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CPS 2010 RFP
FINAL PROJECT REPORT

Project Title

Survival, transfer, and inactivation of *Salmonella* on plastic materials used in tomato harvest

Project Period

January 1, 2011 – December 31, 2012; NCE to February 28, 2013

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Objectives

1: Identify and evaluate surface characteristics of materials used for glove and produce tote/bins. Surface characteristics such as hydrophilicity, wetting behavior, surface energy, surface charge, chemistry, and topography determine the molecular mechanisms behind adhesion of soil and bacteria to plastic materials (glove and tote/bin). Factors such as age, roughness and wear from repeated cleaning, and chemical deterioration from repeated sanitization, can affect surface characteristics. These characteristics therefore greatly impact cleanability and can aid in identifying which materials (composition, texture, and age) are less likely to harbor microbial hazards.

2: Evaluate bacterial survival on and transfer from glove and tote/bin materials used in produce harvest. We will test survival of Salmonella sp. on plastic tote/bin materials under different levels of soil and relative humidity and then evaluate the transfer of Salmonella 3 sp. from inoculated glove and tote materials to tomatoes, as a model system. Tomatoes were selected because of their association to recent outbreaks of food borne illness, and because their uniform size, shape, and surface area facilitate the design of repeatable microbial transfer methodology, as described below. The proposed scientific experimental design enables the results to be transferable to other bacteria and produce systems.

3: Evaluate the cleanability and sanitation efficiency of Salmonella sp. on glove and tote plastics. The proposed study will evaluate cleaning and sanitation methods against a cocktail of Salmonella sp. inoculated on glove and tote materials. The efficiency of these methods will be evaluated by microbial testing and ATP testing. The microbiological tests will give hard scientific data on bacterial reduction after cleaning and sanitation and the ATP tests with help to determine if the use of a rapid test method has the potential to be used in the field to validate HACCP documentation of cleaning and sanitizing.

Funding for this project provided by the Center for Produce Safety through:
UCANR/USDA NIFA grant #2010-34608-20768 (SA7662)

FINAL REPORT

Abstract

In an effort to improve sanitation, growers are increasingly using plastic materials to handle and pack fresh produce, replacing traditional wood crates and paperboard cartons. Further, many workers have begun wearing vinyl or nitrile gloves in an effort to reduce pathogen contamination of hand harvested produce. This project took a multidiscipline approach to determine the chemical and biophysical characteristics of harvest binds and glove materials and their influence upon the bacterial survival, bacterial transfer to tomatoes and sanitation efficiency upon plastic materials.

Background

The most recent estimates indicate that there are 81.9 million instances of food borne illnesses annually, at a cost that has been estimated to be $152 billion dollars each year (Scharff 2010). Furthermore, the CDC has estimated that between 1998 and 2005, at least 13% of food borne outbreaks (and greater than 21% of food borne illnesses) originated from fresh produce (DeWaal and Bhuiya 2007), up from 0.7% in the 1970s (Sivapalasingam, Friedman et al. 2004). The economic cost due attributed to produce-related food borne illness was recently set at $39 billion (Scharff 2010). In comparison to outbreaks originating from other sources one of the fastest growing 'trends' in food-borne illness is produce-related outbreaks (DeWaal and Bhuiya 2007). Indeed, fresh produce represents a unique challenge to food safety as there is often no 'kill step' between harvest and consumption (Wheeler, T. M. Vogt et al. 2005). With increasing consumer demand for minimally processed produce (NAS and USDA 2007) there is a critical need for improving the safety of fresh produce.

Food Safety is of critical importance for fresh produce and a large amount of this burden is placed upon the growers to prevent contamination in the field and during the harvest procedures. The FDA has published a Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (FDA Assessed 4/06/2010) in order to promote safety and sanitation of fresh produce, but at present there are no mandatory farm inspections. The Food Safety Modernization Act was signed into law on 1/4/2011 and currently the FDA has a proposed rule (Standards for the Growing, Harvesting Packing and Holding of Produce for Human Consumption, FDA-2011-N-0921) out for comments. This legislation will influence many aspects of day-to-day farm practices including the requirement of testing of agriculture water and required practices for use of biological soil amendments. In Subpart L or the proposed Produce Rule, the FDA addresses the sanitation of equipment and tools that are intended to come in contact with produce.

The goal of this project was to generate quantitative data to support recommendations of best practices for cleaning and sanitization of harvest buckets to prevent contamination of produce, and to reduce the occurrence of produce-associated food borne illness.

Research Methods and Results

***Objective 1*: Identify and evaluate surface characteristics of materials used for glove and produce tote/bins.**

Research Question 1.1: How do surface characteristics influence the ability of commonly used cleaners and sanitizers to wet plastic materials? Water contact angles were measured on a Krüss DSA100 (Hamburg, Germany) goniometer equipped with a direct dosing system (DO3210, Krüss, Hamburg,

Germany) at room temperature under atmospheric conditions in order to determine the change of wettability induced by the modification of the material surface. The tangent method 2 from the Drop Shape Analysis software version 1.91.0.2 was used for the measurement of contact angles using an automatic dispenser that added the water (HPLC Grade, Fisher, Fair Lawn, NJ) droplet at a rate of 10 µL/min. The contact angle of glove materials were evaluated (Figure 1). In general, a water contact angle greater than 90° is considered to be hydrophobic in nature. Gloves made out of vinyl and nitrile gloves fell under this cut off value so could be considered to be more hydrophilic than the vinyl and LDPE gloves tested in this study (Figure 1).

The ATR-FTIR spectra of glove materials were obtained with an IR Prestige 21 spectrometer (Shimadzu Corporation, Kyoto, Japan) with a diamond ATR crystal. The software for collecting and viewing spectra was IRsolution (v. 1.3, Shimadzu Corp.). Each absorbance spectrum represents 64 scans at 4.0 cm-1 resolution using SqrTriangle apodization, using a clean ATR crystal exposed to the ambient atmosphere as a background. KnowItAll software (v. 8.1, Biorad Laboratories, Inc., Philiadelphia, PA, U.S.A.) aided in spectra visualization and analysis. The chemical spectra of glove materials are shown in Figure 2 indicate chemical differences between the materials.

Surface topography and roughness were evaluated using a Zeta 20 3D optical profiler (Zeta Instruments; San Jose, CA) with bottom illumination, and analyzed using Zeta 3D software (version 1.5). Area roughness was determined using the arithmetic average roughness value (S_a) and the root mean square roughness value (S_q). The results for the gloves are shown in Figure 3, showing that the latex and nitrile gloves have a smoother surface topography than the low density polyethylene or vinyl glove materials.

Worn picking buckets were obtained from a local farm (Figure 4 and 5). In order to study the influence of bin abrasion upon sanitation and transfer, a method was developed to simulate abrasion. Abraded samples were obtained by cutting coupons from the bottom of HDPE picking bins into 2.5 x 7 cm rectangular pieces and agitating samples (18 pieces) in a rock tumbler (Thumbler's Tumbler Model B; Auburn, WA) along with modified MP14 mending plates (1 x 4 inch Simpson Strong-Tie; Home Depot). Mending plates were modified by interlocking and folding two mending plate in such a way as to allow for the barbs to always face upward in a caltrop fashion during shaking (Figure 1). For routine abrasion, samples were agitated with eight modified mending plates (16 total plates) for up to 6 hours. The the process of topography modification can be seen in Figure 6 and 7. The wettability of abraded bins was quantified by water contact angle to determine how scratches alter interactions with water and sanitizing agents. Compared to new bins, abrasion time up to 6 hours did not significantly alter the advancing contact angle (Figure 8a). However, the contact angle hysteresis increased with abrasion time, reaching a maximum change of 35° after 6 hours (Figure 8b). This result is expected as hysteresis is known to increase with surface roughness and irregularity.

