QUANTIFICATION OF SELECT FACTORS INFLUENCING THE RISK OF CONTAMINATION BY *SALMONELLA* ON TOMATOES FROM FARM TO FORK

By

JENNIFER TODD

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ABSTRACT OF THE DISSERTATION

Quantification of Select Factors Influencing the Risk of Contamination by *Salmonella* on Tomatoes from Farm to Fork

By JENNIFER TODD

Dissertation Director:

Dr. Donald W. Schaffner

The occurrence of *Salmonella* on whole, fresh tomatoes presents a risk to human health. The development of a quantitative microbial risk assessment will aid in identifying steps in tomato processing and handling that may either lead to contamination or amplify existing contamination. The findings from Chapter II suggest that growers should harvest dry tomatoes to reduce the risk of contamination since *Salmonella* transfer to tomatoes from soil or mulch is greater when moisture is present. Generally transfer to tomatoes was greater from new or used plastic mulch than from soil. This finding may have profound implications for growers since some believe plastic mulch reduces risk rather than increases it. The results in Chapter III highlight that *Salmonella* survival on tomatoes and plastic mulch is strain dependent, though the direct cause cannot be contributed just to the relative humidity (RH) of the environment or colony morphologies (rdar - rough dry and red) and subsequent biofilm production on tomatoes and plastic mulch. Chapter IV shows that NJ packinghouses implemented very different sanitary procedures that resulted in a wide range of bacterial reductions. Significant ($p<0.05$)

reductions in total plate and coliform counts for any one of five packinghouses typically occurred in half of all visits (2 or 3 visits out of 5). These results suggest that standardization of sanitizing procedures could aid in achieving a more consistent bacterial reductions. The main finding in Chapter V was that minimal *Salmonella* growth (<0.5 log CFU) was predicted on bagged salad during transport from the store to the home. Growth prediction for *Salmonella* on whole tomatoes was not possible (see Chapter VI results below). Growth on whole tomatoes or bagged lettuce is more likely during transport from the packinghouse to retail, or at retail due to expected times and temperatures. The models in Chapter VI demonstrate how currently available data on *Salmonella* transfer to and survival on tomatoes can be used in a full farm to fork model. The survival model demonstrates how unpredictable *Salmonella* survival on tomatoes can be. Further research should lead to a better understanding and potential solution on how to control *Salmonella* on tomatoes.

Dedication

I dedicate this dissertation to three of the most important people in my life:

Arthur, for your love and unwavering support in everything that I do.

Anthony, for being my homework buddy many evenings.

Mom, for never letting anything stop me from achieving academic success.

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Table of Contents

List of Figures and Tables

Chapter I: Literature Review

Salmonella enterica

Salmonella enterica is Gram-negative, facultative, rod-shaped motile bacterium. Some *Salmonella* species are pathogenic and can cause salmonellosis, which is a gastrointestinal disease (Madigan et al. 2009). Typical symptoms of nontyphoidal salmonellosis include fever, vomiting, abdominal cramps, and diarrhea. These symptoms occur between 6 and 72 hours after ingestion (Pegues et al. 2005). These symptoms are generally self-limiting and resolve in 2 to 7 days. Septicemia may occur which can lead to meningitis, osteomyelitis, and pneumonia (Cohen et al. 1987). The more severe manifestations of salmonellosis generally occur in the young, the elderly, and the immunocompromised (Benenson 1995). Serious cases typically require fluoroquinolones or ceftriaxone for treatment (Gilbert et al. 2005). Salmonellosis has historically been linked to the consumption of foods such as eggs, milk, seafood, poultry, beef, pork, fresh produce, and nuts and other dried products (Tauxe 1991; Benenson 1995).

Salmonellae are members of the *Enterobacteriaceae* family and seemed to have diverged from *Escherichia coli* between 100 and 150 million years ago (Dougan et al. 2011). They are very adaptable and can survive in animals, both warm and cold-blooded, as well as outside of a host and in the environment (Dougan et al. 2011). There are over 2,500 *Salmonella* serotypes divided into two species, *S. enterica* and *S. bongori*. *S. enterica* contains more than 99% of the serotypes and includes all of the human pathogens (Brenner and McWhorter-Murlin 1998; Brenner et al. 2000). The classification of various serotypes is based on the O antigen, which is present in the

polysaccharide portion of the lipopolysaccharide layer, and the H antigen, which is present in the filamentous portion of the flagella (Voogt et al. 2002).

Virulence Factors & Pathogenesis

Salmonella is an intracellular pathogen that invades epithelial cells of the intestinal mucosa and survives phagocytosis. *Salmonella* have pathogenicity islands known as *Salmonella* Pathogenicity Island 1 through 10 (SPI) (Hensel 2004). SPI-1 and SPI-2 encode for type III secretion systems (T3SS). The T3SS is a needle-like complex and will directly inject bacterial proteins into the host cell (Hansel et al. 1995; Galen and Curtiss 1989). The T3SS produced by SPI-1 is regulated by HilA, which is mediated by many environmental factors such as temperature (Lostroh and Lee 2001). The SopB effector protein is encoded on SPI-5 and it is transported to the host cell via the T3SS produced by SPI-1. SopB is an inositol phosphatase that will trigger fluid secretion which causes the diarrheal symptoms (Hensel 2004). SipA, SipC, and SopB are translocated to the host cell through the T3SS produced by SPI-1 and cause membrane ruffling by interacting with the actin cytoskeleton. This allows the bacterium to become internalized (Lostroh and Lee 2001; McGhie et al. 2001; Jones and Walker 1993; Goosney et al. 1999). Once inside the host cell, the bacterium then forms a *Salmonella*-containing vacuole (SCV) where it will proliferate (Hensel 2004). The T3SS encoded on SPI-2 is expressed while the bacterium is in the SCV. If the T3SS encoded on the SPI-2 is expressed, that normally means there will be an invasive *Salmonella* infection (Hensel 2000). The SPI-2 T3SS is regulated by SsrA-SsrB 2-component system, which is regulated by the OmpR-EnvZ two-component system (Lee et al. 2000; Garmendia et al. 2003).

Rdar Morphology

Some *Salmonella* form extracellular thin aggregative fimbriae, which interact with synthesized cellulose and other polysaccharides to produce the red, dry, and rough (rdar) phenotype on Congo red agar (White and Surette 2006). Rdar morphology can be influenced by environmental elements such as nutrient depravation, temperature, and oxygen tension (Castelijin et al. 2012; Gerstel and Römling 2001; Romlong et al. 2003). The rdar morphotype as well as non-rdar morphotypes such as brown, dry, and rough (bdar) and smooth and white (SAW) can exist in nature (Cevallos-Cevallos et al. 2012). Gu et al. (2011) found that *Salmonella* expressing the rdar morphotype can colonize both on and in tomatoes better than *Salmonella* with non-rdar morphotypes. Generally, strains with rdar morphology will produce better biofilms than strains with non-rdar morphology, though that is not always the case (Malcova et al. 2008). One study found that 73% of clinical *Salmonella* strains and 84% of strains isolated from meats exhibited the rdar morphotype whereas only 56% of produce isolates had the rdar phenotype (Solomon et al. 2005).

Quorum Sensing

There are three main methods of quorum sensing systems in *Salmonella* (Steenackers et al. 2012). These methods include *n*-acyl-homoserine lactone (AHL), autoinducer-2 (AI-2), and autoinducer-3 (AI-3) signaling. *Salmonella* does not produce AHL, but rather detects AHL from other bacteria through SdiA (Ahmer et al. 1998; Michael et al. 2001; Swift et al. 1999). The sensing molecules for AI-2 and AI-3 signaling are heterocyclic furanosyl-borate and catecholamine-like molecules, respectively (Asad and Opal 2008). There has been no direct correlation between SdiA

and *Salmonella* biofilm production, though studies have shown that the presence of AHLs may influence biofilm production (Bouwman et al. 2003; Crago and Koronakis 1999; Heffernan et al. 1992; Nicholson and Low 2000). Many studies have demonstrated that a mutation in the *luxS* gene, which synthesizes AI-2, leads to impaired biofilm production concluding that AI-2 signaling influences biofilm production (De Keersmaecker et al. 2005; Jesudhasan et al. 2010; Prouty et al. 2002). The two component system PreA/B senses AI-3, as well as epinephrine and norepinephrine, and has been found to effect the motility of *Salmonella* thus influencing biofilm production (Steenackers et al. 2012).

Outbreaks

Non-typhoidal *Salmonella* species are a significant cause of bacterial foodborne illness with approximately 11% of foodborne illnesses in the United States and 28% of the deaths associated with foodborne illnesses linked to nontyphoidal *Salmonella* (CDC 2011a). Between 1988 and 2007, *Salmonella* species were responsible for almost 40% of the foodborne outbreaks due to produce contamination (Greig and Ravel 2009). The past two decades have seen an increase in production and consumption of fresh produce and a corresponding increase in the number of illnesses due to outbreaks concerning fresh produce (Olaimat and Holley 2012). Typhimurium, Enteritidis, and Newport are the most common *Salmonella* serotypes in food. *S.* Newport is a multi-drug resistant serovar that is becoming increasingly common over the past 15 years (CDC 2011b). In the CDC's top five foodborne pathogens in 2011, *Salmonella,* nontyphoidal was ranked the second (after norovirus) causing foodborne illness (1,027,561), but it was ranked first among the foodborne pathogens for both hospital visits (19,336) and deaths (378) in the

US (CDC 2011a). Needless to say, *Salmonella* are important foodborne pathogens of concern to the food industry. *S.* Newport has caused foodborne outbreaks in 2014 due to contamination of bean sprouts, nut butter, chia powder, poultry, and raw cashew cheese (CDC 2014). Table 1 demonstrates the diversity in produce foodborne outbreaks due to *Salmonella* species.

Fresh vegetables and fruits can become contaminated with pathogens and/or spoilage organisms during processing, handling, and cultivation (Tournas 2005). Since there is minimal processing once the produce is picked, there is a risk of contamination and deterioration of the produce (Thomas and O'Beirne 2000). Percentages of foodborne disease outbreaks due to fresh produce in the United States have increased from an estimated 2% between 1973 and 1987 (Bean and Griffin 1990) to 13% today (Doyle and Erickson 2007).

Table 1: Selected *Salmonella* outbreaks between 2005 and 2013. Adapted from CDC (2014).

Produce	Cases	Deaths	Year
Alfalfa sprouts	36	0	2016
Alfalfa sprouts	26	0	2016
Cucumbers	907	6	2015
Cucumbers	275	1	2014
Bean sprouts	115	0	2014
Cucumbers	84	0	2013
Mangoes	127	0	2012
Cantaloupe	261	3	2012
Papaya	106	0	2011
Cantaloupe	20	0	2011
Alfalfa and mixed sprouts	140	0	2011
Alfalfa sprouts	44	0	2010
Alfalfa sprouts	235	0	2009
Cantaloupe	51	0	2008
Basil	32	0	2008
Peppers	1442	2	2008
Alfalfa sprouts	45	0	2007
Basil	51	0	2007
Baby spinach	354	0	2007
Cantaloupe	115	0	2006
Tomatoes	183	0	2006
Alfalfa sprouts	125	0	2006
Tomatoes	459	0	2005

There have been several outbreaks concerning *Salmonella* contamination of fresh, whole tomatoes in the United States. Tomatoes were linked to outbreaks of *S.* Thompson

in 2000, *S*. Braenderup in 2004 and 2005, *S*. Newport in 2004, 2005, and 2006, and *S*. Typhimurium and *S.* Berta in 2006, all in the United States (CDC 2006; CDC 2008; Hanning et al. 2009). Sources of contamination of tomatoes can include domesticated animals in the fields, irrigation water, packinghouses, and food preparation setting (Cummings et al. 2001; Greene et al. 2008; Gupta et al. 2007; Hedberg et al. 1999). Please refer to Table 2 for a list of outbreaks and the cause of them. Of the foodborne outbreaks due to produce between 1998 and 2006, 17% of those outbreaks were due to contaminated tomatoes. Additionally, *Salmonella* is the pathogen of concern in regards to tomato contamination (FDA 2009).

Table 2: *Salmonella* outbreaks due to contaminated tomatoes and contamination source

Year	Serovar	Cause	Source
1990	Javiana	Packinghouse	Hedberg et al. 1999
1993	Montevideo	Packinghouse	Hedberg et al. 1999
1999	Baildon	Farm or packinghouse suspected	Cummings et al. 2001
2002	Newport	Irrigation water	Greene et al. 2007
2004	Braenderup	Packinghouse	CDC 2005
2004	Anatum, Javiana, Muenchen, Thompson, and Typhimurium	Field packing and packinghouse	CDC 2005
2005	Newport	Irrigation water	CDC 2007
2006	Typhimurium	Irrigation water	Behravesh et al. 2012

Salmonella survival on Tomatoes

Research has shown that bacterial survival is better in the stem scar of the tomato versus the smooth fruit surface (Wei et al. 1995). A study by Lang et al. (2004) demonstrated that sampling a tomato 24 h after the inoculum dried had about 1.5 log

CFU/ml lower concentration of *Salmonella* than the tomatoes that were sampled 1 h after the inoculum dried. This is in contrast to a study by Rathinasabapathi (2004) that showed no change in *Salmonella* concentration after 48 h on the surface of a tomato pericarp disc. This discrepancy may be due to the type of the tomatoes used since Lang et al. (2004) used ripe red tomatoes whereas Rathinasabapathi (2004) used mature green tomatoes.

A study by Shi et al. (2007) examined the survival of various *Salmonella* serovars on unripe (green) tomatoes as well as ripe (red) tomatoes, all subjected to three vacuum-release cycles at 10^3 Pa to promote internalization after inoculation. These researchers found that most of the *Salmonella* serovars grew on the unripe tomatoes, but survival of *Salmonella* on ripe tomatoes was more serovar dependent. These *Salmonella* serovar differences could also explain why specific strains are more commonly involved in outbreaks. Shi et al. (2007) demonstrated that *Salmonella* can increase in concentration on the surface of vacuum infused ripe and unripe whole tomatoes to different degrees, depending upon *Salmonella* strain, temperature and relative humidity.

Other research has shown that while *Salmonella* can persist on tomatoes, it does not grow on the surface of inoculated tomatoes (Allen et al. 2005; Beuchat and Mann 2008; Das et al. 2006; Lopez-Velasco et al. 2013). Studies have also shown that *S.* Montevideo has a stronger attachment to tomatoes than *S.* Michigan, Poona, Hartford, and Enteriditis (Guo et al. 2001; Guo et al. 2002). A study by Lopez-Velasco sprayed tomatoes with *Salmonella* contaminated pesticides. There were still *Salmonella*-positive tomato samples 15 days after inoculation including some tomatoes washed in 50 mg/L sodium hypochlorite. Non-inoculated tomatoes were washed with inoculated tomatoes and some were found to be positive for *Salmonella* due to cross-contamination. A study

by Beuchat and Mann (2008) further confirms survival and growth of *Salmonella* in the stem scars of various types of tomatoes in various stages of ripeness. With evidence of the survival of *Salmonella* on tomatoes, prevention of *Salmonella* contamination should start at the farm.

Tomato Safety and Processing

In New Jersey, tomatoes are most commonly cultivated in open field production, harvested, packed in a packinghouse, and then enter distribution channels or are sent directly to the retailer to be purchased by the consumer. Figure 1 shows other possible pathways from production to consumption that can occur in other states or regions of the US and around the world. The FDA has issued guidelines that cover the myriad means of production and distribution of tomatoes to ensure consumer safety (FDA 2008).

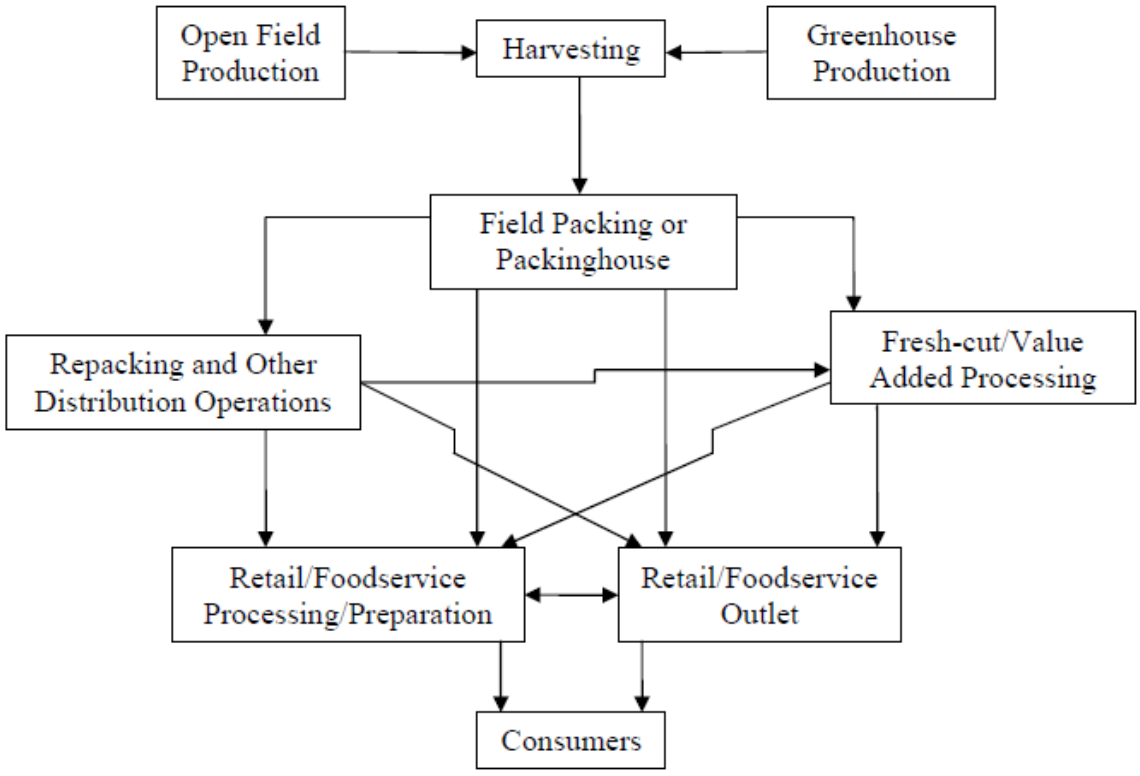


Figure 1. The pathways a tomato may take before it reaches the consumer (FDA 2008)

Open Field Production

There are many aspects in open field production that need to be controlled to manage risk. First, the field itself should be assessed to determine if there are any potential risks. The history of the land usage should be known. For example, if the land was previously used for raising cattle, appropriate steps need to be completed to mitigate risks. If animals are kept on the farm, it is important to ensure that any runoff will not contaminate the tomato field. Any adjacent land use and topography need to be considered as well. Barriers including ditches, fences, or buffer zones may be used to reduce the chance of bacterial contamination. These barriers can help with domestic animals as well as wild animals. Birds are difficult to detour, so if any wildlife activity is detected, proper steps need to be followed to ensure those tomatoes are not harvested. Proper documentation and record keeping are needed to prove adherence to safe practices (FDA 2008).

Production practices

Contamination of water used for tomato crops is documented as a source of contamination in several outbreaks (Greene et al. 2007; CDC 2007; Behravesh et al. 2012). Thus, it is important to ensure the water is microbiologically safe. Any equipment used to store or distribute water should also be well maintained to decrease risks. Irrigation water is not the only type of water that needs to be safe. Any water used in pesticide application needs to be safe as well. Any other chemicals or fertilizers applied to the field or crop can also be a source of contamination. Pathogens, including *Salmonella*, can grow in chemical fertilizers (Lopez-Velasco et al. 2013) so it is imperative to follow the manufacturer's instructions to help mitigate contamination. Of

course any fertilizers containing manure or biosolids needs to have proper documentation that the manure and biosolids were treated properly. Any equipment used in the production of tomatoes must be sanitized and cleaned to minimize the contamination of the crop (FDA 2008).

Another important safety factor is the personnel in the field. This applies to anyone in the field, including visitors or field crewmembers. Toilet facilities with proper hand washing stations need to be readily accessible to employees at all times. Runoff from toilet facilities could cause contamination, thus it is important that all water and waste is contained. Worker health and hygiene are also important. People exhibiting gastrointestinal disease should not have contact with the crop. Additionally, anyone with open wounds cannot touch tomatoes or tomato contact surfaces. Eating and drinking in the field is prohibited. Gloves do not have to be used when the tomatoes are handled, though proper documentation of hand washing procedures is needed. Safety training for employees and visitors is imperative to help lower the risk of contamination (FDA 2008).

Harvest

Many safety aspects that are considered in field production should also to be considered during harvesting. The field should be assessed to ensure no flooding or animal intrusion has occurred. Good hygienic practices should be used during harvest. The equipment and containers used should also be properly cleaned and sanitized. Certain tomatoes should not be harvested. Any tomato touching fecal material or close to areas of animal intrusion should not be harvested. Tomatoes that have evidence of decay or damage should also not be harvested.

If the tomatoes are field-packed, then any sanitation steps must occur in the field. Any sanitation product used needs be capable of producing a 3-log reduction in pathogen concentration. Documentation of proper hygienic practices is needed. Since field-packed tomatoes are generally harvested when fully ripe, it is imperative to remove all damaged tomatoes to prevent cross-contamination. Documentation is also needed to provide traceability. Information to include would be field location, grower, and the crew used for harvest (FDA 2008).

Packinghouse

Tomatoes that are not field-packed are packed in the aptly named packinghouse. As with field production and harvest, the land occupied by the packinghouse needs to be assessed. Packinghouse general maintenance is very important. Food items and non-food items such as sanitizers and pesticides need to be properly separated. Pest control programs are needed to prevent birds, insects, and rodents from contaminating food and food-contact surfaces. Trash and food waste needs to be properly disposed of to ensure that the tomatoes intended for sale do no become contaminated. Records should be kept to ensure that incoming tomatoes come from growers that follow Good Agricultural Practices (GAPs) and that the tomatoes are inspected. Packaging materials (e.g. boxes, containers, etc.) should also be inspected on arrival to assure they are visibly clean and undamaged (FDA 2008).

Water quality is very important for the postharvest washing of fresh tomatoes especially since it is regarded as a control point for pathogens on tomatoes. It is mandated that packinghouses follow Good Manufacturing Practices (GMPs) and that there should be documentation of microbial tests. The water shall be at least 10 °F above

that of the tomato pulp temperature. This is to prevent internalization of bacteria into the stem scar. Internalization can occur when warm tomatoes are put into cooler water. As the tomato shrinks, it can pull water (and bacteria in the water), into the tomato via the stem scar or any damaged skin. If chlorine is utilized as the sanitary treatment, then free chlorine levels and pH must be monitored and recorded at the beginning and every hour. The pH should be between 6.5 and 7.5. If a disinfectant other than chlorine is used, it must be registered with the U.S. EPA.

Control of contamination in the packinghouse includes more than proper sanitizer use. Prior to washing, all damaged/decayed tomatoes should be discarded to help prevent contamination of intact tomatoes. Employees need to be trained on how to safely handle the tomatoes as well as their own hygiene, including proper handwashing. Ripening and/or storage rooms need to be sanitized to lessen the risk of contamination. Transportation vehicles should be inspected prior to transportation to ensure no contamination. As with all other aspects of tomato handling, proper records and traceability need to be kept (FDA 2008).

Retail

Tomatoes may go to a processing center, a redistribution operation or straight to the retail after packing. As with other step in the chain, the operator should inspect the tomatoes as well as verify that the tomatoes came from suppliers with GAP/GMPs. Whole tomatoes should be stored off the floor to prevent contamination and at the proper temperature in accordance with their ripeness stage and variety. Employees should be trained in handling and hygienic practices to ensure no contamination occurs. When displaying whole tomatoes, they should be free from damage and filth (FDA 2008).

Use of Plastic Mulch

When plastic mulch (i.e. plastic sheeting covering the soil) is used, generally the grower also utilizes raised beds, drip irrigation, and fumigation. The raised bed helps provide more uniform soil moisture content. Drip irrigation helps keep moisture under the plastic, where it is available to the growing plant roots and it less subject to evaporation. Fumigation limits growth of weeds and helps prevent insects and disease (Sanders et al. 1995). There are many benefits to using plastic mulch. Research has shown that it can increase crop yield (Downes and Wooley 1966; Jones et al. 1977). It can also allow for production earlier in the growing season since the plastic mulch helps heat the soil (Schales and Sheldrake 1963; Taber 1983). This means the grower can harvest sooner and may have an advantage in the market. The mulch also helps retain the fertilizer in the soil so it is not washed away from rainfall, reducing costs (Sanders et al. 1995). Plastic mulch can also help prevent weed growth as long as black or other light blocking colors are used (Smith 1968). CO_2 can build up under the mulch and may help increase plant growth (Sanders et al. 1995). Plastic mulch also helps prevent evaporation (Lippert and Takatori 1965; Jones et al 1977), which does not mean that the plants need less water (since they are developing faster), but that less water is wasted. The produce cultivated using plastic mulch may be of a higher quality since it does not have contact with the soil. Less rot may occur on the tomatoes grown using plastic mulch (Downes and Wooley 1966). The initial costs of using mulch are greater than not using mulch since specialized equipment is required and disposal costs are incurred when the mulch is removed from the fields. The increased yields and other advantages appear to offset the disadvantages (Sanders et al. 1995).

Research has shown that different color mulches provide different benefits. Black mulch heats the soil and prevents weed growth, while white mulch reflects more light and results in a cooler soil temperature. Red mulch appears to increase yields for some crops (such as tomatoes) compared to black mulch (UMass Extension 2012), and different mulch colors can impact a variety of different growth characteristics of tomato plants (Decoteau et al. 1988). Clear mulch is better at heating the soil as compared to black mulch, but it does not control weeds. Colors such as brown, green, blue, and silver, can be used to increase yield of specific crops (UMass Extension 2012).

The best practice to prevent foodborne outbreaks would be to prevent contamination of the tomatoes in the first place. In an effort to achieve this, FDA has recommended that tomatoes not be harvested if they fall on the ground prior to harvest (FDA 2008), since soil may be a source of pathogens. It is common practice amongst tomato growers to surround growing plants with plastic sheeting (i.e. mulch). It is also common practice to interpret that a tomato which has fallen onto plastic sheeting has not technically touched the ground, so that it can still be harvested in compliance with FDA guidance.

