Evaluating Risks and Mitigation Measures for Foodborne Pathogens on Harvest Bags

Cyril Nsom Ayuk Etaka

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Food Science and Technology

APPROVED
Laura K. Strawn, Chair
Joseph D. Eifert
Renee R. Boyer
Daniel L. Weller

NOT APPROVED
Alexis M. Hamilton

April 29th, 2024
Blacksburg, Virginia

Keywords: *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, Harvest bags

Copyright 2024

Evaluating Risks and Mitigation Measures for Foodborne Pathogens in Harvest Bags

Cyril Nsom Ayuk Etaka

Abstract

Tree fruit growers need information on pathogen dynamics following harvest bags contamination to determine effective sanitation interventions for decontaminating these surfaces. Therefore, the objectives of this research were (i) to determine the survival of generic *E. coli*, *Salmonella*, and *L. monocytogenes* on different harvest bag materials (ii) to quantify the transfer of generic *E. coli*, *Salmonella,* and *L. monocytogenes* from different harvest bag materials to fresh unwaxed apples and (iii) to determine the efficacy of different sanitizers for decontaminating different harvest bag materials. For Obj. 1, harvest bag materials were inoculated with rifampicin-resistant (80ppm; R) *E. coli* (TVS353) or *Salmonella* strain cocktail or *L. monocytogenes* strain cocktail. All surfaces were air-dried and held at 22 °C and either 30 or 80% relative humidity for 90 d (*E. coli*), or at 22 °C and 55% relative humidity (RH) for 21 d (*L. monocytogenes* and *Salmonella*). For Obj. 2, harvest bag materials were inoculated with *E. coli* (TVS353) or *Salmonella* strain cocktail or *L. monocytogenes* strain cocktail and air dried as previously mentioned. For *E. coli* trials, bacterial transfer to unwaxed 'Red Delicious' apples was assessed for 2 inoculum dry times (1 or 4 h), 2 contact times (5 or 25 minutes), and 2 pressure scenarios (0.0 or 0.1kg/cm^2). For *Salmonella* or *L. monocytogenes* trials, transfer was assessed for 1 inoculum dry time (1 h), and 1 contact time (5 minutes). For Obj. 3, coupons were inoculated

with *L. monocytogenes* or *Salmonella* cocktails and were air-dried. Following inoculation, coupons were exposed to different sanitizer treatments: chlorine, peroxyacetic acid (PAA), isopropyl alcohol with quaternary ammonium compounds (IPAQuats), steam, and water. Regression models were fitted, and Tukey's post hoc test was performed at $P<0.05$. *E. coli* exhibited survival for extended durations at 30 % than at 80% RH. In addition, *E. coli* survived at higher concentrations on canvas surfaces than on cordura and nylon surfaces. Generally, *E. coli* survived for more than 21 d across all surfaces and exhibited a triphasic die-off pattern. Similarly, *L. monocytogenes* and *Salmonella* exhibited die-off in phases with an initial rapid die-off followed by more gradual die-off rates up to 21 d. Canvas materials also promoted better *L. monocytogenes* and *Salmonella* survival than cordura surfaces. Contact time did not significantly impact the transfer of *E. coli* from harvest bag surfaces to apples ($P=0.55$). However, pressure, inoculum dry time and material type significantly impacted the transfer of *E. coli* to 'Red Delicious' apples ($P\leq0.03$). The transfer rates of *Salmonella* did not differ between canvas and cordura surfaces ($P=0.46$). However, cordura transferred *L. monocytogenes* at significantly higher rates than canvas surfaces ($P<0.001$). Of the sanitizer treatments that were used on *L. monocytogenes* or *Salmonella* inoculated surfaces, IPAQuats was the most effective achieving over 4.5 log CFU/coupon reduction on both canvas and cordura surfaces. Our studies demonstrated that bacteria could survive for over 21 d under different conditions and could transfer from contaminated harvest bag

surfaces to apples underlining the importance of cleaning and sanitizing harvest bags with sanitizers like IPAQuats.

Evaluating Risks and Mitigation Measures for Foodborne Pathogens in Harvest Bags

Cyril Nsom Ayuk Etaka

General Audience Abstract

Tree fruit growers need information on pathogen behavior on harvest bag surfaces to determine effective sanitation interventions for decontaminating these surfaces. Therefore, the objectives of this research were (i) to determine the survival of generic *E.coli*, *Salmonella*, and *L. monocytogenes* on different harvest bag materials (ii) to quantify the transfer of generic *E. coli*, *Salmonella,* and *L. monocytogenes* from different harvest bag materials to fresh unwaxed apples and (iii) to determine the efficacy of different sanitizers for decontaminating different harvest bag materials. For Obj. 1, harvest bag materials were inoculated with *E. coli* (TVS353) or *Salmonella* strain cocktail or *L. monocytogenes* strain cocktail. All surfaces were air-dried and held for 90 d (*E. coli*), or 21 d (*L. monocytogenes* and *Salmonella*). For Obj. 2, harvest bag materials were inoculated with *E. coli* (TVS353) or *Salmonella* strain cocktail or *L. monocytogenes* strain cocktail and air dried as previously mentioned. For *E. coli* trials, bacterial transfer to unwaxed 'Red Delicious' apples was assessed for 2 drying conditions (wet or visibly dry), 2 contact times (5 and 25 minutes), and 2 pressure scenarios (0.0 and 0.1kg/cm^2). For *Salmonella* or *L. monocytogenes* trials, transfer was assessed for wet surfaces only and apples sat on surfaces for 5 minutes. For Obj. 3, harvest bags were inoculated with *L. monocytogenes* or *Salmonella* cocktails and exposed to different sanitizer treatments including

chlorine, peroxyacetic acid (PAA), isopropyl alcohol with quaternary ammonium compounds (IPAQuats), steam, and water. Regression models were fitted, and Tukey's post hoc test was performed at $P<0.05$. Canvas surfaces promoted better *E. coli* survival compared to cordura and nylon surfaces. Similarly, canvas materials also supported better *L. monocytogenes* and *Salmonella* survival compared to cordura surfaces. Contact time did not significantly impact the transfer of *E. coli* from harvest bag surfaces to apples ($P=0.55$). However, pressure, inoculum dry time and material type significantly impacted the transfer of *E. coli* to 'Red Delicious' apples ($P\leq0.03$). The transfer rates of *Salmonella* did not differ between canvas and cordura surfaces ($P=0.46$). However, cordura transferred *L. monocytogenes* at significantly higher rates than canvas surfaces ($P<0.001$). Of the sanitizer treatments that were used on *L. monocytogenes* or *Salmonella* inoculated surfaces, IPAQuats was the most effective achieving over 4.5 log CFU/coupon reduction on both canvas and cordura surfaces. Our studies demonstrated that bacteria could survive for over 21 d under different conditions and could transfer from contaminated harvest bag surfaces to apples underlining the importance of cleaning and sanitizing harvest bags with sanitizers like IPAQuats.

Dedication

To Etaka Mathias Ayuk, and Munguo Abigail Fulai.

Acknowledgments

It has been a good 3 years since I started my doctoral degree and throughout, I have received guidance, mentorship, and words of encouragement from the members of my committee; Dr. Laura K. Strawn, Dr. Renne R. Boyer, Dr. Joseph D. Eifert, Dr. Daniel L. Weller and Dr. Alexis M. Hamilton. I extend my appreciation to them as they contributed to my growth. Special thanks to Dr. Laura K. Strawn for being a wonderful mentor. She provided me with all the opportunities to learn and grow as a scientist. Through her, I have also made connections with a lot of food safety professionals in industry, government, and academia. I will be forever grateful for the opportunities she has provided me.

Family and friends have also been with me throughout and I appreciate that I have a support system that I always fall back on when things get intense.

The journey has not been without hurdles, but I am grateful that I walked through with support and encouragement from my PI (Dr. Laura K. Strawn) and my other committee members, family, and friends.

Table of Contents

Abstract ii

General Audience Abstract v

Chapter 1: Introduction 1

References 2

Chapter 2: Literature Review 7

Economic value and safety of the apple industry in the US. 7

Apple-related outbreaks and recalls in the US. 7

The outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples. 8

The outbreak of *E. coli* O157:H7 associated with unpasteurized apple cider. 9

The outbreak of *E. coli* O157:H7 infections associated with drinking unpasteurized apple juice. 9

Survival of E. coli, Salmonella, and L. monocytogenes on fresh produce surfaces and food contact surfaces (FCS). 10

Survival of *E. coli*, *Salmonella*, and *L. monocytogenes* on fresh produce. 10

Survival and transfer of *E. coli*, *Salmonella*, and *L. monocytogenes* on FCS. 12

Regulatory framework for fresh produce. 99

Produce safety rule. 99

Good Agricultural Practices. 101

Impact of sanitizer treatments on reduction of Salmonella and Listeria monocytogenes on harvest bag material types. 102

Chapter 3: Generic *Escherichia coli*, *Listeria monocytogenes,* and *Salmonella* survived on different harvest bag material types for 21 days. 138

Chapter 4: Generic *E. coli, Listeria monocytogenes* and *Salmonella* Exhibited Different Transfer Patterns From Harvest Bags to Apples. 196

Chapter 5: Isopropyl Alcohol with Quaternary Ammonium Compounds (IPAQuats) was Effective at Reducing *Salmonella* and *Listeria monocytogenes* on Cordura and Canvas Harvest Bags. 248

Chapter 6: Conclusion 290

Future Studies 291

Chapter 1: Introduction

Fresh produce-related outbreaks have been on the increase over the years due to increased consumer demand for a healthier lifestyle and more nutritious foods (*2*). In the U.S., the consumption of fresh produce is steadily increasing (*16*). With this increase in fresh produce consumption, the likelihood of food-borne illness associated with fresh produce is also expected to increase especially for fresh produce commodities that are consumed in a raw state such as apples (*2*, *10*). Apples are an important crop in the state of Virginia contributing an estimated $235 million annually to Virginia's economy (*17*). While some of the harvested apples are used to make processed products, like juices, ciders, and apple butter, another portion is consumed fresh with no lethality step to kill any bacterial pathogens before consuming the apples (*10*, *17*). Several outbreaks linked to fresh produce have occurred over the years (*1*, *3*, *4*, *6*, *7*, *12*, *13*). For example, in 2015, the Centers for Disease Control and Prevention (CDC) reported an outbreak of listeriosis due to the consumption of *Listeria monocytogenes*-contaminated prepackaged caramel apples (*5*). This multi-ingredient commodity product had a fresh apple component previously considered low risk (*1*). Traceback and environmental investigations resulted in the isolation of outbreak-related strains from food contact surfaces suggesting that this outbreak happened due to cross-contamination from the environment (*1*).

The contamination of fresh produce can occur pre or post-harvest through soil, irrigation water, insects or equipment and tools (*1*, *14*, *15*, *18*). Equipment and tools that come in direct contact with harvested produce may pose a greater risk for fresh produce contamination if not maintained, cleaned, and sanitized (*11*). In the apple packing

industry, these types of equipment include packing containers that are used to convey harvested apples from the tree to the harvest bins in the field (*9*). Packing containers like harvest bags have been recognized as field equipment that can spread contamination (*11*). As such apple growers are required to clean and sanitize these surfaces as a measure to mitigate the risk of cross-contamination of harvested apples that touch the bags (*8*). For these surfaces to be adequately cleaned and sanitized information on their microbial quality that is, the ability of these surfaces to support bacterial growth and transfer, is needed as it can inform sanitation interventions. However, this information is lacking. In addition, food safety auditors have been questioning apple growers about the frequency at which these harvest bags are cleaned and sanitized. Furthermore, there is little information about sanitizers that are effective for decontaminating harvest bag surfaces.

Therefore, the first objective of this dissertation was to determine bacterial survival on harvest bag material surfaces. For this objective, the survival of generic *E. coli*, *Salmonella*, and *Listeria monocytogenes* was evaluated on different harvest bag material types. The second objective of this study was to quantify the transfer of generic *E. coli*, *Salmonella*, and *L. monocytogenes* for different harvest bag material types to fresh unwaxed apples under different transfer scenarios. Finally, we investigated the efficacy of different sanitizer treatments for decontaminating harvest bag material-type surfaces.

References

1. Angelo, K. M., A. R. Conrad, A. Saupe, H. Dragoo, N. West, A. Sorenson, A. Barnes, M. Doyle, J. Beal, K. A. Jackson, S. Stroika, C. Tarr, Z. Kucerova, S.

Lance, L. H. Gould, M. Wise, and B. R. Jackson. 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection*. Cambridge University Press 145:848–856.

2. Carstens, C. K., J. K. Salazar, and C. Darkoh. 2019. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Frontiers in Microbiology* 10.

3. Centers for Disease Control and Prevention. 2023. *Listeria* Outbreak Linked to Leafy Greens. *Centers for Disease Control and Prevention*. Available at: https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/index.html. Accessed 21 March 2024.

4. Centers for Disease Control and Prevention. 2024. *Listeria* Outbreak Linked to Peaches, Nectarines, and Plums. Available at: https://www.cdc.gov/listeria/outbreaks/peaches-11-23/index.html. Accessed 27 February 2024.

5. Centers for Disease Control and Prevention. 2018. Multistate Outbreak of Listeriosis Linked to Commercially Produced, Prepackaged Caramel Apples Made from Bidart Bros. Apples. Available at: https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html. Accessed 6 April 2023.

6. Centers for Disease Control and Prevention. 2018. Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado. Available

at: https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. Accessed 4 April 2023.

7. Centers for Disease Control and Prevention. 2020. Outbreak of *Salmonella* Enteritidis Infections Linked to Peaches. Available at: https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 27 February 2024.
8. Code of Federal Regulations. 2022. Title 21. Food and Drugs. Chapter 1. Food and Drug Administration, Department of Health, and Human Services. Subpart B. Food for Human Consumption. Part 112. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Subpart L. Equipment, Tools, Buildings, and Sanitation. Section 112.123(d)(1).
9. Corelli Grappadelli, L. 2001. Peach handling and marketing in Italy. Proceedings of 60th National Peach Council Convention. p. 39–45. *In*.
10. Economic Research Service. 2021. Apples and oranges remain the top U.S. fruit choices. Available at: http://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=101944. Accessed 2 April 2023.
11. Food And Drugs Administration. 2022. Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables. FDA. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-cut-fruits-and-vegetables. Accessed 2 May 2023.
12. Food And Drugs Administration. 2022. Outbreak Investigation of *Listeria monocytogenes*: Dole Packaged Salad. Available at:

https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-listeria-monocytogenes-dole-packaged-salad-december-2021. Accessed 30 August 2023.

13. Grant, J., A. M. Wendelboe, A. Wendel, B. Jepson, P. Torres, C. Smelser, and R. T. Rolfs. 2008. Spinach-associated Escherichia coli O157:H7 Outbreak, Utah, and New Mexico, 2006. *Emerg Infect Dis* 14:1633–1636.
14. Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on Fresh-Cut Apple Tissue and Its Potential for Transmission by Fruit Flies. *Appl Environ Microbiol* 65:1–5.
15. Park, S., S. Navratil, A. Gregory, A. Bauer, I. Srinath, B. Szonyi, K. Nightingale, J. Anciso, M. Jun, D. Han, S. Lawhon, and R. Ivanek. 2015. Multifactorial effects of ambient temperature, precipitation, farm management, and environmental factors determine the level of generic *Escherichia coli* contamination on preharvested spinach. *Appl Environ Microbiol* 81:2635–2650.
16. Rekhy, R., and R. McConchie. 2014. Promoting consumption of fruit and vegetables for better health. Have campaigns delivered on the goals? *Appetite* 79:113–123.
17. The Virginia Apple Board. 2024. Apple Fun Facts. *Virginia Apples.* Available at: https://www.virginiaapples.net/apple-facts. Accessed 2 April 2024.
18. Weller, D., M. Wiedmann, and L. K. Strawn. 2015. Irrigation Is Significantly Associated with an Increased Prevalence of Listeria monocytogenes in Produce

Production Environments in New York State. *Journal of Food Protection* 78:1132–1141.

Chapter 2: Literature Review

Economic value and safety of the apple industry in the US.

The U. S. has approximately 302,300 acres of apple-bearing acreage with a commercial value of utilized production estimated at approximately $3 billion (*74*). Fifty states in the US produce apples commercially with the top 9 producers being Washington, New York, Michigan, Pennsylvania, California, Virginia, Oregon, West Virginia, and North Carolina (*107*, *110*). In the state of Virginia, the apple industry contributes an estimated $235 million annually to the state's economy and apple growers produce many popular varieties including Gala, Golden Delicious, Granny Smith, Fuji, Red Delicious, Rome, Stayman, Winesap, York, Jonathan and Ginger Gold (*97*). Apples are one of the top 20 fresh produce commodities in Virginia (*119*) and are sold in 15 states and more than 20 countries with 70% of apple production being sold for processing into apple sauce, juice, apple butter, slices, and cider (*97*).
Apples are the most consumed fruit in the US closely followed by oranges (*1*). While 54% of apples produced are processed, about 34% are consumed fresh consumption with minimal processing to further reduce or eliminate harmful microbial pathogens (*14*, *109*). Because a larger proportion of apples are consumed fresh, the safety of this commodity is important.

Apple-related outbreaks and recalls in the US.

Fresh produce may become contaminated with foodborne pathogens pre-harvest, during harvest, and post-harvest. Routes of contamination preharvest include irrigation water, soil, fertilizers, manure, insects, and dust (*10*, *22*, *41*, *56*, *64*, *120*).

During harvest, produce contamination can occur through contact with contaminated surfaces including harvest equipment and tools, knives, transport containers, human hands, and gloves (*12*, *67*, *96*). Post-harvest contamination of fresh produce can occur during processing, storage, and transport (*80*, *92*).

Reported produce-related outbreaks have been traced back to contamination in the pre-harvest and post-harvest environments (*4*, *41*, *66*). The etiologic agents isolated from these outbreaks include *Salmonella*, *Escherichia coli* (*E. coli*), and *Listeria monocytogenes (L. monocytogenes)* (*4*, *16*, *17*, *19*, *40*, *41*, *66*, *115*). While there have been numerous outbreaks for other fresh produce commodities, this section will discuss the outbreak involving apples and apple products.

The outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples.

In 2015, the Centers for Disease Control and Prevention (CDC) reported 35 illnesses in 12 states due to the consumption of *Listeria monocytogenes*-contaminated prepackaged caramel apples (*4*, *18*). This multi-ingredient commodity product had a fresh produce component that was previously considered low risk due to its low internal pH and low water activity of the hot caramel coating (*4*). Further investigation revealed that stick insertion into the core of the apple created a microenvironment that allowed for the survival and growth of *L. monocytogenes* in an otherwise unsupportive environment (*14*, *38*, *81*). Traceback and environmental investigations revealed that this outbreak was the result of cross-contamination from food contact surfaces (FCS) in the produce environment hence, it highlights the importance of implementing mitigation strategies to prevent cross-contamination of produce (*4*).

The outbreak of *E. coli* O157:H7 associated with unpasteurized apple cider. On October 11, 1996, the Connecticut Department of Health (DPH) initiated an investigation into an *E. coli* O157:H7 outbreak after being notified by staff at the Centers for Disease Control and Prevention (CDC) Foodborne Disease Active Surveillance Network (*20*). A matched case-control study by the DPH determined that there was an increased risk for illness associated with drinking "brand A" cider. "Brand A" cider was pressed at a mill from apples purchased from multiple suppliers some of which were "dropped" apples (dropped produce is produce that drops to the ground before harvest). Even though apples were brushed and washed in a municipal wash water system before pressing in a wooden press, pulse-field gel electrophoresis (PFGE) typing still confirmed that 10 of the 12 outbreak-associated isolates of *E. coli* O157:H7 were closely related (*20*). A similar outbreak involving *E. coli* O157:H7 in apple cider was also reported in Oklahoma a few years later (*31*). These outbreaks did not directly involve fresh whole apples but show how the contamination of fresh produce commodities can result in contaminated processed products and illness especially when no lethality step is involved.

The outbreak of *E. coli* O157:H7 infections associated with drinking unpasteurized apple juice.

On October 30, 1996, an outbreak of *E. coli* O157:H7 infections epidemiologically associated with drinking the Odwalla brand unpasteurized apple juice or Odwalla juice mixtures containing apple juice was reported by the Seattle-King County Department of Public Health and the Washington State Department of Health (*21*, *27*). Case-related isolates had an indistinguishable DNA 'fingerprint' pattern (restriction fragment length

polymorphism) as isolates cultured from a previously unopened container of Odwalla juice resulting in a voluntary recall of all Odwalla products distributed nationwide that contained apple juice (*21*, *27*). Traceback investigation revealed that some of the apples used to make the juice responsible for the outbreak came from apple orchards with lots that were frequented by deer (*27*). Additionally, these orchards used seasonal workers who were instructed to not pick dropped apples but had no mechanisms in place to monitor workers (*27*). Although the source of contamination was not identified, this outbreak highlighted the importance of best practices when handling fresh produce commodities that are eaten fresh or processed into products with no lethality step during processing.

Survival of *E. coli*, *Salmonella*, and *L. monocytogenes* on fresh produce surfaces and food contact surfaces (FCS).

Pathogenic bacteria exhibit survival on fresh produce and food contact surfaces (FCS) (*3*, *55*, *72*, *81*, *90*, *121*, *122*, *123*). In addition, studies have shown that pathogenic microorganisms can transfer from contaminated FCS to fresh produce (*12*, *49*, *78*). Since 2010, three bacterial pathogens have been involved in most fresh produce-related outbreaks (*14*, *15*). As a result, this section focuses on *E. coli*, *Salmonella,* and *L. monocytogenes*. Table 1 summarizes the findings of studies on the survival of these 3 pathogens on fresh produce surfaces and FCS.

Survival of *E. coli*, *Salmonella*, and *L. monocytogenes* on fresh produce. Pathogenic bacteria can attach and survive on intact fresh produce surfaces and may grow in bruised, punctured, or cut produce upon contamination (*24*, *55*, *60*, *78*, *81*, *121*).

For example, *Salmonella* has been shown to exhibit varying attachment to apple skins (between 0.03 to 3.18 %) based on apple cultivar (*39*). Another study that examined the survival of *Salmonella* Reading (VI 51763) on apples stored at 22 °C and ca. 70% RH for a total of 12 days observed a noticeable ability of *Salmonella* to survive for six days of storage without exhibiting any significant decrease (*78*). A previous study investigating the survival of *L. monocytogenes* inoculated at the stem end of Gala and Granny Smith apples observed survival for up to 15 days when apples were held at both 5 and 25 °C (*81*). Apple variety impacted the survival of *L. monocytogenes* in this study (*81*). Similar studies with *L. monocytogenes* determined survival on Red Delicious, Fuji, and Granny Smith apples up to 160 days at 3 °C with bacteria exhibiting a lower die-off on waxed apples (*60*). *L. monocytogenes* has also been shown to survive on other fresh produce commodities like peaches, sprouts, and leafy greens (*2*, *55*, *85*). Investigations with *E. coli* have also shown survival on fresh produce commodities. For example, *E. coli* survived on field-inoculated lettuce for more than 10 days post-inoculation (*121*). In this study, detectable levels of 3.64 log MPN/head were recovered at 10 days post-inoculation and *E. coli* exhibited a biphasic die-off rate (*121*). Similar studies with *E. coli* on watermelon surfaces have reported survival for more than 6 days with *E. coli* showing a multiphasic die-off rate (*24*). These studies show that foodborne microorganisms can survive on fresh produce surfaces if a contamination event occurs. As a result, it is important to minimize fresh produce contamination pre- and post-harvest. Table 1 summarizes more findings from some of the studies mentioned in this section.

Survival and transfer of *E. coli*, *Salmonella*, and *L. monocytogenes* on FCS.

The persistence of pathogenic microorganisms on FCS in fresh produce facilities presents a high risk for cross-contamination (*3*, *30*, *53*, *91*, *99*). Instances of outbreak isolates being traced back to FCS in produce packing facilities have been reported extensively (*4*, *63*, *66*, *66*, *80*, *114*).

Food contact surfaces in produce packing facilities are often made of wood, polyvinyl chloride (PVC), high-density polyethylene (HDPE), stainless steel, fabric (canvas, nylon, cordura), and leather (*57*, *80*, *83*, *84*, *90*, *91*, *94*, *118*). Prior studies have extensively reported on the survival of bacteria on these FCS (Table 1). For instance, *E. coli* O157 exhibited survival on stainless steel (S30400) coupons held at 20 °C and 4 °C for more than 28 days with an initial decline by 5 log CFU/coupon 2 days post inoculation from starting levels of ca. 7.7 log CFU/coupon (*122*). *Salmonella* and *L. monocytogenes* have been reported to survive more than 21 days on pressed-card, wood, and plastic surfaces held at 3.2 °C and 22.9% relative humidity (RH), and 22.5 °C and 50.4% RH (*57*). Similar findings have been reported in a study with *L. monocytogenes*, *E. coli, and Salmonella* on cardboard and plastic although survival was impacted by inoculum level, and surface type (*90*).

Because these microorganisms can survive on FCS, there is a risk of cross-contamination or transfer to fresh produce. For example, *Salmonella* can transfer from inoculated FCS like plastic, glass, plastic, and stainless steel to fresh-cut carrots and watermelons at a transfer rate of >80% when surfaces are freshly inoculated (*49*). Similarly, *E. coli* can also transfer from FCS to lettuce although more variable (0.37 to 71.96%) when inoculated surfaces are air-dried for 1 h (*49*). Other studies with

Salmonella or *Enterobacter aerogenes* have observed bacterial transfer from cloth to green tomatoes or from carpet, wood, stainless steel, and tiles to cut watermelon (*29*, *69*). In most of these studies, inoculum size, bacterial species, moisture level, surface type, fresh produce commodity, contact time, pressure, and presence or absence of friction drive the transfer rates from FCS to food products (*12*, *29*, *49*, *53*, *54*, *65*, *69*, *70*). Tables 1 and 2 summarize findings from studies that investigated the survival of microorganisms on FCS (Table 1) and the transfer from contaminated FCS to food products including fresh produce and vice versa (Table 2).

In summary, pathogenic bacteria can survive on produce and FCS in produce-packing facilities. Additionally, survival on FCS could potentially lead to a cross-contamination event. It is therefore essential to minimize the occurrence of these events through sanitation. Sanitation interventions are required by the 'Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (Produce Safety Rule; PSR) as a measure of protecting public health (*25*).

Table 1. Summary of studies on the survival of foodborne pathogens on food and FCS

Commodity, reference	Surface type	Temp[a] (°C)	RH[b] (%)	Study duration and sampling time points	Inoculation method and starting level	Reported outcomes
Nectarines and peaches (*55*)	-[c]	-	-	24 h	Spot inoculated; 6.60 ± 0.03 log CFU/ fruit	The concentrations of *L. monocytogenes* recovered from both peaches and nectarines after inoculum drying were similar (5.15 ± 0.06 log CFU/ fruit).
					Spot inoculated; 5.46 ± 0.05 log CFU/ fruit	The concentration of *L. monocytogenes* recovered from both peaches and nectarines after inoculum drying were similar (3.25 ± 0.05 log CFU/ fruit).
	-	28–30	40 – 50	1, 18 h	Spot inoculated; 5.15 ± 0.06 log CFU/ peach.	Approximately 5.46 ± 0.05 and 5.71 ± 0.22 log CFU/peach, respectively of *L. monocytogenes* were recovered at the unloading and staging of fruit (*P*> 0.05).
				1, 18 h	Spot inoculated; 3.25 ± 0.05 log CFU/ peach.	Approximately 3.75 ± 0.12 and 3.85 ± 0.05 log CFU/peach, respectively of *L. monocytogenes* was recovered at the unloading and staging of fruit (P> 0.05).
	-	18 – 20	40 – 50	1, 18 h	Spot inoculated; 5.15 ± 0.06	At unloading and staging, no significant change in *L. monocytogenes* population was

					log CFU/ peach	observed as ca. 5.66 ± 0.07 log CFU of *L. monocytogenes* was recovered from the peaches at 18 h at both inoculation levels.
				1, 18 h	Spot inoculated; 3.25 ± 0.05 log CFU/ peach	At unloading and staging, no significant change in *L. monocytogenes* population was observed as 3.83 ± 0.04 log CFU of *L. monocytogenes* was recovered from the peaches at 18 h at both inoculation levels.
Fresh-cut apples, cucumbers, cantaloupe, and tomatoes (*43*)	-	4	-	1, 3, 5, 7 d	Spot inoculated; ca. 7 log CFU/g	Both strains of *Salmonella (*Newport and Typhimurium*) were* reduced by < 0.2 log CFU/g (P ≥ 0.05) on cut tomatoes and cantaloupe by 7 d. However, strains were reduced by ca. 0.5 log CFU/g on apples and cucumbers (P < 0.05).
					Spot inoculated; ca. 2 log CFU/g	Population dynamics were like when the high inoculum (ca. 7 log CFU/g) level was used.

Lettuce (*121*)	-	-	-	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 d	Spot inoculated; 8.86 log MPN/head	The mean *E. coli* level decreased from 8.86 to 3.64 log MPN/head by 10 d, a 5.22 log reduction. *E. coli* was still detectable on the lettuce 10 d post-inoculation. The bacterial die-off was biphasic with a mean daily *E. coli* decrease of 0.70 log MPN (95% CI = 0.55, 0.86 log MPN) and 0.19 log MPN (95% CI= 0.05, 0.36 log MPN) from 0 to 106 h and 106 to 240 h, respectively.
Watermelon (*24*)	-	28.8 – 30.5	48 - 94	0, 24, 48, 72, 96, 120 h	Spot inoculated; 3.65 log CFU/cm^2	At 120 h, the total reduction of *E. coli* was 1.94 log CFU/cm^2 (from 3.65 log CFU/cm^2 to 1.71 log CFU/cm^2). The highest daily die-off was 1.30 log CFU/cm^2 at 2 d followed by 0.63 log CFU/cm^2 at 6 d.
Lettuce (*61*)	-	4.5 - 18	-	0, 1, 3, 7, 10, 14, 21, 24, 28 d	Sprayed; 5 - 6 log CFU/g	The concentration of *E. coli* was 1.70 log CFU/g by 14 d. From 21 -28 d, bacterial presence was detected by enrichment.
					Sprayed; 5 log CFU/g	The concentration of *L. innocua* was 3 and 1.70 log CFU/g by 7 and 14 d, respectively. From 21 -28 d, bacterial presence was detected by enrichment.
Fresh-cut romaine lettuce (*48*)	-	-	-	30 s, 1, 2, 5 min	Spot inoculated; 6 log CFU/leaf	No differences in log reduction of *E. coli* at different wash times were significant (*P*=0.05). Overall, a 2.05 ±0.18 log CFU reduction was

						observed after the leaves were washed.
Tomatoes (*99*)	-	25	69.93 ± 2.1	0, 1, 2, 3, 4 d	Spot inoculated; 6.8 log CFU/tomato	*E. coli* concentration was 1.5 log CFU/tomato by 4 d.
	Stainless-steel, high-density polyethylene, polyvinyl belt (PVC)	25	69.93 ± 2.1	0, 1, 2, 3, 4 d	Spot inoculated; 6.1 log CFU/tomato	Surface bacteria were reduced by 4.0, 4.4, and 4.2 log CFU/ml for steel, belt, and plastic upon 90 minutes of drying. Significant reductions in PVC belt, Steel, and plastic were observed during 1-day post-inoculation.
Lettuce (*8*)	-	9 - 39	-	0, 5 d	Watering; 4.5 log CFU/g	Lettuce heads injured at time 0 had a significantly higher count of *E. coli* (P<0.001) than all other treatments. *E. coli* persisted on all lettuce plants for the 5 d of the experiment with all counts >2.3 log CFU/gram. Injury to plants increased persistence especially when fresh.
Apples (*78*)	-	22	70	0, 1, 2, 5, 6, 12 d	Spot inoculation; 6 log CFU/apple	*Salmonella* Reading level declined by 1 log CFU/apple from 6 to 12 d (a reduction rate of 0.16 log cfu/apple).
Apples (*81*)	-	5	-	0, 1, 2, 6, 9, 15 d	Spot inoculation; 6.9 ± 0.6 log CFU/apple	*L. monocytogenes* concentration recovered from the stem end of Gala apples by 15 d was 5.5 ± 0.6 log CFU/apple. Pathogen concentration on the surface of Gala apples was

					below the limit of detection by 2 d. An unexpected concentration of 4.7 ± 0.8 log CFU/apple was observed by 9 d after which bacteria was below the limit of detection.
-	25	-	0, 1, 2, 6, 9, 15 d	Spot inoculation; 6.9 ± 0.6 log CFU/apple	*L. monocytogenes* concentration recovered from the stem end of Gala apples by 15 d was 3.9 ± 0.5 log CFU/apple. Pathogen concentration on the surface of Gala apples was below the limit of detection by 1 d. An unexpected concentration of 5.7 ± 1.5 log CFU/apple was observed by 9 d after which bacteria was below the limit of detection. *L. monocytogenes* concentrations recovered from the surface and stem end of Granny Smith apples (GSA) by 15 d were 3.3 ± 0.6 and 2.9 ± 0.5 log CFU/apple respectively.
Wood, paper, and plastic sticks	5	-	0, 2, 5, 7, 13 d	Spot inoculation; 7 log CFU/stick	After initial inoculation, an approximately 1- to 2-log decrease occurred on both paper and wood sticks. A 3-log CFU decrease occurred on plastic sticks. After 13 d the populations on paper and wood sticks did not decrease significantly, whereas the population on plastic sticks decreased by approximately 1 log CFU/stick.

Apples (*89*)	-	1, 4, 10	-	1, 4, 7, 14 d	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	*L. monocytogenes* increased significantly after 24 h on organic GSA surfaces by 0.5 log CFU/apple for the low inoculation level (3.51 ± 0.08 log CFU/apple; $P < 0.05$). At high inoculation levels, recovery was 6.31 ± 0.06 log CFU/apple after 24 h. The bacterial populations did not significantly change from inoculation levels after 24h during the 14-d storage duration.
	-	22	-	1, 4, 7, 14 d	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	*L. monocytogenes* counts on organic GSA surfaces were reduced by 1.17 and 0.66 log CFU/apple for the high and low inoculation levels, respectively during the 14-d storage duration.
	-	1, 4, 10	-	2, 4, 8, 12 weeks	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	At both inoculation levels, only a 0.5 – 1.5 log CFU/apple reduction of *L. monocytogenes* counts on organic GSA surfaces was observed by 3 months of cold storage.

	-	1, 4, 10	-	1, 4, 7, 14 d	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	For conventional GSA, *L. monocytogenes* maintained the same population size 24 h post-inoculation at the high inoculation level. At the low inoculation level, the bacterial count increased by ca. 0.7 log CFU/apple 24 h post-inoculation. After 2 weeks of cold storage, *L. monocytogenes* counts were slightly decreased for the high inoculation level, while no reduction was observed for the low inoculation level.
	-	22	-	1, 4, 7, 14 d	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	After 2 weeks of cold storage, *L. monocytogenes* counts on conventional GSA reduced by 1.1 and 0.6 log CFU/apple for the high and low inoculation levels, respectively.
	-	1, 4, 10	-	2, 4, 8, 12 weeks	Dipping; 6.29 ± 0.04 and 3.01 ± 0.06 log CFU/apple	After 12-week cold storage, a 0.9 – 2.0 log CFU/apple reduction of *L. monocytogenes* on conventional GSA was achieved for both inoculation levels with 1 °C showing the least reduction and 10 °C the most reduction.
Apples (*58*)	-	8, 20	-	0, 1, 2, 3, 4 d	Dipping; ca. 5 log CFU/disk	*Salmonella* Chester levels increased on cut apple disks by ca. 5 log CFU/disk when disks were held at 20 °C but failed to grow when held at 8 °C. There was more bacterial

						attachment in the stem end and calyx than on the surface.
Apples (*88*)	-	22	ambient	2, 4, 6 weeks	Dipping; ca. 6 log CFU/apple	*L. monocytogenes* (inoculated before waxing) on waxed apples decreased by 1.8–1.9 log CFU/apple at 6 weeks regardless of inoculation level. Wax coating and fungicide had a minor impact on the survival of *L. monocytogenes.*
	-	1	90	2, 4, 6, 9, 12 weeks	Dipping; ca. 6 log CFU/apple or ca. 8 log CFU/apple	*L. monocytogenes* (inoculated before waxing) on waxed apples decreased by 1.8–2.0 log CFU/apple from ca. 6 log CFU/apple at 12 weeks. Wax coating and fungicide had a minor impact on the survival of *L. monocytogenes.* *L. monocytogenes* (inoculated during waxing) decreased by 1.8 log CFU/apple after 12 weeks from ca. 6 log CFU/apple or ca. 8 log CFU/apple.
Apples (*60*)	-	3		0, 1, 3, 7, 16, 31, 62, 93, 160 d	Spot inoculation; ca. 6 log CFU/apple and ca. 5 log CFU/apple	By 62 d, the levels of *L. monocytogenes* on unwaxed GSA, Red Delicious (RD), and Fuji (Fj) apples were 2.79 ± 0.41, 1.89 ± 0.15, and 2.60 ± 0.13 log CFU/apple respectively (close to the limit of detection; 25 CFU/apple. By 160 d, the levels of *L. monocytogenes* on unwaxed GSA,

						Red Delicious (RD), and Fuji (Fj) apples were 0.62 ± 0.50, 0.41 ± 0.30, and 1.30 ± 0.42 log MPN/apple respectively. After 2 months of storage, *L. monocytogenes* populations on waxed apples were consistently higher ($P < 0.05$) than those in un-waxed apples in all three cultivars evaluated.
	-	3	-	0, 1, 3, 7, 16, 31, 62, 93, 160 d	Spot inoculation; ca. 6 log CFU/apple and ca. 5 log CFU/apple	By 62 d, the levels of *L. monocytogenes* on waxed GSA, RD, and Fj apples were 2.36 ± 0.23, 2.08 ± 0.21, and 2.33 ± 0.20 log CFU/apple respectively. By 160 d, the levels of *L. monocytogenes* on waxed GSA, RD, and Fj apples were 2.38 ± 0.24, 2.58 ± 0.17, and 2.43 ± 0.11 log MPN/apple respectively. After 2 months of storage, *L. monocytogenes* populations on waxed apples were consistently higher ($P < 0.05$) than those in un-waxed apples in all three cultivars evaluated.
Cantaloupe (*102*)	-	4, 20	-	0, 1, 3, 6, 9, 15 d	Dipping; ca. 6 log CFU/cm^2	At the end of 15 d of storage at 4 or 20 °C, an approximate 1.5 to 2.0 log reduction in the population of *L. monocytogenes* was observed on the melon surfaces treated with 70%

						ethanol and a 1.0 to 1.5 log reduction on those not treated with ethanol.
Tomatoes (*3*)	-	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.6 log CFU/mL	*Salmonella* populations declined to 1.5 log CFU/mL by 28 d.
	-	20	90	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.6 log CFU/mL	*Salmonella* populations declined to 1.4 log CFU/mL by 28 d.
	-	30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 5.1 log CFU/mL	*Salmonella* populations declined to undetectable levels. However, an unexpected increase to 1.2 log CFU/mL was observed between 21 and d 28 d.
	stainless steel	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.4 log CFU/mL	*Salmonella* populations declined to 0.7 log cfu/mL on 28 d.
		30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.4 log CFU/mL	*Salmonella* was undetectable from 11 to 28 d.
	Conveyor belt	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.3 log CFU/mL	*Salmonella* was undetectable from 21 to 28 d.

	30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.1 log CFU/mL	*Salmonella* was undetectable from 3 to 28 d.
PVC	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 5.1 log CFU/mL	*Salmonella* populations declined to 0.6 log CFU/mL on 28 d.
	30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 5.1 log CFU/mL	*Salmonella* was undetectable from 11 to 28 d.
Sponge rollers	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 4.1 log CFU/mL	*Salmonella* was undetectable from 7 to 28 d.
	30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 5.0 log CFU/mL	*Salmonella* was undetectable from 1 to 28 d.
Wood	20	60	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated; 3.2 log CFU/mL	*Salmonella* populations declined to 1.7 log cfu/mL by 28 d.
	30	80	0, 1, 3, 7, 11, 14, 21, 28 d	Spot inoculated;	*Salmonella* was undetectable by 7 d. Unexpectedly, *Salmonella* at 1.0 log

					4.7 log CFU/mL	CFU/mL was detected by 14 d. However, undetectable levels were again observed on 21 and 28 d.
(*72*)	Stainless steel	12	70	0, 1, 7, 14, 19 d	Spot inoculated; ca. 7 log CFU/mL	All 14 *E. coli* strains survived up to 14 d (<5 log reduction) and 11 strains survived by 19 d. Toxigenic strains survived better than the non-STEC strains ($P = 0.023$). After 7, 14, and 19 d, there was no statistically significant difference in survival between strains ($P > 0.05$).
(*122*)	Stainless steel	4	-	0, 1, 2, 7, 14, 28 d	Spot inoculated; ca. 7.7 log CFU/coupon	*E. coli* initially exhibited a decline by 4.3 log CFU/coupon 7 d post inoculation and stayed constant at ca. 3 log CFU/coupon for the rest of the duration.
		20	-	0, 1, 2, 7, 14, 28 d	Spot inoculated; ca. 7.7 log CFU/coupon	*E. coli* initially exhibited a decline by ca. 5 log CFU/coupon 2 d post inoculation and stayed constant at ca. 2.5 log CFU/coupon for the rest of the duration.
(*123*)	Stainless steel (UNS C30400)	ca. 22	-	0, 90, 180, 270 min	Spot inoculated; ca. 7.7 log CFU/coupon	No significant reduction in *L. monocytogenes* counts over the 90-minute incubation time. *L. monocytogenes* showed a less than 2 log decrease after 270 min exposure at room temperature.

(*57*)	Plastic, pressed-card, wood	3.2	22.9	0, 0.13, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21 d	Spot inoculated and spread uniformly; 5.27–5.53 log CFU/cm^2	*Salmonella* counts on surfaces decreased linearly by 1.11, 1.25, and 2.60 log CFU/ cm^2 on plastic, pressed card, and wood container surfaces, respectively, on the first day. *Salmonella* levels ranged from 2.63 to 3.03 log CFU/cm^2 across three material surfaces by 21 d.
		22.5	50.4	0, 0.13, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21 d	Spot inoculated and spread uniformly; 4.94–5.38 log CFU/cm^2	*Salmonella* levels decreased from ca. 5.0 log CFU/cm^2 to below the detection limit (1.30 log CFU/cm^2) on 3, 9, and 12 d on plastic, wood, and pressed-card surfaces, respectively.
		3.2	22.9	0, 0.13, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21 d	Spot inoculated and spread uniformly; 6.39 to 6.93 log CFU/cm^2	*L. monocytogenes* levels declined significantly by 1.09, 0.52, and 2.26 log CFU/cm^2 on pressed-card, wood, and plastic surfaces, respectively, within the first day. The *L. monocytogenes* population remaining on pressed-card, wood, and plastic surfaces were as high as 4.89 to 5.73 log CFU/cm^2, respectively, by the end of storage.
		22.5	50.4	0, 0.13, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21 d	Spot inoculated and spread uniformly; 6.39 to 6.93 log CFU/cm^2	It took 21 d for *L. monocytogenes* counts to decrease below the detectable limit on wood and plastic surfaces (still present on surfaces after enrichment), whereas there were 2.03 log CFU/cm^2 of the

						pathogen remaining on pressed-card surfaces by the end of storage.
(*90*)	Cardboard, plastic	25	-	1, 8, 24, 48 h	Spot inoculated; between 2 to 5 log CFU/cm^2	At inoculation levels of about 2 and 3 log CFU/cm^2, *Salmonella* Enteritidis reached cell loads under the detection limit (1 log CFU/ cm^2) after 1 and 8 h on cardboard and plastic surfaces, respectively. When inoculation levels were increased to about 5 log CFU/cm^2, the *Salmonella* population was below the limit of detection after 8 and 48 h on cardboard and plastic surfaces, respectively.
		25	-	1, 8, 24, 48 h	Spot inoculated; between 3 log CFU/cm^2	At inoculation levels of about 3 log CFU/cm^2, *L. monocytogenes* reached cell loads under the detection limit (1 log CFU/cm^2) after 1 and 8 h on cardboard and plastic surfaces, respectively. The reduction was significantly higher on cardboard than on the plastic surface.
(*91*)	Laminated and matte paper	23	-	0, 6, 24, 48, 72 h	Spot inoculated and spread; between 6 log CFU/cm^2	*E. coli* and *Salmonella* did not survive on matte paper menus passed 0 h. Approximately 2.7 log CFU/cm^2 of *E. coli* survived on the laminated coupons for 24 h. *Salmonella* survived on the laminated paper coupons from 0 h (ca. 4 log

						CFU/cm^2) to 72 h (ca. 2.7 log CFU/cm^2).
(*53*)	Stainless steel	22 - 25	40 - 45	96 h	Spot inoculation and spreading; ca. 7 log CFU/cm^2	Viable cells of *Salmonella* Enteritidis could still be detected after 96 h.
		22 - 25	40 - 45	96 h	Spot inoculation and spreading; ca. 5 log CFU/cm^2	*Salmonella* Enteritidis cell count decreased within 24 h to levels below the detection limit.
		22 - 25	40 - 45	96 h	Spot inoculation and spreading; ca. 3 log CFU/cm^2	*Salmonella* Enteritidis decreased within 1 h to levels below the detection limit.
(*124*)	Reusable plastic containers	23	55	Cucumber homogenate was sprayed on 5, 15, 25, 35, 45, and 55 d to mimic the introduction of produce residue	Spot inoculation of cocktail at 7 log CFU/mL; cocktail was diluted in cucumber homogenate	The die-off rate was significantly higher (P<0.05) at this RH compared to 85% RH. The level increased when cucumber homogenate was introduced. Further spraying at 5 d intervals did not increase *Salmonella* numbers but resulted in a reduced die-off rate compared to when no homogenate was introduced.

				between uses. Recovery on 0, 2, 5, 10, 20, 40, 60 d.		
		23	85	Cucumber homogenate was sprayed on 5, 15, 25, 35, 45, and 55 d to mimic the introduction of produce residue between uses. Recovery on 0, 2, 5, 10, 20, 40, 60 d.	Spot inoculation of cocktail at 7 log CFU/mL; cocktail was diluted in cucumber homogenate	The die-off rate was significantly lower *(P*<0.05) at this RH compared to 55% RH. There was a decline in *Salmonella* levels within the first 5 d. The level increased when cucumber homogenate was introduced. Further spraying at 5 d intervals did not increase *Salmonella* numbers but resulted in a reduced die-off rate compared to when no homogenate was introduced.
(*30*)	Ceramic tile	21	50	0, 5 min and 2, 4, 8 h and 1, 2, 3, 5, 7, 14, 21, 28 d	-;8 log CFU/mL	Residence time and media type (0.1% peptone water, 1% tryptic soy broth, and 10% tryptic soy broth) had a significant effect on the presence of *Salmonella* Typhimurium (*P*<0.001). 3.5 to 4.5 log CFU/tile surface was recovered at 28 d. Bacterial survival with 10% TSB was 1 log greater than with other diluted media types from 8 h to 28 d. Bacterial die-off was biphasic (0-8 h and 8 h – 28d).
(*53*)	Stainless steel	22-25	40-45	0-100 h	Spot and spreading	The rapid decline in *Salmonella* Enteritidis levels by 1.7 log

					inoculation; ca. 7 log CFU/surface.	CFU/100cm^2 at 4 h post-inoculation. Detectable at least 96 h post-inoculation.
		22-25	40-45	0-100 h	Spot and spreading inoculation; ca. 5 log CFU/surface.	Rapid decline by 3.4 log CFU/100cm^2 in *Salmonella* Enteritidis level at 4 h post-inoculation. Decreased to levels below the limit of detection (0.62log CFU/100cm^2) within 24h.
		22-25	40-45	0-100 h	Spot and spreading inoculation; ca. 2 log CFU/surface.	*Salmonella* Enteritidis decreased to levels below the limit of detection (0.62 log CFU/100cm^2) within 1 h.
(*87*)	Cloths (dry woven 'J-cloth')	18-20	60	0, 1, 4, 24 and 48 h	Spot inoculation; ca. 3-4 log CFU/25 cm^2	On soiled clothes, the laboratory strain of *E. coli* was below the limit of detection by 24 h while the concentration of the wild-type strain ranged from 45 CFU to >250 CFU/25 cm^2 by 48 h. The levels of *Salmonella abony* (3 CFU/25 cm^2), and (below detection limit to 1 CFU/25 cm^2) varied at 48 h. Under clean conditions, the laboratory strain for *E. coli* was below the detection limit at 48 h. The levels for wild-type strains of *E. coli* (3 -18 CFU/25 cm^2), *Salmonella abony* (5-8

						CFU/25 cm^2), and (2-15 CFU/25 cm^2) varied at 48 h.
	laminated surfaces	30	40-45	0, 1, 4, 24, and 48 h	Spot inoculation; 300 CFU/100 µL.	On soiled surfaces, the levels of both strains (laboratory and wild type) of *E. coli* were between 0 and 3 CFU/25 cm^2 at 48 h. Levels of *Salmonella abony* (below detection limit - 3 CFU/25 cm^2), and (13 CFU/25 cm^2) varied at 48 h. On clean surfaces, the levels of both strains (laboratory and wild type) of *E. coli* were between 0 and 1 CFU/25 cm^2 at 48 h. *Salmonella abony* levels were below the detection limit at 48 h. Levels of *Salmonella* (below detection limit – 1 CFU/25 cm^2) varied at 48 h.
(*9*); Spinach and lettuce	-	-	-	0, 4, 8, 24, 48, 72 and 96 h	Spraying; 3.68 to 5.84 log CFU/mL	*E. coli* reduction over 4 d ranged from 3.48 to 4.40 log CFU/100 g of produce in California trials, 2.29 to 4.21 log CFU/100 g of produce in New York trials, and 2.63 to 4.97 log CFU/100 produce in Spain trials. No significant difference in die-off rates (P=0.68) between the starting population level groups was observed; low (<4.8 log CFU/100 g) or high (>4.8 log CFU/100 g). Overall, the log-linear die-off rate across trials, produce type and bacteria was -0.60 (-0.63, -0.58) log/day. Among trials,

						log-linear die-off varied and ranged from -0.20 (-0.27, -0.13) log/day in New York 1 trial to -1.01 (-1.06, -0.95) log/day in Spain 1 trial. Overall segmented log-linear die-off rate across all trials was -4.41(-4.69, -4.12) log/day for 0 to 10 h and -0.3 (-0.33, -0.26) log/day for 10 to 96 h. Among trials, segment 1 die-off rate ranged from -0.46(-0.52, -0.41) log/day in California trial 2 to -6.99(-7.38, -6.59) log/day in California trial 1. Break points ranged from 2.5 h in New York trial 1 to 3.48 d in New York trial 2 and 9/11 trials had breakpoints at 12 h or earlier. Segment 2 die-off rate ranged from 0.28(-0.20, 0.77) log/day in New York trial 2 to -1.00(-1.16, -0.85) log/day in California trial 2. Segmented log-linear die-off was biphasic.
Spinach	-	-	-	0, 4, 8, 24, 48, 72 and 96 h	Spraying; 3.68 to 5.84 log CFU/mL	The mean log-linear die-off rate of *E. coli* on spinach was -0.72 ± 0.29 log CFU/day. Segmented log-linear die-off on lettuce was -5.07 ± 2.80 log CFU/day for segment 1 with a mean break point at 0.77 ± 0.95 d; mean die-off rate for segment 2 was 0.13 ± 2.79 log CFU/day.

Lettuce	-	-	-	0, 4, 8, 24, 48, 72 and 96 h	Spraying; 3.68 to 5.84 log CFU/mL	The mean log-linear die-off rate of *E. coli* on lettuce was -0.77 ± 0.21 log CFU/day. Segmented log-linear die-off on lettuce was -7.07 ± 3.41 log CFU/day for segment 1 with a mean break point at 0.68 ± 0.98 d; mean die-off rate for segment 2 was -0.24 ± 0.70 log CFU/day.
Spinach and lettuce	-	-	-	0, 4, 8, 24, 48, 72 and 96 h	Spraying; 3.77 to 5.84 log CFU/mL	The mean log-linear die-off rate of *Salmonella* on spinach was -0.45 ± 0.38 log CFU/day. Segmented log-linear die-off on lettuce was -2.37 ± 2.10 log CFU/day for segment 1 with a mean break point at 1.09 ± 0.86 d; mean die-off rate for segment 2 was -0.20 ± 0.55 log CFU/day. The mean log-linear die-off rate of *Salmonella* on lettuce was -0.55 ± 0.24 log CFU/day. Segmented log-linear die-off on lettuce was -3.71 ± 2.62 log CFU/day for segment 1 with a mean break point at 0.86 ± 0.84 d; mean die-off rate for segment 2 was -0.48 ± 1.21 log CFU/day.
(*6*)	Soil	-	-	0, 0.17, 1, 2, 4, 7, 10, 14, 21, 28, 56, 84, 112, 168, 210, 252, and 336 d	Spot inoculation, rubbing, and shaking; ca. 4 log CFU/g	Soil type, irrigation regimen, and amendment impacted *Salmonella* survival significantly ($P \leq 0.05$). Bacterial survival ranged between 84 and 210 d. Strain accounted for up to 18% of the variance in survival and 9% of the variance in die-off rate in

						generalized linear models. Log-linear die-off rates for bacterial strains ranged from -0.02 to -0.06 log CFU/day highlighting the varying impact of soil type on different strains. Strains in soil samples that were irrigated weekly survived 128 (101, 154.12); *P*<0.001) d longer than strains in soil samples that were irrigated daily. Similarly, the use of poultry amendment increased survival by 89 d (61.32, 116.02; (*P*<0.001). The mean daily die-off rates in soil type, irrigation, and amendment model were -0.03(-0.03,-0.03; *P*<0.001), -0.02 ((-0.02,-0.02; *P*<0.001), and -0.05(-0.05,-0.04; *P*<0.001), respectively.
Walnut Kernels; (*11*)	-	-	23	21 to 1076 d	Spot Inoculation and massaging; 3.3 to 9.9 log CFU/g	*Salmonella* rate of decline during the first 35 to 49 d of study was 0.25 or 0.29 log CFU/g per month and dropped to 0.05 or 0.10 log CFU/g per month during the remaining 1.5 to 3 years of storage.
	-	-	23	35 to 1065 d	Spot Inoculation and massaging; 2.9 – 9.5 log CFU/g	*E. coli* O157:H7 decline rate per month ranged from 0.21 to 0.86 log CFU/g per month.

