Attachment and Colonization by *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *Staphylococcus aureus* on Stone Fruit Surfaces and Survival through a Simulated Commercial Export Chain

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ABSTRACT

The ability of the foodborne pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *Staphylococcus aureus* to attach, colonize, and survive on stone fruit surfaces was investigated. Fifty microliters of bacterial suspension was spot inoculated onto the sterile intact fructoplane of whole peaches and plums. Minimum time required for initial adhesion and attachment was recorded for different surface contact times. Surface colonization patterns of the four pathogens and survival under simulated commercial export conditions also were evaluated. *L. monocytogenes* and *Salmonella* Typhimurium attached immediately to stone fruit surfaces. *E. coli* O157:H7 and *S. aureus* were visibly attached after 30 s and 1 h, respectively, of direct exposure. Holding freshly harvested stone fruit at 0.5°C to simulate cold storage conditions significantly lowered the titer of *E. coli* O157:H7 on plums and the titers of *L. monocytogenes* and *Salmonella* Typhimurium on stone fruit. *E. coli* O157:H7 and *L. monocytogenes* at a low inoculum level and *S. aureus* and *Salmonella* Typhimurium at high and low levels did not survive the simulated export chain conditions at titers that exceeded the minimum infectious dose. However, *E. coli* O157:H7 and *L. monocytogenes* were able to survive on stone fruit surfaces when inoculated at an artificially high level. In this case, the final titer at the end of the supply chain was higher than the infectious dose. In this laboratory experiment, *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* at potential natural contamination levels were unable to survive simulated export conditions.

The number of human disease outbreaks associated with foodborne pathogens has increased globally, and this change has been linked to increased consumption of contaminated fresh produce *(51)*. The increase in the reported number of outbreaks can be attributed to a various factors, including the shifting focus toward healthier lifestyles and diets in more developed countries. Because of the demand for year-round availability of fresh fruit and vegetables and more exotic produce, these products often are procured from less developed countries with less effectively regulated food control systems. These global procurement patterns have resulted in more extensive supply chains, ultimately involving more complex distribution networks and longer road and sea transit times. More complex distribution systems in turn require more handling. *Staphylococcus aureus* is transmitted through food handlers and therefore is an important consideration in any food safety implementation system. *S. aureus* also has been linked to foodborne outbreaks throughout the world. Foodborne illnesses associated with the consumption of contaminated cantaloupe, cut fruit, strawberries, raspberries, tomatoes, spinach, lettuce, and various other types of fresh produce have been well documented *(8–13, 24, 30, 35, 46)*. Some of the more frequently reported foodborne pathogens associated with fresh produce are *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella (14)*. Both *E. coli* and *Salmonella* have been detected on the surfaces of stone fruits *(1)*.

Microbial contamination of fresh produce can occur within the pre- and postharvest environments. The exposure of fresh produce to contaminated water, handlers, or contact surfaces *(5, 28, 44)* increases the likelihood that foodborne pathogens can successfully attach to the fructoplane. Contamination should therefore be avoided by implementing pre- and postharvest production and distribution practices that prevent contamination. Fresh produce that is traded through extensive supply chains also are exposed to several possible contamination points after leaving the farm. Contamination could therefore occur at any point from the farm and packing house up to the point of handling and consumption within the importing country *(31)*.

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Environmental conditions during transit of the fresh product are important because they can either support microbial growth and survival or contribute to the death of these organisms, thereby either increasing or reducing the food safety risk. Stringent controls at the point of production and dispatch are required to ensure that foodborne pathogens are not introduced into the food chain. When contamination is possible, intervention strategies should be implemented to ensure that the organism cannot survive or proliferate up to the point of consumption. Effective cold chain management systems can prevent the proliferation of foodborne pathogens on fresh produce surfaces. *E. coli, L. monocytogenes, Salmonella,* and *S. aureus* are able to survive refrigeration temperatures, and *L. monocytogenes* is able to grow at the temperatures *(2, 16, 19, 54).*

Adherence, attachment, colonization, and survival of foodborne pathogens on raw fresh produce is a critical element in the fruit contamination cycle *(32).* Understanding the stages of the organisms' contamination cycle will allow the establishment of better prevention strategies within the pre- and postharvest environment. The plant surface and the bacterial cell both have a negative charge *(53).* Adhesion is a physiochemical process that occurs when a bacterial cell is able to overcome the repulsive forces between the cell and the plant surface *(22, 53).* Adhered cells are able to detach if repulsive forces become greater than attractive forces *(22).* Adhered cells become attached by means of exopolysaccharides. Once attached, cells are able to replicate to form microcolonies *(37),* which promote survival. For foodborne pathogens to survive on the plant's phyllo- or fructoplane, the bacteria must transport and utilize available nutrients *(43).* Fett *(20)* suggested that human pathogens may become incorporated into phylloplane biofilms. Microorganims found on the phylloplane often are found in biofilms, which buffer environmental fluctuations *(38, 40, 41).* These biofilms often are associated with sources of nutrients such as the leaf vein and trichomes *(40).*

The aim of this study was to acquire a better understanding of the potential of *E. coli* O157:H7, *L. monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *S. aureus* to adhere, attach, colonize, and survive on stone fruit. Time and temperature exposure conditions that simulated harvesting, packing, transport, cold storage, and export conditions used to retain fruit quality, control decay, and extend shelf life were used to determine the likelihood of foodborne pathogens survival on stone fruit surfaces.