No studies have been conducted concerning pathogen transfer from plastic mulch to whole tomatoes. A study by Soares et al. (2012) examined the transfer of *S*. Entertidis from a plastic cutting board to tomatoes. The starting concentration of *S.* Entertidis on the plastic cutting board was 2.7 log CFU/cm^2 and the ending concentration on the diced tomatoes was 2.7 log CFU/g. Taking into account the total surface area of the cutting board (100 cm^2) and weight of the tomato sample (25 g), roughly 25% of the *Salmonella* transferred from the cutting board to the tomato. Prior studies have suggested that the

presence of organic matter on plastic surfaces can reduce bacterial transfer (Flores et al. 2006; Rusin et al. 2002; Brar and Danyluk 2013), thus "used" plastic mulch containing organic matter would be expected to transfer fewer bacteria to tomatoes compared to new debris-free plastic mulch.

Bacterial Internalization

There have been several studies concerning internalization of *Salmonella* in tomato plants due to exposure in the field. The results are mixed with some studies finding evidence of internalization in the tomato fruit while others showing no evidence of internalization (Guo et al. 2002; Beuchat et al. 2003; Miles et al. 2009; Hintz et al. 2010). These studies all utilized different serovars and possible routes of contamination. Guo et al. (2001) and Shi et al. (2007) demonstrated that if the flowers are contaminated with *Salmonella,* the organism might persist in the fruit. Studies have also shown that *Salmonella* may be aerosolized by rain and can contaminate tomato fruits (Cevallos-Cevallos et al. 2012). Miles et al. (2009) studied the effects of contaminated irrigation water and contaminated seeds on the internalization of *S.* Montevideo in tomatoes. They found that neither condition resulted in internalization of *Salmonella* in the fruit pulp or in the stem scar. Beuchat et al. (2003) utilized a plant-parasitic nematode to create wounds in the tomato plant root to see if internalization would occur in the presence of *Salmonella* contaminated soil. After 4 weeks, 0 of 18 leaf and stem samples were positive for *Salmonella* when the nematode and *Salmonella* were present in the soil. The same results were seen when tomato plants were grown in contaminated soil without the nematode. They concluded it would be unlikely for the fruit to become contaminated. In Hintz et al. (2010), tomato plants were irrigated with contaminated water, which resulted

in 1 out of 4 tomatoes samples testing positive after enrichment for *S.* Newport. The authors concluded it was unlikely that internalization would occur in field conditions since the concentration of *S*. Newport used in the study (7 log CFU/mL) is much higher than would be present in irrigation water (Hintz et al. 2010).

Relative Humidity Studies

Tomatoes are grown across the United States in climates with varying relative humidity. Best practice guidelines suggest that after harvest that tomatoes be stored for 4-7 days between 8-10°C with a relative humidity (RH) between 90-95% (Hardenburg et al. 1986). RH has already been shown to affect the survival of *Salmonella* on tomato plants and unripe tomatoes (Rathinasabapathi 2004; Shi et al. 2007). Tian et al. (2013) demonstrated that *Salmonella* survival on apples was affected by RH, temperature, as well as the surface condition of the fruit (intact vs. bruised). Margas et al. 2014 demonstrated that different *Salmonella* serovars exhibit different reduction kinetics on stainless steel discs at the same RH (33%) and temperature (25°C). A study by Blessington et al. (2014) looked at the survival of *Salmonella* on walnut hull pieces. *S.* Enteritidis was able to survive and grow on walnut hull pieces stored in RH conditions between 38-90% for 14 days. After 1 day, *Salmonella* was not detected on walnut hull pieces stored in an RH environment between 20-43%.

Packinghouse Treatments

Washing can improve produce shelf-life and food safety by removing microorganisms, pesticides residue, as well as cell exudates that could aid in the survival of microbes (Zagory 1999; Gil 2009). When tomatoes pass through a packinghouse, they are typically washed in a sanitizing solution such as hypochlorite or peroxyacetic acid

(FDA 2008). Chlorine is a common treatment, though New Jersey packinghouses also utilize sanitizers containing a mixture of peroxyacetic acid and hydrogen peroxide.

SaniDate 5.0 (BioSafe Systems LLC, East Hartford, CT) can be used to treat the spray or dumptank waters that are used to wash produce. It can also help control bacterial and fungal species that may cause spoilage, however it is not intended to control human pathogens such as *Salmonella* spp. The active ingredients in SaniDate 5.0 are hydrogen peroxide (23.0%) and peroxyacetic acid (5.3%). To treat with SaniDate 5.0, it needs to be added to water at the ratio of 59 to 2010 fl. oz. to 1000 gallons of water. This results in a 462 to 1636 ppm of SaniDate 5.0. The produce should have a minimum contact of 45 seconds with the SaniDate 5.0 solution. In addition to treating wash waters, SaniDate 5.0 could also be utilized to clean surfaces in the packinghouse.

StorOx 2.0 (BioSafe Systems LLC, East Hartford, CT) is different mixture of hydrogen peroxide (27.0%) and peroxyacetic acid (2.0%). StorOx 2.0 can be used to control bacterial and fungal diseases. The label states that it is effective against *S. enterica.* To prepare the solution for use in a dump tank, the sanitizer should be mixed with water at a rate of 1:10000. This results in 1200 to 4355 ppm of StorOx 2.0, which gives the resulting water between 24 to 85 ppm of 100% peroxyacetic acid. For use in a spray bar system, the dilution rate is 1:100 to 1:1000. Like SaniDate 5.0, StorOx 2.0 can also be used to sanitize surfaces in the packinghouse

Peroxyacetic acid and chlorine have been used to reduce the natural microflora on mung bean sprouts in a laboratory setting (Neo et al. 2013). At the highest concentration (70 ppm), peroxyacetic acid only produced a 0.5 log CFU/g reduction when the mung bean sprouts were agitated for 90 sec, though when the dump time was doubled, there

was more than a log CFU/g reduction. A 170 ppm Cl solution produced similar results with a 0.5 log CFU/g reduction after 90 sec and almost a log CFU/g reduction after 180 sec. When tested against *E. coli* O157:H7, 70 ppm of peroxyacetic acid as well as 170 ppm of chlorine yielded a 2 log CFU/g reduction for both times. The 70 ppm of peroxyacetic had similar results against *Salmonella* spp. with a 2 log reduction for 180 sec and just under a 2 log reduction for 90 sec. The 170 ppm solution of chlorine only had a reduction of about 1.5 log CFU/g for *S*. spp. Studies have shown that chlorine and peroxyacetic acid will reduce the level of *Salmonella* on tomatoes (Zhuang et al. 1995; Beuchat et al. 1998; Beuchat and Ryu 1997; Bari et al. 2003; Gurtler et al. 2012), though any EPA-registered chemical is appropriate as long as it has demonstrated a 3 log reduction of *Salmonella* (FDA 2008).

A study by Barrera et al. (2012) looked at three packinghouses that processed spinach and shredded lettuce. They found that high organic load as well as the microbe concentration in the water severely limited the efficacy of the washing. The authors suggest that preventing contamination at the farm level is most important for microbial safety of produce, though a better post-harvest washing method needs to be developed due to the problems with preventing contamination at the farm.

Produce Temperature Increase from Retail to Consumer Home

A literature search reveals very little information about the temperature changes that tomatoes experience during transportation from the retail market to the consumer home. If the tomato were to experience temperature abuse, it may lead to pathogen growth and thus increase the risk for foodborne illness. Figures 2 and 3 demonstrate the temperature increase of prepackaged lunchmeat and ground beef during transport to the

consumer home (Ecosure 2008). Even 30 min outside of temperature control can lead to measurable temperature increase. It is important to see if this trend occurs in fresh produce and determine if it poses a risk for pathogen growth.

Figure 2: The average temperature increase in °C from the time prepackaged lunchmeat is removed from the retail cooler to the time it is placed in the consumer's refrigerator

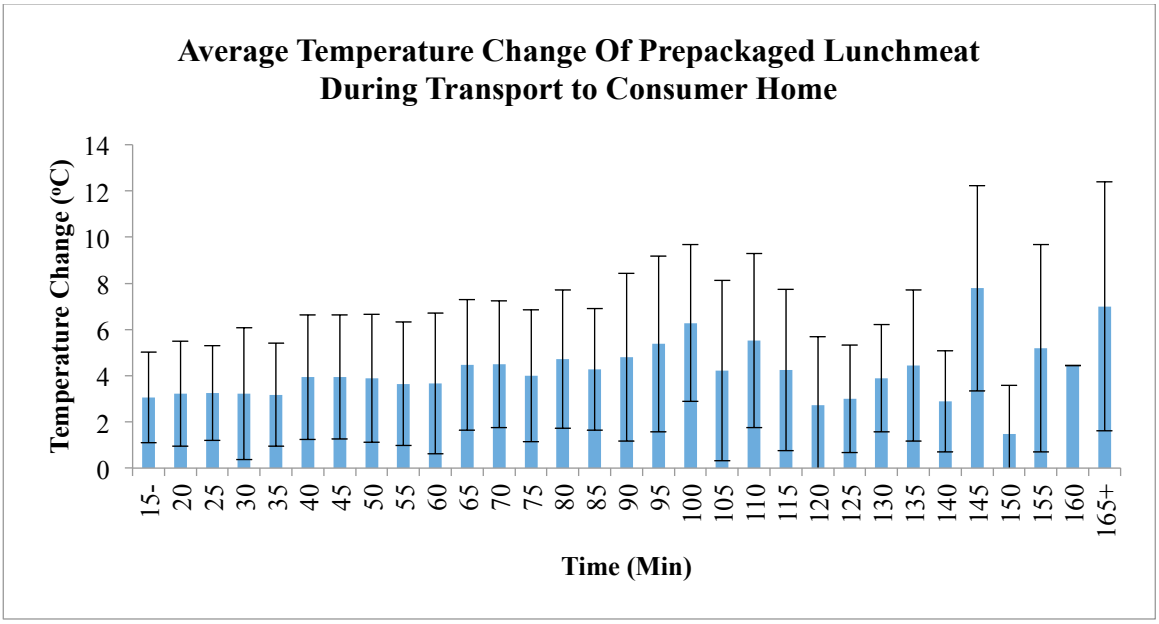
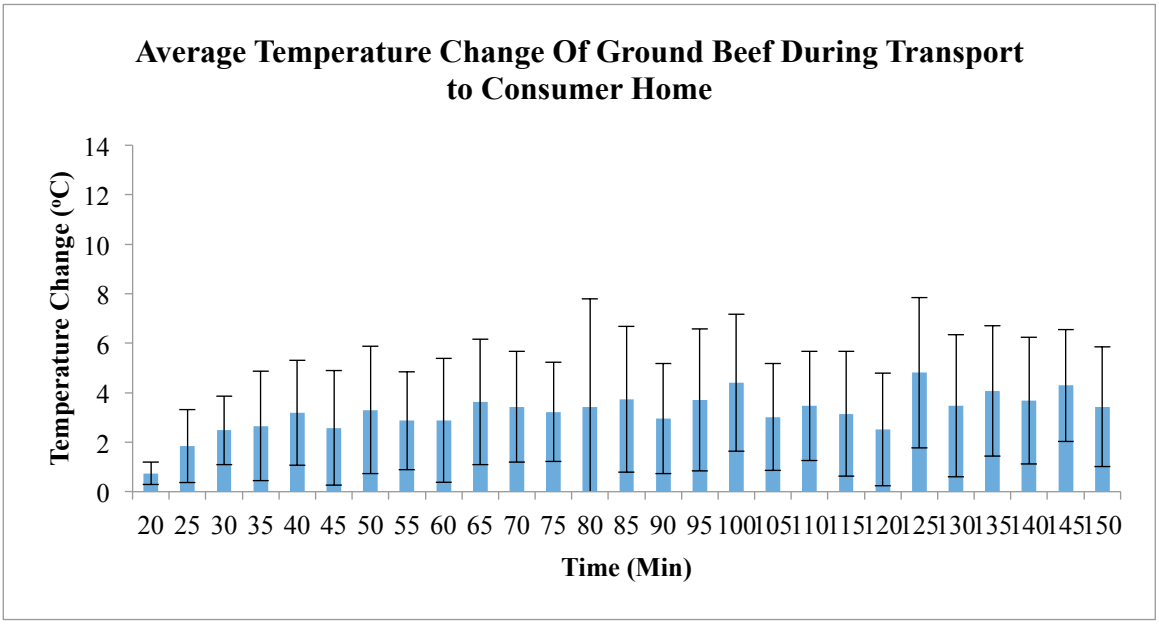


Figure 3: The average temperature increase in °C from the time ground beef is removed from the retail cooler to the time it is placed in the consumer's refrigerator



Quantitative microbial risk assessment

Microbiological risk analysis is comprised of risk assessment, risk management, and risk communication. There are two types of risk assessments: quantitative and qualitative. A qualitative risk assessment uses descriptive language, such as high, medium, or low, to assess risk whereas a quantitative risk assessment uses numerical data to provide a numerical risk estimate (FAO 1999; FAO 2006). There are two types of quantitative risk assessments: deterministic and stochastic. Point values (e.g. means or averages) are used in deterministic models. Stochastic models incorporate variability and uncertainty and express results as the probabilities of given events occurring. Stochastic models are more complex than deterministic models, but better express our knowledge of what may actually occur (FAO 2006).

Quantitative microbial risk assessment is composed of four parts: hazard identification, exposure assessment, hazard characterization, and risk characterization (FAO 1999).

Hazard identification

The agent, whether it be biological, chemical, or physical, is identified of causing harm when people consume a certain food or food type (FAO 1999).

Exposure assessment

The exposure assessment determines the likelihood of a person consuming food that has been contaminated with the biological, chemical, or physical hazard (FAO 1999). Any changes in the level of the hazard, whether it be growth, death, or cross-contamination, needs to be accounted for in this section since the concentration could be very different at time of consumption (FAO 2006). When modeling pathogen change in concentration, it is important to take into consideration environmental changes such as temperature, water activity, relative humidity, pH, etc. (USDA 2012). Microbial hazards are usually characterized as a single exposure (FAO 2006). Variability may need to be characterized in parts such as concentration of hazard initially in the food, environmental changes that in turn affect the concentration of the hazard in the food, the dose of the hazard in a serving of food, and the amount of serving a person may consume (USDA 2012).

Hazard characterization

The hazard characterization typically contains a dose response model if the data is available. This aspect determines the probability of an adverse health effect based on the level of hazard ingestion (FAO 1999). To establish this, epidemiological data from

outbreaks, animal toxicity studies, and clinical human exposure studies can be utilized. For microbial hazards, various types of adverse health effects could be considered, such as infection, morbidity, hospitalization, and death rates, as well as the effect of different doses (FAO 2006). It is important to understand that a hazard may not be distributed uniformly in a matrix, so there different individuals in a population may ingest different doses. Additionally, while a given dose may infect one person, another person may have a higher tolerance and not become infected by that same dose. Some groups that have a lower tolerance include the elderly, young children, individuals with compromised immune systems, and pregnant women (USDA 2012).

Risk characterization

The last part of risk assessment, risk characterization, incorporates hazard identification, hazard characterization, and exposure assessment to determine the probability of illnesses and/or severity of illness in a given population (FAO 1999). Risk characterization can have two components: risk estimation and risk description. Risk estimation gives the actual quantitative risk estimate, while the risk description identifies the variability, uncertainty, and confidence in the risk estimate (USDA 2012). The risk model may be validated with epidemiological data not already used in the risk assessment (FAO 1999).

Risk Management and Risk Communication

Risk management uses the results from risk assessment to determine what control measures need to be taken, including policy or regulatory changes. Risk communication deals with the exchange of information regarding the results of the risk assessment and risk management to actually incorporate the measures to control the risk (FAO 1999).

Chapter II: Quantification of *Salmonella* transfer between tomatoes and tomato bedding

Jennifer Todd-Searle[1], Loretta M. Friedrich[2], Ruth A. Oni[3], Kenneth Shenge[4], Jeffrey T. LeJeune[5], Shirley Micallef[3], Michelle D. Danyluk[2], and Donald W. Schaffner[1*]

[1]Rutgers, the State University of New Jersey, New Brunswick, New Jersey

[2] University of Florida, IFAS, Lake Alfred, Florida

[3] University of Maryland, College Park, Maryland

[4] USDA-ARS, Tucson, Arizona

[5] The Ohio State University, ODARC, Wooster, Ohio

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* Corresponding author

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Abstract

Tomatoes have been linked to *Salmonella* outbreaks in the United States. It is common practice amongst tomato growers to surround growing plants with plastic sheeting (commonly called mulch). US FDA recommends that tomatoes touching the ground not be harvested, but growers may still choose to harvest produce that touches soil or plastic mulch. This research was undertaken to better characterize the risks posed by harvesting tomatoes that touch plastic mulch or soil. Research was conducted in three states (Florida, Maryland and Ohio). Each state utilized tomatoes from their state at the point of harvest maturity most common in that state. Each state used indigenous soil and plastic mulch for transfer scenarios. New plastic mulch obtained directly from the application roll, and used plastic mulch that had been present on beds for a growing season were used. A five strain cocktail of *Salmonella enterica* isolates obtained from tomato outbreaks were used. Mulch, soil or tomatoes were spot inoculated with 100 µl inoculum to obtain a final population of ca. 6 log CFU/surface. Items were either touched immediately (1-2s) allowed to dry at ambient temperature for 1 or 24 hours before contact. The all surfaces were left in brief contact (1-5 s) or for 24 h at ambient temperature. Transfer of *Salmonella* between a tomato and plastic mulch or soil is dependent on factors such as contact time, dryness of the inoculum, type of soil, as well as contact surface. Transfer of *Salmonella* to and from the mulches and tomatoes for wet and 1 h dry inocula were similar with mean % transfers varying from 0.71±0.19-1.85±0.12. The transfer of *Salmonella* between soil or plastic mulch to and from tomatoes was dependent on moisture with wet and 1 h dry inoculum generally producing a higher transfer than the 24 h dry inoculum. Results indicate that harvesting dry

tomatoes reduces the risk of contamination. Transfer was generally greater from new and used plastic mulch to tomatoes than from soil to tomatoes. Since *Salmonella* more readily transfers from mulch to tomatoes (vs. from soil to tomatoes), harvesting produce that has touched mulch is riskier than harvesting produce that has touched soil, if contamination levels on the soil or mulch are equivalent.

Introduction

Non-typhoidal *Salmonella* species are a significant cause of bacterial foodborne illness and *Salmonella* species were the cause of approximately 11% of foodborne illnesses in the United States (CDC 2011a). In the same year, *Salmonella* species were also associated with 35% of hospitalizations and 28% of deaths associated with foodborne illnesses (CDC 2011a). Tomatoes were linked to outbreaks of *Salmonella* Thompson in 2000, *S*. Braenderup in 2004 and 2005, *S*. Newport in 2004, 2005, and 2006, and *S*. Typhimurium and *S.* Berta in 2006, all in the United States (CDC 2006; CDC 2008; Hanning 2009). Sources of contamination of tomatoes may include domesticated animals in the fields, agricultural water, packinghouses, and food preparation setting (Cummings 2001; Greene 2008; Gupta 2007; Hedberg 1999).

The best practice to prevent tomato-borne outbreaks would be to prevent contamination of the tomatoes in the first place. In an effort to achieve this, FDA has recommended that tomatoes not be harvested if they fall on the ground prior to harvest (FDA 2008). It is common practice amongst tomato growers to surround growing plants with plastic sheeting (henceforth referred to as mulch). Since the FDA recommendation is not a requirement, growers may choose to harvest tomatoes that have grown in contact with plastic mulch or soil. Growers may also choose to harvest tomatoes that have fallen and touched the ground or mulch.

Salmonella has been found in the soil and water in agricultural regions (Bell et al. 2015; Gorski et al. 2011; Micallef et al. 2012; Strawn et al. 2013). *Salmonella* has been found in tomato growing environments in the Delmarva region, an area that has produced tomatoes linked to some outbreaks (Bell et al. 2015; Greene et al. 2008). *Salmonella* has

been isolated from seagulls in the Delmarva region (Gruszynski et al. 2014) which could deposit feces on plastic mulch or soil. Tomatoes from Florida and Ohio have also been the cause of *Salmonella* outbreaks (CDC 2007). Visual inspection of used mulch has commonly noted the presence of soil, suggesting that any soil contaminates may be transferred to the plastic mulch. While staking tomato plants is a common practice, this is not required, so tomatoes may grow in contact with plastic mulch or soil. There is also evidence that the rhizoplane and phyllosphere of tomato seedlings can become contaminated with *Salmonella* when grown in contaminated soil (Barak and Liang 2008). Rain may also cause splash dispersal of *Salmonella* from contaminated soil to tomato fruits (Cevallos-Cevallos et al. 2012).

Tomatoes, groundwater, and soil differ microbiologically even between adjacent states (Delaware, Maryland, and New Jersey) on the east coast (Pagadala et al. 2015). Tomatoes touching the ground or mulch had higher total coliform counts than tomatoes on the vine in all three states with Maryland tomatoes having the highest coliform counts and New Jersey having the lowest (Pagadala et al. 2015). Schneider et al. (2017) measured levels of aerobic bacteria, coliforms, and generic *E. coli* on tomatoes before and after packinghouse treatment in Florida, Maryland, and New Jersey. The concentration of aerobic bacteria and coliforms varied significantly between pre-processed tomatoes between all states and in almost all cases for post-processed tomatoes. Strawn et al. (2013) found that drainage class, available water storage, and precipitation were important predictors for *Salmonella* prevalence at 5 NY farms.

Farm size also differs between state and region with small to medium sized farms in the mid-Atlantic region, and larger farms in Florida (Pagadala et al. 2015). Smaller

farms may be at a food safety disadvantage due to costs associated with pathogen testing as compared to larger farms because of economies of scale. This could lead to a higher likelihood of contamination at smaller farms (Parker et al. 2012).

The research presented here was undertaken to improve our understanding of the risk factors associated with cross-contamination between plastic mulch and soil to tomatoes. This study quantified the risk of *Salmonella* transfer from soil and plastic mulches to tomatoes, as well as in the opposite direction under varying contact times and inoculation conditions.

Materials and Methods

Tomatoes

Tomatoes were obtained in each of three states (Florida, Maryland and Ohio, US), at the point of harvest maturity in that state. In Florida, mature green, medium 6/7, tomatoes were obtained from a local grower (Palmetto, FL). In Maryland, ripe red, medium/large tomatoes were procured from two local growers located approximately 17 miles apart in Central Maryland, depending on availability. In Ohio, ripe (pink to light red) tomatoes that were 5.72-7.0 cm in diameter were harvested from the OSU/OARDC Research Farm in Wooster. Fruits in all states were stored at 4˚C prior to use and left overnight at ambient temperature (18-23°C) prior to inoculation.

Soil and plastic mulch

Soil and plastic mulch were obtained from each state, and used for transfer scenarios with tomatoes from those states. Two different conditions of plastic mulch were evaluated; new plastic mulch obtained directly from the application roll and used plastic mulch that had been present on beds for a growing season. In Florida, soil and

plastic were obtained from the University of Florida, Gulf Coast Research and Education Center (Wimauma, FL). In Maryland, plastic mulch was obtained from the University of Maryland's Central Maryland Research and Education Center, Upper Marlboro Facility (Upper Marlboro, MD). In Ohio, bedding plastic mulch and soil were obtained from the Ohio Agricultural Research and Development Center Research Farm in Wooster. All soil was stored at 4 °C prior to use and left overnight at ambient temperature (18-23°C) prior to inoculation. All plastic mulch was cut into 5 cm x 5 cm squares and 10 g of soil was placed into hexagonal polystyrene, 38/25mm weigh boats (ThermoFisher Scientific, Pittsburgh, PA) prior to inoculation. Soil samples were sent to Waters Agricultural Laboratories, Inc. in Camilla, Georgia for analysis.

Selection of Strains

A five strain cocktail of *Salmonella enterica* isolates obtained from tomato outbreaks were used. Their sources and designations are: Montevideo (LJH0519; clinical isolate), Anatum (K2669; clinical isolate), Javiana (ATCC BAA 1593; clinical isolate), Branderup (04E61556-2-99; clinical isolate), Newport (MDD 314; environmental isolate). All strains had previously been adapted to grow in the presence of 80 mg/ml rifampin (ThermoScientific, Waltham, MA) following Parnell et al. (2005).

Inoculum preparation

Prior to each experiment, frozen cultures of each strain were streaked onto tryptic soy agar (TSA; Difco, BD, Sparks, MD) with 80 mg/ml rifampin (TSAR), and incubated at 37°C for 24 h. One isolated colony from each strain was transferred to 10 ml of tryptic soy broth (TSB; Difco, BD, Sparks, MD) with 80 mg/ml rifampin (TSBR), and incubated at 37°C for 24 h. Cultures were subsequently subcultured twice by transferring 0.1 ml of

culture to 10 ml of fresh TSBR and incubated at 37°C for 24 h. Each strain was subjected to centrifugation at 3,000 × g for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). Cells were washed twice by removing the supernatant and suspending the cell pellet in 10 ml of 0.1% peptone (Difco, BD). Washed cells were suspended in 0.1% peptone at half the original culture volume. Strains were diluted and combined in equal volumes to achieve a concentration of ca. 10^7 CFU/ml. Final concentrations were verified for each strain by enumeration on TSAR.

Transfer between Tomatoes and Mulch or Soil

Mulch or soil was spot inoculated with 100 µl of inoculum to obtain a final population of ca. 6 log CFU/surface. The inoculated mulch or soil was either touched immediately (1-2s) to tomatoes (wet) or allowed to dry at ambient temperature for 1 or 24 hours before contact. The tomatoes and surfaces (mulch or soil) were left in contact very briefly (1-5 s) or for 24 h at ambient temperature. Control, inoculated (wet, 1h, or 24h dry) mulch or soil received no tomato contact but received the same drying and incubation times. The reverse transfer direction was measured by spot inoculating the tomato surface with 100 µl of inoculum and allowing contact with the mulch or soil, under the same conditions. Each transfer scenario was replicated 10 times.

Enumeration of cells

Tomato, mulch or soil were placed in a sterile 207 ml Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, USA) and 25 ml of 0.1% peptone, (Difco, BD, Sparks, MD) was added. Tomatoes were shaken for 30 seconds, massaged for 30 seconds, and shaken for 30 seconds. Mulch and soil were macerated in a smasher (AES Laboratories, Chemunex, France) for 90 seconds. Samples were serially diluted in 0.1 % peptone and

surface plated (0.1 ml) onto TSAR. Plates were incubated at 37°C for 24 h. Colonies were counted by hand and *Salmonella* population levels were expressed in CFU/surface following incubation. One ml of the lowest dilution was spread over four plates (0.25 ml per plate) to increase the limit of detection to 25 CFU/item (1.4 log CFU/item).