Objective 2: Evaluate bacterial survival on and transfer from glove and tote/bin materials used in produce harvest.

Research Question 2.1: Can Salmonella sp. survive on plastics used to make produce tote bins? Cocktails of *Salmonella* containing a 5 strains (Table 1) were used for inoculation either suspended in sterile tryptic soy broth (TSB), in the presence organic mass (5% horse serum in TSB), suspended in TSB with both organic (horse serum 5% v/v) and soil (5% w/v) mass, or only soil (5% w/v). Sterile high density polyethylene coupons were inoculated by immersing in cocktails of *Salmonella sp* for 10 min then dried in a biological safety hood. Initial cell numbers Coupons were then transferred to desiccators over saturated salt solutions at 20°C to standardize the equilibrium relative humidities to 94%, 75%, 54%, and 33%. At time points 0, 2, 7, 14, 21 and 28 days triplicate coupons were removed each inoculation cocktail (TSB, serum and serum/soil), shaken with beads for removed (as described above) and plated to determine surviving numbers**.** This work was performed in the McLandsborough laboratory by PD1.

The results in Figure 9 show that *Salmonella* can survive extended periods of times on plastic bin material, although numbers of recoverable bacteria decrease over time. For all inoculation methods and relative humidities tested, a 1.5-3 log reduction was observed after two days of desiccation, with lower numbers recovered as the storage progressed over the 28 day study. In general, the inoculation solution had a significant impact on the number of bacterial cells recovered during the latter two weeks of the experiment (sampling days 21 and 28) at all the relative humidities tested. The greatest survival was seen with *Salmonella* inoculated in a 5% soil in TSB suspension, followed by inoculation in the mixture of soil and horse serum (5% of each), 5% soil and the least amount of survival was seen when *Salmonella* were inoculated in TSB. In general, a similar trend was observed at all relative humidities, except for storage at 33% ERH, which appears to have significantly enhanced the survival of Salmonella inoculated in TSB, and to a lesser extent the samples inoculated in TSB +serum and the TSB + serum/soil mixture. Confocal microscopy using CTC staining for Electron micrographs show that when soil is present, bacteria are associated with soil particles, are visible at the interface between the soil particle and the plastic surface. This is likely the last location that water is present during the drying of the surfaces (Figure 10).

In order to determine if this increase in survival in the presence of soil was an artifact of the high numbers used in the initial experiment, we inoculated disks of high density polyethylene buckets with a surface area of 0.78 cm^2 with the cocktail of five Salmonella strains, so that the initial inoculum was approximately 10^9, 10^4, 10^3,10^2 and 10^1 CFU/chip. Chips were stored at 54% ERH at 20°C. At days 0, 2, 14 and 28, chips were removed and added to test tubes of TSB. Cells were enriched in sterile full-strength $TSBNa_{50}$ for 48 h at 37°C and assessed visually for turbidity. Three independent samples were tested at each time point and the experiment was repeated twice. The results from this experiment are shown in Table 2. As with the previous experiment, there appears to be greater survival of *Salmonella* under desiccation in the presence of soil. When soil was present, salmonella was also detected at day 28 when inoculated at the levels of 10^3 CFU/chip in both trials. There appears to be a trend that the presence of soil increased the detectable levels of *Salmonella* present on the plastic samples. The results from this experiment show that regardless of the inoculation method, at both 10^4 CFU/chip and 10^9 CFU/chip, salmonella were detected after 28 days under desiccated conditions on bin material, indicating, if initial numbers are high enough Salmonella can have long term persistence on bin surfaces, and this survival appears to be enhanced by the presence of soil.

To determine if the enhanced long term survival in the presence of soil was due one or more or all of the five strains in the *Salmonella* cocktail. To do this, individual *Salmonella* strains were inoculated onto bin chips with and without the presence of 5% soil and stored at 54%ERH at 20°C. At time points 0, 2, 7, 14, 21 and 25 days triplicate coupons were removed each inoculation cocktail (TSB, serum and serum/soil), shaken with beads for removed (as described above) and plated to determine surviving numbers**.** The results from this experiment are shown in Figure 11. All five *Salmonella* strains showed similar decrease in numbers with a 6-7 log reduction in numbers observed by 25 days (Fig 11A). In the presence of soil, four of the strains (BAA-708, BAA-709, BAA-710, and BAA-711) showed a similar rate in reduction, with a 5 log reduction after 25 days, which was a moderate (approximately 1 log) increase in survival in the presence of soil. Strain BAA-1045, *Salmonella* Enteritidis Phage type 30, appears to have a slower decrease cell numbers in the presence of soil than the other *Salmonella* sp strains tested (Figure XB), and at times point 7 and 14 days, this strain only had a 3-3.5 log reduction in numbers, compared to the 4.5-5 log reduction observed with the other four strains at these times points, indicating that this strain may have higher desiccation resistance. This strain has been reported by others to show increased heat resistance in peanut butter (Ma, Zhang et al. 2009), and this strain may be in part responsible for recovery of high cell numbers in the initial survival experiments performed with a *Salmonella* cocktail.

Research Question 2.2: How do glove or tote material influence bacterial transfer?

Bacterial transfer can be thought of as a stochastic process, meaning that the physical transfer from one surface to another is dependent on a variety of changing variables. The degree of transfer from one surface (either HDPE bins or gloves materials) to a tomato surface will be dependent upon the adhesion strength the first surface in comparison to the degree of attractive forces to the second surface. The contact surface area and contact forces (both normal and shear) will also influence the degree of transfer. In this study, a shoot method was developed to evaluate transfer. This allowed us to standardize normal and shear forces during transfer, as well as to minimize bruising and damage of produce.

Aluminum shoots with controllable incline were manufactured, and a recessed slot into which inoculated test materials were placed. This method also allowed for standardization of normal pressure and shear pressure between the produce and contaminated surfaces. For glove testing, glove materials were wrapped around HDPE chips and taped on the back. Surfaces were sanitized under UV light for 30 min and then inoculated with 50 µl of the *Salmonella* cocktail in TSB were adjusted to an approximate 1 x 10^9 CFU/ml was spread over the contact area (17.74 cm^2), for an inoculated density of approximately 5 x 10^7 CFU/cm^2. Slides were dried on the bench top for 15 min at room temperature. Tomatoes were rolled down the incline, across the inoculated material, and collected in a petri dish at the bottom of the shoot. Tomatoes were transferred to 15 of sterile buffer with sterile glass beads, bacteria were removed by shaking for 20s, and numbers of transferred bacteria were determined on agar containing 50µg/ml nalidixic acid selection to remove background flora from the tomatoes.