MATERIALS AND METHODS

Cultures. American Type Culture Collection (ATCC) cultures of *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), and *S. aureus* (ATCC 12600) were used as reference cultures in this study. All cultures were maintained lyophilized and stored at −70°C, and subcultures were grown on standard 1 medium (Merck, Johannesburg, South Africa) prepared 24 h before use. Cultures were used to inoculate five replicates of 100 ml of tryptone soy broth (Merck) for each pathogen and were subsequently incubated at 37°C for 18 h to achieve 8 log CFU/ml. Cultures were centrifuged at 2,200 × *g,* washed twice with sterile distilled water, and then resuspended in 1% (wt/vol) peptone buffered water (Merck). Cultures were then serially diluted to obtain a high inoculum level of 7 log CFU/ml and a low inoculum level of 5 log CFU/ml. Levels were confirmed by serial dilution and subsequent plating in duplicate.

Fruit. Peaches (*Prunus persica* cv. Excellence) and plums (*Prunus domestica* cv. Flavour King) were aseptically hand harvested at optimum maturity from two commercial farms in the North West Province and Limpopo Province, respectively, of South Africa. The full experiment was repeated on two separate occasions. Fruits of a uniform size and weight and without pests, disease, and damage were used in this study. Harvested fruits were bagged in paper bags and transported to the laboratory in cooler boxes and stored at 4°C overnight (approximately 12 to 15 h). Collected fruits were divided into three sets. Set 1 was used for scanning electron microscopy (SEM) analysis and consisted of 22 peaches (7 for each pathogen and 1 negative control) and 37 plums (9 for each pathogen and 1 negative control). Set 2 was used to quantify the pathogen titer following high-level inoculation and consisted of 50 peaches (5 replicates for 9 time intervals selected plus 5 negative controls) and 62 plums (5 replicates for 11 time intervals and 7 negative controls). Set 3 was used to quantify the pathogen titer following low-level inoculation and consisted of 30 peaches (5 replicates for 5 day intervals and 5 negative controls) and 42 plums (5 replicates for 7 day intervals and 7 negative controls). Set 1 fruits for SEM studies were surface sterilized with a 30-s dip treatment in 70% ethanol *(49)* followed by air drying. Fruits from sets 2 and 3 were washed with 0.05% (vol/vol) sodium hypochlorite for 30 s, rinsed twice with sterile distilled water, and allowed to air dry.

Spot inoculation. Spot inoculation for SEM studies was done on a surface area (5 by 5 mm) that was marked with a felt pen; 50 μl of prepared culture was used per pathogen for short time intervals (0, 30, and 60 s and 1 h) to determine attachment and for longer time intervals for peaches (1, 14, 20, and 21 days) and plums (1, 6, 13, 18, 25, and 26 days) using the high-level inoculum (7 log CFU/ml) (Fig. 1). For the attachment studies, the culture was put directly onto the fruit at room temperature and aspirated at the respective time intervals. The inoculated fruit surface area was subsequently rinsed with 100 μl of sterile distilled water on the inoculated section. The rinsate was then aspirated and discarded, and the rinsing process was repeated. The inoculated blocks were then aseptically excised and immediately processed for SEM (set 1). Spot inoculation with using 50 μl of culture for determination of pathogen titers was carried out on five set 2 (high-level inoculum) and five set 3 (low-level inoculum) fruit per short time interval (0, 30, and 60 s and 1 and 2 h) and long time intervals (Fig. 1). Spot inoculation was carried out ensuring that cultures were not mixed. After spot inoculation, the final level of each culture on the fruit was confirmed to be 5 (high) and 3 (low) log CFU per fruit with serial dilutions as described before. The titers of viable *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* remaining on these fruits at the various time intervals was determined. Once inoculated, fruit were divided according to replicates (five each for pathogen titer determination and four each for SEM) and were distributed equally into five containers in five areas of the incubation space to allow for temperature variation within the incubation chamber.

Methodology for quantification of microorganisms. Inoculated set 2 and set 3 fruits (five replicates) were used to quantify

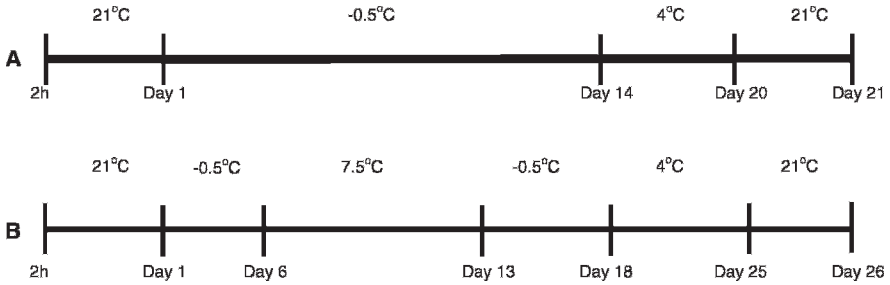

FIGURE 1. *Time regimen for the ship freight export simulation experiment for peaches (A) and plums (B).*

pathogen titers after the various short and long time intervals. Fruits were removed from the incubation cold storage area at the different time intervals and washed to determine the bacterial titer present. Fruits were washed in 500 ml of 0.25× Ringer's solution amended with 0.02% Tween 80 (Sigma, Johannesburg, South Africa) in the Ultrasonic Bath (Labotec, Johannesburg, South Africa) for 30 s. The Ringer's solution was then filtered through a 0.45-nm-pore-size nitrocellulose membrane, and the membrane was used for serial dilution and plating in duplicate onto selective agar specific for the four pathogens: Baird-Parker medium for *S. aureus*, Oxford *Listeria* selective agar for *L. monocytogenes,* Levine eosin–methyl blue agar for *E. coli* O157:H7, and xylose lysine deoxycholate agar for *Salmonella* Typhimurium (all supplied by Merck). Volume displaced (vd) for each fruit was recorded and converted to area (square centimeters) with the following equation *(17)*:

$$A = 4.84\left[(\mathrm{vd})^{1/3}\right]^2$$

Counts were converted to CFU per square centimeter and transformed to $\log(x + 1)$ CFU/cm^2.

