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Metabiosis of proteolytic moulds and *Salmonella* in raw, ripe tomatoes

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ABSTRACT

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Aims: The aim of this study was to determine the survival and growth characteristics of *Salmonella enterica* in sound and chill-injured tomatoes as influenced by co-infection with proteolytic moulds.

Methods and Results: Sound (not chill injured) raw, ripe tomatoes (*Lycopersicon esculentum* Mill. 'Roma') were inoculated with a five-serotype mixture of *S. enterica* and/or *Alternata alternata* (two strains), *Cladosporium herbarum* and *C. cladosporioides*. Simultaneous and delayed (3 days) inoculation of tomatoes with *Salmonella* and each mould was studied. Growth of moulds in sound tomatoes stored at 15 and 25°C for up to 10 days was accompanied by increased pH of radial pericarp tissue (pulp), which enhanced the growth of *Salmonella*. Growth of moulds and *Salmonella* at 25°C was enhanced in chill-injured tomatoes compared with sound tomatoes.

Conclusions: Growth of proteolytic moulds in tomatoes stored at conditions simulating those commonly used in commercial postharvest storage and handling promotes the growth of *Salmonella* that may be an incidental contaminant.

Significance and Impact of the Study: Discarding tomatoes that are infected by moulds is important in handling and minimal processing practices designed to minimize the risk of human salmonellosis.

Keywords: *Alternaria alternata*, *Cladosporium herbarum*, *C. cladosporioides*, proteolytic mould, *Salmonella*, tomato.

INTRODUCTION

An increase in frequency of the number of outbreaks of human infections associated with consumption of fresh fruits and vegetables has occurred in recent years (Mead *et al.* 1999; National Advisory Committee on Microbiological Criteria for Foods 1999; Nguyen-the and Carlin 2000; Beuchat 2002). Factors that may have contributed to this increase include the emergence of new pathogens, adaptation of pathogens to environmental stresses imposed by many fruits and vegetables, increased global travel that facilitates human exposure to pathogens indigenous to certain geographical areas, and agricultural practices that use improperly treated irrigation water or animal manure (Hedberg *et al.* 1994; Beuchat and Ryu 1997).

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High-acid fruits and vegetables (pH < 4·6) have historically been considered to not support the growth of bacteria capable of causing human infections. However, some foodborne pathogens have been determined to possess extraordinary tolerance to stress environments. Salmonellae can grow at temperatures ranging from 2°C (Baker *et al.* 1986) to 54°C (Droffner and Yamamoto 1992) and proliferate at pH 3·99 (Asplund and Nurmi 1991) to 9·5 (Holley and Proulx 1986). Several outbreaks of salmonellosis in the US have been associated with consumption of uncooked tomatoes. In 1990 and 1993, outbreaks of infections caused by *Salmonella enterica* serotypes Javiana and Montevideo, respectively, were linked to the consumption of raw tomatoes (Hedberg *et al.* 1999). In late 1998 and early 1999, there was an outbreak of *S. enterica* serotype Baildon infections associated with raw, diced tomatoes (Cummings *et al.* 2001). A more recent outbreak of *Salmonella* Javiana infections was also linked to diced tomatoes (Toth *et al.* 2002).

The thin epidermis of tomato fruits is easily compromised by mechanical pressure, resulting in punctures, cracks, abrasions, and insect wounds that render the radial pericarp susceptible to preharvest and postharvest microbial invasion. Even fruits devoid of mechanical damage have stem scar tissue that readily absorbs water and microorganisms it may contain. Bartz and Showalter (1981) demonstrated that bacteria can enter stem scar tissue of tomatoes and be drawn into surrounding tissues as a result of exposure to a negative temperature differential, i.e. the temperature of the tomato is higher than the temperature of bacterial suspension in which it is immersed. Internalization of *Salmonella* Montevideo in mature green tomatoes (Zhuang *et al.* 1995; Zhuang and Beuchat 1996) and *Escherichia coli* O157:H7 in apples (Burnett *et al.* 2000) has been demonstrated in fruits exposed to a negative temperature differential. Contamination of raw fruits and vegetables with human pathogens may occur as a result of preharvest contact with contaminated irrigation water or manure, and postharvest contamination can occur during harvesting, washing, minimal processing, distribution, and preparation in foodservice settings or in the home (Beuchat 2002).

Growth of *S.* Montevideo (Zhuang *et al.* 1995), *S. enterica* serotypes Enteritidis, Infantis and Typhimurium (Asplund and Nurmi 1991), and *S. enterica* serotype Baildon (Weissinger *et al.* 2000) in uncooked tomatoes has been reported. The acidic pH of pericarp tissue is the best natural defence against proliferation of many species of bacteria. However, concern arises when the pericarp is infected with fungi that may cause an increase in pH, enhancing the potential for growth of human bacterial pathogens (Draughon *et al.* 1988). Metabolic activities that cause this increase include a breakdown of proteins to form peptides, amines and ammonia. We have isolated 108 proteolytic moulds and yeasts from 215 decayed or damaged tomatoes (Wade and Beuchat 2003). These fungi caused an upward shift in pH of the radial pericarp tissue from a mean of 5·0 in sound tissue to pH 6·2 in decayed tissue. The pH of commercially processed tomato juice increased by an average of 2·3 units (mean pH 6·4) from an initial pH of 4·1 when juice was inoculated with proteolytic fungi. Other researchers have observed that fungi commonly involved in spoilage of tomatoes are capable of raising the pH of tomato juice and tissue to levels that enable *Clostridium botulinum* to grow (Huhtanen *et al.* 1976; Mundt 1978; Odlaug and Pflug 1979; Draughon *et al.* 1988).

The objective of this study was to determine the behavior of *Salmonella* in raw, ripe tomatoes co-infected with proteolytic moulds. Survival and growth of *Salmonella* as affected by co-infection of the radial pericarp tissue of sound (not chill injured) and chill-injured tomatoes were studied.

MATERIALS AND METHODS

Preparation of inocula for sound (not chill-injured) and chill-injured tomatoes

Five serotypes of *S. enterica* (Baildon, Enteritidis, Michigan, Montevideo and Typhimurium DT104, a multi-drug resistant strain) were cultured in 10 ml of tryptic soya broth (TSB) (BBL/Difco, Sparks, MD, USA) supplemented with nalidixic acid (50 μg ml^{-1}) (Sigma Chemical Co., St Louis, MO, USA) (TSBN) at 37°C for 24 h. Cultures were transferred to TSBN by loop inoculation at three successive 24 h intervals. Cells were collected by centrifugation (2000 *g*, 15 min) and then resuspended in 5 ml of sterile deionized water. Suspensions were combined and serially diluted in sterile deionized water to give two inoculum populations, one with twice the number of CFU per millilitre than the other.

Four moulds shown to have proteolytic activity, as evidenced by hydrolysis of gelatin and/or casein (Wade and Beuchat 2003), were examined. Three of the moulds (*Cladosporium herbarum*, *C. cladosporioides* and *Alternaria alternata* 1a2A) were isolated from raw, ripe tomatoes (Wade and Beuchat 2003) and one mould (*A. alternata* ATCC 34958), isolated from sorghum, was obtained from Dr V. Tournas at the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC. Moulds grown on dichloran rose bengal chloramphenicol (DRBC) agar (BBL/Difco) at 25°C for 5 days were streaked onto malt extract agar (BBL/Difco) in 100 mm diameter Petri plates and incubated upright at 25°C for 4 days. Ten millilitres of sterile 0·1% peptone water was deposited on the surface of colonies, which were then gently rubbed with a sterile, bent glass rod. The suspension containing conidia and mycelial fragments was deposited in a sterile screw-capped test tube. This suspension (inoculum) was diluted (1 : 1, v : v) in sterile deionized water to prepare a second inoculum containing half of the number of CFU per millilitre.

Preparation of inocula for temperature differential studies

An experiment was performed to determine if the number of *Salmonella* adhering to or infiltrating tomatoes is influenced by a difference in temperature of tomatoes and cell suspension in which they are immersed. The same five *Salmonella* serotypes used to inoculate sound and chill-injured tomatoes were grown in TSBN at 37°C for 24 h. Ten millilitres of each culture were added to 5 l sterile deionized water at 4 or 37°C and thoroughly mixed. These suspensions were used as inocula for three types of raw, ripe tomatoes.