Transfer of *Salmonella* from different glove materials to tomatoes was evaluated by rolling tomatoes over the inoculated gloves and determining levels of *Salmonella* transferred to tomatoes).Initially experiments on transfer from inoculated glove materials to tomatoes was performed on store purchased grape tomatoes (Figure 12A). With input and assistance from CPS, farm harvested grape tomatoes were obtained from Mike Maxwell at Procacci Brothers Farms in Philadelphia PA which were harvested but not submitted to the standard chlorine wash, therefore accurately represent on farm produce. The results from this experiment are presented in Figure 12B. Transfer was also repeated with green breaker tomatoes (Figure 12C). For all types of tomatoes, statistically lower transfer to tomatoes ($P<0.05$) was observed from vinyl and nitrile gloves, when compared to latex and polyethylene gloves. There was no correlation to the hydrophobicity measurements under objective 1 which concluded that vinyl and nitrile gloves were more hydrophilic than the vinyl and LDPE. For grape tomatoes, vinyl gloves showed the lowest bacterial transfer, (Fig 12A, and B) and for green breaker tomatoes nitrile showed the least amount of transfer. It is likely these differences are due to differences in adhesion strength to glove materials and possibly differences in adhesion to different tomato types. These results indicate that *Salmonella sp.* cellular adhesion to vinyl and nitrile was stronger than the adhesion to latex and polyethylene glove materials.

Objective 3 **Evaluate the cleanability and sanitation efficiency of sanitizers upon *Salmonella* sp. on glove and tote materials.**

The effectiveness of hypochlorite solutions on sanitation of new and worn harvest bucket materials was evaluated with various amounts of soils (either 5% serum or 5% soil) using a modification of the AOAC method 955.14 (AOAC 2013). Our methodology varied in that we inoculated bucket materials and allowed the materials to dry at 20°C and 54% relative humidity for 24 h prior to determining the effectiveness of the sanitizing agents and D/E Neutralization broth (BD Difco, Franklin Lakes, NJ) was used in place of letheen broth for neutralization. Sanitation treatment concentrations

were selected based upon FDA guidance for produce (FDA Assessed 4/06/2010) and the US Food code (FDA 2009).

Worn bucket materials were prepared as described under Objective 1. Both worn and new buckets were cut into small chips (0.635 cm in diameter) and each side was sterilized with UV light for 5 minutes. Chips were immersed in *Salmonella sp.* five strain cocktail with shaking for 5 minutes. Chips were then transferred to sterile petri plates and stored overnight at 20°C at 54% RH. A stock hypochlorite solution was prepared in water and the free HOCl was measured using the N,N dimethyl-p-phenylnediame (DPD) method (Hach Company, Loveland, CO). This solution was then diluted to prepare samples with 50, 100, 150 or 200 ppm total HOCl. Chips were placed into 5 ml HOCl solution and allowed to sit for 2 minutes, after which the chip was transferred to 5ml D/E Neutralization broth (BD Difco, Franklin Lakes, NJ) for 5 min. Numbers on the inoculated slides for calculation of "log reduction" were determined by placing a chip into 5 ml sterile water for 5 min and then 5 ml neutralization broth and vortexing for 30 sec, then dilution and plating using a spiral plating apparatus. Dirty surfaces simulated through the addition of 5% serum (organic matter) or 5% sterilized garden soil to the inoculation medium (TSB) prior to inoculation. Each condition was repeated three times and log reduction was calculated using the following equation (Log reduction = log N_0 – log N), where N_0 were the numbers of bacterial (CFU/cm^2) recovered from the untreated control and N were the numbers (CFU/cm^2) recovered from each treatment. A one way analysis of variance using (Tukey Test) was used to determine statistical differences between treatments.

The influence on the presence of soil or serum upon the sanitation of new materials is shown in Table 3 As expected, a reduction in sanitation efficiency was observed in the presence of soil or serum. No statistical difference in sanitation efficiency was observed when worn materials were compared to new materials. The maximum log observed with a 2 minute contact time with cells inoculated in TSB was 3.5-3.8 log reduction. This was unexpected since by definition sanitation is to reduce the population of microorganisms by 99.999% or a 5 log reduction. In our system, this just under a 4 log reduction was the maximum we observed with a 2 minute contact time, however we were treating organisms that had undergone 24 h of desiccation at 54% RH. As observed in Figure 9, during the initial desiccation, a portion of the organisms die, and the surviving organisms may have turned on a stress response that can make the organisms more resistant to subsequent environmental stress such as encountered during the oxidative stress related to HOCl sanitation. In general, sanitation tests only dry samples for 30 minutes prior to treatment (AOAC 2013), and it is likely that pre-desiccation of the microorganisms contributed to increased sanitizer resistance, but more data is needed to confirm this observation.

Outcomes and Accomplishments

- The surface hydrophobicity, chemical spectra and surface topography were determined for new and worn bucket materials and glove materials. An abrasion method was developed to simulate abraded bucket materials in the laboratory. Abraded materials showed showed changes in contact angle hysteresis.
- Bin materials inoculated with a cocktail of *Salmonella sp.* were observed to have a 1.5-3 log reduction after two days of incubation with lower numbers recovered as the storage progressed over the 28 day study. The level of persistently surviving organisms varied based upon the inoculation method, with the presence of soil having the greater influence of bacterial recovery, with a 3-4 log higher recovery of "persister" cells.
- Bacterial transfer from gloves to tomatoes varied according to glove material type (vinyl, latex, nitrile and low density polyethylene) or tomato type (ripe grape tomatoes or green breaker

tomatoes). In general, vinyl and nitrile showed statistically less transfer to tomatoes than latex or LDPE materials, indicating stronger adhesion to *Salmonella* sp to these materials.

- Sanitation efficiency was observed int eh presence of soil or serum, however no statistical difference in sanitation sensitivity as observed when materials were abraded to simulate worn conditions.

APPENDICES

Publications and Presentations

Talbert, J. N., K. Seto, J. Cotter, L. McLandsborough and J. M. Goddard. Effect of Cleaning and Sanitization Agents on the Surface Characteristics of New and Extended-Wear Produce Picking Bins. *Submitted.* J. Sci Fd Ag. 2013.

Cotter, J. J. Talbert, J. Goddard and L. McLandsborough. Effect of environment upon desiccation survivol of *Salmonella sp* on High Density Polyethylene harvest buckets. *Manuscript in preparation.* J. Food Protection

J. Cotter*, J. Talbert, J. Goddard, W. Autio and L. McLandsborough. Influence of soil particles on the survival of *Salmonella* on plastic tomato harvest containers. IAFP National Meeting 2012. Technical Session 1, T1-04.

Budget Summary (as of April 29, 2013)

Project End Date	Fiscal year to date expenditure	Project to Deate Expenditure	Balance Remaining
30-Jun-2013	53,232.92	190,170.06	44,616.94

Tables and Figures

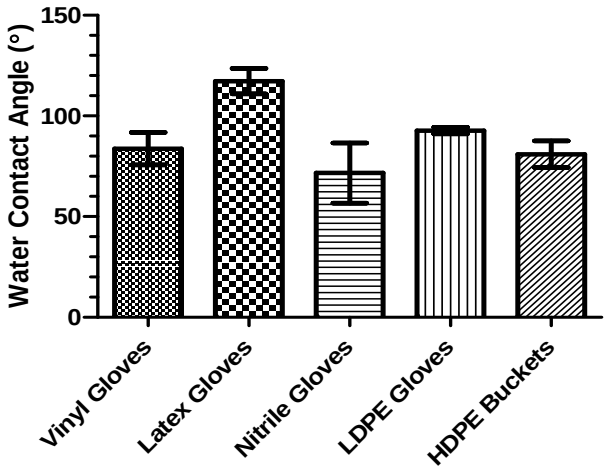


Figure 1: Contact angle of ultra-pure water with disposable gloves and new picking buckets materials used by tomato growers. Error bars represent standard deviation (n=6).