SEM evaluation. Marked and inoculated sections of set 1 fruits were used for SEM evaluation. The uninoculated fruits served as negative controls. Excised sections were stored in 1 ml of fixing solution, which consisted of 1 ml of 25% gluteraldehyde in 0.075 M phosphate buffer mixed according to the method of Coetzee and Van der Merwe *(15)* with a modification of 25% formaldehyde. Samples were stored for a maximum of 1 month. Samples were rinsed three times in 0.075 M phosphate buffer for 15 min each, followed by successive 15-min dehydration rinses in 50, 70, and 90% ethanol and finally three rinses in 100% ethanol. Samples were critical point dried in a Bio-Rad dryer (Bio-Rad, Polaron Equipment, Watford, Hertfordshire, UK) under liquid carbon dioxide. After drying, samples were mounted with nonconductive tape and coated for 2.5 min with 10 mÅ of gold-palladium (Polaron Equipment) and examined under a scanning electron microscope (JSM-840, JEOL Ltd., Tokyo, Japan) operating at 5 or 8 kV. Negative controls were examined first to become familiar with the fructoplane. The next samples examined were those inoculated with the higher titer and allowed to grow for longer time intervals (21 or 26 days) to determine the orientation and size of the bacterial pathogens. The rest of the samples were then examined systematically through all samples from the longest exposure times (highest titers) to the shortest exposure times (lowest titers). Cells were counted on 15 randomly selected areas per sample at ×3,000 magnification. The following equation was generated to calculate the number of pathogen cells per square centimeter of fruit:

$$\text{cells per cm}^2 = (c)(a/y)^{-1}$$

where c is the number of pathogen cells counted, a is the area of the SEM viewing section, and y is the average area per fruit (111.15 cm^2) as determined in the present study (described previously).

Observations were made for 15 areas per sample on each stub to determine adhesion, attachment, replication, colonization, and survival of the organisms on the fruit surfaces. Observations were subsequently transformed into a percentage of observations per sample viewed (i.e., the frequency). Thus, the attachment frequency is the percentage of observed attachment, and the replication frequency is the percentage of observed multiplication.

Statistical analysis. All experiments were repeated. Results obtained for each replicate were analyzed together, for a total of 10 replicates. Statistical analysis was performed on log CFU per square centimeter and log cells per square centimeter. Data were analyzed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC). A one-way analysis of variance was used to determine the difference in pathogen titers on fruit surfaces. Means were analyzed using the least significant difference (using the Fisher test) at a 5% level of significance.

RESULTS

Surface characteristics. The uninoculated control samples examined revealed the fruit surface characteristics, i.e., trichomes, lenticels, wax structures, and epidermal corrugation. Examination of 420 areas revealed that the peach surfaces were extensively covered with trichomes (Fig. 2A). Because of this high incidence of trichomes on this specific cultivar, other surface characteristics were difficult to discern. The peach surface did not appear to have many lenticels. Examination of 540 plum areas revealed a smooth surface without much corrugation and with lenticels and smooth wax plates (Fig. 3A). No microorganisms were found by SEM examination of the control fructoplane, indicating that the ethanol surface sterilization process was successful due to toxic activity on resident microflora *(49)*. The morphological characteristics of the bacterial pathogens on the fruit surfaces were observed for fruits from the high-level inoculation group. Morphological characteristics were consistent for all bacterial pathogens viewed at the simulated longest exposure time period.

All four pathogens preferentially attached to the trichomes on peaches (Fig. 2) and were evenly distributed over the trichome. On plums, all pathogens were able to attach to the smooth surface of the fruit but preferentially attached to areas near lenticels.

Initial adhesion. Initial adhesion was defined as the first time interval for which microbial counts (CFU per square centimeter) for the high- and low-level inoculum groups were obtained and when cells (not attached with