Preparation of tomatoes for pericarp inoculation studies

Raw, ripe tomatoes (*Lycopersicon esculentum* Mill. 'Roma') were obtained from three produce vendors in Griffin, GA, USA, in the Spring of 2002. Tomatoes (*ca* 2 kg) were surface sanitized by immersing in 3 l of 0·05 M potassium phosphate buffer (pH 6·8) containing 200 μg ml^{-1} free chlorine. The source of sodium hypochlorite used to prepare the solution was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, USA. Tomatoes were gently agitated for 1 min, then rinsed in 3 l of deionized water for 1 min, drained, and placed in a laminar hood at 21°C for 2 h to dry. Sets of five tomatoes were placed in disinfected 1·7 or 2·4 l plastic tubs (Rubbermaid, Wooster, OH, USA), covered with a plastic film (S.C. Johnson and Son, Inc., Racine, WI, USA), and stored at 15 or 25°C for 24 h before inoculating with *Salmonella* and/or mould.

In a second experiment, survival and growth of moulds and *Salmonella* inoculated into chill-injured tomatoes was studied. Tomatoes were stored at 4 or 15°C (control) for 13 days, then at 25°C for 24 h before sanitizing and inoculating with *Salmonella* and/or moulds.

Preparation of tomatoes for temperature differential studies

Three types of tomatoes (120 ± 5 g each) were used: round, vine-ripened; round, not vine-ripened; and 'Roma' variety (oblong), not vine-ripened. Tomatoes that were not vine-ripened were ripened postharvest before offering for sale at the retail level. All three types of tomatoes at a red ripe stage of maturity were purchased at supermarkets in Griffin, GA, in the Spring of 2002. Stems were attached to vine-ripened tomatoes but had been detached from the tomatoes that were not vine-ripened at the time of purchase. Tomatoes were not surface sanitized in the laboratory. Portions of each type of tomatoes were held at 4 or 37°C for 16–18 h before immersing into suspensions of salmonellae at 4 or 37°C, using a full-factorial experimental design.

Inoculation of pericarp of sound (not chill-injured) and chill-injured tomatoes with *Salmonella* and/or mould

Five vine-ripened 'Roma' tomatoes in each tub were inoculated with *Salmonella* and/or mould at three separate locations 20 mm apart using a sterile 100-μl syringe (Becton Dickinson, Franklin Lakes, NJ, USA) at a depth of 5 mm below the surface of the skin. The first site of inoculation was 20 mm array from the stem scar tissue. The second and third sites of inoculation were 2 cm apart at locations progressing toward the blossom end. Five inoculation schemes were used:

(i) Salmonella *only*: Tomatoes were inoculated with 20 μl of *Salmonella* suspension (day 0).

(ii) *Mould only*: Tomatoes were inoculated with 20 μl of mould suspension (day 0). Each of the four proteolytic moulds was inoculated into separate tomatoes.

(iii) Salmonella *and mould*: Tomatoes were inoculated at the same site with 10 μl of *Salmonella* suspension containing twice the number of CFU per millilitre of suspension used to inoculate tomatoes with *Salmonella* only and 10 μl mould suspension containing twice the number of CFU per millilitre used to inoculate tomatoes with either *Salmonella* only or mould only.

(iv) Salmonella *and then mould 3 days later*: Tomatoes were inoculated with 10 μl of a *Salmonella* suspension containing twice the number of CFU per millilitre used to inoculate with *Salmonella* only (day 0) (i). After storing tomatoes for 3 days at 15 or 25°C, they were inoculated at the same site with 10 μl of a mould suspension containing twice the number of CFU per millilitre used to inoculate tomatoes with mould only (day 0) (ii).

(v) *Mould and then* Salmonella *3 days later*: Tomatoes were inoculated with 10 μl of a mould suspension containing twice the number of CFU per millilitre used to inoculate with mould only (day 0) (ii). Tomatoes were then stored for 3 days at 15 or 25°C before inoculating at the same site with 10 μl of a *Salmonella* suspension containing twice the number of CFU per millilitre used to inoculate tomatoes with *Salmonella* only (i).

All sound (not chill injured) tomatoes inoculated with *Salmonella* and/or mould were stored at 15°C or 25°C for up to 10 days before analyzing for populations and/or presence of *Salmonella* and presence of moulds.

In another series of studies, tomatoes were chill injured before inoculating with *Salmonella* and/or mould. Only *C. herbarum* and *A. alternata* 1a2A were included in these studies. Tomatoes were stored at 4°C or 15°C (control) for 13 days, then held at 25°C for 24 h before inoculating using the same scheme described for sound (not chill injured) tomatoes. Inoculated tomatoes were stored at 25°C for up to 10 days before subjecting to microbiological analyses.

Inoculation of tomatoes in temperature differential studies

Sets of five tomatoes at 4 or 37°C were immersed into 5050 ml of a five-serotype suspension of *S. enterica* at 4 or 37°C. A full-factorial experimental design was used. Tomatoes were gently agitated for 2 min, removed from the suspension, and surface dried (stem-end down) on a screen in a laminar flow biosafety hood for 3 h. Each tomato was

then placed into a 454 ml plastic bag and stored for 18–20 h before analyzing for populations and presence of *Salmonella* on the surface and in the stem scar tissue.

Microbiological analyses

Sound and chill-injured tomatoes. Inoculated tomatoes were analysed for populations/presence of *Salmonella* and the presence of test mould within 30 min of inoculation (0 day) and at 1- to 4-day intervals of storage at 15 and 25°C (sound tomatoes) or 25°C (chill-injured tomatoes).

At each sampling time, a 2 g sample [1·5 cm (surface diameter), 1·2 cm deep] of skin and radial pericarp tissue at the site of inoculation was removed with a sterile, stainless steel scoopula. The pH of the tissue at each inoculated site, as well as uninoculated tissue, was measured using a 5-mm glass bodied microprobe (Denver Instruments, Arvada, CO, USA). The diameter of visibly decayed tissue on the surface of each tomato was measured using a dial caliper. To determine populations of *Salmonella*, each sample was placed in a stomacher 80 bag (Seward Medical Ltd, London, UK), 18 ml of 0·05 M potassium phosphate buffer (pH 6·8 ± 0·1) was added, and the mixture was pummelled at medium speed for 2 min. Stomachates were surface plated (0·25 ml in quadruplicate and 0·1 ml in duplicate) on tryptic soya agar (BBL/Difco) supplemented with nalidixic acid (50 μg ml^{-1}) (TSAN) and on xylose lysine desoxycholate (XLD) agar (BBL/Difco). Samples (0·1 ml in duplicate) serially diluted in 0·1% peptone were also surface plated on TSAN and XLD agar. Presumptive *Salmonella* colonies formed on plates incubated at 37°C for 24 h were counted. Selected colonies were subjected to latex agglutination and API 20E biochemical assays (bioMérieux and Vitek, Hazelwood, MO, USA) for confirmation. The presence of test mould was confirmed by streaking the stomachate on duplicate DRBC plates. Plates were incubated upright at 25°C for 5 days before examining for colonies of test mould.

Double-strength lactose broth (BBL/Difco) (20 ml) supplemented with nalidixic acid (100 μg ml^{-1}) (LBN) was added to the stomachate, mixed and incubated at 37°C for 24 h. If no presumptive *Salmonella* colonies appeared on TSAN and/or XLD plates, further enrichment steps were carrried out. One loopful of sample enriched in LBN was transferred to 10 ml of selenite cystine broth (BBL/Difco). Bismuth sulphite agar (BBL/Difco) supplemented with nalidixic acid (50 μg ml^{-1}) (BSAN) was streaked with enriched sample. Presumptive *Salmonella* colonies were subjected to latex agglutination and API 20E assays. If presumptive *Salmonella* was not detected, one loopful of selenite cystine broth containing the sample was streaked onto BSAN. Presumptive colonies formed on BSAN after incubation at 37°C for 24 h were inoculated into triple sugar iron agar (BBL/Difco) and lysine iron (LI) agar (BBL/Difco) and incubated at 35°C for 24 h. Development of colonies typical in appearance to that of *Salmonella* was recorded as positive for the pathogen.