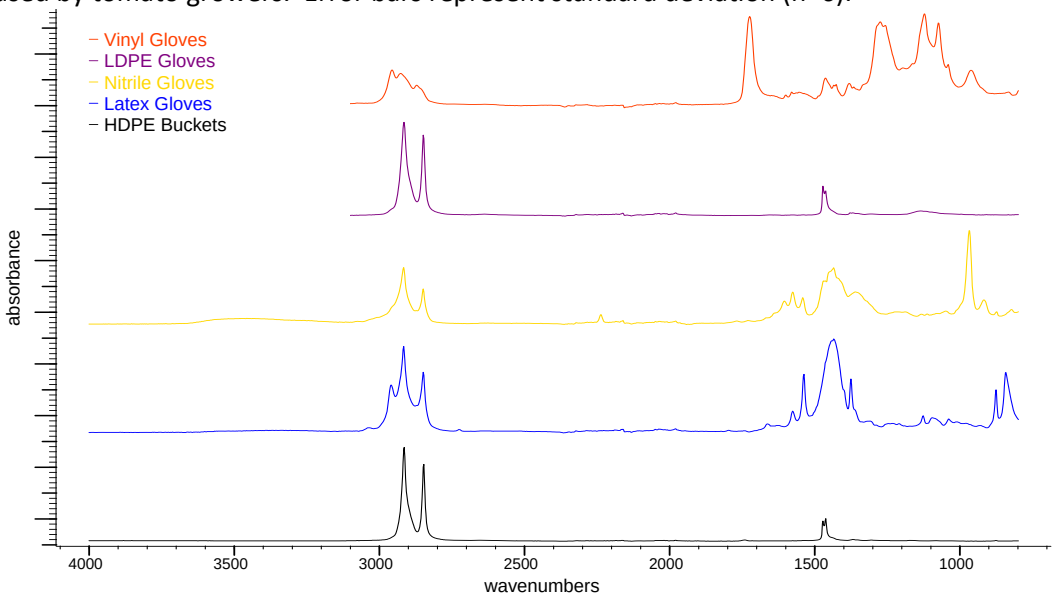


Figure 2: Chemical spectra of disposable gloves and picking buckets used by tomato growers.

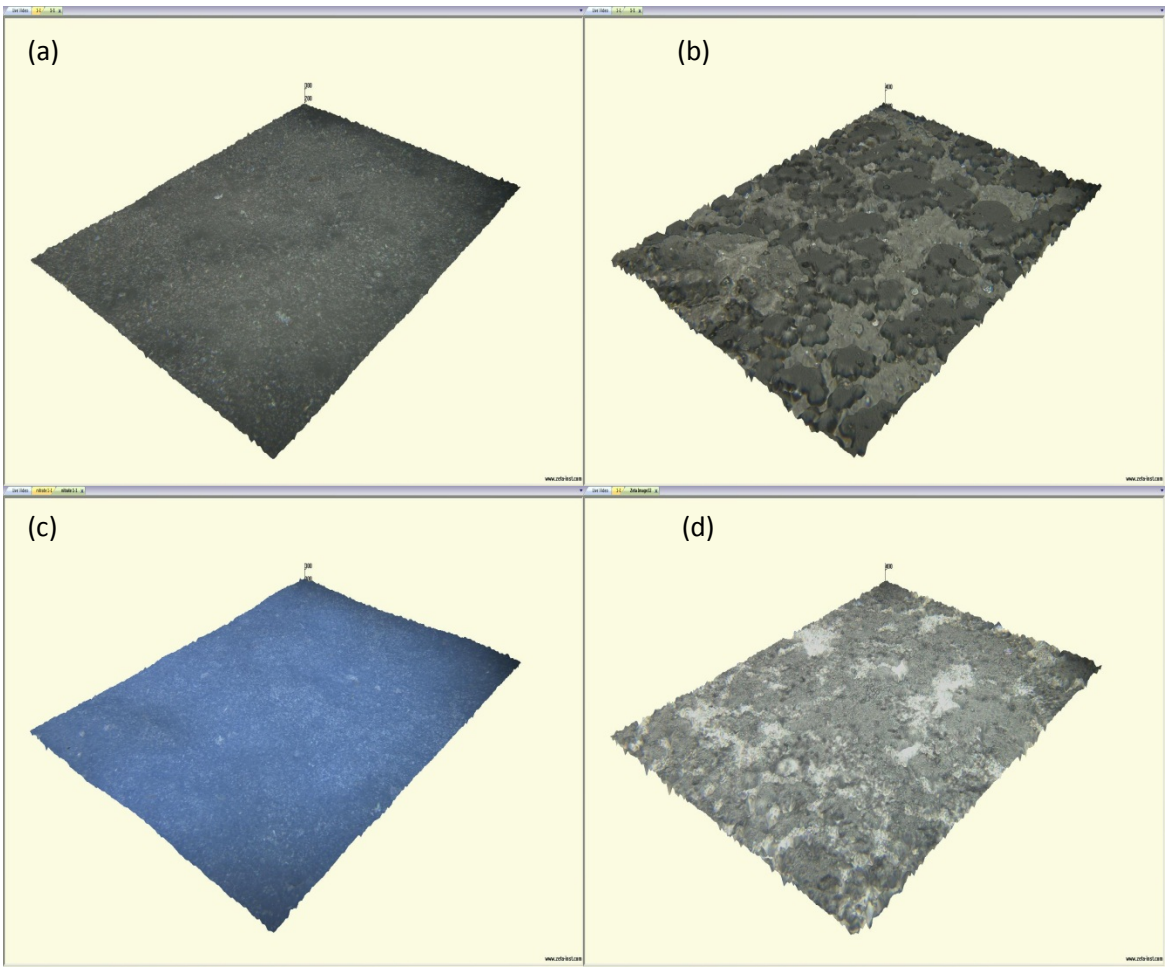


Figure 3: Topographical profiles of (a) Latex, (b) LDPE, (c) Nitrile, and (d) Vinyl gloves.

Figure 4 Comparison of new and used tomato picking buckets.

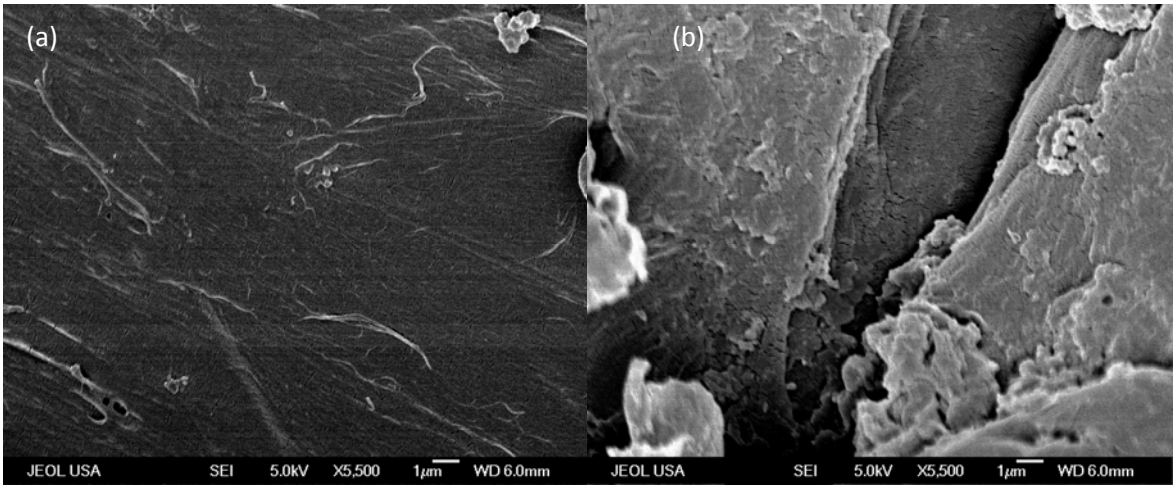


Figure 5: Scanning electron micrographs of the bottoms of (a) new tomato picking buckets and used in the field for six years.