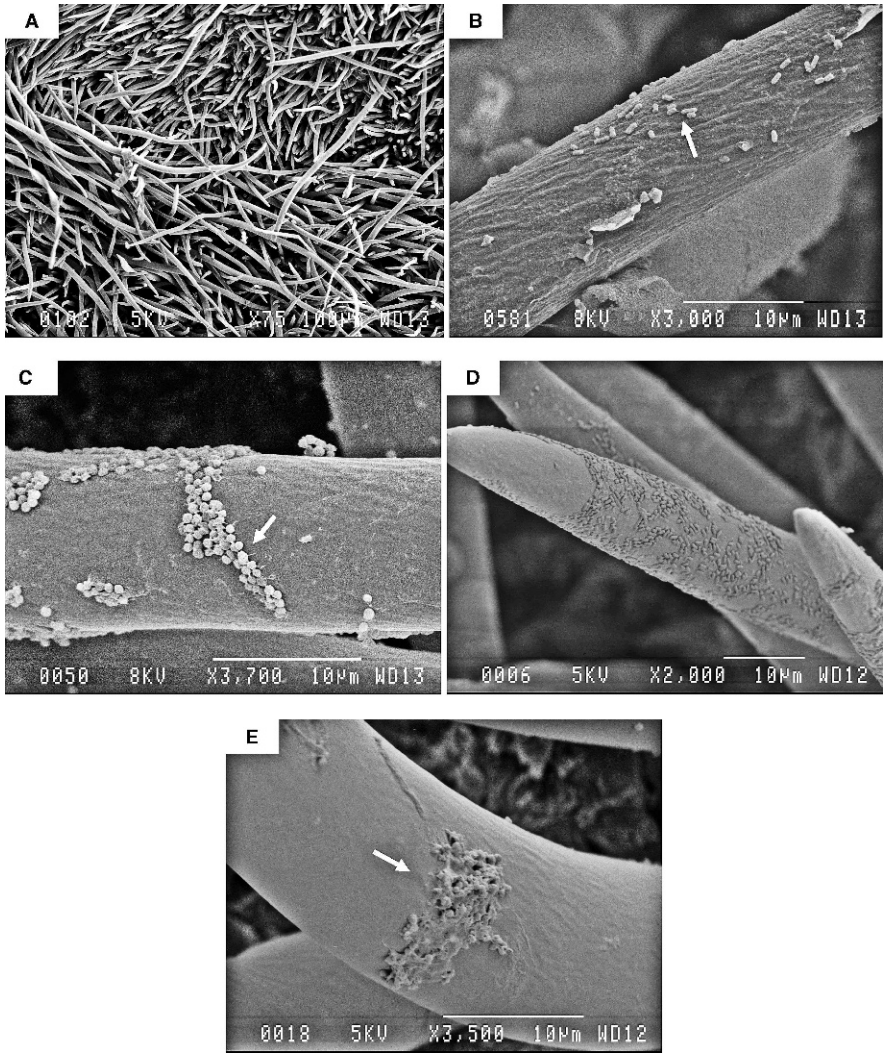

FIGURE 2. *Electron micrographs of foodborne bacterial pathogens on peaches. (A) Trichomes on the surface of peaches (×75 magnification). Arrows indicate the point of attachment with exopolysaccharides by (B)* Listeria monocytogenes *(×3,000 magnification) and (C)* Staphylococcus aureus *(×3,700 magnification). (D) Microcolony formation of* L. monocytogenes *after 1 h on a peach trichome (×2,000 magnification). (E) Microcolony formation of* L. monocytogenes *is indicated with arrows (×3,500 magnification).*

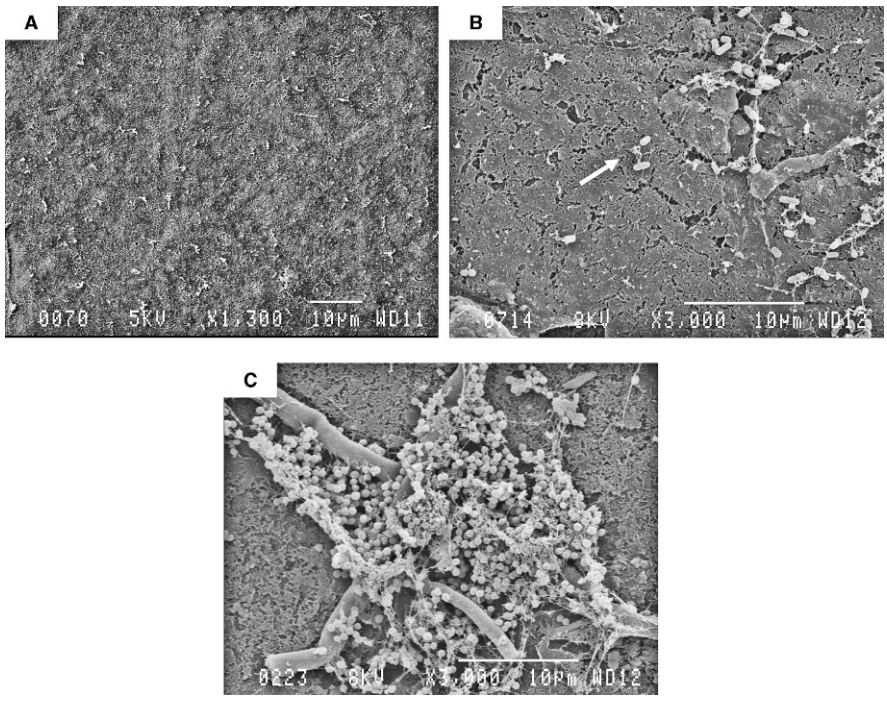

FIGURE 3. *Electron micrographs of foodborne bacterial pathogens on plums. (A) Smooth surface of plums (×1,300 magnification). (B) Arrow indicates the point of attachment with exopolysaccharides of* Salmonella enterica *subsp*. enterica *serovar Typhimurium (×3,000 magnification). (C) Extensive* Staphylococcus aureus *microcolony formation (×3,000 magnification) after 26 days of the export cold chain.*

TABLE 1. *Shortest time required for adhesion and attachment of the high-level inoculum of* Escherichia coli *O157:H7,* Listeria monocytogenes, Salmonella *Typhimurium, and* Staphylococcus aureus *to stone fruits*