Temperature differential studies. Tomatoes inoculated with *Salmonella* in temperature differential studies were analysed for populations and presence of the pathogen. A portion (*ca* 6 g) of the stem scar tissue (core area) penetrating *ca* 1·5 cm into each tomato was removed with a sterile stainless steel scalpel and placed in a stomacher 80 bag. The tissue was mascerated by applying pressure before adding 20 ml of sterile 0·1% peptone water and pummelling for 1 min. Samples of undiluted and diluted stomachate were surface plated on TSAN and BSAN and enriched in LBN as described for analysis of sound and chill-injured tomatoes. Confirmation of presumptive colonies was also as described above.

The surface of the tomatoes from which the stem scar tissue has been removed was analysed for populations and the presence of *Salmonella*. Tomatoes were placed into bags in which they had been stored for 18–20 h. Twenty millilitres of sterile 0·1% peptone was deposited in the bag. Bags were sealed and tomatoes were rubbed by hand for

Table 1 Populations of *Salmonella* and moulds in suspensions used to inoculate tomatoes

	Population ($\log_{10}$ CFU in 10 or 20 μl of inoculum)*			
	Sound tomatoes†		Chilled tomatoes‡	
Inocula§	Day 0¶	Day 3	Day 0	Day 3
Salmonella	2·9	3·4	2·6	2·2
C. herbarum	5·1	5·1	4·7	5·1
Salmonella	2·6	3·4		
C. cladosporioides	4·9	4·4		
Salmonella	2·8	3·4		
A. alternata 34958	2·1	2·6		
Salmonella	2·8	3·4	2·6	3·0
A. alternata 1a2A	2·4	2·7	2·5	2·7

*Tomatoes inoculated only with *Salmonella* or mould were injected with 20 μl of suspension; tomatoes inoculated with both *Salmonella* and mould were injected with 10 μl of each suspension.

†Tomatoes were stored at 25°C for 24 h prior to inoculation, then at 15 or 25°C for up to 10 days after inoculation.

‡Tomatoes were stored at 4 or 15°C (control) for 13 days, then at 25°C for 24 h prior to inoculation and storage at 25°C for up to 10 days; tomatoes were not inoculated with *Cladosporium cladosporioides* or *Alternata alternata* 34958.

§See Materials and methods for description of the inoculation scheme.

¶Day of inoculation.

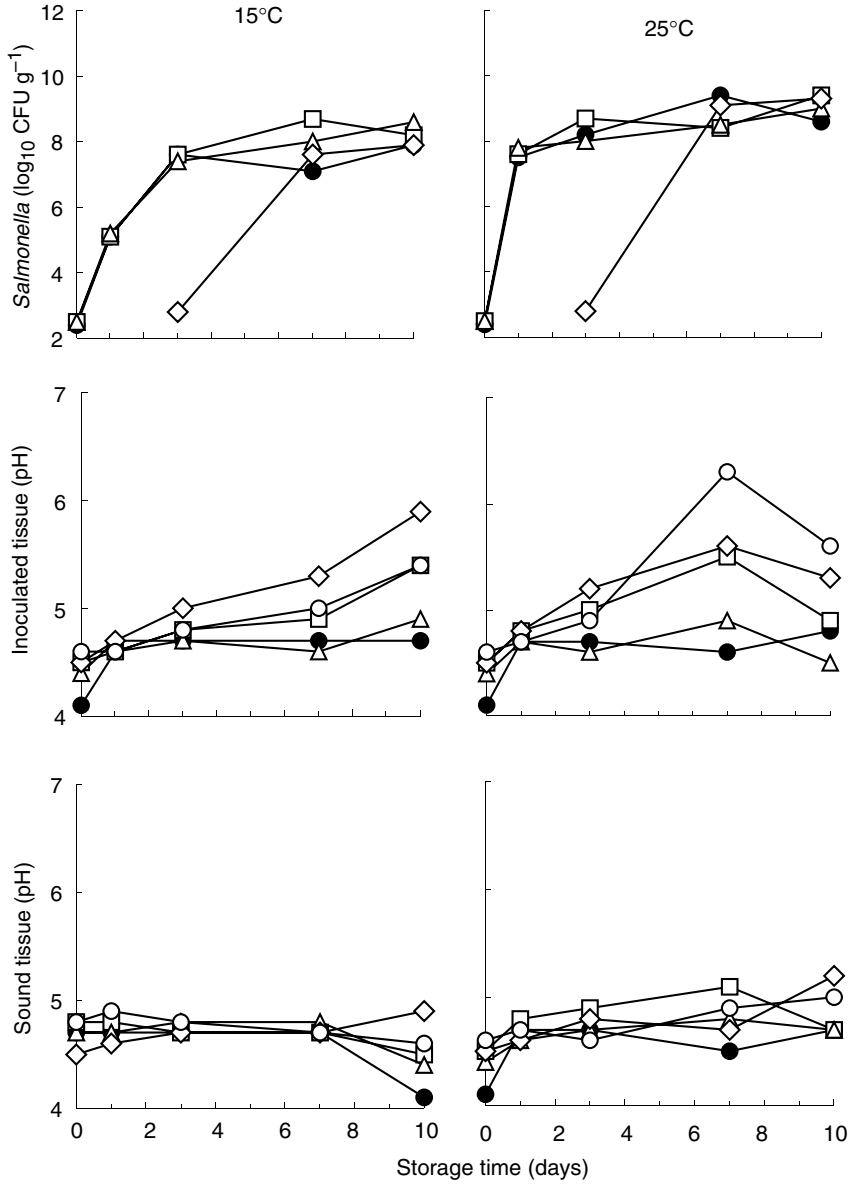

Fig. 1 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of sound and inoculated pericarp tissue of inoculated sound (not chill-injured) tomatoes stored at 15 and 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Cladosporium herbarum* on day 0 (○), *Salmonella* and *C. herbarum* on day 0 (□), *Salmonella* on day 0, then *C. herbarum* on day 3 (△), and *C. herbarum* on day 0, then *Salmonella* on day 3 (◇)

30 s. The peptone wash water was analysed for populations and the presence of *Salmonella* as described above for stem scar tissue.

Measurement of electrolyte leakage

Radial pericarp tissue of uninoculated tomatoes stored at 4 or 15°C for 13 days was analysed for leakage of electrolytes to assess the degree of chill injury. After chill treatment, tomatoes were stored for 0, 1, 3 and 7 days at 25°C before analysis. Six plugs (1·5 cm diameter, 1·2 cm deep) of pericarp tissue from each tomato were removed using a stainless steel scoopula. Excess locular tissue, juice and seeds were removed by washing with a gentle stream of distilled water for 30 s. Each plug was blotted with a paper towel, placed in a 250 ml Erlenmeyer flask containing 100 ml of 0·4 M mannitol solution (Sigma), and gently agitated on a platform shaker for 3 h at 21°C. The conductivity (μ siemens cm^{-1}) of the solution was measured with a conductivity meter (Accument AR50; Fisher Scientific, Fair Lawn, NJ, USA). The amount of electrolytes leached from plugs was determined by measuring the conductivity of the solution (21°C) before and after autoclaving at 121°C for 30 min. The percentage of electrolyte leakage (conductivity of solution after 3 h at 21°C divided by the conductivity of solution after 30 min