	-	-	23	35 to 105 d	Spot Inoculation and massaging; 3.8 – 9.6log CFU/g	*Listeria monocytogenes* decline rate per month ranged from 1.1 to 1.3 log CFU/g per month.
Almonds; (*101*)	-	-	23	161 to 559 d	Spot Inoculation and massaging; 1.2 – 8.5 log CFU/g	The reported log-linear die-off rate with the *Salmonella* cocktail was -0.29 log CFU/month from a 5.8-log CFU/g starting level over a 172-d storage period. The die-off rate for a single strain of *Salmonella* Enteritidis PT 30 ranged from -0.16 log CFU/month to -0.32 log CFU per month.
	-	-	4	171 to 559 d	Spot Inoculation and massaging; 5.8 – 8.5 log CFU/g	The reported log-linear die-off rate with the *Salmonella* cocktail was -0.019 log CFU/month from a 5.8-log CFU/g starting level over a 172-d storage period. The die-off rate for a single strain of *Salmonella* Enteritidis PT 30 ranged from -0.052 log CFU/month to -0.018 log CFU per month.
	-	-	-20	172 to 559 d	Spot Inoculation and massaging;	The reported log-linear die-off rate with the *Salmonella* cocktail was -0.043 log CFU/month from a 5.8 log CFU/g starting level over a 172-d storage period. The die-off rate for a

					5.8 – 7.9 log CFU/g	single strain of *Salmonella* Enteritidis PT 30 was 0.0027 log CFU per month from a starting level of 7.9 log CFU/g over a 559-d trial period.
Basil, Cilantro, Dill, Parsley; (*7*)	-	62 ± 10	21 ± 5	0, 1, 2, 3, 7, 14, 21, 28 d	Spray inoculation; 4.2 ± 0.1 to 5.1 ± 0.1 log CFU/g	*L. monocytogenes* levels reduced to 0.9 ± 0.3, 0.6 ± 0.3, 1.1 ± 0.3, >1.1 ± 0.0 from starting levels of 4.6 ± 0.1, 4.9 ± 0.1, 4.2 ± 0.1 and 5.1 ± 0.1 log CFU/g on basil, cilantro, dill, and Parsley respectively. Bacterial populations on dill fell below the detection limit at 14 and 21 d but regrew at 28 d. From 7 d, bacterial populations on parsley were below the detection limit.
(*59*)	Nylon, Acetal resin, 440C stainless steel, 304 stainless steel, Solid propylene	-	25	14 and 30 d, and monthly thereafter	11 ± 0.5 log CFU/mL	*Salmonella* decline was linear. Storage temperature had a significant effect (*P*<0.05) with a greater decline observed at this temperature. The mean die-off at this temperature was not significant (-0.02 ± 0.01; (*P*>0.05).
	Nylon, Acetal resin, 440C stainless steel,	≤34	4	14 and 30 d, and monthly thereafter	Immersion; 11 ± 0.5 log CFU/mL	*Salmonella* decline was linear. Bacterial decline at this temperature was significantly lower (*P*<0.05). The mean die-off at this temperature was

	304 stainless steel, Solid propylene					not significant (-0.001 ± 0.01; (*P*>0.05).
Onions; (*86*)		-	4	0, 1, 2, 5, 7 d	Immersion; ca. 6.4 log CFU/mL	Avirulent and virulent strains of *L. monocytogenes* survived up to 7 d with recovery rates of 5.2 ± 0.3 and 5.6 ± 0.2 log CFU/onion after 7 d of storage, respectively. The decline of avirulent strains was significant by 7 d (*P*>0.05).
(*75*)	Conveyor belts, nylon brushes, foam pad	-	25	0, 2, 4, 7, 10, 14 d	Spot inoculation; 4.5 log CFU/mL	*L. monocytogenes* declined to levels below the limit of detection (≤0 log CFU/surface) on clean polyvinyl chloride, polyurethane, and nitrile rubber belts by 14 d. Bacterial levels on clean foam pads and nylon brushes were 0.8 ± 1.4 log CFU/surface and 0.6 ± 1.0 log CFU/g respectively by 14 d. Populations on the foam pads and the nylon brushes were similar but significantly higher than populations on other materials (*P*<0.05).
		-	25	0, 2, 4, 7, 10, 14 d	Spot inoculation; 4.5 log CFU/mL	Some soiled surfaces received 1 ml of water 24 hours before enumeration. *L. monocytogenes* populations remained unchanged for both soiled surfaces that did not

								receive any water and those that did. An increase of 1.0-3.0 log CFU/surface was observed in foam pads that received water.

a. Temperature.
b. Relative humidity.
c. No data were available.

Table 2. Summary of studies on the transfer of foodborne pathogens between food and FCS.

Ref[a]	Source	Target surface	Temp[b] (°C)	RH[c] (%)	Contact time	Sampling time points or inoculum dry time	Inoculation method and starting level	Reported outcomes
(*124*)	Reusable plastic containers	Intact cucumber sections	10 or 23	55 or 85	30 s, 8, 24, and 72 h	5 d (incubation at 23°C at 85 % RH for biofilm formation)	Spot inoculation; 3 log CFU/3 cm^2; *Salmonella* biofilm	The three-factor interaction effect of *Salmonella* transfer to cucumbers was not significant ($P = 0.684$). Significant interactions were found between storage temperature and contact time ($P = 0.009$). Contact time extension to 72 h resulted in a significant increase ($P < 0.001$) in transfer relative to the shorter contact time at high RH. There was a significant interaction between

								incubation temperature and RH (higher transfer coefficient at 23 °C and 85%). The transfer coefficient was independent of RH at 10 °C.
	Intact cucumber sections	Reusable plastic containers	10 or 23	55 or 85	30 s, 8, 24, and 72 h	1 h	Immersion; 7 log CFU/mL; *Salmonella* biofilm	The interaction between temperature and contact time was significant (P<0.001). At contact time > 24 h, the transfer coefficient was positively influenced by incubation temperature (P< 0.001) but less so at contact times <24 h. The transfer was impacted by high temperature and RH. The transfer coefficient was independent of temperature at 55 % RH.
(*23*)	Chicken	Hands	Ambient	-[d]	-	15 minutes	Spot inoculation; ca. 8 log CU/mL	*Enterobacter aerogenes* B199A concentration on participants' hands ranged from 5.94 to 8.38 log CFU (0.363 to 100% transfer).
	Hands	Spigots	Ambient	-	-	-	Participants cut previously	After turning on the metal spigot, 2.43 to 5.74 log

						inoculated chicken; NA	CFU (0.001 to 12.303%) of bacteria transferred to the spigot surface.
Spigots	Hands	Ambient	-	-	-	Turning on water faucet using previously contaminated hands from cutting contaminated chicken; 7.43 ± 0.61 log CFU	Participants' hands had some level of bacteria. There was no obvious difference in the transfer rate of bacteria between conventional (hand-operated) and non-conventional (non-hand-operated). Hands before washing contained 7.43 ± 0.61 log CFU (normal distribution). After hand washing, hands contained 4.68 ± 1.17 log CFU (normal distribution). The contaminated spigot had 4.42 ± 1.04 log CFU on the contaminated spigot (normal distribution).
Spigots	Hands	Ambient	-	-	-	Turning on the water faucet using previously contaminated hands from cutting	Participants' hands were clean. The transfer rates ranged from 0.021 to 72.4%. After washing hands, and hand drying with a paper towel, hands contained 1.0 to 4.1 log

							contaminated chicken.	CFU of *E. aerogenes* based on the individual.
	Spigot	Hand	Ambient	-	-	-	Handwashing without handling the spigot.	the transfer to participants' hands after washing and drying ranged from 3.34 to 6.46 log CFU.
	Spigot	Hand	Ambient	-	-	-	Handling contaminated spigots after washing hands.	The transfer to participants' hands after washing and drying ranged from 1.90 to 6.30 log CFU.
	Hand	Lettuce	Ambient	-	-	-	Residual bacteria from washing hands without handling the spigot; ca. 4 log CFU/hand.	When lettuce was handled with washed hands, up to 3.81 log CFU (0.003 to 100 % transfer) of *E. aerogenes* was transferred.
	Cutting board	Lettuce	Ambient	-	-	1 minute	Cutting chicken portion inoculated at ca. 6 log CFU/mL.	Transfer rates between the cutting board and lettuce varied from 0.34 to 54.55%.
(*47*)	Gloved hand	Carrot	-	-	-	1 minute	Spot inoculation	ca. 30% of *E. aerogenes* transferred from gloved

						and rubbing with both hands; 5.5 log CFU/gloved hand.	hands to carrots. Significant differences were observed between transfer rates to carrots and cantaloupe (*P*≤0.05). ca. 3 to 30 % of *E. coli* O157:H7 was transferred to carrots. Significant differences were observed between transfer rates to carrots and cantaloupes or between carrots and celery(*P*≤0.05).
Gloved hand	Celery	-	-	-	1 minute	Spot inoculation and rubbing with both hands; 5.5 log CFU/gloved hand	ca. 10 to 30% of *E. aerogenes* transferred from inoculated gloved hands to celery. No significant differences were observed between transfer rates to celery and cantaloupes, or between transfer rates to carrots and celery (*P*>0.05). 10 % of *E. coli* O157:H7 was transferred to celery. Significant differences were observed between transfer rates to carrots and celery (*P*≤0.05).

	Gloved hand	Cantaloupe	-	-	-	1 minute	Spot inoculation and rubbing with both hands; 5.5 log CFU/gloved hand	ca. 10 to 30% and ca. 30% of *E. aerogenes* and *Salmonella* transferred from inoculated gloved hands to cantaloupe (*P*>0.05). It was confirmed that *E. aerogenes* is a suitable surrogate for *Salmonella* in transfer studies. Significant differences were observed between transfer rates to carrots and cantaloupes (*P*≤0.05). 3% of *E. coli* O157:H7 was transferred to cantaloupes. Significant differences were observed between transfer rates to carrots and cantaloupes or between carrots and celery (*P*≤0.05).
	Carrot	Gloved hand	-	-	-	20 minutes	Spot inoculation in a bag and rubbing;	0.3 to 1% of *E. aerogenes* and *Salmonella* transferred from inoculated cantaloupe to gloved hands. No significant difference among transfer rates from inoculated produce to gloved hands

							was observed (*P*>0.05). 1% of *E. coli* O157:H7 transferred from carrots to gloved hands. No significant difference was detected between transfer rates the transfer rates from carrots to celery (*P*>0.05).
Celery	Gloved hand	-	-	-	20 minutes	Spot inoculation in a bag and rubbing;	0.3 to 1% of *E. aerogenes* and *Salmonella* transferred from inoculated cantaloupe to gloved hands. No significant difference among transfer rates from inoculated produce to gloved hands was observed (*P*>0.05). 0.3 to 1% of *E. coli* O157:H7 transferred from celery to gloved hands. No significant difference was detected between transfer rates the transfer rates from carrots to celery (*P*>0.05).
Cantaloupe	Gloved hand	-	-	-	20 minutes	Spot inoculation in a bag and rubbing;	0.3 to 1% of *E. aerogenes* and *Salmonella* transferred from inoculated

								cantaloupe to gloved hands (*P*>0.05). It was confirmed that *E. aerogenes* is a suitable surrogate for *Salmonella* in transfer studies. No significant difference among transfer rates from inoculated produce to gloved hands was observed (*P*>0.05). 0.3% of *E. coli* O157:H7 transferred from cantaloupe to gloved hands. Transfer rates from cantaloupe were significantly lower than from carrots and celery (*P*≤0.05).
(*98*)	Soil	Tomatoes	Ambient	-	1-5 s or 24 h	0, 1, and 24 h	Spot inoculation ; ca. 6 log CFU/surface ; *Salmonella enterica* cocktail	For wet and 1 h dry time, the transfer rate from Florida soil to tomatoes was significantly higher (*P*<0.05) after a 24 h contact time. At a 24-h dry time in this scenario, the *Salmonella* concentration that transferred to tomatoes was below the limit of detection (1.4 log CFU/tomato) but samples

							were positive for *Salmonella* upon enrichment. The transfer of *Salmonella* from Ohio soil to tomatoes was below the limit of detection for all inoculation conditions and needed enrichment (10/10 from wet inoculated soil to tomatoes at 1-5s contact time; 9/10 at 24 h contact time). At 1 h dry time, 6/10 0r 7/10 were positive at both contact times. At a 24 h dry time, 5/10 were positive.
Plastic mulch	Tomatoes	Ambient	-	1-5 s or 24 h	0, 1, and 24 h	Spot inoculation; ca. 6 log CFU/surface; *Salmonella* cocktail	*New plastic mulch*; In all cases, when new Florida mulch was allowed to dry for 24 h post-inoculation, *Salmonella* concentration was below the limit of detection (1.4 log CFU/tomato). At 24 h contact time, transfer from new mulch to tomatoes was significantly lower

compared to 1 – 5 s touch ($P<0.05$). Transfer from inoculated Maryland new mulch and from inoculated tomatoes to recipient surfaces at wet and 1 h dry times were not significantly different ($P \geq 0.05$). The log percent transfer varied from 1.43 to 1.80 bidirectionally in these scenarios. At a 24 h dry time, new mulch did not transfer any detectable *Salmonella* while transfer occurred in 16 of 20 scenarios when tomatoes were inoculated similarly.

When new Ohio mulch was wet, there was a higher mean log % transfer of *Salmonella* to tomatoes. There was no significant difference in transfer from inocula dried for 1 h or 24 h for either contact time ($P \geq 0.05$).

Used plastic mulch; Inoculated used Florida

mulch that was allowed to dry for 24 h and was in contact with tomatoes for 24 h resulted in varying transfer outcomes (0.40 to 1.85 log %; 3 negative samples upon enrichment with highest transfer at 1.85 log %. Only enrichments at 1-5s contact times were positive. Log % transfer for wet and 1 h did not vary significantly from transfer from mulch or transfer from tomatoes. In the case of Florida, the transfer from used and new mulch to tomatoes and vice versa was similar.

For wet and 1 h dry time, transfer from used Maryland mulch and tomatoes to respective recipient surfaces did not differ significantly ($P \geq 0.05$; 0.96 to 1.66 from used Maryland mulch to tomatoes; 1.27 to 1.57 from Maryland tomatoes to used mulch). At 24h dry time, transfer

							was observed from used mulch to Maryland tomatoes but was not recovered when occurring in the opposite direction. Similar transfer rates (ca. 1.5 log % transfer) were observed at both wet and 1 h inoculum dry time (*P* > 0.05) from used Ohio mulch to tomatoes. At 24 h inoculum dry time, *Salmonella* was only recovered via enrichment (10 positive samples out of 10 samples at 1-5 s contact time and 0/10 for 24 h contact time).
Tomatoes	Soil	Ambient	-	1-5 s or 24 h	0, 1, and 24 h	Spot inoculation; ca. 6 log CFU/surface; *Salmonella* cocktail	At the 0 and 1 h dry times, the transfer from Florida tomatoes to Florida soil was significantly different from equivalent transfers in the opposite direction (*P*<0.05). At a 24 h dry time and 1-5 s contact time, the transfer from Florida tomatoes to soil was below the limit of detection although

							positive upon enrichment in this case but was not when contact time was 24 h. Transfer of wet inoculum from Maryland soil to tomatoes ranged from 0.72 to 1.78% transfer at 1-5 s contact time. There was more variability at a 24 h contact time ranging from negative values upon enrichment to 1.52 log % transfer. At 24 h dry time, no transfer to tomatoes was observed upon enrichment at 1-5 s contact time. At a 1 and 24 h inoculum dry time, 2 and 3 out of 10 enrichments were observed at a 24 h contact time with soil.
Tomatoes	Plastic	Ambient	-	1-5 s or 24 h	0, 1, and 24 h	Spot inoculation ; ca. 6 log CFU/surface ; *Salmonella enterica* cocktail	*New plastic mulch.* At a 24 h contact time, there was a significantly higher transfer from tomatoes to new Florida mulch at 0 and 24 h dry times compared to the brief touch ($P<0.05$).

Transfer of *Salmonella* from Ohio tomatoes to new mulch was like transfer in the opposite direction with a higher mean log % occurring with a wet inoculum. *Used plastic mulch;* The log % transfer rates from Florida tomatoes to mulch for wet and 1 h dry times did not vary significantly. The transfer was 1.27 to 1.57 log % from Maryland tomatoes to used mulch. At 24 h dry time, the transfer did not occur from inoculated tomatoes to used Maryland mulch. Though slightly higher, the transfer of *Salmonella* from Ohio tomatoes to used mulch was like the transfer in the reverse direction. At 24h contact time, 10/10 positive used mulch samples were observed at a 1-5s contact time. At 24 h contact time 4/10 (below the limit of detection; 1.4 CFU/tomato) used mulch

							samples were in the countable range (ca. 1.13 log %), while 6/10 were positive upon enrichment.
Tomatoes	Soil	Ambient	-	1-5 s or 24 h	0, 1, and 24 h	Spot inoculation ; ca. 6 log CFU/surface; *Salmonella enterica* cocktail	There was a wider range of transfer from inoculated tomatoes to Maryland soil. Wet inoculum had the highest transfer frequency at 1.93 mean log % transfer at 1-5 s contact time. At wet or 1-h dry time and 1-5s contact time, transfer from Ohio tomatoes to soil ranged between 1.75 to 2 log. At a 24-h contact time, soil samples (10/10) were positive by enrichment only. At 1-h dry time and 24-h contact time, transfer from tomatoes to soil was at a ratio of 4/10. At 24-h dry time and 1-5 s touch, results were like when tomatoes were inoculated and dried for 1-h and contact time with the soil for 24 h (6/10). At a 24-h dry time and a 24-h contact time, 1/10 was

								positive from tomatoes to soil.
(*30*)	Tile	Bologna	21	50	5, 30 and 60s	0,2,4,8, 24 h	Spot inoculation and spreading; 7-8 log CFU/mL	Residence time (*P*<0.0001), exposure time of bologna (*P*<0.0001), and residence time by exposure time interaction (*P*<0.0001) had significant effects on the transfer of *Salmonella* Typhimurium from tile surfaces. There was a significant decrease (*P*<0.05) in the concentration of bacteria recovered from bologna as residence/drying time increased.
	Wood	Bologna	21	50	5, 30 and 60s	0,2,4,8, 24 h	Spot inoculation and spreading; 7-8 log CFU/mL	Residence time and residence time by exposure time had a significant effect on *Salmonella* Typhimurium (*P*<0.0001). Bologna exposure time was not significant (P = 0.24). There was a significant decrease (*P*<0.05) in the concentration of bacteria recovered from bologna

							as residence/drying time increased.
Carpet	Bologna	21	50	5, 30 and 60s	0,2,4,8, 24 h	Spot inoculation and spreading; 7-8 log CFU/mL	Residence time (*P*<0.0001), exposure time of bologna (*P*=0.0047), and residence time x exposure time interaction (*P*=0.038*)* had significant effects on the transfer of *Salmonella* Typhimurium from the carpet surface. There was no difference in bacterial concentration recovered from bologna as residence/drying time increased at 0, 2, and 4 h. From 8 h to 24 h, the transfer rate declined steadily.
Tile	Bread	21	50	5, 30 and 60s	0,2,4,8, 24 h	Spot inoculation and spreading; 7-8 log CFU/mL	Residence/drying time had a significant effect on the transfer of *Salmonella* Typhimurium to white bread *(P*<0.0001). Contact time did not influence transfer to bread except at an 8 h residence time where >0.5 log CFU/cm^2 less than that recovered at 30

								and 60 s contact times ($P<0.01$).
(*35*)	Hands washed with antimicrobial hand soap	Fresh cantaloupe balls	25 ± 5	-	15 s	-	Touching; ca. 6 log CFU/hand	After a single wash with antimicrobial soap for 15 seconds, 2.46, and 2.84 log CFU/hand of *E. coli* was left on the participants' hands. The mean transfer to cantaloupe balls after washing was 2.00 and 2.36 log CFU/ball, respectively.
	Hands washed with non-antimicrobial hand soap	Fresh cantaloupe balls	25 ± 5	-	15 s	-	Touching; ca. 6 log CFU/hand	After a single wash with non-antimicrobial soap for 30 s, 4.64 and 4.22 log CFU/hand of *E. coli* was left on participants' hands. The mean transfer to cantaloupe balls after washing was 3.48 and 3.43 log CFU/ ball, respectively.
(*49*)	Glass	Peeled carrots, cut celery, cut watermelon, cut lettuce	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	The mean percentage transfer almost always occurred and was very high when surfaces were wet post-inoculation (96.84, 89.88, 86.62% for *Salmonella* in carrots,

							celery, and watermelon and 84.52 for *E.coli* in lettuce). At 1 h drying time, 99.17, 4.93(1.43-16.39%), and 92.52% were reported for *Salmonella* in carrots, celery, and watermelon, and 1.97(0.37-9.09%) for *E. coli* in lettuce.
Ceramic tile	Peeled carrots, cut celery, cut watermelon, cut lettuce	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	Transfer almost always occurred and was very high when surfaces were wet post-inoculation (79.57, 90.40, 92.55% for *Salmonella* in carrots, celery, and watermelon and 91.38 for *E.coli* in lettuce). At 1 h drying time, 69.58(25-90.91%), 48.81(22.22-65.79%), 86.91% was reported for *Salmonella* in carrots, celery, and watermelon, and 4.13(0.49-16.67%) for *E. coli* in lettuce. More variability was observed in carrots, celery, and lettuce at 1 h dry time.

Plastic	Peeled carrots, cut celery, cut watermelon, cut lettuce	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	Transfer was very high when surfaces were wet post inoculation (97.70, 87.72, 97.41% for *Salmonella* in carrots, celery, and watermelon and 86.19 for *E.coli* in lettuce). At 1 h drying time 99.91, 17.44 (12.86-27.59%), 89.39% was reported for *Salmonella* in carrots, celery, and watermelon, and 18.64 (2.27-73.77%), for *E. coli* in lettuce.
Stainless steel	Peeled carrots, cut celery, cut watermelon, cut lettuce	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	Transfer was high when surfaces were wet post-inoculation (91.75, 83.36, 94.64% for *Salmonella* in carrots, celery, and watermelon and 91.28 for *E.coli* in lettuce). At 1 h drying time, 84.75, 0.74(0.20-2.17), and 80.00% were reported for *Salmonella* in carrots, celery, and watermelon, and 39.46(7.09-71.96) for *E. coli* in lettuce.
Cut celery	Glass, Ceramic	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation;	*Salmonella* transfer when surfaces were wet was

	tile, Plastic, and stainless steel					ca. 6 log CFU/surface (*Salmonella* cocktail)	7.72(5.23-9.77%) in ceramic, 8.85(6.74-10.58%) in glass, 8.77(5-13.56%) in plastic, and 15.57 (6.45 -23.73%) in stainless steel. At a 1 h dry-time transfer was 0.01(<0.01-0.02%) in ceramic, 0.01(0.01-0.03) in plastic, 14/20 stainless steel samples below the limit of detection, and 0.29(<0.01-2.02%) in 6/20 stainless steel samples, and 2.96 (1.94-3.54%) in glass.
Cut watermelon	Glass, Ceramic tile, Plastic, and stainless steel	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	Percent transfer of *Salmonella* from freshly inoculated (wet) watermelon was 1.48(0.69 -2.87%) in ceramic, 0.21(0.18-0.24) in glass, 2.64(1.65-4.31) in plastic and 2.86(0.63-8.31%) in stainless steel. At 1 h dry-time, transfer was 1.65(1.04-2.71) in ceramic, 0.74(0.25-1.44%) in glass, 0.73(0.56-1.17%) in plastic and 2.47%(1.17-4.44%) in stainless steel.

Cut romaine lettuce	Glass, Ceramic tile, Plastic, and stainless steel	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6log CFU/surface (*Salmonella* cocktail)	Transfer of *E.coli* from freshly inoculated lettuce was 13.77(10.24-16.98%) in ceramic, 13.97 (10.13-18.23%) in stainless steel, 8.86(1.59-19.19%) in glass and 6.40(3.86-8.92%) in plastic. At 1h dry time, percent transfer was below the detection limit in 3/20 ceramic samples and 0.01(<0.01-0.03%) in 17/20 ceramic samples, 1.54(0.64-4.47%) in stainless steel, 0.47(<0.01 to 1.98) in 14/20 glass samples and below the limit of detection in 6/20 and 0.41(<0.01 to 1.99) with 3/20 plastic samples below the limit of detection.
Peeled carrots	Glass, Ceramic tile, Plastic, and stainless steel	-	-	1-2s (brief)	<5s (wet), 1h	Spot inoculation; ca. 6 log CFU/surface (*Salmonella* cocktail)	High variability in percent transfer of *Salmonella* immediately after inoculation (wet). The transfer was 0.90 (range: 0.11-1.97%) for ceramic surface, 2.32

								(range:0.21-8.25%) for glass, 2.46(range: 0.56-5.04%) for plastic, 1.24% (range: 0.53-1.86%) for stainless steel. At 1 h, transfer was 0.01(<0.01-0.07%) to ceramic, below limit of detection in 12/20 glass samples and <0.01(<0.01-0.01%) in 8/20, <0.01% in plastic and 0.10(0.03-0.31%) in stainless steel.
(*48*)	Unchlorinated municipal tap water	Lettuce	22	-	30s and 1, 2 or 5 min; tryptic soy agar with 80 ppm rifampicin(TSAR)	ca. 10 min	Spot inoculation; ca. 6 log CFU/mL; *E. coli* O157:H7 cocktail	On TSAR, after the inoculated lettuce leaf was washed for 30 seconds, two observations had ca. 2 log reduction and one had ca. 2.5 log reduction. At 1 min wash time, one observation had ca. 2 log reduction and two observations had ca. 2.5 log reduction. At wash times of 2 and 5 mins, all observations had ca. 2.5 log reduction. Log reductions between wash times were not significant (P=0.05). Media type did

							not affect the mean recovery and *E. coli* transferred to wash water in all scenarios (90-99%). At 30 s and 5 min wash times, the most frequent transfer of *E. coli* from inoculated to uninoculated lettuce was 0 log% (ca. 1%). The range was -0.25 – ca. 1 log %(0.6% - ca. 10%). At 1- and 2-min wash times, transfer rates frequently observed were much lower 0.25 log%(ca. 0.6%). Range was 0.0 – 0.5 log% (1 – ca. 3.2%)
Unchlorinated municipal tap water	Lettuce	22	-	30s and 1, 2 or 5 min; sorbitol macConkey agar with 80 ppm rifampicin(SMACR)	ca. 10 min	Spot inoculation; ca. 6 log CFU/mL; *E. coli* O157:H7 cocktail	With SMACR 30 seconds and 5-minute wash times had two instances of ca. 2 log reduction and one instance of 2.5 log reduction while at 1 and 2 min, it was reversed. Log reductions between wash times were not significant (*P*=0.05). Media type did not affect the mean recovery and *E. coli*

								transferred to wash water in all scenarios (90-99%). All wash times frequently showed 0 log % (ca. 1%) transfer from inoculated lettuce to uninoculated lettuce. Range = ca. 0.25 to ca. 0.5 log percent.
(*54*)	Chicken	Stainless steel	-	-	5 min	15 min	ca. 6 log CFU/mL	The mean *Salmonella* Enteritidis transfer rate from chicken to stainless steel was 1.6% (range ca. 1 – ca. 3%).
	Stainless Steel	Sliced cucumber	-	-	10s	-	-	The mean *Salmonella* Enteritidis transfer rate from contaminated stainless steel to sliced cucumber was 34.8% (range ca. 1 – ca. 80%).
(*53*)	Stainless steel	Contact plate	22-25	40-45	10s; 500g/plate pressure	-	50 ± 14 CFU/cm^2	*Salmonella* Enteritidis; 11 ± 2 CFU/cm^2 (23 ± 6 %) recovery with single contact plates and 20 ± 2 CFU/cm^2 (42 ± 6 %)recovery with five contact plates from

							starting contamination levels.
Sponge	Stainless steel	22-25	40-45	-	-	Spreading with a sponge; ca. 9, or ca. 7-8 log CFU/10mL	Test microorganisms (*Salmonella* Enteritidis, *Campylobacter jejuni* *Staphylococcus aureus;* *P* =0.07) and contamination levels (*P* =0.30) did not affect transfer rates. 8 to 9 log CFU/4000 cm^2 and about 7-to 8 log CFU/4000 cm^2 were recovered from surfaces at high and moderate sponge inoculation levels.
Stainless steel	Cucumber	22-25	40-45	10 s; 0 or 500 g/slice pressure	0 minute (cucumber sampling time point post transfer)	Spreading with a sponge; ca. 3-4 log CFU/surface	Sampling time after contamination (0 or 15 min) did not affect the transfer rate to cucumbers (*P*=0.26 with pressure, *P*=0.46 without pressure). Microorganism type did not affect transfer to chicken with pressure but there was an effect without pressure (*P*=0.06 and *P*<0.001, respectively).

							Salmonella Enteritidis: With a starting level of 3.0 ± 0.2 log CFU/cm^2 on stainless steel, 3.0 ± 0.1 log CFU/cm^2 (105 ± 26%) with pressure, and 2.8 ± 0.2 log CFU/cm^2 (65 ± 21 %) transferred to cucumber with no pressure.
Stainless steel	Cucumber	22-25	40-45	10 s; 0 or 500 g/slice pressure	15 min (cucumber sampling time point post transfer)	Spreading with a sponge; ca. 3-4 log CFU/surface	Sampling time after contamination (0 or 15 min) did not affect the transfer rate to cucumbers (P=0.26 with pressure, P=0.46 without pressure). Microorganism type did not affect transfer to chicken with pressure but there was an effect without pressure (P=0.06 and P<0.001, respectively). *Salmonella* Enteritidis: With a starting level of 3.1 ± 0.3 log CFU/cm^2 on stainless steel, 3.0 ± 0.3 log CFU/cm^2 (90 ± 27%) with pressure, and 2.8 ± 0.3 log CFU/cm^2 (50 ± 18 %) transferred to

							cucumber with no pressure.
Stainless steel	Roasted chicken fillet	22-25	40-45	10 s; 0 or 500 g/slice pressure	0 min (chicken sampling time point post transfer)	Spreading with a sponge; ca. 3-4 log CFU/surface	Sampling time after contamination (0 or 15 min) did not affect the transfer rate to chicken (*P*=0.84) without pressure but had an effect with pressure (*P*=0.02). Microorganism type did not affect transfer to chicken with or without pressure (*P*=0.77 and *P*=0.52, respectively). *Salmonella* Enteritidis: With a starting level of 3.1 ± 0.3 log CFU/cm^2 on stainless steel, transfer to chicken was 3.1 ± 0.2 log CFU/cm^2 (94 ± 42%) with pressure and 2.8 ± 0.1 log CFU/cm^2 (49 ± 21%) with no pressure.
Stainless steel	Roasted chicken fillet	22-25	40-45	10 s; 0 or 500 g/slice pressure	15 min (chicken sampling time point post transfer)	Spreading with a sponge; ca. 3-4 log CFU/surface	Sampling time after contamination (0 or 15 min) did not affect the transfer rate to chicken (*P*=0.84) without pressure but had an effect with

								pressure (*P*=0.02). Microorganism type did not affect transfer to chicken with or without pressure (*P*=0.77 and *P*=0.52, respectively). *Salmonella* Enteritidis: With a starting level of 3.0 ± 0.0 log CFU/cm^2 on stainless steel, transfer to chicken was 2.8 ± 0.4 log CFU/cm^2 (55 ± 21%) with pressure and 2.9 ± 0.0 log CFU/cm^2 (32 ± 9%) with no pressure.
(*69*)	Stainless steel	Watermelon	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	*General:* Contact time, food, surface, and food x time interaction had a significant effect on the transfer of *Enterobacter aerogenes* B199A (*E. aerogenes*) (*P*<0.000001). Other significant factors were surface x time (*P* = 0.0019), surface x food (*P* = 0.00019), and surface x matrix (*P* = 0.00005). Inoculum matrix/carrier was less significant TSB or buffer

							(*P* = 0.013) and the food x matrix interaction (*P* = 0.045). The time x matrix interaction effect was not significant (*P* = 0.045). When the inoculum matrix was 0.1% peptone; the mean log transfer of *E. aerogenes* was 1.96 (91.20%) at <1s with a range of 1.96 to 1.99 log % (91.20 to 97.72 %) When the inoculum matrix was tryptic soy broth (TSB); the transfer of *E. aerogenes* was between 1.96 and 1.97 log % (91.20 and 93.33 %). No significant difference in log % transfer between surfaces with this matrix.
Stainless steel	White bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; Had the highest transfer of *E. aerogenes* to bread at 300s (1.91 log %; 81.28%). Mean log percent transfer ranged

							from -1.24 to 1.91 (0.06 to 81.28%). When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* ranged from - 0.95(0.1%) and 1.96 (91.20%).
Stainless steel	Unsalted butter with bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer of *E. aerogenes* was -0.86 and 1.42 (0.14 and 26.30%) from <1s to 300s. When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* was between -1.63 and 1.91 (0.02 and 81.28%).
Stainless steel	Gummy candy	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	The highest mean log % transfer of *E. aerogenes* for any surface was observed in this scenario at 300 s (1.80 log %; 63.10%). Increased over time across all matrices.

							When inoculum matrix was 0.1% peptone; Mean log % transfer of *E. aerogenes* ranged from -1.38 to -1.01(0.04 to 0.10%) When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* ranged from -0.92 to 1.80 (0.12 to 63.10%).
Ceramic glazed tile	Watermelon	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer of *E. aerogenes* was 1.17(14.79 %) and 1.96(91.20%). When the inoculum matrix was tryptic soy broth (TSB); the highest transfer of *E. aerogenes* was at 5 s; 1.99 (97.72%) with a range of 1.98 and 1.99 log % (95.50 to 97.72%).
Ceramic glazed tile	White bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and	When the inoculum matrix was 0.1% peptone; the mean log %

						spreading; ca. 7 log CFU/25cm^2 surface	transfer of *E. aerogenes* ranged from -0.68 – 1.79 (0.21 – 61.66%) with the highest transfer occurring at 300s (1.79 log%). When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* ranged from -0.95 – 1.96 (0.11 – 91.20%) with the highest transfer occurring at 30 s (1.96 log%) which was not significantly different from 1.95 log % at 300s.
Ceramic glazed tile	Unsalted butter with bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone: The transfer of *E. aerogenes* to bread with butter was highest (-0.86 to 1.67 log %; 0.13 to 46.77%) When the inoculum matrix was tryptic soy broth (TSB); the mean log percent transfer of *E. aerogenes* increased by -1.08 and 1.81 (0.08 and 64.57 %) from <1 to 300s.

Ceramic glazed tile	Gummy candy	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone: The mean log percent transfer of *E. aerogenes* ranged from -1.46 to -0.89 (0.04 to 0.12%). The highest mean log % transfer of *E. aerogenes* observed in this scenario at 300s (-0.89 log %; 0.12%) When the inoculum matrix was tryptic soy broth (TSB); the mean log percent transfer of *E. aerogenes* ranged from -0.88 to 0.28 (0.13 to 1.91%).
Maple laminate wood	Watermelon	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log transfer of *E. aerogenes* was 1.93 (86%) at <1s. When the inoculum matrix was tryptic soy broth (TSB); Log % transfer was between 1.96-1.97 (91.20 – 93.33%) across all contact times. No significant difference in the log % transfer of *E.*

							aerogenes between surfaces.
Maple laminate wood	White bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer of *E. aerogenes* ranged from -0.91 – 1.89 (0.12-77.63%) When the inoculum matrix was tryptic soy broth (TSB); log % transfer ranged from -0.64-1.97 (0.23-93.33%) with the highest transfer occurring at 30 s. Transfer at 30s (1.97 log %; highest) was not significantly different from transfer at 300 s (1.95 log %).
Maple laminate wood	Unsalted butter with bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean transfer of *E. aerogenes* was -0.29 to 1.48 (0.51 to 30.20%). When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of of *E. aerogenes* was between

							-1.18 and 1.81 (0.07 and 64.57%).
Maple laminate wood	Gummy candy	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer of *E. aerogenes* -1.20 to -1.13 (0.06 to 0.07%) When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* was -1.04 to 0.34 (0.09 to 2.19 %).
Carpet	Watermel on	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer mean log transfer of *E. aerogenes* ranged from -0.75 (0.2%) to 0.14 (1%) at <1s. When the inoculum matrix was tryptic soy broth (TSB); the log % transfer was between 1.91 – 1.95(81.28-89.13%) across all contact times. No significant difference in the log % transfer of *E.*

							aerogenes between surfaces.
Carpet	White bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone: The mean log % transfer of *E. aerogenes* to bread was between -1.68 to -0.79 (0.02 to 0.16 %) with the highest transfer occurring at 300s. At <1s, 19/20 were below the detection limit. When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* ranged from -0.87(0.1%) to 0.58(4%). Lowest of all 4 target surfaces. At <1s 18/20 were below the detection limit (2 log CFU).
Carpet	Unsalted butter with bread	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	When the inoculum matrix was 0.1% peptone; the mean log % transfer of *E. aerogenes* was -0.56 to 0.19 (0.27 to 1.55%). Lowest transfer compared to other source surfaces.

								When the inoculum matrix was tryptic soy broth (TSB); Transfer from the carpet was less but mean log percent transfer increased with time from -1.15 to 0.9 (0.07 to 7.94%).
	Carpet	Gummy candy	-	-	<1, 5, 30, and 300 s	5 h	Spot inoculation and spreading; ca. 7 log CFU/25cm^2 surface	The lowest mean log % transfer of *E. aerogenes* was observed at 300 s at -0.51 (0.3%). When the inoculum matrix was 0.1% peptone: The mean log percent transfer of *E. aerogenes* ranged from -1.36 to -1.22 log % (0.04 to 0.06 %). When the inoculum matrix was tryptic soy broth (TSB); the mean log % transfer of *E. aerogenes* ranged from -0.63 to -0.42 (0.23 to 0.38%).
(*70*)	Chicken	Cutting board	22	-	-	15min	Spot inoculation ; ca. 6.18 log CFU/source	Data for all activities showed a strong negative linear trend between log CFU of the inoculum on source and log % transfer (high concentration of

							bacteria from source resulted in less transfer to target surface and vice versa). Inoculum size also had a highly significant effect (*P*<0.0001) Had the smallest range for *E. aerogenes* inoculum size (ca. 0.5 log CFU/chicken portion) and the smallest range for log % transfer (ca. 1 log % CFU). The mean log % transfer was 1.05 log % (ranging from 0.48 to 1.49 log %).
Cutting board	Lettuce	22	-	-	-	Touching; 5.33 log CFU/source	The mean log % transfer of *E. aerogenes* was 0.79 log % (ranging from -0.47 to 1.73 log %). The inoculum size effect was significant (*P*<0.0001).
Chicken	Bare hand	22	-	-	-	Spot inoculation ; 8.37 log CFU/source	The mean log % transfer of *E. aerogenes* was 0.59 log % (ranging from -0.44 to 2.00 log %). Significant effect of inoculum size (*P*=0.0006).

Bare hand	Lettuce	22	-	-	-	Touching; 3.97 log CFU/source	The mean log % transfer of *E. aerogenes* was 0.21 log % (ranging from -2.54 to 2.00 log %). The inoculum size effect was significant (P<0.0001).
Spigot	Bare hand	22	-	-	-	Touching; 3.95 log CFU/source	The mean log % transfer of *E. aerogenes* was 0.16 log % (ranging from -1.70 to 2.00 log %). The inoculum size effect on transfer was significant (P=0.0077).
Bare hand	Spigot	22	-	-	-	Touching; 7.16 log CFU/source	The mean log % transfer of *E. aerogenes* was -1.08 log % (ranging from -2.95 to 1.09 log %). The inoculum size effect was significant (P=0.0027).
Gloved hand	Lettuce	22	-	-	-	Touching; 5.08 log CFU/source	Had the largest range for *E. aerogenes* inoculum size (ca. 5 log CFU/hand) and the largest range for log % transfer (ca. 5.5 log % CFU). The mean log % transfer of *E. aerogenes* was -1.26 log % (ranging from -3.98 to 1.53 log %). The inoculum size effect

								was significant (*P*=0.0021).
	Chicken	Gloved hand	22	-	-	-	Spot inoculation; 8.34 log CFU/source	The mean log % transfer of *E. aerogenes* was -2.94 log % (ranging from -4.40 to -0.62 log %). When the effect of inoculum size was determined for each scenario, this was the only case where the inoculum size effect was not significant (*P* = 0.1643). A slight negative linear trend is present regardless.
(*71*)	Chicken	Bare hand	-	-	-	15 min	Spot inoculation; ca. 8 log CFU/150-g portion	The mean % transfer of *E. aerogenes* ranged from 0.61 to 10.43 % (6.01 to 7.27 log CFU). Transfer data was described by a normal distribution.
	Gloved hand	Lettuce	-	-	-	-	Touching; ca. 6.5 log CFU/surface	When participants donned gloves after cutting chicken with bare hands, the mean % transfer of *E. aerogenes* ranged from 0.001 to 0.0545 % (from below the detection limit to 3.23

							log CFU). Transfer data was described by a normal distribution.
Chicken	Gloved hand				15 min	Spot inoculation; ca. 8 log CFU/150-g portion	The mean % transfer of *E. aerogenes* ranged from 0.0001 to 0.079 % (from below the detection limit to 5.45 log CFU). Transfer data was described by a gamma distribution.
Gloved hand	Lettuce	-	-	-	-	Touching; ca. 2-4 log CFU/surface	When participants donned new gloves after cutting chicken with gloved hands, the mean % transfer of *E. aerogenes* ranged from 0.044 to 14.16% (from below the detection limit to 3.74 log CFU). The transfer rate was greatly affected by the initial inoculum on hands. Transfer data was described by a normal distribution.
Chicken	Gloved hand	-	-	-	15 min	Spot inoculation; ca. 8 log	The mean % transfer of *E. aerogenes* ranged from 0.0001 to 0.24 % (from below the detection

							CFU/150-g portion	limit to 5.05 log CFU). Transfer data was described by a gamma distribution.
	Bare hand	Lettuce	-	-	-	-	Touching; ca. 2-4 log CFU/surface	When participants removed gloves after cutting chicken with gloved hands, the mean % transfer of *E. aerogenes* from bare hands ranged from 0.23 to 97% (from below the detection limit to 3.81 log CFU). The transfer rate was greatly affected by the initial inoculum on hands. Transfer data was described by a logistic distribution.
(*82*)	Fabric [Cotton (100%) and Poly-cotton (50% polyester and 50% cotton)]	Finger pad	22-25	47-58	10 s; 0.2 kg/cm^2 pressure	1 h	ca. 5 log CFU/fabric	No significant difference in the transfer of *Staphylococcus aureus* (*P*>0.3) to finger pad was observed between fabric types with or without friction and given the transfer method. Friction had a significant impact (*P*<0.001) on the transfer rate. The method of transfer was also

significant (P<0.001) with the lowest transfer rates observed with dry transfer (<1% cfu). Significant interactions were observed between transfer type x friction and transfer type x fabric type (P<0.001). Transfer from dry cotton to finger pad with no friction was the least (<0.1 % cfu). Transfer from poly-cotton increased with friction but the increase in transfer was more pronounced in the presence of moisture (>0.8 % cfu). A two to five-fold increase in transfer of *Staphylococcus aureus* was observed when friction was used. Transfer from remoistened poly-cotton was higher when no friction was applied (ca. 0.1 % CFU; P<0.01). Application of friction further increased transfer except when polycotton was dry where the

								difference between scenarios with and without friction was not significant (*P*>0.05).
	Fabric	Fabric	22-25	47-58	10s ; 0.2 kg/cm^2 pressure	1 h	ca. 5 log CFU/fabric	The type of fabric and the method of transfer were significant (*P*<0.001). Transfer of *Staphylococcus aureus* from moist source fabric to moist target fabric was highest from poly-cotton to poly-cotton (ca. 0.08 % CFU) followed by transfer from cotton to cotton (ca. 0.02% CFU; *P*<0.05). Dry-to-dry transfer mode had the least transfer(dry cotton to dry cotton transfer was <0.01%). The highest transfer was from moist poly-cotton to dry poly-cotton (>0.12 % CFU). No friction was applied in these scenarios.
(*87*)	Soiled laminate surfaces	Fingertips	30	40-45	30 s	0 (wet), 1, 2 and 24 h	Spot inoculation; ca. 3 log CFU/25 cm^2	The transfer was higher at 1 h post-inoculation. *E. coli* transfer ranged from 55 – 59 CFU/25 cm^2 at 0 h dry time. At 1 h dry

							time, it ranged from 62-69 CFU/25 cm^2 mainly due to regrowth. At 2 h dry time transfer was more variable (25 – 85 CFU/25cm^2) and was minimal at 24 h (1 – 5 CFU/25cm^2). For *Salmonella* spp transfer at 0 h was between 59 to 57 CFU/25cm^2. It was more variable at 1h (55 to 78 CFU/25 cm^2) and dropped from 2 h (<1 to 11 CFU/25 cm^2) to 24 (below detection limit to 6 CFU/25 cm^2).
Soiled laminate surfaces	Stainless steel bowl	30	40-45	30 s; 200g weight (0.01kg/ cm^2 pressure)	0 (wet), 1, 2 and 24 h	Spot inoculation; ca. 3 log CFU/100 µL	Similar transfer rates were observed for *E. coli* at 0 and 1 h post-inoculation (53-59 CFU/25 cm^2 at 0 h; 49-56 CFU/25 cm^2 at 1 h). Transfer levels dropped by 2 h post inoculation (17 to 30 CFU/25cm^2) and were below the detection limit at 24 h. Observations were similar with *Salmonella* spp (49-73 CFU/25 cm^2

							at 0 h; 54-95 CFU/25 cm^2 at 1 h). A reduction in transfer was observed at 2 h post-inoculation (more variable; below detection limit to 30 CFU/25cm^2) and was below detection limit at 24 h post-inoculation.
Soiled cloths	Fingertips	18-20	60	30 s	0 (wet), 1, 4, 24 and 48 h	Spot inoculation; 120 CFU/cm^2	The transfer rate of *E. coli* at 0 and 1 h dry time was similar (ca. 6 CFU/25 cm^2). At 4 h, the transfer rate dropped to levels between 3 and 4 CFU/25 cm^2. At 24 and 48 h dry times, the bacterial levels transferred to fingertips were too numerous to count. Transfer increased due to regrowth.
Soiled clothes	Laminate surface	18-20	60	-	0 (wet), 1, 4, 24 and 48 h	Spot inoculation; 120 CFU/cm^2	The transfer rate of *E. coli* at 0 and 1 h dry time was similar (31 to 37 CFU/25 cm^2 at 0 h; 22 to 31 CFU/25 cm^2 at 1h). At 4 h, the transfer rate dropped to levels between 20 and 22 CFU/25 cm^2. At 24 and

								48 h dry times, the bacterial levels transferred to the stainless steel bowl were too numerous to count. Transfer increased due to regrowth.
(*12*)	Reusable latex gloves and single-use gloves	Tomatoes	22 ± 3	37 ± 10	<5s; light pressure	0 (wet), 1 or 24 h (not used for dirty reusable gloves)	Spot inoculation; ca. 6 log CFU/25 cm^2; *Salmonella* cocktail	At 0 h post-inoculation, *Salmonella* transfer to tomatoes from gloves was not significantly different from transfer to gloves from tomatoes ($P \geq 0.05$). Populations that were recovered from tomatoes 1 h post-inoculation were below the detection limit (1.3 log CFU/surface) in all cases ($P \geq 0.05$). At 0 h dry time, log % transfer to tomatoes did not differ significantly between reusable gloves (clean (0.25 ± 0.1) and dirty (0.41 ± 0.3)) and single-use gloves (0.32 ± 0.1; $P \geq 0.05$). At 1 h drying time, transfer rates did not differ between single-use and clean reusable

								gloves ($P \geq 0.05$). Log % transfer in this scenario was 0.29 ± 0.2 (single use), 0.48 ± 0.5 (clean reusable), and 5/9 positive enrichments (dirty reusable). Transfer from dirty reusable gloves was significantly less ($P \leq 0.05$). At 24 h drying time, transfer rates between single-use (9/9 positive samples) and clean reusable gloves (6/9 positive samples) fell below the detection limit. Transfer rates to single-use gloves and dirty reusable gloves decreased significantly at 1 h dry time but increased significantly with clean reusable gloves when compared with 0 h dry time transfer rates ($P \leq 0.05$).
	Tomatoes	Reusable latex gloves and	22 ± 3	37 ± 10	<5s; light pressure	0 (wet), 1 or 24 h	Spot inoculation; ca. 6 log CFU/25 cm^2;	At 0 h post-inoculation, *Salmonella* transfer to tomatoes from gloves was not significantly different from transfer to

	single-use gloves					*Salmonella* cocktail	gloves from tomatoes (*P*≥ 0.05). At 0 h dry time, the log % transfer from inoculated tomatoes to single-use gloves (0.37 ± 0.2) was significantly higher than to clean reusable gloves (0.18 ± 0.0; *P*≤ 0.05). No significant difference in transfer to single-use (0.39 ± 0.2) and clean reusable gloves (0.38 ± 0.2) was observed at 1 h dry time (*P*≥ 0.05). At 24 h dry time, no *Salmonella* was recovered from single-use gloves (*P*≤ 0.05) but 7/9 clean reusable glove samples were positive. Transfer rates between 0 and 1 h dry-time were not significantly different (*P*≥ 0.05). At 24 h dry time, transfer was significantly less than at 0 and 1 h dry times (*P*≤ 0.05).
Reusable latex gloves and	Tomato (sequential)	22 ± 3	37 ± 10	<5s; light pressure	0 (wet), and 1	Spot inoculation; ca. 6 log CFU/25 cm^2;	*Salmonella was* transferred to 25, 23, and 10 tomatoes after subsequential touches

single-use gloves			*Salmonella* cocktail	onto inoculated single-use, clean, and dirty reusable gloves respectively. No significant differences were observed in the transfer rates at 0 and 1 h dry time for single-use, clean, and dirty reusable gloves (*P*≥ 0.05). Enrichments from tomato 10 to 25 for single use and clean reusable gloves did not differ in results (*P*≥ 0.05). The transfer rate was significantly less for the fourth tomato than the first tomato when touched onto single-use gloves (*P*≤ 0.05). For clean reusable gloves, the transfer to the second tomato was significantly less (*P*≤ 0.05). Single-use gloves transferred fewer bacteria to more tomatoes while clean reusable gloves transferred more bacteria to fewer tomatoes. Drying the inoculum on dirty glove surfaces for 1 h

								reduced the population transfer rates to below detection limits from tomato 1 to tomato 10 with no observable significant differences between all 10 tomatoes ($P \geq 0.05$).
(*50*)	'Valencia' orange peel	Single-use latex gloves and the edible portion of the fruit	-	-	-	24 h	Spot inoculation; ca. 6 log CFU/fruit	A higher concentration of *Salmonella* Typhimurium was recovered from the peel for all inoculation locations (stem (27.06%), styler(23.76%) and equator (9.05%)). Log % transfer of *Salmonella* from inoculated stem or styler regions to the edible portion of the fruit was significantly different from the transfer from the equator to the edible portion of the fruit ($P<0.0001$). *Salmonella* transfer to gloved hands was not significant regardless of the region of inoculation ($P=0.077$). *Salmonella* levels that remained on the peel

							were more variable (0.04 to 122.40%) when the styler was the area of the fruit that was inoculated and the highest percentage of bacteria was recovered from the peel was when the stem area was inoculated (27.06%).
'Satsuma' mandarin peel	Single-use latex gloves and the edible portion of the fruit	-	-	-	24 h	Spot inoculation; ca. 6 log CFU/fruit	The log % transfer to the edible portion was not significantly different when the stem, styler, and equator were not the sources of contamination. However, a significant difference in log % transfer to gloves was observed between the different inoculated regions (*P*<0.0001). Transfer to gloved hands was higher when the equator (4.14%) or styler (3.42%) portions when inoculated than when the stem portion was the source of inoculum. The level of bacteria recovered from the peel (ranging from 53.76 to

							66.30%) of this variety was the highest of all citrus varieties used in this study. Transfer to the edible portion was significantly higher with this variety than with all other citrus varieties used in the experiment (*P*<0.0001).
Navel orange peel	Single-use latex gloves and the edible portion of the fruit	-	-	-	24 h	Spot inoculation; ca. 6 log CFU/fruit	A higher concentration of bacteria was recovered from the peel. The log % transfer to the gloved hand and edible portion were not significantly different regardless of the inoculated region. When the styler was inoculated on the navel orange, a significant portion of contamination remained with the navel portion (14.11%; *P*<0.0001).
‘Minneola’ tangelo	Single-use latex gloves and the edible portion of the fruit	-	-	-	24 h	Spot inoculation; ca. 6 log CFU/fruit	The highest log % transfer to the edible portion was observed when the styler was the inoculated area followed by the stem area (*P*<0.0001). Similarly, log

							% transfer to gloved hands was highest when the styler was the area that was inoculated (*P*<0.0001). Transfer distribution was much more variable for the Minneola experiments with low-frequency peaks. *Salmonella* recovery from the peel was highest (30.33%) when the stem was inoculated followed by the styler and finally the equator. Transfer to the gloves was significantly higher (ranging from (2.68% to 3.53%) with this variety than all other citrus varieties used in the experiment (*P*<0.0001).
'Marsh' grapefruit	Single-use latex gloves and the edible portion of the fruit	-	-	-	24 h	Spot inoculation; ca. 6 log CFU/fruit	No significant difference in transfer to the edible portion was observed no matter the inoculated area of the fruit. The highest transfer to gloved hands was observed when the equator area (1.72%) was inoculated

								followed by the styler (1.50%) and stem areas (0.98%; *P*<0.0001). *Salmonella* that remained on the peel showed a bimodal transfer pattern no matter the area that was inoculated.
(*32*)	beef tissue	beef tissue	23	-	5, 10, 30 minutes	10, 30, 60 minutes	Spot inoculation and spreading; ca. 8 log CFU/tissue	*L. monocytogenes* transfer generally increased with time (*P*<0.01) when the source of contamination was lean meat and decreased over time when the source of contamination was fat. Contact time significantly impacted transfer with a general trend of increasing transfer of *Salmonella* Typhimurium with both lean and fat base tissue surfaces (*P*<0.01)
	Beef tissue	Beef tissue	5	-	15, 30, 60 seconds	18 h	Spot inoculation and spreading; ca. 8 log CFU/tissue	When bacterial cells were cold stressed, the source of contamination did not significantly impact *Listeria monocytogenes* transfer (P>0.10).