Pathogen	Adhesion time		Attachment time	
	Peaches	Plums	Peaches	Plums
Escherichia coli O157:H7	30 s	0 s	60 s	60 s
Listeria monocytogenes	60 s	0 s	1 h	30 s
Salmonella Typhimurium	0 s	0 s	1 h	30 s
Staphylococcus aureus	0 s	0 s	21 days	1 h

polysaccharides) were first observed on the fruit by SEM. Adherence of *Salmonella* Typhimurium and *S. aureus* occurred immediately after inoculation on both fruits, whereas on peaches *E. coli* O157:H7 adhered within 30 s and *L. monocytogenes* adhered within 60 s. For both of these pathogens adherence to plums was immediately (Table 1). High numbers of *S. aureus* cells were observed by SEM immediately after inoculation and at 2 h postinoculation, and colony counts were high on both peaches and plums, indicating the ability of *S. aureus* to adhere to stone fruit when inoculated at both high and low levels (Tables 2 and 3). All four test organisms were able to adhere to stone fruit when inoculated at the low level but had different viable counts initially (Tables 2 and 3).

Attachment. Attachment of bacteria was defined in this study as the organism's ability to produce exopolysaccharide structures. The first attachment of *E. coli* O157:H7 due to exopolysaccharides on peaches and plums was observed after 60 s of exposure (Tables 1 through 3). The first attachment of *L. monocytogenes* and *Salmonella* Typhimurium was observed on plums 30 s postinoculation (Tables 1 and 3 and Fig. 3B) and on peaches 1 h postinoculation (Tables 1 and 2 and Fig. 2B). *S. aureus* was able to visibly attach to the plum surface via attachment structures at 1 h postinoculation (Tables 1 and 3) and to peaches at 21 days postinoculation (Tables 1 and 3 and Fig. 2C). Organisms were able to attach more effectively to the plum than to the peach surface (Tables 1 through 3).

Colonization. For the purpose of this study, colonization was defined as reproduction of the organism on the fruit surface and the formation of extensive attachment structures. Over time, the amount and frequency of exopolysaccharide production by *E. coli, L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* increased on both types of fruit (Tables 2 and 3). Most notable colonization with attachment structures was observed for *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* on the peach surface toward the end of the fruit export chain (Tables 2 and 3). After 21 days, *L. monocytogenes* and *S. aureus* formed microcolonies (Figs. 2E, 3B, and 3C). No *E. coli* O157:H7 replication was observed on the peach surface between 30 s and 1 h (Table 2), and there was no significant difference in the number of cells or CFU per square centimeter recovered from the peaches (Table 2). However, on plums *E. coli* O157:H7 replication was observed by SEM at 60 s and 1 h postinoculation (Table 3), and a significant increase in CFU per square centimeter was noted from 30 s to 1 h (Table 3). *L. monocytogenes* occurred more prominently on peaches at 1 h postinoculation than at 30 and 60 s (Table 2), and the observed replication frequency was 20% at the 1-h time interval (Table 2). A significant increase in the *L. monocytogenes* counts (cells and CFU per square centimeter) was observed on plums from 30 s to 1 h and from 30 s to 1 day, respectively (Table 3), and replication was observed 1 h postinoculation (Table 3 and Fig. 2D). *Salmonella* Typhimurium significantly increased on peaches and plums from 30 s to 1 day postinoculation (Tables 2 and 3), with the highest observed replication occurring 1 h postinoculation on both fruits (Tables 2 and 3). No significant difference was found in *S. aureus* cell counts on peaches, even though a significant increase was observed in CFU per square centimeter (Table 2). No *S. aureus* replication was found on peaches or plums during these time intervals (Tables 2 and 3). An overall increase in *S. aureus* per square centimeter was observed from 30 s to 1 day postinoculation on both fruits (Tables 2 and 3).

Pathogen survival. Survival was defined in this study as the ability of the organism to survive on the fructoplane throughout the simulated export chain. The 0.5°C incubation period of 1 to 13 days had no significant effect on *E. coli* O157:H7 numbers on the peach surface. However, a significant increase was observed following 4°C storage for 13 to 20 days, with no significant difference between 20 and 21 days (Table 2) even though replication was observed (Table 2). No significant difference was seen in *E. coli* O157:H7 titers when inoculated at the low level on plums (Table 3). However, a significant difference was observed in *E. coli* O157:H7 titer on plum surfaces inoculated at the high level and exposed to 0.5°C for 1 to 6 days (Table 3). The number of cells per square centimeter observed on the plum surface by SEM increased slightly but not significantly, as confirmed by consistent replication of *E. coli* O157:H7 from 30 s to 25 days (Table 3). A significant decrease in *L. monocytogenes* and *Salmonella* Typhimurium was observed between 1 and 6 days (at 0.5°C) on both peaches and plums, with no significant difference observed later in the simulated cold chain, reflecting equilibrium reached where the replication and extinction rates were similar (Tables 2 and 3). *S. aureus* titers did not reflect a significant decrease on peaches (Table 2), and no replication was observed by SEM (Table 2). On plums, there was an overall decrease in *S. aureus* numbers with the most significant decrease occurring 13 days after inoculation (Table 3).

