core samples were undoubtedly on the external tissue surface; however, the average external surface area was the same for samples, regardless of the treatment conditions. Thus, differences in the number of *S. montevideo* detected in core samples are attributable to differences in uptake of cells as a result of the treatment conditions. A significantly ($P < 0.05$) higher number of *S. montevideo* cells were taken up by core tissue when tomatoes at 25°C were dipped in suspension at 10°C compared with the number of cells taken up by tomatoes dipped in suspensions at 25 or 37°C. Populations of viable *S. montevideo* cells in the core tissues of tomatoes remained essentially constant throughout subsequent storage for 18 days at 10°C. Storage of tomatoes at 20°C resulted in significant increases in the population of *S. montevideo* in core tissues within 3, 5, and 18 days of storage of tomatoes that had been dipped in suspension at 10, 25, and 37°C.

The effectiveness of chlorine in killing *S. montevideo* on the surfaces and in the core tissues of tomatoes is provided in Table 1. A significant ($P < 0.05$) reduction in the surface population was achieved by dipping the tomatoes in a solution containing 60 ppm free chlorine. An additional significant reduction in population was observed by dipping tomatoes in a solution containing 110 ppm chlorine; however, increased concentrations of up to 320 ppm chlorine did not result in a significant reduction compared with dipping tomatoes in the 110-ppm solution. Dipping in a solution containing 320 ppm chlorine for 2 min resulted in approximately 1.5-$\log_{10}$-unit reduction in the number of viable *S. montevideo* on the surfaces of tomatoes.

TABLE 1. Effectiveness of chlorine on inactivating *S. montevideo* on the surfaces and in the core tissues of mature green tomatoes

Free Cl^- concn (ppm)	Population[a]	
	Surface ($\log_{10}$ CFU/cm^2)	Core ($\log_{10}$ CFU/g)
0	4.81 a	5.97 a
60	4.17 b	5.74 a
110	3.59 c	5.28 b
210	3.58 c	5.21 b
320	3.45 c	4.92 b

[a] Mean values in the same column that are not followed by the same letter are significantly different ($P < 0.05$).

The effectiveness of chlorine in killing *S. montevideo* in core tissue was less than its effectiveness in killing *S. montevideo* causing surface contamination (Table 1). Dipping in a 60-ppm chlorine solution did not result in a significant ($P < 0.05$) reduction in the number of viable *S. montevideo* cells in core tissue. Concentrations of 110 to 320 ppm did, however, significantly reduce the number of viable cells, although there was not a significant difference in the number of cells inactivated in solutions containing this range of chlorine concentrations.

Survival and growth patterns of *S. montevideo* inoculated in chopped, fully ripe tomatoes as well as changes in the population of aerobic mesophiles are illustrated in Fig. 3. Populations of *S. montevideo* remained essentially constant in tomatoes stored at 5°C for 216 h. However, significant ($P < 0.05$) increases occurred by 96 and 22 h in chopped tomatoes stored at 20 and 30°C, respectively. The aerobic mesophile population in chopped tomatoes stored at 5°C remained constant throughout the 216-h test period (Fig. 3). Significant increases in populations were detected in tomatoes stored for 22 h at 20 or 30°C. The initial pH (4.1 ± 0.1) increased to 4.5 ± 0.1, 4.3 ± 0.1, and 4.3 ± 0.2 when tomatoes were stored at 5°C for 216 h,

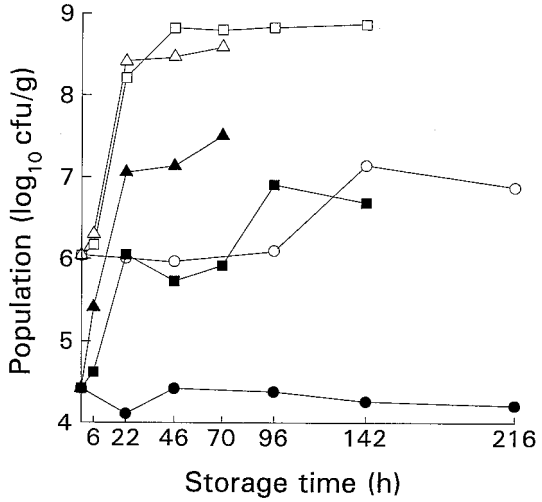

FIG. 3. Effect of temperature on survival and growth of *S. montevideo* and aerobic mesophiles in chopped ripe tomatoes. *S. montevideo* (solid symbols) and aerobic mesophiles (open symbols) were enumerated in tomatoes stored at 5°C (●, ○), 20°C (■, □), and 30°C (▲, △). Each point represents a mean of values from duplicate samples taken from three replicate experiments ($n = 6$).