							Transfer either stayed constant or generally increased with *Listeria monocytogenes*. However, the source of contamination significantly impacted *Salmonella* Typhimurium transfer ($P<0.05$) with higher transfer occurring when the source of contamination had fat. Contact time or recipient surface type did not affect either *L. monocytogenes* or *Salmonella* Typhimurium transfer ($P>0.10$).
Beef tissue	Beef tissue	37	-	5, 10, 30 minutes	18 h	Spot inoculation and spreading; ca. 8 log CFU/tissue	Higher transfer of both *L. monocytogenes* and *Salmonella* Typhimurium happened when the source of contamination was lean tissue ($P<0.05$). No other significant effects were observed for *Salmonella.* There was a significantly higher transfer of *Listeria monocytogenes* when the recipient surface was fat tissue ($P<0.01$). A

								significant interaction between contact time and source of contamination (*P*<0.05) was observed in *Listeria monocytogenes* transfer scenarios even though contact time did not have a significant effect (*P*>0.10).
(*62*)	Donor fabric	Hands	-	-	-	10 s	ca. 6 log CFU/cm^2	Transfer rates of *Staphylococcus saprophyticus*, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes* were 1.67%, 0.47%, 0.36%, and 0.29% respectively, to hands.
	Hands	Recipient fabric	-	-	-	10s	-	Transfer rates of *Staphylococcus saprophyticus*, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes* were 17%, 88%, 76%, and 86% respectively, to recipient fabric.
(*59*)	Polypropyl ene	Flour, cornmeal,	Ambient				ca. 7.8 to 8.5 log CFU/cm^2	Significant interactions between the type of surface contact material,

		sodium chloride						and type of food on *Salmonella* transfer was observed. Transfer from this material was less. Rates of decline from the source of contamination after sequential rinses for flour, cornmeal, and NaCl were -0.02, -0.02, and -0.01 log CFU/cm^2/g, respectively.
	316 Stainless steel	Flour, cornmeal, sodium chloride	Ambient				ca. 7.8 to 8.5 log CFU/cm^2	Significant interactions between the type of surface contact material and type of food on *Salmonella* transfer were observed. Transfer from this material was more. Mean rates of decline from the source of contamination after sequential rinses with flour, cornmeal, and NaCl were -0.57, -0.26, and 1.42 log CFU/cm^2/g, respectively.
(*86*)	Slicer	onions	-	-	-	90 minutes	Immersion; ca. 8.9 log CFU/mL	*Listeria monocytogenes* was recovered from onion 1, 20 and 20 at levels of 6.7 ± 0.2, 3.8 ± 0.1, and

								2.7 ± 0.4 log CFU/onion, respectively
	Slicer	onions	-	-	-	90 minutes	Immersion; ca. 6.4 log CFU/mL	All onion samples were positive for *Listeria monocytogenes* upon enrichment (1 CFU).
	Slicer	onions	-	-	-	90 minutes	Immersion; ca. 5.9 log CFU/mL	All but 1 onion was positive for *L. monocytogenes* upon enrichment.
(*75*)	Conveyor belts	Cantaloupe melons	-	-	-	10 minutes	Spot inoculation; ca 2.5 log CFU/cm^2	No significant difference in the likelihood of contaminating cantaloupe between polyvinyl chloride (52%), polyurethane (43%), and nitrile rubber (49%) materials.
	Foam pads	Cantaloupe melons	-	-	-	10 minutes	Spot inoculation; ca 2.5 log CFU/cm^2	Significantly more likely to contaminate melons than conveyor belts (P<0.05). The percentage of contamination was 78%.

a. Reference
b. Temperature
c. Relative humidity
d. Data not available

min: minutes
s/sec: seconds
h: hour(s)
d: For example, 2 d (2 days)

Regulatory framework for fresh produce.

Produce safety rule.

To implement the Food Safety Modernization Act (FSMA) requirements for fresh produce, the FDA established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption or the produce safety rule (PSR) (*25*). This rule aims to reduce foodborne illnesses associated with the consumption of contaminated produce. The PSR provides key requirements for (i) agricultural water, (ii) biological soil amendments, (iii) sprouts, (iv) domesticated and wild animals, (v) worker training, health, and hygiene, and (vi) equipment, tools, and buildings.

This section focuses on the requirements for equipment, tools, and buildings as it relates to cleaning and sanitation (21 CFR 112 Subpart L). Equipment and tools subject to the PSR are those that are intended to, or likely to, contact covered produce including those instruments or controls used to measure, regulate, or record conditions to control or prevent the growth of microorganisms of public health significance (*25*). Examples of such equipment include knives, implements, mechanical harvesters, waxing machinery, cooling equipment, grading belts, sizing equipment, pelletizing equipment, and equipment used to store or convey harvested covered produce such as containers, bins, food-packing material, dump tanks, flumes, *etc* (21 CFR §112.121). Equipment and tools must be inspected, maintained, and cleaned and when necessary and appropriate, all FCS of the equipment and tools used for covered activities be sanitized as frequently as reasonably necessary to protect against contamination of covered produce (21 CFR §112.123(d)(1)). Additionally, produce growers must maintain and

clean all non-food contact surfaces (NFCS) of equipment and tools subject to subpart L used during harvesting, packing, and holding as frequently as reasonably necessary to protect against contamination of covered produce (21 CFR §112.123(d)(2)).

To implement the requirements of this regulation, produce growers should identify the FCS of equipment and tools by evaluating covered activities (*37*). The evaluation should be based on farm-specific practices and conditions (*37*). Once these FCS of tools and equipment have been identified, produce growers should evaluate and determine appropriate cleaning practices and frequencies for FCS and NFCS including protocols for cleaning and sanitizing FCS and NFCS of equipment and tools based on operations and identified risks in their facilities (*37*, *93*). Additionally, instances when cleaning and sanitizing of FCS and NFCS can occur outside the scheduled frequency should also be determined (*37*). For instance, when potential contamination (e.g., after contact with human or animal excreta, contaminated water, contaminated produce, excluded produce, or contaminated hands) of these surfaces is suspected or discovered (*37*). Overall, compliance with the requirements for cleaning and sanitation of FCS and NFCS of the PSR minimizes the risk of contamination of fresh produce in farms and packing facilities. As such it is important that produce growers develop protocols based on the risks identified in their facilities (*93*). This may require that information on the bacterial survival, and transfer from FCS to fresh produce as well as data on the effectiveness of some sanitizers be made available for produce growers to make data-driven decisions on which interventions suit their specific operations.

Good Agricultural Practices.

Good Agricultural Practices (GAPs) are voluntary guidelines for produce farmers to reduce the risk of microbial contamination related to foodborne illness on their farms (*36*). These guidelines are also aimed at reducing the risk of contamination before it occurs hence minimizing the risk of illness in the public (*36*). GAPs are not intended to sanitize fresh produce or eliminate the risks of contamination (*36*). These guidelines are intended to guide growers to reduce the risk of contamination where possible (*36*). The guidelines are based on the FDA's "Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards of Fresh Fruits and Vegetables" (*36*). Examples of GAPs include worker hygiene and health, manure use, and water quality throughout the production and harvesting process (*36*).

Third-party companies and government agencies provide food safety plan audits for GAPs certification which contributes to reducing both the health and business risks for consumers and growers (*108*). These audits verify that fruits and vegetables are produced, packed, handled, and stored to minimize the risk of microbial contamination (*108*).

While the guidelines for GAPs are voluntary, adhering to these practices and complying with requirements outlined in the PSR will result in a robust food safety program for produce growers.

Impact of sanitizer treatments on reduction of *Salmonella* and *Listeria monocytogenes* on harvest bag material types.

Periodically performing cleaning and sanitizing procedures of FCS, including deep cleaning, and sanitizing procedures, can increase effective soil removal, biofilm prevention, removal, and pathogen inactivation (*37*). Cleaning methods should be effective at removing potential sources of contamination, including visible soil, food residue, grease, and other materials (*37*).

Cleaning can be broadly divided into dry and wet cleaning (*37*). Wet cleaning entails using water and a specific cleaning solution based on specific needs (*37*). It is the most effective method to remove organic material (*37*). Some operations may require dry cleaning which involves mechanical action for the removal of organic material and other residues (*37*).

After cleaning and inspecting FCS and when appropriate, sanitizers may be applied to surfaces based on needs (*25*, *37*). A sanitizer should adequately treat cleaned surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance and substantially reducing numbers of other undesirable microorganisms without adversely affecting the safety of the consumer (21 CFR §112.3). Sanitizers are more effective if the application is preceded by cleaning (*37*). The current regulation requires sanitizing efforts to demonstrate a 5-log reduction for target organisms on hard non-porous FCS within 30 s for non-halide compounds, such as PAA, or equivalency to 50, 100, or 200 ppm free chlorine for halides (*112*). Sanitizing products commonly used on non-porous surfaces may also be used on soft surfaces

such as fabrics provided such products have shown to be effective on non-porous surfaces (*113*).

When preceded by wet cleaning, sanitizing can include the use of agents such as hypochlorite, chlorine dioxide, iodine quaternary ammonium compounds, hot water, steam, dry heat, or ultraviolet light (*25*, *26*, *37*). Prior studies have reported the efficacy of sanitizers for the decontamination of different material types of FCS (Table 3). In apple packing operations, FCS can be made with materials like stainless steel, nylon, wood, canvas or fabric, cardboard, polyvinyl chloride, *etc* (*44*, *46*, *80*). The efficacy of sanitizers can be impacted by the specific properties of these FCS and sanitizer efficacy varies based on the bacterial genera (*13*, *68*, *77*, *79*). As an example, it has been determined that chlorine (sodium hypochlorite solution) sanitizer can significantly reduce the levels of *L. innocua* (a surrogate for *L. monocytogenes)* on stainless steel, Teflon®-wrapped and polypropylene FCS by >5 logs when preceded by cleaning (*79*). However, a 3.91 log CFU/cm reduction of *Salmonella enterica* has been observed with chlorine sanitizing solution (bleach) (*116*). Similarly, Byun et al. (*13*) observed variable reductions ranging from 3.79 to 4.91 log CFU/coupon when stainless steel, silicone, and plastic surfaces inoculated with *Salmonella* Enteritidis were sanitized with sodium hypochlorite. Within the produce industry, the use of chlorine as an effective sanitizer is an established practice (*34*, *76*, *104*). However, the antimicrobial activity of chlorine dependence on pH and organic residue conditions can easily become a hindrance in a large processing or packing facility (*34*, *95*). Additionally, sodium hypochlorite may react with halogenated compounds to form possibly carcinogenic trihalomethanes (THM) and haloacetic acid (HAA) by-products, which are being monitored by the Environmental

Protection Agency as disinfection by-products (*44*, *117*). As a result, alternative antimicrobials are being tested or increasingly being used (*37*, *104*). Examples of these alternatives include chlorine dioxide (ClO_2), peroxyacetic acid (PAA), quaternary ammonium compounds (QACs), and alcohols (*37*, *104*).

While previous studies have investigated the sanitation of hard surfaces including stainless steel, wood, plastic, and glass, (*13*, *44*, *73*, *77*, *79*) there is limited data on the sanitation of FCS such as tree fruit harvest bags. Tree fruit harvest bags can be made with nylon, cordura, and canvas (*33*, *94*). These bags differ in design and have adjustable canvas shoulder straps that encircle the upper body and arms and hold the bag at chest height. Bag sizes range from 32 to 45 lbs., and based on the design, they can have leather, metals, and plastic (*94*). The interior surfaces of these bags are coated with a PVC coating that makes materials waterproof and protects them from dirt, mildew, oil, chemicals, and salt (*94*). Additionally, the coating provides added strength and durability (*94*, *100*, *106*). Harvest bags are employed during harvest operations to convey hand-picked fruits from trees to harvest bins in the field (*28*). Because the harvested fruit comes in direct contact with the surface of these bags, it may present a risk for fruit contamination (*106*). As such, sanitation efforts should prioritize these surfaces because these bags are used for tree fruit commodities like apples that are mostly eaten fresh or minimally processed (*111*). There are fewer options available for sanitizing harvest bags as most sanitizers are labeled for use on hard and non-porous FCS (*105*). Thus, investigations on the efficacy of different sanitizers on harvest bags are needed to provide more options for sanitizing these equipment (*37*, *103*, *104*).

Table 3. Summary of studies on the efficacy of sanitizers for decontaminating pathogens on FCS

References	Surface/ condition	Rate	Sanitizer	Contact time	Inoculation method and starting level	Reported outcomes
(*13*)	Stainless steel	100µg/ mL	NaOCl	1 minute	6.64 ± 0.12 log CFU/cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 2.87 ± 0.08 log CFU/cm^2.
				5 minutes	6.56 ± 0.03 log CFU/cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 1.65 ± 0.35 log CFU/cm^2.
			$ClO_{2(liquid)}$	1 minute	6.51 ± 0.34 log CFU/cm^2	*Salmonella* Enteritidis was not detected after sanitizer treatment.
				5 minutes	6.55 ± 0.09 log CFU/cm^2	*Salmonella* Enteritidis was not detected after sanitizer treatment.
	Silicone rubber	100µg/ mL	NaOCl	1 minute	6.71 ± 0.19 log CFU/cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 3.50 ± 0.26 log CFU/cm^2.
				5 minutes	6.62 ± 0.08 log CFU/cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 2.83 ± 0.13 log CFU/cm^2.
			$ClO_{2(liquid)}$	1 minute	6.74 ± 0.13 log CFU/ cm^2	Salmonella Enteritidis concentration after sanitizer

						application was 1.54 ± 0.08 log CFU/cm^2
				5 minutes	6.68 ± 0.10 log CFU/cm^2	*Salmonella* Enteritidis was not detected in concentration after sanitizer treatment.
	Plastic	100μg/ mL	NaOCl	1 minute	6.71 ± 0.08 log CFU/ cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 3.21 ± 0.03 log CFU/cm^2.
				5 minutes	6.60 ± 0.16 log CFU/cm^2	*Salmonella* Enteritidis concentration after sanitizer application was 2.40 ± 0.06 log CFU/cm^2.
			$ClO_{2(liquid)}$	1 minute	6.71 ± 0.11 log CFU/cm^2	Salmonella Enteritidis concentration after sanitizer application was 1.22 ± 0.14 log $CFU/cm^{2.}$
				5 minutes	6.60 ± 0.09 log CFU/cm^2	*Salmonella* Enteritidis was not detected in concentration after sanitizer treatment
(*79*)	100% nylon polishing brush/waxed	200 ppm	Chlorine	15 minutes	6.5 log CFU/ surface	*L. innocua* reduced by ca. 2 log CFU/surface.
		200 ppm + 15.6 mL/1000 mL	Chlorine + detergent	15 minutes	6.5 log CFU/ surface	*L. innocua* reduced by ca. 3 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent.

	500 ppm	PAA	15 minutes	6.5 log CFU/ surface	*L. innocua* reduced by ca. 3.5 log CFU/surface.
	500 ppm + 15.6 mL/1000 mL	PAA + detergent	15 minutes	6.5 log CFU/ surface	*L. innocua* reduced by ca. 3.5 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent
Stainless steel rollers/waxed	200 ppm	Chlorine	15 minutes	7.9 log CFU/ surface	*L. innocua* reduced by ca. 1.5 log CFU/surface.
	200 ppm + 15.6 mL/1000 mL	Chlorine + detergent	15 minutes	7.9 log CFU/ surface	*L. innocua* reduced by ca. 6 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent.
	500 ppm	PAA	15 minutes	7.9 log CFU/ surface	*L. innocua* reduced by ca. 1.5 log CFU/surface.
	500 ppm + 15.6 mL/1000 mL	PAA + detergent	15 minutes	7.9 log CFU/ surface	*L. innocua* reduced by ca. 6 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent
Plastic interlocking belt/waxed	200 ppm	Chlorine	15 minutes	7.8 log CFU/ surface	*L. innocua* reduced by ca. 2 log CFU/ surface.
	200 ppm + 15.6 mL/1000 mL	Chlorine + detergent	15 minutes	7.8 log CFU/ surface	*L. innocua* reduced by > 5 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent.

		500 ppm	PAA	15 minutes	7.8 log CFU/ surface	*L. innocua* reduced by ca. 4 log CFU/surface
		500 ppm + 15.6 mL/1000 mL	PAA + detergent	15 minutes	7.8 log CFU/ surface	*L. innocua* reduced by > 5 log CFU/surface when sanitizer treatment was preceded by scrubbing with a detergent
(*116*)	Bamboo surfaces	200 ppm	Bleach	3 minutes	5.37 ± 0.03 log CFU/cm^2	*Salmonella enterica* was reduced by 1.14 log CFU/cm^2.
				5 minutes	5.37 ± 0.03 log CFU/cm^2	*Salmonella enterica* was reduced by 1.46 log CFU/cm^2.
		400 ppm	PAA	3 minutes	5.37 ± 0.03 log CFU/cm^2	*Salmonella enterica* was reduced by 1.36 log CFU/cm^2.
				5 minutes	5.37 ± 0.03 log CFU/cm^2	*Salmonella enterica* was reduced by 2.26 log CFU/cm^2.
(*51*)	Clean stainless steel	Ready-to-use	IPAQuat	30 seconds	6.93 log CFU/25 cm^2	*Salmonella* Hartford reduced by 6.18 log CFU/25 cm^2.
				1 minute	6.93 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
				5 minutes	6.93 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
	Soiled stainless steel	Ready-to-use	IPAQuat	30 seconds	6.72 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
				1 minute	6.72 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.

				5 minutes	6.72 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
	Clean conveyor belt material	Ready-to-use	IPAQuat	30 seconds	6.99 log CFU/25 cm^2	*Salmonella* Hartford was reduced by 5.68 log CFU/25 cm^2.
				1 minute	6.99 log CFU/25 cm^2	*Salmonella* Hartford reduced by 6.27 log CFU/25 cm^2.
				5 minutes	6.99 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
	Soiled conveyor belt material	Ready-to-use	IPAQuat	30 seconds	6.71 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
				1 minute	6.71 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
				5 minutes	6.71 log CFU/25 cm^2	*Salmonella* Hartford was below the limit of detection.
Unpublished	Nylon	500 ppm	PAA	1 minute	7 log CFU/coupon	*L. monocytogenes* reduced by 4 log CFU/coupon.
				2 minutes	7 log CFU/coupon	*L. monocytogenes* reduced by 4.5 log CFU/coupon.
				1 minute	ca. 8 log CFU/coupon	*Salmonella enterica* reduced by 3 log CFU/coupon.
				2 minutes	ca. 8 log CFU/coupon	*Salmonella enterica* was reduced by 3.5 log CFU/coupon.

		500 ppm	Chlorine	1 minute	7 log CFU/coupon	*L. monocytogenes* reduced by 2 log CFU/coupon.
				2 minutes	7 log CFU/coupon	*L. monocytogenes* reduced by <2 log CFU/coupon.
				1 minute	ca. 8 log CFU/coupon	*Salmonella enterica* was reduced by 1.5 log CFU/coupon.
				2 minutes	ca. 8 log CFU/coupon	*Salmonella enterica* reduced by <1.5 log CFU/coupon.
(*45*)	Stainless steel	100 °C	Saturated steam	6, 30, 60, 90, 120, 180 seconds	6.8 -7.3 log CFU/coupon	*L. innocua* in the 7-day-old biofilm reduced by 3.1, 4.0, 4.6, 5.0, 5.7, and 6.4 log CFU/coupon respectively.
	Polyethylene terephthalate (PET)					*L. innocua* in the 7-day-old biofilm reduced by 2.5, 3.0, 3.3, 4.2, 4.5, and 4.8 log CFU/coupon respectively.
	Low-density polyethylene (LDPE)					*L. innocua* in the 7-day-old biofilm reduced by 2.4, 2.8, 3.1, 3.3, 3.9, and 4.2 log CFU/coupon respectively.
	PVC					*L. innocua* in the 7-day-old biofilm reduced by 2.5, 2.7, 3.3, 3.6, 3.8, and 4.5 log CFU/coupon respectively.

rubber					*L. innocua* in the 7-day-old biofilm reduced by 2.6, 2.6, 2.7, 2.8, 3.0, and 3.3 log CFU/coupon respectively.
Clean stainless steel	100 °C	Saturated steam	6, 30 seconds	6.8 -7.3 log CFU/coupon	*L. innocua* in the 7-day-old biofilm reduced by 3.2 and 3.8 log CFU/coupon respectively.
Clean polyethylene terephthalate					*L. innocua* in the 7-day-old biofilm reduced by 2.5 and 2.8 log CFU/coupon respectively.
Clean low-density polyethylene					*L. innocua* in the 7-day-old biofilm reduced by 2.4 and 2.9 log CFU/coupon respectively.
Clean PVC					*L. innocua* in the 7-day-old biofilm reduced by 2.5 and 2.7 log CFU/coupon respectively.
Clean rubber					*L. innocua* in the 7-day-old biofilm reduced by 2.6 and 2.6 log CFU/coupon respectively.
Soiled stainless steel	100 °C	Saturated steam	6, 30 seconds	6.8 -7.3 log CFU/coupon	*L. innocua* in the 7-day-old biofilm reduced by 4.1 and 4.4 log CFU/coupon respectively.
Soiled polyethylene terephthalate					*L. innocua* in the 7-day-old biofilm reduced by 2.8 and 3.5 log CFU/coupon respectively

	Soiled low-density polyethylene.					*L. innocua* in the 7-day-old biofilm reduced by 2.9 and 3.0 log CFU/coupon respectively
	Soiled PVC					*L. innocua* in the 7-day-old biofilm reduced by 2.8 and 2.8 log CFU/coupon respectively.
	Soiled rubber					*L. innocua* in the 7-day-old biofilm reduced by 2.5 and 2.6 log CFU/coupon respectively.
(*73*)	Plastic	22.5 ppm	Peroxyacetic acid	5 minutes	ca. 6 log CFU/cm^2	*Salmonella enterica* serovar Thompson reduced by 1.49 ± 0.06 log CFU/cm^2.
		45 ppm				*Salmonella enterica* serovar Thompson reduced by 2.77 ± 0.03 log CFU/cm^2.
		90 ppm				*Salmonella enterica* serovar Thompson reduced by 3.08 ± 0.10 log CFU/cm^2.
		145 ppm				*Salmonella enterica* serovar Thompson reduced by 3.29 ± 0.04 log CFU/cm^2.
(*42*)	Stainless steel	Ready-to-use	IPA Quat	5, 10, 15 30 minutes	5.4 log CFU/cm^2	The concentrations of *L. monocytogenes* recovered were ca. 4.2, 4.2, 4.0, and 2.1 log CFU/cm^2, respectively.

	Polypropylene	Ready-to-use	IPA Quat	5, 10, 15 30 minutes	6 log CFU/cm^2	The concentrations of *L. monocytogenes* recovered were ca. 5.9, 5.1, 4.9, and 4.0 log CFU/cm^2, respectively.
(*5*)	PVC	100 °C	Saturated steam	5, 10, 20, 30 minutes	6.51 log CFU/ coupon	*Salmonella* Typhimurium concentrations recovered after treatment were 5.16 ± 0.13, 4.36 ± 0.16, 3.61 ± 0.52, and 3.08 ± 0.76 log CFU/coupon respectively.
	Stainless steel	100 °C	Saturated steam	5, 10, 20, 30 minutes	6.49 log CFU/ coupon	*Salmonella* Typhimurium concentrations recovered after treatment were 4.79 ± 0.28, 3.96 ± 0.23, 2.90 ± 0.77, and 2.39 ± 0.74 log CFU/coupon respectively.
	PVC	100 °C	Saturated steam	5, 10, 20, 30 minutes	6.32 log CFU/ coupon	*L. monocytogenes* concentrations recovered after treatment were 4.98 ± 0.07, 4.38 ± 0.12, 4.15 ± 0.12, and 3.80 ± 0.29 log CFU/coupon respectively.
	Stainless steel	100 °C	Saturated steam	5, 10, 20, 30 minutes	6.17 log CFU/ coupon	*L. monocytogenes* concentrations recovered after treatment were 4.79 ± 0.10, 4.47 ± 0.26, 3.74 ± 0.19, and 3.16 ± 0.37 log CFU/coupon respectively.

(*52*)	Stainless steel	100 ppm	PAA	5 minutes	6.57 ± 0.10 log CFU/cm^2	*Salmonella* Typhimurium concentration was below the limit of detection after treatment.
			NaOCl	5 minutes	6.57 ± 0.10 log CFU/cm^2	*Salmonella* Typhimurium concentration recovered after treatment was 3.43 ± 0.38 log CFU/cm^2.
			PAA	5 minutes	6.19 ± 0.13 log CFU/cm^2	*L. monocytogenes* concentration was below the limit of detection after treatment.
			NaOCl	5 minutes	6.19 ± 0.13 log CFU/cm^2	*L. monocytogenes* concentration recovered after treatment was 3.24 ± 0.06 log CFU/cm^2.
(*44*)	Stainless steel (SS), PVC, LDPE, PET, Silicon rubber (SR)	160 ppm	PAA	1 or 5 minutes	ca. 8 log CFU/coupon	A 1-minute exposure to PAA reduced *L. monocytogenes* biofilms by ca. 4.3, 3.5, 3.8, 4.1, and 3.7 log CFU/coupon *L. monocytogenes* biofilms on SS, LDPE, PVC, PET, and SR, respectively. No significant improvement in efficacy was observed when exposure time increased to 5 minutes.
	SS, PVC, LDPE, PET, SR	200 ppm	PAA	1 or 5 minutes	ca. 8 log CFU/coupon	Similar reductions were observed between 1- and 5-minute exposure time. In general, *L. monocytogenes* biofilms were reduced by 4.5, 4.0, 4.4, 4.3, and

						4.4 log CFU/coupon reductions on SS, PET, PVC, LDPE, and SR, respectively.
	SS, PVC, LDPE, PET, SR	100 ppm	Chlorine	1 or 5 minutes	ca. 8 log CFU/coupon	At 1 minute treatment with chlorine caused 1 to 2 log CFU/coupon reduction of *L. monocytogenes* biofilms on all tested surfaces. At 5 minutes of exposure, the reduction SS, LDPE, and PVC were ca. 2.5 log CFU/coupon. A ca. 3-and 2-log reductions were observed after exposing PET and rubber to chlorine respectively.
	SS, PVC, LDPE, PET, SR	200 ppm	Chlorine	1 or 5 minutes	ca. 8 log CFU/coupon	At 1 minute exposure, the reduction across surfaces ranged from ca. 2 to 2.5 log CFU/coupon across all surfaces. At 5 minutes of exposure, chorine caused 3.8, 2.7, 3.3, 3.6, and 3.0 log CFU/coupon reduction of *L. monocytogenes* on SS, LDPE, PVC, PET, and rubber surfaces, respectively.

CONCLUSION

Fresh produce-related outbreaks are increasing, and the risk of its occurrence increases when the fresh produce commodity is eaten raw with no kill step to control for foodborne microorganisms. As such, it is important to mitigate the risk of contamination with harvested produce. Harvest bags are an FCS that is commonly used during apple harvest operations to transport harvested fruits from trees to bins. Data on the microbial quality of these surfaces is lacking and this data can inform cleaning and sanitizing interventions. Additionally, most available sanitizers are labeled for use on hard non-porous FCS whereas harvest bags are not hard and are porous. Furthermore, the PSR requires that these surfaces being an FCS, be cleaned and adequately sanitized if necessary. Therefore, data on the microbial quality of harvest bags and information on the effectiveness of sanitizers for decontaminating these bags is needed.

REFERENCES

1. Agricultural Marketing Resource Center. 2021. Apples. Available at: https://www.agmrc.org/commodities-products/fruits/apples. Accessed 2 April 2023.
2. Alegbeleye, O., and A. S. Sant'Ana. 2022. Survival and growth behaviour of *Listeria monocytogenes* in ready-to-eat vegetable salads. *Food Control* 138:109023.
3. Allen, R. L., B. R. Warren, D. L. Archer, K. R. Schneider, and S. A. Sargent. 2005. Survival of *Salmonella* spp. on the Surfaces of Fresh Tomatoes and Selected Packing Line Materials. *HortTechnology*. American Society for Horticultural Science 15:831–836.
4. Angelo, K. M., A. R. Conrad, A. Saupe, H. Dragoo, N. West, A. Sorenson, A. Barnes, M. Doyle, J. Beal, K. A. Jackson, S. Stroika, C. Tarr, Z. Kucerova, S. Lance, L. H. Gould, M. Wise, and B. R. Jackson. 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection*. Cambridge University Press 145:848–856.
5. Ban, G.-H., H. Yoon, and D.-H. Kang. 2014. A comparison of saturated steam and superheated steam for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* biofilms on polyvinyl chloride and stainless steel. *Food Control* 40:344–350.
6. Bardsley, C. A., D. L. Weller, D. T. Ingram, Y. Chen, D. Oryang, S. L. Rideout, and L. K. Strawn. 2021. Strain, Soil-Type, Irrigation Regimen, and Poultry Litter

Influence *Salmonella* Survival and Die-off in Agricultural Soils. *Frontiers in Microbiology* 12.

7. Bardsley, C. A., R. R. Boyer, S. L. Rideout, and L. K. Strawn. 2019. Survival of *Listeria monocytogenes* on the surface of basil, cilantro, dill, and parsley plants. *Food Control* 95:90–94.
8. Barker-Reid, F., D. Harapas, S. Engleitner, S. Kreidl, R. Holmes, and R. Faggian. 2009. Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. *J Food Prot* 72:458–464.
9. Belias, A. M., A. Sbodio, P. Truchado, D. Weller, J. Pinzon, M. Skots, A. Allende, D. Munther, T. Suslow, M. Wiedmann, and R. Ivanek. 2020. Effect of Weather on the Die-Off of *Escherichia coli* and Attenuated *Salmonella enterica* Serovar Typhimurium on Preharvest Leafy Greens following Irrigation with Contaminated Water. *Appl Environ Microbiol* 86:e00899-20.
10. Berry, E. D., J. E. Wells, J. L. Bono, B. L. Woodbury, N. Kalchayanand, K. N. Norman, T. V. Suslow, G. López-Velasco, and P. D. Millner. 2015. Effect of Proximity to a Cattle Feedlot on *Escherichia coli* O157:H7 Contamination of Leafy Greens and Evaluation of the Potential for Airborne Transmission. *Applied and Environmental Microbiology*. American Society for Microbiology 81:1101–1110.
11. Blessington, T., E. J. Mitcham, and L. J. Harris. 2012. Survival of Salmonella enterica, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Walnut Kernels during Storage. *Journal of Food Protection* 75:245–254.

12. Brar, P., and M. Danyluk. 2013. *Salmonella* Transfer Potential during Hand Harvesting of Tomatoes under Laboratory Conditions. *Journal of Food Protection* 76:1342–9.

13. Byun, K.-H., S. H. Han, J. Yoon, S. H. Park, and S.-D. Ha. 2021. Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on *Salmonella* Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. *Food Control* 123:107838.

14. Carstens, C. K., J. K. Salazar, and C. Darkoh. 2019. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Frontiers in Microbiology* 10.

15. Centers for Disease Control and Prevention. 2023. List of Multistate Foodborne Outbreak Notices. Available at: https://www.cdc.gov/foodsafety/outbreaks/lists/outbreaks-list.html. Accessed 16 April 2023.

16. Centers for Disease Control and Prevention. 2023. *Listeria* Outbreak Linked to Leafy Greens. Available at: https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/index.html. Accessed 21 March 2024.

17. Centers for Disease Control and Prevention. 2024. *Listeria* Outbreak Linked to Peaches, Nectarines, and Plums. Available at: https://www.cdc.gov/listeria/outbreaks/peaches-11-23/index.html. Accessed 27 February 2024.

18. Centers for Disease Control and Prevention. 2018. Multistate Outbreak of *Listeriosis* Linked to Commercially Produced, Prepackaged Caramel Apples Made

from Bidart Bros. Apples. Available at: https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html. Accessed 6 April 2023.

19. Centers for Disease Control and Prevention. 2020. Outbreak of *Salmonella* Enteritidis Infections Linked to Peaches. Available at: https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 27 February 2024.
20. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 Infection and *Cryptosporidiosis* Associated with Drinking Unpasteurized Apple Cider -- Connecticut and New York, October 1996. Available at: https://www.cdc.gov/mmwr/preview/mmwrhtml/00045558.htm. Accessed 6 April 2023.
21. Centers for Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice--British Columbia, California, Colorado, and Washington, October 1996. *MMWR Morb Mortal Wkly Rep* 45:975.
22. Cernicchiaro, N., D. L. Pearl, S. A. McEwen, L. Harpster, H. J. Homan, G. M. Linz, and J. T. LeJeune. 2012. Association of Wild Bird Density and Farm Management Factors with the Prevalence of *E. coli* O157 in Dairy Herds in Ohio (2007–2009). *Zoonoses and Public Health* 59:320–329.
23. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. *Journal of Food Protection* 64:72–80.

24. Chhetri, V. S., K. Fontenot, R. Strahan, V. K. Yemmireddy, C. Cason, K. Kharel, and A. Adhikari. 2019. Attachment strength and on-farm die-off rate of *Escherichia coli* on watermelon surfaces. *PLOS ONE*. Public Library of Science 14:e0210115.

25. Code of Federal Regulations. 2022. Title 21. Food and Drugs. Chapter 1. Food and Drug Administration, Department of Health and Human Services. Subpart B. Food for Human Consumption. Part 112. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Subpart L. Equipment, Tools, Buildings, and Sanitation. Section 112.123(d)(1).

26. Code of Federal Regulations. 2023. Title 21. Food and Drugs. Chapter 1. Food and Drug Administration, Department of Health and Human Services. Subpart B. Food for Human Consumption. Part 120. Hazard Analysis and Critical Control Point (HACCP) Systems. Available at: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-120. Accessed 5 April 2023.

27. Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, J. H. Lewis, B. Swaminathan, R. G. Bryant, and D. J. Vugia. 1999. An Outbreak of *Escherichia coli* O157:H7 Infection from Unpasteurized Commercial Apple Juice. *Ann Intern Med*. American College of Physicians 130:202–209.

28. Corelli Grappadelli, L. 2001. Peach handling and marketing in Italy. Proceedings of 60th National Peach Council Convention. p. 39–45. *In*.

29. Danyluk, M. 2012. Pathogen transfer risks associated with specific tomato harvest and packing operations. Available at:

https://www.centerforproducesafety.org/amass/documents/researchproject/311/2010-Danyluk_Pathogen%20transfer%20risks.pdf. Accessed 10 October 2021.

30. Dawson, P., I. Han, M. Cox, C. Black, and L. Simmons. 2007. Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood, and carpet: testing the five-second rule. *Journal of Applied Microbiology* 102:945–953.
31. Diallo, M. O., K. K. Bradley, J. M. Crutcher, M. Lytle, A. Lee, and R. L. Moolenaar. 1999. An Outbreak of *Escherichia coli* O157:H7 Infection Linked to Unpasteurized Apple Cider in Oklahoma, 1999. *Food Protection Trends* 31:88–92.
32. Dickson, J. S. 1990. Transfer of *Listeria monocytogenes* and *Salmonella* Typhimurium Between Beef Tissue Surfaces1. *Journal of Food Protection* 53:51–55.
33. DuPont. 2011. Cordura®. Available at: https://web.archive.org/web/20110731140129/http://www2.dupont.com/Heritage/en_US/related_topics/condura.html. Accessed 16 March 2022.
34. Fatica, M., and K. Schneider. 2009. The use of chlorination and alternative sanitizers in the produce industry. *CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources* 4:10.
35. Fischler, G. E., J. L. Fuls, E. W. Dail, M. H. Duran, N. D. Rodgers, and A. L. Waggoner. 2007. Effect of Hand Wash Agents on Controlling the Transmission of Pathogenic Bacteria from Hands to Food. *Journal of Food Protection* 70:2873–2877.

36. Food And Drugs Administration. 1998. Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. *Federal Register*. Available at: https://www.federalregister.gov/documents/1998/10/29/98-29022/guidance-for-industry-guide-to-minimize-microbial-food-safety-hazards-for-fresh-fruits-and. Accessed 17 April 2023.
37. Food and Drugs Administration. 2018. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption: Guidance for Industry Small Entity Compliance Guide. Available at: https://www.fda.gov/media/107298/download. Accessed 9 March 2024.
38. Glass, K. A., M. C. Golden, B. J. Wanless, W. Bedale, and C. Czuprynski. 2015. Growth of *Listeria monocytogenes* within a Caramel-Coated Apple Microenvironment. *mBio*. American Society for Microbiology 6:e01232-15.
39. González-López, C., R. A. Martínez-Peniche, M. H. Iturriaga, and S. M. Arvizu-Medrano. 2019. Attachment and colonization of *Salmonella* on 'Rayada', 'Golden Delicious', and 'Red Delicious' apples. *Journal of the Science of Food and Agriculture* 99:1166–1171.
40. Grant, J., A. M. Wendelboe, A. Wendel, B. Jepson, P. Torres, C. Smelser, and R. T. Rolfs. 2008. Spinach-associated *Escherichia coli* O157:H7 Outbreak, Utah and New Mexico, 2006. *Emerg Infect Dis* 14:1633–1636.
41. Greene, S. K., E. R. Daly, E. A. Talbot, L. J. Demma, S. Holzbauer, N. J. Patel, T. A. Hill, M. O. Walderhaug, R. M. Hoekstra, M. F. Lynch, and J. A. Painter. 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes

from contaminated fields, 2005. *Epidemiology & Infection*. Cambridge University Press 136:157–165.

42. Harada, A. M. M., and M. S. Nascimento. 2021. Efficacy of dry sanitizing methods on *Listeria monocytogenes* biofilms. *Food Control* 124:107897.
43. He, Y., R. Chen, Y. Qi, J. K. Salazar, S. Zhang, M. L. Tortorello, X. Deng, and W. Zhang. 2021. Survival and transcriptomic response of *Salmonella enterica* on fresh-cut fruits. *International Journal of Food Microbiology* 348:109201.
44. Hua, Z., A. M. Korany, S. H. El-Shinawy, and M.-J. Zhu. 2019. Comparative Evaluation of Different Sanitizers Against *Listeria monocytogenes* Biofilms on Major Food-Contact Surfaces. *Frontiers in Microbiology* 10.
45. Hua, Z., F. Younce, J. Tang, D. Ryu, B. Rasco, I. Hanrahan, and M.-J. Zhu. 2021. Efficacy of saturated steam against *Listeria innocua* biofilm on common food-contact surfaces. *Food Control* 125:107988.
46. Hua, Z., and M.-J. Zhu. 2024. Unlocking the Hidden Threat: Impacts of Surface Defects on the Efficacy of Sanitizers Against *Listeria monocytogenes* Biofilms on Food-contact Surfaces in Tree Fruit Packing Facilities. *Journal of Food Protection* 87:100213.
47. Jensen, D. A., M. D. Danyluk, L. J. Harris, and D. W. Schaffner. 2017. Quantifying Bacterial Cross-Contamination Rates between Fresh-Cut Produce and Hands. *J Food Prot* 80:213–219.
48. Jensen, D. A., L. M. Friedrich, L. J. Harris, M. D. Danyluk, and D. W. Schaffner. 2015. Cross-contamination of *Escherichia coli* O157:H7 between lettuce and wash water during home-scale washing. *Food Microbiology* 46:428–433.

49. Jensen, D. A., L. M. Friedrich, L. J. Harris, M. D. Danyluk, and D. W. Schaffner. 2013. Quantifying Transfer Rates of *Salmonella* and *Escherichia coli* O157:H7 between Fresh-Cut Produce and Common Kitchen Surfaces. *Journal of Food Protection* 76:1530–1538.
50. Jung, J., L. M. Friedrich, M. D. Danyluk, and D. W. Schaffner. 2017. Quantification of Transfer of *Salmonella* from Citrus Fruits to Peel, Edible Portion, and Gloved Hands during Hand Peeling. *Journal of Food Protection* 80:933–939.
51. Kane, D. M., K. J. K. Getty, B. Mayer, and A. Mazzotta. 2016. Sanitizing in Dry-Processing Environments Using Isopropyl Alcohol Quaternary Ammonium Formula. *J Food Prot* 79:112–116.
52. Kim, J.-M., B.-Z. Zhang, and J.-M. Park. 2023. Comparison of sanitization efficacy of sodium hypochlorite and peroxyacetic acid used as disinfectants in poultry food processing plants. *Food Control* 109865.
53. Kusumaningrum, H. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology* 85:227–236.
54. Kusumaningrum, H. D., E. D. van Asselt, R. R. Beumer, and M. H. Zwietering. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. *J Food Prot* 67:1892–1903.
55. Kuttappan, D., M. S. Muyyarikkandy, E. Mathew, and M. A. Amalaradjou. 2021. *Listeria monocytogenes* Survival on Peaches and Nectarines under Conditions Simulating Commercial Stone-Fruit Packinghouse Operations. *Int J Environ Res Public Health* 18:9174.

56. Lahou, E., and M. Uyttendaele. 2014. Evaluation of three swabbing devices for detection of *Listeria monocytogenes* on different types of food contact surfaces. *International journal of environmental research and public health*. Multidisciplinary Digital Publishing Institute 11:804–814.

57. Li, K., H. Khouryieh, L. Jones, X. Etienne, and C. Shen. 2018. Assessing farmer's market produce vendors’ handling of containers and evaluation of the survival of *Salmonella* and *Listeria monocytogenes* on plastic, pressed-card, and wood container surfaces at refrigerated and room temperature. *Food control*. Elsevier Ltd.

58. Liao, C.-H., and G. M. Sapers. 2000. Attachment and Growth of *Salmonella* Chester on Apple Fruits and In Vivo Response of Attached Bacteria to Sanitizer Treatments. *Journal of Food Protection* 63:876–883.

59. Liu, S., S. E. Keller, and N. M. Anderson. 2022. Transfer of *Salmonella* from Inert Food Contact Surfaces to Wheat Flour, Cornmeal, and NaCl. *Journal of Food Protection* 85:231–237.

60. Macarisin, D., I. Sheth, M. Hur, A. Wooten, H. J. Kwon, Z. Gao, A. De Jesus, W. Jurick, and Y. Chen. 2019. Survival of outbreak, food, and environmental strains of *Listeria monocytogenes* on whole apples as affected by cultivar and wax coating. *Sci Rep* 9:12170.

61. Machado-Moreira, B., K. Richards, F. Abram, F. Brennan, M. Gaffney, and C. M. Burgess. 2021. Survival of *Escherichia coli* and *Listeria innocua* on Lettuce after Irrigation with Contaminated Water in a Temperate Climate. 9. *Foods*. Multidisciplinary Digital Publishing Institute 10:2072.

62. Mackintosh, C. A., and P. N. Hoffman. 1984. An extended model for the transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection. *J Hyg (Lond)* 92:345–355.

63. Marus, J. R. 2019. Notes from the Field: Outbreak of Listeriosis Likely Associated with Prepackaged Caramel Apples — United States, 2017. *MMWR Morb Mortal Wkly Rep* 68.

64. Materon, L. A., M. Martinez-Garcia, and V. McDonald. 2007. Identification of sources of microbial pathogens on cantaloupe rinds from pre-harvest to post-harvest operations. *World J Microbiol Biotechnol* 23:1281–1287.

65. Mbithi, J. N., V. S. Springthorpe, J. R. Boulet, and S. A. Sattar. 1992. Survival of *Hepatitis* A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J Clin Microbiol* 30:757–763.

66. McCollum, J. T., A. B. Cronquist, B. J. Silk, K. A. Jackson, K. A. O'Connor, S. Cosgrove, J. P. Gossack, S. S. Parachini, N. S. Jain, P. Ettestad, M. Ibraheem, V. Cantu, M. Joshi, T. DuVernoy, N. W. Fogg, J. R. Gorny, K. M. Mogen, C. Spires, P. Teitell, L. A. Joseph, C. L. Tarr, M. Imanishi, K. P. Neil, R. V. Tauxe, and B. E. Mahon. 2013. Multistate Outbreak of *Listeriosis* Associated with Cantaloupe. *N Engl J Med*. Massachusetts Medical Society 369:944–953.

67. McEvoy, J. L., Y. Luo, W. Conway, B. Zhou, and H. Feng. 2009. Potential of *Escherichia coli* O157:H7 to grow on field-cored lettuce as impacted by postharvest storage time and temperature. *International Journal of Food Microbiology* 128:506–509.

68. Megahed, A., B. Aldridge, and J. Lowe. 2018. The microbial killing capacity of aqueous and gaseous ozone on different surfaces contaminated with dairy cattle manure. *PLOS ONE*. Public Library of Science 13:e0196555.

69. Miranda, R. C., and D. W. Schaffner. 2016. Longer Contact Times Increase Cross-Contamination of Enterobacter aerogenes from Surfaces to Food. *Appl Environ Microbiol* 82:6490–6496.

70. Montville, R., and D. W. Schaffner. 2003. Inoculum Size Influences Bacterial Cross Contamination between Surfaces. *Applied and Environmental Microbiology*. American Society for Microbiology 69:7188–7193.

71. Montville, R., Y. Chen, and D. W. Schaffner. 2001. Glove Barriers to Bacterial Cross-Contamination between Hands to Food. *Journal of Food Protection* 64:845–849.

72. Møretrø, T., E. Heir, K. R. Mo, O. Habimana, A. Abdelgani, and S. Langsrud. 2010. Factors affecting the survival of Shigatoxin-producing *Escherichia coli* on abiotic surfaces. *International Journal of Food Microbiology* 138:71–77.

73. Nahar, S., H. L. Jeong, A. J. Cho, J.-H. Park, S. Han, Y. Kim, S.-H. Park, and S.-D. Ha. 2022. Efficacy of ficin and peroxyacetic acid against *Salmonella enterica* serovar Thompson biofilm on plastic, eggshell, and chicken skin. *Food Microbiology* 104:103997.

74. National Agricultural Statistics Service. 2024. Statistics by Subject Results. Available at: https://www.nass.usda.gov/statistics_by_subject/result.php?4897e19c-92c1-313b-8bd1-

5ffb63464d10§or=crops&group=fruit%20%26%20tree%20nuts&comm=apples. Accessed 2 April 2024.

75. Nyarko, E., K. E. Kniel, B. Zhou, P. D. Millner, Y. Luo, E. T. Handy, C. East, and M. Sharma. 2018. *Listeria monocytogenes* persistence and transfer to cantaloupes in the packing environment is affected by surface type and cleanliness. *Food Control* 85:177–185.
76. Ölmez, H., and U. Kretzschmar. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology* 42:686–693.
77. Park, S.-H., and D.-H. Kang. 2017. Influence of surface properties of produce and food contact surfaces on the efficacy of chlorine dioxide gas for the inactivation of foodborne pathogens. *Food Control* 81:88–95.
78. Perez-Rodriguez, F., M. Begum, and G. S. Johannessen. 2014. Study of the cross-contamination and survival of *Salmonella* in fresh apples. *International Journal of Food Microbiology* 184:92–97.
79. Ruiz-Llacsahuanga, B., A. M. Hamilton, K. Anderson, and F. Critzer. 2022. Efficacy of cleaning and sanitation methods against *Listeria innocua* on apple packing equipment surfaces. *Food Microbiology* 107:104061.
80. Ruiz-Llacsahuanga, B., A. Hamilton, R. Zaches, I. Hanrahan, and F. Critzer. 2021. Prevalence of *Listeria* Species on Food Contact Surfaces in Washington State Apple Packinghouses. *Appl. Environ. Microbiol.* American Society for Microbiology 87.

81. Salazar, J. K., C. K. Carstens, V. M. Bathija, S. S. Narula, M. Parish, and M. L. Tortorello. 2016. Fate of *Listeria monocytogenes* in Fresh Apples and Caramel Apples. *Journal of Food Protection* 79:696–702.

82. Sattar, S. a., S. Springthorpe, S. Mani, M. Gallant, R. c. Nair, E. Scott, and J. Kain. 2001. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. *Journal of Applied Microbiology* 90:962–970.

83. Schmidt, R. H., and D. J. Erickson. 2006. Sanitary Design and Construction of Food Equipment. *EDIS* 2005.

84. Schmidt, R. H., D. J. Erickson, S. Sims, P. Wolff, and H. Wainness. 2012. Characteristics of Food Contact Surface Materials: Stainless Steel. *Food Protection Trends* 32:574-584.

85. Schoeller, N. P., S. C. Ingham, and B. H. Ingham. 2002. Assessment of the Potential for *Listeria monocytogenes* Survival and Growth during Alfalfa Sprout Production and Use of Ionizing Radiation as a Potential Intervention Treatment. *Journal of Food Protection* 65:1259–1266.

86. Scollon, A. M., H. Wang, and E. T. Ryser. 2016. Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control* 65:160–167.

87. Scott, E., and S. F. Bloomfield. 1990. The survival and transfer of microbial contamination via cloths, hands, and utensils. *Journal of Applied Bacteriology* 68:271–278.

88. Shen, X., Y. Su, Z. Hua, H. Zhu, G. Ünlü, C. Ross, M. Mendoza, I. Hanrahan, J. Tang, and M.-J. Zhu. 2023. *Listeria monocytogenes* cross-contamination during

apple waxing and subsequent survival under different storage conditions. *Food Microbiol* 110:104166.

89. Sheng, L., K. Edwards, H.-C. Tsai, I. Hanrahan, and M.-J. Zhu. 2017. Fate of *Listeria monocytogenes* on Fresh Apples under Different Storage Temperatures. *Front. Microbiol.* 8:1396.

90. Siroli, L., F. Patrignani, D. I. Serrazanetti, C. Chiavari, M. Benevelli, L. Grazia, and R. Lanciotti. 2017. Survival of Spoilage and Pathogenic Microorganisms on Cardboard and Plastic Packaging Materials. *Frontiers in Microbiology* 8.

91. Sirsat, S. A. 2020. The Persistence of Foodborne Pathogens on Produce Box Cartons. *Journal of Environmental Health*. National Environmental Health Association 82:16–21.

92. Smolinski, H. S., S. Wang, L. Ren, Y. Chen, B. Kowalcyk, E. Thomas, J. V. Doren, and E. T. Ryser. 2018. Transfer and Redistribution of *Salmonella* Typhimurium LT2 and *Escherichia coli* O157:H7 during Pilot-Scale Processing of Baby Spinach, Cilantro, and Romaine Lettuce. *Journal of Food Protection* 81:953–962.

93. Strawn, A. L. K., V. Tech, R. C. Williams, and R. R. Boyer. 2019. Packinghouse Best Practices To Support the FSMA Produce Safety Rule. *Virginia Cooperative Extension*. Available at: https://vtechworks.lib.vt.edu/server/api/core/bitstreams/01c7bec6-86f8-4dc7-aff9-a6950834959f/content. Accessed 17 April 2023.

94. Superior Fruit Equipment. 2022. Product Guide: Prunning tools and harvest equipment for the professional fruit industry. Available at: https://store-

n7xn0zsz.mybigcommerce.com/content/2022%20product%20guide.pdf. Accessed 29 November 2022.

95. Suslow, T. 1997. Basic Properties and Key Points for Effective Disinfection. Available at: https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/26414.pdf. Accessed 30 March 2024.

96. Taormina, P. J., L. R. Beuchat, M. C. Erickson, L. Ma, G. Zhang, and M. P. Doyle. 2009. Transfer of *Escherichia coli* O157:H7 to Iceberg Lettuce via Simulated Field Coring. *Journal of Food Protection* 72:465–472.

97. The Virginia Apple Board. 2024. Apple Fun Facts. *Virginia Apples*. Available at: https://www.virginiaapples.net/apple-facts. Accessed 2 April 2024.

98. Todd-Searle, J., L. M. Friedrich, R. A. Oni, K. Shenge, J. T. LeJeune, S. A. Micallef, M. D. Danyluk, and D. W. Schaffner. 2020. Quantification of *Salmonella enterica* transfer between tomatoes, soil, and plastic mulch. *International Journal of Food Microbiology* 316:108480.

99. Tokarskyy, O., M. Korda, and H. Lukyantseva. 2020. Transfer efficacy of *Escherichia coli* O157:H7 between surfaces of green mature tomatoes and common food processing materials. *Potravinarstvo Slovak Journal of Food Sciences* 14:1027–1034.

100. Trinetta, V., U. Yucel, F. Critzer, M. Bhullar, and L. Nwadike. 2022. Validation study for the tree-fruit industry: effective strategies to sanitize harvest bins and picking bags. Available at: https://www.centerforproducesafety.org/amass/documents/researchproject/482/trinetta_final.pdf. Accessed 29 November 2022.

101. Uesugi, A. R., M. D. Danyluk, and L. J. Harris. 2006. Survival of *Salmonella* enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23, and 35 degrees C. *J Food Prot* 69:1851–1857.

102. Ukuku, D. O., and W. Fett. 2002. Behavior of *Listeria monocytogenes* Inoculated on Cantaloupe Surfaces and Efficacy of Washing Treatments to Reduce Transfer from Rind to Fresh-cut Pieces. *Journal of Food Protection* 65:924–930.