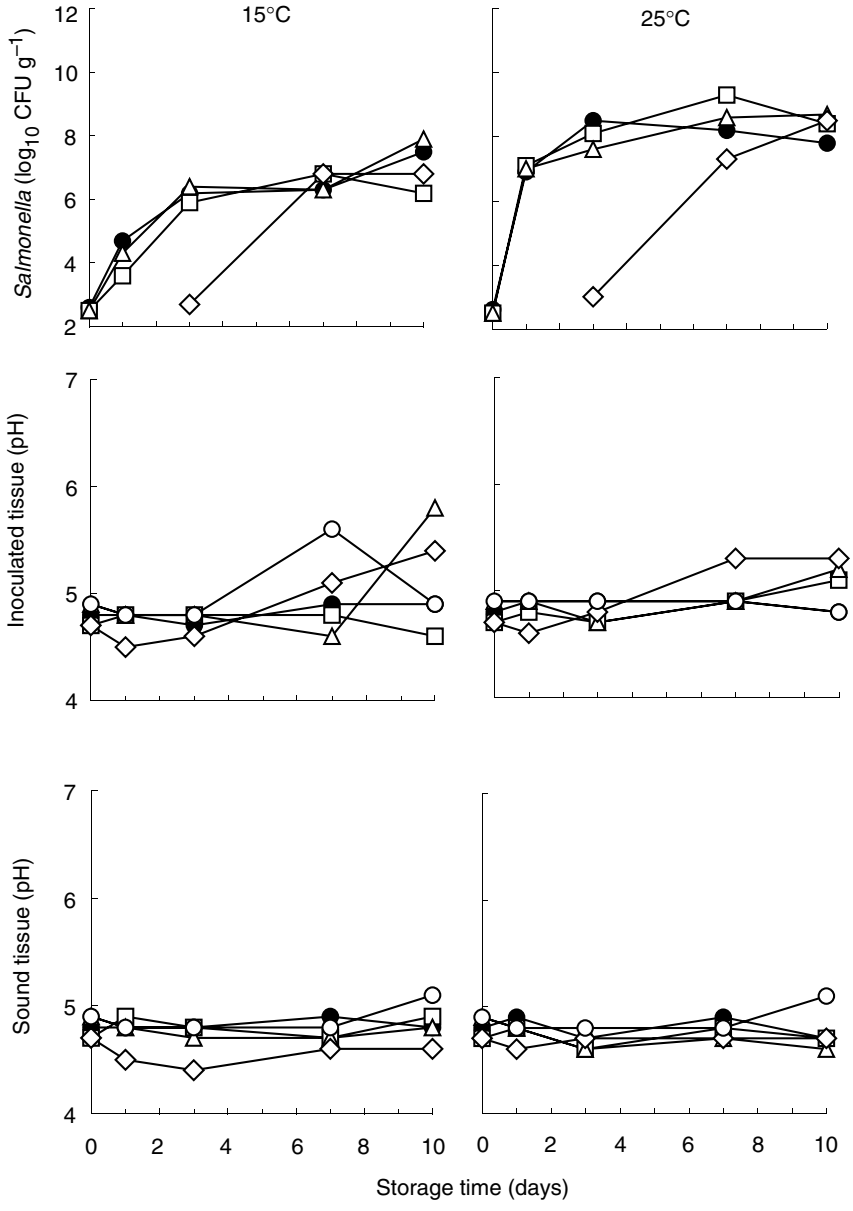

Fig. 2 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of sound and inoculated pericarp tissue of inoculated sound (not chill-injured) tomatoes stored at 15 and 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Cladosporium cladosporioides* on day 0 (○), *Salmonella* and *C. cladosporioides* on day 0 (□), *Salmonella* on day 0, then *C. cladosporioides* on day 3 (△), and *C. cladosporioides* on day 0, then *Salmonella* on day 3 (◇)

at 121°C) was calculated to assess the extent of chill injury (King and Ludford 1983).

Statistical analysis

All experiments were replicated three or four times. Each replicate consisted of five tomatoes analysed at each sampling time. Data were analysed using the general linear model procedure of the Statistical Analysis Software (SAS Institute, Cary, NC, USA). Significant differences ($\alpha = 0{\cdot}05$) between mean values were determined using Duncan's multiple range test.

RESULTS

Recovery on TSAN vs XLD agar and BSAN

Populations of *Salmonella* recovered on TSAN were consistently and often significantly ($\alpha = 0{\cdot}05$) higher than populations recovered on XLD agar and BSAN (data not shown). The lower counts on XLD agar and BSAN are attributed to the inability of stressed cells to resuscitate and form colonies when exposed to selective chemicals in these media. Only counts obtained by plating samples on TSAN are reported in the following tables and figures.

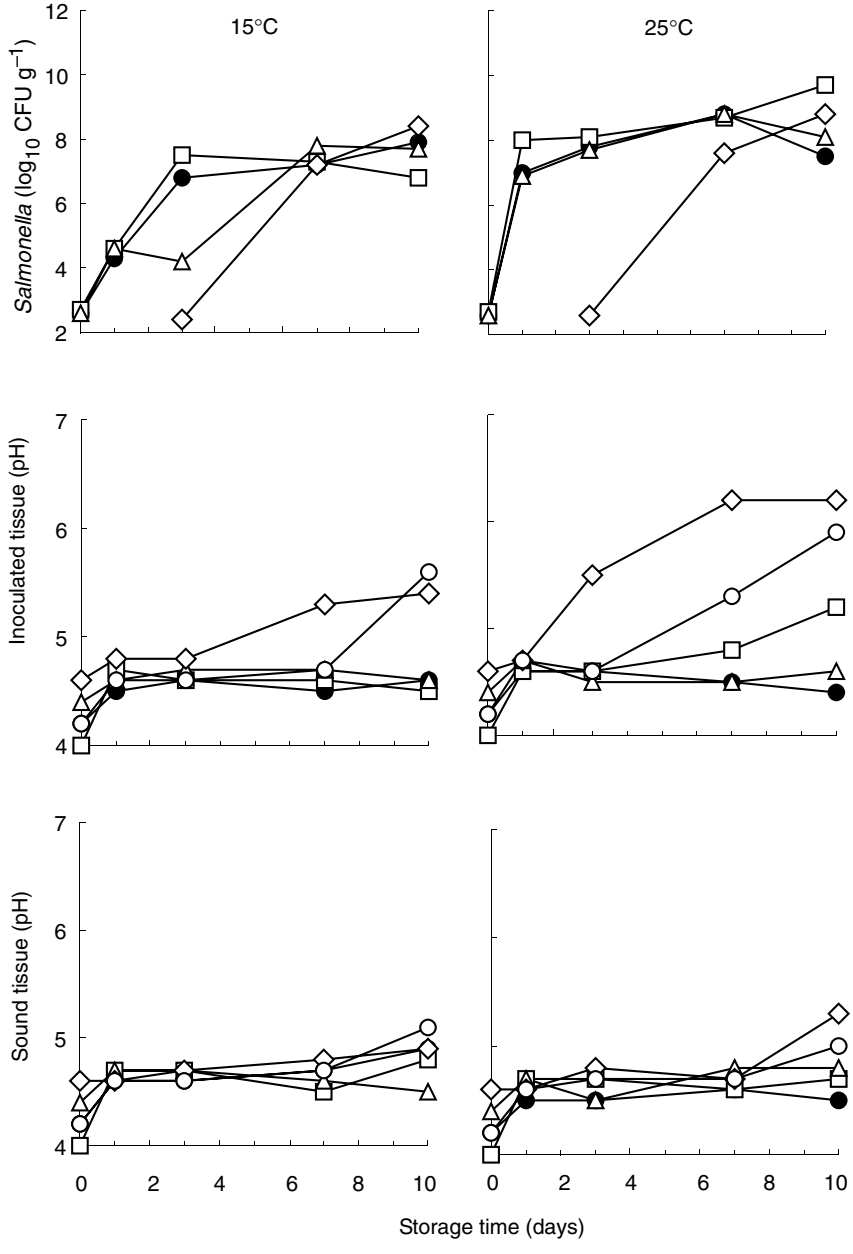

Fig. 3 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of sound and inoculated pericarp tissue of inoculated sound (not chill-injured) tomatoes stored at 15 and 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Alternata alternata* 34958 on day 0 (○), *Salmonella* and *A. alternata* 34958 on day 0 (□), *Salmonella* on day 0, then *A. alternata* 34958 on day 3 (△), and *A. Alternata* 34958 on day 0, then *Salmonella* on day 3 (◇)

Populations and presence of *Salmonella* and moulds inoculated into sound- (not chill-injured) and chill-injured tomatoes

Populations of *Salmonella* in suspensions (10 or 20 μl) inoculated into sound (not chill-injured) tomatoes on days 0 and 3 were 2·6–3·3 and 3·4–3·6 $\log_{10}$ CFU, respectively (Table 1). Approximately equal populations of each of the five serotypes were in each suspension. Decreases in populations ranging from 0·6 to 1·9 $\log_{10}$ CFU g^{-1} of the 2 g sample within 30 min (day 0) of inoculation of pericarp tissue are attributed to stress imposed by the acidic pH of pericarp. Populations in mould suspensions (10 or 20 μl) used to inoculate sound tomatoes varied in the range of 2·1 $\log_{10}$ CFU of *A. alternata* to 5·1 $\log_{10}$ CFU of *C. herbarum*.