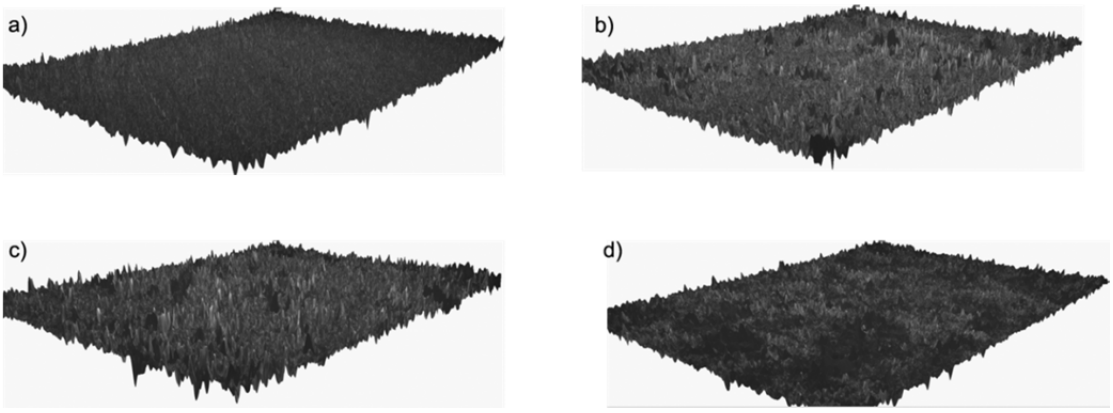


Figure 6. Optical profilometer images of HDPE picking bin samples after abrasion for (a) 0 hours, (b) 0.5 hours, (c) 2 hours, and (d) 6 hours.

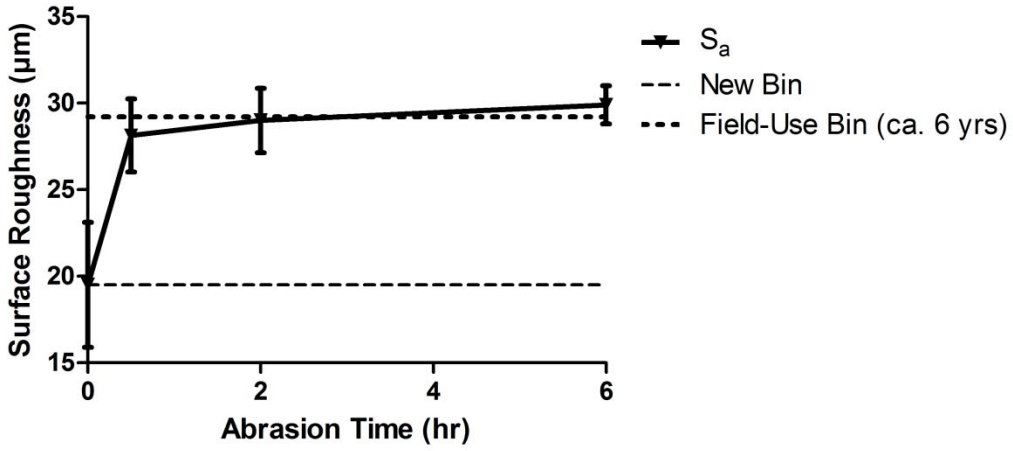


Figure 7. Arithmetic average (S_a) surface roughness values of new HDPE tomato picking bins, field-use bins (ca. 6 years of usage), and bins after abrasion for 0, 0.5, 2, 6 hours. Values represent a sample size of n = 3 with error bars indicating standard deviation.

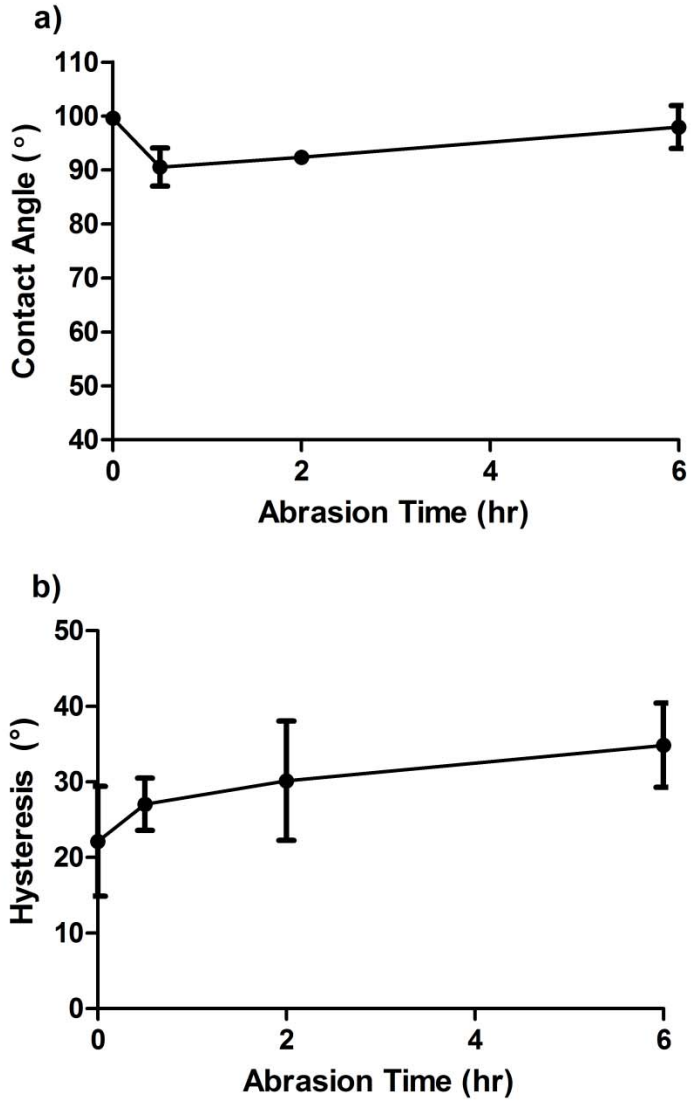


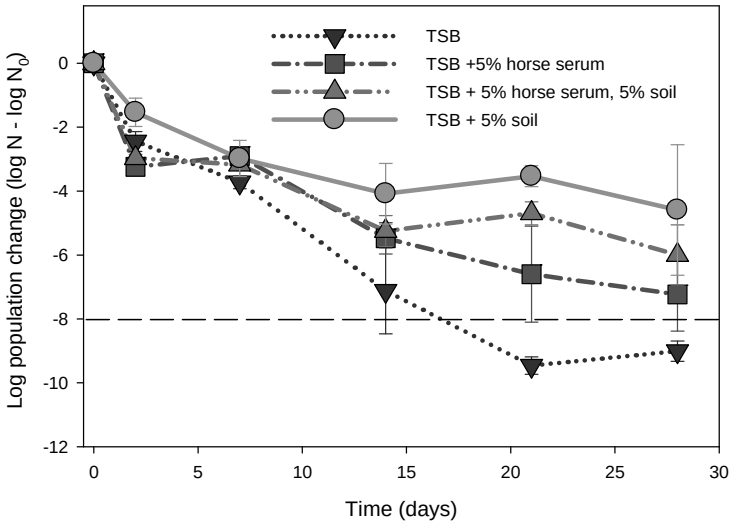
Figure 8. Effect of abrasion time on the (a) advancing contact angle and (b) hysteresis values of HDPE picking bins. Values represent a sample size of n = 3 with error bars indicating standard deviation.