103. United Fresh Food Safety & Technology Council. 2013. Guidance on Environmental Monitoring and Control of Listeria for the Fresh Produce Industry.

104. United Fresh Food Safety and Technology Council. 2018. Strategies for Listeria Control in Tree Fruit Packaging Houses. Available at: https://info.unitedfresh.org/hubfs/food%20safety%20pubs/final-ufpa-listeria-guidance5.pdf. Accessed 20 April 2023.

105. United States Environmental Protection Agency. Product Performance Test Guidelines Ocspp 810.2300: Sanitizers for Use on Hard Surfaces Efficacy Data Recommendations. Available at: https://nepis.epa.gov/exe/zynet.exe/p100ijau.txt?zyactiond=zydocument&client=epa&index=2011+thru+2015&docs=&query=&time=&endtime=&searchmethod=1&tocrestrict=n&toc=&tocentry=&qfield=&qfieldyear=&qfieldmonth=&qfieldday=&intqfieldop=0&extqfieldop=0&xmlquery=&file=d%3a%5czyfiles%5cindex%20data%5c11thru15%5ctxt%5c00000010%5cp100ijau.txt&user=anonymous&password=anonymous&sortmethod=h%7c-&maximumdocuments=1&fuzzydegree=0&imagequality=r75g8/r75g8/x150y150g16/i425&display=hpfr&defseekpage=x&searchback=zyactionl&back=zyactions&backd

esc=results%20page&maximumpages=1&zyentry=1&seekpage=x&zypurl#. Accessed 28 March 2024.

106. University of Maine Cooperative Extension. 2022. Harvesting and Storage of Maine Tree Fruits. Available at: https://extension.umaine.edu/fruit/harvest-and-storage-of-tree-fruits/harvesting-procedures/. Accessed 29 November 2022.

107. US Apple Association. 2023. Industry at a Glance. Available at: https://usapple.org/industry-at-a-glance. Accessed 2 April 2023.

108. U.S Department of Agriculture. 2023. Good Agricultural Practices (GAP) Audits | Agricultural Marketing Service. Available at: https://www.ams.usda.gov/services/auditing/gap-ghp. Accessed 2 May 2023.

109. U.S Department of Agriculture. 2023. More than half of apples available for U.S. consumption are used in juices. Available at: http://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=107383. Accessed 2 April 2024.

110. U.S. Department of Agriculture. 2011. U.S. apple production, by State. Available at: http://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=75112. Accessed 2 April 2024.

111. U.S Department of Agriculture-National Agriculture Statistics Service. 2021. Noncitrus Fruits and Nuts. Available at: https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/sf269213r/6t054c23t/ncit0521.pdf. Accessed 1 December 2022.

112. US Environmental Protection Agency. 2012. Product Performance Test Guidelines Ocspp 810.2300: Sanitizers for Use on Hard Surfaces Efficacy Data

Recommendations. Available at: https://nepis.epa.gov/exe/zynet.exe/p100ijau.txt?zyactiond=zydocument&client=epa&index=2011%20thru%202015&docs=&query=&time=&endtime=&searchmethod=1&tocrestrict=n&toc=&tocentry=&qfield=&qfieldyear=&qfieldmonth=&qfieldday=&useqfield=&intqfieldop=0&extqfieldop=0&xmlquery=&file=d%3a%5czyfiles%5cindex%20data%5c11thru15%5ctxt%5c00000010%5cp100ijau.txt&user=anonymous&password=anonymous&sortmethod=h%7c-&maximumdocuments=1&fuzzydegree=0&imagequality=r75g8/r75g8/x150y150g16/i425&display=hpfr&defseekpage=x&searchback=zyactionl&back=zyactions&backdesc=results%20page&maximumpages=1&zyentry=4. Accessed 23 April 2023.

113. US Environmental Protection Agency, O. 2022. EPA Releases Interim Efficacy Guidance and Test Methods for Disinfection of Soft Surfaces. News Release. Available at: https://www.epa.gov/pesticides/epa-releases-interim-efficacy-guidance-and-test-methods-disinfection-soft-surfaces. Accessed 23 April 2023.

114. U.S. Food and Drug Administration. 2020. Country Fresh Expands Voluntary Recall. FDA. Available at: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/country-fresh-expands-voluntary-recall. Accessed 12 April 2023.

115. U.S. Food And Drugs Administration. 2018. FDA Investigated Multistate Outbreak of *E. coli* O157:H7 Infections Linked to Romaine Lettuce from Yuma Growing Region. Available at: https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigated-multistate-outbreak-e-coli-o157h7-infections-linked-romaine-lettuce-yuma-growing. Accessed 21 March 2024.

116. Vanith, H. 2018. Efficacy of cleaning and sanitation methods in reducing foodborne pathogens on common food contact surfaces in Cambodian fresh food markets. Available at: https://krex.k-state.edu/bitstream/handle/2097/43060/VannithHay2023.pdf?sequence=1&isAllowed=y. Accessed 20 April 2023.

117. Vidić, R. D. 1996. Control of Disinfection By-Products in Drinking Water: Regulations and Costs, p. 259–273. *In* Č. Maksimović, F. Calomino, and J. Snoxell (eds.), Water Supply Systems. Springer, Berlin, Heidelberg.

118. Vijayakumar, P., B. Brady, and B. Wolff. 2017. Produce Food Safety: Packing & Storing.

119. Virginia Department of Agriculture and Consumer Services. 2023. Virginia's Top 20 Farm Commodities. Available at: https://www.vdacs.virginia.gov/agriculture-top20.shtml. Accessed 1 September 2023.

120. Wasala, L., J. L. Talley, U. DeSilva, J. Fletcher, and A. Wayadande. 2013. Transfer of Escherichia coli O157:H7 to Spinach by House Flies, Musca domestica (Diptera: Muscidae). *Phytopathology®*. Scientific Societies 103:373–380.

121. Weller, D. L., J. Kovac, S. Roof, D. J. Kent, J. I. Tokman, B. Kowalcyk, D. Oryang, R. Ivanek, A. Aceituno, C. Sroka, and M. Wiedmann. 2017. Survival of Escherichia coli on Lettuce under Field Conditions Encountered in the Northeastern United States. *Journal of Food Protection* 80:1214–1221.

122. Wilks, S. A., H. Michels, and C. W. Keevil. 2005. The survival of Escherichia coli O157 on a range of metal surfaces. *International Journal of Food Microbiology* 105:445–454.

123. Wilks, S., H. Michels, and C. Keevil. 2006. Survival of Listeria monocytogenes Scott A on metal surfaces: Implications for cross-contamination. *International journal of food microbiology* 111:93–8.

124. Zhu, Y., F. Wu, A. Trmcic, S. Wang, and K. Warriner. 2020. Microbiological status of reusable plastic containers in commercial grower/packer operations and risk of Salmonella cross-contamination between containers and cucumbers. *Food Control* 110:107021.

Chapter 3: Generic *Escherichia coli*, *Listeria monocytogenes,* and *Salmonella* survived on different harvest bag material types for 21 days.

ABSTRACT

Harvest containers can be a source of microbial contamination when they are not cleaned and sanitized; thus, data on pathogen survival on these surfaces can inform sanitation best practices. The objective of this study was to determine the survival of generic *E. coli, L. monocytogenes,* and *Salmonella* survival on different harvest bag material types (100% each canvas, nylon, and cordura). Coupons of each material type were inoculated with a single strain of rifampicin-resistant (80ppm; R) *E. coli* or with 5-strain cocktails of rifampicin-resistant *L. monocytogenes* or *Salmonella* at approx. 7.3 ± 0.1 log CFU/coupon. Coupons were air-dried for approx.1 h and held at 22 ºC and 30 or 80% relative humidity (*E. coli*) for 90 days (d) or 22 ◦C and 55% RH (*L. monocytogenes* or *Salmonella*) for 21 d. *E. coli* concentration was enumerated at 12-time points: 0, 1.5, 4, and 8 h, and 1, 2, 3, 7, 14-, 30-, 60-, and 90 d post-inoculation (dpi). *L. monocytogenes* and *Salmonella* levels were enumerated at 10-time points: 0, 1, 4, and 8 h, and 1, 2, 3-, 7-, 14-, and 21 d. Coupons were rubbed and shaken for 60 seconds with 20 mL of 0.1% peptone and plated in duplicate on selective and non-selective media in triplicate experiments with triplicate replicates (n=9). Regression models were fitted to describe bacterial die-off in log CFU/coupon over time. The die-off of *E. coli* was described by a segmented linear model (R^2 = 0.92), with breakpoints at 0.47 d (95% CI = 0.35, 0.58) and 20.05 d (95% CI = 18.3, 21.8). The segmented model also described a biphasic die-off pattern with a breakpoint at 0.37 d (95% CI = 0.27, 0.47) for *L. monocytogenes* (R^2=0.82) and a triphasic die-off pattern with breakpoints at 1.13 d

(95% CI = 0.97, 1.29) and 9.04 d (95% CI = 4.13, 13.96) for *Salmonella* (R^2= 0.92). Findings showed that bacterial survival was impacted by RH, time, and material type, with bacterial microorganisms exhibiting the slowest die-off on canvas material. Frequent cleaning of harvest bag materials is recommended.

Keywords: Survival, *E. coli*, *L. monocytogenes*, *Salmonella*, Bags

HIGHLIGHTS

- *E. coli* survived for an extended duration at 30% than at 80% RH.
- Canvas materials promoted bacterial survival at higher levels compared to cordura and nylon.
- Bacterial die-off occurred in phases with an initial rapid die-off.
- Frequent cleaning of harvest bags is recommended as bacteria survive up to 21 d.

INTRODUCTION

Foodborne illness linked to the presence of pathogenic bacteria in fresh produce has increased over the years (*7*, *11*). For instance, the US Interagency Food Safety Analytics Collaboration attributed approx. 40% of salmonellosis, 50% of listeriosis, and 65% of *E. coli* O157:H7 infections in 2021 to the consumption of contaminated produce (*12*).

Pathogens can transfer from environmental (e.g., soil, wildlife or domestic animal feces, irrigation water) or human (e.g., contaminated harvest equipment, ill workers) to produce before, during, and after harvest (*3*, *29*, *43*, *67*). Clearly understanding

pathogen dynamics in pre-harvest, harvest, and post-harvest is important for managing pathogen contamination risks from field to packinghouse.

Equipment and tools that touch produce commodities may constitute a risk for contamination if not properly cleaned, sanitized, and maintained to mitigate cross-contamination. Such was the case with the listeriosis outbreak linked to commercially produced, prepackaged apples in 2015 that was traced back to food contact surfaces such as polishing and, drying brushes, the conveyor, and the inside of a wooden harvest bin (*3*). Harvest equipment was a likely source of contamination in this outbreak, underscoring the need to manage these implements to prevent produce contamination.

In apple harvest operations, apples touch different food contact surfaces including packing containers like tree fruit harvest bags that are used pre-harvest to transport harvested fruit from the trees to harvest bins (*16*, *55*). These bags can be made with nylon, cordura, and canvas materials and have adjustable canvas shoulder straps that encircle the upper body and arms and hold the bag at chest height (*19*, *55*). Bag sizes range from 32 to 45 lbs., and based on the design, they can have leather, metals, and plastic (*55*). The interior surfaces of these bags are coated with a polyvinyl chloride coating that makes materials waterproof and protects them from dirt, mildew, oil, chemicals, and salt (*55*). Additionally, the coating provides added strength and durability (*55*, *59*, *61*). In the tree fruit industry, harvest bags are employed during harvest operations to transport hand-picked fruits to harvest bins in the field (*16*, *22*). Because the fruits touch the harvest bag surfaces, they constitute a food contact surface that can present a risk for fruit contamination if not cleaned and sanitized adequately (*61*). As such, sanitation efforts should prioritize these surfaces because

these bags are used for tree fruit commodities like apples that are eaten fresh (*20*, *62*). As a food contact surface, these bags have not been studied extensively to assess the survival of bacterial microorganisms following contamination.

The U.S Food and Drugs Administration's 'Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables' describes picking baskets, buckets, or bags as field equipment that can easily spread contamination to fresh produce and recommends keeping such equipment clean (*21*). Additionally, the 'Standards for Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (the Produce Safety Rule)' requires all food contact surfaces of equipment and tools used in covered activities (including equipment used to store or convey harvested covered produce such as harvest bags) to be inspected, maintained, cleaned and where necessary and appropriate, sanitized to protect against the contamination of covered produce (*14*). For produce growers in the tree fruit industry to comply with regulatory requirements for cleaning, sanitizing, and maintaining their harvest bags or containers, more data on the dynamics of bacterial microorganisms following the contamination of this equipment needs to be made available.

Therefore, the objective of this study was to determine the survival of generic *E. coli*, *L. monocytogenes,* and *Salmonella* on harvest bags made from different material types. For this study, we wanted to investigate survival on different material types at different holding conditions.

MATERIALS AND METHODS

Study design. This study consisted of three trials. Each trial included three technical replicates per treatment (9 replicates per treatment).

For trials with *E. coli*, each treatment was defined by the harvest bag material used (100% canvas, 100% cordura, or 100% nylon), the relative humidity (RH) at which the coupons were held (30% and 80%), and time between inoculation and enumeration (12-time points; 3 material types * 12-time points * 2 RH * 3 replicates * 3 trails = 648 samples total). The RH values were selected to reflect the minimum and maximum RH during the apple-picking season (which begins in July and extends through early November) in central Virginia using data from the University of Virginia WeatherSTEM station (*37*, *63*, *65*). Commercial harvest bags:100% multipurpose canvas bags (Amazon, Seattle WA), 40 lb capacity 100% nylon bags (Wells and Wade, East Wenatchee, WA), and 42 lb capacity 100% cordura padded bags (Wells and Wade, East Wenatchee, WA) were cut into 25 cm^2 coupons using sterile scissors. Coupons were inoculated with rifampicin-resistant generic *E. coli* (TVS 353; University of California, Davis). Following inoculation, coupons were air dried for 1.5 h in a biosafety cabinet (Labconco Co., Kansas City, MO) at ambient temperature and RH. After 1.5 h, coupons were held at either 30% or 80% RH and 22 °C for up to 90 d. Bacterial enumeration was performed at each of twelve time points; 0,1.5, 4, and 8 h, and 1, 2, 3, 7, 14, 30, 60, and 90 d. All conditions except RH were kept constant throughout the study and were designed to mirror the storage conditions of harvest bags during the harvest season. Storage parameters (temperature and RH) were monitored and recorded using ‘WatchDog’ B-Series button data loggers (Spectrum Technologies Inc., Thayer Court, Aurora, IL) to verify that there were little to no deviations.

Trials with *L. monocytogenes* and *Salmonella* were defined by harvest bag material used (canvas and cordura) and the time between inoculation and enumeration (10-time points; 2 material types * 10-time points * 3 replicates * 3 trails * 2 bacterial pathogens = 360 samples total). A RH of 55% was selected to reflect the average in central Virginia between September 2021 to March 2023 (WeatherSTEM 2023). Material types were cut into 25 cm^2 coupons with sterile scissors and inoculated with a five-strain cocktail of rifampicin-resistant *L. monocytogenes* or a five-strain cocktail of rifampicin-resistant *Salmonella*. At 1 h post-inoculation, material types were held at 55% RH and 22 °C for 21 d. *L. monocytogenes* and *Salmonella* were enumerated at ten time points: 0, 1, 4, 8 h and 1, 2, 3, 7, 14, and 21 d. Temperature and RH conditions were maintained using a growth chamber (Percival Scientific, Inc., Perry, IA), where parameters were monitored with the 'WatchDog B-Series' button data loggers (Spectrum Technologies Inc., Thayer Court, Aurora, IL).

Culture. A single strain of generic *E. coli* (TVS 353; University of California, Davis) isolated from surface irrigation water was used in this study. This strain was adapted to 80 ppm rifampicin (Fisher Scientific, Fair Lawn, NJ) and stored with 15% glycerol at -80 °C. *L. monocytogenes* cocktail was prepared using five different strains (Scott A, 4b, 1983 pasteurized milk outbreak-reference strain for many challenge studies; V7, 1/2a, raw milk isolate-reference strain for many challenge studies; LM390-6, 1/2a, environmental isolate for 2011 cantaloupe outbreak; LM390-2, 1/2b, environmental isolate for 2011 cantaloupe outbreak; LM573-035, 4b, clinical isolate for 2014-2015 caramel apple outbreak). The *Salmonella* cocktail was prepared using five different strains (*Salmonella* enterica Enteriditis 2020AM-1539, peach outbreak, clinical

isolate; *Salmonella enterica* Newport 2020AM-0919, onion outbreak, clinical isolate; *Salmonella* Agona, alfalfa outbreak; *Salmonella* Montevideo, tomato outbreak; *Salmonella* St. Paul, pepper outbreak).

Inoculum Preparation. Before each experiment, 10 µL of frozen culture of *E. coli* was streaked from 15 % glycerol stocks stored at -80 °C onto tryptic soy agar containing 80 ppm of rifampicin (TSAR; Difco, Becton Dickinson Co., Sparks, MD). After incubating the plates at 37 ± 2 °C for 24 h, an isolated colony was transferred with a 1-µL sterile loop to 10 mL tryptic soy broth with 80 ppm rifampicin (TSBR; Difco, Becton Dickinson Co., Sparks, MD). After incubating the broth at 37 ± 2 ºC for 24 h, 10 µL of broth was transferred into 10 mL of fresh TSBR, which was then incubated at 37 ± 2 ºC for 24 h. One milliliter of the resulting bacterial suspension was pipetted onto a large (150 × 15 mm) TSAR plate and spread with a sterile spreader to cover the entire plate. The TSAR plate was held in the biosafety cabinet for 30 minutes with the vent on until all moisture was absorbed. The plate was then transferred to an incubator and incubated for 24 h at 37 ± 2 °C. After incubation, the bacterial lawn was collected by adding 9 mL of 0.1% peptone water (Fisher Scientific, Fair Lawn, NJ) to the plate and suspending the lawn with a sterile spreader. The bacterial slurry was pipetted into 10 mL Falcon tubes, and the inoculum concentration was determined by performing serial dilutions in 0.1% peptone water. Each serial dilution was spread plate onto TSAR and MacConkey agar with 80 ppm rifampicin (MACR; Difco, Becton Dickinson Co., Sparks, MD), and the plates were incubated at 37± 2 °C for 24 h. The bacterial slurry was then adjusted by serial dilution to approx. 8.44 ± 0.34 log CFU/ml. The target level was verified by spread plating on both TSAR and MACR.

A 5-strain cocktail of *L. monocytogenes* or *Salmonella* was prepared by streaking the frozen culture of individual strains onto separate TSAR plates followed by two successive transfers in TSBR as previously described. A 100 uL aliquot of each overnight culture was spread plated on TSAR and incubated for 24 h at 35 °C. After incubation, each agar plate was flooded with 5 mL of buffered peptone water (BPW; Difco, Becton Dickinson Co., Sparks, MD), and the bacterial lawns were suspended in the BPW using a sterile L-shaped spreader. The resulting bacterial slurries per strain were combined in equal volumes to create 5-strain cocktails for each bacterial pathogen (2 mL of each strain suspension). Cocktails were adjusted to an absorbance of 0.05 or 0.1 at an optical density of 600nm for *L. monocytogenes* and *Salmonella*, respectively to a mean level of 8.18 ± 0.1 log CFU/mL for both bacterial pathogens. The adjusted levels were always verified by spread-plating appropriate serial dilutions onto selective and non-selective media.

Coupon Sterilization. Before inoculation, coupon surfaces were placed in weighing boats (United Scientific Supplies Inc., Libertyville, IL) and sterilized following a modified version of the methods previously described by Reinhard et al., (2020). Briefly, each side of the coupons was treated with UV light for 5 minutes using a biosafety cabinet.

Surface inoculation. Coupon inoculation was conducted in a laboratory biosafety cabinet at ambient temperature and humidity. Briefly, 100 µL of inoculum was distributed in 15 to 20 droplets over the entire coupon surface excluding the area <2mm from the edge. This method was designed to ensure a mean starting concentration of 7.3 ± 0.1 log CFU/coupon. Coupons inoculated with *E. coli* were held in a biosafety

cabinet with the vent on for a 1.5 h dry time then transferred to sterile Whirl-Pak bags with filter (Nasco, Fort Atkinson, WI) and held in a walk-in incubator (Harris Environmental Solution, Andover, MA) at a 30% RH or held in weighing boats for storage in a growth chamber (Percival Scientific, Inc., Perry, IA) at an 80% RH. All material types of coupons were held at 22 °C for 90 d. Coupon surfaces inoculated with *L. monocytogenes* or *Salmonella* cocktails were held in a biosafety cabinet with the vent on for a 1 h dry-time, and subsequently transferred to and held in a growth chamber (Percival Scientific, Inc., Perry, IA) at 22 °C and 55% RH for 21 d.

Bacterial enumeration. Coupon surfaces in While-Pak bags were enumerated by adding 20 mL of 0.1% w/v peptone water containing 0.1% (v/v) Tween80 and subjecting to rub-shake-rub for 60 seconds (*10*). The resulting suspension was serially diluted in 9 mL of 0.1% peptone water, and appropriate dilutions were spread plated onto selective (MACR for *E. coli*, modified Oxford agar supplemented with 80 ppm of rifampicin (MOXR) for *L. monocytogenes* and xylose lysine deoxycholate supplemented with 80 ppm of rifampicin (XLDR) for *Salmonella* (Difco, Becton Dickinson Co., Sparks, MD) and non-selective media supplemented with 80 ppm of rifampicin (TSAR). *E. coli* plates were incubated at 37 ± 2 °C for 24 h *L. monocytogenes* and *Salmonella* were incubated at 35 ± 2 °C. Colonies were counted, and counts were expressed as log CFU/coupon.

When *E. coli* counts fell below the limit of detection (1.3 log CFU/coupon), enrichments were performed following a modified procedure previously reported by Akhil R., (2018). Briefly, 1 ml of liquid suspension from each sample was transferred into test tubes containing 9 ml of modified trypticase soy broth with 80 ppm of rifampicin

(mTSBR; Oxoid, Basingstoke, Hants, UK). These were incubated for 24 h at 37 ± 2 °C. After incubation, enriched samples were streaked onto MACR and incubated at 37 ± 2 °C for 24 h “modification”. Typical *E.coli* colonies on MACR were reported as “presence” and “absence” if typical colonies were not observed. A count value of 0.5 was assigned when bacterial counts were below the limit of detection.

Data analysis. The die-off of generic *E.coli* was visualized by plotting mean log CFU/coupon against time for each material and RH using the gpplot package (*68*). Using the lmtest() package in R (*58*), a linear regression model was fit to describe the bacterial die-off rate on each coupon type. The model outcome was log CFU/coupon, while days post-inoculation (dpi), material type, relative humidity, and trial were included as fixed effects. A separate model that included an interaction between time and material type was also implemented. Akaike’s Information Criterion (AIC) was used to determine if the model with or without the interaction better fit the data. Using the segmented package in R, a *Davies* test was conducted to determine if the die-off rate changed during the study (i.e., if there was a breakpoint) (*64*). The segmented model was then fit using this breakpoint. To determine if there was a second breakpoint, a second Davies test was then conducted. AIC was used to determine if the linear model or the segmented model best fit the data.

For survival trials with *L. monocytogenes* and *Salmonella*, bacterial levels at each time point were subtracted from starting levels at inoculation to obtain reduction values (log CFU/coupon). Using the *lmtest()* package (*27*), linear models were fitted with time (days), and material type as fixed factors and log CFU/coupon as the outcome. Trial was also included to make the model more robust and interactions between time, and

material type were also added. Akaike’s Information Criterion (AIC) was used to select the model with the best fit. Using the *segmented()* package (*64*), a Davies test was performed to determine non-constant regression parameters (breakpoints) in the linear model. These breaks-points were used to develop the segmented model which was retested until no more breakpoints were determined.

RESULTS

On average, *E. coli* survived for an extended duration on coupons held at 30% RH across all material types. Because counts on TSAR and MACR were significantly different ($P<0.01$), only results obtained with TSAR plates were further discussed as our worst-case scenario. By 90 dpi, reductions by >5.8 ± 0.4, 5.4 ± 1.0, and 5.2 ± 1.1 were observed on nylon, canvas, and cordura respectively (Table 1). Bacterial die-off was greatest on nylon, followed by cordura and then canvas (Figure 1 and Table 1). For example, by 90 d, 0/9 nylon samples were positive for *E. coli* upon enrichment compared to 1/9 and 4/9 for cordura and canvas respectively (Figure 1 and Table 1). At 80% RH, *E. coli* was below the limit of detection (<1.3 log CFU/coupon) on cordura surfaces and was not detected upon enrichment of this material type from 60 to 90 d (0/9 samples were positive; Figure 2 and Table 2). At 90 dpi, *E. coli* had decreased below the limit of detection on all material types (Figure 2 and Table 2).

The mean times to the last detection of bacteria on nylon, cordura, and canvas surfaces held at 30% were 40, 60, and 73.3 d, respectively (Table 3). At 80% RH, the mean times to the last detection of *E. coli* on nylon, cordura, and canvas surfaces were 31.6,

22.1, and 60 d respectively (Table 3). Overall, canvas held at 30 and 80 % RH supported *E. coli* survival for a longer duration than nylon and cordura.

The segmented model explained 92% of the variation in log CFU of *E. coli* per coupon. To examine the effects of time, material type, and RH, linear and segmented linear regression models were fitted. The linear regression model captured a mean daily die-off rate of 0.06 log CFU/coupon (95% CI = -0.07, -0.06) and explained 83% of all variation in log CFU/coupon (R^2 = 0.83; Table 4). However, *E. coli* die-off has been previously reported to happen in phases (*40*, *66*) and is better represented by a segmented linear model. As such only, the results of the segmented model were discussed in more detail. Using the Davies test, three nonconstant regression parameters were determined in the linear model (P<0.001). Therefore, a segmented linear model was fitted (Table 4) with breakpoints at 0.47 d (95% CI=0.35 d, 0.58 d) and 20.05 d (95% CI=18.27 d, 21.83 d). A third breakpoint was identified at approx. 66.7 d. However, the data collected did not suffice in estimating the slope of this breakpoint. As such, this breakpoint was not included in the final segmented linear regression model (Table 4). The segmented model captured a mean daily die-off rate of 1.73 log CFU/coupon (95% CI = -2.30, -1.17), 0.14 log CFU/coupon (95% CI = -0.15, -0.13), and 0.03 log CFU/coupon (95% CI = -0.04, -0.03) for time points between 0.0 to 0.47 d, 0.47 to 20.05 d, and 20.05 to 90 d, respectively (Table 4). The segmented linear regression model accounted for 92% of all variation in log CFU/coupon (R^2 = 0.92; Table 4). According to the segmented linear regression model, material type had a significant effect on bacterial die-off (P<0.001; Table S1). For example, the mean die-off rate was 0.73 log CFU/coupon (95% CI=-0.83, -0.64) on cordura and 0.57 log

CFU/coupon (95% CI=-0.67, -0.47; Table S1). These rates were significantly different when compared with the die-off rate on canvas surfaces (*P*<0.001; Table S1). In addition, RH was significant with a mean die-off rate of 0.30 log CFU/coupon (95% CI=-0.36, -0.23) at 80% RH (*P* < 0.001; Table S1). The interaction between material type and time was also examined in the segmented linear regression model. The interaction effect of time and cordura was not significant *(P* = 0.91; Table S1). Conversely, the interaction effect of time and nylon was significant (*P* < 0.001; Table S1).

***L. monocytogenes* and *Salmonella* survived on different harvest bag material types up to 21 d and exhibited die-off in phases.** Counts on TSAR were significantly higher different from counts on MOXR hence only results obtained with TSAR plates were further discussed (*P*<0.001). Bacterial levels on material types inoculated with *L. monocytogenes* were reduced by 2.89 ± 0.96 and 3.15 ± 0.93 log CFU/coupon from starting levels of 7.32 ± 0.08 and 7.36 ± 0.07 log CFU/coupon on canvas and cordura materials respectively (Table 5 and Figure 4). To describe the effect of time and material type on *L. monocytogenes* die-off, linear and segmented linear regression models were fitted with log CFU/coupon as the outcome. The linear regression model captured a mean daily die-off rate of 0.13 (95% CI= -0.14,-0.12) log CFU/coupon and accounted for 79% of the observed variation in log CFU/coupon described by parameters of time and material type (Table 6). Because bacterial die-off is best described by a segmented linear model, a Davies test was performed to determine non-constant regression parameters (breakpoints) in the previously fitted linear model with log CFU/coupon as the outcome. One breakpoint was determined at 0.37 d (95% CI= 0.27, 0.47) and was included in a segmented model which captured

mean daily die-off rates of 1.79 (95% CI= -2.13, -1.27) and 0.11 (95% CI= -0.13, -0.10) log CFU/coupon between 0 to 0.37 d and 0.37 to 21 d, respectively (Table 6). According to the segmented model, material type had a significant effect on the daily die-off rate of *L. monocytogenes* with reduction rates by 0.18 (95% CI= -0.27, -0.09) log CFU/coupon on cordura surfaces. The segmented model accounted for 82% of the observed variation in log CFU/coupon (Table 6 and Figure 4).

Salmonella counts decreased significantly when samples were plated on XLDR compared to TSAR ($P<0.001$). Hence, further discussion was only on results obtained with TSAR plates. By 21 d, the levels of *Salmonella* had reduced by 2.08 ± 0.36 and 2.43 ± 0.35 log CFU/coupon from starting levels of 7.29 ± 0.07 and 7.29 ± 0.06 log CFU/coupon on canvas, and cordura respectively (Table 7 and Figure 5). To describe bacterial die-off over time, linear and segmented linear models were also fitted with log CFU/coupon as model outcome (Table 8). The observed mean daily die-off rate of *Salmonella* with the linear model was 0.09 (95% CI= -0.10, -0.08) log CFU/coupon. The linear model accounted for 71% of the observed variation in log CFU/coupon. With the Davies test, 2 breakpoints were determined at 1.13 (95% CI= 0.97, 1.29) and 9.04 (95% CI= 4.13, 13.96) d. The segmented model that was subsequently fitted with these breakpoints captured mean daily die-off rates of 0.93 (95% CI= -1.05, -0.82), 0.07 (95% CI= -0.10, -0.04), and 0.03 (95% CI= -0.05, -0.01) log CFU/coupon and accounted for 92% of the observed variation in log CFU/coupon (Table 8 and Figure 5). Additionally, the material type had a significant effect with bacterial levels reducing by a rate of 0.22 (95% CI= -0.29, -0.15) log CFU/coupon when the material type was cordura. A

significant interaction between time and material type ($P<0.001$) was observed indicating significant differences in log CFU/coupon between material types.

DISCUSSION

Survival of *E. coli* on material types was better at 30% RH than at 80% RH when the temperature was maintained at 22 °C. Prior studies have reported relative humidity, temperature, and surface material to impact bacterial survival (*2*, *36*, *41*, *56*). For example, Møretrø et al. (*41*) reported higher reductions at 20 °C compared to 12 °C when stainless steel materials and polyoxymethylene copolymer coupons were held at 70 and 85% RH. Further investigation on the effect of RH on the survival of *E. coli* showed that when temperatures were maintained at 20 °C, survival was higher at 35% RH than at 85% RH (*41*). In our study, harvest bag materials were held at 22 °C at both high and low humidity conditions similar to the ones reported by (*41*) and observations were similar. It was a surprise that the survival of *E. coli* was better in drier holding conditions with little nutrients. Previous studies have shown similar tolerance levels for desiccation between certain *E. coli* strains (*26*, *41*). Also, because of prior foodborne outbreaks involving dry foods, it has been suggested that *E. coli* can persist in low moisture environments (*18*, *30*, *44*, *48*). While most of the strains used in these studies were pathogenic and survived in nutrient-rich environments, generic *E. coli* was used in our study and surfaces were not nutrient-rich. This may imply that *E. coli* can persist in dry surfaces like harvest bags resulting in an increased risk of cross-contamination. More investigations are needed to confirm this finding.

On average, *E. coli* cells survived for more than 21 d under all holding conditions on all harvest bag material types. In general, the duration of *E. coli* survival on canvas surfaces was longer than for other material types under both RH conditions. The differences in surface characteristics of individual material types may have contributed to observed differences in survival. Canvas harvest bags are made from cotton (*55*). Fabrics made from cotton have been shown to contribute to the extended survival of bacteria (*15*, *54*). For example, Silla Varghese and K. Gopalakrishna (*54*) reported *E.coli* survival on cotton materials for up to 43 d. Survival can be extended if temperatures are below 25 °C and cotton materials are stored in the dark (*15*). In addition, water absorption and retention by cotton fibers and bacterial attachment are enhanced when cotton is treated with alkali before processing into fabrics (*6*, *34*, *39*, *51*). A wet inoculum was used in this study and materials were held in the dark at 22 °C which may have promoted *E. coli* survival on canvas. Cordura and nylon are synthetic materials that tend to be hydrophobic and have poor adsorbing capacity compared to canvas which is made of natural fibers (*19*, *23*, *24*, *50*). These properties may have contributed to the decreased survival of *E. coli* on nylon and cordura. Other properties of the fabric (*i.e.*, woven, nonwoven, knitted, thickness, etc.) may have also impacted moisture and heat retention which may have impacted resident microorganisms resulting in observed differences in survival across material types (*38*, *46*). The findings of this study suggest that canvas may present a greater risk for contamination. It should be noted that the structural properties of the fabric was not a focus in this study. Therefore, future studies should examine the surface characteristics of harvest bags and how bacteria interact with these surfaces.

The segmented model provided a better description of the die-off of *E. coli* on harvest bags. Linear regression and segmented linear models were fitted to describe the die-off of bacteria. However, because the parameters of the segmented model presented a more accurate picture of *E. coli* die-off, we focused on this model for our discussion. The segmented model indicated an initial rapid die-off over the first 0.47 d (1.73; 95% CI= 2.30, 1.17) followed by a reduction in die-off rate up to 20.05 d (0.14; 95% CI= 0.15, 0.13) and then a more gradual die-off thereafter (0.03; 95% CI= 0.04, 0.03). A rapid initial die-off of *E. coli* has been observed in prior studies (*25*, *42*, *57*, *66*, *70*). Our findings that die-off was triphasic and was best represented by a segmented linear model are consistent with previous studies performed in agricultural environments (*8*, *40*, *53*, *66*). Weller et al. (*66*) evaluated different models that best describe *E.coli* die-off on lettuce under field conditions encountered in the northeastern United States and found the segmented model parameters to have a more intuitive interpretation of die-off. Similar findings were reported by McKellar et al. (*40*). In our study, two breakpoints were used to fit the segmented linear model although 3 breakpoints were determined. The third breakpoint (66.7 d) was not included because there was not enough data after that time point to allow for the slope to be calculated. Future studies should therefore include more data points after 66 d. Overall, our data indicate that the time between 0.0 d and 0.47 dpi is the most important for *E. coli* reduction due to rapid die-off. It also suggests that survival could be quantified up to 21 d as we observed a second breakpoint at 20.04 d after which die-off was minimal (Figure 3). The triphasic pattern observed in our study may be explained by several mechanisms including the use of heterogenous bacterial populations in the stationary phase and the adaptation of

microbial populations to conditions they are subject to such as surface type, the wetness of the surfaces, or the humidity of environment (*45*, *66*). The findings from our study are consistent with previous studies because we observed that material type and relative humidity were associated with bacterial die-off. A significant interaction between time and the nylon material ($P = 0.01$) was also observed which suggest that daily die-off on nylon was significantly different from die-off on canvas and cordura. Although the survival of *E. coli* decreased at 80% RH, it may be tricky for the produce industry to make use of this information as harvest bags are usually stored in open face sheds and bags may be subject to varying humidity levels based on weather.

The initial die-off rate of 1.73 log CFU/coupon from 0.0 to 0.47 d was slightly above the higher end of the range reported in previous studies (*28*, *42*, *66*, *70*). However, the die-off rates of 0.14 and 0.03 log CFU/coupon from 0.47 to 20.05 d and 20.05 to 90 d respectively were at the lower end of the range reported in previous studies (0.4 to 1.64 log MPN/day) (*28*, *42*, *66*, *70*). A variety of factors may explain these differences. McKellar et al. (*40*) reported die-off rates to be positively associated with inoculum levels. Additionally, differences in study design, the type of strain(s) used (multi versus single strain or pathogenic versus non-pathogenic), inoculation procedure, surface type, or environmental conditions may have contributed to these differences in die-off rate (*5*, *8*, *42*, *66*). It is worth noting that inoculum levels used here and in previous studies may not reflect natural contamination events which are likely lower (*28*, *42*, *66*, *70*). As such, die-off rates following natural contamination may be lower. While die-off rates reported here may overestimate die-off following natural contamination, they are similar to those reported in previous studies. The studies on the survival of

generic *E. coli* on harvest bags lacking so more studies are needed for us to determine if the rates in our study are comparable and can be used in quantitative risk assessments to determine preharvest risk management strategies associated with contaminated harvest containers.

***L. monocytogenes* and *Salmonella* were detected on harvest bag surfaces at 21 d.** Twenty-one days post-inoculation, *L. monocytogenes,* and *Salmonella* were still detectable on canvas and cordura material types. By comparison, prior studies with inoculum levels between ~3 and 7 log CFU/surface have reported different survival patterns based on methods and surface characteristics (*17*, *33*, *35*, *49*, *52*, *71*). For example, Scott and Bloomfield reported *Salmonella* levels ranging from below detection limit to 1 CFU/25 cm^2 on soiled 'J-woven' cloth 48 h post-inoculation from starting levels of approx. 3 to 4 log CFU/25 cm^2. Under clean conditions, *Salmonella* levels were between 2 and 15 CFU/25 cm^2. Similarly, Li et al. (*35*) reported a reduction in inoculated *Salmonella* and *L. monocytogenes* levels on plastic, pressed card, and wood surfaces held at ~23 ^oC and 50% RH. For instance, by 21 d, *Salmonella* levels fell below the detection limit (1.30 log CFU/cm^2) from starting levels of approx. 5.5 log CFU/cm^2 on plastic, wood, and pressed-card surfaces by 3, 9, and 12 d, respectively. In survival studies with *L. monocytogenes* Li et al. (*35*) observed a decline *L. monocytogenes* to levels below the detection limit by 21 d on plastic and wood surfaces held at ~23 ^oC and 50% RH but recovered bacteria from pressed card surfaces (2.03 log CFU/cm^2). Different survival outcomes have been observed in other studies due to differences in surface properties, temperature, RH, bacterial strain, and inoculum concentration on surfaces (*2*, *17*, *33*, *49*, *69*). To our knowledge, this is the first study that looks at the

survival of *L. monocytogenes* and *Salmonella* on reusable harvest bags made with canvas and cordura material types. Further investigations with varying RH, temperature, and inoculum levels will be needed to further assess bacterial survival on these surfaces and to make comparisons.

Segmented linear models indicated biphasic and triphasic die-off patterns for *L. monocytogenes* and *Salmonella* respectively. The linear model that was fitted with data from *L. monocytogenes* trials predicted a mean daily die-off of 0.13 log CFU/coupon (95% CI= -0.14, -0.12). Studies have reported bacterial die-off to occur in patterns (*13*, *40*, *66*). As such non-constant regression parameters were determined by the Davies test and a segmented model was fitted for *L. monocytogenes* data. With the segmented linear model, *L. monocytogenes* die-off followed a biphasic pattern with an initial rapid mean daily die-off by 1.79 log CFU/coupon (95% CI= -2.32, -1.27) occurring from 0 to 0.37 d followed by a gradual die-off between by 0.11 log CFU/coupon (95% CI= -0.13, -0.10) from 0.37 to 21 d. By comparison, *L. monocytogenes* in walnut kernels declined by a mean daily die-off rate of approx. 0.04 log CFU/g per day (*9*). Also, Kimber et al (*32*) reported a daily decline for *L. monocytogenes* of 0.02 and 0.03 log CFU/g in almonds and pistachios respectively, still lower than the rates observed in our study.

More studies have quantified *Salmonella* die-off rates with log-linear models although the surfaces or matrices are not similar to the ones used in our study (*4*, *9*, *31*, *32*, *60*). For instance, Bardsley et al (*4*) reported a mean daily linear die-off rate for *Salmonella* ranging from 0.02 to 0.05 log CFU in poultry-amended soils that were irrigated daily or weekly. Another study by Uesugi et al (*60*) reported a mean daily die-

off rate of approx. 0.01 log CFU of *Salmonella* in almonds held at ambient temperature for 172 d. Keller et al (*31*) observed a mean daily decline for *Salmonella* by 0.002 log CFU/g in black pepper held at 25 ºC. In our study, a mean log-linear die-off rate of 0.09 log CFU/coupon (95% CI= -0.10, -0.08) was observed. The mean daily die-off rates observed with the segmented model were 0.93 (95% CI= 1.05, -0.82), 0.07 (95% CI= -0.10, -0.04), and 0.03 (95% CI= -0.05, -0.01) log CFU/coupon. The die-off rate from 9.04 to 21 d (approx. 0.03) is comparable to die-off rates observed in soil or almonds (*4*, *60*). However, die-off rates for the linear model or prior phases of the segmented (0.00 to 1.13 d and 1.13 to 9.04 d) were higher.

These results are expected because the surfaces used in our study were inert and had no nutrients for bacterial survival compared to almonds, black pepper, walnuts, or irrigated poultry amended spoil. In addition, prior literature has suggested that bacterial die-off rate can be impacted by inoculum level, the heterogeneity within microbial populations in the inoculum, and the adaption of surviving microorganisms to environmental conditions resulting in changes in die-off rates over time (*40*, *66*). This may be the case with the observed bacterial die-off patterns in our study. More studies modeling the die-off of *L. monocytogenes* and *Salmonella* on harvest bag material types are needed so that comparisons can be made to determine if die-off rates are reasonable and can be used for quantitative microbial risk assessment.

CONCLUSION

Overall, our findings show that *E. coli*, *L. monocytogenes,* and *Salmonella* survived for 21 days on all material types regardless of the holding conditions used in

our trials. In addition, the duration of survival on canvas was longer than all other material types across conditions indicating that this material may pose a greater risk for produce contamination. As such, growers should use harvest bags with minimal canvas or transition out. Additionally, bacterial die-off occurred in phases with different die-off rates. The die-off rates reported in this study were generally not comparable to rates reported in other studies. More studies on the die-off of *E. coli*, *L. monocytogenes,* and *Salmonella* on harvest bags are needed for us to make comparisons to determine if the die-off rates in our study can be used in quantitative risk assessments to determine preharvest risk management strategies associated with contaminated harvest containers. Overall, our study underlines the importance of cleaning and sanitizing harvest bags.

ACKNOWLEDGMENTS

This work is supported by the Specialty Crops Research Initiative [2020-51181-32157] from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture

CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

LS, CE, and AH contributed to the study conception and design. LS, CE, AH, KW, and TL contributed to the acquisition of the data. LS, CE, AH, and DW contributed to the analysis and interpretation of the data. LS, CE, AH, and DW contributed to drafting the manuscript. LS, CE, AH, and DW contributed to the critical revisions of the manuscript.

DATA AVAILABILITY

The datasets for this article are not publicly available. Requests to access the datasets should be directed to Laura Strawn, laurakstrawn@vt.edu.

REFERENCES

1. Akhil R., B. 2018. Identification of a single selective enrichment media for the simultaneous recovery of *Salmonella* and *E. coli* O157 on Beef Hide Swabs and fecal samples. Texas Tech University.

2. Allen, R. L., B. R. Warren, D. L. Archer, K. R. Schneider, and S. A. Sargent. 2005. Survival of *Salmonella* spp. on the Surfaces of Fresh Tomatoes and Selected Packing Line Materials. *HortTechnology*. American Society for Horticultural Science 15:831–836.

3. Angelo, K. M., A. R. Conrad, A. Saupe, H. Dragoo, N. West, A. Sorenson, A. Barnes, M. Doyle, J. Beal, K. A. Jackson, S. Stroika, C. Tarr, Z. Kucerova, S. Lance, L. H. Gould, M. Wise, and B. R. Jackson. 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection*. Cambridge University Press 145:848–856.

4. Bardsley, C. A., D. L. Weller, D. T. Ingram, Y. Chen, D. Oryang, S. L. Rideout, and L. K. Strawn. 2021. Strain, Soil-Type, Irrigation Regimen, and Poultry Litter Influence Salmonella Survival and Die-off in Agricultural Soils. *Frontiers in Microbiology* 12.

5. Barker-Reid, F., D. Harapas, S. Engleitner, S. Kreidl, R. Holmes, and R. Faggian. 2009. Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. *J Food Prot* 72:458–464.

6. Begum, H. A., T. R. Tanni, and M. A. Shahid. 2021. Analysis of Water Absorption of Different Natural Fibers. 4. *Journal of Textile Science and Technology*. Scientific Research Publishing 7:152–160.

7. Bennett, S. D., S. V. Sodha, T. L. Ayers, M. F. Lynch, L. H. Gould, and R. V. Tauxe. 2018. Produce-associated foodborne disease outbreaks, USA, 1998–2013. *Epidemiology & Infection* 146:1397–1406.

8. Bezanson, G., P. Delaquis, S. Bach, R. McKellar, E. Topp, A. Gill, B. Blais, and M. Gilmour. 2012. Comparative examination of *Escherichia coli* O157:H7 survival on romaine lettuce and in soil at two independent experimental sites. *J Food Prot* 75:480–487.

9. Blessington, T., E. J. Mitcham, and L. J. Harris. 2012. Survival of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Walnut Kernels during Storage. *Journal of Food Protection* 75:245–254.

10. Brar, P., and M. Danyluk. 2013. *Salmonella* Transfer Potential during Hand Harvesting of Tomatoes under Laboratory Conditions. *Journal of food protection* 76:1342–9.

11. Carstens, C. K., J. K. Salazar, and C. Darkoh. 2019. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Frontiers in Microbiology* 10.

12. Centers for Disease Control and Prevention. 2023. Annual Reports on Foodborne Illness Source Attribution Estimates | Interagency Food Safety Analytics Collaboration | Food Safety | CDC. Available at: https://www.cdc.gov/foodsafety/ifsac/annual-reports.html. Accessed 13 May 2024.

13. Chhetri, V. S., K. Fontenot, R. Strahan, V. K. Yemmireddy, C. Cason, K. Kharel, and A. Adhikari. 2019. Attachment strength and on-farm die-off rate of *Escherichia coli* on watermelon surfaces. *PLOS ONE*. Public Library of Science 14:e0210115.

14. Code of Federal Regulations. 2022. Title 21. Food and Drugs. Chapter 1. Food and Drug Administration, Department of Health and Human Services. Subpart B. Food for Human Consumption. Part 112. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Subpart L. Equipment, Tools, Buildings, and Sanitation. Section 112.123(d)(1).

15. Colclasure, V. J., T. J. Soderquist, T. Lynch, N. Schubert, D. S. McCormick, E. Urrutia, C. Knickerbocker, D. McCord, and J. H. Kavouras. 2015. Coliform bacteria, fabrics, and the environment. *American Journal of Infection Control* 43:154–158.

16. Corelli Grappadelli, L. 2001. Peach handling and marketing in Italy, Proceedings of 60th National Peach Council Convention. p. 39–45. *In*.

17. Dawson, P., I. Han, M. Cox, C. Black, and L. Simmons. 2007. Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule. *Journal of Applied Microbiology* 102:945–953.

18. Deng, Y., J. H. Ryu, and L. R. Beuchat. 1998. Influence of temperature and pH on survival of *Escherichia coli* O157:H7 in dry foods and growth in reconstituted infant rice cereal. *Int J Food Microbiol* 45:173–184.

19. DuPont. 2011. Cordura®. Available at: https://web.archive.org/web/20110731140129/http://www2.dupont.com/Heritage/en_US/related_topics/condura.html. Accessed 16 March 2022.

20. Economic Research Service. 2021. Apples and oranges remain the top U.S. fruit choices. Available at: http://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=101944. Accessed 2 April 2023.

21. Food and Drug Administration. 2022. Guidance for Industry: Juice Hazard Analysis Critical Control Point Hazards and Controls Guidance, First Edition. FDA. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-juice-hazard-analysis-critical-control-point-hazards-and-controls-guidance-first. Accessed 14 September 2023.

22. Freivalds, A., C. Lee, G. Earle-Richardson, C. Mason, and J. May. 2006. Effect of belt/bucket interface in apple harvesting. *International Journal of Industrial Ergonomics - Int J Ind Ergonomic* 36:1005–1010.

23. Grishanov, S. 2011. Structure and properties of textile materials. Woodhead Publishing Limited. p. 28-63.

24. Gupta, D., and S. Bhaumik. 2007. Antimicrobial treatments for textiles. *Indian Journal of Fibre & Textile Research* 32:254–263.

25. Harapas, D., R. Premier, B. Tomkins, P. Franz, and S. Ajlouni. 2010. Persistence of *Escherichia coli* on injured vegetable plants. *Int J Food Microbiol* 138:232–237.

26. Hiramatsu, R., M. Matsumoto, K. Sakae, and Y. Miyazaki. 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Appl Environ Microbiol* 71:6657–6663.

27. Hothorn, T., A. Zeileis, R. W. Farebrother, C. Cummins, G. Millo, D. Mitchell, and M. A. Zeileis. 2015. Package 'lmtest'. *Testing linear regression models. https://cran. r-project. org/web/packages/lmtest/lmtest. pdf. Accessed* 24 March 2024.

28. Hutchison, M. l., S. m. Avery, and J. m. Monaghan. 2008. The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. *Journal of Applied Microbiology* 105:848–857.

29. Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on Fresh-Cut Apple Tissue and Its Potential for Transmission by Fruit Flies. *Appl Environ Microbiol* 65:1–5.

30. Keene, W. E., E. Sazie, J. Kok, D. H. Rice, D. D. Hancock, V. K. Balan, T. Zhao, and M. P. Doyle. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *JAMA* 277:1229–1231.

31. Keller, S. E., J. M. VanDoren, E. M. Grasso, and L. A. Halik. 2013. Growth and survival of *Salmonella* in ground black pepper (Piper nigrum). *Food Microbiol* 34:182–188.

32. Kimber, M. A., H. Kaur, L. Wang, M. D. Danyluk, and L. J. Harris. 2012. Survival of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Almonds and Pistachios Stored at −19, 4, and 24°C. *Journal of Food Protection* 75:1394–1403.

33. Kusumaningrum, H. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology* 85:227–236.

34. Lawson, R., and K. L. Hertel. 1975. Changes in Cotton Fiber Properties Caused by Three Sodium Hydroxide Concentrations at Three Temperatures. *Textile Research Journal*. SAGE Publications Ltd STM 45:76–79.

35. Li, K., H. Khouryieh, L. Jones, X. Etienne, and C. Shen. 2018. Assessing farmers market produce vendors' handling of containers and evaluation of the survival of *Salmonella* and *Listeria monocytogenes* on plastic, pressed-card, and wood container surfaces at refrigerated and room temperature. *Food Control* 94:116–122.

36. Machado-Moreira, B., K. Richards, F. Abram, F. Brennan, M. Gaffney, and C. M. Burgess. 2021. Survival of *Escherichia coli* and *Listeria innocua* on Lettuce after Irrigation with Contaminated Water in a Temperate Climate. 9. *Foods*. Multidisciplinary Digital Publishing Institute 10:2072.

37. Marini, R. P. 2020. Growing Apples in Virginia. Available at: https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/422/422-023/SPES-233.pdf. Accessed 3 December 2022.

38. Matusiak, M. 2006. Investigation of the thermal insulation properties of multilayer textiles. *Fibres and Textiles in Eastern Europe* 14:98–102.

39. McDonald, A. W., R. S. Orr, G. C. Humphreys, and J. N. Grant. 1957. Physical Properties of Chemically Modified Cottons: Part III: Effects of Mercerization. *Textile Research Journal*. SAGE Publications Ltd STM 27:641–648.

40. McKellar, R. C., F. Peréz-Rodríguez, L. J. Harris, A.-L. Moyne, B. Blais, E. Topp, G. Bezanson, S. Bach, and P. Delaquis. 2014. Evaluation of different approaches for modeling *Escherichia coli* O157:H7 survival on field lettuce. *Int J Food Microbiol* 184:74–85.

41. Møretrø, T., E. Heir, K. R. Mo, O. Habimana, A. Abdelgani, and S. Langsrud. 2010. Factors affecting survival of Shigatoxin-producing *Escherichia coli* on abiotic surfaces. *International Journal of Food Microbiology* 138:71–77.

42. Moyne, A., M. R. Sudarshana, T. Blessington, S. T. Koike, M. D. Cahn, and L. J. Harris. 2011. Fate of *Escherichia coli* O157:H7 in field-inoculated lettuce. *Food Microbiology* 28:1417–1425.

43. Park, S., S. Navratil, A. Gregory, A. Bauer, I. Srinath, B. Szonyi, K. Nightingale, J. Anciso, M. Jun, D. Han, S. Lawhon, and R. Ivanek. 2015. Multifactorial effects of ambient temperature, precipitation, farm management, and environmental factors determine the level of generic *Escherichia coli* contamination on preharvested spinach. *Appl Environ Microbiol* 81:2635–2650.

44. Paton, A. W., R. M. Ratcliff, R. M. Doyle, J. Seymour-Murray, D. Davos, J. A. Lanser, and J. C. Paton. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *J Clin Microbiol* 34:1622–1627.

45. Peleg, M. 2006. Advanced Quantitative Microbiology for Foods and Biosystems: Models for Predicting Growth and Inactivation, 0 ed. CRC Press.

46. Premkumar, S., and K. Thangamani. 2017. Study of woven and non-woven fabric on water retention property for effective curing of concrete. *The Journal of The Textile Institute*. Taylor & Francis 108:962–970.

47. Reinhard, R. G., R. M. Kalinowski, P. W. Bodnaruk, J. D. Eifert, R. R. Boyer, S. E. Duncan, and R. H. Bailey. 2020. Fate of *Listeria* on various food contact and noncontact surfaces when treated with bacteriophage. *Journal of Food Safety* 40:e12775.