Populations of *Salmonella* in 10 or 20 μl of suspension used to inoculate chill-injured tomatoes on days 0 and 3 were 2·5–2·6 and 2·2–3·0 $\log_{10}$ CFU, respectively (Table 1). Populations of moulds in suspensions (10 or 20 μl) used to inoculate tomatoes ranged from of 2·5 $\log_{10}$ CFU of *A. alternata* 1a2A to 5·1 $\log_{10}$ CFU of *C. herbarum*.

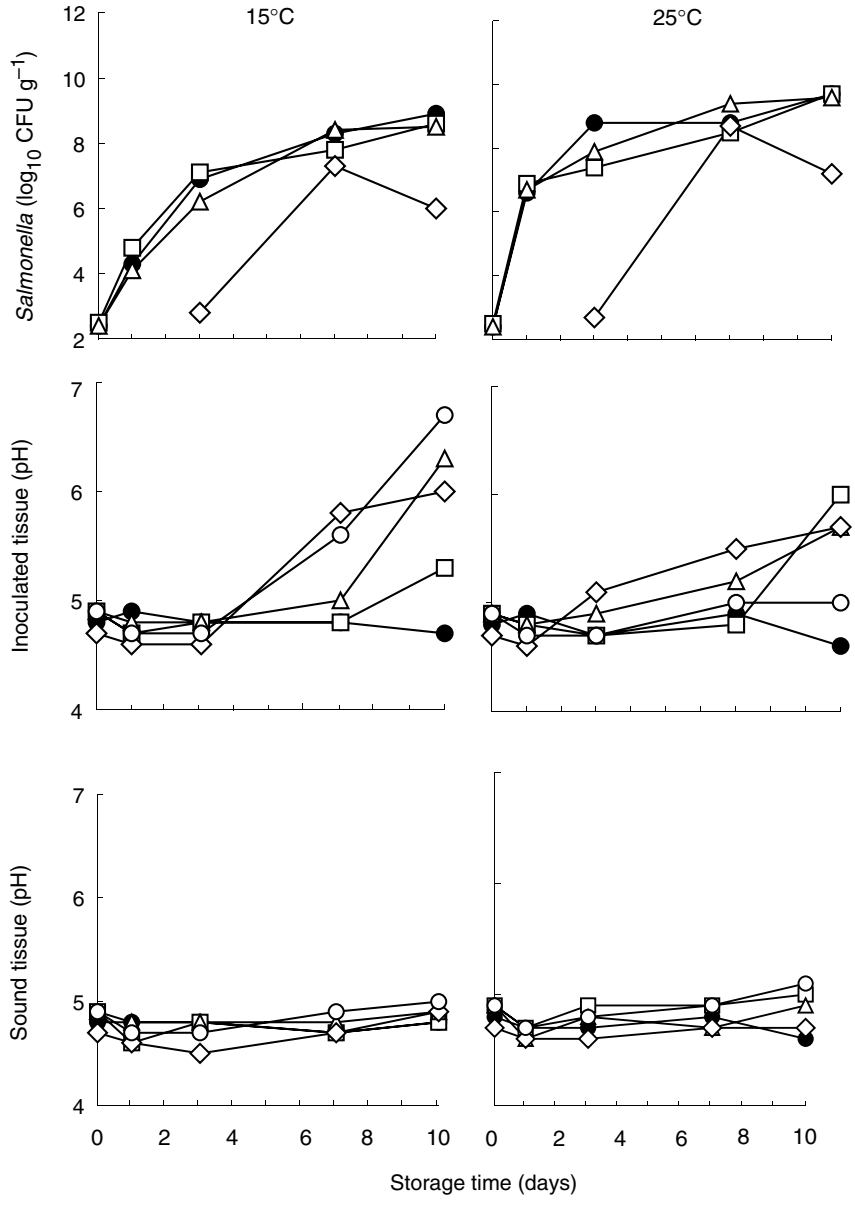

Fig. 4 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of sound and inoculated pericarp tissue of inoculated sound (not chill-injured) tomatoes stored at 15 and 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Alternata alternata* 1a2A on day 0 (○), *Salmonella* and *A. alternata* 1a2A on day 0 (□), *Salmonella* on day 0, then *A. alternata* 1a2A on day 3 (△), and *A. alternata* 1a2A on day 0, then *Salmonella* on day 3 (◇)

Populations of *Salmonella* detected in inoculated (not chill-injured) tomatoes

Changes in populations of *Salmonella* and pH of inoculated and sound tissues of sound tomatoes inoculated with *Salmonella*, *C. herbarium*, or both *Salmonella* and *C. herbarum* are shown in Fig. 1. Sound tomatoes inoculated with only *Salmonella* on day 0 supported more rapid growth at 25°C than at 15°C, with populations increasing significantly ($\alpha = 0{\cdot}05$) by 6·8 and 4·7 $\log_{10}$ CFU g^{-1}, respectively, within 7 days. Tomatoes inoculated with *Salmonella* and *C. herbarum* on day 0 and tomatoes inoculated with *Salmonella* on day 0, then *C. herbarum* on day 3, increased by 6·2 and 6·1 $\log_{10}$ CFU of *Salmonella* per gram, respectively, within 7 days at 15°C storage. Tomatoes inoculated with both *Salmonella* and *C. herbarum* supported growth of *Salmonella* to higher populations at 25°C than at 15°C.

Increases in the pH of inoculated tissues were smallest in tomatoes inoculated with only *Salmonella* on day 0 and in tomatoes inoculated with *Salmonella* on day 0, then *C. herbarum* on day 3, both at 15 and 25°C storage (Fig. 1). Tomatoes inoculated with *C. herbarum* on day 0, then *Salmonella* on day 3 showed the greatest increase in pH when storage was at 15°C, reaching pH 5·9 on day 10. At 25°C, pericarp tissue inoculated with only *C. herbarum* on day 0 had the highest pH (6·3) after 7 days of storage. There