Enrichment

When counts fell below the limit of detection (25 CFU/item), enrichments were conducted by following protocols from the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA BAM, 2007). Twenty-five milliliters double strength lactose broth (Difco, BD) was added to stomached or massaged samples (tomato, mulch, or soil and 25 ml of 0.1% peptone) as the pre-enrichment step and incubated at 37˚C for 24 h. One hundred microliters and 1 ml of the broth were transferred to 10 ml of Rappaport-Vassiliadis R10 (RV, Difco, BD) and tetrathionate (TT, Difco, BD) broths, respectively. Test tubes were incubated for 48 h at 42 °C for RV broth and 24 h at 37 °C for TT broth. Ten-microliter loopfuls were streaked onto three selective agars supplemented with rifampin (80 µg/ml): Bismuth sulfite agar (BSA; Difco, BD), xylose lysine deoxycholate agar (XLD; Difco, BD), and Hektoen enteric agar (HE; Difco, BD) and incubated at 37 °C for 24 h. Plates were examined for the presence of colonies with typical *Salmonella* morphology.

Data Analysis

Microsoft Excel (Microsoft, Redmond, WA) was used to compile and analyze data and to create histograms. Percent transfer data was log transformed to normalize the data (Chen 2001; Schaffner 2003). The frequency of a certain transfer rate occurring within a specific range, or bin, was plotted against the log percent transfer rates. The

ranges were in increments of 0.25 log percent transfer, as previous research that has shown ranges of 0.25 to 0.50 to be satisfactory (Chen 2001; Jensen 2013; Montville 2001; Schaffner and Schaffner 2007).

The following equation was used when calculating transfer rate to tomato from soil, used plastic mulch, or new plastic mulch:

$$\text{Percent Transfer (\%)} = \frac{CFU_{tomato}}{CFU_{total}} \text{ x } 100\ \%$$

The following equation was used when calculating transfer rate to soil, used plastic mulch, or new plastic mulch from tomato:

$$\text{Percent Transfer (\%)} = \frac{CFU_{soil}}{CFU_{total}} \text{ x } 100\ \%$$

CFU_{total} is defined as followed:

$$CFU_{total} = CFU_{tomato} + CFU_{bedding}$$

The mean, standard deviation, median, minimum, maximum, and range for the transfer of *Salmonella* to and from tomato and plastic mulch or soil were calculated using Microsoft Excel. Significant differences between scenarios within a state for a specific transfer as well as between states for a certain inoculum conditions and transfer between states were determined using a one way ANOVA with StatTools 7 (Palisade Corporation).

Results

Statistical analysis

Figures 1 and 2 show log percent transfer histograms for bedding to tomato, and tomato to bedding, respectively. Tables 1 and 2 shows the mean, standard deviation, median, minimum, maximum, and range of the transfer of *Salmonella* from mulch or soil to tomato, and tomato to mulch or soil, respectively. These will be discussed in detail below.

Soil Analysis

The results of the soil analysis are shown in Table 3. The soil from Maryland was deficient in 4 nutrients (phosphorus, potassium, zinc, and manganese), and was high in magnesium and very high in calcium. The Florida soil had an abundance of phosphorus, calcium, zinc, and manganese, but was below suggested levels for potassium and manganese. The soil from Ohio was rich in magnesium, calcium, manganese, and zinc, but slightly lacking potassium and phosphorus. Florida and Maryland soil had pH values close to neutral (7.3 and 6.5, respectively). Ohio soil had a slightly acidic pH value (5.4). Ohio and Maryland soils were primarily composed of loam while Florida soil was primarily composed of sand.

Salmonella *transfer - Florida mulch, soil, and tomatoes*

Figure 1 and 2, panel a shows the log percent transfer data for Florida tomatoes and new mulch. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. In all cases when new mulch was allowed to dry for 24 h after inoculation, *Salmonella* concentration on tomatoes contacting that mulch were below the detection limit (1.4 log CFU/tomato). A 24 h contact time resulted in significantly less transfer from new mulch to the tomato for the wet inocula as compared

to a brief touch contact ($p<0.05$). Conversely, a 24 h contact time had a significantly higher transfer rate from tomatoes to new mulch for a wet inocula and a 24 h dry inocula as compared to a brief touch contact ($p<0.05$)(Table 2). The log percent transfer rates for the wet and 1 h dried inoculum were between 1.5 and 2 for tomato to new mulch (Figure 2.a-c).

Figure 1 and 2, panel b show the log percent transfer data for Florida tomatoes and used mulch. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. Inoculated used mulch allowed to dry for 24 h and then contacted tomatoes for 24 h resulted in a wide range of transfer with 3 samples negative for *Salmonella* on enrichment with the highest transfer at 1.85 log % while the touch contact only was positive for *Salmonella* via enrichments. The log percent transfer rates for the wet and 1 h dried inoculum did not vary significantly for either transfer from the mulch (1.21±0.20-1.47±0.25 log % transfer; Table 1) or transfer from the tomato (1.04±0.91-1.69±0.09 log % transfer; Table 2)($p<0.05$).

Figure 1 and 2, panel c show the log percent transfer data for Florida tomatoes and soil. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. The mean log percent transfer from Florida soil for wet and 1 h dried inoculums were significantly higher after a 24 h contact as compared to the touch contact ($p<0.05$)(0.13±0.46-0.28±0.06 log % vs -1.74±0.62- -1.38±0.42 log %, respectively; Table 1). When Florida soil was inoculated and allowed to dry for 24 h, all tomatoes contacting that soil had *Salmonella* concentrations below the detection limit (1.4 log CFU/tomato), though all samples were positive for *Salmonella* after enrichment (Table 1). The percent transfer of *Salmonella* from Florida tomatoes to Florida soil for

both the wet and 1 h dried inoculum was significantly higher than the corresponding percent transfer in the other direction ($p<0.05$)(Figures 1.c and 2.c). When a tomato was inoculated and allowed to dry for 24 h and the tomato was briefly touched to Florida soil, the *Salmonella* concentration was below the detection limit (1.4 log CFU/gm soil) but the soil was always positive for *Salmonella* after enrichment for a touch contact and negative for a 24 h contact (Table 2). Generally we observed that when quantifying *Salmonella* transfer from Florida tomatoes to Florida soil, a brief touch contact time led to more pathogen transfer than when the contact time lasted 24 h.

The transfer of *Salmonella* from new mulch (Figure 1.a) and used mulch (Figure 1.b) to Florida tomatoes was similar. When inoculum was wet or had dried for 1 h, mean log % transfers ranged from 0.98±0.26 to 1.52±0.21, with less transfer occurring for a 24 h dry inoculum. The transfer from Florida soil to tomatoes (Figure 1.c) was lower than from new or used mulch to tomatoes. The transfer of *Salmonella* from Florida tomatoes to new and used mulches were similar to the transfer from plastic mulches to tomato (1.04±0.91-1.85±0.12; Table 2). Florida tomatoes that were inoculated, dried for 24 h, and had a 24 h contact time transferred more *Salmonella* to new plastic mulch than to used plastic mulch (Figure 2.a-b; Table 2), though some mulch samples were below the detection limit (1.4 log CFU/ 5 cm by 5 cm piece of mulch). When this occurred, Florida new and used mulch sample were always positive for *Salmonella* on enrichment (Table 2).

Salmonella *transfer - Maryland mulch, soil, and tomatoes*

Figures 1 and 2, panels d summarize *Salmonella* transfer using Maryland tomatoes and new mulch. Figure 1 shows transfer from mulch to tomato, while Figure 2

shows transfer from tomato to mulch. Both new mulch and tomatoes with wet inocula or 1 h dry inocula had similar transfer to their recipient surface ($p<0.05$; Tables 1 and 2). Overall, mean log % transferred varied from 1.43±0.24 to 1.80±0.15 for both direction of transfer in those scenarios (Tables 1 and 2). While new mulch that was inoculated and allowed to dry for 24 h did not transfer detectable *Salmonella* to any tomatoes, tomatoes inoculated in the same way lead to transfer in 17 out of 20 trials.

Figures 1 and 2, panels e summarize *Salmonella* transfer using Maryland tomatoes and used mulch. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. For the fresh and 1 h dried inocula, mean % transfer was not significantly different from used mulch to tomatoes (0.96±0.64-1.66±0.23; $p<0.05$) or from tomatoes to used mulch (1.27±0.56-1.57±0.30; $p<0.05$). While *Salmonella* was detectable when transferred from used mulch to Maryland tomatoes (Table 1), *Salmonella* was not recovered when it was transferred from tomato to the used mulch for the 24 h dried inoculum (Table 2).

Figures 1 and 2, panels f summarize *Salmonella* transfer using Maryland tomatoes and soil. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. Transfer of wet inoculum from soil to tomatoes had a range of 0.72 to 1.78 log % transfer for a touch contact. The range of transfer for 24 h contact ranged from one sample that was negative on enrichment to 1.52 log % transfer. When Maryland soil was inoculated with *Salmonella* and allowed to dry for 24 h and then had a touch contact with tomatoes, there was no detectable *Salmonella* even after enrichment (Table 1). Maryland soil inoculated with *Salmonella* for 1 and 24 h and in contact with tomatoes for 24 h had only 2 and 3 out of 10 positive enrichments respectively, while the

rest were negative for *Salmonella* (Table 1). The transfer of *Salmonella* from tomato to soil (Figure 2.f) was greater than from Maryland soil to tomato (Figure 1.f) with all the conditions having a large range of log % transfer. The fresh, wet inoculum on tomato with a brief touch to Maryland soil had the highest frequency at 2 log % transfer (Figure 2.f).

The log % transfer for new and used Maryland mulches or tomatoes with wet or 1 h dry inocula were similar with the highest frequencies generally occurring between 1.5 and 2 log % transfer (Figures 1.d-e; Figures 2.d-e). There was no recovery of *Salmonella* from tomatoes that contacted new Maryland mulch inoculated and dried for 24 h, but there was recovery (10/10 for the touch contact and 7/10 for the 24 h contact) from Maryland tomatoes that contacted used mulch that was inoculated and dried for 24 h (Table 1). Transfer of *Salmonella* from new and used plastic mulch to tomatoes was typically higher than the transfer of *Salmonella* from soil to tomatoes. The results of the transfer of *Salmonella* from Maryland tomatoes to used mulch were very similar to that of used mulch to tomatoes for the wet and 1 h dry inocula.

Salmonella *transfer – Ohio mulch, soil, and tomatoes*

Figures 1 and 2, panels g summarize the log % transfer data using new Ohio mulch and tomatoes. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. When new mulch had a wet inoculum, there was a higher mean percent transfer of *Salmonella* to tomatoes. There was no significant difference in transfer from inocula dried for 1 h or 24 h and for either contact time (Table 1). The log percent transfer of *Salmonella* from Ohio tomatoes to new Ohio mulch was

similar to transfer in the reverse direction with a higher mean percent transfer occurring with a wet inoculum (Table 2).

Figures 1 and 2, panels h summarize the log % transfer data using used Ohio mulch and tomatoes. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. Both wet and 1 h dried inocula exhibited similar log % transfers with most falling near ~1.5 log percent transfer (Figure 1.h), with no significant differences when inoculated used mulch contacted tomatoes, ($p<0.05$; Table 1). When used Ohio mulch was inoculated and allowed to dry for 24 h, *Salmonella* concentrations were below the detection limit (1.4 CFU/tomato), while enrichment recovered *Salmonella* from 10 out of 10 samples after a touch contact and from 0 out of 10 samples after a 24 h contact (Table 1). The transfer of *Salmonella* from Ohio tomatoes to used mulch was similar to transfer in the reverse direction, though an examination of Figures 1.h and 2.h indicate the log % transfer rate was slightly higher from Ohio tomato to used Ohio mulch. When tomatoes were inoculated and allowed to dry for 24 h, *Salmonella* concentrations were below the detection limit (1.4 CFU/tomato) but enrichment recovered *Salmonella* from 10 out of 10 samples after a touch contact. Six out of 10 samples for the 24 h contact were positive after enrichment with the remaining 4 samples in the countable range (1.13±0.53 mean log %; Table 2).

Figures 1 and 2, panels i summarize the log % transfer data using Ohio soil and tomatoes. Figure 1 shows transfer from mulch to tomato, while Figure 2 shows transfer from tomato to mulch. The samples for all inoculation conditions were below detection limit for the transfer of *Salmonella* from Ohio soil to tomatoes, so all samples needed enrichment, as denoted in Table 1. The wet inoculated soil that tomatoes briefly touched

had the highest number of positive samples (10 out of 10) while a freshly inoculated soil with 24 h of contact with tomatoes had 9 out of 10 samples positive by enrichment. When Ohio soil was inoculated and allowed to dry for 1 h, 6 or 7 out of 10 tomato samples were positive by enrichment. When the soil was inoculated with *Salmonella* and allowed to dry for 24 h only 5 out of 10 tomato samples were positive by enrichment (Table 1). The transfer of *Salmonella* from Ohio tomatoes to soil was different than transfer in the opposite direction. When tomatoes were either freshly inoculated and briefly touched to soil or inoculated and allowed to dry for 1 h with a touch contact, percent log transfers were high, ranging between 1.75 and 2 log % transfer for both conditions (Figure 2.i). When tomatoes were freshly inoculated and placed in contact with soil for 24 h touch, no soil samples were countable, but all were positive by enrichment (Table 2). When Ohio tomatoes were inoculated, allowed to dry for 1 h dry, and placed in contact with soil for 24 h contact, enrichment was negative 6 time and positive 4 times as seen in Table 2. When tomatoes were inoculated with *Salmonella* and allowed to dry for 24 h and then briefly touched soil, results were the same as when tomatoes were inoculated and dried for 1 h, and then placed in contact with soil for 24 h (with both experiments indicating 4 samples negative and 6 positive after enrichment). When tomatoes were inoculated, dried for 24 h and placed in contact with soil for 24 h, only one sample was positive by enrichment (Table 2).

Ohio transfer results for wet and 1 h dry inocula were similar for both mulches to and from tomatoes with frequencies typically peaking between 1 and 2 log % (Figure 1.g-h and Figure 2.g-h). For the 24 h dry inoculum, there was more transfer from tomatoes to the mulches than the mulches to the tomatoes (Tables 1 and 2). Transfer of *Salmonella*

from tomatoes to soil for the wet and 1 h dry inocula with a touch contact was higher than the reverse transfer. All other transfer scenarios were similar despite the transfer direction.

Comparison of state-to-state differences in Salmonella *transfer*

The mean transfer rates from wet inoculum on new mulch to tomatoes with a touch contact did not vary significantly between states ($p<0.05$; Table 1). This mean transfer rate did vary significantly between states when new mulch and tomatoes were in contact for 24 h with the wet inoculum ($p<0.05$; Table 1). There was more variance in mean log % transfer when the inoculum was dried for 1 h or 24 h. There was generally no significant difference between transfer scenarios from tomatoes to new mulch (Table 2).

Mean % transfer rates were similar for transfer from used mulch to tomatoes with a wet and 1 h dry inoculum, though Ohio had a significantly higher transfer than Maryland with a 1 h dry inoculum and 24 h contact ($p<0.05$; Table 1). Mean % transfer rates were similar for transfer from tomatoes to used mulch with a wet and 1 h dry inoculum, though Ohio had significantly more transfer than Florida with a wet inoculum ($p<0.05$; Table 2). There was detectible transfer of *Salmonella* from tomatoes to used mulch with a 24 h inoculum in Florida and Ohio, but not in Maryland.

Transfer of *Salmonella* from soil to tomato always occurred in Florida, though some transfer was only detectable upon enrichment. No tomato samples in Ohio that contacted inoculated soil were ever above the detection limit for *Salmonella* (1.4 CFU/tomato). The log % *Salmonella* transfer from tomato to soil for the wet and 1 h dry

inocula was higher compared to transfer from soil to tomato in all three states for the same conditions.

The mean percent transfer from the mulches and soil to and from tomatoes following a touch contact with the wet and 1 h dried inocula was typically higher in all states than when the inoculum had dried for 24 h (Tables 1 and 2). The lowest overall transfer occurred from soil to tomatoes in all states.

Discussion

Differences between soil and plastic mulch

The calculated log % transfer between new mulch or used mulch and tomatoes was similar between the 3 states, as might be expected because of the similarity in the mulch composition. There was usually less transfer for any inoculum that had dried for 24 h compared to wet inocula, or inocula dried for 1 h, which is not surprising given the importance of moisture in facilitating bacterial transfer (Jensen et al. 2013; Miranda and Schaffner 2016; Sreedharan et al. 2014). The log % transfer of *Salmonella* from any soil from the three states to the corresponding tomato was less than the log % transfer of *Salmonella* from any states clean or used plastic mulch to tomatoes. The log % transfer of *Salmonella* from tomatoes to any states soil was higher than the log percent transfer of *Salmonella* from any states soil to the tomato. Prior studies have suggested that organic matter on porous, plastic surfaces can reduce bacterial transfer to another surface (Flores et al. 2006; Rusin et al. 2002; Brar and Danyluk 2013). We would have expected a lower transfer from the used plastic mulch to tomatoes as compared to the new plastic mulch due to the presence of soil on the used mulch, but our results show that this was not the case; the new and used plastic mulches had similar transfer rates to tomatoes. Rusin et al.

(2002) found that the highest bacterial transfers were from hard, non-porous surfaces which could explain why there was higher *Salmonella* transfer from new and used plastic mulch to tomatoes than from inoculated soil to tomatoes.

Effect of soil characteristics on bacterial transfer

The transfer of *Salmonella* between soil and tomatoes (and vice versa) in Ohio was less than that observed in the experiments in Maryland and Florida. This difference cannot be explained solely by soil composition, as the Ohio and Maryland soils had similar compositions (loam soil) whereas the soil in Florida was composed primarily of sand. The difference may be due to soil pH. The Maryland and Florida soils had a pH of 6.5 and 7.3, respectively, which creates a neutral environment optimal for *Salmonella* growth (Stokes and Bayne 1957). The pH of the Ohio soil used was more acidic (pH 5.4). Though *Salmonella* can grow in acidic conditions, growth is not as rapid as a more neutral pH (Stokes and Bayne 1957). This lower pH may have stressed the organism and affected transfer.

Salmonella *transfer from plastic to tomatoes*

A study by Soares et al. (2012) examined the transfer of *S*. Entertidis from a plastic cutting board to tomatoes that were cut on the board. The starting concentration of *S.* Entertidis on the plastic cutting board was 2.73 log CFU/cm^2 and the ending concentration on the diced tomatoes was 2.71 log CFU/g. Taking into account the total surface area of the cutting board (100 cm^2) and weight of the tomato sample (25 g), there was roughly a 1.40 log % transfer from the cutting board to the tomato with a wet inoculum and the tomatoes were in contact with the cutting board briefly as they were

being cut. The transfer from the used and new mulch to the whole tomatoes in our study is comparable to the transfer reported by Soares et al. (2012).

The mean transfer of *E. coli* O157:H7 and *Salmonella* from a plastic surface to celery, lettuce, and watermelon varied from 86.19-97.41% for a wet inoculum and 17.44-89.39% for a 1 h dry inoculum (Jensen et al. 2013). This is consistent with the findings in our study with typically less transfer when the inoculum was dry.

Salmonella *attachment and survival on Tomatoes*

In Lang et al. (2004), tomatoes sampled 24 h after the inoculum dried had about 1.5 log CFU/ml lower concentration of *Salmonella* than the tomatoes that were sampled 1 h after the inoculum dried. This is in contrast to a study by Rathinasabapathi (2004) who found that there was no change of *Salmonella* concentration after 48 h on the surface of a tomato pericarp disc. This discrepancy may be due to starting concentration used. Lang et al. (2004) used a higher starting concentration than Rathinasabapathi (2004), but the ending concentration found on the tomatoes were similar for both studies (~6.0 log CFU/tomato) suggesting that a higher *Salmonella* concentration may not be supported on tomatoes which would explain the death in one study while the *Salmonella* population was maintained in the other study. In our study, there was generally about a log decrease on the tomatoes after 24 h dry period (data not shown). Additionally, we found a lower percent transfer to the mulches or soil if the inoculum was allowed to dry for 24 h on the tomato rather than the wet or 1 h dry inoculum. While *Salmonella* populations may decline over 24 h period, remaining cells may be more firmly attached to the tomato resulting in less transfer. Iturriaga et al. (2003) reported that the attachment of *Salmonella* to tomatoes was greater after 90 min of storage vs. 0 min (2.1-6.6% and 07-

0.7% respectively), which supports our suggestion that less transfer occurs at longer times due to greater attachment. Studies have shown that *S.* Montevideo has a stronger attachment to tomatoes than *S.* Michigan, Poona, Hartford, and Enteriditis (Guo 2001; Guo 2002), so it may be the Montevideo strain in our cocktail primarily influencing transfer between the surfaces.

Survival of *Salmonella* in soil in contact with tomatoes was better compared to *Salmonella* in either just soil or just on tomatoes (Guo et al. 2002), and our research indicate that transfer can occur at long (24 h) contact times. These two points suggest that tomatoes that grow in contact with contaminated soil could have higher levels of *Salmonella* than tomatoes that had a brief contact with soil.

Conclusions

Our study demonstrates that the transfer of *Salmonella* between tomatoes and plastic mulch or soil is dependent on factors such as contact time, dryness of the inoculum, soil characteristics, as well direction of transfer. The transfer of *Salmonella* between soil or plastic mulch to and from tomatoes was dependent on moisture with wet and 1 h dried inoculum generally producing a higher transfer than the 24 h dry inoculum. This suggests that harvesting dry tomatoes reduce the risk of contamination. In most cases, the transfer was greater from new and used plastic mulch to the tomatoes vs. from soil to the tomatoes. These data suggest that if contamination levels in soil and on mulch are similar, that harvesting tomatoes in contact with the soil poses a lower risk than harvesting tomatoes in contact with new or used plastic mulch.

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Tables

Table 1. Transfer of a 5-strain cocktail of *Salmonella* from mulches and soil to tomatoes with characteristics common in three states.

Inoculated Surface	Location	Drying Time (Hours)	Contact Time (Hours)	Countable	Enrichment Negative	Enrichment Positive	Mean (log %)	Stats[b]	Standard Deviation (log %)	Median (log %)	Maximum (log %)	Minimum (log %)	Range (log %)[a]
New Mulch	FL	0	0	10	-	-	1.52	a,x	0.21	1.54	1.89	1.08	0.81
			24	10	-	-	0.98	b,x	0.26	0.95	1.39	0.56	0.83
		1	0	10	-	-	1.40	a,xy	0.16	1.45	1.53	0.99	0.54
			24	10	-	-	1.40	a,x	0.37	1.49	1.73	0.42	1.30
		24	0	0	3	7	-	-	-	-	-	-	-
			24	0	8	2	-	-	-	-	-	-	-
	MD	0	0	10	-	-	1.43	a,x	0.24	1.49	1.72	0.90	0.81
			24	10	-	-	1.72	a,y	0.20	1.76	1.97	1.28	0.69
		1	0	10	-	-	1.62	a,x	0.20	1.68	1.83	1.24	0.59
			24	10	-	-	1.65	a,x	0.30	1.74	1.87	0.87	1.00
		24	0	0	10	0	-	-	-	-	-	-	-
			24	0	10	0	-	-	-	-	-	-	-
	OH	0	0	10	-	-	1.64	a,x	0.21	1.70	1.88	1.20	0.68
			24	10	-	-	1.40	ab,z	0.34	1.50	1.73	0.61	1.13
		1	0	10	-	-	1.02	bc,y	0.60	1.12	1.72	-0.05	1.77
			24	10	-	-	0.71	c,y	0.19	0.70	0.93	0.33	0.60
		24	0	10	-	-	1.02	bc,-	0.33	0.89	1.52	0.57	0.95
			24	10	-	-	1.16	bc,-	0.17	1.18	1.46	0.89	0.57
Used Mulch	FL	0	0	10	-	-	1.38	a,x	0.40	1.43	1.81	0.44	1.37
			24	10	-	-	1.47	a,x	0.25	1.51	1.87	0.94	0.93
		1	0	10	-	-	1.35	a,x	0.19	1.33	1.61	1.07	0.54
			24	10	-	-	1.21	a,xy	0.20	1.22	1.50	0.81	0.69
		24	0	0	0	10	-	-	-	-	-	-	-
			24	5	3	2	1.36	a,x	0.58	1.52	1.85	0.40	1.45
	MD	0	0	10	-	-	1.57	a,x	0.10	1.57	1.74	1.42	0.32
			24	10	-	-	1.66	a,x	0.23	1.68	2.00	1.33	0.67
		1	0	10	-	-	0.96	ab,x	0.64	1.04	1.66	0.01	1.65
			24	7	-	2	1.03	ab,x	0.58	1.03	1.89	0.36	1.53
		24	0	10	-	-	1.39	ab,-	0.70	1.67	1.92	-0.34	2.26
			24	6	3	1	0.59	b,x	0.99	0.51	1.60	-0.60	2.20
	OH	0	0	10	-	-	1.33	a,x	0.22	1.31	1.76	0.96	0.80
			24	10	-	-	1.39	a,x	0.38	1.49	1.84	0.42	1.42
		1	0	10	-	-	1.39	a,x	0.13	1.34	1.64	1.26	0.38

			24	10	-	-	1.56	a,y	0.20	1.56	1.95	1.27	0.68
		24	0	0	0	10	-	-	-	-	-	-	-
			24	0	10	0	-	-	-	-	-	-	-
Soil	FL	0	0	10	-	-	-1.74	a,x	0.62	-1.97	-0.08	-2.15	2.06
			24	5	0	5	0.13	b,x	0.46	0.10	0.59	-0.52	1.10
		1	0	10	-	-	-1.38	a,x	0.42	-1.42	-0.60	-1.77	1.17
			24	3	0	7	0.28	b,-	0.06	0.29	0.32	0.21	0.11
		24	0	0	0	10	-	-	-	-	-	-	-
			24	0	0	10	-	-	-	-	-	-	-
	MD	0	0	6	-	-	1.19	a,y	0.38	1.15	1.78	0.72	1.06
			24	2	1	7	1.34	a,y	0.26	1.34	1.52	1.15	0.37
		1	0	7	0	3	0.22	b,y	0.43	0.15	0.77	-0.40	1.17
			24	0	8	2	-	-	-	-	-	-	-
		24	0	0	10	0	-	-	-	-	-	-	-
			24	0	7	3	-	-	-	-	-	-	-
	OH	0	0	0	0	10	-	-	-	-	-	-	-
			24	0	1	9	-	-	-	-	-	-	-
		1	0	0	3	7	-	-	-	-	-	-	-
			24	0	4	6	-	-	-	-	-	-	-
		24	0	0	5	5	-	-	-	-	-	-	-
			24	0	5	5	-	-	-	-	-	-	-

[a] Range was calculated as the difference between the maximum and the minimum

[b]a,b,c,d denotes significant difference within a transfer scenario (i.e. *Salmonella* from new mulch to tomato) within a state and x,y,z denotes significant difference between states for a specific transfer scenario (i.e. wet inocula with touch contact from new mulch to tomato)($p<0.05$)

Table 2. Transfer of a 5-strain cocktail of *Salmonella* from tomatoes to mulches and soil with characteristics common in three states.