20°C for 142 h, and 30°C for 70 h, respectively. Microbial growth was evident in tomatoes stored for 22 h at 20 or 30°C. Subjective observations of aroma revealed that tomatoes were inedible after 22 h of storage at 20 or 30°C and after 96 h at 5°C.

DISCUSSION

The retention of viability of *S. montevideo* on the surfaces and in the cores of tomatoes held at 10°C suggests a potential for survival for the duration of transport and storage preceding ripening and consumption. The observation that *S. montevideo* grows on the surfaces and in the core tissues of tomatoes stored at 20 or 30°C precludes the notion that the pathogen will rapidly die during the transportation, storage, and ripening period.

The presence of microorganisms in otherwise healthy tissues of vegetables was documented in the early 1960s (19, 20). In tomatoes, populations are highest close to the stem scar and the central core of the fruit, decreasing toward the fruit periphery. Lactobacilli are known to penetrate brined tomatoes primarily through the stem tissue (20). Bartz and Showalter (5) demonstrated that warm (26 to 40°C) tomatoes immersed for 10 min or longer in cool (20 to 22°C) suspensions of *Serratia marcesens*, *Erwinia carotovora*, *Pseudomonas marginalis*, or *Pseudomonas aeruginosa* were infiltrated by water and these bacteria. Infiltration was associated with a negative temperature difference (differential) between the water and the tomatoes, i.e., when the water temperature was less than the tomato temperature. Because the differential was shifted to a positive relationship (water temperature higher than tomato temperature), the extent of infiltration was reduced (2, 4). The observations reported here on *S. montevideo* confirm this phenomenon. Uptake of a significantly higher number of *S. montevideo* cells by tomato core tissue occurred when the temperature differential was −15°C, i.e., when the temperature of the water was 15°C less than the temperature of the tomato, than when differentials were 0 or +12°C. Bartz (4) reported that tomatoes that resist infiltration are less likely to undergo soft rot as quickly compared with those that readily absorb water and *E. carotovora*.

The recommended concentration of 100 to 150 ppm free chlorine in packinghouse wash water (22) would, on the basis of the observations in the present study, reduce the level of *S. montevideo* that might contaminate the surfaces and core tissues of mature green tomatoes. However, chlorination should be considered only as disinfection treatment since, even at 320 ppm, elimination of viable *S. montevideo* from inoculated tomatoes was not achieved. Bartz (3) reported that the incidence of tomato fruit disease associated with infiltration with contaminated water can be reduced but not eliminated by adding 50 to 100 ppm free chlorine to wash water, thus providing further evidence that treatment with chlorine is not totally effective in killing the wide range in the types of microorganisms that may be naturally present in or on tomatoes in the packinghouse (21).

Asplund and Nurmi (1) reported that three *Salmonella* serotypes (*S. enteritidis*, *S. infantis*, and *S. typhimurium*) can grow in cut tomatoes at 22 and 30°C to reach populations exceeding 10^6/g. The initial pH of the tomatoes used in these studies varied from 3.99 to 4.37, which bracketed the pH (4.1 ± 0.1) of the tomatoes used in our study. The pHs of ripe and overripe tomatoes range from 3.4 to 4.7 and 4.0 to 4.8, respectively (25). Our observations indicate that *S. montevideo* can grow well in chopped, ripe tomatoes held at 20 or 30°C, thus further demonstrating the ability of an additional *Salmonella* serotype to pose a risk of causing salmonellosis associated with the consumption of improperly handled tomatoes.

The present study clearly demonstrates the possibility for tomatoes to serve as a vehicle of salmonellae under current handling procedures. On the basis of these findings, we recommend that tomato packinghouses maintain their dip tanks at a temperature higher than the temperature of the tomatoes and at a free chlorine concentration greater than 110 ppm and preferably near 200 ppm. Tomatoes should be cooled to 10°C as rapidly as possible after harvesting and should be held at 10°C until they are ripened immediately before consumption.

ACKNOWLEDGMENTS

We acknowledge the South Carolina Tomato Association for providing financial support for this study.

We are also grateful to Herb Bryan at the Tropical Research and Education Center, University of Florida, Homestead, for arranging the supply of mature green tomatoes and to Peggy Hayes at the Centers for Disease Control and Prevention for supplying *S. montevideo* G4639 used in the study.

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