48. Ryu, J.-H., Y. Deng, and L. R. Beuchat. 1999. Survival of *Escherichia coli* O157:H7 in dried beef powder as affected by water activity, sodium chloride content and temperature. *Food Microbiology* 16:309–316.

49. Salazar, J. K., C. K. Carstens, V. M. Bathija, S. S. Narula, M. Parish, and M. L. Tortorello. 2016. Fate of *Listeria monocytogenes* in Fresh Apples and Caramel Apples. *Journal of Food Protection* 79:696–702.

50. Sanders, D., A. Grunden, and R. R. Dunn. 2021. A review of clothing microbiology: the history of clothing and the role of microbes in textiles. *Biology Letters*. Royal Society 17:20200700.

51. Schindler, W. D., and P. J. Hauser. 2004. Chemical Finishing of Textiles. Elsevier.

52. Scott, E., and S. F. Bloomfield. 1990. The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Bacteriology* 68:271–278.

53. Seidu, R., I. Sjølander, A. Abubakari, D. Amoah, J. A. Larbi, and T. A. Stenström. 2013. Modeling the die-off of *E. coli* and *Ascaris* in wastewater-irrigated vegetables: implications for microbial health risk reduction associated with irrigation cessation. *Water Sci Technol* 68:1013–1021.

54. Silla Varghese, T., and B. K. Gopalakrishna. 2022. Comparison of Survival of Healthcare Associated Bacteria on Materials used for Making White Coat. *Journal of Pure and Applied Microbiology*. Available at: https://microbiologyjournal.org/comparison-of-survival-of-healthcare-associated-bacteria-on-materials-used-for-making-white-coat/. Accessed 2 February 2023.

55. Superior Fruit Equipment. 2022. Product Guide: Pruning tools and harvest equipment for the professional fruit industry. Available at: https://store-n7xn0zsz.mybigcommerce.com/content/2022%20Product%20Guide.pdf. Accessed 29 November 2022.

56. Tokarskyy, O., M. Korda, and H. Lukyantseva. 2020. Transfer efficacy of *Escherichia coli* O157:H7 between surfaces of green mature tomatoes and common food processing materials. *Potravinarstvo Slovak Journal of Food Sciences* 14:1027–1034.

57. Tomás-Callejas, A., G. López-Velasco, A. B. Camacho, F. Artés, F. Artés-Hernández, and T. V. Suslow. 2011. Survival and distribution of *Escherichia coli* on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions. *International Journal of Food Microbiology* 151:216–222.

58. Torsten, H., Z. Achim, W. F. Richard, C. Clint, M. Giovanni, and M. David. 2021. Package 'lmtest": Testing Linear Regression models. Available at: https://cran.r-project.org/web/packages/lmtest/lmtest.pdf. Accessed 11 December 2022.

59. Trinetta, V., U. Yucel, F. Critzer, M. Bhullar, and L. Nwadike. 2022. Validation study for the tree-fruit industry: effective strategies to sanitize harvest bins and picking bags.

https://www.centerforproducesafety.org/amass/documents/researchproject/482/trinetta_final.pdf. Accessed 29 November 2022.

60. Uesugi, A. R., M. D. Danyluk, and L. J. Harris. 2006. Survival of Salmonella enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23, and 35 degrees C. *J Food Prot* 69:1851–1857.

61. University of Maine Cooperative Extension. 2022. Harvesting and Storage of Maine Tree Fruits. Available at: https://extension.umaine.edu/fruit/harvest-and-storage-of-tree-fruits/harvesting-procedures/. Accessed 29 November 2022.

62. U.S Department of Agriculture-National Agriculture Statistics Service. 2021. Noncitrus Fruits and Nuts. Available at: https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/sf269213r/6t054c23t/ncit0521.pdf. Accessed 1 December 2022.

63. Virginia Apple Board. 2022. Virginia Apples. Available at: https://www.virginiaapples.net/virginia-apples. Accessed 3 December 2022.

64. Vito M. R., M. 2022. Package ‘segmented’: Regression Models with Break-Points/ Change-Points (with Possibly Random Effects) Estimation. Available at: https://cran.r-project.org/web/packages/segmented/segmented.pdf. Accessed 11 December 2022.

65. WeatherSTEM. 2022. The WeatherSTEM Starting Lineup. Available at: https://www.weatherstem.com. Accessed 2 December 2022.

66. Weller, D. L., J. Kovac, S. Roof, D. J. Kent, J. I. Tokman, B. Kowalcyk, D. Oryang, R. Ivanek, A. Aceituno, C. Sroka, and M. Wiedmann. 2017. Survival of *Escherichia coli* on Lettuce under Field Conditions Encountered in the Northeastern United States. *Journal of Food Protection* 80:1214–1221.

67. Weller, D., M. Wiedmann, and L. K. Strawn. 2015. Irrigation Is Significantly Associated with an Increased Prevalence of *Listeria monocytogenes* in Produce Production Environments in New York State. *Journal of Food Protection* 78:1132–1141.

68. Wilkinson, L. 2011. ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H. *Biometrics* 67:678–679.

69. Wilks, S., H. Michels, and C. Keevil. 2006. Survival of *Listeria monocytogenes* Scott A on metal surfaces: Implications for cross-contamination. *International journal of food microbiology* 111:93–8.

70. Wood, J. D., G. S. Bezanson, R. J. Gordon, and R. Jamieson. 2010. Population dynamics of *Escherichia coli* inoculated by irrigation into the phyllosphere of spinach grown under commercial production conditions. *Int J Food Microbiol* 143:198–204.

71. Zhu, Y., F. Wu, A. Trmcic, S. Wang, and K. Warriner. 2020. Microbiological status of reusable plastic containers in commercial grower/packer operations and risk of Salmonella cross-contamination between containers and cucumbers. *Food Control* 110:107021.

Table 1. Reduction[a] of generic *E. coli* on harvest bag material types held at 30% RH[b]

Humidity(%)	Time (days)	Material type		
		Nylon	**Canvas**	**Cordura**
30	0.17	0.1± 0.2[c]	0.1 ± 0.2	0.3 ± 0.2
	0.33	0.2 ± 0.3	0.3 ± 0.2	0.4 ± 0.4
	1	0.7 ± 0.2	0.4 ± 0.3	0.8 ± 0.3
	2	1.2 ± 0.4	0.7 ± 0.3	1.2 ± 0.3
	3	1.4 ± 0.3	0.8 ± 0.3	1.3 ± 0.7
	7	2.0 ± 0.3	1.5 ± 0.4	1.8 ± 0.2
	14	2.8 ± 0.4	1.9 ± 0.4	2.2 ± 0.4
	30	3.0 ± 1.8	2.2 ± 1.0	2.2 ± 1.6
	60	5.6 ± 0.6 (3/9)[d]	4.3 ± 0.6	4.7 ± 0.8 (6/9)
	90	>5.8 ± 0.4 (0/9)[e]	5.4 ± 1.0 (4/9)	5.2 ± 1.1 (1/9)

a. Bacterial concentrations 1.5 h post inoculation were 6.9 ± 0.4, 7.1 ± 0.3, and 6.7 ± 0.4 log CFU/coupon for nylon, canvas, and cordura respectively.

b. Mean log reduction (± Standard Deviation) of generic *E.coli* per coupon from day zero (counted on TSAR plates).

c. Numbers represent positive samples following the enrichment of coupons from the harvest bag material types. If nothing is in parentheses (), it was 9/9 enrichment positive.

d. When counts fell below the limit of detection (1.3 log CFU/coupon), a count value of 0.5 was assigned.

Table 2. Reduction[a] of generic *E. coli* on harvest bag material types held at 80% RH[b]

Humidity (%)	Time (days)	Material type		
		Nylon	Canvas	Cordura
80	0.17	0.4 ± 0.2[c]	0.5 ± 0.2	0.5 ± 0.3
	0.33	0.6 ± 0.2	0.6 ± 0.2	0.9 ± 0.3
	1	0.7 ± 0.3	0.7 ± 0.4	0.9 ± 0.7
	2	0.9 ± 0.2	1.0 ± 0.4	1.3 ± 0.6
	3	1.3 ± 0.5	1.0 ± 0.2	1.2 ± 0.4
	7	1.9 ± 0.6	1.3 ± 0.4	1.9 ± 0.6
	14	3.4 ± 0.8	2.1 ± 0.2	3.7 ± 0.9 (8/9)
	30	4.9 ± 0.8 (8/9)	2.7 ± 0.6	5.3 ± 0.5 (5/9)
	60	5.9 ± 0.1 (1/9)	5.2 ± 0.8 (8/9)	>5.8 ± 0.3 (0/9)
	90	>5.9 ± 0.1 (0/9)	>6.1 ± 0.1 (0/9)	>5.9 ± 0.3 (0/9)

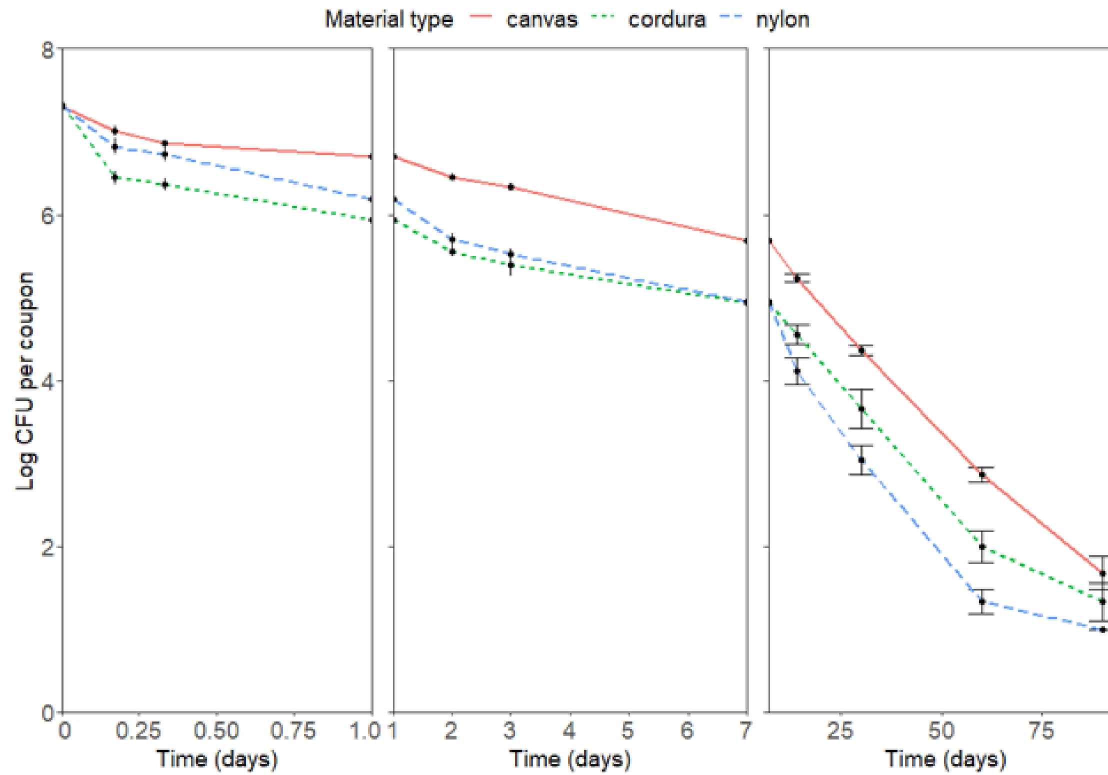
a. Bacterial concentrations 1.5 h post inoculation were 6.9 ± 0.4, 7.1 ± 0.1, 6.9 ± 0.3 log CFU/coupon for nylon, canvas and cordura respectively.

b. Mean log reduction (± Standard Deviation) of generic *E.coli* per coupon from day zero (counted on TSAR plates).

c. Numbers represent positive samples following the enrichment of coupons from the harvest bag material types. If nothing is in parentheses (), it was 9/9 enrichment positive.

d. When counts fell below the limit of detection (1.3 log CFU/coupon), a value of 0.5 was assigned.

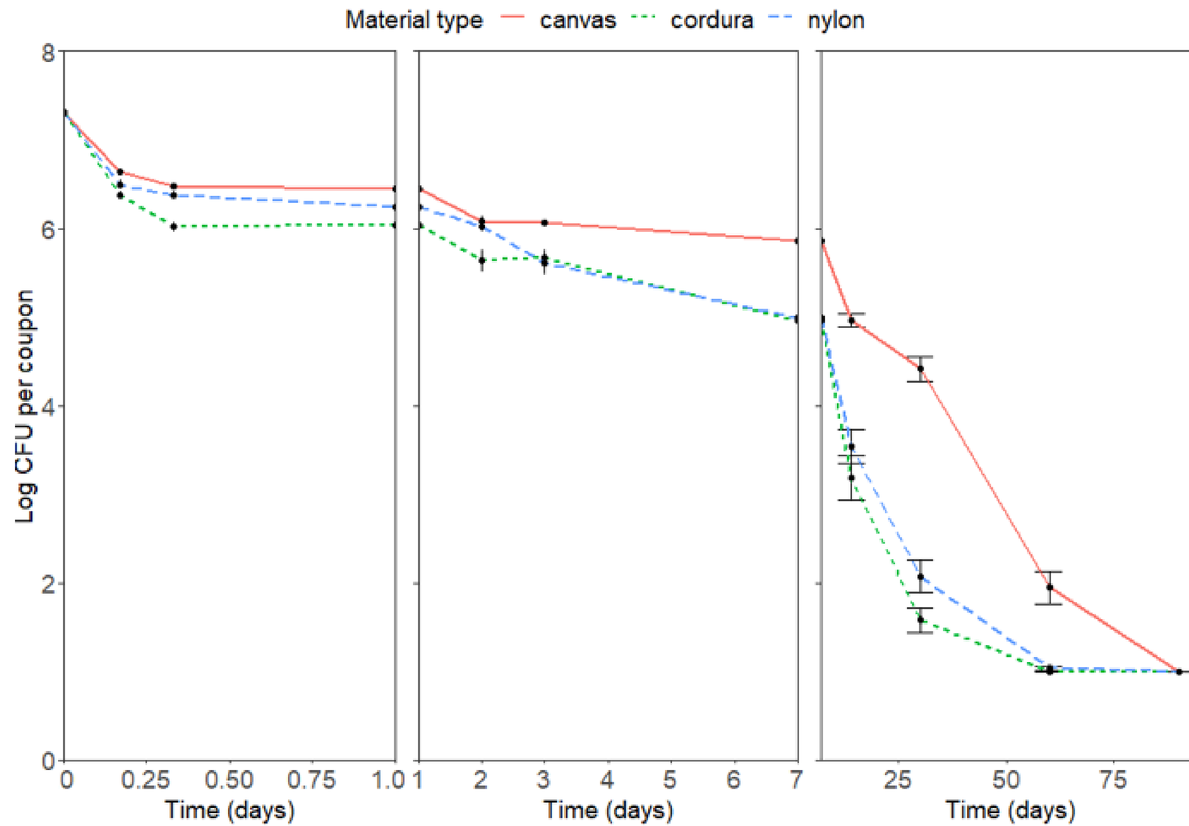
Figure 1. Die-off of generic *E. coli* on different tree fruit picking bag material types held at 30% RH[ab]



a. Bacterial concentrations 1.5 h post inoculation were 6.9 ± 0.4, 7.1 ± 0.3, and 6.7 ± 0.4 log CFU/coupon for nylon, canvas, and cordura, respectively.

b. The line graph was split into 3 grids from 0 to 1 d, 1 to 7 d and 7 to 90 d.

Figure 2. Die-off of generic *E. coli* on different harvest bag material types held at 80% RH[ab]



a. Bacterial concentrations 1.5 h post inoculation were 6.9 ± 0.4, 7.1 ± 0.1, 6.9 ± 0.3 log CFU/coupon for nylon, canvas, and cordura respectively.

b. To better observe die-off, the line graph was split into 3 grids from 0 to 1 d, 1 to 7 d and 7 to 90 d.

Table 3. Time to the last detection of generic *E. coli* on different tree fruit picking bag material types[a]

Material type	Humidity	Mean	Median	Minimum	Maximum	Range
nylon	30	40.0	30	30	60	30
canvas		73.3	60	60	90	30
cordura		53.3	60	30	90	60
nylon	80	31.6	30	14	60	46
canvas		60.0	60	30	90	60
cordura		22.1	30	7	30	23

Table 4: Parameters for the linear and segmented models that characterize the relationship between time (days) from inoculation and concentration of generic *E.coli* in log CFU per coupon.

Model	**Breakpoints (days)[a]**	**Intercept**	**Daily die-off rate**	**95% CI[b]**	***P* value**	**AIC[c]**	**R^{2} [d]**
Linear	-	6.69	-0.06	-0.07, -0.06	<0.001	2967.97	0.83
Segmented	0.0 - 0.47	7.62	-1.73	-2.30, -1.17	<0.001	2075.94	0.92
	0.47 - 20.05	6.88	-0.14	-0.15, -0.13			
	20.05 - 90	4.65	-0.03	-0.04, -0.03			

a. Estimated breakpoint 1 was at 0.47 days CI (0.35, 0.58) and breakpoint 2 was at 20.05 days CI (18.29, 21.81)

b. Confidence intervals

c. Akaike's Information Criterion

d. R squared

Figure 3: Segmented model split by material type and RH (*E. coli*)

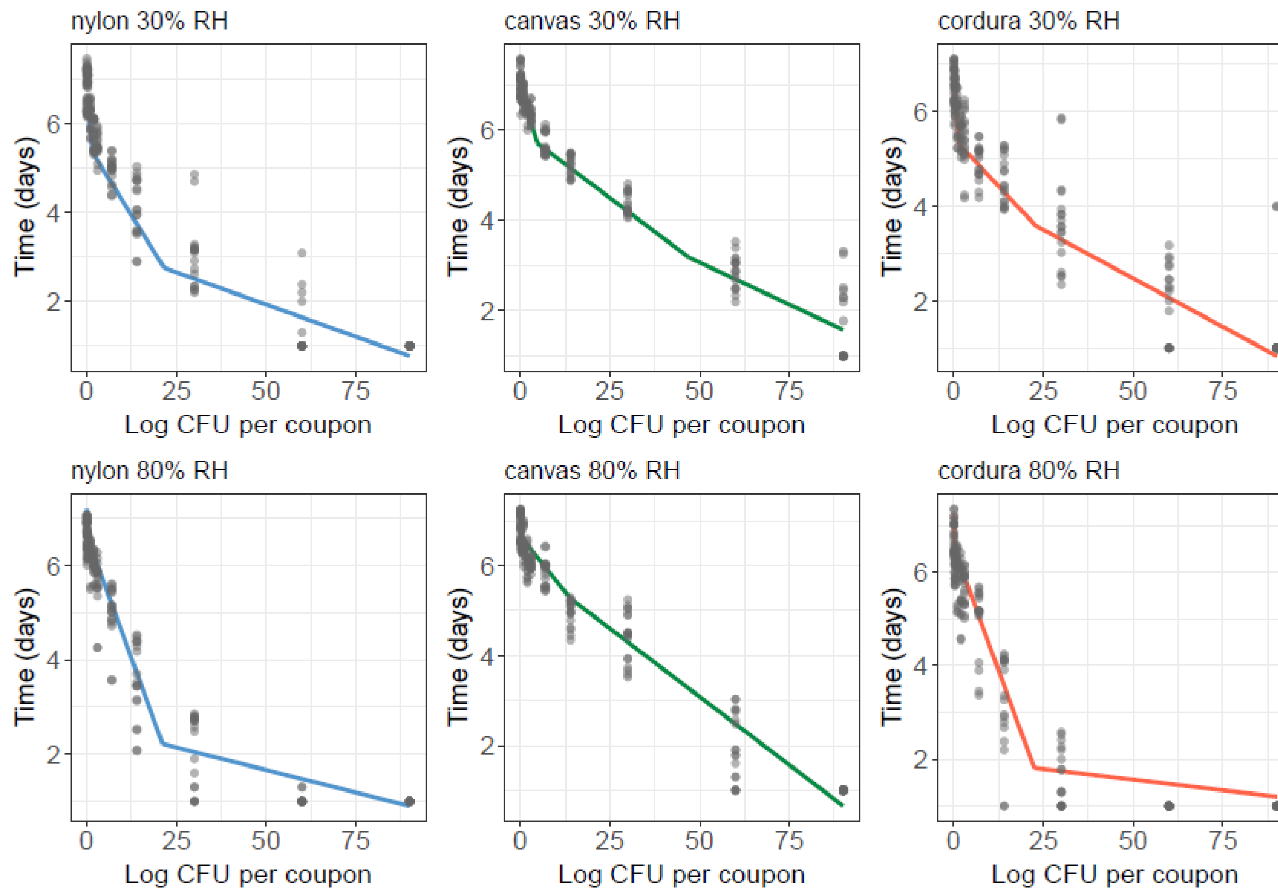


Table 5. Reduction[a] of *L. monocytogenes* on different harvest bag material types (TSAR)[b]

Time (days)	Material type	
	Canvas	**Cordura**
0.17	0.11 ± 0.19	0.24 ± 0.27
0.33	0.36 ± 0.17	0.42 ± 0.26
1	0.50 ± 0.27	0.55 ± 0.45
2	0.60 ± 0.14	0.85 ± 0.23
3	0.59 ± 0.15	1.00 ± 0.26
7	1.28 ± 0.39	1.65 ± 0.49
14	1.83 ± 0.63	1.89 ± 0.62
21	2.89 ± 0.96	3.15 ± 0.93

a. The starting level of *L. monocytogenes* at inoculation was 7.29 ± 0.09 and 7.32 ± 0.08 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation bacterial levels were was 7.07 ± 0.23 and 6.96 ± 0.19 log CFU/coupon on canvas, and cordura respectively.

b. Mean log reduction (± standard deviation) of *L. monocytogenes* per coupon from day zero counted on TSAR plate

Table 6. Parameters for the linear and segmented models describing the die-off of *L. monocytogenes* (log CFU/coupon) over time (TSAR)[ab].

Model	Breakpoints (days)	Intercept	Daily die-off rate	95% CI[c]	*P*-value	AIC[d]	R^2
Linear	-	6.57	-0.13	-0.14, -0.12	<0.001	604.70	0.78
Segmented	0 – 0.37	6.90	-1.79	-2.32, -1.27	<0.001	518.02	0.82
	0.37 - 21	6.32	-0.11	-0.13, -0.10			

Breakpoint	Estimate
1	0.37 (0.27, 0.47)

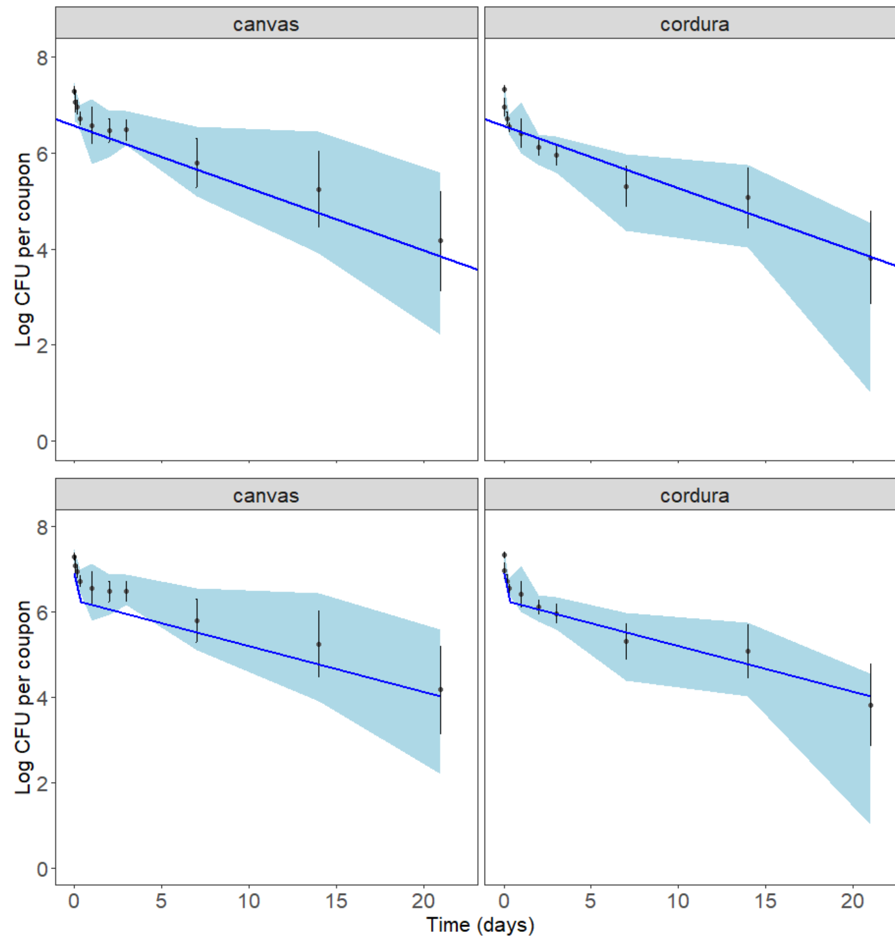
a. The starting level of *L. monocytogenes* at inoculation was 7.29 ± 0.09 and 7.32 ± 0.08 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation bacterial levels were was 7.07 ± 0.23 and 6.96 ± 0.19 log CFU/coupon on canvas, and cordura respectively.

b. Log CFU/coupon for *L. monocytogenes* on TSAR plates.

c. 95 % confidence interval.

d. Akaike's Information Criterion.

Figure 4. Linear (first row) and segmented (second row) models for canvas and cordura describing *L. monocytogenes* die-off over time (TSAR)[ab].



a. *L. monocytogenes* levels (log CFU/coupon) were also reported as mean (dark points), standard deviation (dark bars) and minimum and maximum (blue shading).

b. Log CFU/coupon for *L. monocytogenes* on TSAR plates.

Table 7. Reduction[a] of *Salmonella* on different harvest bag material types (TSAR)[b].

Time (days)	Material type	
	Canvas	Cordura
0.17	0.47 ± 0.12	0.49 ± 0.11
0.33	0.50 ± 0.13	0.64 ± 0.16
1	0.71 ± 0.17	1.11 ± 0.16
2	1.05 ± 0.15	1.39 ± 0.25
3	1.10 ± 0.15	1.45 ± 0.15
7	1.33 ± 0.18	1.93 ± 0.25
14	1.66 ± 0.18	2.31 ± 0.30
21	2.08 ± 0.36	2.43 ± 0.35

a. The starting level of *Salmonella* at inoculation was 7.29 ± 0.07 and 7.29 ± 0.06 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation bacterial levels were 7.14 ± 0.10 and 7.11 ± 0.09 log CFU/coupon on canvas, and cordura respectively.

b. Mean log reduction (± standard deviation) of *Salmonella* per coupon from day zero counted on TSAR plates.

Table 8: Parameters for the linear and segmented models describing the die-off of *Salmonella enterica* (log CFU/coupon) over time (TSAR)[ab].

Model	Breakpoints (days)	Intercept	Daily die-off rate	95% CI[c]	*P*-value	AIC[d]	R^2
Linear	-	6.45	-0.09	-0.10, -0.08	<0.001	297.47	0.71
Segmented	0.00 - 1.13	6.93	-0.93	-1.05, -0.82	<0.001	-7.32	0.92
	1.13 - 9.04	5.96	-0.07	-0.10, -0.04			
	9.04 - 21	5.56	-0.03	-0.05, -0.01			

Breakpoints	Estimate
1	1.13 (0.97, 1.29)
2	9.04 (4.13, 13.96)

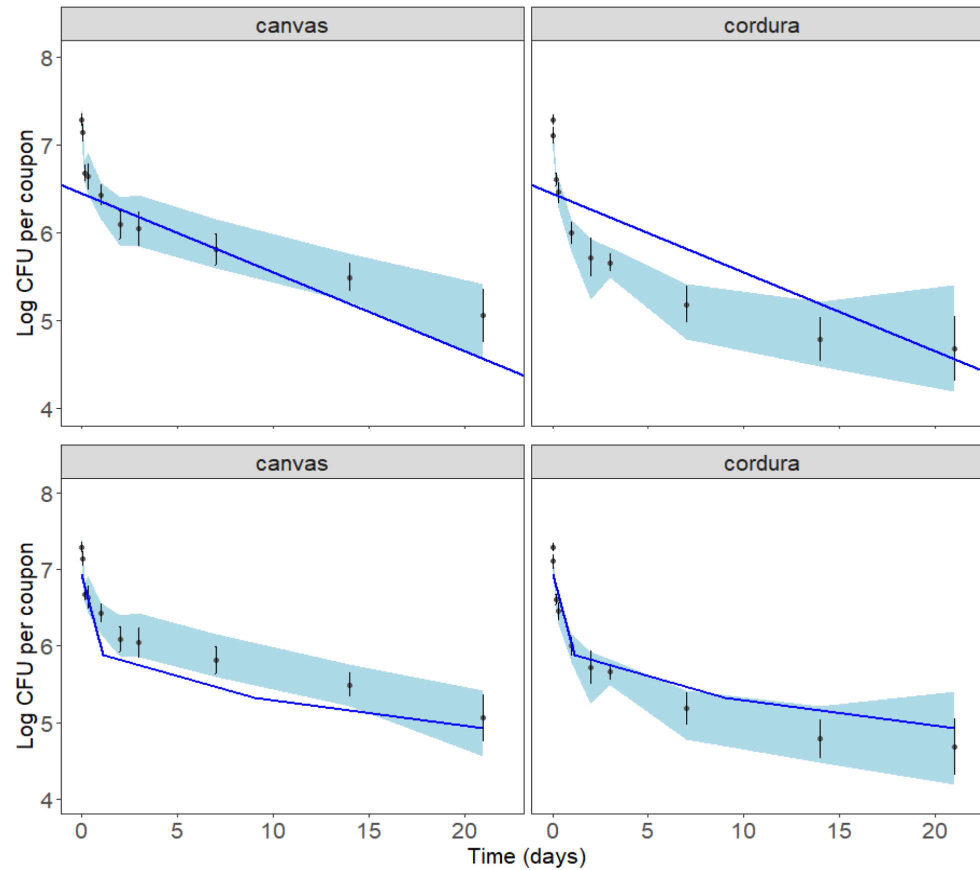
a. The starting level of *Salmonella* at inoculation was 7.29 ± 0.07 and 7.29 ± 0.06 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation bacterial levels were was 7.14 ± 0.10 and 7.11 ± 0.09 log CFU/coupon on canvas, and cordura respectively.

b. Log CFU/coupon for *Salmonella* on TSAR plates

c. 95 % confidence interval

d. Akaike's Information Criterion

Figure 5. Linear (first row) and segmented (second row) models for canvas and cordura describing *Salmonella* die-off over time (TSAR)[ab].



a. *Salmonella* levels (log CFU/coupon) were also reported as mean (dark points), standard deviation (dark bars) and minimum and maximum (blue shading).

b. Log CFU/coupon for *Salmonella* on TSAR plates.

SUPPLEMENTAL MATERIALS

Table S1: Parameters of segment model the die-off of generic *E.coli* in log CFU/coupon on TSAR plates

Parameters	**Effect estimate**	**95% confidence interval**	***P* value**
Intercept	7.56	7.40, 7.72	<0.001
Time (days)			
0.0-0.47	-1.73	-2.30, -1.17	<0.001
0.47-20.05	-0.14	-0.15, -0.13	<0.001
20 – 90	-0.03	-0.04, -0.03	<0.001
Material type (reference =Canvas)			
Cordura	-0.73	-0.83, -0.64	<0.001
Nylon	-0.57	-0.67, -0.47	<0.001
80% humidity (reference = 30% humidity)	-0.30	-0.36, -0.23	<0.001
Trial	-0.02	-0.06, 0.03	0.47
Interaction between material type and time (reference =time x canvas)			
Time x cordura	<0.001	-0.003, 0.003	0.91
Time x nylon	-0.01	-0.008, -0.002	<0.001

Table S2. Reduction[a] of generic *E. coli* on harvest bag material types held at 30% RH[b]

Humidity (%)	Time (days)	Material type		
		Nylon	Canvas	Cordura
30	0.17	0.1 ± 0.2[c]	0.1 ± 0.2	0.3 ± 0.3
	0.33	0.2 ± 0.3	0.3 ± 0.2	0.4 ± 0.5
	1	0.7 ± 0.3	0.5 ± 0.3	0.8 ± 0.3
	2	1.3 ± 0.4	0.7 ± 0.3	1.2 ± 0.4
	3	1.4 ± 0.3	0.9 ± 0.3	1.4 ± 0.8
	7	2.1 ± 0.3	1.6 ± 0.4	1.9 ± 0.3
	14	2.9 ± 0.4	2.1 ± 0.4	2.4 ± 0.2
	30	4.1 ± 1.1	2.9 ± 0.3	3.3 ± 1.1
	60	5.5 ± 0.5 (3/9)	4.5 ± 0.6	4.9 ± 0.7 (6/9)
	90	>5.7 ± 0.5 (0/9)	5.7 ± 0.9 (3/9)	5.3 ± 1.0 (1/9)

a. Bacterial concentrations 1.5 h post inoculation were 6.7 ± 0.5, 7.0 ± 0.3, and 6.6 ± 0.4 log CFU/coupon for nylon, canvas, and cordura respectively.

b. Mean log reduction (± Standard Deviation) of generic *E.coli* per coupon from day zero (counted on MACR plates).

c. Numbers represent positive samples following the enrichment of coupons from the harvest bag material types. If nothing is in parentheses (), it was 9/9 enrichment positive.

d. When counts fell below the limit of detection (1.3 log CFU/coupon), a count value of 0.5 was assigned.

Table S3. Reduction[a] of generic *E. coli* on harvest bag material types held at 80% RH[b]

Humidity (%)	Time (days)	Material type		
		Nylon	Canvas	Cordura
80	0.17	1.0 ± 0.2	0.7 ± 0.1	1.1 ± 0.2
	0.33	1.1 ± 0.2	0.8 ± 0.1	1.5 ± 0.2
	1	1.4± 0.3	1.0 ± 0.2	1.7 ± 0.6
	2	1.6 ± 0.3	1.2 ± 0.3	1.9 ± 0.4
	3	2.2 ± 0.6	1.4 ± 0.3	2.1 ± 0.4
	7	3.2 ± 0.4	1.6 ± 0.4	2.9 ± 0.7
	14	4.5 ± 1.0 (8/9)	2.7 ± 0.3	4.9 ± 0.6 (1/9)
	30	4.7 ± 1.9 (3/9)	3.0 ± 1.3	4.4 ± 2.4 (2/9)
	60	>6.1 ± 0.1 (0/9)	5.9 ± 0.4 (3/9)	>6.1 ± 0.2 (0/9)
	90	>6.1 ± 0.1 (0/9)	>6.1 ± 0.1 (0/9)	>6.1 ± 0.2 (0/9)

a. Bacterial concentrations 1.5 h post inoculation were 6.6 ± 0.2, 6.9 ± 0.1, and 6.6 ± 0.3 log CFU/coupon for nylon, canvas, and cordura respectively.

b. Mean log reduction (± Standard Deviation) of generic *E.coli* per coupon from day zero (counted on MACR plates).

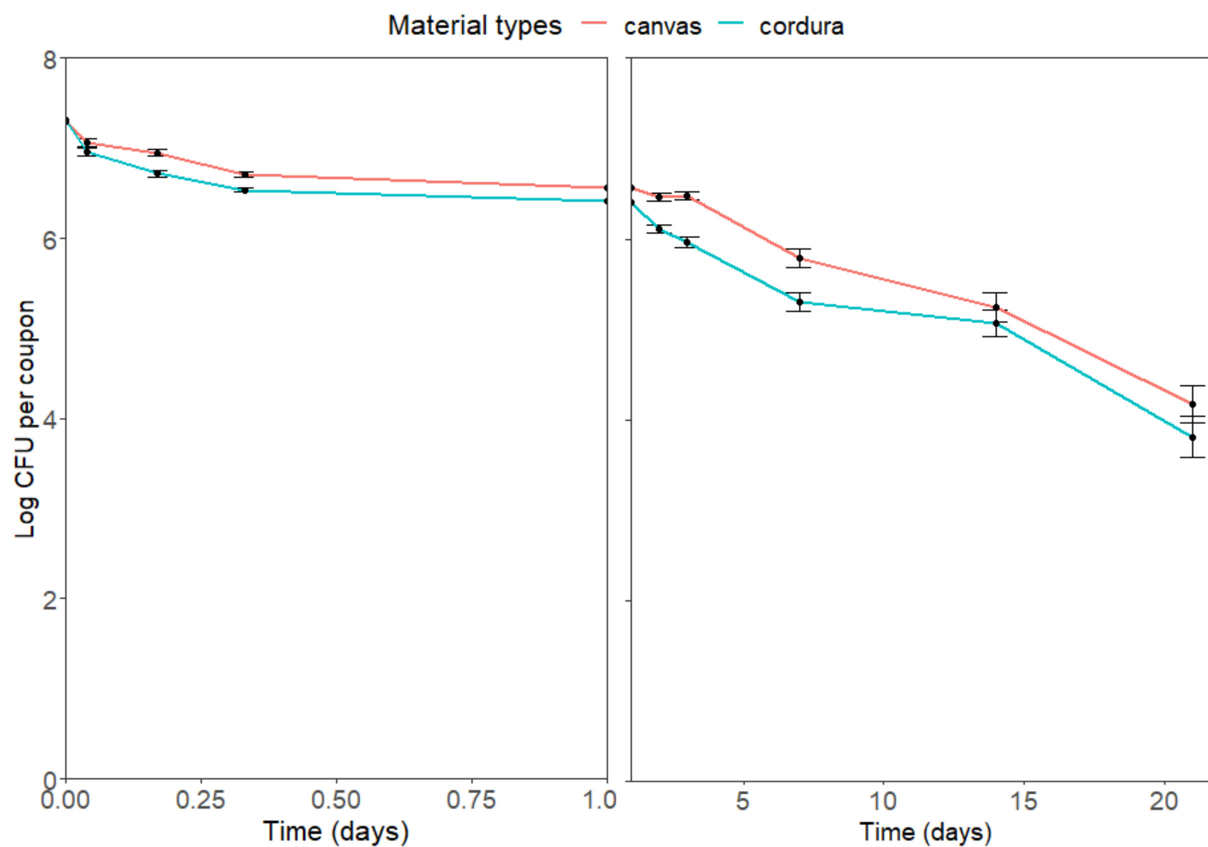
c. Numbers represent positive samples following the enrichment of coupons from the harvest bag material types. If nothing is in parentheses (), it was 9/9 enrichment positive.

d. When counts fell below the limit of detection (1.3 log CFU/coupon), a count value of 0.5 was assigned.

Table S4. Parameters of segment model the die-off of generic *E.coli* in log CFU/coupon on MACR plates

Parameters	**Effect estimate**	**95% confidence interval**	***P* value**
Intercept	7.96	7.79, 8.12	<0.001
Time (days)			
0.0-0.41	-2.70	-3.17, -2.23	<0.001
0.41-19.10	-0.16	-0.17, -0.16	<0.001
19.10 – 90	-0.02	-0.03, -0.02	<0.001
Material type (reference =Canvas)			
Cordura	-0.82	-0.91, -0.73	<0.001
Nylon	-0.67	-0.76, -0.58	<0.001
% RH	-0.01	-0.01, -0.01	<0.001
Trial	0.02	-0.02, 0.06	0.29
Interaction between material type and time (reference =time x canvas)			
Time x cordura	0.004	0.001, 0.0073	<0.01
Time x nylon	-0.001	-0.004, -0.002	0.48

Figure S1. Die-off (±SE) of *L. monocytogenes* on different harvest bag material types (TSAR)[ab]

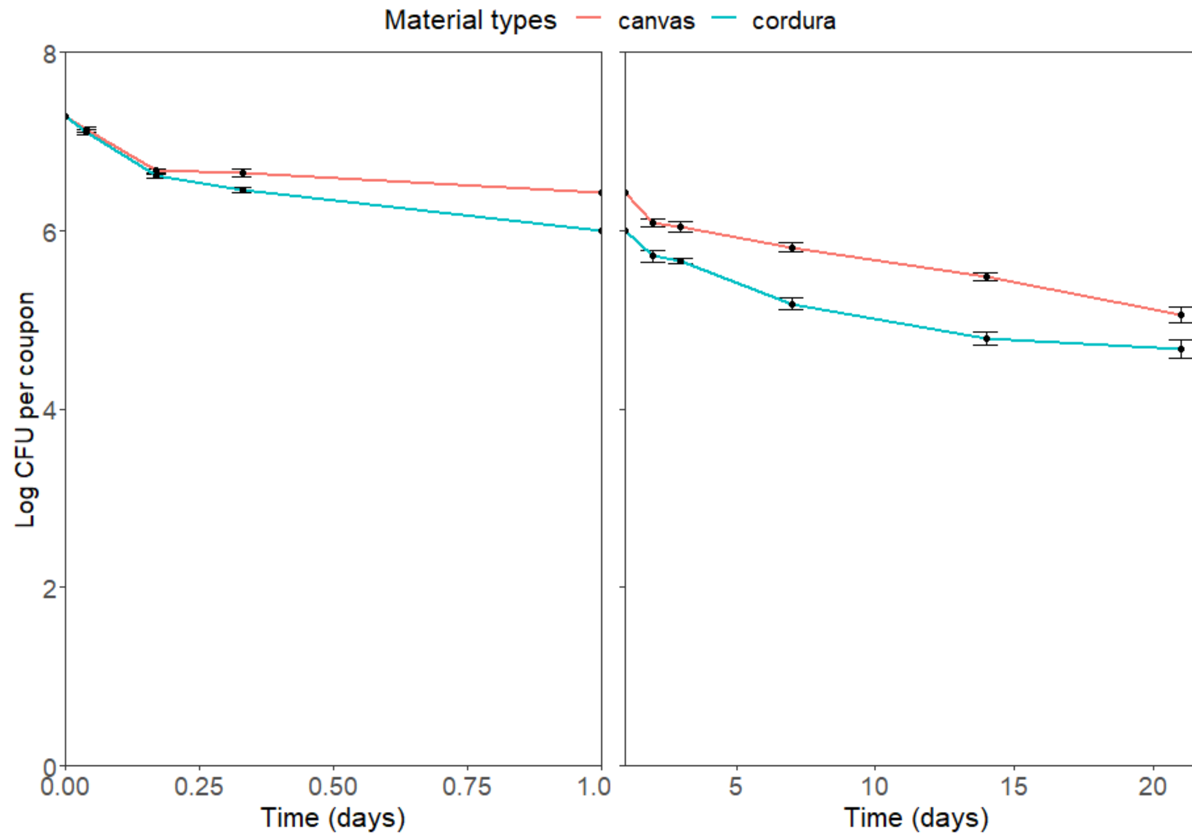


a. The starting level of *L. monocytogenes* at inoculation was 7.29 ± 0.09 and 7.32 ± 0.08 log CFU/coupon on canvas, and cordura respectively.

b. Mean log CFU/coupon (± standard error) of *L. monocytogenes* on TSAR plates.

c. The plot was facetted into 2 grids to show die-off at before 24 h (0 – 1 d) and after 24 h (1 -21 d).

Figure S2. Die-off (±SE) of *Salmonella* on different harvest bag material types (TSAR)[a*]



a. The starting level of *Salmonella* at inoculation was 7.29 ± 0.07 and 7.29 ± 0.06 log CFU/coupon on canvas, and cordura respectively.

b. Mean log CFU/coupon (± standard error) of Salmonella on TSAR plates.

c. The plot was facetted into 2 grids to show die-off at before 24 h (0 – 1 d) and after 24 h (1 -21 d).

Table S5. Reduction[a] of *Salmonella* on different harvest bag material types (XLDR)[b].

Time (days)	Material type	
	Canvas	Cordura
0.17	0.60 ± 0.11	0.64 ± 0.18
0.33	0.63 ± 0.13	0.80 ± 0.16
1	0.80 ± 0.14	1.16 ± 0.18
2	1.29 ± 0.20	1.73 ± 0.18
3	1.32 ± 0.23	1.79 ± 0.21
7	1.59 ± 0.25	2.15 ± 0.22
14	1.88 ± 0.15	2.65 ± 0.33
21	2.57 ± 0.48	3.74 ± 0.69

a. The starting concentration of *Salmonella* was 7.21 ± 0.07 and 7.22 ± 0.07 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation, bacterial levels were 6.89 ± 0.09 and 6.95 ± 0.15 log CFU/coupon on canvas, and cordura respectively.

b. Mean log reduction (± standard deviation) of *Salmonella* from day zero counted on XLDR plates.

Table S6. Parameters for the linear and segmented models describing the die-off of *Salmonella* (log CFU/coupon) over time (XLDR)[ab].

Model	Breakpoints (days)	Intercept	Daily die-off rate	95% CI[c]	*P*-value	AIC[d]	R^2
Linear	-	6.49	-0.12	-0.12, -0.09	<0.001	377.63	0.76
Segmented	0.00 - 1.25	7.03	-1.03	-1.18, -0.87	<0.001	135.25	0.91
	1.25 – 21	5.82	-0.06	-0.07, -0.04			

Breakpoint	Estimate
1	1.25 (1.07, 1.44)

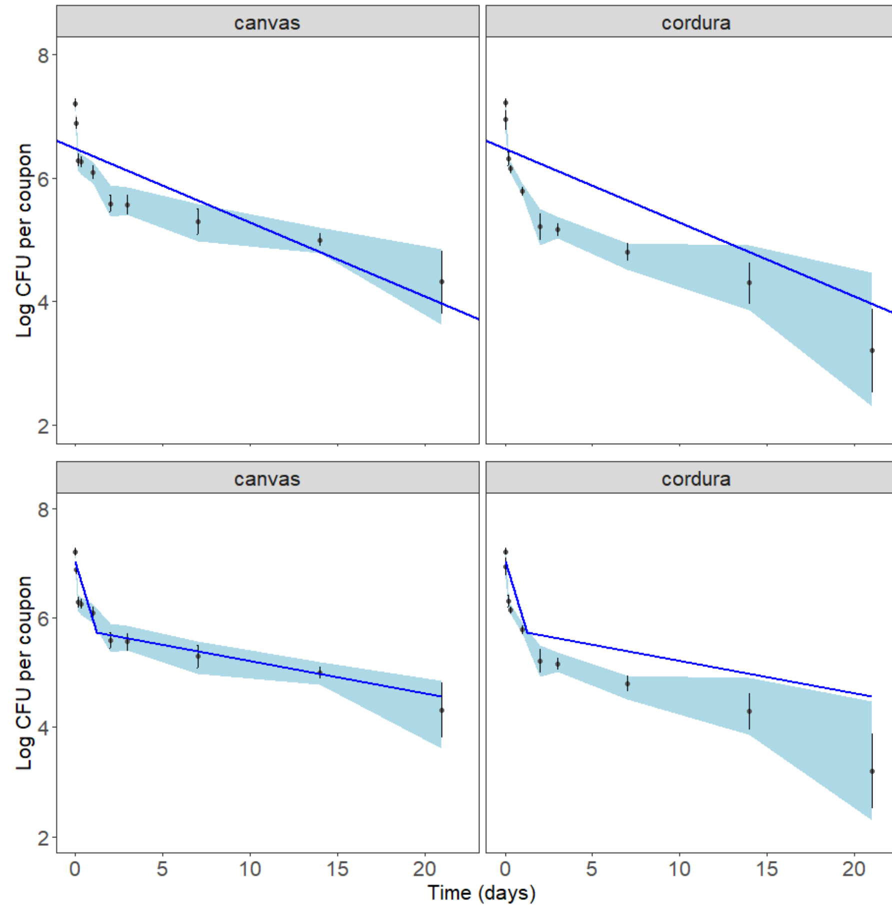
a. The starting concentration of *Salmonella* was 7.21 ± 0.07 and 7.22 ± 0.07 log CFU/coupon on canvas, and cordura respectively. At 1 h post inoculation, bacterial levels were 6.89 ± 0.09 and 6.95 ± 0.15 log CFU/coupon on canvas, and cordura respectively.

b. Log CFU/coupon for *Salmonella* on XLDR plates

c. 95 % confidence interval

d. Akaike's Information Criterion

Figure S3. Linear (first row) and segmented (second row) models for canvas and cordura describing *Salmonella* die-off over time (XLDR)[ab].



a. *Salmonella* levels (log CFU/coupon) were also reported as mean (dark points), standard deviation (dark bars) and minimum and maximum (blue shading).

b. Log CFU/coupon for *Salmonella* on XLDR plates.

Table S7. Reduction[a] of *L. monocytogenes* on different harvest bag material types (MOXR)[b].

Time (days)	Material type	
	Canvas	**Cordura**
0.17	0.22 ± 0.23	0.44 ± 0.35
0.33	0.69 ± 0.48	0.91 ± 0.63
1	0.50 ± 0.22	0.65 ± 0.55
2	0.77 ± 0.18	1.06 ± 0.31
3	0.78 ± 0.18	1.24 ± 0.40
7	1.78 ± 0.82	2.22 ± 0.81
14	2.09 ± 0.69	2.14 ± 0.64
21	3.33 ± 1.10	3.34 ± 0.88

a. The starting level of *L. monocytogenes* at inoculation was 7.25 ± 0.08 and 7.31 ± 0.07 log CFU/coupon canvas, and cordura respectively. At 1 h post inoculation, bacterial levels were 6.91± 0.18 and 6.80 ± 0.25 log CFU/coupon on canvas, and cordura respectively.

b. Mean log reduction (± standard deviation) of *L. monocytogenes* per coupon from day zero counted on MOXR plates.

Table S8: Parameters for the linear and segmented models the die-off of *L. monocytogenes* (log CFU/coupon) over time (MOXR)[ab].

Model	Breakpoints (days)	Intercept	Daily die-off rate	95% CI[c]	*P*-value	AIC[d]	R^2
Linear	-	6.32	-0.14	-0.14, -0.12	<0.001	819.97	0.70
Segmented	0 – 0.25	7.08	-3.84	-5.24, -2.45	<0.001	743.02	0.76
	0.25 - 21	6.12	-0.12	-0.13, -0.12			

Breakpoint	Estimate
1	0.25 (0.17, 0.34)

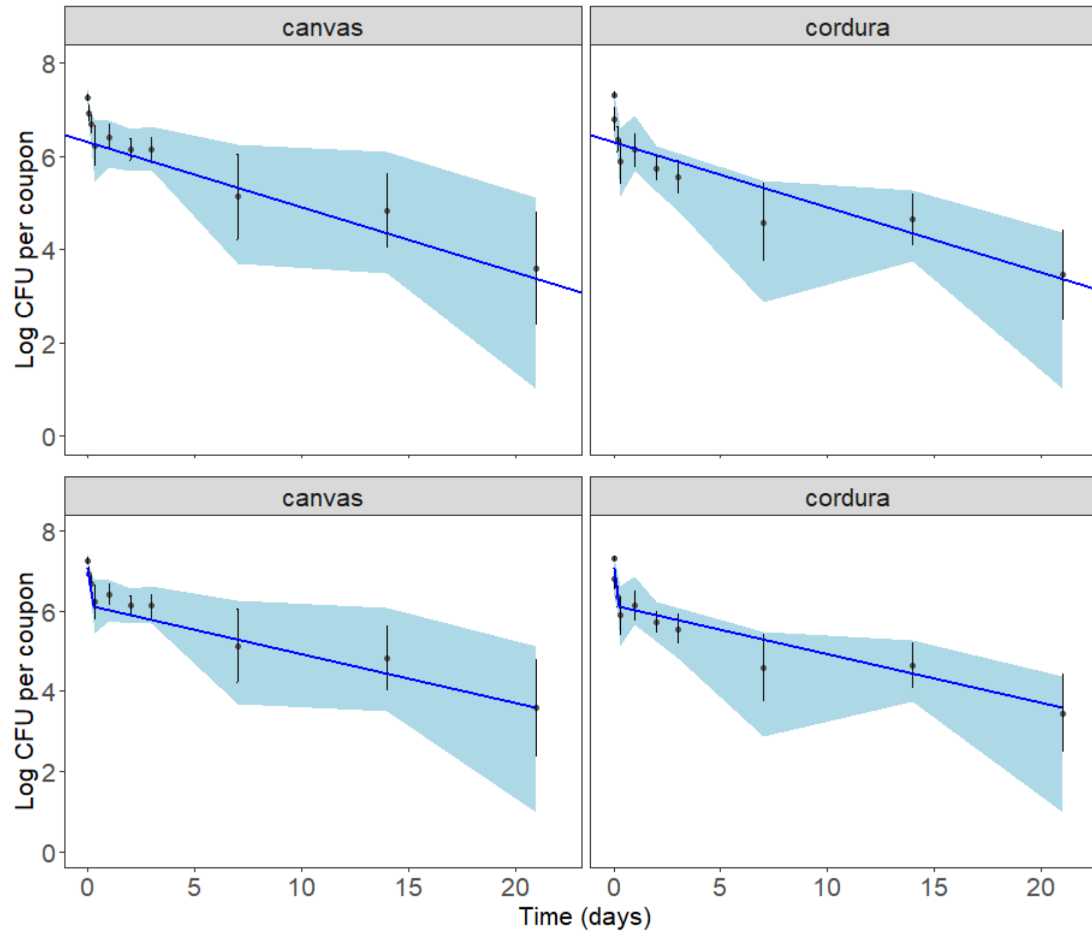
a. The starting concentration of *L. monocytogenes* at inoculation was 7.25 ± 0.08 and 7.31 ± 0.07 log CFU/coupon canvas, and cordura respectively. At 1 h post inoculation, bacterial levels were 6.91± 0.18 and 6.80 ± 0.25 log CFU/coupon on canvas, and cordura respectively.

b. Log CFU/coupon for *L. monocytogenes* on MOXR plates

c. 95 % confidence interval

d. Akaike's Information Criterion

Figure S4. Linear (first row) and segmented (second row) models for canvas and cordura describing *L. monocytogenes* die-off over time (MOXR)[ab].



a. *L. monocytogenes* levels (log CFU/coupon) were also reported as mean (dark points), standard deviation (dark bars) and minimum and maximum (blue shading).
b. Log CFU/coupon for *L. monocytogenes* on MOXR plates

Chapter 4: Generic *E. coli, Listeria monocytogenes* and *Salmonella* Exhibited Different Transfer Patterns from Harvest Bags to Apples.