Recipient Surface	Location	Drying Time (Hours)	Contact Time (Hours)	Countable samples	Enrichment Negative	Enrichment Positive	Mean (log %)	Stats	Standard Deviation (log %)	Median (log %)	Maximum (log %)	Minimum (log %)	Range (log %)[a]
New Mulch	FL	0	0	10	-	-	1.54	a,x	0.10	1.53	1.72	1.39	0.32
			24	10	-	-	1.85	b,x	0.12	1.88	1.95	1.56	0.39
		1	0	10	-	-	1.61	ab,x	0.07	1.58	1.75	1.51	0.24
			24	10	-	-	1.82	b,x	0.08	1.82	1.99	1.71	0.29
		24	0	8	0	2	0.25	c,x	0.31	0.16	0.79	-0.10	0.89
			24	3	0	7	1.15	d,x	0.57	0.89	1.80	0.76	1.04
	MD	0	0	10	-	-	1.70	a,x	0.24	1.81	1.90	1.27	0.63
			24	10	-	-	1.80	a,x	0.15	1.82	1.96	1.45	0.51
		1	0	10	-	-	1.49	a,xy	0.34	1.61	1.81	0.71	1.10
			24	9	-	-	1.70	a,x	0.22	1.75	2.00	1.25	0.75
		24	0	3	-	5	0.45	b,x	0.54	0.75	0.77	-0.18	0.95
			24	0	3	7	-	-	-	-	-	-	-
	OH	0	0	10	-	-	1.58	ab,x	0.27	1.63	1.85	1.03	0.82
			24	10	-	-	1.81	a,x	0.04	1.82	1.87	1.74	0.13
		1	0	10	-	-	1.28	bc,y	0.17	1.24	1.59	1.05	0.54
			24	10	-	-	1.19	bcd,y	0.11	1.17	1.33	1.06	0.28
		24	0	10	-	-	0.87	cd,x	0.70	0.54	1.74	0.03	1.71
			24	10	-	-	0.72	d,x	0.44	0.79	1.55	0.05	1.50
Used Mulch	FL	0	0	10	-	-	1.69	a,x	0.09	1.69	1.80	1.53	0.27
			24	10	-	-	1.04	a,x	0.91	1.46	1.93	-0.88	2.81
		1	0	10	-	-	1.62	a,x	0.11	1.63	1.77	1.47	0.31
			24	10	-	-	1.48	a,x	0.41	1.49	1.98	0.86	1.13
		24	0	10	-	-	0.05	b,-	0.55	0.17	0.95	-0.95	1.90
			24	2	0	8	0.54	ab,x	0.51	0.54	0.90	0.17	0.72
	MD	0	0	10	-	-	1.55	a,xy	0.21	1.59	1.81	1.10	0.71
			24	10	-	-	1.57	a,xy	0.30	1.65	1.94	0.93	1.01
		1	0	10	-	-	1.27	a,x	0.56	1.46	1.79	0.10	1.69
			24	9	-	-	1.32	a,x	0.45	1.43	1.83	0.39	1.44
		24	0	0	10	0	-	-	-	-	-	-	-
			24	0	10	0	-	-	-	-	-	-	-
	OH	0	0	10	-	-	1.49	ab,y	0.12	1.51	1.69	1.32	0.37
			24	10	-	-	1.77	a,y	0.39	1.89	1.98	0.68	1.30
		1	0	10	-	-	1.54	ab,x	0.12	1.57	1.70	1.33	0.37
			24	10	-	-	1.56	ab,x	0.27	1.57	1.87	1.09	0.78
		24	0	0	0	10	-	-	-	-	-	-	-
			24	4	0	6	1.13	b,x	0.53	1.19	1.71	0.42	1.29

Soil	FL	0	0	10	-	-	1.92	a,x	0.10	1.97	2.00	1.71	0.29
			24	10	-	-	1.43	b,x	0.23	1.51	1.68	1.00	0.67
		1	0	10	-	-	1.79	a,x	0.03	1.79	1.83	1.72	0.11
			24	10	-	-	1.56	b,x	0.07	1.57	1.67	1.44	0.24
		24	0	0	0	10	-	-	-	-	-	-	-
			24	0	10	0	-	-	-	-	-	-	-
	MD	0	0	9	-	-	1.93	a,x	0.07	1.96	1.98	1.74	0.24
			24	9	-	1	0.71	bc,y	0.77	0.97	1.44	-0.97	2.41
		1	0	8	-	2	0.99	bc,y	0.87	1.16	1.95	-0.35	2.30
			24	9	-	-	0.17	b,x	0.45	0.21	0.78	-0.44	1.22
		24	0	10	-	-	1.09	ac,-	0.67	1.18	1.86	-0.03	1.89
			24	10	-	-	0.65	bc,-	0.69	0.74	1.54	-0.64	2.18
	OH	0	0	10	-	-	1.94	a,x	0.14	1.99	2.00	1.54	0.46
			24	0	0	10	-	-	-	-	-	-	-
		1	0	10	-	-	1.68	b,x	0.19	1.67	1.92	1.29	0.63
			24	0	6	4	-	-	-	-	-	-	-
		24	0	0	6	4	-	-	-	-	-	-	-
			24	0	9	1	-	-	-	-	-	-	-

[a] Range was calculated as the difference between maximum and minimum percent transfer

[b]a,b,c,d denotes significant difference within a transfer scenario (i.e. *Salmonella* from new mulch to tomato) within a state and x,y,z denotes significant difference between states for a specific transfer scenario (i.e. wet inocula with touch contact from new mulch to tomato)($p<0.05$)

Table 3. Chemical analysis of soils from each of three states used in cross-contamination experiments

	State					
Soil attribute	Florida		Ohio		Maryland	
Phosphorus (lbs/acre)	244	Very high	50	Moderate	22	Low
Potassium (lbs/acre)	34	Low	125	Moderate	46	Low
Magnesium (lbs/acre)	114	Moderate	417	Very high	193	High
Calcium (lbs/acre)	1257	Very high	2436	Very high	1339	Very high
Zinc (lbs/acre)	24	Very high	6.8	Adequate	1	Low
Manganese (lbs/acre)	46	High	49	High	17	Low
Estimated Nitrogen Release (lbs/acre)	7.8		33.4		30.8	
Soil pH	7.3	High	5.4	Low	6.5	Adequate
Buffer pH	7.9		7.85		7.85	
Soluble Salts (mmhos/cm)	0.04		0.59		0.08	
Percent Organic Matter (%)	0.39		1.67		1.54	
Cation Exchange Capacity (meq/100g)	4.5		9.2		5.4	
Potassium (%)	1		1.7		1.1	
Magnesium (%)	10.6		18.9		14.9	
Calcium (%)	70.4		66.3		61.9	
Hydrogen (%)	17.9		13.1		22.2	
Soil type	Sand		Loam		Loam	
Sand (%)	97.2		35.2		42.3	
Clay (%)	2.0		24.0		16.8	
Silt (%)	0.8		40.8		40.0	

Figures

Figure 1. Log Percent Transfer of *Salmonella* from Mulch or Soil to Tomato with panel a representing Florida, new mulch, b representing Florida, used mulch, c representing Florida soil, d representing Maryland, new mulch, e representing Maryland, used mulch, f representing Maryland soil, g representing Ohio, new mulch, h representing Ohio, used mulch, and i representing Ohio soil. The following six conditions were used for each transfer scenario: wet inoculum with touch contact ( ), wet inoculum with 24 h contact ( ), 1 h dry inoculum with touch contact ( ), 1 h dry inoculum with 24 h contact ( ), 24 h dry inoculum with touch contact ( ), 24 h dry inoculum with 24 hour contact ( ).

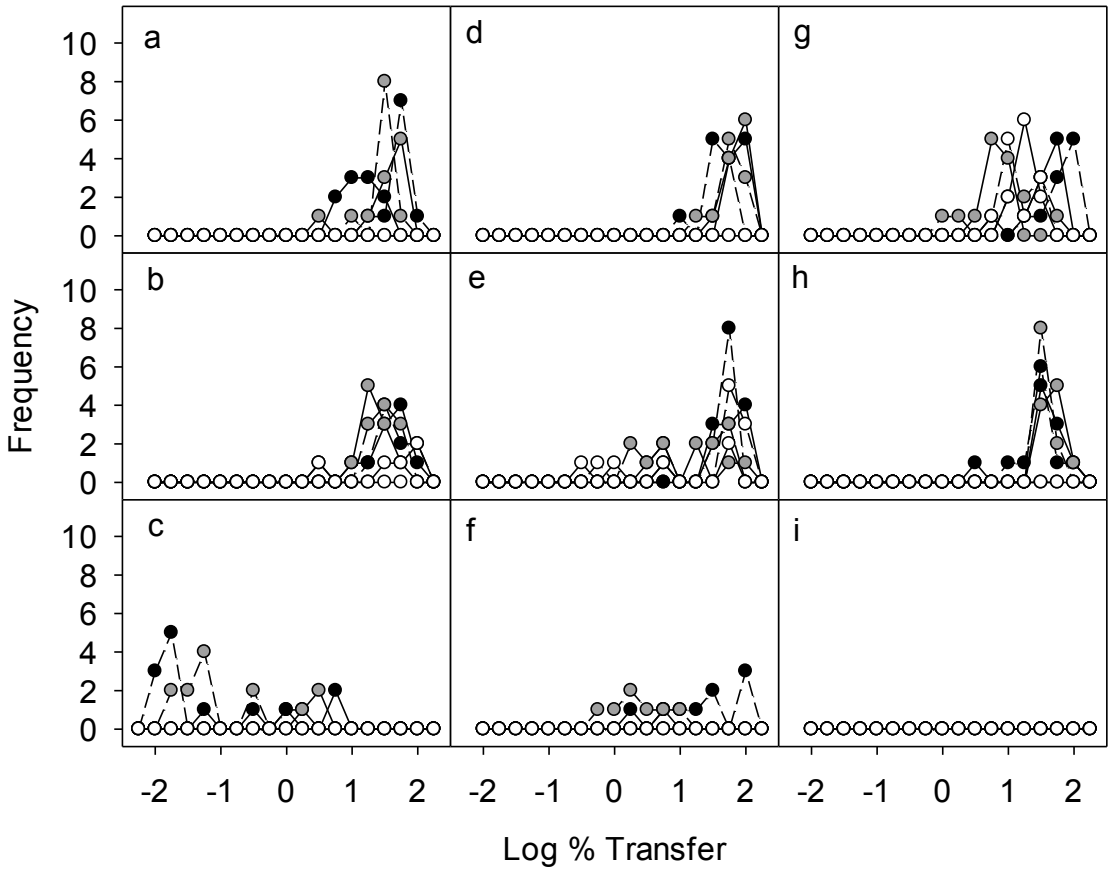
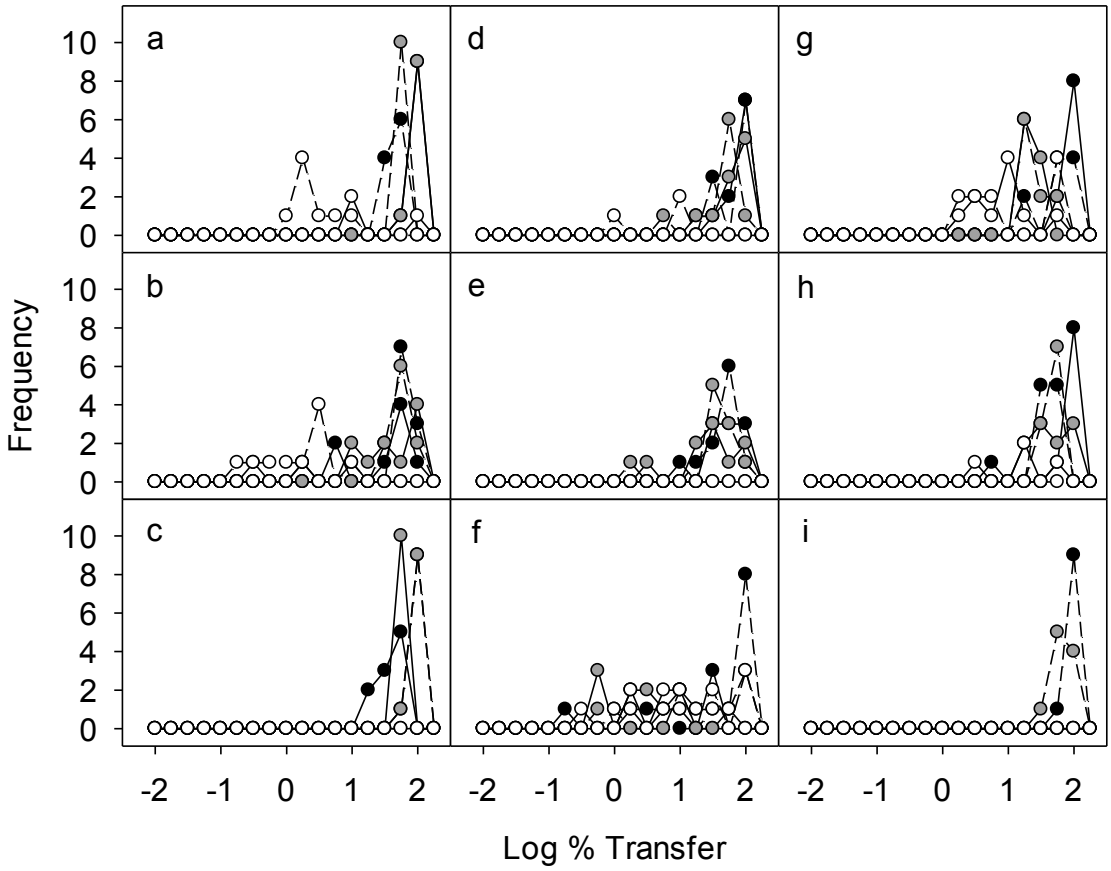


Figure 2. Log Percent Transfer of *Salmonella* from Tomato to Mulch and Soil with panel a representing Florida, new mulch, b representing Florida, used mulch, c representing Florida soil, d representing Maryland, new mulch, e representing Maryland, used mulch, f representing Maryland soil, g representing Ohio, new mulch, h representing Ohio, used mulch, and i representing Ohio soil. The following six conditions were used for each transfer scenario: wet inoculum with touch contact ( ), wet inoculum with 24 h contact ( ), 1 h dry inoculum with touch contact ( ), 1 h dry inoculum with 24 h contact ( ), 24 h dry inoculum with touch contact ( ), 24 h dry inoculum with 24 hour contact ( ).



Chapter III: The effects of relative humidity on *Salmonella* biofilm production, quorum sensing, and subsequent survival on tomatoes and plastic mulch

Jennifer Todd-Searle[1] and Donald W. Schaffner[1]

[1]Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States

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Abstract

Tomatoes have been linked to many United States *Salmonella* outbreaks but little is known about the environmental and physiological factors that influence the survival of *Salmonella* on tomatoes and in the field. This project seeks to understand how *Salmonella* biofilm production *in vitro* and survival on tomatoes and plastic ground cover (mulch) is affected by relative humidity and explained by *Salmonella* rdar (red dry and rough) morphology, biofilm production, and quorum sensing. Desiccators with salt slurries (lithium chloride, potassium carbonate, and potassium sulfate) created controlled RH environments (~15, 50, and 100% RH). Biofilm production by six *Salmonella* strains was screened in microtiter plates using the crystal violet assay. The rdar positive morphotype MAE110, and rdar negative morphotypes MAE119 and J1890 were chosen for further testing based on biofilm production. *Salmonella* from tomatoes stored at 100% RH were also tested for quorum sensing. Biofilm production in microtiter plates was greatest by strain MAE110 and least by strain J1890 across all RH conditions. Strain MAE119 showed the widest variability in biofilm production across all RH conditions. The MAE110, MAE119 and J1890 strains had the best survival at 100% RH at the end of 14 days on plastic mulch (4.2±0.4 to 6.0±0.7 log CFU/square) and 7 days on tomatoes (5.0±0.9 to 7.1±0.2 log CFU/tomato). No biofilm production was observed on plastic mulch at 100% RH. MAE110 was the only strain to show potential biofilm production on tomatoes. Quorum sensing was not observed for any of the 3 *Salmonella* strains on tomatoes stored at 100% RH. *Salmonella* survival on tomatoes and plastic mulch is strain dependent, but more complex that the environmental RH, rdar morphotype, and biofilm production can explain. High RH conditions support better *Salmonella* survival,

but specific conditions that might allow *Salmonella* growth on whole tomatoes are still unclear.

Introduction

Several *Salmonella* outbreaks linked to tomatoes were traced back to contamination occurring on the farm (CDC 2006; CDC 2008; Hanning et al. 2009). When contamination originates on the farm, *Salmonella* must survive on the tomato skin until these fresh tomatoes are consumed. The literature on *Salmonella* survival on the surface of tomato fruit contains conflicting results. Iturriaga et al. (2007) found a 2 log CFU/tomato increase of *S.* Montevideo on tomatoes stored at 97% relative humidity (RH) and 22°C after 10 days. These researchers also noted an increase between 0.5 and 2 log CFU/tomato after 10 days at 30°C in RH environments ranging from 60-97%. Shi et al. (2007) found that *Salmonella* concentration increased on the surface of vacuum infused ripe and unripe whole tomatoes to different degrees, depending upon *Salmonella* strain, temperature, and RH. Conversely, Rathinasabapathi (2004) demonstrated no change in *Salmonella* concentration after 48 h at 37°C and an RH of 100% on the surface of a tomato pericarp disc. Many other studies further demonstrate the complexities of *Salmonella* survival by using a variety of tomatoes and storage environments resulting in a range of *Salmonella* concentrations on tomatoes (Allen et al. 2005; Beuchat and Mann 2008; Das et al. 2006; Lang et al. 2004; Lopez-Velasco et al. 2013, Pao et al. 2012; Zhuang et al. 1995). Clear correlations between the environmental and physiological factors that influence the survival of *Salmonella* on tomatoes remain to be discovered.

Some *Salmonella* form extracellular thin aggregative fimbriae, which interact with synthesized cellulose and other polysaccharides to produce a red, dry, and rough (rdar) morphotype on Congo red agar (White and Surette 2006). Gu et al. (2011) found that *Salmonella* expressing the rdar morphotype can better colonize tomatoes than

Salmonella with non-rdar morphotypes. Generally, strains with rdar morphology also produce better biofilms than strains with non-rdar morphology, although not universally (Malcova et al. 2008). One study found that 73% of clinical *Salmonella* strains and 84% of strains isolated from meats exhibited the rdar morphotype whereas only 56% of produce isolates had the rdar morphotype (Solomon et al. 2005).

Quorum sensing plays a role in biofilm formation, which can in turn influence bacterial survival. There are three main methods of quorum sensing systems in *Salmonella* (Steenackers et al. 2012). These include *n*-acyl-homoserine lactone (AHL), autoinducer-2 (AI-2), and autoinducer-3 (AI-3) signaling. *Salmonella* does not produce AHL, but rather detects AHL from other bacteria (Ahmer et al. 1998; Michael et al. 2001; Swift et al. 1999). The presence of AHLs may have a positive influence on biofilm production (Bouwman et al. 2003; Crago and Koronakis 1999; Heffernan et al. 1992; Nicholson and Low 2000). Many studies have demonstrated that a mutation in *luxS* gene, which synthesizes AI-2, leads to impaired biofilm production concluding that AI-2 signaling influences biofilm production (De Keersmaecker et al. 2005; Jesudhasan et al. 2010; Prouty et al. 2002). The two component system PreA/B senses AI-3, as well as epinephrine and norepinephrine, and has been found to effect the motility of *Salmonella* thus influencing biofilm production (Steenackers et al. 2012).

This research seeks to better understand how *Salmonella* biofilm production *in vitro* and survival on tomatoes and plastic mulch is affected by RH and potentially explained by rdar morphology and quorum sensing. The information gained in this study may help understand and predict the risks of *Salmonella* survival on tomatoes and in the production environment.

Materials and Methods

Strain selection and preparation

Six *Salmonella* strains were used in this project. Four *Salmonella enterica* strains Newport (J1890), Anatum (K2669), Javiana (K2674), and Braenderup (K2680), previously studied in our lab have all been associated with tomato linked outbreaks (Pan and Schaffner, 2010). Two other *S.* Typhimurium strains (MAE110 and MAE119) were kindly provided by Dr. Ariena van Bruggen from the University of Florida. All strains were grown in tryptic soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, NJ) overnight at 37°C prior to experimentation.

Vibrio harveyi strains used in quorum sensing assays BB120, BB152, BB170, and BB886 (ATCC BAA-1116-1118) were grown in marine broth (MB; BD) overnight at 37°C. The *E. coli* TEVS232 strain used in the β-galactosidase assay was kindly provided by Dr. Vanessa Sperandio from the University of Texas Southwestern Medical Center. TEVS232 was grown overnight in Luria-Bertani (LB; Thermo Fisher Scientific) broth at 37°C.

Morphotype studies

Salmonella strains were plated on LB agar without salt supplemented with Congo red and Coomassie brilliant blue to study rdar morphology (Römling et al. 1998). Samples were incubated at room temperature at various RH conditions.

Biofilm production in microtiter plates

Desiccators with saturated salt slurries (lithium chloride, potassium carbonate, and potassium sulfate) were used to create controlled RH environments (~15, 50, and 100% RH). Biofilm production of various *Salmonella* strains were tested using a

microtiter plate assay with crystal violet according to a modified procedure from Wang et al. (2013). Ten microliters of an overnight culture was inoculated in 90 µl of fresh TSB in a sterile microtiter plate. The plates were incubated at the desired RH for 72 h at room temperature (~22°C). After 3 days, the excess liquid present in the 100% RH environment was dumped and the microplates in all RH environments were rinsed thrice with sterile, deionized water. Plates were air-dried for 1 h in a biosafety cabinet (SterilGARD Hood, the Baker Company, Inc., Sanford, Maine) after which 125 µl of 0.25% crystal violet was added to the cells for 30 min. The plates were emptied and rinsed thrice with sterile, deionized water. Plates were dried again for 1 hr. The dye was solubilized with 125 µl of 95% ethanol for 30 min. Absorbance was then measured at 570 nm with a THERMOmax microplate reader (Molecular Devices, Sunnyvale, CA). Three of the six *Salmonella* strains (MAE110, MAE119 and J1890) were selected for further studies on tomatoes and plastic mulch.

Salmonella *Survival on Plastic Mulch and Tomatoes*

Plastic mulch is commonly used around growing tomatoes to aid in weed control and conserve water. Tomatoes are commonly harvested if they have fallen from the vine but are resting on the plastic mulch. Plastic mulch and ripe plum tomatoes were obtained from a NJ grower. The plastic mulch was cut into 5 by 5 cm squares. *Salmonella* cells from an overnight culture were washed thrice at 3,000 g for 10 min with 0.1% peptone (BD). The plastic mulch and tomatoes were spot inoculated with 100 µl of each *Salmonella* strain. Plastic mulch and tomatoes were allowed to dry for 2.5 h in a biosafety cabinet before being placed in the desiccators at 15, 50, or 100% RH. Tomatoes and plastic mulch were sampled on days 0, 1, 3, and 7. Tomatoes showed

signs of decomposition after 7 days, but plastic mulch was also sampled on day 14. Cells were removed from the tomatoes and mulch with 10 ml of 0.1% peptone water and hand massaged for 1 m. Samples were diluted and plated on TSA (BD) for enumeration. After 2 hours, an XLT4 (BD) overlay was poured over the TSA to select for *Salmonella*. Plates were incubated at 37°C for 24 h.

Biofilm Production on Tomatoes and Plastic Mulch

Salmonella strains grown on tomatoes and plastic mulch were studied for biofilm production at 100% RH since this condition was most conducive to survival. The crystal violet assay from Adetunj and Isola (2011) was used to quantify biofilm production. Briefly, loose cells were rinsed from the surface and remaining cells fixed with methanol. The surface was then stained with crystal violet. Excess crystal violet was washed off and the dye re-solubilized with glacial acetic acid and absorbance was measured at 570 nm.

Quorum sensing

Salmonella samples from inoculated tomatoes were assayed for quorum sensing. Tomatoes were inoculated as described above and placed in desiccators at 100% RH for 3 days. A modified procedure from Surette and Bassler (1998) was followed to obtain cell-free supernatants. The sensor *Vibrio* strains were grown in autoinducer broth and the control strains grown in MB, as above. Plastic mulch and tomatoes were massaged with 10 ml of 0.1% peptone water to remove *Salmonella* cells. *Salmonella* and *Vibrio* samples were centrifuged for 5 min at 23,000 g and the supernatant passed through a 0.22 um filter (EMD Millipore, Billerica, MA).

The procedure from Surette and Basler (1998) was followed to determine if the quorum sensing molecules AHL and/or AI-2 could be detected in the supernatants. *V. harveyi* B886 and BB170 detect AHL and AI-2, respectively. *V. harveyi* strain BB120 produces both AHL and AI-2 and BB152 only produces AI-2. Supernatants from BB120 and BB152 were used as positive controls. The cell-free supernatant from the *Salmonella* and *Vibrio* strains were combined with *V. harveyi* BB170 (for AI-2 detection) or *V. harveyi* BB886 (for AHL detection) in 96-well plates to measure the bioluminescence. The microtiter plate (Nunc MicroWell White Polystyrene Plate, Thermo Fisher Scientific, Waltham, WA) was shaken for 8 hours at 37 °C with the light production measured every hour with a Luminoskan Ascent (Labsystems, Thermo Fisher Scientific). Activity is expressed relative to the control, which is assigned a value of 1.

The β-galactosidase assay was utilized following the procedure in Walters et al (2006) to test for AI-3 production. The TEVS232 reporter strain from Sperandio et al. (1999) was grown overnight in LB broth. The *E. coli* TEVS232 reporter strain was combined with the supernatant from *Salmonella* samples grown on tomatoes at 100% RH. The β-galactosidase activity is measured with *o*-nitrophenyl β-D-galactopyranoside as a substrate. If AI-3 is present, it will activate transcription of *LEE1*. This will lead to the production of β-galactosidase, which can cleave *o*-nitrophenyl β-D-galactopyranoside resulting in *o*-nitrophenyl, which creates a yellow color. The yellow color was quantified by measuring the absorbance at 420 nm and 550 nm with a Spectronic 501 (Milton Roy, Thermo Fisher Scientific) and transformed into units as per Miller (1972).