DISCUSSION

The attachment of foodborne pathogens to surfaces of various types of fresh produce has not been widely studied

TABLE 2. *Scanning electron microscopy examination results and total foodborne pathogen counts in the simulated peach export chain experiment*

Pathogen	Time	Attachment frequency (%)[a]	Replication frequency (%)[b]	Log counts[c] SEM (x + 1 cells/cm^2)	Log counts[c] High (x + 1 CFU/cm^2)	Log counts[c] Low (x + 1 CFU/cm^2)
E. coli O157:H7	0 s[d]	0.00	0.00	0.00 D	1.81 BC	NI[e]
	30 s	0.00	0.00	0.49 CD	1.66 BC	NI
	60 s	6.67	0.00	1.58 CD	2.03 ABC	NI
	1 h	6.67	0.00	1.47 CD	2.47 AB	NI
	2 h[f]	NI	NI	NI	3.21 A	0.51 AB
	1 day	NI	NI	NI	2.02 ABC	0.00 B
	13 days	0.00	0.00	2.18 BC	0.96 C	0.00 B
	20 days	6.67	6.67	3.99 B	2.23 AB	1.03 A
	21 days	33.33	6.67	5.91 A	1.86 BC	0.07 B
L. monocytogenes	0 s[d]	0.00	0.00	0.00 CD	2.41 BC	NI
	30 s	0.00	0.00	0.00 C	2.11 BC	NI
	60 s	0.00	0.00	4.10 B	2.49 BC	NI
	1 h	13.33	20.00	6.11 B	3.82 A	NI
	2 h[f]	NI	NI	NI	3.74 A	2.30 A
	1 day	NI	NI	NI	2.34 BC	0.58 BC
	13 days	0.00	20.00	6.40 A	1.61 C	0.07 C
	20 days	26.67	33.33	6.66 A	1.88 BC	0.81 B
	21 days	6.67	20.00	6.39 A	3.09 AB	0.99 B
Salmonella Typhimurium	0 s[d]	0.00	0.00	0.49 C	0.54 AB	NI
	30 s	0.00	0.00	0.52 C	0.52 AB	NI
	60 s	0.00	6.67	4.10 B	0.87 AB	NI
	1 h	20.00	13.33	6.11 A	1.53 A	NI
	2 h[f]	NI	NI	NI	1.43 A	0.31 A
	1 day	NI	NI	NI	0.88 AB	0.00 A
	13 days	0.00	0.00	6.40 A	0.23 B	0.01 A
	20 days	0.00	6.67	6.71 A	0.75 B	0.04 A
	21 days	40.00	26.67	6.26 A	0.38 B	0.23 A
S. aureus	0 s[d]	0.00	0.00	1.60 C	2.77 CD	NI
	30 s	0.00	0.00	4.58 B	2.61 CD	NI
	60 s	0.00	0.00	5.61 AB	2.43 D	NI
	1 h	0.00	0.00	5.98 AB	3.48 BC	NI
	2 h[f]	NI	NI	NI	4.55 A	2.29 A
	1 day	NI	NI	NI	4.19 AB	1.74 A
	13 days	0.00	0.00	6.77 A	4.47 AB	1.61 AB
	20 days	0.00	0.00	1.19 C	3.55 BC	2.21 A
	21 days	20.00	0.00	6.70 A	3.18 CD	0.78 B

[a] Percentage of observed attachment.
[b] Percentage of observed multiplication.
[c] Letters indicate the least significant difference according to the Fisher test ($P < 0.05$).
[d] After inoculation, culture was immediately aspirated.
[e] NI, not included. Value was not included because it was not an important consideration within this study.
[f] After initial inoculation and drying for the export chain experiment.

but is of great significance to the food industry *(47)*. Most studies focus on processed fruit in the postharvest environment and not on preharvest contamination or on fruits within the supply chain *(6)*.

Colonization and survival on fresh produce is dependent on the ability of foodborne pathogens to adapt to ecological niches outside the host. Traditionally, foodborne pathogens were of little importance on fresh produce, but recent reports of survival and colonization of *E. coli* and *S. enterica* have provided evidence that contamination with these pathogens might represent a food safety risk *(6)*. Pathogens tested in this study survived on peach and plum surfaces. The promotion of growth due to postharvest treatments allows for the pathogen population to be sustained to levels above the minimum infectious dose.

In this study, *S. aureus* adhered to stone fruit surfaces within 30 s. *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium required 1 min to attach to peach surfaces, and *L. monocytogenes* and *Salmonella* Typhimurium required 1 h to adhere to plum surfaces. In general, adhesion occurred more rapidly on peach than on plum surfaces. Solomon and Matthews *(47)* found that heat-killed

TABLE 3. *Scanning electron microscopy examination results and total foodborne pathogen counts in the simulated plum export chain experiment*