ABSTRACT

Pathogen transfer from food contact surfaces to fresh produce has been linked to foodborne outbreaks and recalls. This study quantified generic *Escherichia coli, L. monocytogenes,* and *Salmonella* transfer from harvest bags to unwaxed apples. The percentage of *E. coli* that transferred from harvest bag materials to 'Red Delicious" apples were assessed for 4 material types (canvas, cordura, leather, and nylon), 2 inoculum dry times (1 and 4 h), 2 contact times (5 and 25 minutes), and 2 pressure scenarios (0.0 and 0.1kg/cm^2), totaling 32 scenarios. The percentage of *L. monocytogenes* and *Salmonella* transfer was assessed for 2 material types (canvas and cordura), 1 inoculum dry-time (1 h), and 1 contact time (5 minutes). Materials were cut into 25 cm^2 coupons and inoculated with a single strain of rifampicin-resistant *E. coli* (TVS353) or a 5-strain cocktail of *L. monocytogenes* or *Salmonella* and air-dried in a biosafety cabinet. The concentration of bacterial microorganisms on coupons post-drying was determined (approx. 7 log CFU/coupon) after which 'Red Delicious' unwaxed whole apples were placed on inoculated materials (this step is henceforth referred to as transfer). Post-transfer, coupons, and apples were placed in separate, sterile Whirl-bags, and massaged for 60 seconds in 0.1% peptone with 0.1% Tween 80. Using selective and non-selective media the concentrations of *E. coli, L. monocytogenes,* and *Salmonella* on apples and coupons were determined by spread-plating appropriate dilutions of the coupon and apple samples. The log percent of *E. coli, L. monocytogenes,* and *Salmonella* that were transferred from the coupons to the

apples was then calculated. To characterize differences in transfer between bag materials linear mixed models were fitted followed by a post hoc Tukey's analysis. Based on the model, contact time (P=0.55) was not significantly associated with the percent of *E. coli* that transferred from the coupon to the apple but inoculum dry time, material type, pressure, and apple weight were significantly associated (P<0.05). We found evidence of significant interactions between inoculum dry-time and pressure, and dry-time and coupon material. Harvest bag materials transferred more *E. coli* when the inoculum was allowed to dry for less time and *E. coli* transfer varied based on the harvest bag material types. The transfer of *L. monocytogenes* was significantly associated with material type (P<0.001), although not significantly associated with apple weight (P=0.46). Neither material type (P=0.46) nor apple weight (P=0.32) was significantly associated with *Salmonella* transfer. Because bacteria transferred from harvest bag surfaces to apples regardless of the scenario, it is important to clean and sanitize these surfaces.

Keywords. *Escherichia coli, Listeria monocytogenes*, *Salmonella*, Apple, Bag

HIGHLIGHTS

- Contact time did not significantly impact the transfer of generic *E. coli* from harvest bags to apples.
- Bacterial transfer levels decreased significantly when the inoculum on harvest bags was allowed to dry for 4 h.
- The transfer of *E. coli* was impacted by pressure.
- Transfer patterns were different based on the bacterial microorganism.

INTRODUCTION

During production and harvest, fresh produce comes in direct contact with various equipment, tools, and containers (*10*, *13*, *18*, *26*, *54*). For example, peach and apple growers utilize harvest bags or bushel buckets that can weigh up to 19 kg (42 lbs.) to transport fruit from the orchard to bins (*10*, *18*). In addition, to tools and equipment used during harvest to collect and transport produce, workers often use clothes to wipe produce, gloves, and their bare hands (*5*, *16*, *26*, *27*, *30*, *33*, *42*, *49*, *51*, *52*). Because fresh produce comes in direct contact with the surfaces of these tools and equipment, there is the potential for pathogens on produce to transfer to tools or equipment during harvest and vice-versa.

Previous studies have shown that human pathogens can survive for a prolonged period on materials, that harvest equipment, tools, and containers are made from (*1*, *28*, *32*, *39*, *49*, *50*, *57*, *58*). Allen et al. (*1*) recovered 0.7 log CFU/mL of *Salmonella* from stainless steel 28 days post-inoculation (dpi) when the steel was held at 20 °C and 60% relative humidity (RH). When the steel was held at 30 °C and 80% RH, *Salmonella* was undetectable by 11 dpi. Although Allen et al. (*1*) found a similar discrepancy in *Salmonella* survival on Polyvinyl chloride (PVC) and wood held at 20 °C and 60% RH versus 30 °C and 80% RH, *Salmonella* concentrations, were on average, a log higher for wood compared to steel after being held at 20 °C and 60% RH for 28 days. Scott and Bloomfield (*48*) demonstrated the survival of *E. coli* and *Salmonella* on clean and soiled fabric. In this study, the laboratory strain of *E. coli* survived up to 4 h on inoculated soiled fabric surfaces while the wild-type strains of *E. coli* and *Salmonella* were recovered at concentration levels >250 CFU/25cm^2 and 3 CFU/25cm^2 respectively, 48 h post-inoculation. When clean fabric surfaces were inoculated, both

the wild-type strains of *E. coli* and *Salmonella* were still present on the surfaces after 48 h. More studies have reported survival on paper, plastic, and cardboard material surfaces (*32*, *49*, *50*). These studies show the ability of bacterial microorganisms to survive under different conditions on materials commonly used to make harvest equipment, tools, and containers. Where there is survival, there may be a risk for cross-contamination if fresh produce touches the contaminated surfaces.

Bacterial transfer has been quantified from food contact surfaces to fresh produce and vice versa, highlighting the inherent risk of cross-contamination (*5*, *9*, *11*, *12*, *21*, *21*, *24*, *25*, *28*, *29*, *36*, *55*). Miranda and Schaffner (*36*) reported bacterial transfer from different surfaces (wood, ceramic tile, carpet, and stainless steel) to different food products including watermelon, white bread, white bread with butter, and gummy bears under different transfer scenarios with moisture having a significant impact on the transfer rate of bacteria. About 3 log CFU/cm^2 of *Salmonella* Enteritidis was transferred to cucumbers after contacting contaminated stainless steel materials for 10 seconds (*28*). Sattar et al. (*45*) observed the transfer of *Staphylococcus aureus* from contaminated cotton and polycotton fabric under varying conditions of moisture and friction and noted transfer rates greater than 0.8% CFU.

Despite the risks posed by the survival of microorganisms on food contact surfaces and the potential for cross-contamination, scientific studies quantifying bacterial transfer from contaminated harvest bag surfaces are lacking. These bags or bushel buckets come in different sizes, and designs, and are made from different fabric types. The bags can be reused during harvest operations to carry fruit from trees to bins making them food contact surfaces (*18*, *53*). Though there have been no outbreaks and

traceback investigations that have resulted from cross-contamination from harvest bags, outbreaks involving whole tree fruits have occurred (*2*, *6*, *7*). Preharvest conditions are often targeted as a likely source of contamination during these outbreaks even though traceback investigations rarely identify the source of pathogen contamination. With reusable harvest bags being used in the preharvest environment, there are many avenues for surface contamination and potential transfer to harvested fruits. Hence, quantifying the risk of bacterial transfer to fruit from contaminated bags will provide critical data that may be useful in developing risk management strategies to mitigate or eliminate cross-contamination from harvest bags. This study aimed to quantify the transfer of *E. coli*, *L. monocytogenes,* and *Salmonella* from different harvest bag material types to fresh apples.

MATERIALS AND METHODS

Culture. A single strain of generic *E. coli* (TVS 353; University of California, Davis) that was isolated from surface irrigation water was used in this study. This strain was adapted to 80 ppm rifampicin (Fisher Scientific, Fair Lawn, NJ) and stored with 15% glycerol at -80 °C. *L. monocytogenes* cocktail was prepared using five different strains (Scott A, 4b, 1983 pasteurized milk outbreak-reference strain for many challenge studies; V7, 1/2a, raw milk isolate-reference strain for many challenge studies; LM390-6, 1/2a, environmental isolate for 2011 cantaloupe outbreak; LM390-2, 1/2b, environmental isolate for 2011 cantaloupe outbreak; LM573-035, 4b, clinical isolate for 2014-2015 caramel apple outbreak). The *Salmonella* cocktail was prepared using five different strains (*Salmonella* enterica Enteriditis 2020AM-1539, peach outbreak, clinical isolate; *Salmonella enterica* Newport 2020AM-0919, onion outbreak, clinical isolate;

Salmonella Agona, alfalfa outbreak; *Salmonella* Montevideo, tomato outbreak; *Salmonella* St. Paul, pepper outbreak).

Inoculum Preparation. Bacterial inoculum was prepared as described in the previous chapter. Briefly, 10 µL of frozen culture of *E. coli* was streaked from 15 % glycerol stocks stored at -80 °C onto tryptic soy agar containing 80 ppm of rifampicin (TSAR; Difco, Becton Dickinson Co., Sparks, MD). After incubating the plates at 37 ± 2 °C for 24 h, a single colony was collected from the plates and 2 consecutive transfers were performed in 10 mL tryptic soy broth with 80 ppm rifampicin (TSBR; Difco, Becton Dickinson Co., Sparks, MD). After incubating the broth at 37 ± 2 ºC for 24 h, 1mL of the resulting bacterial suspension was pipetted onto a large (150 × 15 mm) TSAR plate and spread with a sterile spreader to cover the entire plate. The TSAR plate was held in the biosafety cabinet for 30 minutes with the vent on until all moisture was absorbed. The plate was then transferred to an incubator and incubated for 24 h at 37 ± 2 °C. After incubation, the bacterial lawn was collected by adding 9 mL of 0.1% peptone water (Fisher Scientific, Fair Lawn, NJ) to the plate and suspending the lawn with a sterile spreader. The resulting slurry was pipetted into 10-mL Falcon tubes, and the bacterial concentration was enumerated by performing serial dilutions in 0.1% peptone water and plating onto TSAR and MacConkey agar with 80 ppm rifampicin (MACR; Difco, Becton Dickinson Co., Sparks, MD). The TSAR and MACR plates were incubated at 37± 2 ºC for 24 h, and bacterial counts were reported in log CFU/mL (*4*). The bacterial slurry was adjusted to a mean starting level of 8.48 ± 0.25 log CFU/mL. The adjusted levels were always verified by spread plating appropriate serial dilutions of the inoculum on TSAR and MACR.

A 5-strain cocktail of *L. monocytogenes* or *Salmonella* was prepared as described in the previous chapter. Briefly, individual strains were streaked from frozen culture onto separate TSAR plates followed by two successive transfers in TSBR as previously described. A 100 uL aliquot of each overnight culture was spread plated on TSAR and incubated for 24 h at 35 °C. After incubation, each plate was flooded with 5 mL of buffered peptone water (BPW; Difco, Becton Dickinson Co., Sparks, MD), and the bacterial lawns were suspended in the BPW using a sterile L-shaped spreader. The resulting bacterial slurries per strain were combined in equal volumes to create 5-strain cocktails for each bacterial pathogen (2 mL of each strain suspension). Cocktails were adjusted to an absorbance of 0.05 or 0.1 at an optical density of 600nm for *L. monocytogenes* and *Salmonella*, respectively to a mean level of 8.18 ± 0.1 log CFU/mL for both bacterial pathogens. The adjusted levels were always verified by spread-plating appropriate serial dilutions onto selective and non-selective media.

Tree fruit commodity. Mature, unwaxed, whole 'Red Delicious' apples were obtained from a local packer (Crown Orchards., Covesville, VA) and stored at 4 ºC in a walk-in cooler (Harris Environmental Solution, Andover, MA) until use. Before trials, apples were taken out of cool storage, rinsed with tap water, and stored overnight at room temperature (approx.22 ± 3 ºC) (*5*, *19*). A circle (3.5 cm in diameter) was printed on the equator of the apples using a permanent marker after the apples came to room temperature (*5*). Each apple was weighed (weight ranging from 126.64 g to 339.39 g). To verify the absence of rifampicin-resistant bacteria, separate negative control trials were conducted by transferring uninoculated unwashed apples to sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI), adding 30 mL (*L. monocytogenes* and *Salmonella*) or 50 mL

(*E. coli*) of 0.1% peptone water with 0.1% (v/v) Tween 80 (Fisher Scientific, Fair Lawn, NJ) and plating appropriate dilutions on TSAR.

Harvest bag surface preparation. Four harvest bag material types were selected: multipurpose canvas tote bags (CTB; 100% cotton, purchased from Amazon), jumbo nylon picking buckets (Wells and Wade 7P104, East Wenatchee, WA; 32 lb fruit capacity, obtained from a local apple packing house), cordura fruit picking bag (Wells and Wade 73250, East Wenatchee, WA; 42 lb fruit capacity, obtained from a local apple packing house), and leather (Wells and Wade 73250, East Wenatchee, WA; 42 lb fruit capacity, obtained from a local apple packing house). Each of these was cut using scissors into 25 cm^2 coupons and placed on media pour rings (approx. 3.7 cm in diameter; PYREX, San Nicolas de los Garza, Mexico) (*5*, *21*). Before inoculation, both sides of the coupons were sterilized by UV light in a biosafety cabinet first for 10 minutes (5 minutes on each side) (*44*).

Transfer between harvest bag materials and apples. The percentage of *E. coli* that transferred from each coupon material type was assessed for 2 inoculum dry times (1 and 4 h), 2 contact times (5 and 25 minutes), and 2 pressure scenarios (0.0 and 0.1kg/cm^2), totaling 32 unique treatments. Each treatment was replicated 9 times (N=9 replicates x 32 treatments=totaling 288 samples). All coupon inoculations were performed in a biosafety cabinet. Coupons were inoculated by distributing 100 µL of bacterial inoculum in 15 to 20 droplets on the entire coupon surface excluding areas <2 mm from the edge. The final *E. coli* concentration on the coupons at inoculation was between approx. 7 and 7.5 log CFU/coupon (Table 1). The inoculated coupons were held in the biosafety cabinet with the vent on at ambient temperature (22 ± 3 ^oC) and

humidity for either 1- or 4-h. Apples were placed on the coupons so that the printed circle touched the coupon for either 5 or 25 minutes. Apples were held in place using media pour rings underneath the inoculated coupon surfaces. For scenarios with pressure, a 0.10 kg/cm^2 pressure was achieved by adding 2.27 kg Olympic weights (Dick's Sporting Goods, Christiansburg, VA) to the apple after placing it on the inoculated coupon surface. This pressure value was determined by using the weight of 13.64 kg (30 lbs.) of apples in a picking bag with a bottom length and width of 53.34 cm x 2.54 cm (21 x 1 in). After either 5 or 25 minutes, coupons and apples were transferred to separate sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) for *E. coli* enumeration.

Transfer rates of *L. monocytogenes* and *Salmonella* were evaluated for 2 material types (canvas and cordura), 1 inoculum dry-time (1 h), and 1 contact time (5 minutes). Each transfer scenario was replicated 30 times per material type totalling 60 measurements per bacterial pathogen (30 measurements per material type x 2 material types). Coupon surfaces were prepared, and sterilized and transfer was performed as previously described.

Enumeration. *E. coli* enumeration on apples was performed by adding 50 mL of 0.1% peptone water with 0.1% (v/v) Tween 80 to bags containing fruits. For apples used in *L. monocytogenes* and *Salmonella* trials, 30 mL of 0.1% peptone water with 0.1% (v/v) Tween 80 was added to bags containing fruits. Twenty milliliters of 0.1% peptone water with 0.1% (v/v) Tween 80 was added to bags with coupons. All samples were massaged for 60 seconds. The rinsate was then serially diluted in 9 mL of 0.1% peptone water, and appropriate dilutions were plated onto selective media (MACR for *E. coli*, modified Oxford agar supplemented with 80 ppm of rifampicin (MOXR) for *L.*

monocytogenes and xylose lysine deoxycholate supplemented with 80 ppm of rifampicin (XLDR) for *Salmonella*) and non-selective media (TSAR; all bacterial microorganisms). Plates were incubated at 35 °C (*L. monocytogenes* and *Salmonella*) or 37 ± 2 ºC (*E. coli*) for 24 h. Bacterial populations recovered from coupons were expressed in CFU/coupon and the populations on apples were expressed in CFU/apple. A value of 0.5 was assigned when bacterial counts fell below the detection limit (1.3 log CFU/coupon; 1.5 or 1.7 log CFU/apple).

Statistical analyses. The percentage of bacteria that transferred from coupon surfaces to apples in each scenario was calculated following the equation TC = Pt/Pi, where Pt was the bacterial concentration on the apple and Pi is the sum of bacterial concentration on the coupon and apple surfaces post-transfer (*5*, *21*). The transfer rate was expressed as a percentage as shown below.

$$\%\,Transfer = \left(\frac{Population\ on\ produce}{(Population\ on\ coupon\ +\ Population\ on\ produce)}\right) x\ 100$$

Because transfer rates were strongly skewed, log percent transfer was calculated as previously described (*9*, *46*).

All statistical analyses were performed using R Studio (version 4.2.3).

Statistical analyses of *E. coli* data. First, log-linear mixed-effects models were fitted using the *lme4* package (*3*). Inoculum dry time (1 or 4 h)', 'pressure (0.0 or 0.1 kg/cm^2)', 'material type (canvas, cordura, nylon or cordura)' and 'contact time (5 or 25 minutes)' were included fixed effects, while 'trial' was a random effect and 'log % CFU transfer' was the outcome. Interactions between 'inoculum dry time and material type', and 'pressure and material type' were also considered. To determine if including an interaction improved model fit, Akaike's Information Criterion (AIC) was used. Initial

regression analysis showed that contact time was not significant (Table S1). Hence, another linear mixed effects model was fitted with ‘inoculum dry time’, ‘pressure’, and ‘material type’ as fixed effects and ‘trial’ as a random effect. The model with a fixed effect of ‘contact time’ that was initially fitted was compared to the model without a fixed effect of ‘contact time’ using the AIC to determine if there was any significant improvement by dropping the ‘contact time’. Because the AIC showed an improvement when ‘contact time’ was dropped, subsequent statistical analysis was performed with the second model. The normality of our data was assessed using the *qqnorm()* function (*40*). After regression analysis, Tukey's post hoc analysis was performed using the *emmeans()* and *multcomp()* packages with significance at $P<0.05$ (*20*, *31*). The result from the post hoc analysis was included in a summary table with mean, median, minimum, maximum, and range for log % transfer values for each treatment combination. The number of positive apple samples per treatment combination (presence; n=18) was also included in the summary table. Finally, frequency distribution plots were created using the *ggplot2()* package to visualize the distribution log % percent transfer under different transfer scenarios (*56*). A bin width of 0.5 log % was selected for the plots as prior research has shown bin widths from 0.25 to 0.50 to be generally satisfactory (*8*, *23*, *38*). All plots were faceted by material type and pressure.

Statistical analyses of *L. monocytogenes* and *Salmonella* data. Log-linear mixed-effects models were fitted using the *lme4()* package (*4*) with material type, and apple weight as fixed effects, trial as a random effect, and ‘log % CFU transfer’ as the outcome. Frequency distribution curves were created using the *ggplot2()* package (*52*) to visualize the distribution of log % transfer for each transfer scenario.

RESULTS

No rifampicin-resistant *E. coli* were recovered upon plating negative controls onto TSAR. Preliminary analysis also showed that media type had a significant effect on the log % transfer (P=0.03). Therefore, only results obtained with TSAR plates were further discussed. Results from MACR plates are provided in the supplemental material section.

Contact time was not significantly associated with the transfer of *E. coli* from harvest bags to apples. Initial regression analysis showed that contact time did not have a significant effect on log % transfer of *E. coli* (P = 0.55; Table S1). Therefore, another model was fitted without contact time. This model was compared with the initial model using AIC. The AIC showed an improvement in our model when contact time was dropped. Hence, subsequent analysis was performed with the second model (Table 2). Inoculum dry time had a significant effect on log % transfer (P<0.001). At a 4-h dry time, the mean log % transfer rate to apples decreased significantly by 3.05 (95% CI=-3.42, -2.68) when compared to the 1-h dry time. The mean log % transfer rate also decreased significantly by 2.43 (95% CI=-2.88, -1.99) and 1.16 (95% CI=-1.61, -0.72) when leather and nylon material types were the sources of contamination, respectively (P<0.001; Table 2). However, when the source of contamination was cordura, the decrease in the mean log % transfer rate was not significant (P=0.13). When a 0.10 kg/cm^2 pressure was used in transfer scenarios, the mean log % transfer rate increased significantly by 0.41 (95% CI=0.05, 0.78). The mean effect of apple weight on log % transfer rate was very minimal (-0.001; -0.01, 0.00) although significant (P<0.001). Significant interactions between inoculum dry time and material type and pressure and material type were

observed (Table 2). For instance, the mean log % transfer rates increased significantly by 1.53 (95% CI=1.01, 2.04) at a 4-h dry time when leather was the source of *E. coli* contamination (*P*<0.001). In contrast, the mean bacterial log % transfer rates decreased significantly by 0.67 (95% CI=-1.18, -0.16) when cordura was the source of *E. coli* contamination and the dry time was 4 h (*P*=0.01). For interactions between pressure and material type, the mean log % transfer rates decreased significantly by 0.88 (95% CI=-1.39.-0.36) when nylon was the source of contamination.

The transfer of *E. coli* frequently occurred when canvas materials were the source of contamination at a 1-h dry time and 0.0 kg/cm^2 pressure. When the dry time was 1 h and the pressure was 0.0 kg/cm^2 pressure, the mean transfer log % rates from canvas differed significantly from rates with leather and nylon materials (*P*<0.001; Table 3). However, no significant difference was observed between transfer rates from canvas and cordura (*P*=0.81). The mean log % transfer of *E. coli* from canvas to apples was 1.27 log % (18.62%) and ranged from 1.04 log % (10.97%) to 1.51 log % (32.36%). The mean log % transfer rates of leather and nylon materials were -1.27 log % (range= <1.7 log CFU/apple to 1.49 log %(30.90%)) and 0.63 log % (range=<1.7 log CFU/apple to 1.54 log % (36.67%)), respectively. The mean log % transfer from cordura was 0.55 log % (3.55%) and ranged from below the detection limit (<1.7 log CFU/apple) to 1.77 log % (58.88%). More variability in log % transfer rate was observed when cordura, nylon, and leather were the sources of contamination (Table 3 and Figure 1). Additionally, the most frequent transfer to apples occurred with the canvas material type (18/18; >1.7 log CFU/apple), while the least transfer occurred with leather (11/18; >1.7 log CFU/apple).

Canvas and cordura materials transferred *E. coli* frequently at a 1-h dry time and 0.1 kg/cm^2 pressure. When the dry time was 1 h and the 0.1 kg/cm^2 pressure, the transfer rates from canvas and cordura were similar (P>0.05; Table 3, Figure 1). However, compared to leather and nylon, these log percent transfer rates were significantly higher (P<0.001). No significant difference was observed between the log % transfer rates of nylon and leather material types (P= 0.06). When canvas materials were the source of bacterial contamination, the mean log percent transfer was 1.22 log % (16.59%) and ranged from 0.51 (3.24%) to 1.50 log % (31.62%). With cordura, the mean log % transfer was 1.52 log % (33.11%) and ranged from 1.28 (19.06%) to 1.84 log % (69.18%). The mean log % transfer level on leather was -1.40 log % (0.04%) and ranged from below the detection limit (<1.7 log CFU/apple) to 1.51 log % (32.36%). The mean transfer rate from nylon materials was -1.43 log % (0.04%) and ranged from below the detection limit (<1.7 log CFU/apple) to 1.30 log % (19.95%). There was more variability in log % transfer rates from nylon and leather than from canvas and cordura (Table 3 and Figure 1). In addition, canvas and cordura transferred *E. coli* to apples more frequently (18/18) than nylon (9/18) and leather (12/18), respectively. Increasing pressure from 0.0 to 0.1 kg/cm^2 did not significantly change transfer rates from canvas, leather, and nylon materials (P≥0.2; Table 3 and Figure 1). A significant increase with less variability in the log % transfer rate was observed with cordura at 0.1 kg/cm^2 (P=0.004; Figure 1).

***E. coli* transfer occurred frequently with canvas surfaces at a 4-h dry time and 0.0 kg/cm^2 pressure.** When dry time increased to 4 h, the mean log % transfer rates decreased significantly (P<0.001; Tables 2 and 3). When there was no pressure

(0.0 kg/cm^2), the log percent transfer rates between canvas and nylon did not differ significantly (P=0.08; Table 3). Compared with cordura and leather, the mean log % transfer from the canvas was significantly higher (P<0.001; Table 3). Among nylon, cordura, and leather, the mean log % transfer rates were not significant (P≥0.77). The mean log % transfer from canvas was -2.13 log % (0.007%) and ranged from -3.24 log % (0.001%) to -0.60 log % (0.25%). On cordura, the mean log % transfer to apples was -2.93% (0.002%) and ranged from below the detection limit to -1.72 log % (0.02%). The mean transfer rate from leather was similar to cordura with a mean of -2.94% (0.001%) and ranged from below the detection limit to -1.36 log % (0.04%). The mean log % transfer rate from nylon was the lowest with a mean of -3.29 (<0.001%) and a range from below the detection limit to -1.70 log % (0.02%). Cross contamination was most frequent when the source was canvas (18/18: Table 3 and Figure 2) but was less frequent with leather (7/18; Table 3 and Figure 2).

The transfer of *E. coli* from leather materials occurred less frequently at 4 h dry time and 0.1 kg/cm^2 pressure. When pressure was used (0.1 kg/cm^2) at a 4 h dry time, the transfer rates between canvas and cordura were not significantly different (P=0.16; Table 3). In contrast, there were significant differences in transfer rates between leather and nylon compared to canvas (P<0.001; Table 3). The mean log % transfer from canvas was -1.10 log % (0.08%) with a range of -2.44 (0.004%) to -0.47 log % (0.34%). With cordura, the mean was -1.94 log % (0.01%) and ranged from -3.57 (0.0003%) to -0.01 log % (1.02%). The mean log % transfer rate from leather was -2.34 (0.005%) and ranged from below the detection limit to -0.53 log % (0.30%). The mean transfer rate from nylon was -2.10 log % (0.008%) and ranged from below the detection

limit to -0.73 log % (0.19%). While all other material types transferred bacteria to all apple samples (n = 18), the number of positive apples with leather was less than when no pressure was used (5/18; Table 3 and Figure 2). Using pressure during transfer significantly increased the log % transfer rate from cordura materials (P=0.004; Table 3). Increased variability was also observed with cordura, leather, and nylon materials when pressure was added to apples during transfer trials (Figure 2).

***L. monocytogenes* and *Salmonella* transferred differently from canvas and cordura materials to apples**. Significant differences in transfer rates were observed between TSAR and MOXR media (P<0.001). Hence, only results from analysis with counts from TSAR plates were further discussed. The mean log % transfer rate of *L. monocytogenes* increased significantly by 0.55 (95% CI= 0.48, 0.61) when the source of contamination was cordura (Table 5). Apple weight did not have a significant effect on log % transfer of *L. monocytogenes* (P=0.46; Table 5). The mean log % transfer from canvas was 1.17 ± 0.29 (14.79 ± 1.95%) and ranged from 0.52 (3.31%) to 1.61 log % (40.74%; Table 4 and Figure 3). The mean log % transfer from cordura was 1.71 ± 0.11 (51.29 ± 1.29%) and ranged from 1.55 (35.48%) to 1.87 log % (74.13%; Table 4 and Figure 3). Very minimal variation in transfer rate was observed when cordura materials were the source of contamination (Figure 3).

No significant difference in counts on TSAR and XLDR media types was observed (P=0.90). As such, further analysis was performed on combined data. When cordura was the source of *Salmonella* contamination, the mean log % transfer rate increased by 0.12 (95% CI= -0.19, 0.44) log % although not significantly when compared with canvas (Table 5). Similarly, apple weight did not significantly impact the transfer of *Salmonella*

(*P*=0.32; Table 5). The mean log % transfer from canvas materials was 0.53 ± 1.07 (3.39 ± 11.75%) and ranged from -3.19 (0.001%) to 1.39 (24.55%) log % (Table 4 and Figure 4). The mean log % transfer from cordura materials was 0.58 ± 1.69 (3.80 ± 48.98%) and ranged from -3.59 (<0.001%) to1.77 (58.88%) log % (Table 4 and Figure 4). There was more variability in the distribution of log % transfer in these trials especially when cordura surfaces were the source of contamination (Figure 4).

DISCUSSION

Our study showed that *E. coli* transfer was impacted by moisture (inoculum dry time), pressure, and material type (source of contamination) but not by contact time. Different studies have arrived at different conclusions due to differences in methods including transfer calculations, the number of replicates, contact time, source of contamination, recipient surface, inoculum concentration, bacterial strain, and moisture content (*9*, *21*, *22*, *28*, *29*, *34*, *36*, *37*, *55*, *59*). In our study, the total level of bacteria recovered from the source surfaces was determined by calculating the sum of bacteria recovered from the source of contamination (material types) and bacteria that were recovered from the recipient surface (apples) as shown in the methods section. Other studies have used only the level of bacteria recovered from the source surface for calculating transfer or only levels on the recipient surface as the transfer values, resulting in varying outcomes (*29*, *29*, *45*, *48*).

***E. coli* transfer was significantly impacted by moisture (inoculum dry time).** There was higher transfer when surfaces were wet versus when visibly dry. Published literature has reported moisture to be a significant driver for transfer regardless of the origin of the moisture (the source of contamination or from the recipient surface) (*24*, *28*,

36, *43*, *45*, *48*, *55*). Jensen et al. (*24*) observed an 89.88%, transfer of *Salmonella* to celery, and 84.53% transfer of *E. coli* to lettuce from freshly inoculated glass surfaces (less than 5 seconds post inoculation). When the inoculum on the glass surface was allowed to dry for 1 h, % transfer dropped to 4.93 % for *Salmonella* in celery and 1.97 % for *E. coli* in lettuce. Another study by Todd-Searle et al. (*55*) reported significantly higher transfer rates of *Salmonella* at less than 5 seconds and 1 h inoculum dry time. However, transfer rates decreased significantly and, in some cases, to levels below detection limits when the inoculum was allowed to dry for 24 hours on source surfaces. Research by Kusumaningrum (*28*) showed a 3.0 ± 0.3 log CFU/cm^2 (90 ± 27%) transfer of *Salmonella* Enteritidis to cucumbers from stainless steel surfaces inoculated at 3.1 ± 0.3 log CFU/cm^2. Almost all bacteria inoculated to the surface transferred to cucumbers and it was suggested that the moisture from the cucumbers significantly impacted transfer. Similarly, Miranda and Schaffner (*36*) observed transfer rates up to 1.99 log % (97.72%) when cut watermelons were the recipient surface. Although there was some variation based on the source of contamination and the inoculum matrix, transfer always occurred with watermelons with the highest transfers seen at 300 seconds contact time. It was also suggested that moisture influenced the transfer to watermelons. Scott and Bloomfield (*48*) observed similar transfer rates of *E. coli* at 0 and 1 h post-inoculation (53-59 CFU/25 cm^2 at 0h; 49-56 CFU/25 cm^2 at 1h). Transfer levels dropped by 2 h post inoculation (17 to 30 CFU/25cm^2) and were below the detection limit at 24 h.

Although not highly significant, the use of pressure resulted in increased transfer of *E. coli* from material types such as cordura regardless of inoculum dry time (approx. 1 log % difference). Different outcomes have been observed in transfer

experiments that include pressure as a factor. Kusumaningrum (*28*) demonstrated an increase in transfer by approx. 0.3 log CFU/cm^2 when a pressure of 500 g/slice of cucumber was used. Mbithi et al. (*35*) used 0.2 or 1.0 kg/ cm^2 pressure with or without friction and observed that transfer was significantly impacted (approx. 0.5 log PFU increase in transfer when pressure was increased; $P<0.05$). A study by Escudero et al. (*17*) used approx. 10g/cm^2 or approx. 100g/cm^2 and observed a significant increase in log % transfer of viruses when ceramic surfaces were the source of contamination. This was not the case when stainless steel and Formica, a composite laminate material, were the sources of contamination. Similar studies in the same lab had previously reported that pressure of approx. 1 or approx. 100g/cm^2 did not affect the transfer of viruses (*15*). These studies show that the effect of pressure can result in minimal or no increase in transfer and pressure effect may be impacted by the nature of the source of contamination. In our study, transfer increased significantly for cordura when pressure was applied although the increase in transfer was accompanied by an increase in variability at a 4 h dry time. In contrast, this increase in pressure did not significantly change transfer rates from canvas, nylon, and leather materials during dry times. These results support findings in previous studies and suggest that transfer may vary in scenarios with pressure based on the properties of the source of contamination.

The log % transfer of *E. coli* varied based on material type with higher transfer rates from canvas and cordura material types. Transfer occurred less frequently with leather materials than with other materials demonstrating that the source of contamination can impact the transferability of bacteria (*5*, *36*, *48*). For instance, Miranda and Schaffner (*36*) reported mean log % transfer rates on bread in a range

from -1.24 to 1.97 log % (0.06 to 93.33%) when the sources of contamination were wood, ceramic tile, and stainless steel regardless of inoculum matrix. When the source of contamination was the carpet, log % transfer values with bread decreased and ranged from -1.68 to 0.58 log % (0.02 to 3.80%). The absorbance of carpet surfaces resulted in less available bacteria for transfer. Scott and Bloomfield (*48*) recovered *E. coli* on fingertips in the range of 55 – 59 CFU/25 cm^2 at 0 h dry time when the source surface was a soiled laminated surface. When the source surface was soiled cloth, *E. coli* transfer to fingertips dropped to approx. 6 CFU/25 cm^2 at 0 h dry time. Similarly, Brar and Danyluk (*5*) saw differences in transfer rates from single-use, clean reusable, and soiled reusable gloves at 1 h post-inoculation in ‘glove to tomato’ transfer scenarios. It should be noted that different factors may impact the transferability of bacteria from the source to the recipient surface and not just one factor is responsible for transfer. The leather materials used in our study were ca 0.2 cm thick and made from animal skin. These properties may have increased absorption and adherence of bacteria resulting in less bacteria available for transfer.

Contact time did not have a significant impact on *E. coli* transfer. The expectation in our study was that increasing contact time from 5 to 25 minutes would result in increased transfer of bacteria. Different observations have been made in the published literature regarding the impact of contact time on bacterial transfer. As an example, Dawson et al. (*12*) reported that contact time significantly ($P<0.0001$) impacted the transfer of *Salmonella* from tile and carpet to bologna. In this study, contact time had no significant impact on % transfer when wood was the source of *Salmonella* contamination and bologna was the recipient surface. However, when the

source of continuation was tile and the recipient surface was bread, contact time only had an effect at 8 h post-inoculation (fewer bacteria were recovered at 5-second contact time; approx. 3 log CFU/cm^2) than at 30-and 60-second contact times (approx. 3.5 log CFU/cm^2). The contact times used in this study were less than or equal to 1 minute. Zhu et al. (*59*) reported a significant increase ($P < 0.001$) in transfer coefficients when contact time increased to 72 h in transfer scenarios between reusable plastic containers and intact cucumber sections at 85% RH. This study also reported significant interactions between contact time and temperature especially at contact times above 24h. Miranda and Schaffner (*36*) reported instances when increasing contact time resulted in more transfer but also instances where transfer did not increase or change with increasing contact time. For example, with bread, the mean log percent transfer of *Enterobacter aerogenes* from wood increased from -0.91 to 1.89 log % (0.12% to 77.63%) as contact time increased from below 1 to 300 seconds. Contrarily, when the recipient surface was watermelon, and the inoculum matrix was tryptic soy broth, increasing contact time (<1, 5, 30, and 300 seconds) did not result in a considerable increase in transfer from wood (range=1.96 to 1.97 log %), stainless steel (range=1.96 to 1.97 log %), tile (range=1.98 to 1.99 log %), and carpet (range=1.91 to 1.95%). When 0.1 % peptone buffer was the inoculum matrix and the recipient surfaces had less moisture, the expected increase in transfer with increasing contact time could be observed. These studies show that different factors could impact the number of bacterial cells that transfer from the source to the recipient surface and that increasing contact time does not always result in an increase or a change in transfer. Overall,

these highlight the complex nature of transfer and emphasize the need for more investigations to better understand variability under specific transfer scenarios.

Transfer patterns were different for *L. monocytogenes* and *Salmonella*. In our study, the mean log % transfer rate of *L. monocytogenes* was significantly different ($P<0.001$) with cordura materials exhibiting higher transfer rates with minimal variation (1.71 ± 0.11 log %) compared to canvas (1.17 ± 0.29 log %). In contrast, transfer scenarios with *Salmonella* did not show any significant difference between materials ($P=0.46$), and transfer in this case was more variable than scenarios with *L. monocytogenes.* Previous studies have reported increased and more frequent transfer with canvas material types in transfer investigations with generic *E. coli*. These findings suggest that there are differences in the transfer patterns of different bacterial genera. Very few studies have examined differences in transfer patterns between different bacteria (*14*, *34*). For example, Dickson (*14*) observed increased *L. monocytogenes* transfer from lean beef tissue with increasing time and decreased transfer with increasing time when the source of contamination was fatty beef tissue ($P<0.01$). Trials performed with *Salmonella* Typhimurium in this same study showed increased transfer over time with both lean and fatty beef tissue. Similarly, Mackintosh and Hoffman (*34*) demonstrated transfer rates of *Staphylococcus saprophyticus*, *E. coli, Pseudomonas aeruginosa*, and *Klebsiella aerogenes* by 1.67%, 0.47%, 0.36%, and 0.29% respectively, to hands. These studies show that different bacteria have different transfer patterns. The transfer rates for *L. monocytogenes* in our study are similar to transfer rates observed in trials investigating *L. monocytogenes* transfer from conveyor belt materials to whole cantaloupes (1.63 (43%) to 1.72 (52%) log %) even though the

transfer was evaluated for consecutive cantaloupes (*41*). Similarly, the calculated mean transfer rate to onions from a contaminated onion slicer was approx. 1.69 log % (49. 44%) which is also similar to transfer rates observed in this study (*47*). Hence transfer rates observed with *L. monocytogenes* are comparable to rates in other studies.

Prior studies that have investigated the transfer of *Salmonella* from inert surfaces have reported different results. For example, in transfer trials involving gloves and tomatoes, *Salmonella* transferred at rates of 0.29 ± 0.2 log % (1.95 ± 1.59%), 0.48 ± 0.5 (3.02 ± 3.16%), and 5 of 9 by enrichments to tomatoes from single-use, clean reusable and dirty gloves respectively (*5*). Only the transfer rate from clean reusable gloves was comparable to the transfer values of *Salmonella* observed in our study. Another study that looked at transfer rates of *Salmonella* from new and used plastic mulch to tomatoes reported mean log % transfer at 1 h dry time ranging from 0.96 (9.12%) to 1.62 (41.69%) log % which was higher than values observed in this study (*55*). These differences between transfer rates in literature and the ones in this study may be due to differences in methods, surfaces used (source of contamination and recipient surfaces), inoculum levels, and the way transfer rates were calculated.

A similar pattern of variability in the transfer of *Salmonella* (left tail end of the distribution curve) could be observed for both material types. The exact reason for this observation is not known. Because we used a strain cocktail in our study, the observed variation pattern may be the result of differences in attachment characteristics between bacterial strains in the cocktail. Further studies might want to explore this hypothesis.

CONCLUSION

The presence or absence of moisture greatly impacted the transfer of *E. coli* from harvest bag material surfaces to fresh unwaxed whole apples. Transfer rates varied between material types based on further the transfer scenario. Canvas was found to transfer bacteria more frequently than any other material type while leather materials transferred less bacteria. Though pressure impacted transfer, it was not highly significant since transfer still occurred regardless of pressure application. This study highlighted the role that moisture plays during cross-contamination from harvest bags.

In transfer studies with *L. monocytogenes,* cordura materials promoted a greater transfer compared to canvas. However, both materials transferred *Salmonella* at similar rates suggesting that different bacterial microorganisms exhibit different transfer patterns.

The findings of our study emphasize the importance of always keeping harvest bags constructed with these material types dry. Because bacteria can transfer from these surfaces, produce growers would want to make sure that harvest bags are handled and stored in ways that keep bags dry and reduce contamination. Harvest bags should be cleaned frequently and dried properly before use. As concerns cleaning frequency, each operation should have a sanitation standard operating procedure with cleaning tailored to fit the operation. Additionally, dry cleaning approaches should be emphasized during cleaning and sanitizing. Overall, data from this study will be useful in risk assessment for the development of risk mitigation measures for controlling food-borne pathogens in tree fruit harvest bags.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the corresponding author.

AUTHOR CONTRIBUTION

LS, AH, and CE contributed to the study conception and design. LS, AH, TL, KW, and CA contributed to the acquisition of data. LS, DS, DW, AH, and CA contributed to the analysis and interpretation of the data and drafting of the manuscript. LS, DS, DW, AH, and CA contributed to the critical revisions of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work is supported by the Specialty Crops Research Initiative 2020-51181-32157 from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

We are grateful to Crown Orchard and Turkey Knob apple packing houses for providing us with apples and apple harvest bags for this study. We also thank Faith Critzer and Michelle Danyluk for thoughtful discussions on data interpretation. We appreciate the revisions by Rebekah Miller and Isa Maria Reynoso.

REFERENCES

1. Allen, R. L., B. R. Warren, D. L. Archer, K. R. Schneider, and S. A. Sargent. 2005. Survival of *Salmonella* spp. on the Surfaces of Fresh Tomatoes and Selected Packing Line Materials. *HortTechnology*. American Society for Horticultural Science 15:831–836.
2. Angelo, K. M., A. R. Conrad, A. Saupe, H. Dragoo, N. West, A. Sorenson, A. Barnes, M. Doyle, J. Beal, K. A. Jackson, S. Stroika, C. Tarr, Z. Kucerova, S. Lance, L. H. Gould, M. Wise, and B. R. Jackson. 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection*. Cambridge University Press 145:848–856.
3. Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, G. Grothendieck, P. Green, and M. B. Bolker. 2015. Package 'lme4'. *convergence*. Available at: http://dk.archive.ubuntu.com/pub/pub/cran/web/packages/lme4/lme4.pdf. Accessed 24 March 2024.
4. Blessington, T., C. G. Theofel, and L. J. Harris. 2013. A dry-inoculation method for nut kernels. *Food Microbiology* 33:292–297.
5. Brar, P., and M. Danyluk. 2013. Salmonella Transfer Potential during Hand Harvesting of Tomatoes under Laboratory Conditions. *Journal of food protection* 76:1342–9.
6. Centers for Disease Control and Prevention. 2024. *Listeria* Outbreak Linked to Peaches, Nectarines, and Plums. Available at:

https://www.cdc.gov/listeria/outbreaks/peaches-11-23/index.html. Accessed 27 February 2024.

7. Centers for Disease Control and Prevention. 2020. Outbreak of *Salmonella* Enteritidis Infections Linked to Peaches. Available at: https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 27 February 2024.

8. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J Food Prot* 64:72–80.

9. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. *Journal of Food Protection* 64:72–80.

10. Corelli Grappadelli, L. 2001. Peach handling and marketing in Italy. Proceedings of 60th National Peach Council Convention. p. 39–45. *In* .

11. Danyluk, M. 2012. Pathogen transfer risks associated with specific tomato harvest and packing operations. Available at: https://www.centerforproducesafety.org/amass/documents/researchproject/311/2010-Danyluk_Pathogen%20transfer%20risks.pdf. Accessed 10 October 2021.

12. Dawson, P., I. Han, M. Cox, C. Black, and L. Simmons. 2007. Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule. *Journal of Applied Microbiology* 102:945–953.

13. DeFlorio, W., S. Liu, A. R. White, T. M. Taylor, L. Cisneros-Zevallos, Y. Min, and E. M. A. Scholar. 2021. Recent developments in antimicrobial and antifouling coatings to

reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Compr Rev Food Sci Food Saf* 20:3093–3134.

14. Dickson, J. S. 1990. Transfer of *Listeria monocytogenes* and *Salmonella* Typhimurium Between Beef Tissue Surfaces1. *Journal of Food Protection* 53:51–55.

15. D'Souza, D. H., A. Sair, K. Williams, E. Papafragkou, J. Jean, C. Moore, and L. Jaykus. 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. *International Journal of Food Microbiology* 108:84–91.

16. Erickson, M. C., J.-Y. Liao, C. C. Webb, M. Y. Habteselassie, and J. L. Cannon. 2018. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* deposited on gloves in a liquid state and subjected to drying conditions. *Int J Food Microbiol* 266:200–206.

17. Escudero, B. I., H. Rawsthorne, C. Gensel, and L. A. Jaykus. 2012. Persistence and Transferability of *Noroviruses* on and between Common Surfaces and Foods. *Journal of Food Protection* 75:927–935.

18. Freivalds, A., C. Lee, G. Earle-Richardson, C. Mason, and J. May. 2006. Effect of belt/bucket interface in apple harvesting. *International Journal of Industrial Ergonomics - INT J IND ERGONOMIC* 36:1005–1010.

19. He, Y., R. Chen, Y. Qi, J. K. Salazar, S. Zhang, M. L. Tortorello, X. Deng, and W. Zhang. 2021. Survival and transcriptomic response of *Salmonella enterica* on fresh-cut fruits. *International Journal of Food Microbiology* 348:109201.

20. Hothorn, T., F. Bretz, P. Westfall, R. M. Heiberger, A. Schuetzenmeister, and S. Scheibe. 2023. Package 'multcomp'. Available at: https://cran.r-project.org/web/packages/multcomp/multcomp.pdf. Accessed 10 April 2024.

21. Jensen, D. A., M. D. Danyluk, L. J. Harris, and D. W. Schaffner. 2017. Quantifying Bacterial Cross-Contamination Rates between Fresh-Cut Produce and Hands. *J Food Prot* 80:213–219.

22. Jensen, D. A., M. D. Danyluk, L. J. Harris, and D. W. Schaffner. 2017. Quantifying Bacterial Cross-Contamination Rates between Fresh-Cut Produce and Hands. *Journal of Food Protection* 80:213–219.

23. Jensen, D. A., L. M. Friedrich, L. J. Harris, M. D. Danyluk, and D. W. Schaffner. 2015. Cross contamination of *Escherichia coli* O157:H7 between lettuce and wash water during home-scale washing. *Food Microbiology* 46:428–433.

24. Jensen, D. A., L. M. Friedrich, L. J. Harris, M. D. Danyluk, and D. W. Schaffner. 2013. Quantifying Transfer Rates of *Salmonella* and *Escherichia coli* O157:H7 between Fresh-Cut Produce and Common Kitchen Surfaces. *Journal of Food Protection* 76:1530–1538.

25. Jung, J., L. M. Friedrich, M. D. Danyluk, and D. W. Schaffner. 2017. Quantification of Transfer of *Salmonella* from Citrus Fruits to Peel, Edible Portion, and Gloved Hands during Hand Peeling. *Journal of Food Protection* 80:933–939.

26. Karl R Matthews. 2013. Sources of enteric pathogen contamination of fruits and vegetables: future directions of research. *Stewart Postharvest Review* 9:1–5.

27. Killinger, K. 2014. Assessment of sanitation techniques for tree fruit storage bins. Available at: https://www.centerforproducesafety.org/researchproject/345/awards/Assessment_of_sanitation_techniques_for_tree_fruit_storage_bins.html. Accessed 1 January 2024.

28. Kusumaningrum, H. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology* 85:227–236.

29. Kusumaningrum, H. D., E. D. van Asselt, R. R. Beumer, and M. H. Zwietering. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. *J Food Prot* 67:1892–1903.

30. Leaman, S. M., J. Kerr, S. Salas, A. Malik, T. V. Suslow, M. Wiedmann, and D. A. Davis. 2023. Fresh Produce Harvesting Equipment – A Review of Cleaning and Sanitizing Practices and Related Science 43:126–143.

31. Lenth V., R., B. Bolker, P. Buerkner, I. Gine-Vazquez, M. Herve, M. Jung, J. Love, F. Miguez, H. Riebl, and H. Singmann. 2024. Package ‘emmeans’. Available at: https://cran.r-project.org/web/packages/emmeans/emmeans.pdf. Accessed 10 April 2024.

32. Li, K., H. Khouryieh, L. Jones, X. Etienne, and C. Shen. 2018. Assessing farmers market produce vendors’ handling of containers and evaluation of the survival of *Salmonella* and *Listeria monocytogenes* on plastic, pressed card, and wood container surfaces at refrigerated and room temperature. *Food control*. Elsevier Ltd.

33. López-Gálvez, F., L. Rasines, E. Conesa, P. A. Gómez, F. Artés-Hernández, and E. Aguayo. 2021. Reusable Plastic Crates (RPCs) for Fresh Produce (Case Study on Cauliflowers): Sustainable Packaging but Potential *Salmonella* Survival and Risk of Cross-Contamination. 6. *Foods*. Multidisciplinary Digital Publishing Institute 10:1254.

34. Mackintosh, C. A., and P. N. Hoffman. 1984. An extended model for transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection. *J Hyg (Lond)* 92:345–355.

35. Mbithi, J. N., V. S. Springthorpe, J. R. Boulet, and S. A. Sattar. 1992. Survival of *hepatitis* A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J Clin Microbiol* 30:757–763.

36. Miranda, R. C., and D. W. Schaffner. 2016. Longer Contact Times Increase Cross-Contamination of *Enterobacter aerogenes* from Surfaces to Food. *Appl Environ Microbiol* 82:6490–6496.

37. Montville, R., and D. W. Schaffner. 2003. Inoculum Size Influences Bacterial Cross Contamination between Surfaces. *Applied and Environmental Microbiology*. American Society for Microbiology 69:7188–7193.

38. Montville, R., Y. Chen, and D. W. Schaffner. 2001. Glove Barriers to Bacterial Cross-Contamination between Hands to Food. *Journal of Food Protection* 64:845–849.

39. Møretrø, T., E. Heir, K. R. Mo, O. Habimana, A. Abdelgani, and S. Langsrud. 2010. Factors affecting survival of Shigatoxin-producing *Escherichia coli* on abiotic surfaces. *International Journal of Food Microbiology* 138:71–77.

40. Nagle, C. 2018. An Introduction to Fitting and Evaluating Mixed-Effects Models in R. 1. *Pronunciation in Second Language Learning and Teaching Proceedings*. Iowa State University Digital Press 10.

41. Nyarko, E., K. E. Kniel, B. Zhou, P. D. Millner, Y. Luo, E. T. Handy, C. East, and M. Sharma. 2018. *Listeria monocytogenes* persistence and transfer to cantaloupes in the

packing environment is affected by surface type and cleanliness. *Food Control* 85:177–185.

42. Patrignani, F., L. Siroli, F. Gardini, and R. Lanciotti. 2016. Contribution of Two Different Packaging Material to Microbial Contamination of Peaches: Implications in Their Microbiological Quality. *Frontiers in Microbiology* 7.

43. Pérez-Rodríguez, F., A. Valero, E. Carrasco, R. M. García, and G. Zurera. 2008. Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science & Technology* 19:131–144.

44. Reinhard, R. G., R. M. Kalinowski, P. W. Bodnaruk, J. D. Eifert, R. R. Boyer, S. E. Duncan, and R. H. Bailey. 2020. Fate of *Listeria* on various food contact and noncontact surfaces when treated with bacteriophage. *Journal of Food Safety* 40:e12775.

45. Sattar, S. A., S. Springthorpe, S. Mani, M. Gallant, R. C. Nair, E. Scott, and J. Kain. 2001. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. *Journal of Applied Microbiology* 90:962–970.

46. Schaffner, D. W. 2003. Challenges in cross contamination modelling in home and food service settings. *FOOD TECHNOL.AUST.* Australian Institute of Food Science and Technology 55:583–586.

47. Scollon, A. M., H. Wang, and E. T. Ryser. 2016. Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control* 65:160–167.

48. Scott, E., and S. F. Bloomfield. 1990. The survival and transfer of microbial contamination via cloths, hands, and utensils. *Journal of Applied Bacteriology* 68:271–278.

49. Siroli, L., F. Patrignani, D. I. Serrazanetti, C. Chiavari, M. Benevelli, L. Grazia, and R. Lanciotti. 2017. Survival of Spoilage and Pathogenic Microorganisms on Cardboard and Plastic Packaging Materials. *Frontiers in Microbiology* 8.

50. Sirsat, S. A. 2020. The Persistence of Foodborne Pathogens on Produce Box Cartons. *Journal of Environmental Health*. National Environmental Health Association 82:16–21.

51. Sreedharan, A., K. R. Schneider, and M. D. Danyluk. 2014. *Salmonella* transfer potential onto tomatoes during laboratory-simulated in-field debris removal. *J Food Prot* 77:1062–1068.

52. Sullivan, G. B., and M. Wiedmann. 2020. Detection and Prevalence of *Listeria* in U.S. Produce Packinghouses and Fresh-Cut Facilities. *Journal of Food Protection* 83:1656–1666.

53. Superior Fruit Equipment. 2022. Product Guide: Pruning tools and harvest equipment for the professional fruit industry. Available at: https://store-n7xn0zsz.mybigcommerce.com/content/2022%20Product%20Guide.pdf. Accessed 29 November 2022.

54. Suslow, T. V., M. P. Oria, L. R. Beuchat, E. H. Garrett, M. E. Parish, L. J. Harris, J. N. Farber, and F. F. Busta. 2003. Production Practices as Risk Factors in Microbial Food Safety of Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety* 2:38–77.

55. Todd-Searle, J., L. M. Friedrich, R. A. Oni, K. Shenge, J. T. LeJeune, S. A. Micallef, M. D. Danyluk, and D. W. Schaffner. 2020. Quantification of *Salmonella*

enterica transfer between tomatoes, soil, and plastic mulch. *International Journal of Food Microbiology* 316:108480.