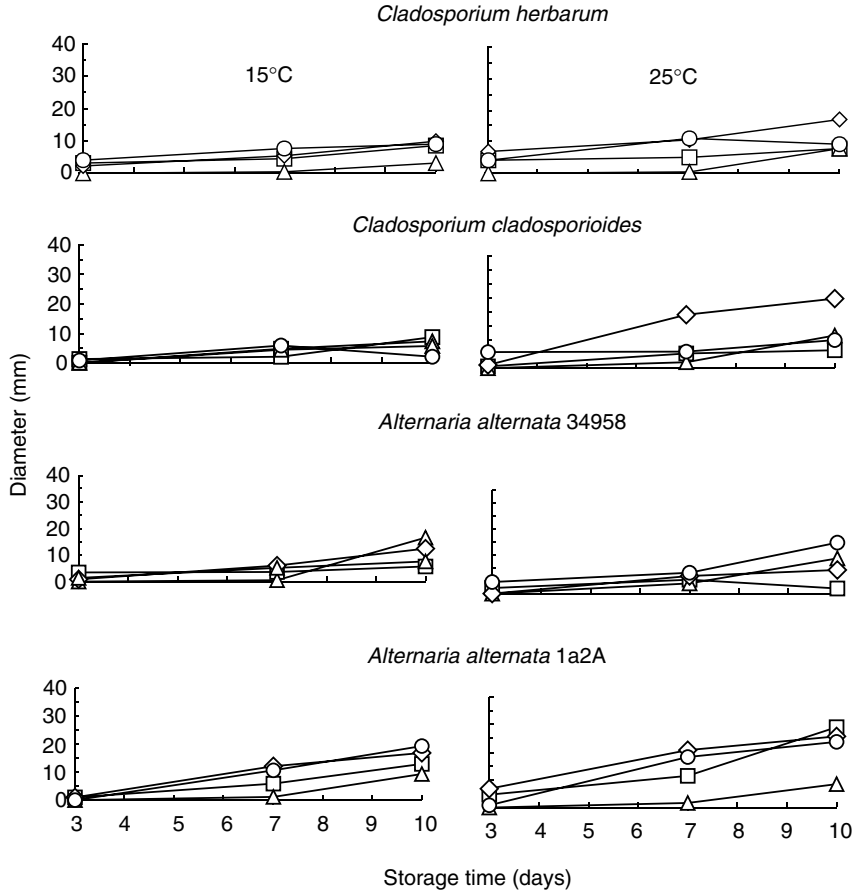

Fig. 5 Diameter (mm) of decayed pericarp tissue on the surface of sound (not chill-injured) tomatoes inoculated with *Cladosporium herbarum*, *C. cladosporioides*, *A. alternata* 34958, and *A. alternata* 1a2A and stored at 25°C for up to 10 days. Tomatoes were inoculated with mould on day 0 (○), *Salmonella* and mould on day 0 (□), *Salmonella* on day 0, then mould on day 3 (△), and mould on day 0, then *Salmonella* on day 0 (◇)

was an increase of pH in tissues co-infected with *Salmonella* and *C. herbarum* when tomatoes were stored at 15 or 25°C, although increases were most pronounced at 25°C.

Changes in populations of *Salmonella* in inoculated and sound tissues of sound tomatoes inoculated with *Salmonella*, *C. cladosporioides*, or both *Salmonella* and *C. cladosporioides* are shown in Fig. 2. At 15°C, increases in populations of *Salmonella* followed a similar trend, regardless of inoculation sequence, growing up to 4·3 $\log_{10}$ CFU g^{-1} within 7 days. At 25°C, *Salmonella* reached the highest population, 6·8 $\log_{10}$ CFU g^{-1} within 7 days in tomatoes inoculated with *Salmonella* and *C. cladosporioides* on day 0. Compared with tomatoes inoculated with *Salmonella* and *C. herbarum* (Fig. 1), populations of *Salmonella* in tomatoes co-infected with *Salmonella* and *C. cladosporioides* were generally lower.

The pH of tomato pulp inoculated with only *C. cladosporioides* on day 0 increased to 5·8 within 7 days at 15°C (Fig. 2). Unlike the other proteolytic moulds tested, tomatoes co-infected with *Salmonella* and *C. cladosporioides* did not reach higher pH levels at 25°C compared with 15°C.

Changes in populations of *Salmonella* in inoculated and sound tissues of sound tomatoes inoculated with *Salmonella*, *A. alternata* 34958, or both *Salmonella* and *A. alternata* 34958 are shown in Fig. 3. At 15°C, tomatoes were inoculated with *A. alternata* 34958 on day 0, then *Salmonella* on day 3 supported the highest population of *Salmonella* within 7 days. At 25°C, *Salmonella* populations, regardless of inoculation scheme, were similar within 10 days.

The highest pH values were observed in pericarp tissue inoculated with only *A. alternata* 34958 on day 0 and in tissue inoculated with *A. alternata* 34958 on day 0 and *Salmonella* on day 3, reaching pH 5·6 and 5·4, respectively, at 15°C and 5·9 and 6·2, respectively, at 25°C. There was an increase in pH of pericarp tissue inoculated with both *Salmonella* and *A. alternata* 34958 and stored at 15 or 25°C, although increases were most pronounced at 25°C.

Changes in populations of *Salmonella* and pH of inoculated and sound tissues of sound tomatoes inoculated with *Salmonella*, *A. alternata* 1a2A, or both *Salmonella* and *A. alternata* 1a2A are shown in Fig. 4. Growth patterns of *Salmonella* at 15 and 25°C were similar, regardless of inoculation scheme, with the exception of tomatoes inoculated with *A. alternata* 1a2A on day 0, then *Salmonella* on day 3. Populations of *Salmonella* in these tomatoes decreased

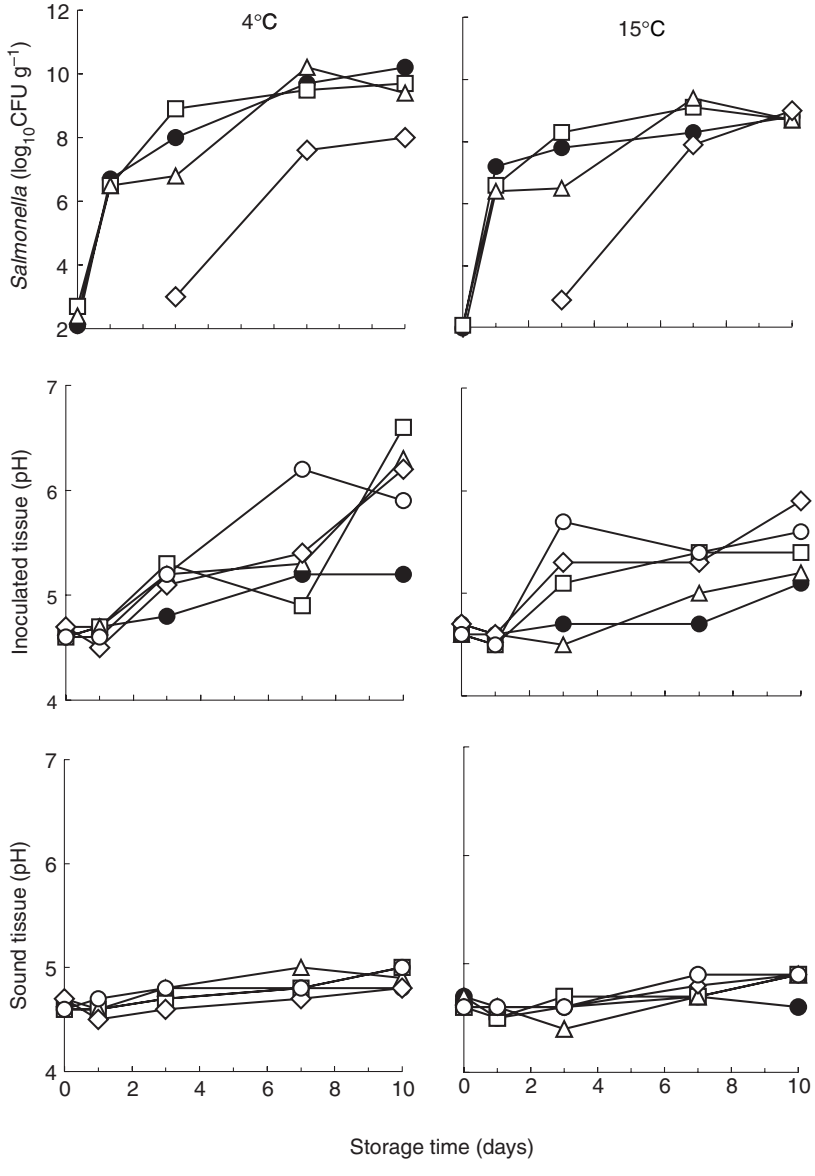

Fig. 6 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of uninoculated (sound) and inoculated pericarp tissue of inoculated, chill-injured tomatoes stored at 4 or 15°C for 13 days, then at 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Cladosporium herbarum* on day 0 (○), *Salmonella* and *C. herbarum* on day 0 (□), *Salmonella* on day 0, then *C. herbarum* on day 3 (△), and *C. herbarum* on day 0, then *Salmonella* on day 3 (◇)

by 1·4 $\log_{10}$ CFU g^{-1} between 4 and 7 days after inoculation. Decreases in populations of *Salmonella* in tomatoes co-infected with *A. alternata* 34958 were also observed in the final days of storage (Fig. 3).