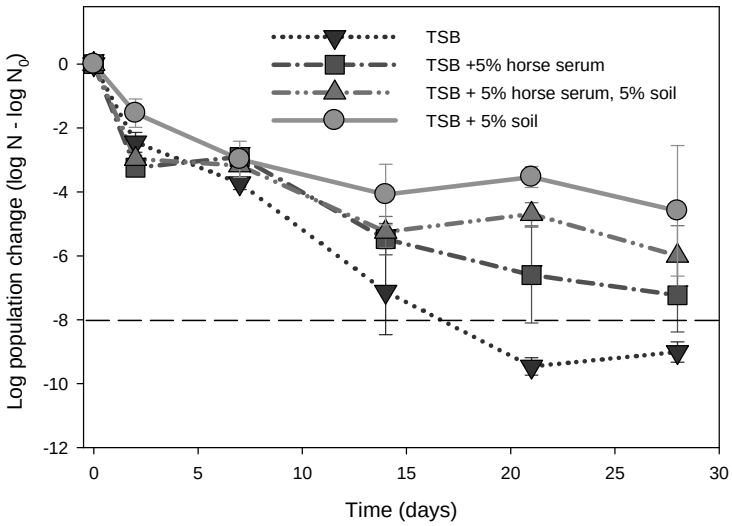
Table 1. *Salmonella enterica* serovars used in this study

Strain number	Name	Source
BAA-708	*Salmonella enterica* serovar Enteritidis	ATCC; clinical, egg associated
BAA-709	*Salmonella enterica* serovar Michigan	ATCC; cantalope
BAA-710	*Salmonella enterica* serovar Montevideo	ATCC; clinical, tomato associated
BAA-711	*Salmonella enterica* serovar Gaminara	ATCC; dried orange juice
BAA-1045	*Salmonella enterica* serovar Enteritidis	ATCC; Phage type 30 raw almonds

Lynne McLandsborough, University of Massachusetts

Survival, transfer, and inactivation of Salmonella on plastic materials used in tomato harvest





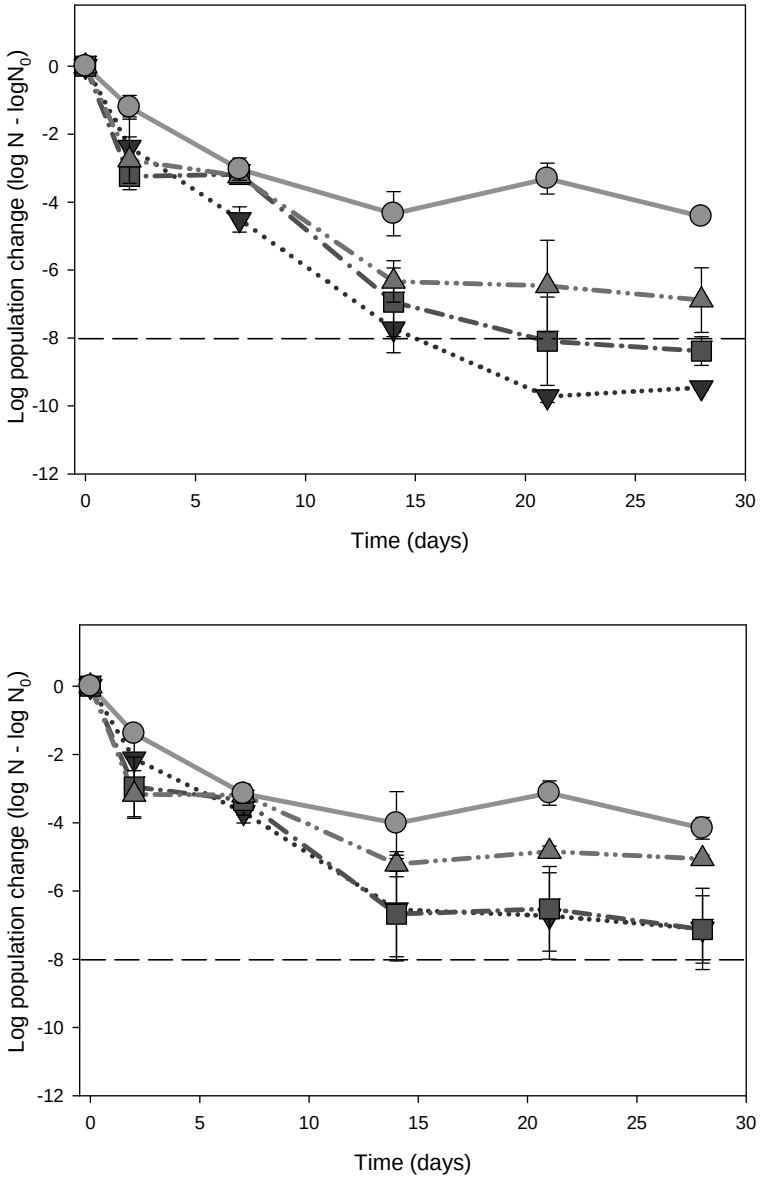


Figure 9 Influence of inoculation medium upon survival of *Salmonella sp.* 5 strain cocktail over the course of 28 days at 95, 75, 54 and 33% RH. Inoculated HDPE harvest bucket chips were inoculated with a *Salmonella sp.* 5 strain cocktail in TSB (····▼····), TSB + 5% horse serum (–·–■–·–), TSB + 5% horse serum and 5% soil (—··▲··—), or TSB + 5% soil (—○—). The chips were incubated in at 20°C over saturated salt solutions to create a humidity level of 95% (A), 75% (B), 54% (C) or 33% (D). Data points at or below the dotted line, represent MPN numbers. The data represent the mean and the standard error of three independently run experiments.