56. Wilkinson, L. 2011. ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H. *Biometrics* 67:678–679.

57. Wilks, S. A., H. Michels, and C. W. Keevil. 2005. The survival of *Escherichia coli* O157 on a range of metal surfaces. *International Journal of Food Microbiology* 105:445–454.

58. Wilks, S., H. Michels, and C. Keevil. 2006. The survival of *E. coli* O157 on a range of metal surfaces. *International journal of food microbiology* 105:445–54.

59. Zhu, Y., F. Wu, A. Trmcic, S. Wang, and K. Warriner. 2020. Microbiological status of reusable plastic containers in commercial grower/packer operations and risk of *Salmonella* cross-contamination between containers and cucumbers. *Food Control* 110:107021.

Material type	Inoculation	1 h post-inoculation	4 h post-inoculation
Canvas	7.59 ± 0.21	7.30 ± 0.11	7.11 ± 0.33
Cordura	7.53 ± 0.27	6.78 ± 0.40	6.78 ± 0.41
Leather	7.52 ± 0.27	7.30 ± 0.14	6.65 ± 0.70
Nylon	7.59 ± 0.35	7.31 ± 0.19	7.00 ± 0.32

TABLES AND FIGURES

Table 1. Mean *E. coli* levels (log CFU/coupon ± SD) on each material type at inoculation and 1 and 4 h post-inoculation

Parameters	Effect estimate[b]	95 % CI[c]	*P* value
Intercept	2.37	1.60, 3.14	<0.001
Inoculum dry time (reference = 1 h)			
4 h	-3.05	-3.42, -2.68	<0.001
Pressure (reference = 0.0 kg/cm^2)			
Pressure (0.10 kg/cm^2)	0.41	0.05, 0.78	0.03
Material type (reference = Canvas)			
Cordura	-0.35	-0.79, 0.10	0.13
Leather	-2.43	-2.88, -1.99	<0.001
Nylon	-1.16	-1.61, -0.72	<0.001
Apple weight (g)	-0.01	-0.01, 0.00	<0.001
Inoculum dry time x material type (reference = 4h x Canvas)			
4 h x Cordura	-0.67	-1.18, -0.16	0.01
4 h x Leather	1.53	1.01, 2.04	<0.001
4h x Nylon	0.51	0.00, 1.02	0.05
Pressure x material type (reference = 0.10 kg/cm^2 x Canvas)			
0.10 kg/cm^2 x Cordura	0.42	-0.09, 0.93	0.11
0.10 kg/cm^2 x Leather	-0.28	-0.79, 0.23	0.28
0.10 kg/cm^2 x Nylon	-0.88	-1.39, -0.36	<0.001

Table 2. Regression model parameters describing the log % transfer rates of *E. coli* from different harvest bag material types to apples[a].

a. Akaike's Information Criterion (AIC) =1819.28
b. The effect of contact time was not significant in initial data analyses. Therefore, another model was fitted without contact time as determined by the AIC. The results displayed were obtained from counts on TSAR plates
c. 95 % confidence interval

Table 3. Mean (±SD) log % transfer rates of generic *E. coli* from different harvest bag material types to apples (TSAR)[a]

Dry time (h)	Pressure (kg/cm^2)	Material type	Mean[b]		SD[c]	Median	Minimum	Maximum	Range	Presence[d]
1	0.0	canvas	1.27	ab, x	0.13	1.30	1.04	1.51	0.47	18/18
1	0.0	cordura	0.55	b, x	1.72	1.39	-3.60	1.77	5.37	17/18
1	0.0	leather	-1.27	e, x	1.68	-2.26	-3.02	1.49	4.51	11/18
1	0.0	nylon	0.63	c, x	1.49	1.24	-3.58	1.54	5.12	17/18
1	0.1	canvas	1.22	a, x	0.23	1.30	0.51	1.50	0.99	18/18
1	0.1	cordura	1.52	a, x	0.14	1.49	1.28	1.84	0.56	18/18
1	0.1	leather	-1.40	de, x	1.68	-2.53	-3.15	1.51	4.66	9/18
1	0.1	nylon	-1.43	cd, x	1.90	-2.55	-3.55	1.30	4.85	12/18
4	0.0	canvas	-2.13	ab, y	0.69	-2.18	-3.24	-0.60	2.64	18/18
4	0.0	cordura	-2.93	d, y	0.54	-2.95	-4.02	-1.72	2.30	16/18
4	0.0	leather	-2.94	d, y	0.61	-3.07	-3.82	-1.36	2.46	7/18
4	0.0	nylon	-3.29	bcd, y	0.68	-3.59	-4.06	-1.70	2.36	13/18
4	0.1	canvas	-1.10	a, y	0.48	-0.93	-2.44	-0.47	1.97	18/18
4	0.1	cordura	-1.94	abc, y	0.95	-2.10	-3.57	-0.01	3.56	18/18
4	0.1	leather	-2.34	cd, y	0.60	-2.37	-3.38	-0.53	2.85	5/18
4	0.1	nylon	-2.10	d, y	0.80	-2.14	-3.94	-0.73	3.21	18/18

a. Data represents mean (±SD) including median, minimum, maximum, and range of log % transfer of generic *E. coli* (±SD) from material types to apples at 1 and 4 h dry times and pressure 0.0 and $0.1 kg/cm^2$. For example, the mean transfer of generic *E. coli* from canvas bags to apples at a 1 h dry time and $0.0 kg/cm^2$ pressure was 1.27 ± 0.13 log % CFU. The results displayed were obtained from counts on TSAR plates.

b. a, b, c, d, e denote significant differences (P<0.05) for transfer scenarios within each dry time. x, y denote significant differences (P<0.05) within pressure levels between dry times.

c. Standard deviation.

d. Number of apple samples per scenario that had counts above the detection limit (1.7 log CFU/apple).

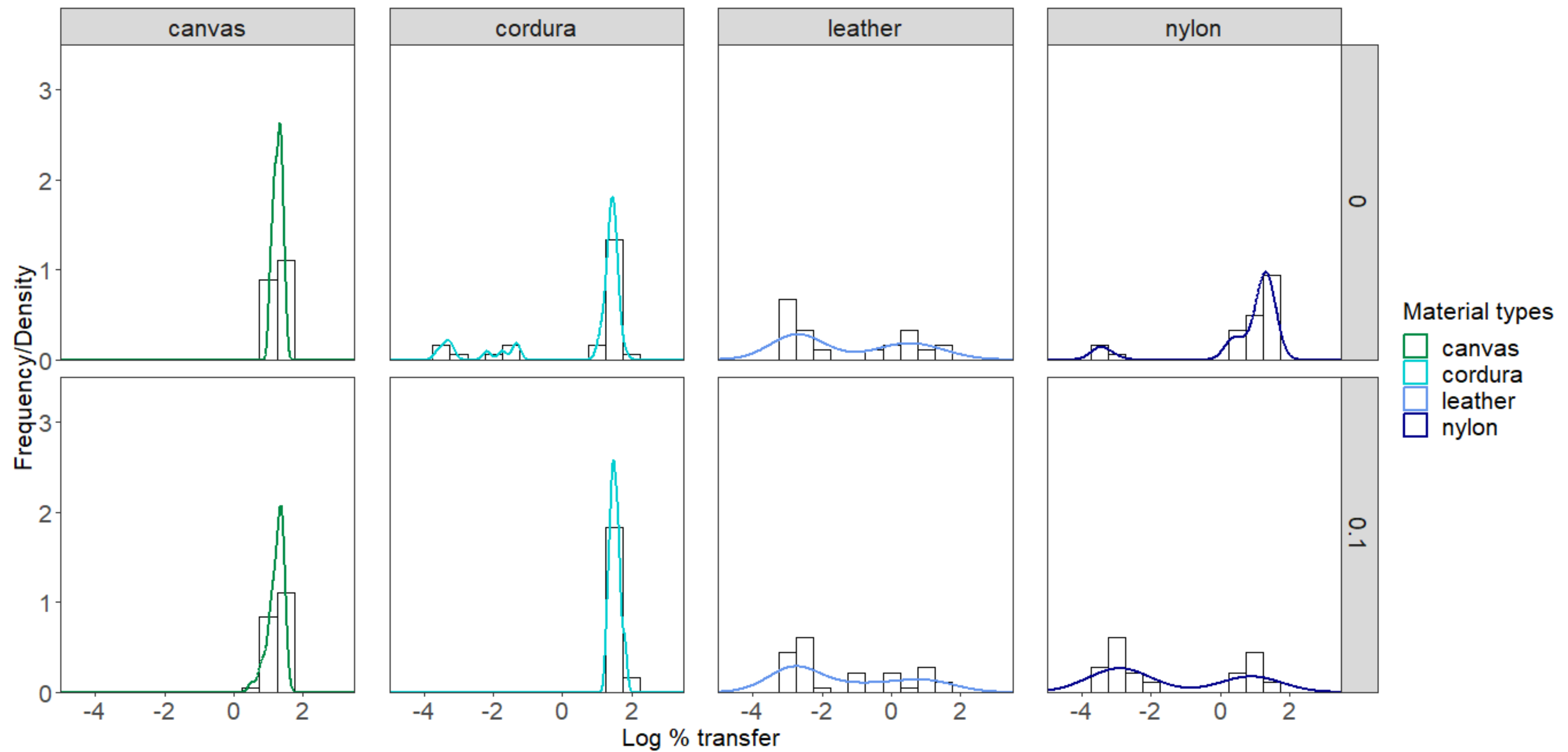


Figure 1. Distribution of log % transfer of E. coli for different harvest bag material types at 1 h dry time (TSAR)

The results displayed were obtained from counts on TSAR plates. Plots were facetted by material type and pressure (0.0 and 0.1kg/cm^2). The starting levels for *E. coli* 1 h post-inoculation are shown in Table 1.

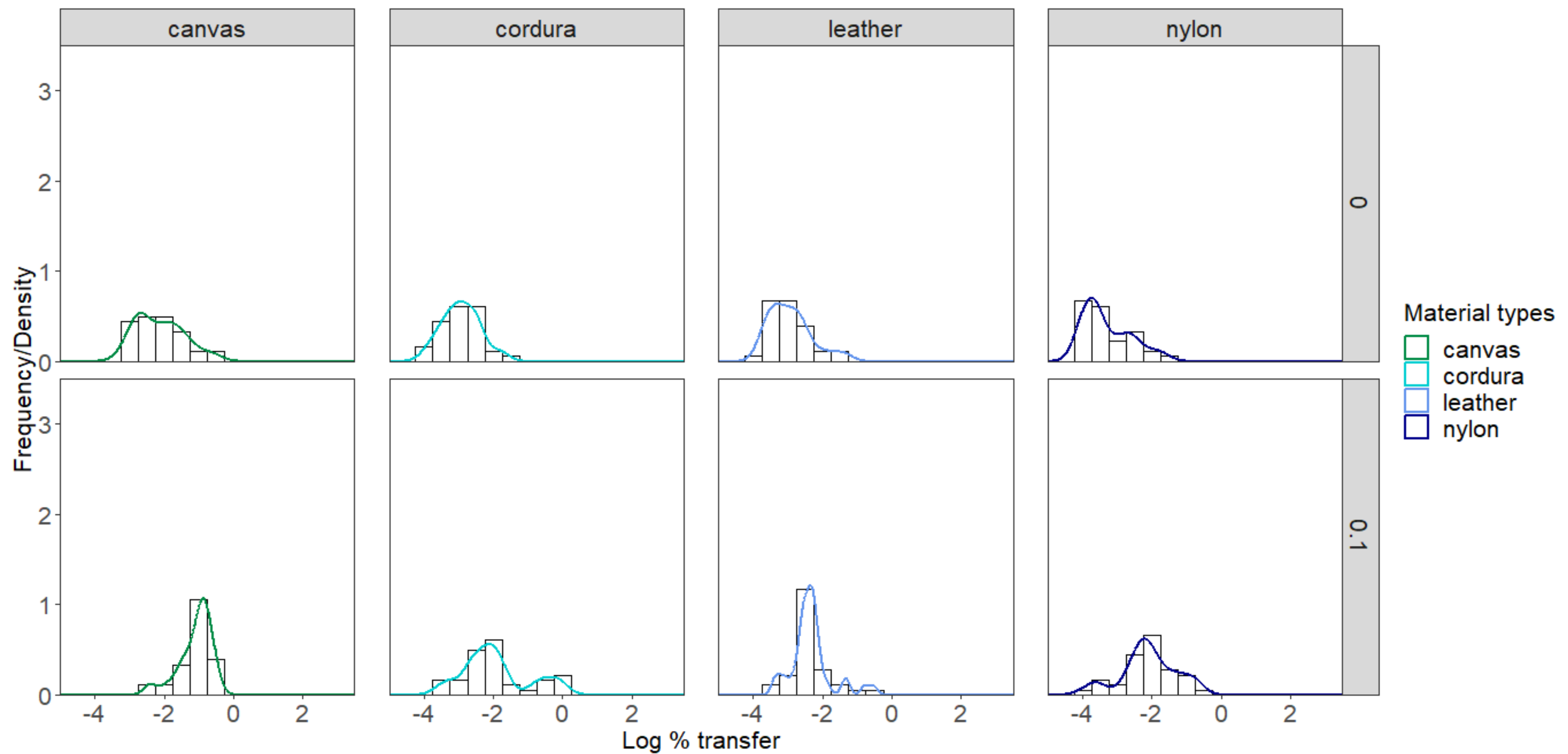


Figure 2. Distribution of log % transfer of *E. coli* for different harvest bag material types at 4 h dry time (TSAR). The results displayed were obtained from counts on TSAR plates. Plots were facetted by material type and pressure (0.0 and 0.1kg/cm^2). The starting levels for *E. coli* 4 h post-inoculation are shown in Table 1.

Table 4. Mean transfer (log % transfer) of *L. monocytogenes* and *Salmonella* from different material types to apples[a]

	Material type	
	Canvas	**Cordura**
L. monocytogenes	1.17 ± 0.29 A	1.71 ± 0.11 B
Salmonella sp	0.53 ± 1.07 A[b]	0.58 ± 1.69 A

a. Means ± SD (standard deviation) with the same upper-case letter between material types were not significantly different ($P<0.05$).
b. The mean transfer values for *Salmonella* were obtained from counts on both TSAR and XLDR while mean transfer values for *L. monocytogenes* were obtained from counts on TSAR only.

Table 5. Linear mixed models describing the transfer (log % CFU/coupon) of *L. monocytogenes* and *Salmonella* from harvest bag material types.

Bacteria	Parameters	Estimate	95% CI[a]	*P* value
L. monocytogenes	Intercept	1.08	0.83, 1.33	<0.001
	Material type (reference = Canvas)			
	Cordura	0.55	0.48, 0.61	<0.001
	Apple weight (g)	0.000	-0.001, 0.002	0.46
Salmonella[b]	Intercept	1.09	-0.07, 2.26	0.07
	Material type (reference = Canvas)			
	Cordura	0.12	-0.20, 0.44	0.46
	Apple weight (g)	-0.003	-0.009, 0.003	0.32

a. 95 % confidence interval
b. Transfer values for *Salmonella* were obtained from counts on both TSAR and XLDR and transfer values for *L. monocytogenes* were obtained from counts on TSAR only.

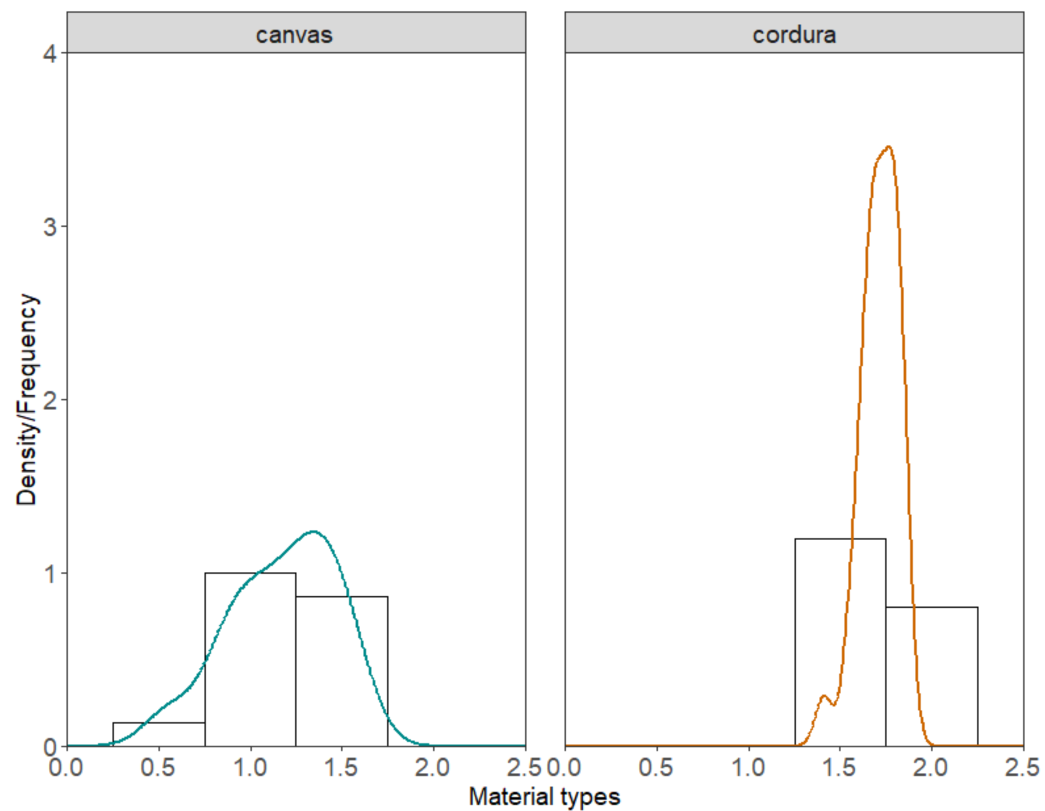


Figure 3. Distribution of log percent transfer of *L. monocytogenes* from different harvest bag material types to apples (TSAR). Plots were facetted by material type. The bin width used for this plot was 0.5.

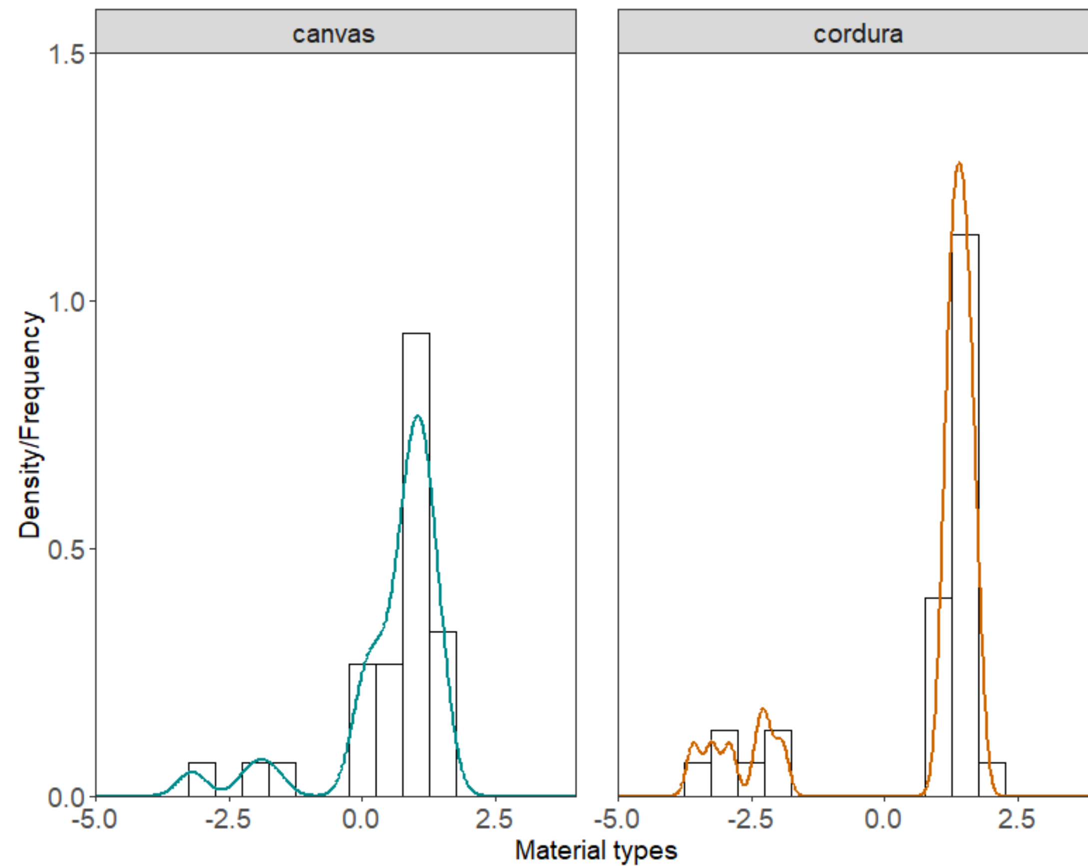


Figure 4. Distribution of log percent transfer of *Salmonella* from different harvest bag material types to apples. Plots were facetted by material type. The bin width used for this plot was 0.5. Data was combined for both TSAR and XLDR as their effect was not significant (P=0.90).

SUPPLEMENTAL MATERIALS

Table S1. Linear regression model parameters describing the log % transfer rates of *E. coli* from different harvest bag material types to apples (with contact time; TSAR).*

Parameters	**Effect estimate**	**95 % CI[a]**	***P* value**
Intercept	2.34	1.58, 3.12	<0.001
Inoculum dry time (reference = 1 h)			
4 h	-3.05	-3.43, -2.68	<0.001
Contact time (reference = 5 mins)			
25 mins	0.06	-0.12, 0.24	0.55
Pressure (reference = 0.0 kg/cm^2)			
Pressure (0.10 kg/cm^2)	0.41	0.05, 0.78	0.03
Material type (reference = Canvas)			
Cordura	-0.35	-0.79, 0.10	0.13
Leather	-2.43	-2.88, -1.99	<0.001
Nylon	-1.16	-1.61, -0.72	<0.001
Apple weight (g)	-0.01	-0.01, 0.00	<0.001
Inoculum dry time x material type (reference = 4h x Canvas)			
4 h x Cordura	-0.67	-1.18, -0.16	0.01
4 h x Leather	1.53	1.01, 2.04	<0.001
4h x Nylon	0.51	0.00, 1.02	0.05
Pressure x material type (reference = 0.10 kg/cm^2 x Canvas)			
0.10 kg/cm^2 x Cordura	0.42	-0.09, 0.93	0.11
0.10 kg/cm^2 x Leather	-0.28	-0.79, 0.23	0.29
0.10 kg/cm^2 x Nylon	-0.88	-1.39, -0.36	<0.001

*: The effect of contact time (0.06 (-0.12, 0.24); $P = 0.55$) was not significant in initial data analyses. Therefore, contact time data was pooled and another linear mixed model was fitted. Akaike's information Criterion =1823.83.
a: 95 % CI: 95 % confidence interval

Table S2. Linear regression model parameters describing the log % transfer rates of *E. coli* from different harvest bag material types to apples (MACR).*

Parameters	**Effect estimate**	**95 % CI[a]**	***P* value**
Intercept	3.27	2.46, 4.09	<0.001
Inoculum dry time (reference = 1 h)			
4 h	-0.99	-1.11, -0.87	<0.001
Contact time (reference = 5 mins)			
25 mins	0.003	-0.01, 0.01	0.57
Pressure (reference = 0.0 kg/cm^2)			
Pressure (0.10 kg/cm^2)	0.09	0.02, 0.16	0.02
Material type (reference = Canvas)			
Cordura	-0.03	-0.58, 0.52	0.91
Leather	-2.64	-3.19, -2.10	<0.001
Nylon	-1.31	-1.85, -0.76	<0.001
Apple weight (g)	-0.01	-0.01, 0.00	<0.001
Inoculum dry time x material type (reference = 4 h x Canvas)			
4 h x Cordura	-0.26	-0.42, -0.09	<0.001
4 h x Leather	0.53	0.37, 0.70	<0.001
4h x Nylon	0.16	-0.01, 0.33	0.07
Pressure x material type (reference = 0.10 kg/cm^2 x Canvas)			
0.10 kg/cm^2 x Cordura	0.07	-0.03, 0.17	0.17
0.10 kg/cm^2 x Leather	-0.02	-0.12, 0.08	0.66
0.10 kg/cm^2 x Nylon	-0.18	-0.28, -0.08	<0.001

*: The effect of contact time (0.003 (-0.01, 0.01); $P = 0.57$) was not significant in initial data analyses. Therefore, contact time data was pooled and another linear mixed model was fitted. The results displayed were obtained from counts on MACR plates.
a: 95 % CI: 95 % confidence interval

Table S3. Linear regression model parameters describing the log % transfer rates of *E. coli* from different harvest bag material types to apples (MACR)*

Parameters	**Effect estimate**	**95 % CI[a]**	***P* value**
Intercept	2.31	1.58, 3.07	<0.001
Inoculum dry time (reference = 1 h)			
4 h	-2.96	-3.33, -2.60	<0.001
Pressure (reference = 0.0 kg/cm^2)			
Pressure (0.10 kg/cm^2)	0.44	0.09, 0.80	0.02
Material type (reference = Canvas)			
Cordura	-0.29	-0.72, 0.15	0.20
Leather	-2.11	-2.54, -1.67	<0.001
Nylon	-1.15	-1.58, -0.71	<0.001
Apple weight (g)	-0.01	-0.01, 0.00	<0.001
Inoculum dry time x material type (reference = 4h x Canvas)			
4 h x Cordura	-0.77	-1.27, -0.27	0.003
4 h x Leather	1.60	1.10, 2.10	<0.001
4h x Nylon	0.48	-0.03, 0.98	0.07
Pressure x material type (reference = 0.10 kg/cm^2 x Canvas)			
0.10 kg/cm^2 x Cordura	0.35	-0.15, 0.85	0.17
0.10 kg/cm^2 x Leather	-0.11	-0.61, 0.39	0.66
0.10 kg/cm^2 x Nylon	-0.91	-1.41, -0.41	<0.001

*: The effect of contact time (0.003 (-0.01, 0.01); $P = 0.57$) was not significant in initial data analyses. Therefore, contact time data was pooled and another linear mixed model was fitted. The results displayed were obtained from counts on MACR plates.
a: 95 % CI: 95 % confidence interval

Table S4. Mean and median log percent transfer rates of *E. coli* from different harvest bag material types to apples (MACR)[a]

Dry time (h)	Pressure (kg/cm^2)	Material type	Mean		SD	Median	Minimum	Maximum	Range	Presence (n=18)
1	0	canvas	1.29	ab, x	0.14	1.29	1.06	1.57	0.51	18/18
1	0	cordura	0.58	b, x	1.63	1.42	-3.09	1.62	4.71	17/18
1	0	leather	-0.84	d, x	1.41	-1.61	-2.58	1.62	4.20	10/18
1	0	nylon	0.61	c, x	1.43	1.16	-3.42	1.60	5.03	17/18
1	0.1	canvas	1.20	a, x	0.15	1.24	0.82	1.41	0.58	18/18
1	0.1	cordura	1.53	a, x	0.12	1.53	1.29	1.74	0.45	18/18
1	0.1	leather	-1.00	cd, x	1.58	-1.77	-2.77	1.72	4.50	10/18
1	0.1	nylon	-1.42	cd, x	1.82	-2.49	-3.38	1.23	4.61	11/18
4	0	canvas	-2.09	ab, y	0.79	-2.25	-3.52	0.35	3.86	18/18
4	0	cordura	-2.89	cd, y	0.56	-2.96	-3.80	-1.66	2.14	14/18
4	0	leather	-2.58	bd, y	0.90	-2.58	-4.05	-1.10	2.95	4/18
4	0	nylon	-3.23	bcd, y	0.68	-3.44	-3.98	-1.53	2.45	11/18
4	0.1	canvas	-0.98	a, y	0.51	-0.85	-2.35	-0.22	2.13	18/18
4	0.1	cordura	-1.97	b, y	0.74	-2.06	-3.12	-0.31	2.81	17/18
4	0.1	leather	-1.56	ab, y	0.80	-1.64	-2.91	0.63	3.54	6/18
4	0.1	nylon	-2.07	d, y	0.85	-1.93	-3.73	-0.63	3.09	16/18

a: Data represents mean (±SD) including median, minimum, maximum, and range of log % transfer of *E. coli* (±SD) from material types to apples at 1 and 4 h dry times and pressure 0.0 and 0.1kg/cm^2. The results displayed were obtained from counts on MACR plates. Because the effect of contact time (0.003 (-0.01, 0.01); $P = 0.57$) was not significant, contact time data was pooled (n= 18).
b: a, b, c, d, e denote significant differences ($P<0.05$) for transfer scenarios within each dry time. x, y denote significant differences ($P<0.05$) within pressure levels between dry times.
c: Range was calculated as the difference between the maximum and the minimum log % transfer values for each scenario.
SD: Standard deviation.
d: Number of apple samples per scenario that had counts above the detection limit (1.7 log CFU/apple).

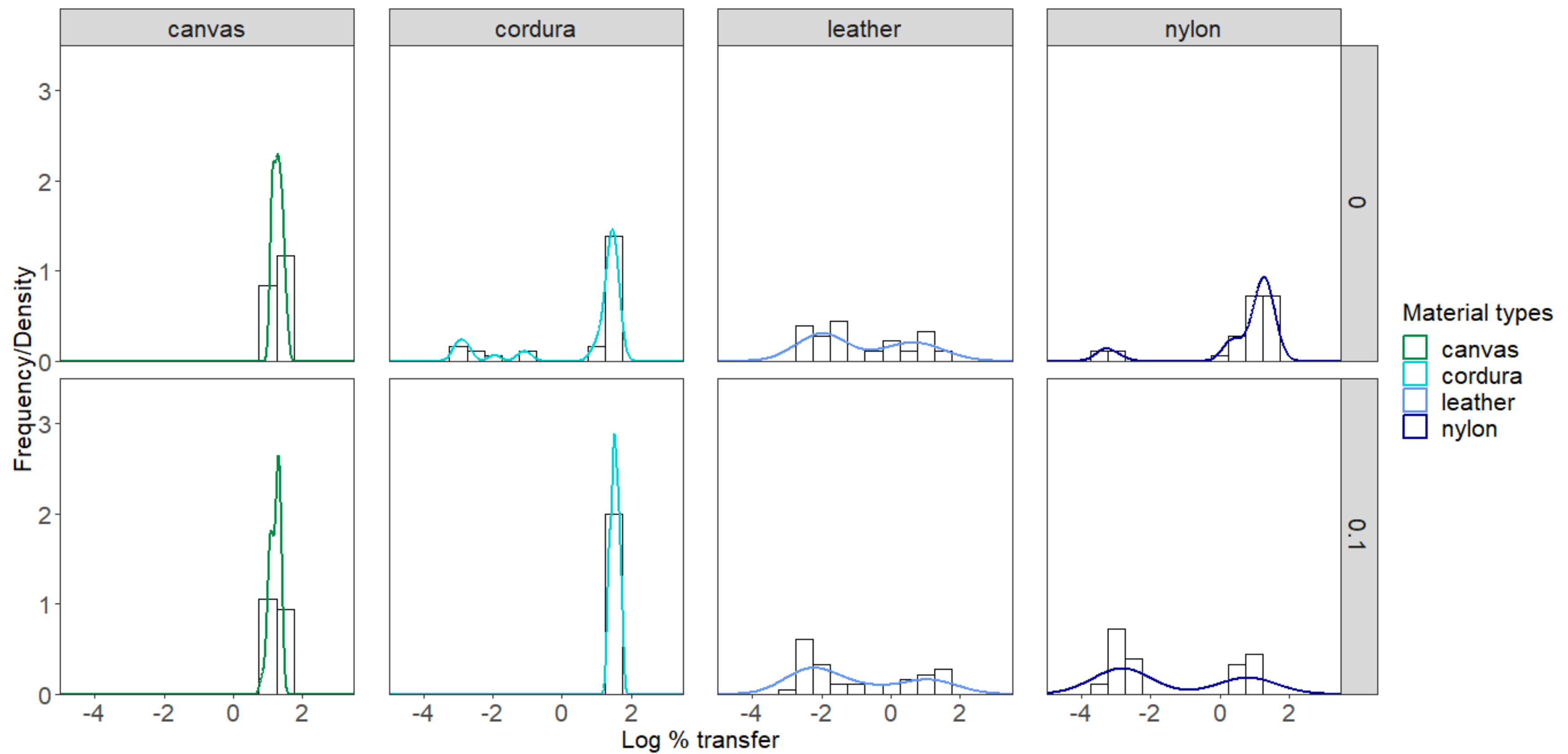


Figure S1. Distribution of log % transfer of *E. coli* for different harvest bag material types at 1 h dry time (MACR). The results displayed were obtained from counts on MACR plates. Plots were facetted by material type and pressure (0.0 and 0.1kg/cm^2). The starting levels of *E. coli* 1 h post-inoculation are shown in Table 1.

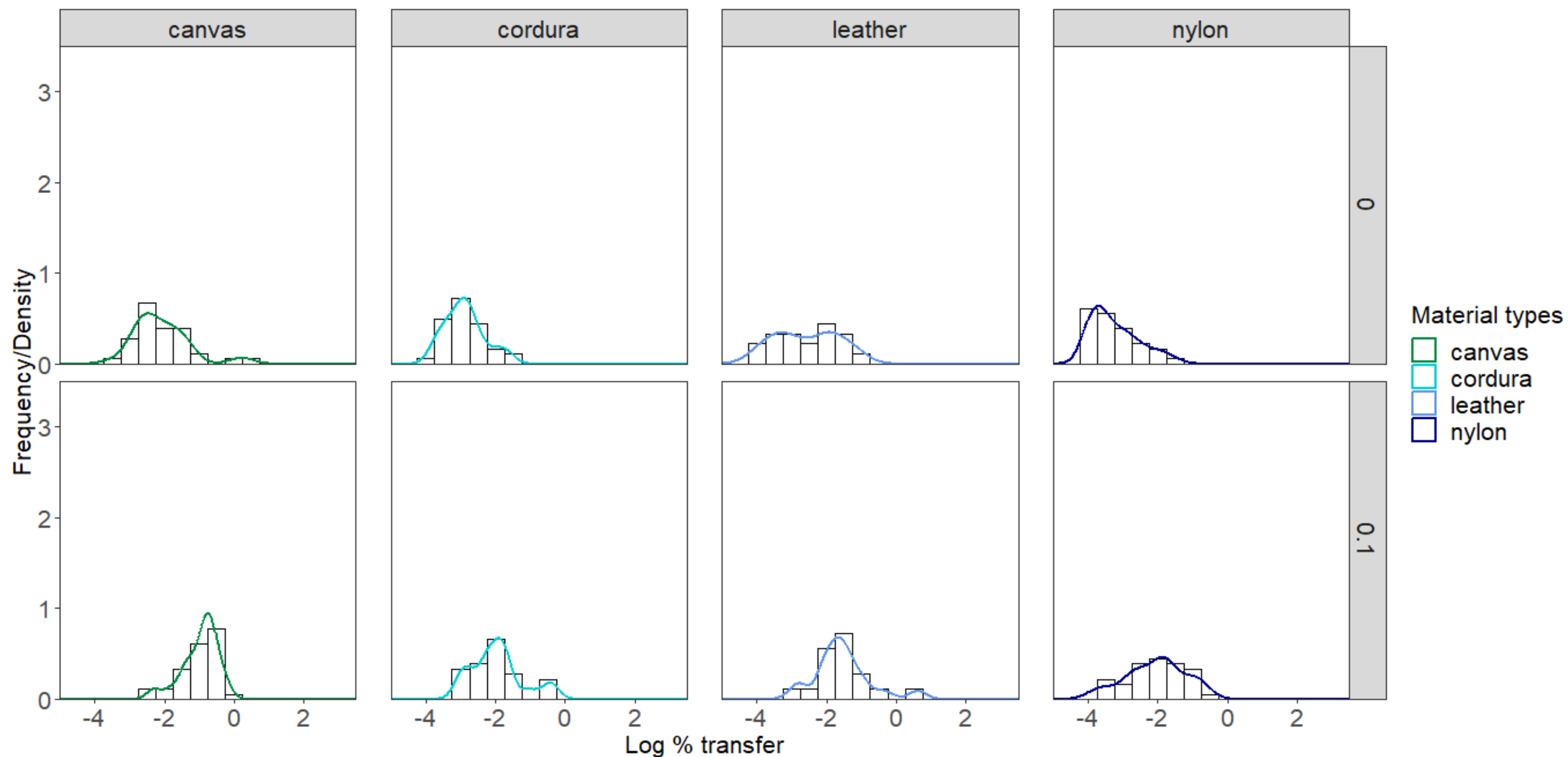


Figure S2. Distribution of log % transfer of *E. coli* for different harvest bag material types at 4 h dry time (MACR). The results displayed were obtained from counts on MACR plates. Plots were facetted by material type and pressure (0.0 and 0.1kg/cm^2). The starting level of *E. coli* 4 h post-inoculation is shown in Table 1.

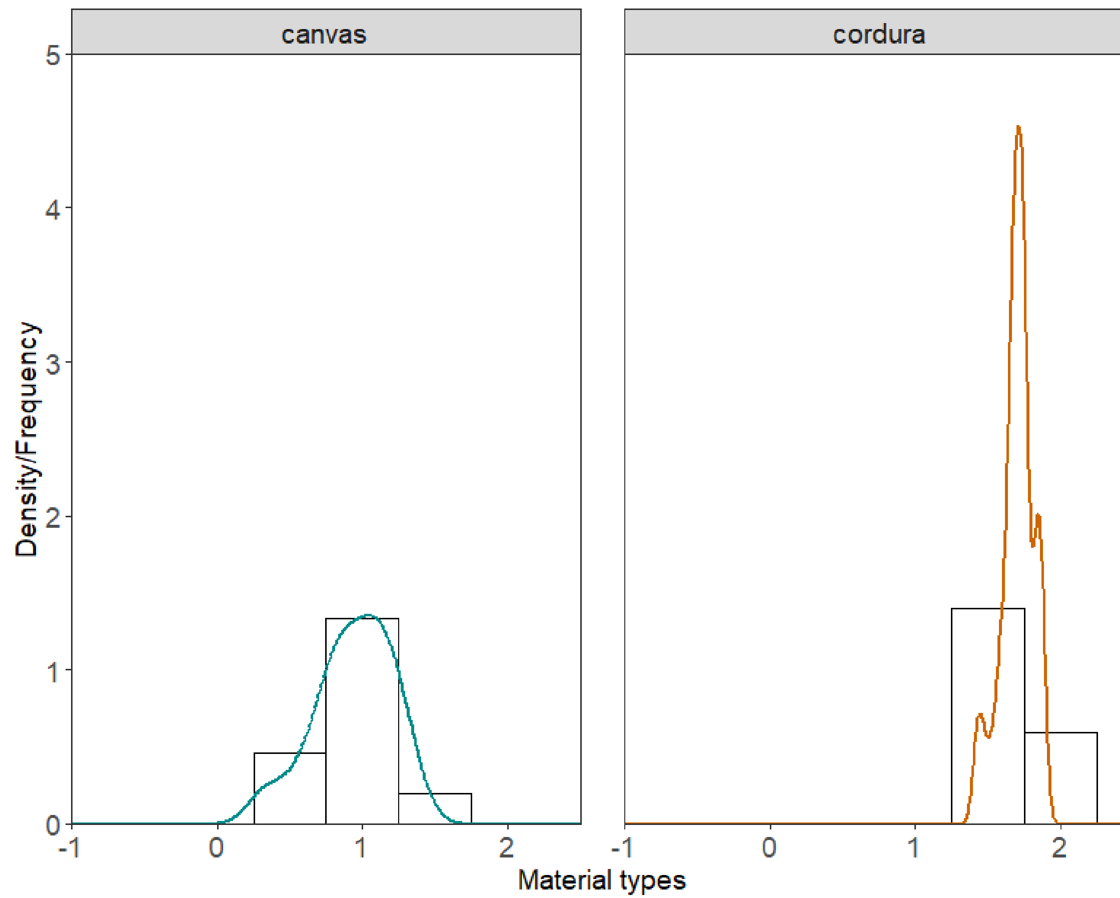


Figure S3. Distribution of log percent transfer of *L. monocytogenes* from different harvest bag material types to apples (MOXR). Plots were faceted by material type. The bin width used for this plot was 0.5.

Table S5. Linear mixed model describing the transfer (log % CFU/coupon) of *L. monocytogenes* from harvest bag material types (MOXR)[a].

Parameters	**Estimate**	**95% CI[b]**	***P* value**
Intercept	0.86	0.62, 1.10	<0.001
Material type (reference = Canvas)			
Cordura	0.76	0.70, 0.83	<0.001
Apple weight (g)	0.00	-0.001, 0.002	0.52

a. Transfer values for *L. monocytogenes* were obtained from counts on MOXR only.
b. 95 % confidence interval

Chapter 5: Isopropyl Alcohol with Quaternary Ammonium Compounds (IPAQuats) was Effective at Reducing *Salmonella* and *Listeria monocytogenes* on Cordura and Canvas Harvest Bags.

ABSTRACT

Food contact surfaces, including harvest bags, should be cleaned, and sanitized to prevent potential foodborne contamination events. Recommendations for sanitation best practices of harvest bags are lacking. The main objective of this study was to assess the efficacy of sanitizers on the reduction of *L. monocytogenes* and *Salmonella* on harvest bag material types (canvas and cordura). Material types were cut to 25 cm^2 coupons and inoculated with 5-strain cocktails of *L. monocytogenes* or *Salmonella* at approx. 7.42 ± 0.14 log CFU/coupon. The surfaces were air-dried in a biosafety cabinet for 1.5 hours until visibly dry. Inoculated surfaces were treated with sanitizers: chlorine (200 ppm and pH 10.35), chlorine (200 ppm and pH 7), peroxyacetic acid (PAA; 200 ppm), isopropyl alcohol with quaternary ammonium compounds (IPAQuats; ready-to-use), steam and water. Additional trials with *Salmonella* were performed by treating cordura surfaces with chlorine (500 ppm and pH 7). All sanitizers were applied following the manufacturer's instructions for a 1-minute treatment time. Surfaces were rubbed and shaken for 60 seconds in 20 mL of D/E neutralizing broth + 1% Tween80 and plated on selective (Modified Oxford-RP; *L. monocytogenes*) or (Xylose Lysine

Deoxycholate-RP; *Salmonella*) and non-selective (Tryptic Soy Agar-RP; both pathogens) media for enumeration. Duplicate experiments were conducted with five replicates per treatment combination (n=10/treatment combination). Regression models were fitted, and significant differences were evaluated by Tukey's HSD test at $P<0.05$ in R-Studio (version 4.3.2). *L. monocytogenes* and *Salmonella* reduction was significantly higher on cordura surfaces treated with IPAQuats, and PAA compared to canvas ($P<0.02$). IPAQuats was the most effective sanitizer resulting in reductions ≥4.61 log CFU/coupon on both material types for both pathogens. When surfaces were treated with PAA, *L. monocytogenes* concentrations were reduced by 2.63 ± 0.56 and 3.92 ± 0.81 log CFU/coupon while *Salmonella* concentrations were reduced by 3.68 ± 0.79 and 3.21 ± 1.14 log CFU/coupon on canvas and cordura, respectively. With 200 ppm chlorine (pH 7 or 10.35), steam, and water, reductions were <2 log CFU/coupon for *Salmonella*, and reductions ranged from 0.90 to 2.74 log CFU/coupon for *L. monocytogenes*. Chlorine levels of 500 ppm at pH 7 reduced *Salmonella* by >3 log CFU/coupon on cordura. Generally, material type impacted sanitizer efficacy ($P<0.01$). IPAQuats was the most effective treatment for sanitizing canvas and cordura harvest bags. Our study provides apple growers with information on sanitizer options for sanitizing harvest bags.

Keywords: Sanitation, *Salmonella*, *Listeria monocytogenes*, Harvest bags

HIGHLIGHTS

- IPAQuats reduced bacteria by ≥4.61 log CFU/coupon.
- PAA was more effective at decontaminating surfaces inoculated with *Salmonella*
- Higher concentrations of chlorine (500 ppm and pH 7) were more effective than chlorine at 200 ppm (pH 7 or 10.35).
- The study provides sanitizer options for decontaminating tree fruit harvest bags.

INTRODUCTION

The ‘Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables’ recognizes harvest containers as equipment that can spread bacterial microorganisms to fresh produce and recommends keeping harvest containers clean (*14*). In addition, the ‘Standards for Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (the Produce Safety Rule)’ requires produce growers to use food packing materials that are adequate for intended use which includes being cleanable or designed for single use and unlikely to support growth or transfer of pathogenic microorganisms (*11*). These materials must be inspected, maintained, cleaned and where necessary and appropriate, sanitized as frequently as reasonably necessary to protect against the contamination of covered produce (*11*). While these regulations allow for flexibility in terms of the frequency of cleaning and sanitization of food contact surfaces (FCS), there is limited information on effective sanitation interventions to decontaminate porous food packing materials like harvest bags to protect against produce contamination from the produce handling environment. *Salmonella* and *L. monocytogenes* are foodborne microorganisms that have been increasingly involved in fresh produce-associated outbreaks (*1*, *6*, *7*, *8*, *9*, *10*, *29*).

Illnesses associated with these pathogens cost the U.S. over 2.8 billion U.S. dollars in monetary loss each year (*16*). As a result, produce growers are encouraged to observe best practices for sanitation to mitigate the risk of pathogens contaminating their products, especially with fresh produce commodities that are eaten raw and do not receive any lethality or kill step.

Several studies have examined the effectiveness of different sanitizer treatments against *L. monocytogenes* and *Salmonella* on hard, non-porous FCS such as stainless steel, high-density polyethylene, polyvinyl chloride, polyethylene terephthalate, *etc.,* (*4*, *15*, *17*, *20*, *26*, *27*). For example, Harada and Nascimen (*15*) recovered *L. monocytogenes* from stainless steel surfaces treated with isopropyl alcohol with quaternary ammonium compounds at levels of approx. 4.2, 4.2, 4.0, and 2.1 log CFU/cm^2 after 5, 10, 15, and 30 minutes of exposure respectively from inoculation levels of 5.4 log CFU/cm^2. Ban et al. (*2*) treated *Salmonella* Enteritidis inoculated polyvinyl surfaces with saturated steam (100 °C) for 5, 10, 20, or 30 minutes and recovered 5.16 ± 0.13, 4.36 ± 0.16, 3.61 ± 0.52, and 3.08 ± 0.76 log CFU/coupon respectively after surface treatment. Studies that have examined the efficacy of sanitizers on porous surfaces are limited (*25*, *32*). Additionally, most available sanitizers are labeled for use on non-porous FCS, and requirements for demonstrating the efficacy of these sanitizers are specified for hard non-porous FCS even though porous materials such as harvest bags of different fabric types, wood, rubber, and foam are used in fresh produce operations. The produce safety rule requires that these materials be maintained, cleaned, and where necessary sanitized to reduce the risk of cross-contamination of fresh produce commodities. Therefore, it is critical to evaluate

the efficacy of sanitizers on porous FCS such as harvest bags made with cordura and canvas. Current regulations on sanitizer efficacy on non-porous FCS require a 5-log reduction in the number of the target organisms within 30 seconds for non-halide sanitizers like PAA or IPAQuats or equivalency to 50, 100, or 200 free chorine for halide compounds (*31*). For non-FCS that are porous such as textiles and fabric, the EPA requires that sanitizers demonstrate a more than 99.9% (a 3-log reduction) in the number of each test microorganism (*30*). Therefore, one objective of this study was to determine sanitizers that can be effective at decontaminating harvest bags, especially ones that can be used for dry sanitation. This is because studies performed by Etaka et al. (*12*) have shown that moisture is a significant driver for bacterial cross-contamination from apple harvest bags. Another objective was to explore the impact of chlorine pH and concentration on the reduction of bacterial contamination on harvest bag surfaces.

MATERIALS AND METHODS

Study design. The study evaluated two foodborne pathogens (*L. monocytogenes* and *Salmonella*), two FCS (canvas and cordura), and six sanitizer treatments including chlorine (12.5% sodium hypochlorite; 200 ppm and pH 10.35), chlorine (12.5% sodium hypochlorite; 200 ppm and pH 7), SaniDate 5.0 (5.3% Peracetic Acid + 23% Hydrogen Peroxide), isopropyl alcohol with quaternary ammonium compounds (IPAQuats), saturated steam, and sterile deionized water (DI water; treated control). Treatments were applied at various concentrations specific to their labels for intended use (Table 2). The total number of treatment combinations was 24 (2 pathogens * 2 material types * 6 sanitizer treatments). To further explore the impact of chlorine concentration on bacterial reduction, additional trials were performed with a

higher concentration of 500 ppm and pH 7. In these trials, cordura materials were inoculated with *Salmonella*

During trials, five samples were analyzed per treatment combination and all experiments were replicated 2 times (n= 10 coupons per treatment combination). Hence, a total of 240 coupons were treated in trials with *L. monocytogenes* (24 treatment combinations * 10 coupons per treatment combination). For trials with *Salmonella*, 250 coupons were treated (24 treatment combinations * 10 coupons per treatment combination) + 10 additional coupons for trials with chlorine at 500 ppm and pH 7).

***L. monocytogenes* and *Salmonella* strains and inoculum preparation.** The bacterial strains used for this study are shown in Table 1. Cocktails of *L. monocytogenes* and *Salmonella* were prepared as described in previous chapters. Briefly, all strains were streaked from frozen culture and grown at 35 °C for 24 h with two successive transfers in tryptic soy broth containing 80 ppm of rifampicin (TSBR; Difco, Becton Dickinson Co, Sparks, MD). A 100 uL aliquot of an overnight culture of each strain was spread plated onto TSAR and incubated for 24 h at 35°C. After incubation, the slurry of bacterial each strain was collected by flooding the agar plates with 5 mL of buffered peptone water (BPW; Difco, Becton Dickinson Co, Sparks, MD), suspending cells using an L-shaped spreader (Avantor delivered by VWR, Radnor, PA), and transferring the bacterial suspension in separate centrifuge tubes. Each pathogen strain was combined in equal volumes to create a 5-strain cocktail (2 mL of each strain suspension). Ten-fold serial dilutions were made in 0.1% w/v peptone (Difco, Becton Dickinson Co, Sparks, MD) and plated in duplicate on TSAR to determine the slurry

concentration. The slurry was adjusted by spectrophotometer to an absorbance of 0.05 OD600 nm (*L. monocytogenes*) and 0.1 OD600 nm (*Salmonella*) to obtain a mean inoculum concentration of 8.23 ± 0.09 log CFU/mL before inoculation. The inoculum concentration was verified by enumeration using TSAR and MOXR or XLDR plates.

Harvest bag materials. Canvas (purchased on Amazon), and cordura 73250 padded bags (73250 padded bags; Wells and Wade 73250, East Wenatchee, WA) were selected as these materials are commonly used to make apple harvest bags. Coupons of each material type were cut with sterile scissors into 25 cm^2 coupons. Before inoculation, each coupon surface was placed in a sterile weigh boat and sterilized with UV light in a biosafety cabinet for 10 minutes (5 minutes on each side).

Coupon surface inoculation. Coupon surface inoculation was conducted in a biosafety cabinet at ambient temperature and humidity. Briefly, surfaces were placed in weighing boats (Avantor delivered by VWR, Radnor, PA) in a biosafety cabinet and 100 µL of each pathogen cocktail suspension was distributed in 15 to 20 droplets on the coupon surfaces excluding <2 mm from the edge to a target concentration of approx. 7.42 ± 0.14 log CFU/coupon. Each coupon surface was held in a biosafety cabinet with the vent on for a 1.5 h dry time before sanitizer treatments.

Sanitizer treatments. Inoculated coupon surfaces were treated with sanitizers at various concentrations (Table 2). All sanitizer treatments except for steam were applied by spraying (1 L capacity plastic spray bottles; Avantor delivered by VWR, Radnor, PA) on the surfaces and the weight of coupon materials before and after treatment for each sanitizer was recorded (Table S7). For the steam treatment, inoculated coupon surfaces were placed in an enclosed plexiglass box (73 x 49 x 43 cm; length x width x height)

and steam was injected into the box using a steam machine (Table 2). The temperature of the jet of steam hitting the coupon surface and the temperature of the box were recorded using the Omega 12-Channel Temperature Recorder (Type T thermocouples; Omega Engineering Inc., Norwalk, CT). The concentration of PAA was verified using the AquaPhoenix Peracetic Acid test kit (Hanover, PA, United States), and free chlorine levels were confirmed using the CHEMets® Chlorine Kit (AquaPhoenix Scientific, Midland, VA). Chlorine pH levels were adjusted with citric acid (Fisher Scientific, Fair Lawn, NJ) and verified with a pH meter (Fisher Scientific, Fair Lawn, NJ). For all treatments, a timer was started as the sprayed sanitizer treatment was deposited onto the test surfaces.

Pathogen enumeration. Each treated coupon sample was transferred to a sterile While-Pak bag (Nasco, Fort Atkinson, WI) and enumeration was performed by adding 20 mL of Dey/Engley (D/E) neutralizing broth (Difco, Becton Dickinson Co, Sparks, MD) containing 1% (v/v) Tween80 (Fisher Scientific, Fair Lawn, NJ). All samples were rubbed and shaken for 60 seconds and the suspension was serially diluted in 9 mL of 0.1% peptone water and plated onto both TSAR + 1 g/L of sodium pyruvate (TSARP; Difco, Becton Dickinson Co, Sparks, MD) and modified oxford agar supplemented with 80 ppm of rifampicin + 1 g/L of sodium pyruvate (MOXRP; Difco, Becton Dickinson Co, Sparks, MD) for *Listeria monocytogenes* or both TSARP and xylose lysine deoxycholate supplemented with 80 ppm of rifampicin + 1 g/L of sodium pyruvate (XLDRP; Difco, Becton Dickinson Co, Sparks, MD) for *Salmonella*. Sodium pyruvate (C3H3NaO3) was added to molten forms of selective and non-selective media types, as previous studies have reported it to facilitate the resuscitation of sub-lethally

injured cells (*22*, *23*, *34*). Plates were incubated at 35 °C for 24 h (*Salmonella* non-selective and selective agars) or 48h (*L. monocytogenes* non-selective and selective agars). After incubation bacterial colonies were counted, and pathogen concentration was expressed in log CFU/coupon.