Statistical analysis

Experiments were performed in triplicate with means and standard deviations calculated in Excel (Microsoft, Redmond, WA). StatTools (Palisade Corporation, Ithaca, NY) was used to calculated significance difference ($p<0.05$).

Results

Morphotype studies

The only *Salmonella* strain observed to grow with an rdar morphotype at any RH was MAE110. The smooth and white (saw) morphotype was observed for MAE119, J1890, K2669, K2674, and K2680 in all RH environments.

Biofilm production in 96-well plates

The TSB created 100% RH conditions in all three environments due to the liquid in the broth. The 15 and 50% RH environments reestablished themselves within 3 days. Strain MAE110 had the most (A_{570} 2.14±0.48-2.60±0.09) and J1890 had the least (A_{570} 0.29±0.11-0.79±0.16) amount of biofilm production in all RH environments (Fig 1). Strains K2669, K2674, and K2680 produced similar amounts of biofilm at 15 (A_{570}1.37±0.11-1.52±0.33), 50 (A_{570} 1.07±0.10-1.28±0.26), and 100% RH (A_{570} 0.79±0.13-1.06±0.27) as shown in Fig 1. Strain MAE119 had the largest range in biofilm production between the various RH environments (A_{570} 0.42±0.07-1.93±0.11) as indicated in Fig 1. Overall, strains exposed to 15% RH produced the most biofilm with 100% producing the least (A_{570} 0.79±0.16-2.58±0.11 and A_{570} 0.29±0.11-2.14±0.48 respectively) as presented in Fig 1. Strains MAE110, MAE119, and J1890 were chosen for further testing based on their varying biofilm production and morphotypes.

Survival on plastic mulch

Figure 2 indicates that strain MAE110 showed the best survival at 100% RH (4.2±0.4 log CFU/square after 14 days), then at 15% RH (2.6±0.8 log CFU/square after 14 days), then at 50% (1.4±0.6 log CFU/square after 14 days). Strain MAE119 also had the best survival at 100% (6.0±0.6 log CFU/square after 14 days), then at 50% (0.7±0.6 log CFU/square after 14 days), then 15% (0.6±1.1 log CFU/square and least at). Survival of strain J1890 was similar to MAE119 with the best survival at 100% (6.0±0.7 log CFU/square after 14 days), then at 50% (3.4±0.1 log CFU/square after 14 days), and least at 15% (2.7±0.7 log CFU/square after 14 days). Survival at 100% RH at day 14 was significantly higher for all strains than survival at 50% or 15% ($p<0.05$). Strains MAE119 and J1890 showed significantly more survival at 100% RH at day 14 than MAE110 ($p<0.05$). Strains MAE110 and J1890 had significantly higher survival than MAE119 at 50 and 15% RH ($p<0.05$).

Survival on tomatoes

The tomato experiments used the same desiccator and saturated salt systems as the plastic mulch experiments with one important difference. The added moisture from the tomatoes themselves raised the 15% RH system to ~40-60% RH and 50% RH system to ~60-80% RH. The 100% RH system was unaffected by the presence of the tomatoes. For clarity the results are reported using the target RH values of 15, 50, and 100%, and are shown in Fig 3. Strain MAE110 showed the most survival at 100% RH (7.1±0.2 log CFU/tomato after 7 days), then at 15% RH (4.4±0.8 log CFU/tomato after 7 days), then 50% (2.6±1.3 log CFU/tomato after 7 days). Strain MAE119 had the best survival at 100% as well (5.0±0.9 log CFU/tomato after 7 days), followed by 50% (2.2±0.7 log

CFU/tomato after 7 days), then 15% (1.6±0.9 log CFU/tomato after 7 days). Strain J1890 showed similar survival patterns as strain MAE110 with the best survival at 100% RH (7.1±0.1 log CFU/tomato after 7 days), then at 15% RH (5.1±0.3 log CFU/tomato after 7 days), and 50% (2.6±0.4 log CFU/tomato after 7 days). Survival at 100% RH at day 7 was significantly better than survival at 15 and 50% for all strains ($p<0.05$). Strain J1890 was the only strain investigated that showed significantly less survival at 50% RH than 15% RH ($p<0.05$). Strains MAE110 and J1890 had statistically better survival at day 7 than strain MAE119 when the RH was 15 or 100% ($p<0.05$).

Biofilm production

Figure 4 shows that there was no significant difference in biofilm production between the uninoculated plastic mulch control and all 3 *Salmonella* strains on plastic mulch ($p>0.05$; A_{570} 0.012±0.003-0.027±0.009). There was also no significant difference in biofilm production between the uninoculated control tomato and strains J1890 and MAE119 ($p>0.05$; A_{570} 0.14±0.02-0.19±0.02). There was a statistically significant difference ($p<0.05$) between the uninoculated control tomato (A_{570} 0.14±0.02) and strain MAE110 (A_{570} 0.23±0.01).

Quorum sensing

Figure 5a illustrates that the control (no *Salmonella*), MAE110, MAE119, and J1890 strains all showed significantly less relative luminescence (0.24±0.09 to 0.44±0.13) than BB886 + BB152 and BB886 + BB120 strains ($p<0.05$) indicating a lack of AI-1 production by *Salmonella* on tomatoes. Figure 5b likewise indicates that the control (no *Salmonella*), MAE110, MAE119, and J1890 strains all showed significantly less relative luminescence (0.37±0.16 - 0.40±0.16) than the sensor strain (BB170) with

BB120 (1) and BB152 (0.84±0.43) indicating a lack of AI-2 production. Figure 6 shows that the *Salmonella* strains (A_{420} 256.8±31.1-276.4±11.1) were not significantly different from the TEVS232 (A_{420} 244.9±21.3) and tomato only (A_{420} 261.8±9.22) controls indicating a lack of AI-3 production. None of the quorum sensing tests suggest any of the 3 *Salmonella* strains produced quorum sensing molecules on tomatoes stored at 100% RH.

Discussion

Salmonella concentration declined on plastic mulch by 2 to 7 log CFU/square over 14 days, depending upon %RH and strain. This is considerably faster than the declines see in soil, where for example Guo et al. (2002) saw only a ~1 log CFU/g reduction in soil over 45 days. These declines are in line with those reported by Margas et al (2014) for *Salmonella* on stainless steel at 25°C and 33% RH, by Kusumaningrum et al. (2003) on stainless steel at 22-25 °C and 40-45% RH, and by Allan et al (2004) on stainless steel, acetal resin, and plastic wall paneling at 10 °C and high humidity.

While we expected that *Salmonella* decline in concentration on plastic mulch at any RH due to a lack of nutrients, some investigators have reported apparent *Salmonella* growth on tomatoes at high RH (Iturriaga et al. 2007). While it appeared that *Salmonella* population increased on both tomatoes and plastic mulch from days 1 to 3 in our experiments, especially at 100% RH, it is not clear that growth actually occurred. Our results may be different from those of Iturriaga et al. (2007) due to difference in starting concentrations. Iturriaga et al. (2007) used a 4.0 log CFU/tomato starting concentration while we used an 8.0 log CFU/tomato starting concentration. Many studies have also found the limit of *Salmonella* on tomatoes to be roughly 8.0 log CFU/tomato (Iturriaga et

al. 2007; Guo et al. 2002; Shi et al. 2007; Yuk et al. 2007). Iturriaga et al. (2007) found a ~1.0 log CFU/tomato difference for tomatoes stored at 97% vs. 85% RH after 7 days, while our results show a ~3-5 log CFU/tomato difference for tomatoes stored at 100% vs. 60-80% RH. Both Iturriaga et al. (2007) and our study used similar methods to create the specific RH environments. It is likely that the tomatoes influenced the environmental RH in Iturriaga et al. (2007), as they did in our study, so their reported 85% RH may be higher and very close 100%.

Guo et al. (2002) inoculated mature green tomatoes with 6.5-7 log CFU *Salmonella*/tomato and stored them at 20°C and ~70% RH. At the end of 14 days, their tomatoes had ~3 log CFU *Salmonella*/tomato, while at 7 days, they recovered between 3.25 and 4.25 log CFU/tomato. Our 50% RH target conditions (with actual RH of 60-80%) is the closest to those used by Guo et al. (2002), and our results show lower *Salmonella* recovery (~2-3 log CFU/tomato) despite a higher starting concentration. These differences may be due to the tomatoes (mature green vs. red ripe) or to one or more especially hardy *Salmonella* serovars in their 5-strain cocktail.

Allen et al. (2005) found about 2 log CFU/ml difference between tomatoes at 90% vs. 60% RH after 7 days, similar to 100% and 15% RH target (40-60% RH actual) found in our study. The 90% RH environment at 20°C was used by Allen et al. (2005) to imitate standard ripening room conditions. While Allen et al. (2005) saw a steady decline of *Salmonella* on tomatoes over 28 days, survival was best at this ripening room condition. Allen et al. (2005) used a different cocktail of 5 *Salmonella* serovars, and also started with mature green tomatoes making direct comparison difficult. Shi et al. (2007) used a vacuum chamber and subjected dip inoculated tomatoes to three vacuum-release

cycles to facilitate internalization prior to performing storage studies similar to ours. These researchers found that a majority of *Salmonella* can grow and become established both on and within unripened tomatoes, but growth on ripened fruit was serovar dependent. Shi et al. (2007) also noted that 8 out of 10 serovars had higher concentrations after 7 days at an RH of 95% than at 75% at 25°C, supporting our observation that RH values closer to 100% results in higher *Salmonella* populations on tomatoes.

Much of the work discussed above (as well as our own work) indicates that high RH supports better *Salmonella* survival, or may increase the chance of *Salmonella* growth on whole tomatoes. Despite these observations, an RH of 85-95% is recommended for produce storage, including tomatoes, to prevent water loss and prolong the shelf-life (Florida Tomatoes; Hardenburg et al. 1986).

Though our biofilm test results suggest that strain MAE110 may have produced biofilms on tomatoes, the quorum sensing was not evident. It may be that quorum sensing molecules were produced, but levels were too low to be detected. Iturriaga et al. (2007) also found evidence of extracellular material production with developed biofilms by day 4 for *Salmonella* Montevideo on tomatoes stored in 97% RH and 22°C. Future research with more sensitive assays or which allow longer times for biofilm development may allow detection of quorum sensing molecules.

Zaragoza et al. (2012) noted that non-rdar *Salmonella* strains were more commonly isolated from produce outbreaks than rdar strains. They found that an rdar morphotype strain had better attachment to tomato surfaces, but the non-rdar mutants had better fitness within the tomatoes. In our study, strains MAE110 and J1890 survived

similarly on tomatoes even though only MAE110 has the rdar morphotype. J1890 had better survival than MAE119 on tomatoes even though both strains have the saw morphotype. This suggests that other factors beyond rdar morphology may play a role in aiding *Salmonella* survival on tomatoes. Note that J1890 was isolated from a tomato outbreak whereas MAE110 and MAE119 were not.

Malcova et al. (2008) screened ninety-four strains of *S.* Typhimurium for their ability to form biofilm and found a correlation between morphotype and biofilm production, where rdar strains tended to form biofilms more readily than saw strains. This is in contrast the lack of correlation we observed for the small number of strains we studied. This difference may be due to growth conditions (Malcova et al. used minimal media while we used a nutrient rich medium) or because a majority of *Salmonella* strains screened by Malcova et al. (2008) came from animal hosts while our saw strains were isolated from tomatoes.

Conclusions

It is clear that *Salmonella* survival on tomatoes and plastic mulch is strain dependent, although the factors that influence survival are more complex that just the environmental RH, rdar morphotype, and biofilm production can explain. Our research does support the consensus that high RH conditions support better *Salmonella* survival, although specific conditions that might allow *Salmonella* growth on whole tomatoes are still unclear. Further testing of *Salmonella* strains isolated from tomato outbreaks may lead to better understanding as to how they survive and perhaps grow on tomatoes and potentially lead to improved control measures.

Acknowledgements

This work was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture under award number 2011-51181-30767. Special thanks to Dr. Wesley Kline and the NJ growers for the tomatoes and plastic mulch used in this experiment.

Figures

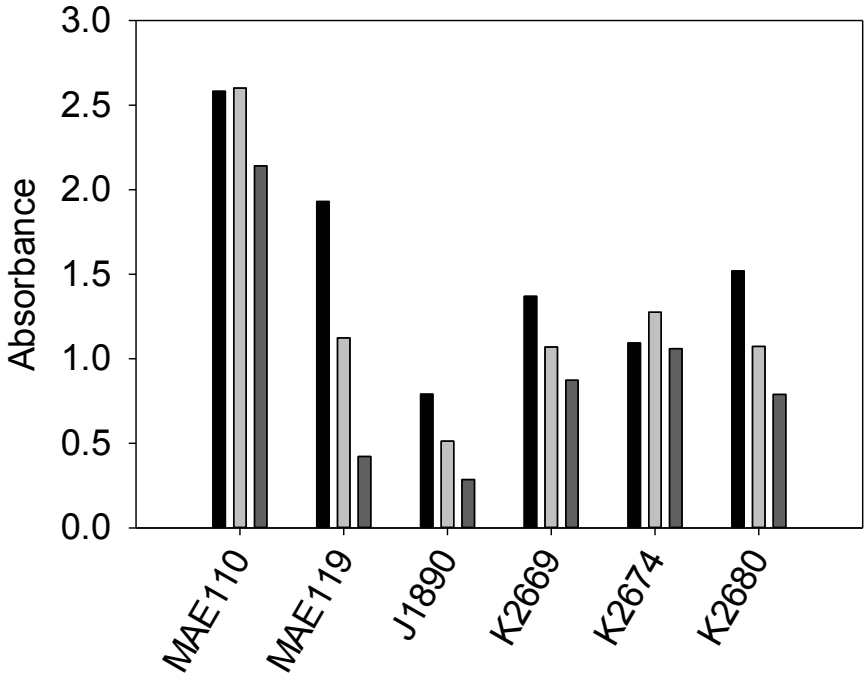

Figure 1: Absorbance at 570 nm as a measure of biofilm production by six *Salmonella* strains in 96 well plates in 3 different RH environments: 15% (▬), 50% (▬), and 100% (▬).

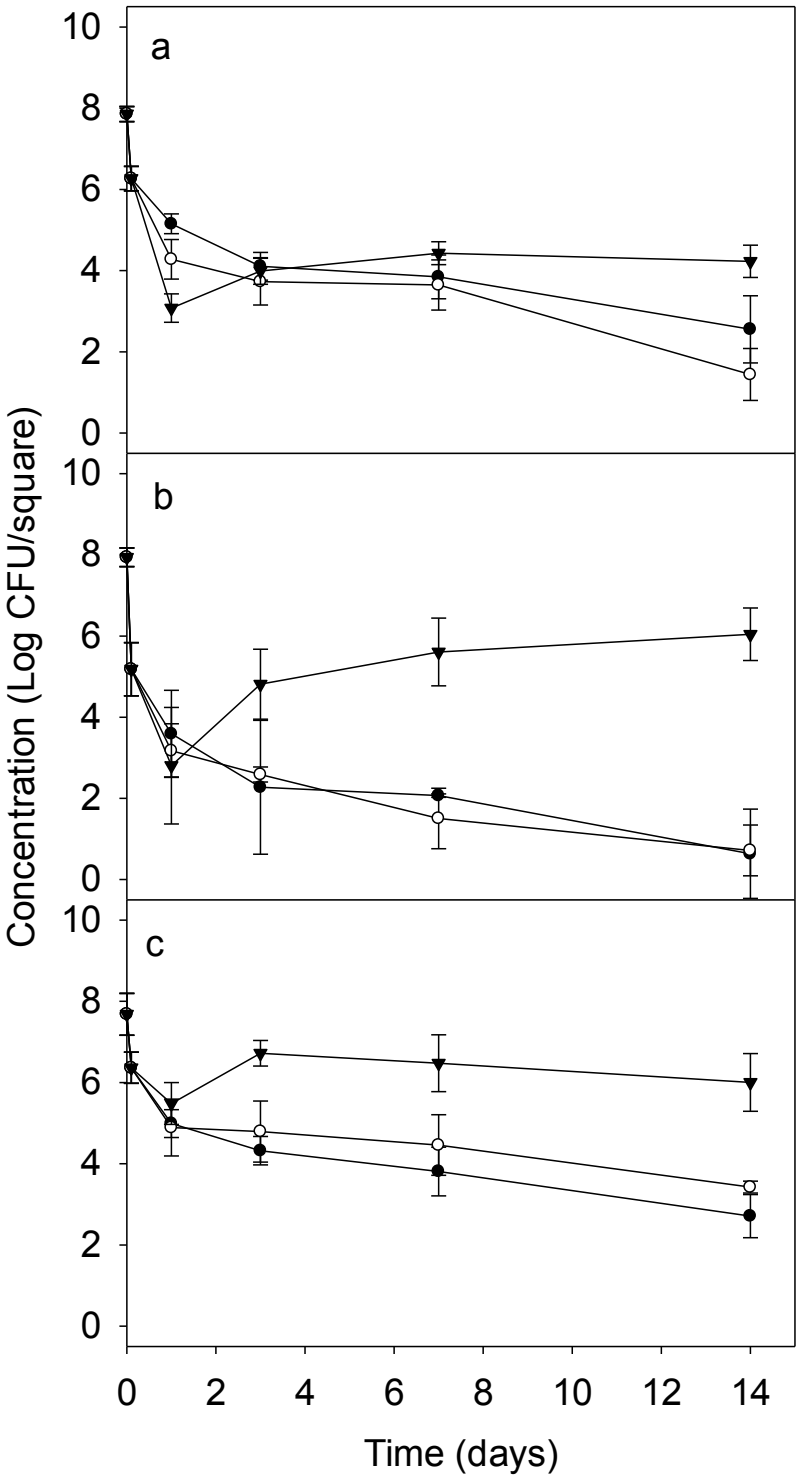

Figure 2: Survival of *Salmonella* on plastic mulch by *Salmonella* strains MAE110 (A), MAE119 (B), J1890 (C) at 15% (–●–), 50% (–○–), and 100% (–▼–) relative humidity.

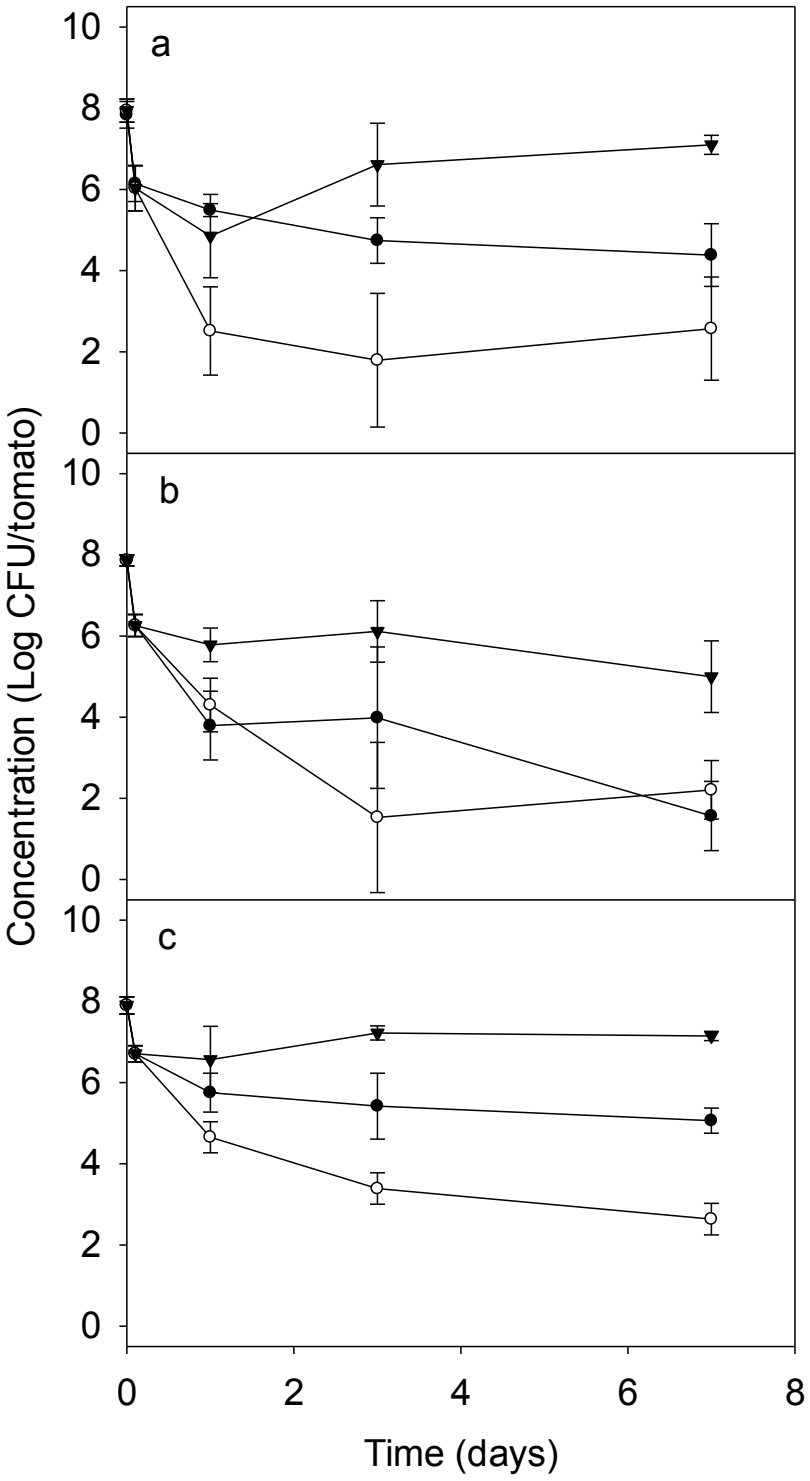

Figure 3. Survival of *Salmonella* on tomatoes by *Salmonella* strains MAE110 (A), MAE119 (B), J1890 (C) at 15% (—●—), 50% (—○—), and 100% (—▼—) relative humidity.

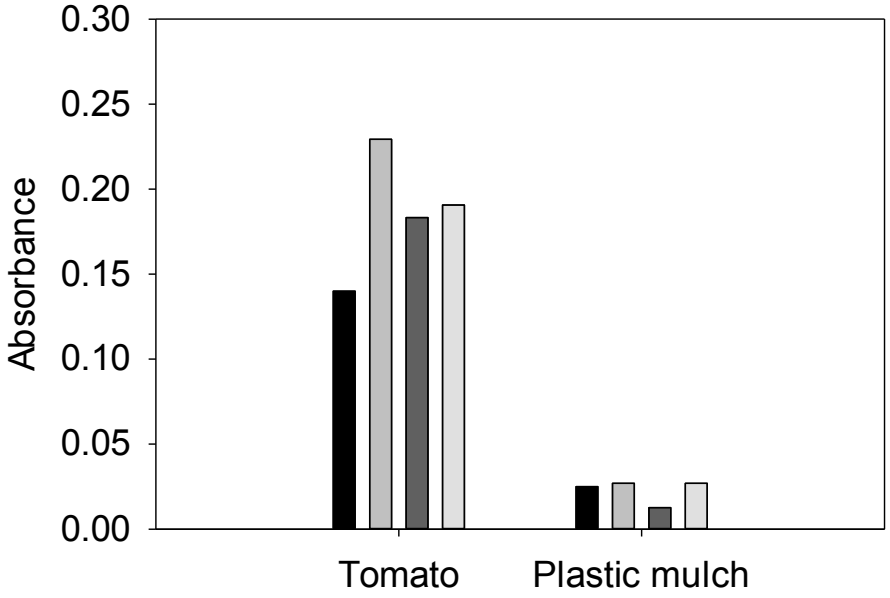

Figure 4. Biofilm production by *Salmonella* on tomatoes and plastic mulch at 100% RH as measured by A_{570}. An uninoculated tomato or plastic mulch square were used as the control (▬), and *Salmonella* strains MAE110 (▬), MAE119 (▬), J1890 (▬).

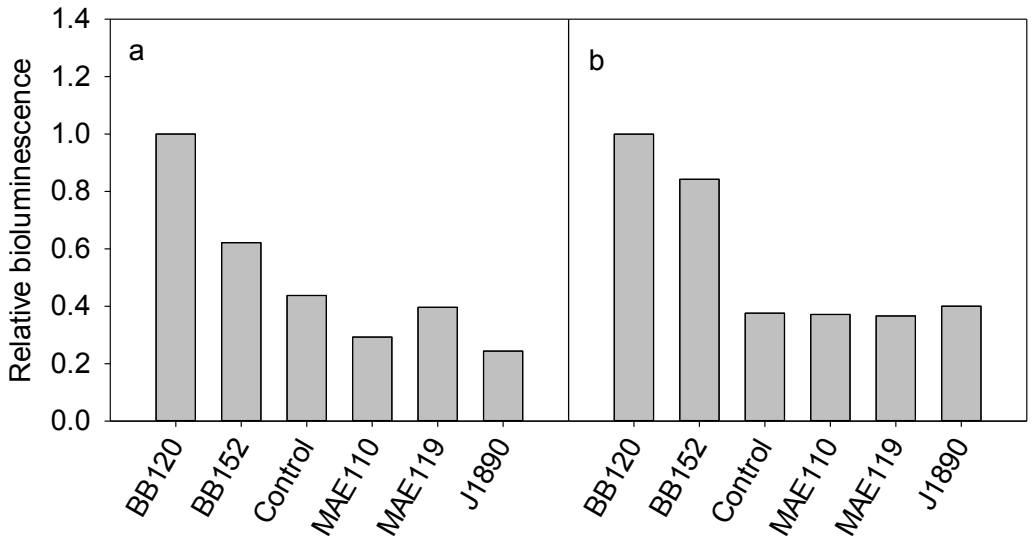

Figure 5: Relative bioluminescence of *Salmonella* on tomatoes after 2 hours. Panel A shows bioluminescence relative to sensing strain BB886 that detects Auto Inducer 1 (AHL). Panel B shows bioluminescence relative to sensing strain BB170 that detects Auto Inducer 2 (AI-2). Control samples are from tomatoes with no *Salmonella* and *Salmonella* strains are MAE110, MAE119 and J1890. *V. harveyi* strain BB120 produces both AHL and AI-2 and BB152 only produces AI-2.