The pH of tissues inoculated with *A. alternata* 1a2A only was highest among tomatoes stored at 15°C, reaching 6·7 within 10 days (Fig. 4). The highest pH in tomatoes stored at 25°C was 6·0, which was observed after 10 days in pericarp tissue inoculated with *Salmonella* and *A. alternata* 1a2A on day 0. The behaviour of *A. alternata* 1a2A differed from that of *A. alternata* 34958 in that it caused greater increases in pH at 25°C than at 15°C.

Shown in Fig. 5 are diameters of decayed areas on the surface of sound tomatoes inoculated with proteolytic moulds and stored at 15 or 25°C for 3, 7 and 10 days. Growth of all moulds was more extensive at 25°C than at 15°C. *Cladosporium herbarum* and *C. cladosporioides* grew slowest among the four test moulds. Growth of the two *A. alternata* strains was most evident in the locular cavity. Diameters of decayed areas were measured on the surface of tomatoes, so comparison of growth of *A. alternata* with other moulds, in terms of biomass produced and extent of decay of pericarp, is difficult.

Populations of *Salmonella* detected in inoculated chill-injured tomatoes

Changes in populations of *Salmonella* and pH of pericarp tissue of chill-injured tomatoes inoculated with *Salmonella*, *C. herbarum*, or both micro-organisms are shown in Fig. 6.

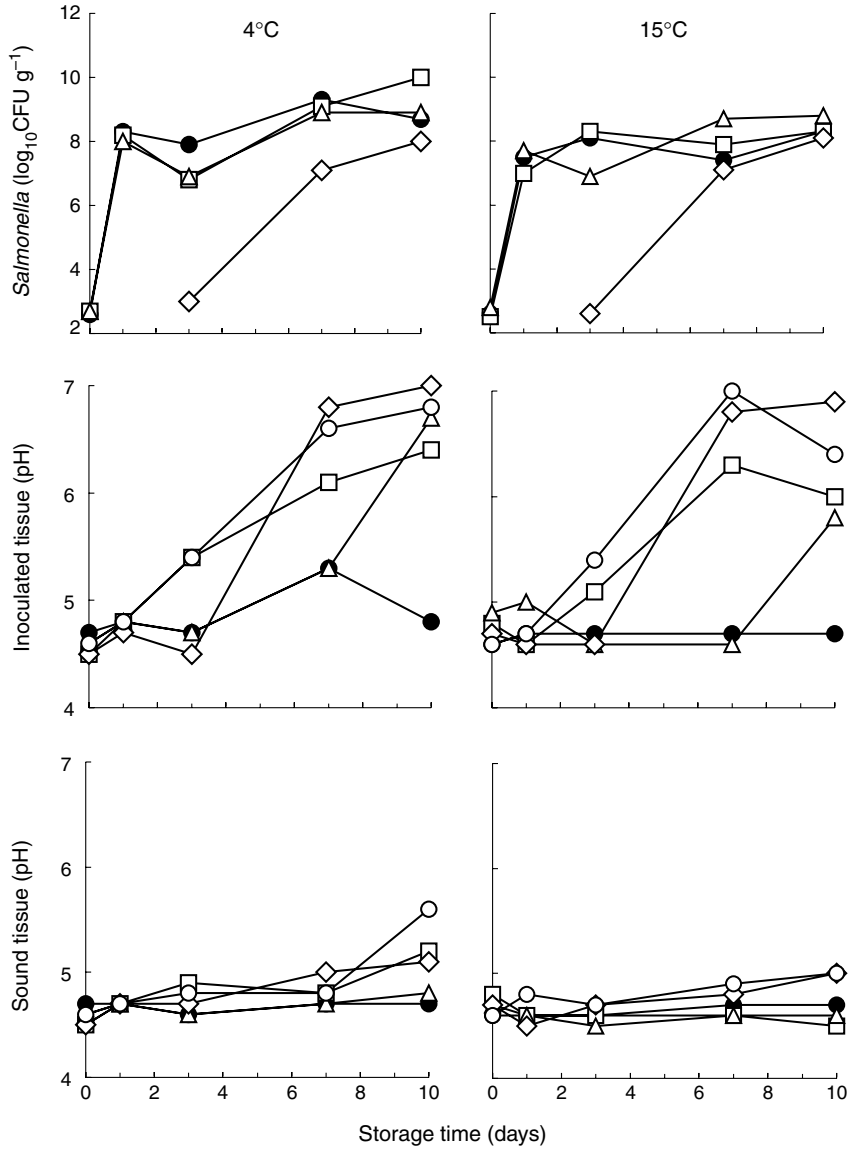

Fig. 7 Populations of *Salmonella* ($\log_{10}$ CFU g^{-1} of tomato sample) and pH of uninoculated (sound) and inoculated pericarp tissue of inoculated, chill-injured tomatoes stored at 4 or 15°C for 13 days, then at 25°C for up to 10 days. Tomatoes were inoculated with *Salmonella* on day 0 (●), *Alternata alternata* 1a2A on day 0 (○), *Salmonella* and *A. alternata* 1a2A on day 0 (□), *Salmonella* on day 0, then *A. alternata* 1a2A on day 3 (△), and *A. alternata* 1a2A on day 0, then *Salmonella* on day 3 (◇)

Salmonella reached higher populations after 7 days in tomatoes that had been chilled for 13 days at 4°C compared with tomatoes held at 15°C and tomatoes that had not been chill injured (Fig. 1). In chill-injured tomatoes co-infected with *Salmonella* and *C. herbarum*, *Salmonella* grew more rapidly in tomatoes that had been chilled at 4°C compared with 15°C, then stored at 25°C for up to 10 days (Fig. 6).

Tomatoes chill-injured at 4°C, then co-inoculated with *Salmonella* and *C. herbarum*, underwent greater increases in pH (up to 6·7) compared with changes in pH of tomatoes that had been held at 15°C (Fig. 6) and the pH of tomatoes that were not held at 4 or 15°C before inoculation (Fig. 1).

Salmonella reached higher populations in tomatoes that had been chilled for 13 days at 4°C than in tomatoes held at 15°C (Fig. 7) or tomatoes that were not held at these temperatures (Fig. 4) when co-inoculated with *A. alternata* 1a2A. Populations of *Salmonella* decreased between 4 and 7 days in sound (not chill-injured) tomatoes inoculated with *A. alternata* 1a2A on day 0 and *Salmonella* on day 3 (Fig. 4). In contrast, populations of *Salmonella* in chill-injured tomatoes subjected to the same inoculation scheme increased during this period (Fig. 7).

Tomatoes co-infected with *Salmonella* and *A. alternata* 1a2A, when previously chilled at 4°C, underwent a greater increase in pH (up to pH 6·9) compared with tomatoes that had been held at 15°C (pH 6·8) (Fig. 7) or sound (not chill-injured) tomatoes (pH 6·7) (Fig. 4).

Figure 8 shows diameters of decayed tissue on the surface of tomatoes that were held at 4 or 15°C for 13 days, then inoculated with *C. herbarum* or *A. alternata* 1a2A and stored