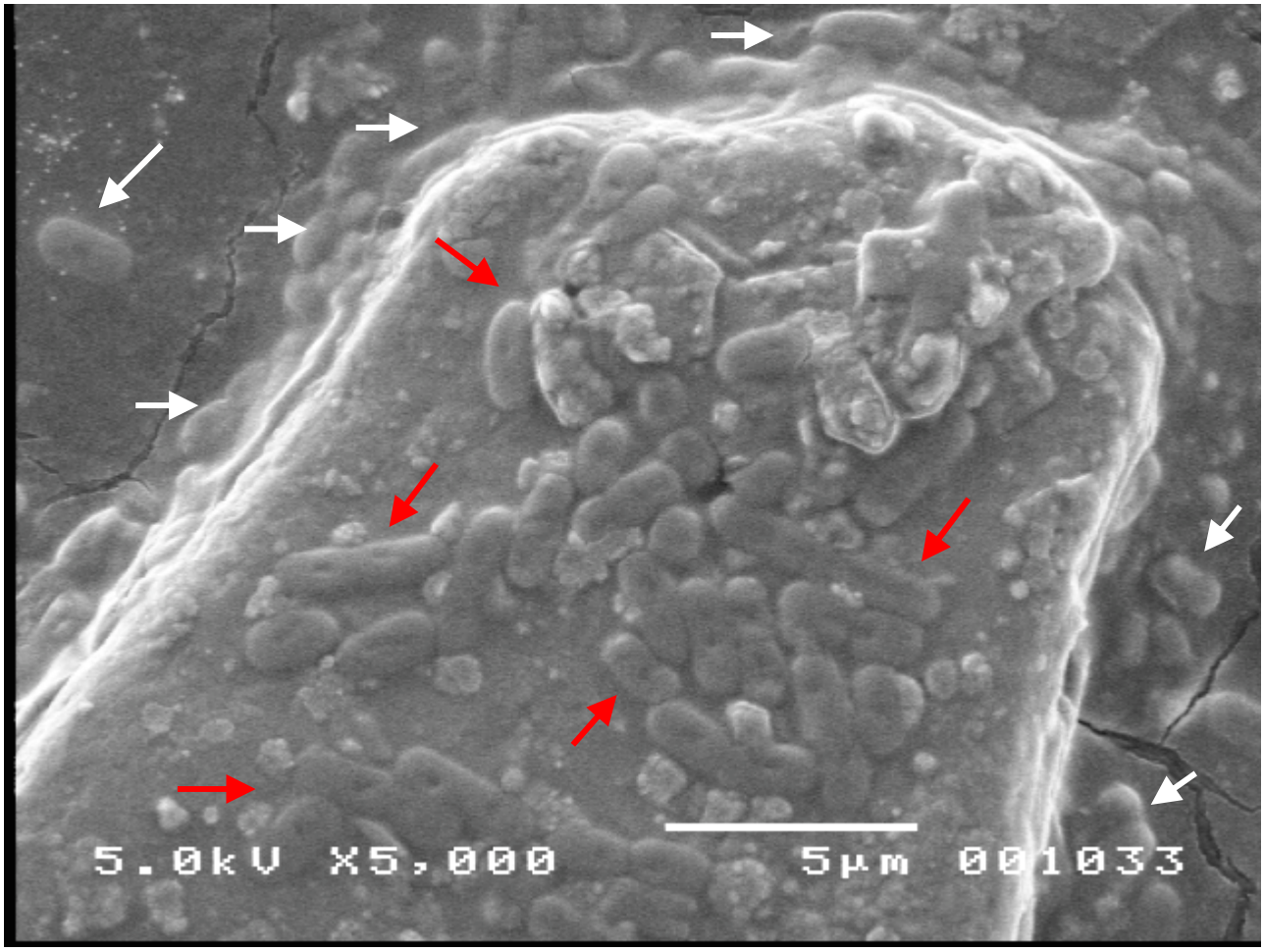


Figure 10 *Salmonella* sp cells associated with a soil particle (red arrows) and harvest bucket (white arrows) after 14 days of incubation at 53% RH. Cells showed indented morphology indicating water loss

Table 2. Survival of *Salmonella sp.* cocktail inoculated at various levels in TSB with and without the addition of 5% soil onto HDPE bucket chips and held at 54%RH at 20°C.

	Day 0		Day 2		Day 14		Day 28	
Inoculum	TSB	TSB+Soil	TSB	TSB+Soil	TSB	TSB+Soil	TSB	TSB+Soil
				Trial 1				
10^9	+ + +[1]	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
10^4	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ - +	+ + +
10^3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	+ + +
10^2	+ + +	+ + +	+ - +	+ + +	- - -	- + -	- - -	- - -
10^1	+ + +	+ + +	- - -	- - -	- - -	- - -	- - -	- - -
				Trial 2				
10^9	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
10^4	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- + -	+ + +
10^3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	+ + +
10^2	+ + +	+ + +	- - +	+ + +	- - -	+ + -	- - -	- - -
10^1	+ + +	+ + +	- - -	- - -	- - -	- - -	- - -	- - -

[1]+ inoculated were enriched in sterile full-strength TSB + nalidixic acid for 48 h at 37°C and assessed visually for growth.

A "+" indicates a positive for growth in that sample, and a "-" indicates no growth. Three independent samples were tested at each time point.