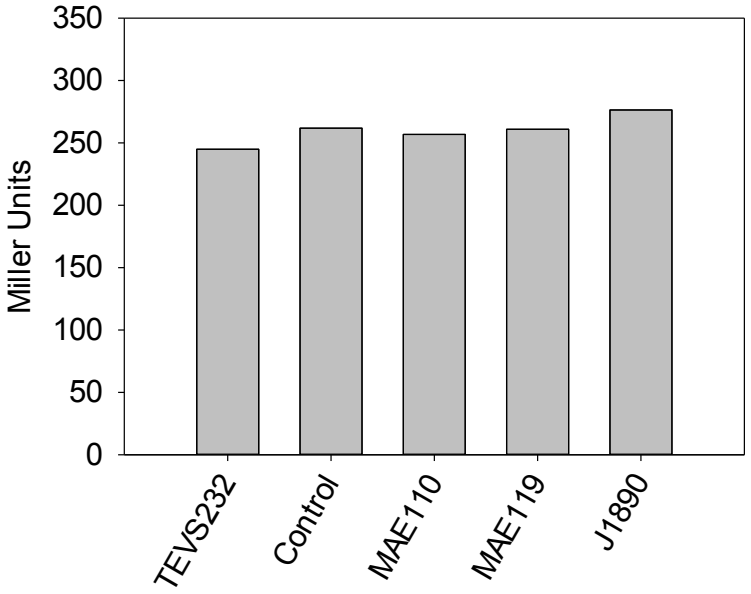

Figure 6: Detection of Auto Inducer 3 using the *E. coli* TEVS232 sensing strain compared to control (uninoculated tomato) and three *Salmonella* strains (MAE110, MAE119, and J1890).

Chapter IV: Effectiveness of sanitation procedures in controlling indicator organisms in five New Jersey tomato packinghouses

Jennifer Todd-Searle[1], Wesley Kline[2], and Donald W. Schaffner[1]

[1]Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States

[2]Rutgers Cooperative Extension, Cumberland County, Millville, NJ 08332, United States

Some data from this manuscript has been previously published:

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Abstract

Information regarding the effectiveness of sanitizers in controlling pathogens and indicator organisms under real-world fresh produce packinghouse conditions is limited. The goal of this work was to survey sanitation methods in New Jersey packinghouses and quantify their effectiveness against indicator organisms on whole, fresh tomatoes. Twenty samples of 5 tomatoes each were collected before and after sanitary treatment from 5 NJ packinghouses at 5 different times over a 3-year period (1,000 samples, 5,000 total tomatoes). Chlorine or peroxyacetic acid was applied to tomatoes via a dump tank or spray bars. Sanitizer concentrations varied between packinghouses and over the course of the study. Total plate, coliform, and presumptive *E. coli* counts were determined by plating samples on plate count agar and CHROMagar ECC. Colonies were enumerated and bacterial populations were expressed in log CFU/tomato or percentage of positive samples. Significant reductions in total plate and coliform counts typically occurred on 2-3 out of 5 visits for each packinghouse. Generally when a significant reduction in total plate count was observed, a significant reduction in coliforms also occurred during that same visit. *E. coli* prevalence was unchanged or declined after packinghouse treatment in 4 or 5 out of 5 visits for all packinghouses except for the only packinghouse that did not report having an equipment cleaning or sanitizing program. None of the 5 packinghouses had a program for monitoring tomato pulp temperature and average pulp temperature almost always exceeded dump tank temperature. NJ packinghouses can improve control of sanitizer concentrations and develop more consistent treatment times. Research is needed on shorter treatment times,

use of sanitizers in spray bar type application, and combination dump/spray application with different sanitizers.

Introduction

Tomatoes have been the source of foodborne disease outbreaks in the United States in 1990, 1993, 1999, 2002, 2004, 2005, and 2006 (Behravesh et al. 2012, Cummings et al. 2001, Greene et al. 2008, Hedberg et al. 1999, CDC 2005, 2006, & 2007). The ultimate source of contamination in these outbreaks ranged from contaminated irrigation water and field packing at the farm to cross-contamination at the packinghouse (CDC 2005, 2006, & 2007). *Salmonella* has been found in some tomato production environments, so effective post-harvesting procedures that reduce contamination and prevent cross-contamination are of critical importance (Greene et al. 2008, CDC 2015).

The FDA has published non-regulatory guidelines for United States tomato producers (FDA 2008). Florida is the only state to have regulations concerning tomato production and has developed Tomato Good Agricultural Practices (T-GAP) and Tomato Best Management Practices (T-BMP) that went into effect in 2008 (FDACS 2012). Florida T-BMP requirements state that dump tank water temperatures must be 10 °F (5.5 °C) above the incoming fruit pulp temperature to minimize the risk of intrusion of microorganisms into the tomatoes. The Florida T-BMP list free chlorine as an approved sanitizer for dump tanks at a minimum concentration of 150 ppm for a maximum of 2 minutes with frequent monitoring of the dump water for free-chlorine, water temperature, and pH. The Florida T-BMP also allows peroxyacetic acid, aqueous chlorine dioxide, gas-phase and aqueous ozone as alternatives to chlorine. Florida regulations state that any other chemicals used for pathogen control in Florida packinghouses must prove a 3 log reduction of *Salmonella* (FDACS 2012). Dump or flume tank systems as well as

spray bar and roller systems are common washing methods for tomatoes. Sanitizers are thought to reduce cross-contamination in these washing systems (Chang and Schneider 2012).

Studies have demonstrated the efficacy of many of these sanitizers against pathogens. When chlorine concentration ranged from 100 to 200 ppm and treatment time varied between 0.5 and 5 minutes, *Salmonella* reductions ranged between 1 and 6 log CFU/tomato (Bari et al. 2003, Chaidez et al. 2007, Iturriaga and Esartín 2010, Mattson et al. 2011, Sapers and Jones 2006, Xu and Wu 2014, Yuk et al. 2005, Zhuang 1995). Chang and Schneider (2012) found that 80 ppm peroxyacetic acid applied by spray bar with 5 and 60 s application times gave a 3 to 6 log CFU/tomato reduction in *Salmonella* concentration. Yuk et al. 2005 showed that 87 ppm peroxyacetic acid applied via dump tank reduced *Salmonella* concentration 2 and 6 log CFU/tomato.

Indicator organisms are thought to "indicate" the presence of pathogens and can be used when pathogen concentrations are low (Bell et al. 2015; Micallef et al. 2012; Mukherjee et al. 2004), as well as when testing for pathogens would not be cost effective, would be too time consuming, or would not be practical. Efstratiou et al. (1998) found that total coliforms, fecal coliforms, and fecal streptococci all had strong associations with *Salmonella* in sea water. McEgan et al. (2013) found weak correlations between indicator organisms (*E. coli* and coliforms) and *Salmonella* in Florida surface waters.

Studies have demonstrated the efficacy of sanitizers against indicator organisms and pathogens on tomatoes under laboratory conditions. Few studies have addressed use of these chemicals in real world packinghouse conditions. The goal of this study was to

characterize how sanitizers were being used in 5 New Jersey tomato packinghouses and determine their effectiveness on indicator organisms.

Materials and Methods

Tomato sampling

Tomatoes were sampled from 5 New Jersey farms (12-50 acres in size) at 5 different times over 3 consecutive years. A total of 100 tomatoes were sampled before treatment at the packinghouse as well as after treatment for each packinghouse visit. Tomatoes were grouped into 20 samples of 5 tomatoes per sample. The samples taken from before the dump tank were selected from the top as well as the bottom of the bulk harvest bin. Pulp temperature was measured prior to sanitation at the packinghouse by taking the average of 5 tomato pulp temperatures. The probe of a FoodPro Plus thermometer (Fluke, Everett, WA) was inserted into the center of the tomato. Samples were stored on ice during transport to the lab where microbiological analysis was completed within 24 hours of sampling.

Information about packinghouse sanitary procedures was also taken during each visit, including dump tank temperature, sanitation step time and sanitizer concentration. Peroxyacetic acid concentration was measured using BioSafe Systems test strips (BioSafe Systems, East Hartford, CT) and free chlorine concentration was measured using Water Works Free Chlorine Check test strips (Industrial Test Systems, Inc., Rock Hill, SC). Each packinghouse was surveyed during the last visit to assess management knowledge and understanding of food safety. The survey included questions about sanitizer level monitoring, microbiological testing, and safety protocols used to prevent microbial, chemical, and physical contaminants.

Microbiological analysis

Each sample of 5 tomatoes was massaged in a sterile filter bag containing 100 ml of 0.1% peptone water (Becton Dickinson, Franklin Lakes, NJ) to remove bacteria. If the tomatoes had been washed with chlorine at the packinghouse, the peptone water contained 0.6% sodium thiosulfate (Acros Organics, Thermo Fisher Scientific, Waltham, MA) to deactivate the chlorine. Serial dilutions were made in 0.1% peptone water and samples were duplicate plated on total plate count agar (PCA: BD) and CHROMagar ECC (DRG International, Inc., Springfield, NJ) to determine total plate, coliform, and presumptive *E. coli* counts. PCA was incubated for 48 h at 30°C and CHROMagar ECC was incubated for 24 h at 37°C.

Colonies were counted after incubation and the log CFU/tomato was determined. Generic *E. coli* counts were low, so *E. coli* results were presented as percentage of positive samples. Blue colonies on ECC were isolated on tryptic soy agar (TSA) (Thermo Fisher Scientific) and confirmed as generic *E. coli* using BD Enterotube^{TM} II (BD).

Statistical analysis

Excel (Microsoft, Redmond, WA) was used to calculate means and standard deviations. The Excel add-in, StatTools 7 (Palisade Corporation, Ithaca, NY) was used to calculated statistical difference ($p<0.05$).

Results

Packinghouse A utilized Solution 1 in a dump tank for sanitation (Table 1). Solution 1 contained a mixture of 23.0% hydrogen peroxide and 5.3% peroxyacetic acid. The label for Solution 1 indicates it is for control of spoilage organisms, not organisms of

public health concern. The label indicates it should be diluted to yield a solution of 24 to 85 ppm peroxyacetic acid, and should be in contact with tomatoes for at least 45 seconds. Solution 1 was found at concentrations between 20 and >160 ppm during the five visits at Packinghouse A, with contact times between 2 min 20 sec and 4 min 26 sec. The temperature in the packinghouse varied between 23 and 30°C with the temperature in the dump tank between 19 and 24°C. Average pulp temperature was between 20.5±0.2 and 27.8±0.7°C.

Packinghouse A (Figure 1) had a mean reduction in total plate count between 0.39±0.84 and 1.05±0.65 log CFU/tomato and for coliforms between 0.05±0.71 and 1.43±0.63 log CFU/tomato. Statistically significant ($p<0.05$) reductions occurred in August and September 2014 and September 2015. Packinghouse A had a consistently higher *E. coli* positive samples after the wash (0-20% vs. 5-85%), which suggests that either contamination was introduced at the packinghouse or that *E. coli* contamination was spread from a few contaminated tomatoes to previously uncontaminated tomatoes.

Packinghouse B had a spray bar and dump tank setup with Solution 1 at a concentration between 0 and >160 ppm (Table 1), and contact times from 2 min 17 sec to 13 min. The average pulp temperature of the tomato was between 22.9±0.2 and 37.6±2.7°C with the average temperature of the dump tank between 22 and 29°C. The temperature in the packinghouse was between 20 and 34°C.

The mean total plate count reduction for packinghouse B (Figure 2) was between 0.35±1.00 and 1.67±0.91 log CFU/tomato and coliform reduction was between 0.59±1.49 to 1.68±1.22 log CFU/tomato. Significant ($p<0.05$) reductions in total plate count and coliforms occurred in August 2013 and 2014 and July 2015. While some tomato samples

were positive for *E. coli* before the wash, there was always a reduction in the number of positive samples at the end of the sanitation procedure, and no *E. coli* detected on 4 or 5 sampling days.

Packinghouse C employed a dump tank and spray bar setup similar to Packinghouse B (Table 1). Solution 1 was used during the first 4 sampling times and sodium hypochlorite was in use at the last sampling time. Contact times ranged from 45 sec to 4 min 33 sec. Dump tank sanitizer concentration was between 10 and 85 ppm peroxyacetic acid or 100 ppm free chlorine. Chlorine was reportedly utilized in the spray bar, although a measureable residual was never detected. The average temperature in the dump tank was 25 to 30°C with average tomato pulp temperature ranging from 24.5±0.5 and 32.9±1.4°C. The temperature in the packinghouse ranged from 21 to 32°C.

Packinghouse C (Figure 3) had a mean reduction in total plate count between 0.38±1.04 and 0.74±0.89 log CFU/tomato and in coliform counts between -0.14±0.83 to 0.56±0.97 log CFU/tomato. Total plate count reductions were statistically significant ($p<0.05$) in August 2013, 2014, and 2015. A statistically significant reduction in coliform counts was never detected. In the first year, *E. coli* positive samples were detected before washing on one visit (20%), but the percentage was decreased after sanitation at the packinghouse (5%). On three visits no *E. coli* were detected before or after packing. In July 2015, *E. coli* positive samples increased from 0% to 35% after packinghouse treatment.

Packinghouse D used a spray bar and roller system with chlorine (10 ppm) at the first sampling time (Table 1). Packinghouse D switched to Solution 2 for the next 3 sampling periods (50-85 ppm peroxyacetic acid), and then to Solution 1 for the final

sampling (85 ppm peroxyacetic acid). Contact times for Packinghouse D ranged from 1 min 46 sec to 3 min 30 sec. Solution 2 contains 27.0% hydrogen peroxide and 2.0% peroxyacetic acid. Directions for Solution B are to dilute it to yield 24 to 85 ppm peroxyacetic acid. Average tomato pulp temperature ranged from 18.0±1.9 and 34.8±3.6°C with the temperature of the water in spray bar ranging from 14 to 25°C. The temperature in the packinghouse ranged from 20 to 25°C over the 5 sampling periods.

Packinghouse D (Figure 4) had mean reductions in total plate count between 0.09±0.66 and 1.15±0.86 log CFU/tomato. Mean reductions for coliform count were between 0.29±0.86 and 1.36±0.68 CFU/tomato. Significant ($p<0.05$) reductions in total plate and coliform counts occurred twice at the same two visits (August 2013 and September 2015). The percentage of *E. coli* positive samples declined after packing in three instances (5-65 to 0-25%), but increased in two other instances (0 to 15% and 25 to 40%).

Packinghouse E used a spray bar and roller system like Packinghouse D (Table 1). Chlorine was used in 2013, but the concentration was not determined. Packinghouse E used Solution 1 at a concentration between 20 and 160 ppm was used in 2014 and 2015 and contact times ranged from 2 min 10 sec to 6 min 50 sec. The temperature in the packinghouse ranged from 16 to 21°C. The temperature of the water in the spray bar ranged from 15 to 21°C with average tomato pulp temperature ranging from 18.1±0.9 and 25.8±0.4°C.

Packinghouse E (Figure 5) had mean reductions in total plate count from -0.10±0.50 to 0.75±0.64 log CFU/tomato and between 0.43±0.83 and 1.18±0.68 log CFU/tomato for coliforms. These reductions were only statistically significant on two of

the 5 visits ($p<0.05$). One pre wash sample was positive for *E. coli* in July 2015, but *E. coli* was not recovered on any tomatoes after treatment. *E. coli* was not detected on any other samples.

Food safety survey results revealed that packinghouses B, D, and E were actively monitoring sanitizer levels and doing routine microbiological testing on their water supplies. Packinghouse C had no documented food safety plan or training program for their workers while the other four facilities did have documented food safety plans and were training their workers. Packinghouse D used a third party auditor, but the other four facilities did not. Packinghouse B and E reported cleaning their packinghouse equipment every day, Packinghouse D reported sanitized their equipment every two weeks. Packinghouse C reported cleaning their equipment, but did not disclose how often. Packinghouse A did not comment on equipment cleaning frequency. None of the 5 packinghouses had a protocol for monitoring temperature differential between the tomatoes and wash water. It is therefore not surprising that in the 22 instances where water and average tomato temperatures were available, only 4 times were average tomato temperatures below water temperature (two only very slightly), and in only one case was average tomato temperature more than 5.6 °C below water temperature (Packinghouse D, 9/30/14 visit), as required by the T-BMP.

Discussion

Sanitizer concentration varied widely between packinghouses and within a packinghouse at different times over the course of the study. Low sanitizer concentrations can lead to poor pathogen control and can facilitate cross-contamination between tomatoes (Chang and Schneider 2012). In some cases sanitizers were found at

or above maximum concentrations permitted on the label, which is a violation of federal law. NJ packinghouses used 4 different sanitizers: calcium hypochlorite, sodium hypochlorite, and two commercially formulated sanitizers each containing hydrogen peroxide and peroxyacetic acid (identified as solution 1 and solution 2 here). The two commercially formulated sanitizers were both designed to be diluted to yield 24 to 85 ppm peroxyacetic acid, with solution 2 having approximately 2 fold greater concentration of hydrogen peroxide after dilution. Solution 1 is labeled for control of plant pathogens, while solution 2 is labeled for control of human and plant pathogens.

The results we observed in NJ packinghouses were consistent with or lower than observations reported in the published literature, similar to what has been observed in citrus packinghouses in Florida (Pao and Brown 1998). Beuchat et al. (1998) found significant reductions in aerobic mesophiles from 0.5 and 1 log CFU/cm^2 when 200 ppm chlorine was applied to tomatoes for treatment times from 3 to 10 minutes. Keeratipibul et al. (2011) report that soaking tomatoes in chlorine (25-75 ppm) for 10 minutes led to reductions between 2.25 and 3 log CFU/g for coliforms and generic *E. coli*. Soaking tomatoes in peroxyacetic acid (30-50 ppm) for 10 minutes led to reductions between 3.5 and 4.5 log CFU/g for coliforms and *E. coli* (Keeratipibul et al. 2011). Our data from NJ packinghouses do reveal areas where packinghouses can improve their sanitizer use practices including better control of sanitizer concentrations and more consistent treatment times. Our findings also indicate that research is needed using more realistic (i.e. shorter) treatment times, use of sanitizers in spray bar type application as well as combination dump/spray application and dual sanitizer use (e.g. peroxyacetic acid dump tank and chlorine in spray bar).

The FDA recommends that dump tank water be at least 5.6 °C (10 °F) above the pulp temperature of the tomatoes (FDA 2008), and Florida requires that dump tank water meet this same criterion (FDACS 2012). This recommendation/requirement stems from concern over the internalization of *Salmonella* (Zhuang et al. 1995), which can occur because warm tomatoes contract in volume when placed in cold dump tank water. As the tomatoes contract, and their volume decreases water is drawn into the fruit. Any bacteria present in the water are drawn into the fruit. Internalized bacteria may multiply and are also more resistant to control by sanitizers.

None of the 5 packinghouses measured tomato pulp temperature, and none had protocols in place to address the need for a temperature differential. As noted above, in the 22 instances where water and average tomato temperatures were available, average tomato temperatures were below water temperature only four times (and of those, twice only slightly, 0.1 and 0.2 °C). In only one case was average tomato temperature more than 5.6 °C below water temperature. Interestingly in the one instance where average tomato temperature more than 5.6 °C below water temperature (Packinghouse D, September 30, 2014 visit) neither total count nor coliform count reductions were significant, and post-wash *E. coli* prevalence increased (Fig 4). In the other instance where average tomato temperature was 2.7 °C below water temperature (Packinghouse A, September 22, 2014), bacterial reductions were significant, but post-wash *E. coli* prevalence also increased (Fig 1).

Schneider et al. (2017) found that tomatoes from Florida packinghouses had significantly lower aerobic plate and total coliform counts compared to tomatoes from

Maryland and New Jersey, both before and after packinghouse treatment. This difference may be due to the mandatory T-GAP and T-BMP requirements in Florida.

Conclusion

The New Jersey packinghouses studied had very different sanitary procedures from one another and perhaps as a result, also showed a wide range of bacterial reductions. Significant reductions in total plate and coliform counts typically occurred on 2-3 out of 5 visits for each packinghouse. Generally when a significant reduction in total plate count was observed, a significant reduction in coliforms also occurred during that same visit, except for Packinghouse C, which never observed a significant reduction in coliform counts. *E. coli* prevalence was unchanged or declined after packinghouse treatment in 4 or 5 out of 5 visits for all packinghouses except Packinghouse A. *E. coli* prevalence increased after treatment in all 5 visits to Packinghouse A which did not monitor sanitizer concentration or regularly perform microbiological tests on water samples. Packinghouse A did not report having any equipment cleaning or sanitizing program when surveyed. None of the 5 packinghouses surveyed reported a program for monitoring tomato pulp temperature and average pulp temperature almost always exceeded dump tank temperature. NJ packinghouses can improve their sanitizer use practices including better control of sanitizer concentrations and more consistent treatment times. More research is needed using more realistic (i.e. shorter) treatment times, use of sanitizers in spray bar type application as well as combination dump/spray application and dual sanitizer use (e.g. peroxyacetic acid dump tank and chlorine in spray bar).

Acknowledgements

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Figures

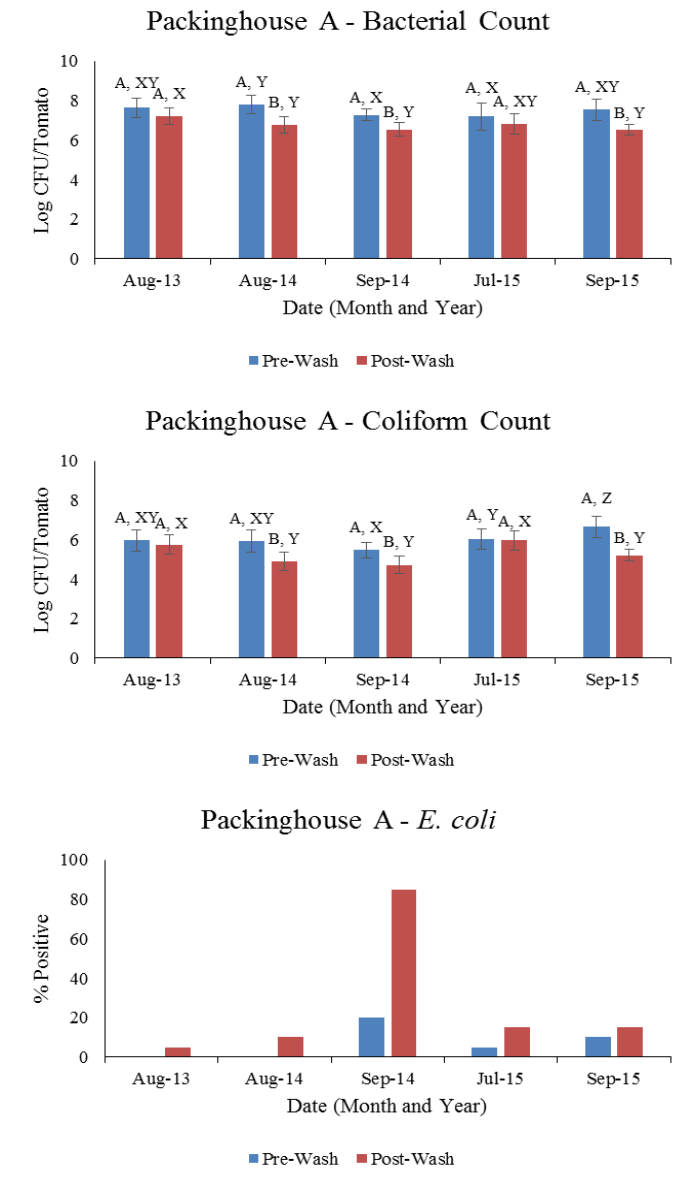

Figure 1: Microbial results for Packinghouse A. Top: Pre and post wash levels for total aerobic plate count. Middle: pre and post wash levels for coliform count. Bottom: Pre and post wash percent positive for generic *E. coli*. A and B denote a significant ($p<0.05$) difference in bacterial counts between pre- and post-wash tomatoes in the same sampling period. X, Y, and Z denote significant ($p<0.05$) difference within pre- or post-wash counts over all 5 sampling days.

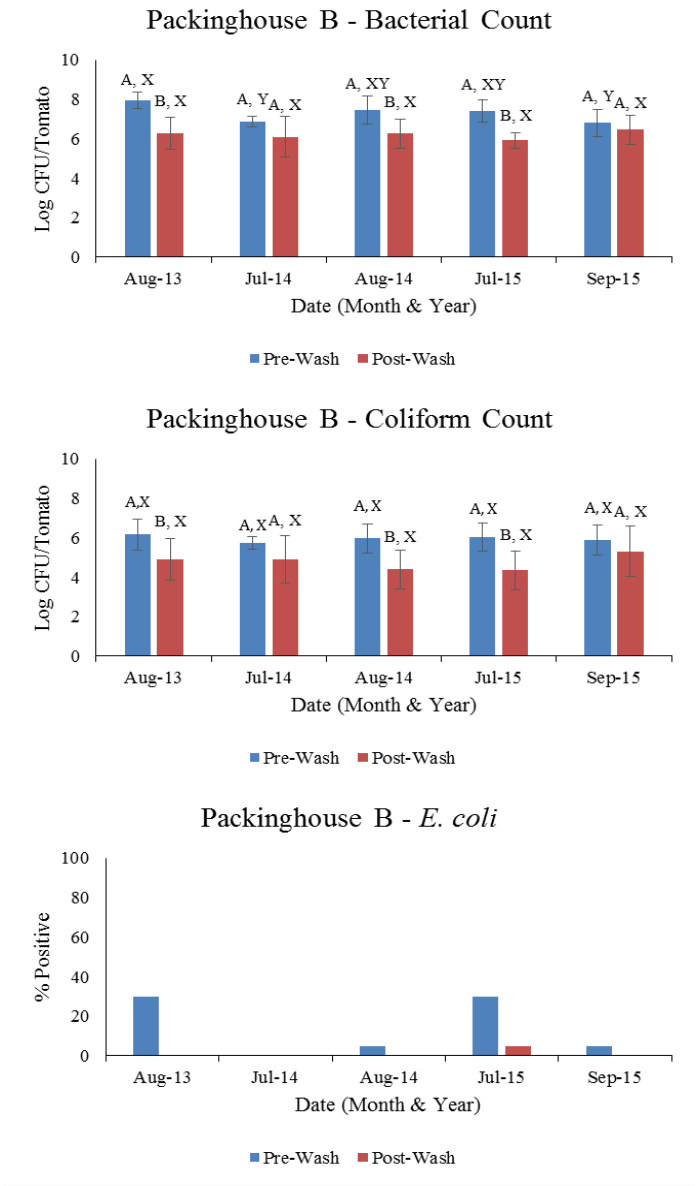

Figure 2: Microbial results for Packinghouse B. Top: Pre and post wash levels for total aerobic plate count. Middle: pre and post wash levels for coliform count. Bottom: Pre and post wash percent positive for generic *E. coli*. A and B denote a significant ($p<0.05$) difference in bacterial counts between pre- and post-wash tomatoes in the same sampling period. X, Y, and Z denote significant ($p<0.05$) difference within pre- or post-wash counts over all 5 sampling days.