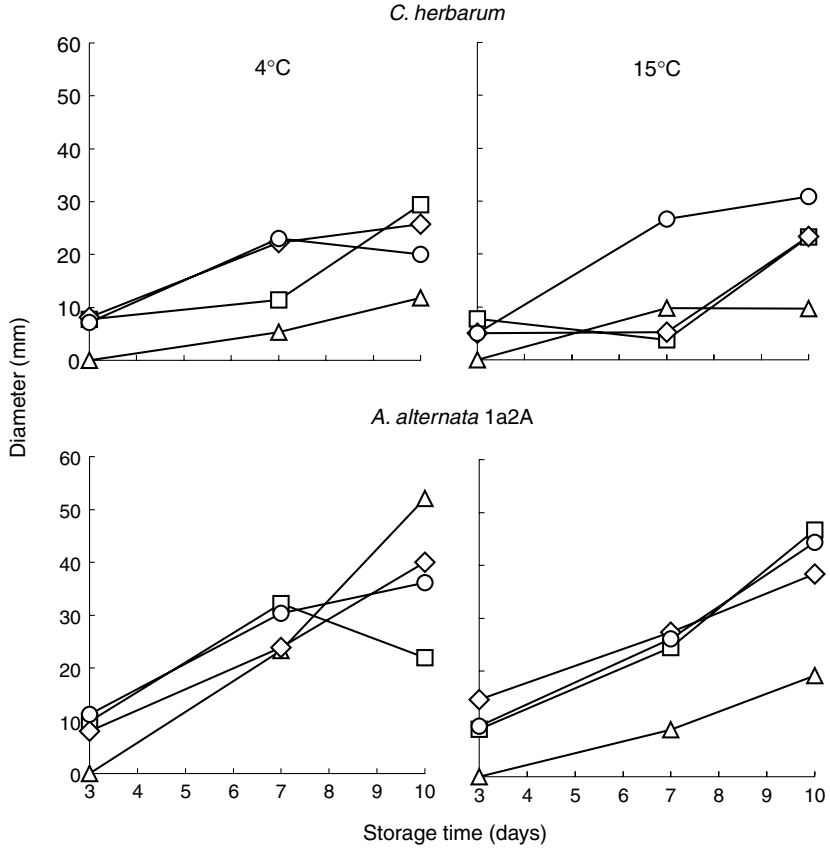

Fig. 8 Diameter (mm) of decayed pericarp tissue on the surface of chill-injured tomatoes inoculated with *C. herbarum*, or *A. alternata* 1a2A, then stored at 25°C for 10 days. Tomatoes were stored at 4 or 15°C for 13 days, then 1 day at 25°C before inoculating with mould on day 0 (○), *Salmonella* and mould on day 0 (□), *Salmonella* on day 0, then mould on day 3 (△), and mould on day 0, then *Salmonella* on day 0 (◇)

at 25°C for up to 10 days. *Cladosporium herbarum* grew slower than *A. alternata*, as evidenced by visual appearance of the external surface of tomatoes. Growth of *A. alternata* 1a2A was observed in the locular cavity. The diameter of decayed tissue was measured on the surface of tomatoes, thereby not necessarily reflecting the amount of mycelial growth at various depths in the pericarp and locular tissues. Diameters of decayed tissue of chill-injured tomatoes inoculated with *C. herbarum* or *A. alternata* were larger than those on sound tomatoes (Fig. 5).

The percentage of electrolytes leaked from tissues of uninoculated tomatoes held at 4°C for 13 days, then at 25°C for up to 7 days, ranged from 18 to 26%, indicating that chill injury results from this treatment.

Populations of *Salmonella* detected on and in tomatoes inoculated in temperature differential studies

Populations of *Salmonella* recovered from the surface and stem scar tissue of tomatoes dip-inoculated under positive and negative temperature differentials are listed in Table 2. Within each type of tomato tested, the temperature of the tomato and the inoculum did not have a significant effect

Table 2 Populations of *Salmonella* on the surface and in stem scar tissue of tomatoes as influenced by difference in temperature of tomatoes and inoculum

	Temp. (°C)		$\log_{10}$ CFU*	
Tomato type†	Tomato	Inoculum	Surface	Scar tissue
Round				
Vine-ripened	4	37	2·01 A	2·04 B
	37	4	1·98 A	2·50 A
Not vine-ripened	4	37	3·87 A	4·29 A
	37	4	4·30 A	4·11 A
Oblong ('Roma')				
Not vine-ripened	4	37	2·80 A	2·69 B
	37	4	2·78 A	3·04 A

*Mean values indicate $\log_{10}$ CFU detected on the entire surface of tomato or $\log_{10}$ CFU g^{-1} of stem scar tissue. Values within the same sample type (surface or scar tissue) and the same tomato type (round, vine-ripened; round, not vine-ripened; or Roma, not vine-ripened) followed by the same letter are not significantly different ($\alpha = 0{\cdot}05$).
†Tomatoes were harvested when red ripe (vine-ripened) or mature green (not vine-ripened); the latter type was ripened (postharvest) before using in experiments.

($\alpha = 0{\cdot}05$) on the number of *Salmonella* adhering to the surface of the fruit. A negative temperature differential, i.e. the temperature of the tomato was higher (37°C) than the temperature of the inoculum (4°C), however, resulted in significantly higher numbers of *Salmonella* infiltrating the stem scar tissue of round, vine-ripened tomatoes and Roma tomatoes that were not vine-ripened, but not round tomatoes that were not vine-ripened, compared with tomatoes inoculated under a positive temperature differential.

DISCUSSION

Awareness of the ability of salmonellae to grow in uncooked, diced tomatoes is not new. Asplund and Nurmi (1991) reported that *S. enterica* serotypes Enteritidis, Infantis and Typhimurium grew in tomatoes stored at 22 or 30°C for 24 h. The pH of tomatoes before inoculation varied from 3·99 to 4·37. The population of *S.* Montevideo increased significantly in inoculated chopped tomatoes (initial pH $4{\cdot}1 \pm 0{\cdot}1$) stored for 4 days or 22 h at 20 or 30°C, respectively (Zhuang *et al.* 1995). An initial population of 0·79 $\log_{10}$ CFU g^{-1} of diced tomatoes (pH 4·4) increased to 5·3 $\log_{10}$ CFU g^{-1} during storage at 21°C for 24 h (Weissinger *et al.* 2000). Results of our study show that growth of salmonellae is promoted in tomato pulp co-infected with moulds often responsible for preharvest and postharvest decay. *Alternaria* and *Cladosporium* rots are often associated with chill-injured tomatoes and other solanaceous fruits (Dennis 1983; Snowdon 1991). Our study shows that growth of salmonellae is enhanced in pulp of chill-injured tomatoes co-infected with these moulds.

Treatment of raw fruits and vegetables with chlorine or other sanitizers cannot be relied upon for elimination of human pathogens (Beuchat 1998). Reductions of pathogens (CFU g^{-1} or CFU cm^{-2}) on treated produce are generally in the range of 10- to 100-fold. The efficacy of sanitizer would be expected to decrease when cells of pathogens grow and are enmeshed in tomato pulp tissue, a situation promoted by growth of moulds. Thus, preventing pre and postharvest fungal decay of tomato is critical to minimizing microbiological safety risks at every step of production and postharvest handling of tomatoes. Removal of decayed tomatoes during processing and preparation in foodservice and home settings from those intended for consumption without cooking is also an essential intervention to reduce safety risks.

Huhtanen *et al.* (1976) reported that growth of *Cladosporium* and *Penicillium* species in tomato juice caused an increase in the pH, facilitating germination of *C. botulinum* spores, growth and toxin production. Odlaug and Pflug (1979) observed a similar phenomenon using *Aspergillus gracilis* as a test mould. Growth of *Fusarium*, *Alternaria* and *Rhizoctonia* co-inoculated with *C. botulinum* in fresh tomatoes increases the pH of healthy (sound) tissues ($4{\cdot}4 \pm 0{\cdot}1$) to levels as high as 8·0 (Draughon *et al.* 1988). Macerates of tomatoes inoculated with *C. botulinum* and *Alternaria* or *Fusarium* were toxic to mice. Hotchkiss *et al.* (1992) reported that growth of *Alternaria* on raw tomato stored under modified and controlled atmospheres at 13 or 23°C increased the pH to >4·6 but concluded that failure of *C. botulinum* to grow and produce toxin before extreme spoilage indicates the lack of a significant risk of botulism. Our results indicate that co-infection of uncooked tomatoes with *Salmonella* and *Cladosporium* or *Alternaria* promotes the growth of the bacterium, thereby increasing the risk of salmonellosis.

Others have shown that microbial infection of fruits and vegetables affects the survival and growth of bacterial foodborne pathogens. Apple tissue infected with *Glomerella cingulata* supported the growth of *E. coli* O157:H7 (Riordan *et al.* 2000) and *Listeria monocytogenes* (Conway *et al.* 2000). Higher levels of incidence of *Salmonella* have been associated with bacterial soft rot of fresh fruits and vegetables (Wells and Butterfield 1997). These observations, and those on metabiotic associations of *Salmonella* and moulds and enhancement of infiltration of *Salmonella* into stem scar tissue of tomatoes subjected to a negative temperature differential observed in the present study, point out the need to remove decayed fruits and vegetables from lots intended for minimal processing if the risk of diseases associated with consumption of raw produce is to be minimized.

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