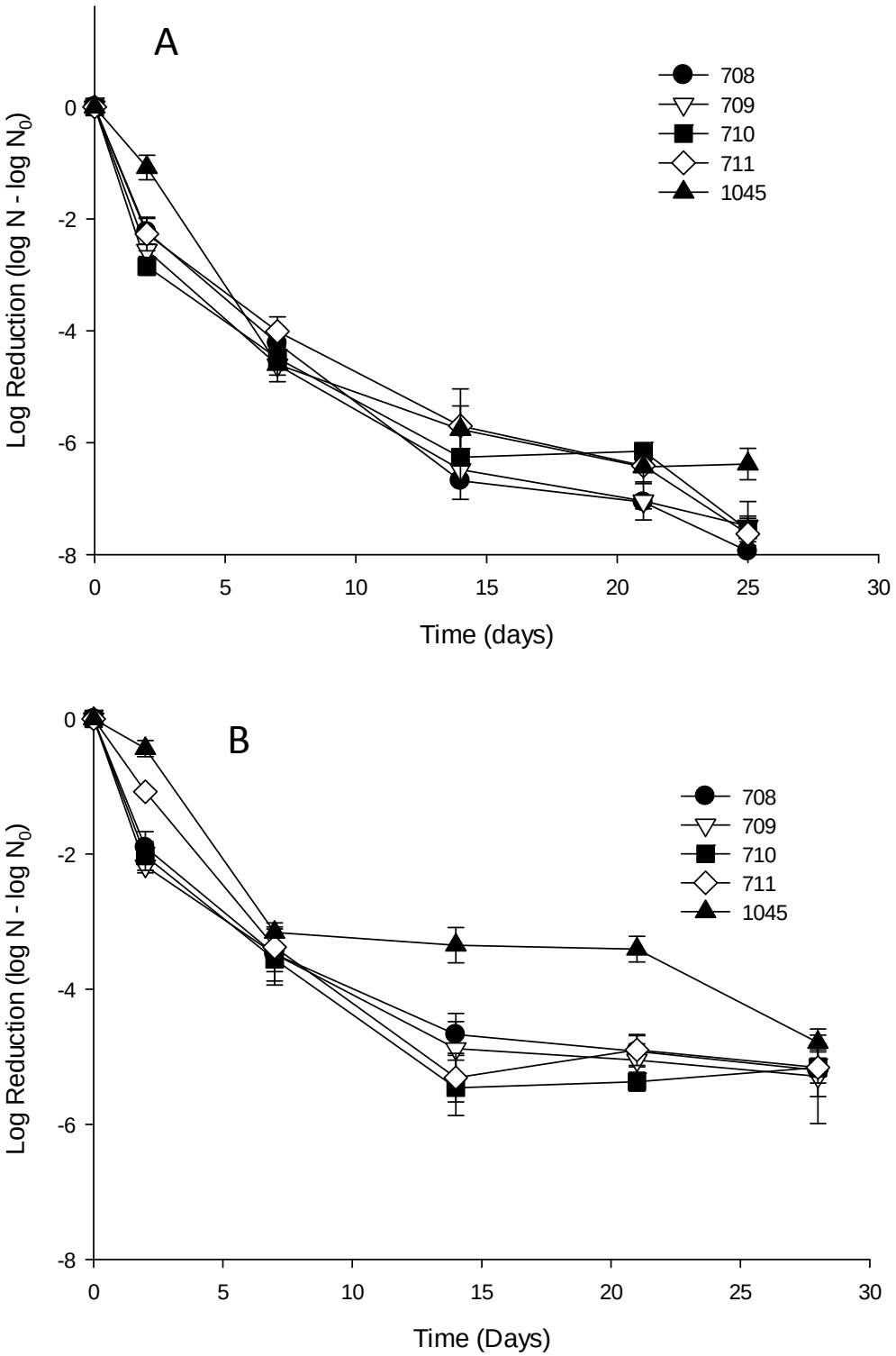


Figure 11. Influence of inoculation medium (cells suspended in TSB (A) or cells suspended in TSB+5% soil (B) upon survival of individual *Salmonella sp.* strains over the course of 28 days 54% RH.

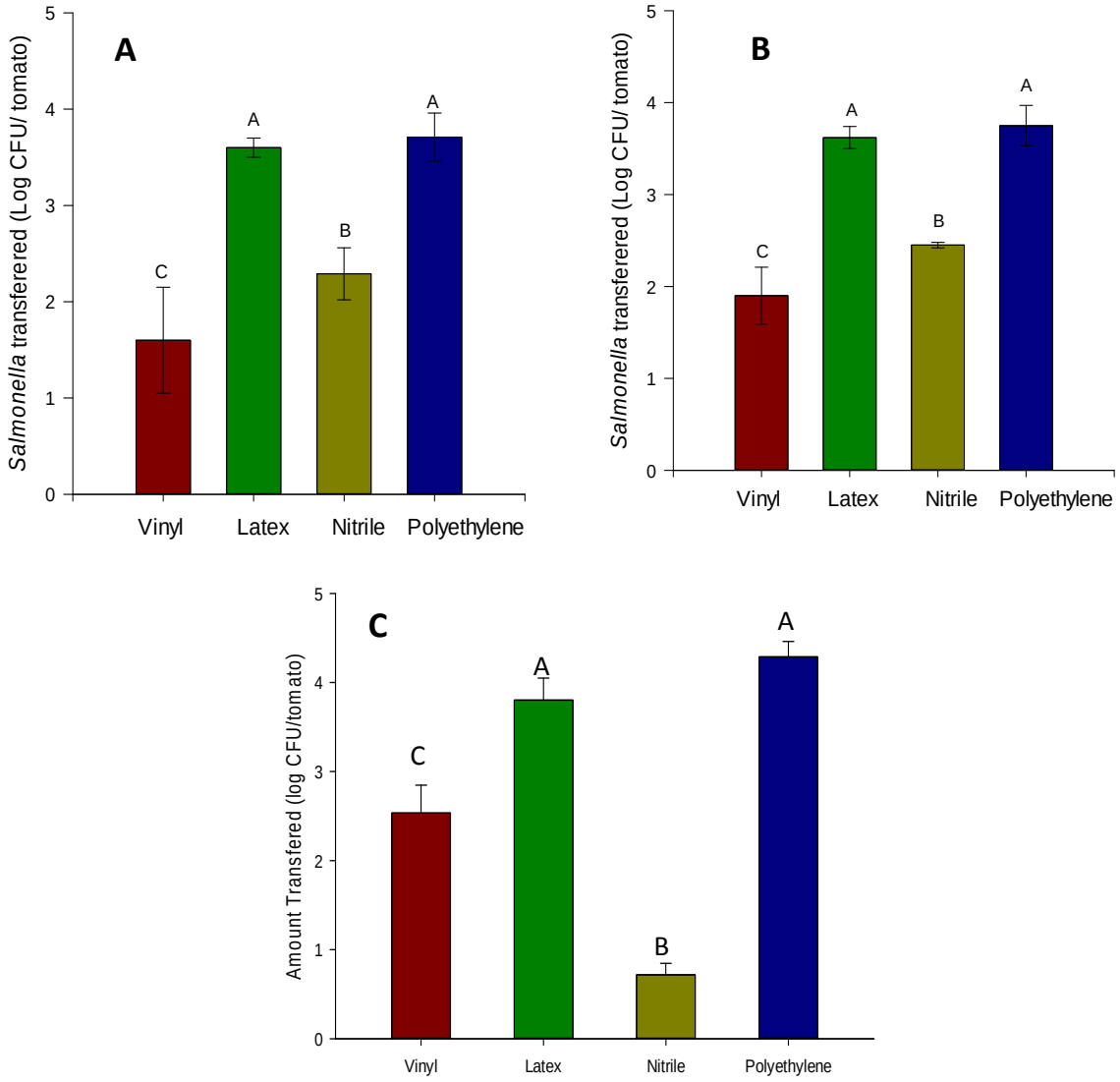


Figure 12. Transfer *Salmonella sp* from glove materials to grape tomatoes. A) Transfer of *Salmonella* to store purchased grape tomatoes from glove materials. B) Transfer of *Salmonella* to farm harvested (not treated) grape tomatoes from glove materials. C) Transfer of *Salmonella* from gloves to green breaker tomatoes. Salmonella was inoculated onto surfaces and dried at room temperature for 10 min to transfer. Bacteria were transferred by rolling over inoculated surfaces and transferred bacteria were removed from the tomato by vortexing with glass beads in 15ml buffer. Cell numbers were determined by plating on TSB with naladixic acid. Samples with different letters are statistically different ($P<0.05$).

Table 3. Effectiveness of sodium hypochlorite on reducing *Salmonella* sp. on harvest new bucket materials with varying amounts of soil.

	Total HOCl concentration Log Reduction (st. dev)			
Inoculation conditions	50 ppm	100 ppm	150 ppm	200 ppm
TSB	3.70 (0.61)^{A}	3.83 (0.50)^{A}	3.50 (0.27)^{A}	3.47 (1.33)^{A}
TSB + 5% Soil	1.65 (0.22)^{B}	2.59 (1.21)^{A}	2.39 (0.176)^{B}	2.68 (0.09)^{A}
TSB+5% Serum	1.91 (0.30)^{B}	2.10 (0.42)^{A}	2.05 (0.40)^{B}	2.66 (0.51)^{A}

Data points with different letters indicate significant differences within each bleach treatment (P<0.05).

Table 4. Effectiveness of sodium hypochlorite on reducing *Salmonells sp.* on new and worn harvest bucket materials

		Treatment HOCl concentration		
Inoculation conditions	Harvest bucket condition	50 ppm	100 ppm	200 ppm
TSB	New	3.70 (0.61)^{A}	3.83 (0.50)^{A}	3.47 (1.33)^{A}
TSB	Worn	2.93 (0.40)^{AB}	2.50 (0.24)^{A}	2.66 (0.53)^{A}
TSB + 5% soil	New	1.65 (0.22)^{C}	2.59 (1.21)^{A}	2.68 (0.09)^{A}
TSB+5% soil	Worn	1.93 (0.26)^{BC}	2.51 (0.47)^{A}	2.58 (0.53)^{A}

Data points with different letters indicate significant differences within each bleach treatment (P<0.05).

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