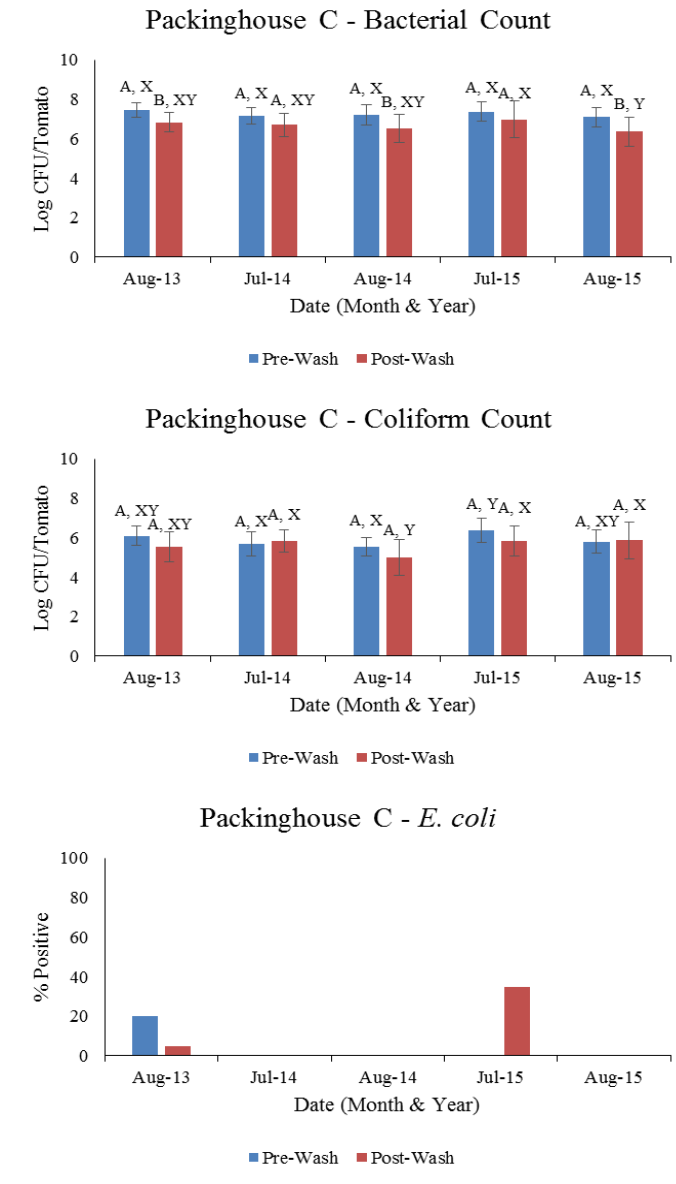

Figure 3: Microbial results for Packinghouse C. Top: Pre and post wash levels for total aerobic plate count. Middle: pre and post wash levels for coliform count. Bottom: Pre and post wash percent positive for generic *E. coli*. A and B denote a significant ($p<0.05$) difference in bacterial counts between pre- and post-wash tomatoes in the same sampling period. X, Y, and Z denote significant ($p<0.05$) difference within pre- or post-wash counts over all 5 sampling days.

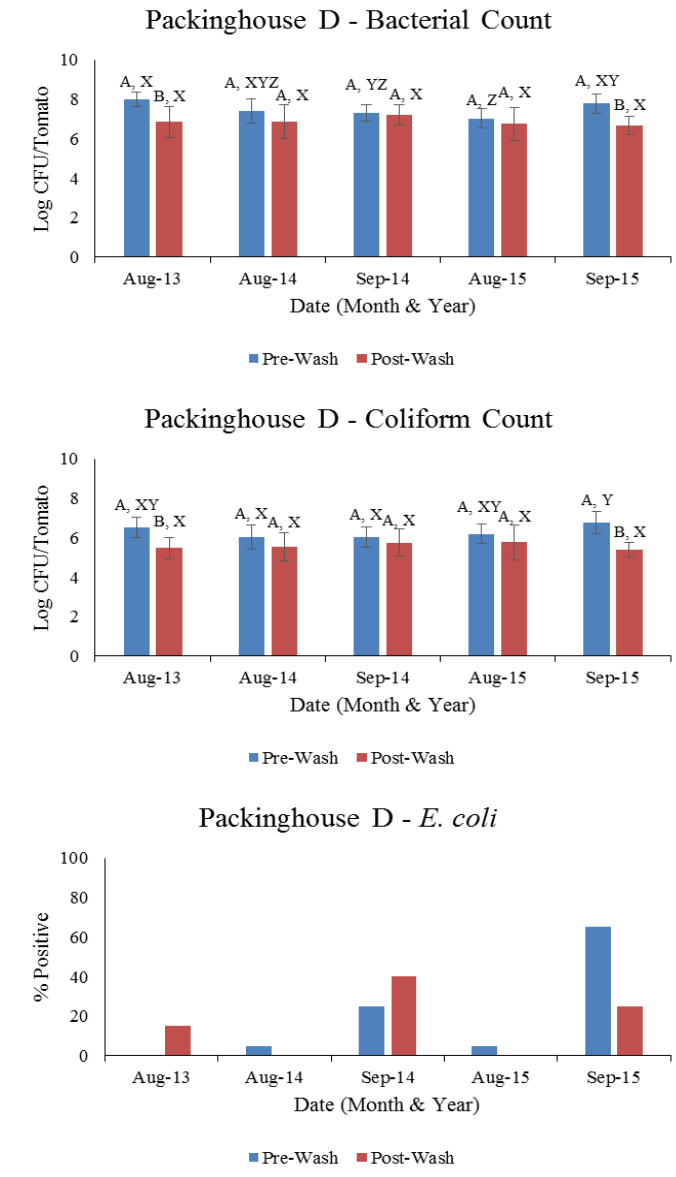

Figure 4: Microbial results for Packinghouse D. Top: Pre and post wash levels for total aerobic plate count. Middle: pre and post wash levels for coliform count. Bottom: Pre and post wash percent positive for generic *E. coli*. A and B denote a significant ($p<0.05$) difference in bacterial counts between pre- and post-wash tomatoes in the same sampling period. X, Y, and Z denote significant ($p<0.05$) difference within pre- or post-wash counts over all 5 sampling days.

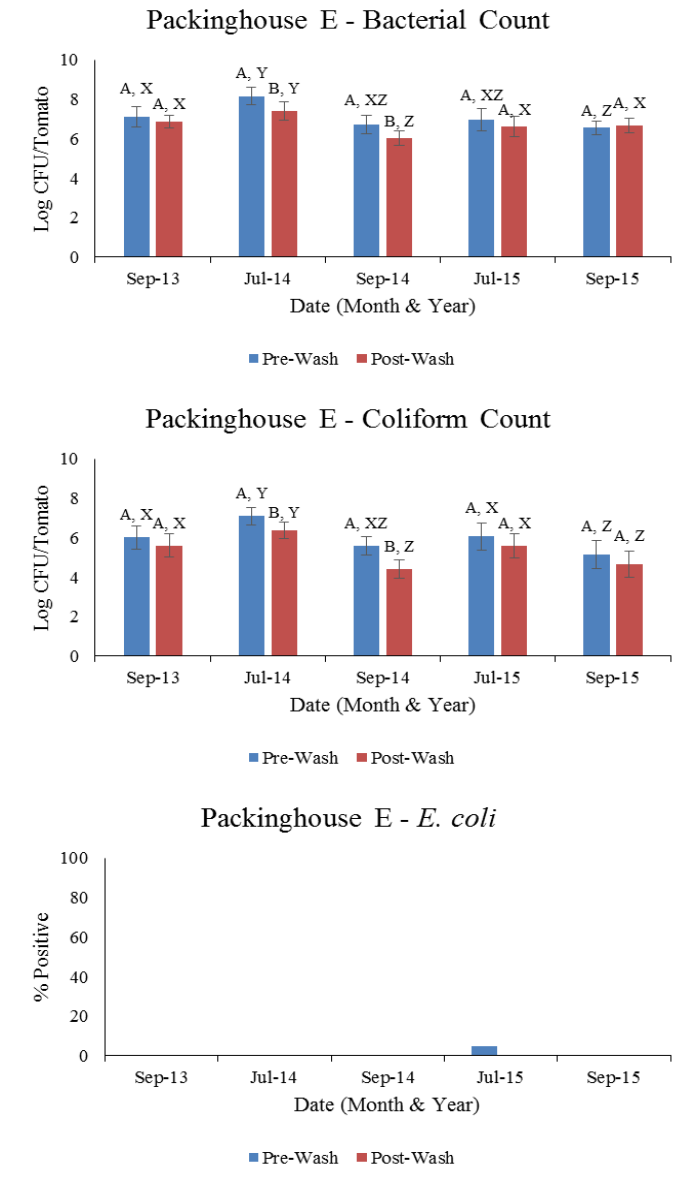

Figure 5: Microbial results for Packinghouse E. Top: Pre and post wash levels for total aerobic plate count. Middle: pre and post wash levels for coliform count. Bottom: Pre and post wash percent positive for generic *E. coli.* A and B denote a significant ($p<0.05$) difference in bacterial counts between pre- and post-wash tomatoes in the same sampling period. X, Y, and Z denote significant ($p<0.05$) difference within pre- or post-wash counts over all 5 sampling days.

Tables

Table 1: Summary of sanitation interventions and other relevant data at five New Jersey tomato packinghouses.

Packinghouse	Visit	Temperature (°C)				Water treatment		Concentration (ppm)		Contact Time
	Date	Ambient	Dump tank	Spray bar	Pulp[1]	Dump tank	Spray bar	Dump tank	Spray bar	(min:sec)
A	8/7/13	22.8	18.9	N/A[2]	20.5 ± 0.2	S1[3]	N/A	50-60 PA[4]	N/A	2:30
	8/19/14	23.9	20.9	N/A	22.6 ± 2.4	S1	N/A	50-85 PAA	N/A	2:35-3:40
	9/22/14	24.1	24.2	N/A	**21.5 ± 2.8**	S1	N/A	>160 PAA	N/A	2:20
	7/8/15	30.2	20.8	N/A	27.8 ± 0.7	S1	N/A	50-85 PAA	N/A	4:08
	9/10/15	25.3	21.2	N/A	24.5 ± 0.3	S1	N/A	20-50 PAA	N/A	4:26
B	8/8/13	20.3	24.8	-[5]	29.5 ± 1.7	S1	S1	-	-	5:00-13:00
	7/8/14	33.4	28.4	-	37.6 ± 2.7	S1	S1	85-160 PAA	>160 PAA	3:06
	8/22/14	26.3	24.6	-	25.8 ± 1.2	S1	S1	50-85 PAA	85-160 PAA	3:45-4:00
	7/15/15	24.4	22.2	-	22.9 ± 0.2	S1	S1	0 PAA	160 PAA	2:17
	9/9/15	33.7	28.7	-	**28.6 ± 1.3**	S1	S1	0-10 PAA	160 PAA	6:35
C	8/6/13	21.1	24.7	-	**24.5 ± 0.5**	S1	NaClO	-	-	0:45
	7/11/14	31.4	29.2	-	32.1 ± 2.8	S1	NaClO	10-20 PAA	0 Cl[6]	1:17-4:33
	8/28/14	26.8	29.1	-	31.7 ± 4.8	S1	NaClO	50-85 PAA	0 Cl	1:44
	7/16/15	26.9	24.7	-	32.4 ± 0.9	S1	NaClO	50-85 PAA	0 Cl	2:12
	8/25/15	28.3	30.0	-	32.9 ± 1.4	NaClO	NaClO	100 Cl	0 Cl	2:36
D	8/13/13	20.9	N/A	14.3	21.8 ± 0.6	N/A	$Ca(ClO)_2$	N/A	10 Cl	3:30
	8/5/14	24.9	N/A	22	34.8 ± 3.6	N/A	S2[7]	N/A	>85 PAA	1:46-1:51
	9/30/14	19.6	N/A	25.1	**18.0 ± 1.9**	N/A	S2	N/A	50-85 PAA	2:15
	8/13/15	22.11	N/A	-	22.8 ± 1.8	N/A	S2	N/A	85 PAA	1:53
	9/30/15	22.7	N/A	16.8	23.1 ± 0.5	N/A	S1	N/A	85 PAA	2:01
E	9/30/13	18.4	N/A	15.2	18.1 ± 0.9	N/A	$Ca(ClO)_2$	N/A	-	2:41-3:19
	7/17/14	21.4	N/A	21.2	25.8 ± 0.4	N/A	S1	N/A	85-160 PAA	2:10-6:50
	9/25/14	15.8	N/A	-	24.7 ± 0.4	N/A	S1	N/A	50-85 PAA	4:14
	7/8/15	27.6	N/A	-	24.9 ± 0.9	N/A	S1	N/A	85 PAA	2:46
	9/28/15	22.8	N/A	16.3	21.2 ± 0.6	N/A	S1	N/A	20 PAA	2:42

1: Mean pulp temperatures in bold are below corresponding water temperature

2: N/A: packinghouse did not have that component (i.e. dump tank or spray bar).

3: S1: 23.0% hydrogen peroxide and 5.3% peroxyacetic acid, diluted to yield 24 to 85 ppm peroxyacetic acid

4: PAA: Peroxyacetic acid

5: –: measurement not taken

6: Cl: Chlorine

7: S2: 27.0% hydrogen peroxide and 2.0% peroxyacetic acid, diluted to yield 24 to 85 ppm peroxyacetic acid

Chapter V: Temperature changes in whole tomatoes and bagged lettuce from retail to home

Jennifer Todd-Searle[1] and Donald W. Schaffner[1]

[1]Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States

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Abstract

Fresh produce is at risk for temperature abuse during transportation from retail store to the consumers' home. The risk for pathogen growth depends upon transport time, transport temperature, and the nature of the produce. The temperature changes of bagged, leafy greens and whole, fresh tomatoes purchased from four different locations from store to home was measured 25 times over a 1 year period. Measurements included temperatures recorded during the 1 h period after arrival at the home (where leafy greens were refrigerated or tomatoes remained on the counter). *Salmonella* growth on leafy greens (assuming no lag phase) was predicted using a previously published growth model. *Salmonella* growth on tomatoes was not likely given published data and other data elsewhere in this dissertation. Predicted increases in *Salmonella* concentration in leafy greens varied from 0.008 to 0.064 log CFU/g. Maximum temperatures of leafy greens varied from 6.20±1.37 to 16.76±5.40, 9.81±2.70 to 17.69±7.32, 8.52±2.08 to 15.46±3.52, and 6.11±2.74 to 13.15±2.49 for the spring, summer, fall, and winter, respectively, and showed no significant difference between seasons ($p<0.05$). While pathogen growth in some fresh produce over its shelf life is possible, this research suggests that increases in *Salmonella* concentration from retail to home are unlikely to contribute significantly to risk.

Introduction

The increase in production and consumption of fresh produce has been linked to an increase in the number of illnesses due to outbreaks concerning fresh produce (Olaimat and Holley 2012). *Salmonella* species were responsible for almost 40% of the foodborne outbreaks due to produce contamination between 1988 and 2007 (Greig and Ravel 2009). Fresh produce can become contaminated at any point from farm to plate and includes sources such as domesticated animals in nearby fields, irrigation water, contamination in the packinghouse, and during food preparation (Beuchat and Ryu 1997, Cummings et al. 2001; Greene et al. 2008; Gupta et al. 2007; Hedberg et al. 1999).

Fresh produce is at risk for temperature abuse during its entire shelf life, including transport from retail locations to consumer's homes. This rise in temperature may lead to pathogen growth. Studies have modelled *Salmonella* growth in cut leafy greens (Danyluk and Schaffner 2011; Mishra et al 2017a; Sant'Ana et al. 2012), however, real world data for leafy greens temperature changes from retail to consumer home has not been previously documented. Temperature changes in fresh meat, fish, deli, and cheese products during transport from retail to a home have been documented (Ecosure 2008).

Studies have investigated the growth of *Salmonella* in cut tomatoes (Asplund and Nurmi 1991; Beuchat and Mann 2008), and Pan and Schaffner (2010) developed a model predicting the growth of *Salmonella* in cut tomatoes. While many studies have looked at the survival and potential growth of *Salmonella* on whole tomatoes (Allen et al. 2005; Das et al. 2006; Guo et al. 2002; Iturriaga et al. 2010; Pao et al. 2012; Shi et al. 2007; Todd-Searle et al.; Yuk et al. 2005; Zhuang et al. 1995), there are currently no published models for *Salmonella* growth or survival on whole tomatoes.

The temperature change of bagged lettuce and tomatoes during transport from grocery store to home at multiple times over the course of a year was monitored to determine the risk of *Salmonella* growth.

Materials and Methods

Collection of temperature data

Four different stores were visited at least six times per season for a total of 25 trials. Trials were roughly 2 weeks apart, and seasons were defined as: Winter (December, January, and February), Spring (March, April, and May), Summer (June July, and August), and Fall (September, October, and November).

EL-USB-TC Thermocouple Data Loggers (DATAQ Instruments, Akron, OH) were used to monitor temperatures during transport for bagged lettuce and whole tomatoes. Dataloggers were started prior to entering the supermarket. The following time points were noted: arrival at the store, inserting data logger into bagged lettuce, inserting data logger into the tomato, standing in line for checkout, exiting the store, arriving at car, leaving parking lot, parking at home, entering the home, and placing lettuce in home refrigerator.

Three bagged, chopped lettuce samples (6.5-12 oz) and 3 individual roma tomatoes were selected during each shopping trip and a data logger was placed in each. The store produce storage temperature was recorded if visible from the store thermometer. One additional data logger was used to record ambient temperature during the shopping trip and transport. Tomato samples were placed on the counter after arrival at home and the bagged lettuce was placed in the fridge. All sample temperatures were recorded for 1 additional hour at home before the data logging was complete.

Growth model for leafy greens

The growth model for *Salmonella* on leafy greens presented in Mishra et al. (2017a) was used to calculate potential pathogen growth for each trial. Growth was predicted for the time interval from pick up at retail through one hour in the fridge. The prediction assumed lag phase did not occur. Mishra et al. (2017a) modified the square root model from Ratkowsky et al. (1982) which resulted in the following equation: $\sqrt{\mu} = b((T_a - (T_a - T_o)e^{-Bt}) - T_{min})$, where μ is the specific growth rate (ln CFU/(g*h)). T_a is the air temperature around the produce with T_o the temperature of the produce. T_{min} and b are constants with values of -0.57 and 0.02, respectively (Mishra et al. 2017b). The value for B was determined to be 0.017 min^{-1} and was taken from Mishra et al. (2017a). The environmental temperature in the refrigerator was assumed to be 4°C based on FDA recommendations (FDA, 2014).

Growth model for tomatoes

One goal of this dissertation was to develop a model for *Salmonella* growth and/or survival on whole tomatoes (chapter 6). That model found that *Salmonella* survival depends on multiple factors and did not find a clear association between *Salmonella* survival and temperature. The model developed in chapter 6 uses a combination of triangular distributions based on the number of days after a contamination event. Growth and/or survival of *Salmonella* on whole tomatoes from retail to the consumer home due to temperature changes were not calculated because of the unexpected complexities in developing this model.

Results & Discussion

Grocery store C refrigerated tomatoes while stores A, B, and D stored tomatoes at room temperature. Despite that, the tomatoes from grocery store C did not always have the lowest initial temperature. Tomato initial temperatures ranged from 6.76±1.63 to 21.94±0.39°C at the store (Table 1). Maximum tomato temperature during transportation ranged from 6.85±0.58 to 25.28±2.37°C. Tomato temperatures ranged from 14.72±0.48 to 27.96±1.31°C after 1 h on the counter at home. Tomatoes generally had a higher temperature on the counter in the home than during transport.

The FDA recommends that the dump tank water be at least 10°F above the pulp temperature of the tomatoes (FDA 2008), but this is not common in NJ packinghouses (Todd-Searle et al.). Cooler dump tank water may lead to internalization of *Salmonella* cells that are on the tomatoes or in the wash waters (Zhuang et al. 1995). Since tomatoes are commonly left on the counter and may remain there for several days, this may promote better survival and/or growth of *Salmonella* associated with whole tomatoes (Asplund and Nurmi 1991; Beuchat and Mann 2008).

Leafy green temperatures ranged from 4.72±1.21 to 12.13±0.89°C at the store (Table 2). The maximum leafy green temperatures observed during transport to the home varied from 6.11±2.74 to 17.69±7.32°C, above the 5°C recommended by the FDA model food code for leafy green storage at retail (FDA 2009). Temperatures ranged from 3.61±0.48 and 11.67±2.78°C after 1 h of home refrigeration. Only 4 of 25 trials ended with a home storage temperature meeting FDA's food code recommendation after 1 hr.

Predicted *Salmonella* growth varied from 0.008 to 0.064 log CFU/g (Figure 1). Maximum temperatures of leafy greens varied from 6.20±1.37 to 16.76±5.40, 9.81±2.70

to 17.69±7.32, 8.52±2.08 to 15.46±3.52, and 6.11±2.74 to 13.15±2.49 for the spring, summer, fall, and winter, respectively. The maximum leafy green temperature was slightly higher in the summer, but there was no significant difference in predicted *Salmonella* growth between seasons ($p<0.05$). Average predicted increase in *Salmonella* concentration growth were 0.03±0.02, 0.03±0.02, 0.3±0.01, and 0.02±0.01 log CFU/g, for the spring, summer, fall, and winter months respectively (Figure 2).

Total time for leafy green transport varied from 21 to 59 min. Figure 3 shows a slight correlation ($R = 0.38$) with longer times leading to more growth. Other data on retail to home transport (Ecosure 2008) reported a larger range in transport times for prepackaged lunchmeat and ground beef (12-230 minutes), which would be expected with an increased number of study participants. Longer transport time and higher final temperatures would likely lead to a greater predicted increase in *Salmonella*.

Mishra et al. (2017a) predicted that *Salmonella* would not exit lag phase if the air temperature was less than 21°C over a period of 10 hr. For air temperatures of 21 and 40°C, Mishra et al (2017a) predicted that lag time was 5.5 to 1.7 h with *Salmonella* growth between 0.43 and 2.12 log CFU/g after 10 h. Mishra et al (2017a) predicted that at temperatures of 20 and 40°C, *Salmonella* growth would be 0.62 to 2.44 log CFU/g after 10 h if *Salmonella* experienced lag time.

Mishra et al (2017a) made their predictions at constant temperature, but the data we collected were for changing temperature conditions. Studies have found conflicting results about bacteria lag phases in food systems when the temperature fluctuates (McKellar and Delaquis 2011; Mishra et al. 2015b; Munoz-Cuevas et al. 2010). In any event, by assuming no lag phase, our results represent worst-case (fail-safe) assumptions.

Although we studied *Salmonella*, other researchers have investigated the growth of other organisms. Zeng et al. (2014) monitored temperature changes in packaged salad during transportation to the store as well as storage and display at the store. Growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* was then predicted based on those temperature changes. Maximum growth was found to be ~3 log CFU/g for each pathogen, though growth was typically <2 log CFU/g. Time periods varied from 1 to 3 days for each phase (transport, storage and display). Given that Zeng et al. (2014) found that it took 9 days to achieve up to 3 log CFU/g growth, it is not surprising that our study predicted such little growth in short time from acquisition in the store to refrigeration in the home.

Conclusions

Overall, predicted *Salmonella* growth was less than 0.1 log CFU. This suggests that transportation between store and home and subsequent cooling in the home refrigerator would not lead to significant *Salmonella* growth. If *Salmonella* growth were to occur, it would likely happen during periods when longer times for temperature abuse are possible such as transport from packinghouse to the retail store and retail storage.