Pathogen	Time	Attachment frequency (%)[a]	Replication frequency (%)[b]	Log counts[c]		
				SEM (x + 1 cells/cm^2)	High (x + 1 CFU/cm^2)	Low (x + 1 CFU/cm^2)
E. coli O157:H7	0 s[d]	0.00	6.67	0.33 DE	0.57 CD	NI[e]
	30 s	0.00	0.00	0.57 DE	0.65 CD	NI
	60 s	33.30	33.33	2.75 B	2.08 AB	NI
	1 h	46.67	20.00	4.22 A	2.90 A	NI
	2 h[f]	NI	NI	NI	2.68 AB	0.74 A
	1 day	0.00	6.67	NI	2.01 B	0.12 A
	6 days	NI	NI	0.34 DE	0.43 CD	0.18 A
	13 days	33.33	6.67	1.29 CD	0.05 CD	0.07 A
	18 days	6.67	13.33	2.33 BC	0.10 CD	0.16 A
	25 days	80.00	26.67	3.05 B	0.92 C	0.57 A
	27 days	0.00	0.00	0.00 E	0.03 D	0.13 A
L. monocytogenes	0 s[d]	0.00	0.00	1.29 BC	0.71 FG	NI
	30 s	6.67	0.00	0.77 C	1.62 DE	NI
	60 s	0.00	0.00	2.36 B	2.45 CD	NI
	1 h	13.33	66.67	3.93 A	3.62 AB	NI
	2 h[f]	NI	NI	NI	3.04 BC	0.60 B
	1 day	NI	NI	NI	4.19 A	1.70 A
	6 days	20.00	33.33	3.90 A	1.41 EF	0.13 C
	13 days	60.00	20.00	4.83 A	1.07 EF	0.12 C
	18 days	93.33	13.33	4.75 A	1.05 EF	0.10 C
	25 days	53.33	6.67	4.15 A	0.09 G	0.02 C
	27 days	26.67	6.67	1.69 BC	0.69 FG	0.03 C
Salmonella	0 s[d]	0.00	0.00	0.94 C	2.08 A	NI
Typhimurium	30 s	20.00	26.67	3.20 AB	1.96 A	NI
	60 s	0.00	0.00	2.19 BC	2.10 A	NI
	1 h	33.33	40.00	3.08 AB	2.36 A	NI
	2 h[f]	NI	NI	NI	2.40 A	0.49 A
	1 day	NI	NI	NI	2.81 A	0.22 A
	6 days	20.00	0.00	2.33 ABC	0.76 B	0.04 A
	13 days	0.00	0.00	2.58 AB	0.93 B	0.06 A
	18 days	40.00	13.33	3.70 A	2.08 B	0.00 A
	25 days	6.67	0.00	2.19 BC	0.61 B	0.00 A
	27 days	33.33	20.00	2.41 AB	0.11 B	0.11 A
S. aureus	0 s[d]	0.00	0.00	3.50 DEF	1.85 D	NI
	30 s	0.00	0.00	2.98 F	2.20 D	NI
	60 s	0.00	0.00	3.27 EF	2.19 D	NI
	1 h	13.33	0.00	4.69 BC	3.64 BC	NI
	2 h[f]	NI	NI	NI	4.54 AB	2.51 A
	1 day	NI	NI	NI	4.97 A	1.93 AB
	6 days	100.00	0.00	5.67 A	4.44 ABC	1.63 BC
	13 days	60.00	13.33	4.89 AB	3.57 C	0.75 D
	18 days	26.67	0.00	3.92 CDE	1.94 D	1.23 BCD
	25 days	26.67	0.00	4.27 BCD	2.49 D	1.07 CD
	27 days	66.67	6.67	3.64 DEF	1.99 D	0.62 D

[a] Percentage of observed attachment.
[b] Percentage of observed multiplication.
[c] Letters indicate the least significant difference according to the Fisher test ($P < 0.05$).
[d] After inoculation, culture was immediately aspirated.
[e] NI, not included. Value was not included because it was not an important consideration within this study.
[f] After initial inoculation and drying for the export chain experiment.

bacteria could adhere to lettuce leaves, demonstrating that no physiological activity was required for adhesion.

Adhesion and attachment are essential for colonization and survival. Attachment is a mechanism that ensures that the bacterial cells are not dislodged from the surface once colonization is triggered. Following the initial interaction (adhesion) between the bacteria and the plant, attachment follows if the bacteria are able to utilize the surface nutrients. Even though initial adhesion occurred more quickly on peaches, attachment by means of observed

attachment structures was observed earlier on plums than on peaches. In this study, attachment occurred as early as 1 min for *E. coli* O157:H7 and *L. monocytogenes*. *Salmonella* Typhimurium was better able to attach and grow on plums than was *E. coli* O157:H7, a finding in agreement with that of Barak et al. *(4)*. However, the same trend was not seen with peaches, on which *E. coli* O157:H7 was better able to attach and grow than was *Salmonella* Typhimurium. Barak et al. *(3)* and Jeter and Matthysee *(29)* found that *E. coli* O157:H7 and *Salmonella* produced fibrils and aggregative polymers for attachment. Plant pathogens produce similar fibrils to attach to plant hosts. Latham et al. *(33)* found that *Ruminococcus flavefaciens* attached to ryegrass after 30 min of exposure, *Pseudomonas lachrymans* attached to young cucumber leaves after 10 min *(34),* and *Bacillus subtilis* attached to the surface of avocado leaves within 2 h *(18)*. Bacteria in the present study attached to one another, forming typical microcolonies. Barak et al. *(4)* also found that *S. enterica* used colonization niches on sprout surfaces, and cells attached to one another, therefore increasing the possible attachment surface area.