Statistical analysis. For analyses, “before treatment (t0)” was defined as the average concentration of pathogens on the coupon surfaces at inoculation. “After treatment (t1)” was defined as the concentration of pathogens on the coupon surfaces after sanitizer treatments were applied. All analyses were performed in R Studio (version 4.3.2). Log reduction (log CFU/coupon) was determined as shown by the formula below.

$$Log\ reduction\ (CFU/coupon) = t0 - t1$$

Log-linear mixed models were fitted using the *lme4()* package (*3*) and the best fit was selected using Akaike’s Information Criterion (AIC). The change in log CFU/coupon from t0 was the outcome while treatment and materials were fixed effects and trial was the random effect. For the analysis of *Salmonella* data, an interaction between material type and treatment was also included in the linear mixed model as determined with the AIC. Tukey’s post hoc analyses were performed with the *emmeans()* package (*24*) after fitting the models for each pathogen, and the results were included in tables that show the mean log reduction of bacteria (log CFU/coupon) per material type and treatment ($P<0.05$). Finally, levels of bacteria (log CFU/coupon) pre- and post-sanitizer treatment were visualized in bar plots created with *ggplot2()* (*33*). Data from trials with higher levels of chlorine was analyzed by fitting a linear model with chlorine concentration as

the independent variable and a change in log CFU/coupon from t0 as the outcome as determined with AIC. Finally, Tukey's post hoc analysis at *P*<0.05.

RESULTS

IPAQuats and PAA achieved *L. monocytogenes* reductions greater than 2 log CFU/coupon. Only results obtained using TSAR plates were further discussed as there was a significant difference reduction (log CFU/coupon) between TSAR and MOXR (*P*<0.001). The reduction of *L. monocytogenes* on cordura was significantly higher compared to canvas after sanitizer treatments (*P*<0.001; Tables 3 and 4). Among the sanitizers, the highest reduction was achieved with IPAQuats (*P*<0.001; Table 1). For instance, reductions of *L. monocytogenes* by 5.16 ± 0.93 and 6.01 ± 0.49 log CFU/coupon were achieved with IPAQuats from starting levels of 7.47 ± 0.19 and 7.50 ± 0.20 log CFU/coupon on canvas and cordura materials respectively (Table 4 and Figure 1). Significant reductions were also achieved with PAA and Chlorine (200 ppm and pH 7) between material types (*P*<0.001; Tables 3 and 4). On canvas and cordura, reductions of 2.63 ± 0.56 and 3.92 ± 0.81 log CFU/coupon respectively, were observed when PAA was used to treat the harvest bag surfaces (Table 4 and Figure 1). Bacterial reductions with IPAQuats and PAA were significantly higher than reductions after surfaces were exposed to 200 ppm of chlorine at pH 7 (*P*<0.001; Table 4 and Figure 1). Lower reductions were observed with steam, water, and chlorine (200 ppm and pH 10.35) compared to IPAQuats, PAA, and chlorine (200 ppm and pH 7) even though between materials, reductions on cordura were still significantly higher (*P*<0.001; Table 4 and Figure 1).

***Salmonella* reductions greater than 3 log CFU/coupon were achieved with IPAQuats and PAA.** Only results obtained with counts on TSAR plates were further discussed (P<0.001). IPAQuats was the most effective sanitizer for decontaminating *Salmonella* on canvas and cordura materials (P<0.001; Table 5). Reductions by 4.61 ± 1.03 and 5.90 ± 0.57 log CFU/coupon from starting levels of 7.37 ± 0.07 and 7.36 ± 0.10 log CFU/coupon were observed on canvas and cordura materials respectively when this sanitizer was used (Table 5 and Figure 2). Additionally, bacterial counts on some harvest bag samples treated with IPAQuats were below the detection limit (Table 6). When PAA was applied to surfaces, significant reductions were also observed although lower than IPAQuats (P<0.001; Tables 5 and 6 and Figure 2). Surprisingly, the reductions after exposure to PAA were significantly higher on canvas than on cordura (P = 0.02; Table 6). The reductions observed with chlorine (200 ppm and pH 10.35 or 7), steam, and water on canvas were similar (P>0.05) and significantly lower than those observed with IPAQuats and PAA (P<0.001; Tables 5 and 6 and Figure 2). On cordura materials, the reductions with chlorine (200 ppm and pH 10.35 or 7), and steam sanitizers were similar (P>0.05) whereas the reduction with water was the lowest among all sanitizers used on cordura surfaces (P<0.004; Tables 5 and 6 and Figure 2). To further explore chlorine efficacy, a concentration of 500 ppm at pH 7 was used to sanitize cordura surfaces inoculated with *Salmonella* Reductions obtained with this higher chlorine concentration were compared with reductions of *Salmonella* observed when coupon surfaces were exposed to chlorine at 200 ppm and pH 7 or 10.35. When a concentration of 500 ppm of chlorine at pH 7 was used, a significant reduction of 3.22 ± 0.89 log CFU/coupon on cordura surfaces was observed (Table 7). This reduction was

significantly higher ($P<0.001$) than reductions achieved with chlorine at 200 ppm and pH 7 or 10.35 (Tables 7 and 8). Between chlorine at 200 ppm and pH 7 or 10.35, reductions were not significant ($P=0.80$; Tables 7 and 8).

DISCUSSION

IPAQuats was the most effective at decontaminating harvest bag surfaces inoculated with *L. monocytogenes*. More than 5-log reduction in *L. monocytogenes* levels was observed on both canvas and cordura surfaces after treating surfaces with IPAQuats. This meets the EPA's requirement for sanitizer efficacy although that requirement is specific for sanitizers labeled for use on hard non-porous FCS (*31*). Surprisingly the reductions in our study are higher than reductions previously observed on stainless steel and polypropylene surfaces treated with IPAQuats for longer contact times (*15*). *L. monocytogenes* on polypropylene surfaces reduced by approx. 0.1, 0.9, 1.1, and 2 log CFU/cm^2 from starting levels of 6 log CFU/cm^2 after treatment with IPAQuats for 5, 10, 15, and 30 minutes, respectively (*15*). In another study with IPAQuats, more than 4 log CFU/g of *L. monocytogenes* was recovered (approx. 2- log reduction) on peanut shells from initial levels of 6.1 ± 0.5 log CFU/g (*25*). The reason for these differences in observation may be the result of differences in methods and surface properties. PAA was the next most effective sanitizer achieving a 2.63 ± 0.56 and 3.92 ± 0.81 log reduction on canvas and cordura. The reduction on cordura was significantly higher than on canvas and met the EPA's requirement for sanitizer efficacy on fabric and textiles even though that requirement is for non-FCS (*30*). By comparison, the reductions of *L. monocytogenes* on PAA-treated surfaces were lower than reductions of *L. monocytogenes* previously observed on stainless steel, polyvinyl

chloride, low-density polyethylene, polyethylene terephthalate, and silicon rubber (*18*). When materials were exposed to 200 ppm of chlorine, the reductions at pH 10.35 were lower than reductions at pH 7 on both cordura and canvas. In addition, reductions on cordura were significantly higher than on canvas. This was expected as a pH greater than 7.5 yields more hypochlorite ions which are less effective at destroying bacterial cells than hypochlorous acid which is more available between pH 6.5 and 7.5 and more effective at destroying bacterial cells (*28*). Bacterial reductions on cordura upon treatment with chlorine (pH 7 or 10.35) were comparable to bacterial reductions previously observed on hard surfaces (*18*). In contrast, reductions on canvas upon exposure to chlorine (pH 7 or 10.35) were lower than reductions in a prior study where a reduction by 1.55 ± 0.05 log CFU/cm^2 was observed on stainless steel surfaces exposed to 200 ppm of chlorine for 1 minute (*21*). Differences in the properties of surfaces may have impacted chlorine efficacy. The steam treatment was least effective and achieved similar reductions as water across harvest bag surfaces. Previous studies that have treated FCS with steam have demonstrated reductions higher than the reductions observed in the present study (*2*, *19*). This was mainly because the mean temperature of the jet of steam hitting the coupons was over 3 times lower (31.23 ± 3.91 °C) than the temperature used in previous studies that used steam at temperatures of 100 °C steam (*2*, *19*). Additionally, the exposure time of materials to steam was less than the exposure time used in these studies (31.23 ± 3.91 °C).

The most effective sanitizer for decontaminating *Salmonella* on harvest bag surfaces was IPAQuats. *Salmonella* reduced by 4.61 ± 1.03 and 5.90 ± 0.57 log CFU/coupon on canvas and cordura surfaces respectively. By comparison, these

reduction levels were lower than reductions (≥6.18 log CFU/25 cm^2) observed in similar studies with clean and soiled stainless steel surfaces inoculated with *Salmonella* Hartford and treated with IPAQuats at the same exposure time (*20*). Just like with *L. monocytogenes* trials, IPAQuats was more effective on cordura than on canvas surfaces which is not uncommon as differences between surfaces can impact sanitizer efficacy (*4*, *18*, *27*, *32*). PAA was the next most effective sanitizer with reductions greater than 3 log CFU/coupon across both harvest bag surface types. The reduction levels on canvas were significantly higher than the reduction on cordura after exposure to PAA. This outcome was unexpected given a trend of more reductions on cordura than canvas in trials with *L. monocytogenes*. By comparison, the reduction of *Salmonella* on PAA-treated surfaces was similar to reductions reported in previous studies (*5*, *26*). These studies however used exposure times greater than 1 minute (*5*, *26*). More studies are needed to determine *Salmonella* reductions at longer treatment times. The exposure of canvas and cordura materials to chlorine 200 ppm at pH 7 or 10.35 resulted in a similar reduction. It was expected that the reductions of *Salmonella* on materials treated with 200 ppm of chlorine at a pH level of 7 will be significantly higher than 200 ppm of chlorine at a pH of 10.35 due more free chlorine at the lower pH level (*28*). The treatment time or the concentration of chlorine may have been resulted in this observeation. To further explore the chlorine concentration, additional trials were performed by exposing cordura surfaces inoculated with *Salmonella* to chlorine at a concentration of 500 ppm and a pH of 7.0. A significantly higher reduction of *Salmonella* (3.22 ± 0.89 log CFU/coupon) was observed which was comparable to reductions at lower concentrations on hard non-porous surfaces (200 ppm) and also meets the EPA

requirements for surface sanitizers of textiles and fabric (*4*, *30*). For example Byun et al. (*4*) reported approx. 3 log reduction on silicon rubber surfaces exposed to 100 ppm of chlorine for 1 minute. More studies with longer treatment times and lower concentrations of chlorine may be needed to assess the impact of treatment time on sanitizer efficacy on harvest bag surfaces.

CONCLUSION

The present study examined the efficacy of different sanitizers for inactivating bacteria on harvest bag surfaces. IPAQuats was the most effective at destroying *L. monocytogenes* and *Salmonella* cells on canvas and cordura surfaces. PAA was the next most effective sanitizer, especially for harvest bag surfaces inoculated with *Salmonella* A chlorine concentration of 200ppm was not as effective as expected especially when surfaces were inoculated with *Salmonella* However, significantly higher reductions were observed when chlorine concentrations were increased from 200 to 500 ppm at pH 7. Produce growers may use IPAQuats for decontaminating harvest bags in their produce operations. This sanitizer is also adequate for dry sanitizing surfaces. Alternatively, PAA may be used but harvest bags must be dry before using them for harvest operations. Chlorine may also be used but growers should manage the pH of the sanitizing solution. Based on our study higher concentrations may be adequate for harvest bags although the effects of this concentration on the wear and tear of the harvest bags is not known. Overall, our study provides produce growers with sanitizer options for sanitizing harvest bags of different material types.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the corresponding author.

AUTHOR CONTRIBUTION

LS, AH, RB, JE, DW, FC, and CE contributed to the study conception and design. LS, MB, IR, and CE contributed to the acquisition of data. LS, DW, AH, and CE contributed to the analysis and interpretation of the data and drafting of the manuscript. LS, AH, RB, JE, DW, FC, and CE contributed to the critical revisions of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work is supported by the Specialty Crops Research Initiative 2020-51181-32157 from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

We are grateful to Kim Waterman and Alyssa Rosenbaum for their contributions to data acquisition.

REFERENCES

1. Angelo, K. M., A. R. Conrad, A. Saupe, H. Dragoo, N. West, A. Sorenson, A. Barnes, M. Doyle, J. Beal, K. A. Jackson, S. Stroika, C. Tarr, Z. Kucerova, S. Lance, L. H. Gould, M. Wise, and B. R. Jackson. 2017. Multistate outbreak of

Listeria monocytogenes infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection*. Cambridge University Press 145:848–856.

2. Ban, G.-H., H. Yoon, and D.-H. Kang. 2014. A comparison of saturated steam and superheated steam for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and Listeria monocytogenes biofilms on polyvinyl chloride and stainless steel. *Food Control* 40:344–350.
3. Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, G. Grothendieck, P. Green, and M. B. Bolker. 2015. Package ‘lme4’. *convergence*. Available at: http://dk.archive.ubuntu.com/pub/pub/cran/web/packages/lme4/lme4.pdf. Accessed 24 March 2024.
4. Byun, K.-H., S. H. Han, J. Yoon, S. H. Park, and S.-D. Ha. 2021. Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on *Salmonella* Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. *Food Control* 123:107838.
5. Byun, K.-H., K. W. Na, M. Ashrafudoulla, M. W. Choi, S. H. Han, I. Kang, S. H. Park, and S.-D. Ha. 2022. Combination treatment of peroxyacetic acid or lactic acid with UV-C to control *Salmonella* Enteritidis biofilms on food contact surface and chicken skin. *Food Microbiology* 102:103906.
6. Carstens, C. K., J. K. Salazar, and C. Darkoh. 2019. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Frontiers in Microbiology* 10.

7. Centers for Disease Control and Prevention. 2023. Listeria Outbreak Linked to Leafy Greens. *Centers for Disease Control and Prevention*. Available at: https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/index.html. Accessed 21 March 2024.

8. Centers for Disease Control and Prevention. 2024. Listeria Outbreak Linked to Peaches, Nectarines, and Plums. Available at: https://www.cdc.gov/listeria/outbreaks/peaches-11-23/index.html. Accessed 27 February 2024.

9. Centers for Disease Control and Prevention. 2018. Multistate Outbreak of *Listeriosis* Linked to Whole Cantaloupes from Jensen Farms, Colorado. Available at: https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. Accessed 4 April 2023.

10. Centers for Disease Control and Prevention. 2020. Outbreak of *Salmonella* Enteritidis Infections Linked to Peaches. Available at: https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 27 February 2024.

11. Code of Federal Regulations. 2022. Title 21. Food and Drugs. Chapter 1. Food and Drug Administration, Department of Health and Human Services. Subpart B. Food for Human Consumption. Part 112. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Subpart L. Equipment, Tools, Buildings, and Sanitation. Section 112.123(d)(1).

12. Etaka, C. A., K. Waterman, A. Hamilton, T. Le, D. W. Schaffner, and L. K. Strawn. Unpublished results. Quantification of generic *E.coli* Cross-Contamination from Different Tree Fruit Harvest Bag Material Types to Apples.
13. Etaka, C. A., K. Waterman, A. Hamilton, T. Le, D. L. Weller, and L. K. Strawn. Unpublished results. Survival and Transfer of *Listeria monocytogenes* and Salmonella from Different Tree Fruit Harvest Bag Material Types to Apples.
14. Food and Drug Administration. 2022. Guidance for Industry: Juice Hazard Analysis Critical Control Point Hazards and Controls Guidance, First Edition. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-juice-hazard-analysis-critical-control-point-hazards-and-controls-guidance-first. Accessed 14 September 2023.
15. Harada, A. M. M., and M. S. Nascimento. 2021. Efficacy of dry sanitizing methods on Listeria monocytogenes biofilms. *Food Control* 124:107897.
16. Hoffman, S., B. Maculloch, and M. Batz. 2015. Economic Burden of Major Foodborne Illnesses Acquired in the United States. 205081. Economic Information Bulletin, United States Department of Agriculture, Economic Research Service.
17. Hua, G., and D. A. Reckhow. 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. *Water Research* 41:1667–1678.
18. Hua, Z., A. M. Korany, S. H. El-Shinawy, and M.-J. Zhu. 2019. Comparative Evaluation of Different Sanitizers Against *Listeria monocytogenes* Biofilms on Major Food-Contact Surfaces. *Frontiers in Microbiology* 10.

19. Hua, Z., F. Younce, J. Tang, D. Ryu, B. Rasco, I. Hanrahan, and M.-J. Zhu. 2021. Efficacy of saturated steam against *Listeria innocua* biofilm on common food-contact surfaces. *Food Control* 125:107988.

20. Kane, D. M., K. J. K. Getty, B. Mayer, and A. Mazzotta. 2016. Sanitizing in Dry-Processing Environments Using Isopropyl Alcohol Quaternary Ammonium Formula. *J Food Prot* 79:112–116.

21. Kim, M., S. Y. Park, and S.-D. Ha. 2016. Synergistic effect of a combination of ultraviolet–C irradiation and sodium hypochlorite to reduce *Listeria monocytogenes* biofilms on stainless steel and eggshell surfaces. *Food Control* 70:103–109.

22. Knudsen, D. M., S. A. Yamamoto, and L. J. Harris. 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. *J Food Prot* 64:1483–1488.

23. Lang, M. M., L. J. Harris, and L. R. Beuchat. 2004. Survival and Recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on Lettuce and Parsley as Affected by Method of Inoculation, Time between Inoculation and Analysis, and Treatment with Chlorinated Water. *Journal of Food Protection* 67:1092–1103.

24. Lenth V., R., B. Bolker, P. Buerkner, I. Gine-Vazquez, M. Herve, M. Jung, J. Love, F. Miguez, H. Riebl, and H. Singmann. 2024. Package ‘emmeans’. Available at: https://cran.r-project.org/web/packages/emmeans/emmeans.pdf. Accessed 10 April 2024.

25. McEgan, R., and M. D. Danyluk. 2015. Evaluation of aqueous and alcohol-based quaternary ammonium sanitizers for inactivating *Salmonella* spp., *Escherichia coli*

O157:H7, and *Listeria monocytogenes* on peanut and pistachio shells. *Food Microbiology* 47:93–98.

26. Nahar, S., H. L. Jeong, A. J. Cho, J.-H. Park, S. Han, Y. Kim, S.-H. Park, and S.-D. Ha. 2022. Efficacy of ficin and peroxyacetic acid against *Salmonella enterica* serovar Thompson biofilm on plastic, eggshell, and chicken skin. *Food Microbiology* 104:103997.
27. Ruiz-Llacsahuanga, B., A. M. Hamilton, K. Anderson, and F. Critzer. 2022. Efficacy of cleaning and sanitation methods against *Listeria innocua* on apple packing equipment surfaces. *Food Microbiology* 107:104061.
28. Suslow, T. 1997. Basic Properties and Key Points for Effective Disinfection. Available at: https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/26414.pdf. Accessed 30 March 2024.
29. Townsend, A., L. K. Strawn, B. J. Chapman, and L. L. Dunn. 2021. A Systematic Review of *Listeria* Species and *Listeria monocytogenes* Prevalence, Persistence, and Diversity throughout the Fresh Produce Supply Chain. 6. *Foods*. Multidisciplinary Digital Publishing Institute 10:1427.
30. United States Environmental Protection Agency. 2012. Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles Efficacy Data Recommendations. Available at: https://nepis.epa.gov/exe/zynet.exe/p100shai.txt?zyactiond=zydocument&client=epa&index=2011+thru+2015&docs=&query=&time=&endtime=&searchmethod=1&tocrestrict=n&toc=&tocentry=&qfield=&qfieldyear=&qfieldmonth=&qfieldday=&intqfieldop=0&extqfieldop=0&xmlquery=&file=d%3a%5czyfiles%5cindex%20data%5c11thr

u15%5ctxt%5c00000027%5cp100shai.txt&user=anonymous&password=anonymous&sortmethod=h%7c-&maximumdocuments=1&fuzzydegree=0&imagequality=r75g8/r75g8/x150y150g16/i425&display=hpfr&defseekpage=x&searchback=zyactionl&back=zyactions&backdesc=results%20page&maximumpages=1&zyentry=1&seekpage=x&zypurl#. Accessed 28 March 2024.

31. US Environmental Protection Agency. 2012. Product Performance Test Guidelines Ocspp 810.2300: Sanitizers for Use on Hard Surfaces Efficacy Data Recommendations. Available at: https://nepis.epa.gov/exe/zynet.exe/p100ijau.txt?zyactiond=zydocument&client=epa&index=2011%20thru%202015&docs=&query=&time=&endtime=&searchmethod=1&tocrestrict=n&toc=&tocentry=&qfield=&qfieldyear=&qfieldmonth=&qfieldday=&useqfield=&intqfieldop=0&extqfieldop=0&xmlquery=&file=d%3a%5czyfiles%5cindex%20data%5c11thru15%5ctxt%5c00000010%5cp100ijau.txt&user=anonymous&password=anonymous&sortmethod=h%7c-&maximumdocuments=1&fuzzydegree=0&imagequality=r75g8/r75g8/x150y150g16/i425&display=hpfr&defseekpage=x&searchback=zyactionl&back=zyactions&backdesc=results%20page&maximumpages=1&zyentry=4. Accessed 23 April 2023.
32. Vanith, H. 2018. Efficacy of cleaning and sanitation methods in reducing foodborne pathogens on common food contact surfaces in Cambodian fresh food markets.
33. Wilkinson, L. 2011. ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H. *Biometrics* 67:678–679.

34. Yamamoto, S. A., and L. J. Harris. 2001. Phosphate buffer increases recovery of *Escherichia coli* O157:H7 from frozen apple juice. *J Food Prot* 64:1315–1319.

TABLES AND FIGURES

Bacteria	Strain
L. monocytogenes	390-2, 1/2b; environmental isolate, 2011 cantaloupe outbreak
L. monocytogenes	390-6, 1/2a; environmental isolate, 2011 cantaloupe outbreak
L. monocytogenes	573-035, 4b; clinical isolate 2014 caramel apple outbreak

L. monocytogenes	Scott A, 4b; pasteurized milk outbreak
L. monocytogenes	V7, 1/2a; raw milk isolate
Salmonella	Agona, LJH 517; alfalfa sprouts outbreak
Salmonella	Enteritidis, ATCC BAA-1045; peach outbreak
Salmonella	Montevideo; Tomato outbreak
Salmonella	Newport, ATCC 6962; clinical isolate, onion outbreak
Salmonella	St Paul; Pepper outbreak

Table 1. Bacterial strains used for cocktail preparation

Table 2. Sanitizer treatments, application method, rates, contact time, and justification.

Treatment	Method of application	Contact time (minutes)	Rate	Justification for selected application rate	Labels	Company
Chlorine (12.5% sodium hypochlorite)	Spraying with a hand pump spray bottle till visibly wet	1 minute	200ppm/pH 7 or 10.35	Previously used by (*27*) at a rate of 200 ppm. Labelled for use at 200 ppm to FCS for at least 2 minutes of contact time	https://www.paceint.com/wp-content/uploads/2015/08/Pace-Pac-Chlor-12.5-Spec-Label.pdf.	Pace International, Wapato, WA.
Chlorine (12.5% sodium hypochlorite)	Spraying with a hand pump spray bottle till visibly wet	1 minute	500ppm/pH 7	Previously used by Faith Critzer (unpublished data). Labeled for use at 200 ppm to FCS for at least 2 minutes of contact time	https://www.paceint.com/wp-content/uploads/2015/08/Pace-Pac-Chlor-12.5-Spec-Label.pdf.	Pace International, Wapato, WA.
Saturated steam	Enclosed plexiglass box (73 x 49 x 43 cm) with thermocouples attached to coupon surface and inside the box	1 minute	31.23 ± 3.91 °C on coupon surface and 29.13 ± 7.23 °C in the box, 67.44 psi	Previously used by Faith Critzer (unpublished data) and (*19*, *27*)	https://www.solutionsstores.com/amerivap-system.	AmeriVap Systems, Dawsonville, GA.

SaniDate 5.0 (5.3% Peracetic Acid + 23% Hydrogen Peroxide)	**Spraying with a hand pump spray bottle till visibly wet**	**1 minute**	**200 ppm**	**Used at concentration between 145-500 ppm for sanitizing FCS as specified on the label.**	**https://cdn.shopify.com/s/files/1/0559/1256/2893/files/sanidate-5_label.pdf?v=1659727595.**	**Seven Springs Farm Supply, Blacksburg, VA.**
Isopropyl alcohol with quaternary ammonium compounds (IPAQuats)	Spraying with a hand pump spray bottle till visibly wet	1 minute	Sanitizer is ready to use.	Labelled for use on food contact surfaces and previously used in published literature (*15*, *20*)	https://www.bestsanitizers.com/wp-content/uploads/2019/07/Alpet-D2-Tech-Sheet.pdf.	Alphet D2 Surface Sanitizer, Best Sanitizers Inc., Penn Valley, CA
Sterile deionized water	Spraying with a hand pump spray bottle till visibly wet	1 minute	-	Previously used by Faith Critzer (unpublished data) and (*27*)	-	-

Table 3. Parameters of the linear mixed model describing the reduction of *L. monocytogenes* on harvest bag material types post sanitizer treatments (TSAR)[a]

Parameters	Effect	95% CI[b]	*P* value
Intercept	0.91	0.67, 1.16	0.01
Material (reference = Canvas)			
Cordura	1.15	1.03, 1.26	<0.001
Treatment (reference = Chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7.00	0.67	0.47, 0.87	<0.001
IPAQuats	4.09	3.89, 4.30	<0.001
PAA	1.79	1.59, 1.99	<0.001
Steam	0.14	-0.06, 0.34	0.18
Water	-0.003	-0.20, 0.20	0.98

a. The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.47 ± 0.19 and 7.50 ± 0.20 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.98 ± 0.17 and 6.86 ± 0.20 log CFU/coupon on canvas and cordura materials respectively.
b. 95% confidence interval.

Table 4. Mean reduction (log CFU/coupon ± SD) of *L. monocytogenes* on canvas and cordura materials post-sanitizer treatment (TSAR)[a]

Treatment[b]	Material types			
	Canvas		Cordura	
Chlorine_pH 7.00	1.58 ± 0.10[c]	a, x	2.74 ± 0.13	a, y
Chlorine_pH 10.35	0.90 ± 0.29	d, x	2.08 ± 0.30	d, y
IPAQuats	5.16 ± 0.93 (8/10)	b, x	6.01 ± 0.49 (5/10)	b, y
PAA	2.63 ± 0.56	c, x	3.92 ± 0.81	c, y
Steam	0.95 ± 0.23	d, x	2.31 ± 0.47	d, y
Water	0.96 ± 0.21	d, x	2.01 ± 0.24	d, y

a. The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.47 ± 0.19 and 7.50 ± 0.20 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.98 ± 0.17 and 6.86 ± 0.20 log CFU/coupon on canvas and cordura materials respectively.
b. The chlorine concentration was 200 ppm.
c. Significant differences within material types are indicated with letters a, b, c, and d, and significant differences between material types are represented with letters x and y. Numbers in parentheses represent the total number of samples that were above the detection limit (>1.3 log CFU/coupon).

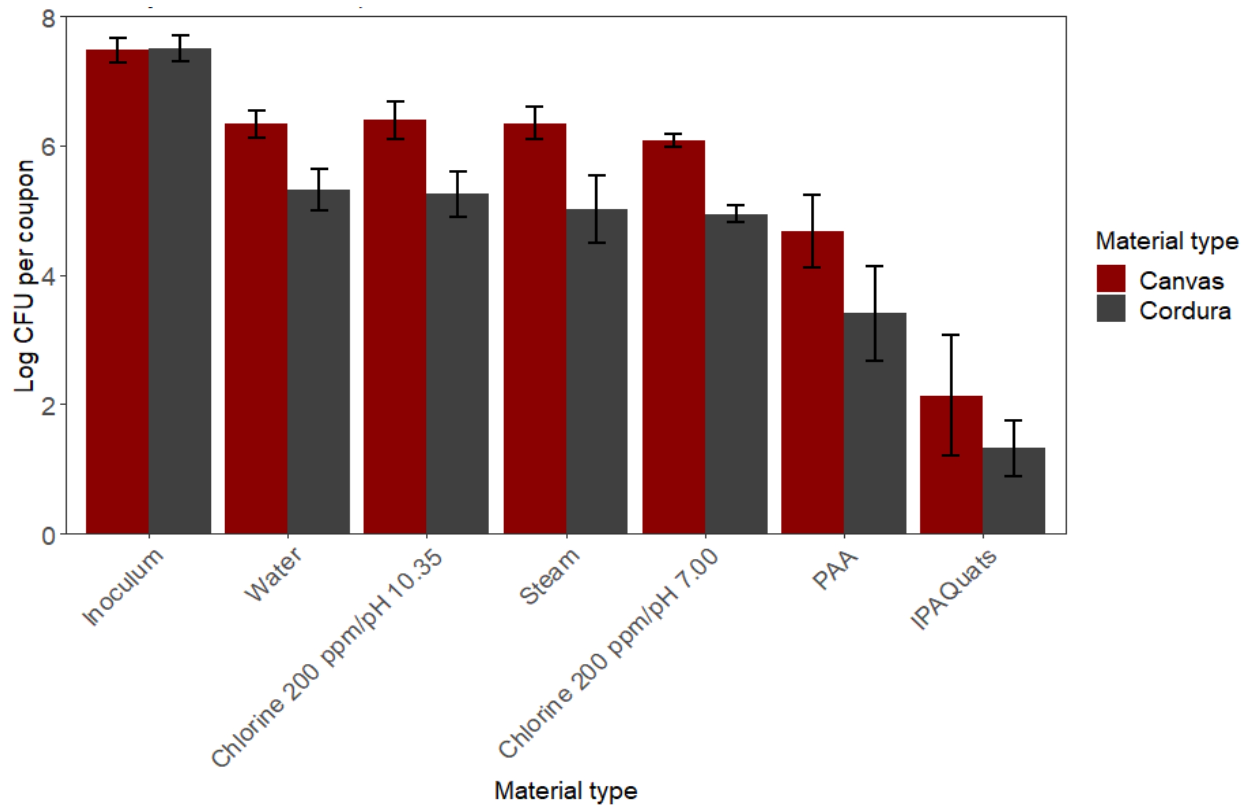


Figure 1. Mean log CFU/coupon ± SD of *L. monocytogenes* on canvas and cordura materials post-sanitizer treatment (TSAR). The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.47 ± 0.19 and 7.50 ± 0.20 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.98 ± 0.17 and 6.86 ± 0.20 log CFU/coupon on canvas and cordura materials respectively.

Table 5. Parameters of the linear mixed model describing the reduction of *Salmonella* on harvest bag material types post-sanitizer treatment (TSAR)[a]

Parameters	Effect	95% CI[b]	*P* value
Intercept	0.57	0.31, 0.83	<0.001
Material (reference = Canvas)			
Cordura	0.52	0.15, 0.88	0.01
Treatment (reference = Chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7.00	0.48	0.12, 0.85	0.01
IPAQuats	4.04	3.67, 4.41	<0.001
PAA	3.11	2.74, 3.48	<0.001
Steam	0.45	0.09, 0.82	0.02
Water	-0.04	-0.41, 0.33	0.82
Material type x Treatment			
Cordura x Chlorine 200 ppm/pH 7.00	-0.28	-0.80, 0.24	0.30
Cordura x IPAQuats	0.78	0.26, 1.30	<0.001
Cordura x PAA	-0.99	-1.51, -0.47	<0.001
Cordura x Steam	0.23	-0.29, 0.76	0.38
Cordura x Water	-0.64	-1.16, -0.12	0.02

a. The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.37 ± 0.07 and 7.36 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, levels on canvas and cordura materials were 6.83 ± 0.39 and 6.88 ± 0.33 log CFU/coupon respectively.
b. 95% confidence interval.

Table 6. Mean reduction (log CFU/coupon ± SD) of *Salmonella* on canvas and cordura materials post-sanitizer treatment (TSAR)[a]

Treatment[b]	Material types			
	Canvas		Cordura	
Chlorine_pH 7.00	1.05 ± 0.11[c]	a, x	1.29 ± 0.15	ad, x
Chlorine_pH 10.35	0.57 ± 0.18	a, x	1.08 ± 0.42	a, y
IPAQuats	4.61 ± 1.03[(9/10)]	b, x	5.90 ± 0.57[(8/10)]	b, y
PAA	3.68 ± 0.79	c, x	3.21 ± 1.14	c, y
Steam	1.02 ± 0.62	a, x	1.77 ± 0.56	d, y
Water	0.53 ± 0.14	a, x	0.40 ± 0.09	e, x

a. The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.37 ± 0.07 and 7.36 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, levels on canvas and cordura materials were 6.83 ± 0.39 and 6.88 ± 0.33 log CFU/coupon respectively.
b. The chlorine concentration was 200 ppm.
c. Significant differences within material types are indicated with letters a, b, c, d, and e, and significant differences between material types are represented with letters x and y. Numbers in parentheses represent the total number of samples that were above the detection limit (>1.3 log CFU/coupon).

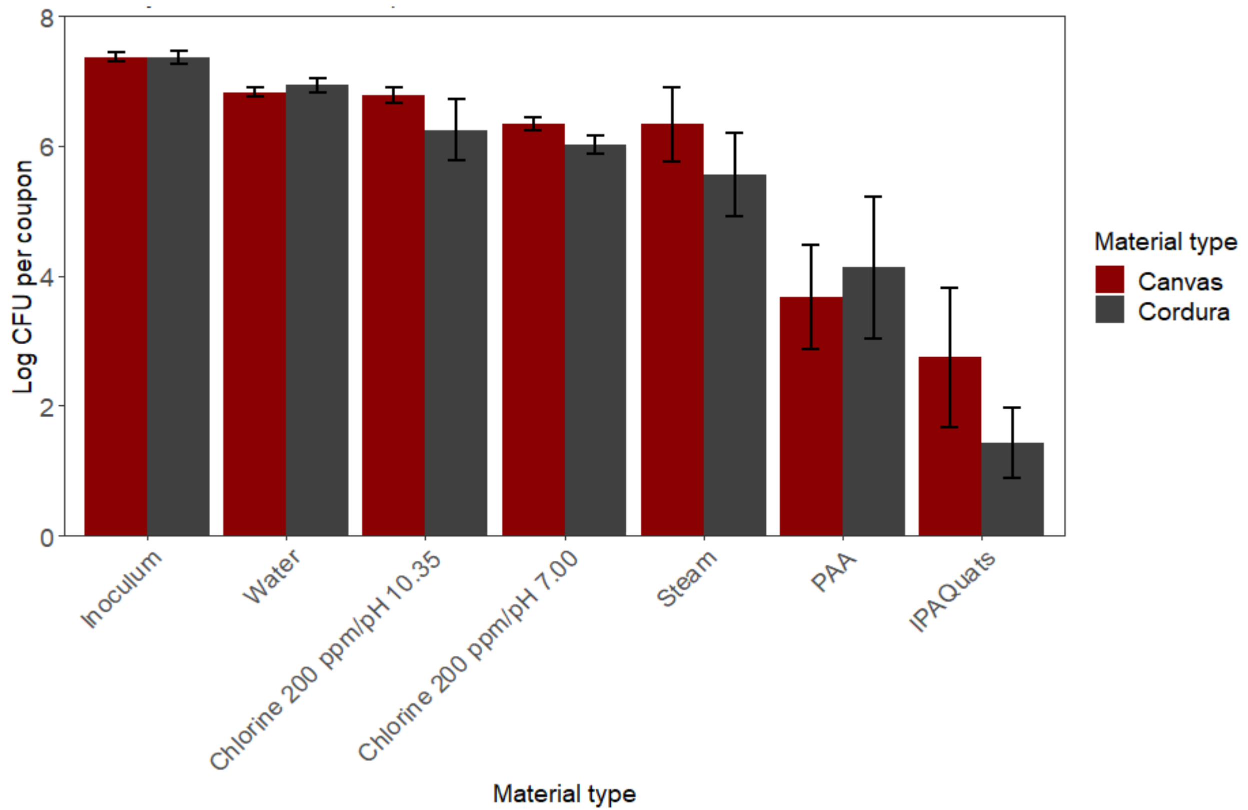


Figure 2. Mean log CFU/coupon ± SD of *Salmonella* on canvas and cordura materials post-sanitizer treatment (TSAR). The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.37 ± 0.07 and 7.36 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, levels on canvas and cordura materials were 6.83 ± 0.39 and 6.88 ± 0.33 log CFU/coupon respectively.

Table 7. Reduction of *Salmonella* on cordura coupons treated with chlorine at different pH levels and concentrations (TSAR)[a]

Material	pH	Concentration (ppm)	Reduction (log CFU/coupon)
Cordura	10.35	200	1.08 ± 0.42 a
Cordura	7.00	200	1.29 ± 0.15 a
Cordura	7.00	500	3.22 ± 0.89 b

a. The mean starting level of *Salmonella* on cordura was 7.36 ± 0.10 log CFU/coupon.

Table 8. Parameters of the linear model describing the reduction of *Salmonella* on cordura surfaces post-chlorine treatment (TSAR)[a]

Parameter	Estimate	95% CI[b]	*P* value
Intercept	1.08	0.74, 1.43	0.01
Treatment (reference = chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7	0.05	-0.36, 0.47	0.80
Chlorine 500 ppm/pH 7	2.47	2.06, 2.88	<0.001

a. The mean starting level of *Salmonella* on cordura was 7.36 ± 0.10 log CFU/coupon.
b. 95% confidence interval.

SUPPLEMENTAL MATERIALS

Table S1. Parameters of the linear mixed model describing the reduction of *L. monocytogenes* on harvest bag material types post sanitizer treatments (MOXR)[a]

Parameters	Effect	95% CI[b]	*P* value
Intercept	1.14	0.98, 1.30	<0.001
Material (reference = Canvas)			
Cordura	1.08	0.96, 1.20	<0.001
Treatment (reference = Chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7.00	0.53	0.31, 0.74	<0.001
IPAQuats	4.00	3.79, 4.22	<0.001
PAA	1.91	1.70, 2.12	<0.001
Steam	0.08	-0.13, 0.29	0.47
Water	-0.04	-0.25, 0.17	0.70

a. The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.39 ± 0.15 and 7.34 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.56 ± 0.46 and 6.60 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.
b. 95% confidence interval.

Table S2. Mean reduction (log CFU/coupon ± SD) of *L. monocytogenes* on canvas and cordura materials post-sanitizer treatment (MOXR)[a]

Treatment	Material types			
	Canvas		Cordura	
Chlorine_pH 7.00[b]	1.53 ± 0.16[c]	a, x	2.88 ± 0.38	a, y
Chlorine_pH 10.35	1.29 ± 0.12	d, x	2.32 ± 0.25	d, y
IPAQuats	6.09 ± 0.28 (6/10)	b, x	5.69 ± 0.57 (5/10)	b, y
PAA	3.26 ± 0.48	c, x	3.43 ± 0.42	c, y
Steam	1.24 ± 0.21	d, x	2.52 ± 0.24	d, y
Water	1.23 ± 0.13	d, x	2.23 ± 0.22	d, y

a. The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.39 ± 0.15 and 7.34 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.56 ± 0.46 and 6.60 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.
b. The chlorine concentration was 200 ppm.
c. Significant differences within material types are indicated with letters a, b, c, and d, and significant differences between material types are represented with letters x and y. Numbers in parentheses represent the total number of samples that were above the detection limit (>1.3 log CFU/coupon).

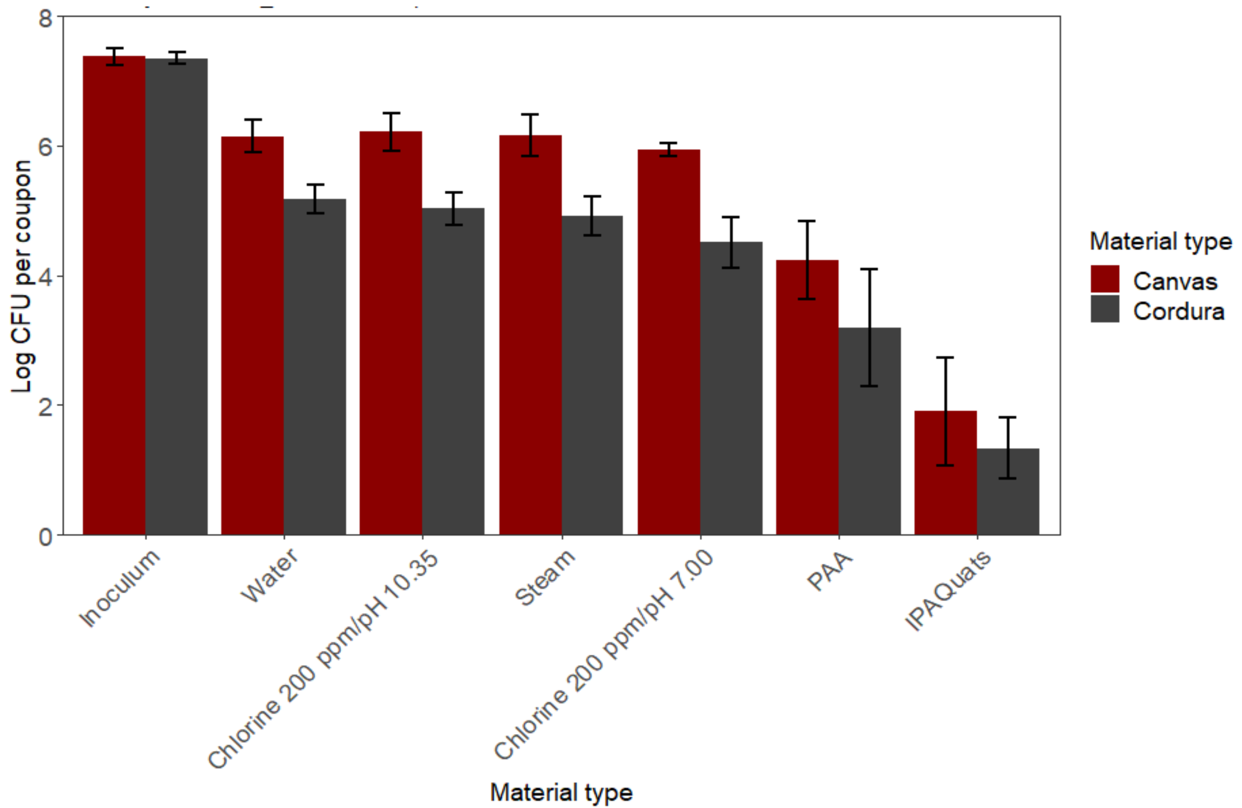


Figure S1. Mean log CFU/coupon ± SD of *L. monocytogenes* on canvas and cordura materials post-sanitizer treatment (MOXR). The starting inoculum levels of *L. monocytogenes* on canvas and cordura materials were 7.39 ± 0.15 and 7.34 ± 0.10 log CFU/coupon respectively. At 1.5 h post-inoculation, bacterial levels were 6.56 ± 0.46 and 6.60 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.

Table S3. Parameters of the linear mixed model describing the reduction of *Salmonella* on harvest bag material types post sanitizer treatments (XLDR)[a]

Parameters	Effect	95% CI[b]	*P* value
Intercept	0.80	0.51, 1.09	<0.001
Material (reference = Canvas)			
Cordura	0.42	0.04, 0.80	0.03
Treatment (reference = Chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7.00	1.07	0.68, 1.46	<0.001
IPAQuats	3.86	3.48, 4.24	<0.001
PAA	3.16	2.78, 3.54	<0.001
Steam	1.09	0.71, 1.47	<0.001
Water	-0.27	-0.65, 0.11	0.17
Material type x Treatment			
Cordura x Chlorine 200 ppm/pH 7.00	-0.29	-0.83, 0.25	0.29
Cordura x IPAQuats	0.87	0.33, 1.41	<0.001
Cordura x PAA	-1.09	-1.63, -0.54	<0.001
Cordura x Steam	0.26	-0.28, 0.80	0.35
Cordura x Water	-0.54	-1.08, 0.001	0.05

a. The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.33 ± 0.12 and 7.29 ± 0.08 log CFU/coupon respectively. At 1.5 h post-inoculation, levels were 6.78 ± 0.47 and 6.77 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.
b. 95% confidence interval

Table S4. Mean reduction (log CFU per coupon ± SD) of *Salmonella* on canvas and cordura materials post-sanitizer treatment (XLDR)[a]

Treatment	Material types			
	Canvas		Cordura	
Chlorine_pH 7.00[b]	1.83 ± 0.20[c]	a, x	1.96 ± 0.18	a, x
Chlorine_pH 10.35	0.80 ± 0.22	b, x	1.22 ± 0.39	b, y
IPAQuats	4.66 ± 1.04$^{(8/10)}$	c, x	5.95 ± 0.48$^{(3/10)}$	c, y
PAA	3.96 ± 0.93	d, x	3.29 ± 0.96	d, y
Steam	1.89 ± 0.64	a, x	2.57 ± 0.86	a, y
Water	0.53 ± 0.26	b, x	0.41 ± 0.10	e, x

a. The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.33 ± 0.12 and 7.29 ± 0.08 log CFU/coupon respectively. At 1.5 h post-inoculation, levels were 6.78 ± 0.47 and 6.77 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.
b. The chlorine concentration was 200 ppm.
c. Significant differences within material types are indicated with letters a, b, c, d, and e, and significant differences between material types are represented with letters x and y. Numbers in parentheses represent the total number of samples that were above the detection limit (>1.3 log CFU/coupon).

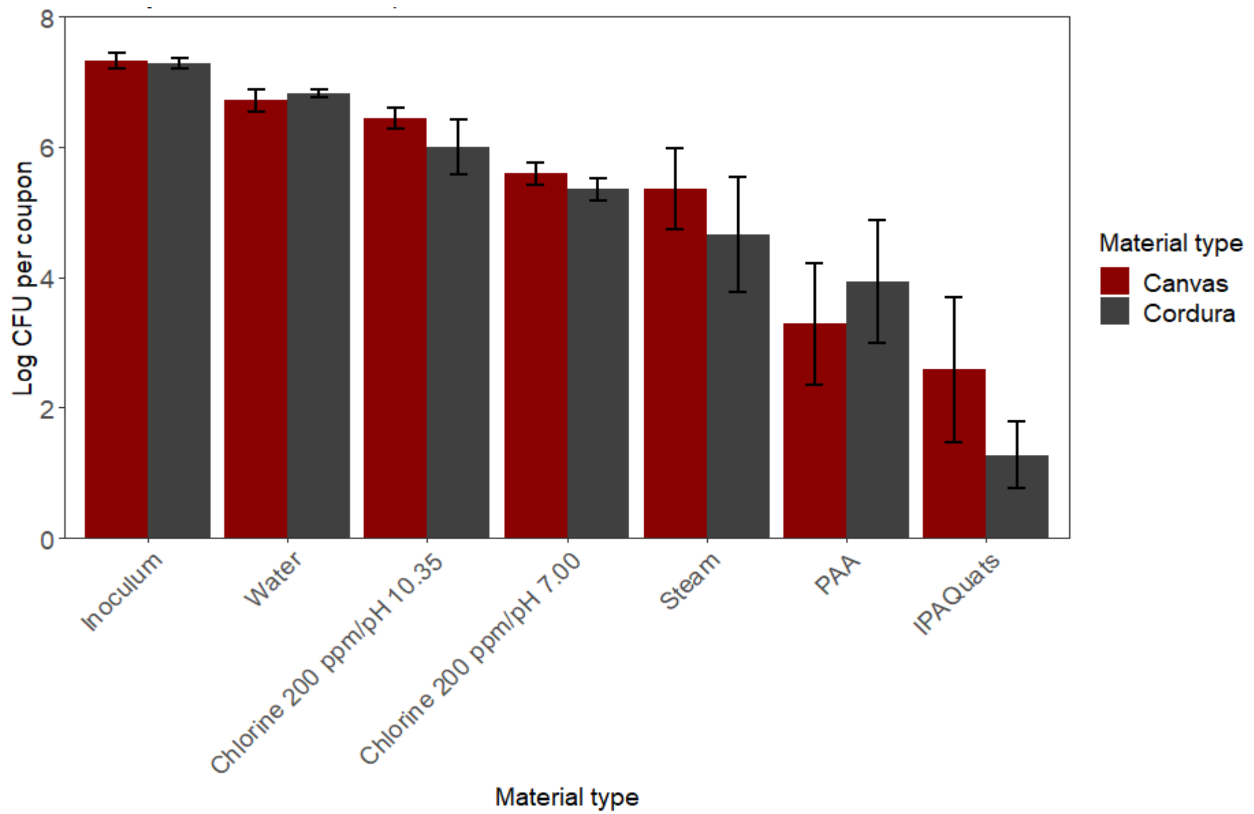


Figure S2. Mean log CFU/coupon ± SD of *Salmonella* on canvas and cordura materials post-sanitizer treatment (XLDR). The starting inoculum levels of *Salmonella* on canvas and cordura materials were 7.33 ± 0.12 and 7.29 ± 0.08 log CFU/coupon respectively. At 1.5 h post-inoculation, levels were 6.78 ± 0.47 and 6.77 ± 0.39 log CFU/coupon on canvas and cordura materials respectively.

Table S5. Reduction of *Salmonella* on cordura coupons treated with chlorine at different pH levels and concentrations (XLDR)[a]

Material	**pH**	**Concentration (ppm)**	**Reduction (log CFU/coupon)**
Cordura	10.35	200	1.22 ± 0.39 a
Cordura	7.00	200	1.96 ± 0.18 b
Cordura	7.00	500	3.05 ± 0.79 c

a. The mean starting level of *Salmonella* on cordura was 7.29 ± 0.08 log CFU/coupon.

Table S6. Parameters of the linear model describing the reduction of on cordura surfaces post-chlorine treatment (XLDR)[a]

Parameter	**Estimate**	**95% CI[b]**	***P* value**
Intercept	1.22	0.99, 1.45	<0.001
Treatment (reference = chlorine 200 ppm/pH 10.35)			
Chlorine 200 ppm/pH 7	0.72	0.33, 1.12	<0.001
Chlorine 500 ppm/pH 7	1.83	1.66, 2.45	<0.001

a. The mean starting level of on cordura was 7.29 ± 0.08 log CFU/coupon.
b. 95% confidence interval

Table S7. Weight before and after treatment

Mode of application	**Material types**	**Mean weight ± SD[a] (g) before treatment**	**Mean weight ± SD (g) after treatment**
Spray bottles	Canvas	0.34 ± 0.29	0.96 ± 0.55
	Cordura	0.61± 0.10	1.20 ± 0.56
Steam			
	Canvas	0.35 ± 0.07	5.23 ± 1.36
	Cordura	0.64 ± 0.06	4.21 ± 0.68

a. Standard deviation

Chapter 6: Conclusion

The first objective of this study was to determine the survival of generic *E. coli*, *Salmonella*, and *Listeria monocytogenes* on different harvest bag material types. Next, we wanted to quantify the transfer of generic *E. coli*, *Salmonella*, and *Listeria monocytogenes* from different harvest bag material types to fresh unwaxed apples under different transfer scenarios. Finally, we investigated the efficacy of different sanitizer treatments for decontaminating harvest bag material-type surfaces. Our findings showed that *E. coli* survived for more than 21 d on all material types under all holding conditions. In addition, canvas supported the survival of *E. coli* more than cordura and nylon. The die-off of *E. coli* was triphasic with an initial rapid die-off rate followed by more gradual die-off rates that were comparable to rates reported in other studies. *L. monocytogenes* and *Salmonella* survived up to 21 d on canvas and cordura surfaces. These bacterial pathogens exhibited die-off rates in biphasic (*Listeria monocytogenes*) and triphasic (*Salmonella*) patterns. The transfer of *E. coli* was impacted by the presence of moisture on harvest bag surfaces. The most frequent transfer of *E. coli* occurred when canvas surfaces were the source of contamination. Pressure impacted the transfer of *E. coli*. The transfer of *L. monocytogenes* from cordura surface was significantly higher than transfer rates from canvas surfaces. However, *Salmonella* transferred from both canvas and cordura surfaces at similar rates. Of all the sanitizing agents that were applied, IPAQuats was the most effective sanitizer treatment for reducing *L. monocytogenes* and on canvas and cordura surfaces. PAA was more effective on harvest bag surfaces inoculated with *Salmonella* Chlorine was more effective at a concentration of 500 ppm. Overall, these studies

showed that bacterial pathogens can survive and transfer from harvest bag surface to tree fruit in case of contamination. Harvest bags should have a sanitation standard operating procedure. While cleaning and sanitizing the surfaces of harvest bags depends on specific activities in the operation, apple growers must maintain these bags to prevent contamination. In case of a known contamination event, harvest bags should be cleaned, sanitized, and fully dried before using. As it relates to sanitization, apple growers should emphasize dry cleaning of harvest bags as moisture is a driver for bacterial transfer. This can be achieved with IPAQuats which is very effective at decontaminating harvest bags and is applicable in dry sanitizing interventions. In case of the use of other sanitizing agents like PAA, harvest bags should be properly dried before use.

Future Studies

- More studies are needed to examine the surface characteristics of harvest bags and how bacteria interact with these surfaces at a microscopic level.
- More studies modeling the die-off of *E. coli*, *L. monocytogenes,* and *Salmonella* on harvest bag material types are needed so that comparisons can be made to determine if die-off rates are reasonable and can be used for quantitative microbial risk assessment.
- The observed variation pattern in transfer studies with *Salmonella* may have been the result of differences in attachment characteristics between bacterial strains in the cocktail. More studies are needed to confirm this suggestion.
- More studies are needed to determine the efficacy of sanitizers in reducing *Salmonella* and *Listeria monocytogenes* at treatment times >1 minute.