Foodborne pathogens used in this study colonized peach surfaces more effectively than plum surfaces, and this difference was attributed to the increased surface area of the peach due to the presence of trichomes. The trichomes on peaches provide a niche for bacteria, making detachment more difficult during washing. Trichomes also serve as additional colonization sites for microorganisms, thereby increasing the surface area that can be used for adherence, attachment, and eventual colonization. The colonization studies using the four selected foodborne pathogens indicated preferential attachment sites on the peach trichomes and nearby lenticels. Seo and Frank *(45)* found that *E. coli* O157:H7 and epiphytes that attached to the intact surface of lettuce leaves attached to areas located near stomata, on trichomes, and on veins. Takeuchi and Frank *(50)* found that plant pathogens may be better adapted than human foodborne bacterial pathogens to the phyllosphere. In the present study, *Salmonella* Typhimurium and *E. coli* O157:H7 were the least effective colonizers of stone fruit surfaces. *Salmonella* Typhimurium was able to produce microcolonies, but survival was poor. On cilantro leaves, *S. enterica* formed colonies 2 days postinoculation, and larger colonies were observed at 9 days postinoculation *(7)*. In the present study, *Salmonella* Typhimurium produced fewer microcolonies than did *S. aureus* and *L. monocytogenes* on the sections examined. Microcolony formation is one of the survival strategies used by bacteria cells to provide protection through the production of exopolysaccharides *(36)*.

Another important requirement for microbial colonization is successful reproduction. In this study, multiplication of all four foodborne bacterial pathogens was observed on the stone fruit surface areas, indicating that the bacteria were able to utilize nutrients available on the surface of the fruit. The increase in number of *S. aureus* cells also demonstrated this organism's ability to reproduce on peach surfaces. Colonization and survival of enteric bacteria has been noted on plants by various authors *(7, 25–27, 42, 48)*, mainly on leaves and roots.

After storage at the initial export temperature of 0.5°C, titers of all pathogens (except *S. aureus*) on peaches and plums decreased over time due to the inhospitable environmental conditions. Survival of foodborne pathogens can therefore be reduced by careful management and by maintaining export temperatures for stone fruit at 0.5°C. However, pathogen titers increased again once the fruit was removed from cold storage, simulating the export chain. Similarly, Francis and O'Beirne *(21)* found a decrease in titers of *E. coli* O157:H7 and *L. monocytogenes* when temperatures changed from 8 to 4°C. Most often, colonization is more successful at higher temperatures. Temperature and water activity play important roles in survival of bacteria on a plant surface. At high conducive temperatures (optimum temperatures) and high relative humidity, *S. enterica* was able to multiply rapidly in the phyllosphere *(7)*. The results of the present study indicate that *E. coli* O157:H7 inoculated onto stone fruit at realistic contamination loads will not survive the entire export chain when contaminated at the point of harvest when the correct cold chain regimens are followed. Survival of *E. coli* O157:H7 in this study was poor, even though the organism was able to adhere, attach, and colonize. Mitra et al. *(39)* found that *E. coli* spot inoculated onto spinach leaves followed the same trend, but Solomon et al. *(48)* found that the *E. coli* O157:H7 population declined but survived on lettuce seedlings for up to 30 days postinoculation. Temperature also influenced *E. coli* O157:H7 survival in the present study, with titers decreasing at ultralow temperatures (0°C) and slight recovery at higher (refrigeration) temperatures (for the high-level inoculation group).

L. monocytogenes and *S. aureus* survived on the stone fruit surfaces more effectively than did the other two pathogens studied. *L. monocytogenes* is able to survive freezing temperatures, and *S. aureus* can withstand a number of environmental stresses in its natural habitat (human skin). A fluctuation in bacterial numbers was observed from immediately after inoculation at 21°C, with a decrease in titer during storage at 12°C (peaches) or 7.5°C (plums). *L. monocytogenes* down-regulated attachment at 37°C; optimal colonization and survival occurred at 20°C, followed by 30°C and then 10°C *(23)*.

In the present study, *S. aureus* was unable to survive in high enough numbers to produce toxins when inoculated at a realistic level. However, when inoculated at an unnaturally high level the pathogen was able to survive and grow to populations large enough to potentially produce toxins. The likelihood of this scenario happening under natural circumstances is low, but these results shows the potential for the organism to maintain initial high titers. At the end of the simulated export chain, *E. coli* O157:H7 survived at titers that theoretically could lead to illness. This pathogen can cause disease at a level of 10^1 cells. *L. monocytogenes* survived at 10^3 cells, which may be high enough to cause illnesses *(52)*. In contrast, *S. aureus* populations must be at least 10^5 cells to produce toxins *(52)*. Future research should focus on the likelihood that contaminated fruit could cause consumer illnesses at the end of the supply chain.

In conclusion, for illness to result from the consumption of contaminated fresh produce, foodborne pathogens must adhere, attach, colonize, and proliferate to a level above the minimum infectious dose. When fresh produce is contaminated preharvest, the organism must survive through postharvest treatments, including export cold chain storage conditions. If the organism is able to survive on the fruit surface under export environmental conditions and then proliferate before consumption, the level of risk increases. *Salmonella* Typhimurium and *S. aureus* in pure culture inoculated onto fruit under laboratory conditions are low-risk foodborne pathogens on stone fruit. The results of this study indicate that *E. coli* O157:H7 and *L. monocytogenes* can survive at levels high enough above the minimum infectious dose on stone fruit surfaces under controlled conditions to be a food safety concern. However, these high levels were attained only when the fruits were artificially contaminated with an unnatural high inoculum load and not with a lower load. The time-temperature regimen therefore cannot suppress *E. coli* O157:H7 and *L. monocytogenes* at high levels on stone fruit, and preventative intervention strategies would therefore be required. However, it is unrealistic to assume that such artificially high levels of contamination would occur when basic good agricultural practices are followed.

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