Figures

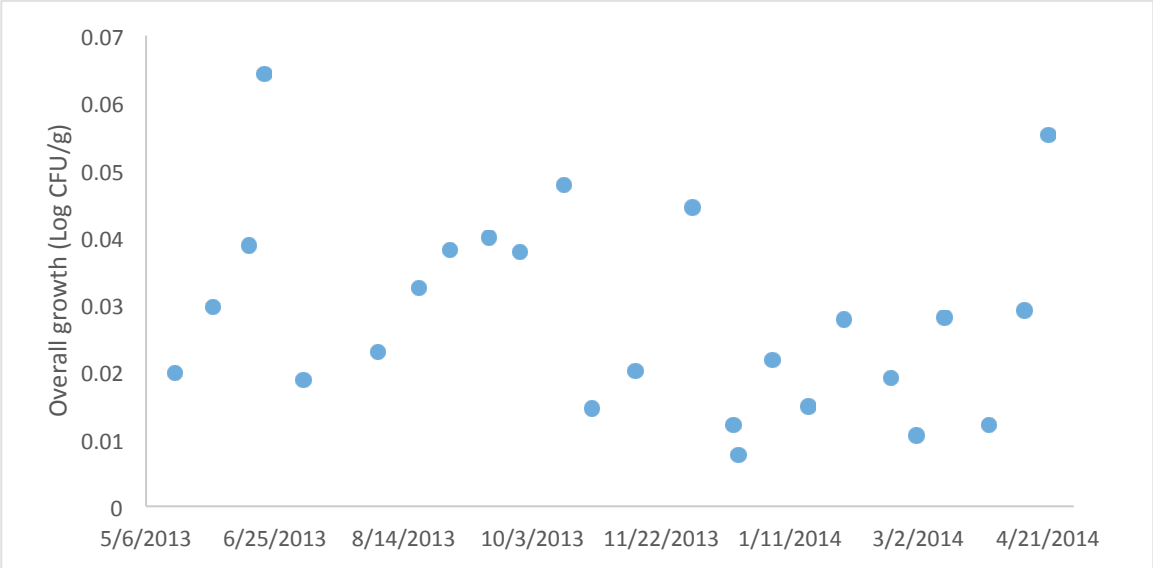

Figure 1: Predicted *Salmonella* growth log CFU/g) on leafy greens by purchase date**.**

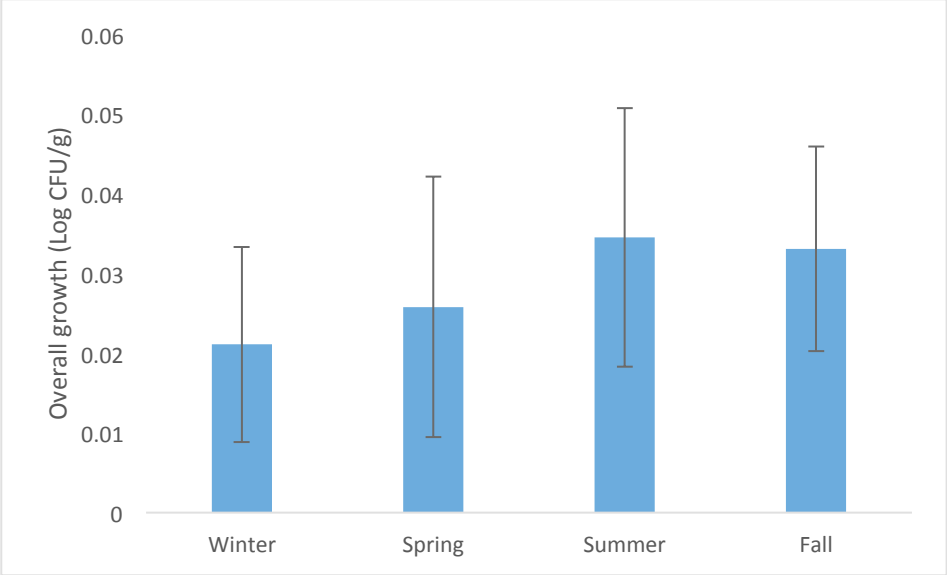

Figure 2: Average predicted *Salmonella* growth (log CFU/g) on leafy greens by season**.**

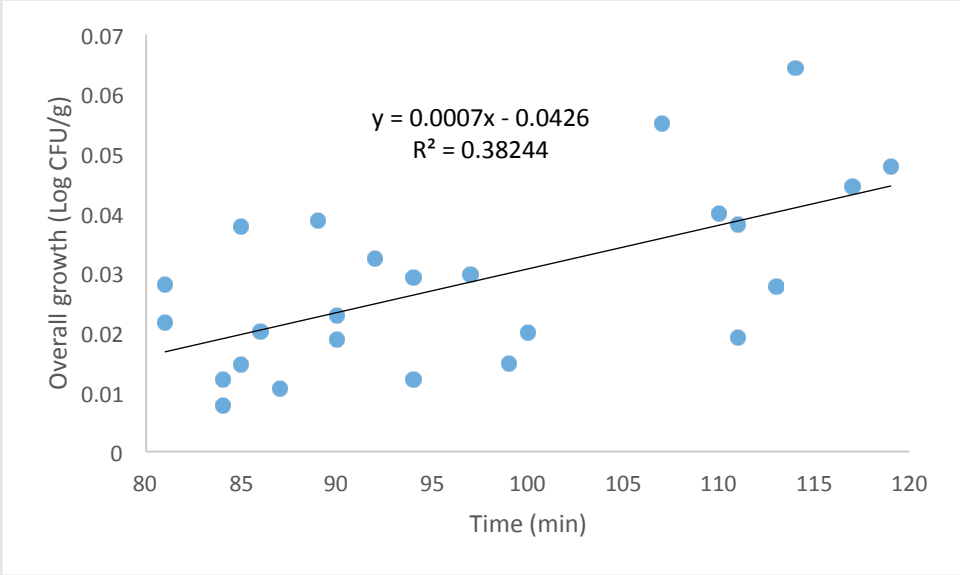

Figure 3: Overall predicted *Salmonella* growth (log CFU/g) on leafy greens by time from acquisition to home storage**.**

Tables

Table 1. Transport times and temperatures for tomatoes from four New Jersey supermarkets to consumers' homes over four seasons.

Date	Store	Travel time (min)	Initial temperature	Maximum temperature during travel	Final temperature
3/1/2014	B	24	10.46±0.32	9.44±0.48	16.39±0.83
3/12/2014	C	19	10.74±0.81	11.2±0.70	16.76±1.63
3/29/2014	B	22	13.8±0.16	13.43±0.80	20.37±2.89
4/12/2014	A	29	18.06±2.17	18.24±1.43	23.15±1.53
4/21/2014	D	46	17.13±1.05	17.69±0.89	21.2±0.16
5/17/2013	A	47	17.41±1.25	21.48±0.42	23.61±1.00
6/1/2013	B	32	19.35±0.16	21.11±1.27	26.48±2.63
6/15/2013	C	27	16.85±2.12	15.56±1.11	24.82±1.25
6/21/2013	A	53	14.72±0.56	25.28±2.37	27.78±0.28
7/6/2013	B	27	21.39±2.41	24.72±3.13	27.59±1.97
8/4/2013	C	28	17.41±4.83	17.13±3.47	27.96±1.31
8/20/2013	D	29	21.94±0.39	20.56±0.79	23.19±0.20
9/1/2013	A	47	14.26±0.89	15.56±1.21	22.78±1.27
9/16/2013	D	49	12.59±0.89	13.89±0.83	17.96±0.89
9/28/2013	C	22	9.44±0.48	13.98±1.85	18.33±0.83
10/15/2013	A	57	17.41±0.16	17.78±0.73	21.39±0.28
10/26/2013	B	23	18.06±0.73	17.22±0.83	20.93±0.42
11/12/2013	C	23	9.07±0.42	9.07±0.32	17.59±1.70
12/4/2013	D	55	14.26±2.36	14.82±1.37	18.7±1.37
12/20/3013	A	33	15.93±0.58	15.37±0.16	19.07±0.16
12/22/2013	B	22	18.61±1.21	18.7±1.31	22.04±0.80
1/4/2014	C	17	6.76±1.63	6.85±0.58	14.72±0.48
1/18/2014	A	35	17.13±0.16	13.52±2.16	21.02±0.64
2/1/2014	A	48	17.5±0.48	14.63±1.37	21.57±0.16
2/19/2014	D	50	13.98±0.98	14.35±0.58	17.22±0.73

Table 2. Transport times and temperatures for leafy greens from four New Jersey supermarkets to consumers' homes over four seasons.

Date	Store	Travel time (min)	Size (oz)	Initial temperature	Maximum temperature during travel	Final temperature
3/1/2014	B	27	10	5.83±1.27	6.2±1.37	4.72±1.67
3/12/2014	C	21	12	11.2±0.42	13.8±2.80	7.04±3.00
3/29/2014	B	24	10	6.76±1.16	7.31±1.12	5.28±0.73
4/12/2014	A	34	10	8.06±1.47	12.69±1.85	7.31±2.67
4/21/2014	D	48	6.5	8.7±2.97	16.76±5.40	10.93±0.32
5/17/2013	A	41	9	7.78±3.89	11.3±2.74	3.61±0.48
6/1/2013	B	37	10	9.44±2.10	12.78±1.55	6.57±3.35
6/15/2013	C	30	12	9.35±0.58	14.72±2.17	8.7±1.25
6/21/2013	A	56	6	8.15±3.06	17.69±7.32	11.67±2.78
7/6/2013	B	31	12	6.48±2.36	9.81±2.70	6.76±1.12
8/4/2013	C	30	12	7.04±2.33	10.00±1.94	7.31±1.76
8/20/2013	D	33	6.5	7.5±0.79	14.44±2.75	5.69±1.37
9/1/2013	A	51	10	8.8±2.36	14.82±2.84	6.85±1.97
9/16/2013	D	51	6.5	6.94±1.27	12.78±1.82	8.98±1.95
9/28/2013	C	25	12	12.13±0.89	15.46±3.52	8.98±0.89
10/15/2013	A	59	9	6.94±0.56	14.44±2.90	9.63±2.23
10/26/2013	B	26	12	6.57±1.67	8.52±2.08	5.65±0.58
11/12/2013	C	26	12	9.07±0.58	10.00±1.21	6.76±0.58
12/4/2013	D	58	6.5	6.2±0.89	13.15±2.49	10.00±1.73
12/20/3013	A	35	10	5.28±1.55	6.67±0.83	5.74±0.32
12/22/2013	B	25	12	4.72±1.21	6.11±2.74	4.17±0.73
1/4/2014	C	20	12	8.52±3.63	9.81±0.42	7.87±1.40
1/18/2014	A	39	10	5.46±1.05	9.07±1.97	3.61±1.21
2/1/2014	A	53	10	6.85±0.85	11.67±2.47	5.83±1.11
2/19/2014	D	52	10	5.93±0.89	9.07±0.70	5.46±0.98

Chapter VI: Models for *Salmonella* contamination of whole tomatoes on the farm and in packinghouses for use in risk assessment

Jennifer Todd-Searle[1], Michelle D. Danyluk[2], Donald W. Schaffner[1]

[1]Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States

[2]Department of Food Science and Human Nutrition, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, United States

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Abstract

Salmonella outbreaks have been linked to fresh tomatoes in the US. Stochastic models were built in Excel to predict *Salmonella* behavior on tomatoes from farm-to-fork and are suitable for use with the Monte Carlo modeling software @Risk add-in. The models were focused on three main aspects in tomato processing: in field contamination by *Salmonella* (via irrigation water and cross-contamination from soil or mulch), *Salmonella* survival on tomatoes, and the effect of packinghouse sanitizing chemicals. The literature surveyed on *Salmonella* growth and survival on tomatoes suggesting multiple factors including but not limited to temperature, RH, and *Salmonella* serovar play a role. Time post inoculation was the only factor investigated that appeared to have a clear effect on *Salmonella* survival suitable for modeling. Three triangular distributions were used depending on the length of time post inoculation. Models for chlorine application in a dump tank and peroxyacetic acid in a spray bar were developed. The models developed here can be used in a farm-to-fork quantitative microbial risk assessment.

Introduction

The CDC estimates that *Salmonella enterica* causes more illnesses (1,000,000), hospitalizations (19,000), and deaths (380) than any other foodborne bacterial pathogen every year (Scallan et al. 2011). *Salmonella* caused outbreaks linked to eggs, alfalfa sprouts, poultry, pistachios, and powdered meal replacement shakes in just 2016 alone (CDC 2016). *Salmonella* species were responsible for almost 40% of the foodborne outbreaks occurring between 1988 and 2007 linked to fresh produce (Greig and Ravel 2009). The increase in illnesses from fresh produce appears to correspond to increase in production and consumption of these foods (Olaimat and Holley 2012). The three most common *Salmonella* serotypes in food are Typhimurium, Enteritidis, and Newport with *S.* Newport becoming an increasingly common multi-drug resistant serovar in the past 15 years (CDC 2011b).

Outbreaks due to *Salmonella* contamination of tomatoes have occurred in 1990, 1993, 1999, 2002, 2004, 2005, and 2006 in the United States (Behravesh et al. 2012; Cummings et al. 2001; Greene et al. 2007; Hedberg et al. 1999; CDC 2005, 2006, and 2007). The FDA estimates that 17% of foodborne disease outbreaks between 1998 and 2006 linked to produce were specifically from contaminated tomatoes (FDA 2015). Contamination can occur at the farm from contaminated irrigation water or at the packinghouse due to cross-contamination or contaminated wash water (CDC 2005, 2006, and 2007). *S.* Newport has been found in produce growing environments including the Delmarva region and has been linked to outbreaks involving tomatoes and cucumbers (CDC 2015). Because *Salmonella* can be present in the farm environment, post-

harvesting treatments that control and reduce *Salmonella* contamination on the tomatoes is of critical importance.

Washing produce in dump tanks or via spray bars using water with sanitizing solutions can help remove dirt and control foodborne pathogens. Unfortunately these procedures can also spread contamination to non-contaminated produce if sanitizer levels are inadequate (Allende et al. 2008; Bartz et al. 2001; Danyluk and Schaffner 2011; López-Gálvez et al. 2009; Pao et al. 2007 and 2009; Rana et al. 2010; Tomás-Callejas et al. 2012). Some research has suggested that sanitizers are more important in reducing risk of cross-contamination than in reducing microbial contamination already on the produce (Zhang et al. 2009; López-Gálvez et al. 2009).

Quantitative microbial risk assessment (QMRA) can be useful in estimating foodborne disease risk and identifying optimal points in the process where risk can be reduced (Boone et al. 2010). QMRA can be divided into two types: deterministic and stochastic. A stochastic model uses probability distributions to account for variability and uncertainty whereas a deterministic model uses single points (Vose 2000). QMRA can be divided into four steps: hazard identification, exposure assessment, hazard characterization, and risk characterization (CAC 2014). This manuscript presents models of in-field contamination of tomatoes by *Salmonella*, stochastically modeling several modes of contamination of tomatoes in the field, as well as reduction of *Salmonella* on tomatoes due to common packinghouse sanitation procedures. This manuscript also presents a preliminary assessment of the factors influencing survival of *Salmonella* on tomatoes.

Materials and Methods

Overview

A literature search was conducted to obtain relevant data on *Salmonella* contamination of tomatoes in the field. Data on control of *Salmonella* on tomatoes from to common packinghouse sanitation procedures and on factors influencing survival and/or growth on whole, fresh tomatoes were also collected.

Field contamination

Data for three separate in-field routes of contamination on the farm were collected: via contaminated irrigation water, via contact with contaminated soil, and via contact with contaminated plastic mulch.

Data and calculations for tomato contamination from irrigation water are shown in Table 1. In this table and most of the tables that follow, the first column indicates the spreadsheet cell containing the variable. The second column provides an English language description of variable within that row. The third column is the variable, which can be a value or an equation. The fourth column provides the units for the variable in the third column. The fifth column gives the source of the value or equation (user input, expert opinion, calculated from other cells or based on literature data. Since the expected *Salmonella* concentration in irrigation water is largely unknown, this variable was via user input. Prevalence of *Salmonella* in water was extracted from Bell et al. (2015), Gorski et al. (2011), Micallef et al. (2012), and Strawn et al. (2013). The amount of water applied and the number of plants to be water were set via user input. It was assumed that each plant yielded 40 tomatoes. The remaining entries in Table 1 show

subsequent calculations eventually yielding the concentration on the tomato after irrigation.

Table 2 shows the data and calculation for tomato contamination via the transfer of *Salmonella* from contaminated soil to tomatoes. As above, since the expected *Salmonella* concentration in soil is largely unknown, this variable was set via user input. Prevalence of *Salmonella* in soil was extracted from Bell et al. (2015), Gorski et al. (2011), Micallef et al. (2012), and Strawn et al. (2013). The chance of a tomato contacting the soil was set at 5% based on expert opinion. Data from Guo et al. (2002) were used to model *Salmonella* survival in soil. Time from contamination to harvest was between 1 and 20 weeks, and was based on expert opinion. The maximum level of *Salmonella* on a tomato was set at 9 log CFU/tomato based on expert opinion. Data for transfer from soil to tomato were based on Guo et al. (2002) and used in combination with unpublished data from Todd-Searle et al. to model transfer of *Salmonella* from soil to tomatoes. The remaining entries in Table 2 show subsequent calculations yielding the concentration on the tomato after contact with the soil.

Table 3 shows the data and calculation for tomato contamination via the transfer of *Salmonella* from contaminated plastic mulch to tomatoes. As above, since the expected *Salmonella* concentration on mulch is largely unknown, this variable was set via user input. Prevalence of *Salmonella* on mulch was assumed to be the same as prevalence in soil as shown in Table 2. The chance of tomatoes contacting mulch was set at 5% based on expert opinion. Data for transfer from soil to tomato were based on unpublished data from Todd-Searle et al. to model transfer of *Salmonella* from soil to

tomatoes. The remaining entries in Table 3 show subsequent calculations yielding the concentration on the tomato after contact with the soil.

Salmonella *Survival on Tomatoes*

Table 4 shows the data and calculation for *Salmonella* survival on tomatoes. The structure of table 4 is slightly different from the other all tables in this manuscript, as the account for the differential concentration change occurring on days one through three in the simulation. While the cell number, variable, value, unit and source columns are the same there are three additional columns to the left of the value column that calculate change in concentration at different days in the simulation. The number of days in the field varied from 1 to 85 based on expert opinion. As above, the expected *Salmonella* concentration was set via user input. In future versions of the model this variable could be set from upstream components of the model (e.g. Tables 1-3). Data from Allen et al. (2005), Das et al. (2006), Guo et al. (2002), Iturriaga et al. (2007), Pao et al. (2012), Shi et al. (2007), Todd-Searle et al., Yuk et al. (2005), and Zhuang et al. (1995) was used to model survival of *Salmonella* on tomatoes as a function of days post inoculation.

Packinghouse sanitation

Models were developed for two sanitation methods: chlorine in a dump tank and peroxyacetic acid applied in a spray bar and roller system. Data was extracted from Iturriaga and Escartín (2010) and Sapers and Jones (2006) for chlorine in a dump tank (Table 5) and data from Pao et al (2009) was analyzed for a spray bar and roller system (Table 6).

Simulation modeling

Extract data, models, and user inputs were entered into an Excel (Microsoft, Redmond, WA) spreadsheet as described in Tables 1-6 above.

Results and Discussion

Field Contamination

The model for *Salmonella* contamination of tomatoes via contaminated irrigation water is shown in Table 1. The prevalence of *Salmonella* in irrigation water assumed to be 3% per L according to data extracted from Bell et al. (2015), Gorski et al. (2011), Micallef et al. (2012), and Strawn et al. (2013). The transfer of *Salmonella* from contaminated water to tomatoes was based calculations and assumption of 40 tomatoes per plant, which was validated using data from Lopez-Velasco et al. (2013). This model is almost entirely deterministic, with the exception of the binomial distribution using the prevalence of *Salmonella* in water.

Table 2 shows the model for transfer of *Salmonella* from soil to tomatoes. The prevalence of *Salmonella* in irrigation water assumed to be 3.6% per g according to data extracted from Bell et al. (2015), Gorski et al. (2011), Micallef et al. (2012), and Strawn et al. (2013). Survival of *Salmonella* in the soil was based on Guo et al. (2002) who studied the survival of *Salmonella* on tomatoes in contact with soil. Data extracted from Guo et al. (2002) are shown in Figure 1, and Table 2 assumes the actual rate of decline to be uniformly distributed between the slopes of the two lines. A triangular distribution derived from Guo et al. 2002 and Todd-Searle et al. for the log % transfer of *Salmonella* from soil to the tomatoes is shown in Figure 2A. This data is expressed in the model as a

triangular distribution with a minimum, mode, and maximum at -3, -1, and 3 log % transfer respectively.

The model for the transfer of *Salmonella* from plastic mulch to tomatoes (Table 3) is similar to the transfer from soil to tomatoes. Survival of *Salmonella* in the soil was based on Todd Searle et al. who studied the survival of *Salmonella* on plastic mulch at different relative humidities. Table 3 assumes the actual rate of survival to be uniformly distributed between the slopes of two lines based on data extracted from Todd-Searle et al. (data not shown). Todd-Searle et al. was used to derive a triangular distribution for the transfer of *Salmonella* from plastic mulch to tomatoes (Figure 2B), and this data is expressed in the model in Table 3 as a triangular distribution with a minimum, mode, and maximum of 0.5, 1.5, 2.5 log percent transfer respectively. The prevalence for *Salmonella* in soil was also used for plastic mulch as well due to a complete lack of published data on the prevalence of *Salmonella* on plastic mulch.

Salmonella *Survival on Tomatoes*

Figure 3 shows a summary of data collected as part of the literature search revealed conflicting results as to whether *Salmonella* grows or dies on tomatoes (Allen et al. 2005; Das et al. 2006; Guo et al. 2002; Iturriaga et al. 2007; Pao et al. 2012; Shi et al. 2007; Yuk et al. 2005; Zhuang et al. 1995; Todd-Searle et al.). While multiple factors probably influence the survival of *Salmonella* including serovar, relative humidity, and temperature, Figure 3 shows that by themselves temperature and RH do not offer much explanatory power for predicting *Salmonella* growth and survival. A plot of temperature and RH on growth and survival was also not revealing (data not shown). The relative change in population of *Salmonella* on tomatoes (both increase and decrease) did show a

clear time dependence. Figure 4 shows frequency distributions for survival of *Salmonella* on tomatoes in the first day after inoculation (A), in days 2 and 3 after inoculation (B) and for 4 or more days after inoculation (C). These frequency distributions for survival of *Salmonella* are represented by triangular distributions in Table 4.

Packinghouse

Chlorine application via dump tank and peroxyacetic acid in a spray bar and roller application are two common forms of sanitation observed at tomato packinghouses, and published in the literature. Data extracted from Iturriaga and Esartin 2009 and Sapers and Jones 2006 for the effect of chlorine applied via dump tank on *Salmonella* on tomatoes are shown in Fig. 5. Initial application is highly effective, with as much as a 5-log reduction, however *Salmonella* populations eventually recover and the treatment has a net log reduction of less than 2 logs after 10 days. Given this observation, a normal distribution for the effect of chlorine was calculated using reduction data that occurred 24 hr post treatment.

Figure 6 shows data from Pao et al. (2009) and Chang and Schneider (2012) for the spray bar and roller system application of peroxyacetic acid. Since the reductions for water alone (at a high-flow rate) were similar to 80 ppm peroxyacetic acid, normal distributions for *Salmonella* reduction based on water flow, not on sanitizer concentration or treatment time, were included in Table 6.

The proposed models are designed to be linked together with a model for cross-contamination at the packinghouse due to the dump tank or spray bar and roller system, but data gaps remain that need to be filled to create a comprehensive farm-to-fork model.

Examples of data gaps include information on length of time between tomato harvest and consumption. Because *Salmonella* prevalence in the environment is low (Bell et al. 2015; Gorski et al. 2011; Micallef et al. 2012; Strawn et al. 2013), data on concentration of *Salmonella* in irrigation water, soil, and on plastic mulch are not readily available. While models for the effect of chlorine in dump tanks and peroxyacetic acid in spray bars have been developed, models based on other methods of sanitation are needed. Data on sanitizers besides chlorine and peroxyacetic acid as well as the application of combinations of sanitizers (e.g. dump tank and spray bar application of different agents) is needed.

Conclusions

The information presented here highlights the current data available to model *Salmonella* transfer to and survival on tomatoes in a full farm-to-fork model. The survival models developed here indicate how potentially unpredictable *Salmonella* survival and/or growth on tomatoes can be, and the importance of understanding both known and unknown factors governing *Salmonella* behavior on tomatoes. Further research is needed on sanitizers besides chlorine and peroxyacetic acid and on sequential application of sanitizers to develop a better understanding and potential means to control *Salmonella* risk on tomatoes.

Acknowledgements

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Tables

Table 1: Overview of simulation variables and parameters for *Salmonella* transfer from irrigation water to tomatoes.

Cell	Variable	Value	Units	Source
C2	Starting concentration in water	-	CFU/L	User input
C3	Prevalence of *Salmonella* in water	0.030	% (0-1)	Bell et al. 2015, Gorski et al. 2011,' Micallef et al. 2012,' Strawn et al. 2013
C4	Amount of water applied	-	L	User input
C5	Number of tomato plants	-	Plants	User input
C6	Concentration/plant	=C2*C4/C5	CFU/plant	Calculated
C7	Concentration/tomato	=C6/40	CFU/tomato	Expert opinion
C8	Log CFU/contaminated tomato	=LOG(C7)	Log CFU/tomato	Calculated
C9	Log CFU/non-contaminated tomato	-100	Log CFU/tomato	Calculated
C10	Chance of contaminated tomato	=C3	% (0-1)	Calculated
C11	Chance of non-contaminated tomato	=1-C10	% (0-1)	Calculated
C12	Choose contaminated or non-contaminated	=RiskBinomial(1,C10)	No Units	Calculated
C13	Concentration on tomato	=IF(C12=1,C8,C9)	Log CFU/tomato	Calculated

Table 2: Overview of simulation variables and parameters for the transfer of *Salmonella* from soil to tomatoes.

Cell	Variable	Value	Units	Source
C2	Starting concentration in soil	-	CFU/g	User input
C3	Starting concentration in soil	=LOG(C2)	Log CFU/g	Calculated
C4	Prevalence of *Salmonella* in soil	0.036	% (0-1)	Bell et al. 2015, Gorski et al. 2011, Micallef et al. 2012, Strawn et al. 2013
C5	Chance of tomato in contact with soil	0.05	% (0-1)	Expert opinion
C6	Log change in soil	=RiskUniform(-0.2639, -0.2212)	Log CFU/g/week	Guo et al. 2002
C7	Weeks in field	=RiskUniform(1,20)	Weeks	Expert opinion
C8	Concentration at time of contact	=C3+(C6*C7)	Log CFU/g	Calculated
C9	Limit of level if >10^9	=IF(C8<9,C8,9)	Log CFU/g	Expert opinion
C10	CFU at time of contact	=10^C9	CFU/g	Calculated
C11	Log percent transfer to tomato	=RiskTriang(-3,-1,3)	Log % transfer	Guo et al. 2002, Todd-Searle et al.
C12	Percent transfer to tomato	=10^C11	% transfer	Calculated
C13	Amount on contaminated tomato	=C10*(C12/100)	CFU/tomato	Calculated
C14	Log on contaminated tomato	=LOG(C13)	Log CFU/tomato	Calculated
C15	Amount on non-contaminated tomato	-100	Log CFU/tomato	Calculated
C16	Chance of contaminated tomato	=C4*C5	% (0-1)	Calculated
C17	Chance of non-contaminated tomato	=1-C16	% (0-1)	Calculated
C18	Choose contaminated or non-contaminated	=RiskBinomial(1,C16)	No units	Calculated
C19	Concentration on tomato	=IF(C18=1,C14,C15)	Log CFU/tomato	Calculated

Table 3: Overview of simulation variables and parameters for the transfer of *Salmonella* from plastic mulch to tomatoes.

Cell	Variable	Value	Units	Source
C2	Concentration on plastic mulch	-	CFU/cm^2	User Input
C3	Concentration on plastic mulch	=LOG(C2)	Log CFU/cm^2	
C4	Prevalence of *Salmonella* on plastic mulch	0.036	% (0-1)	Bell et al. 2015, Gorski et al. 2011, Micallef et al. 2012, Strawn et al. 2013
C5	Chance of tomato in contact with plastic mulch	0.05	% (0-1)	Expert opinion
C6	Log change in soil	=RiskUniform(-2.6355, 0.2856)	Log CFU/cm^2/week	Todd-Searle et al.
C7	Weeks in field	=RiskUniform(1,20)	Weeks	Expert opinion
C8	Concentration at time of contact	=C3+(C6*C7)	Log CFU/cm^2	Calculated
C9	Limit of level if >10^9	=IF(C8<9,C8,9)	Log CFU/cm^2	Expert opinion
C10	CFU at time of contact	=10^C9	CFU/cm^2	Calculated
C11	Log percent transfer to tomato	=RiskTriang(0.5,1.5,2.5)	Log % transfer	Todd-Searle et al.
C12	Percent transfer to tomato	=10^C11	% transfer	Calculated
C13	Amount on contaminated tomato	=C10*(C12/100)	CFU/tomato	Calculated
C14	Log on contaminated tomato	=LOG(C13)	Log CFU/tomato	Calculated
C15	Amount on non-contaminated tomato	-100	Log CFU/tomato	Calculated
C16	Chance of contaminated tomato	=C4*C5	% (0-1)	Calculated
C17	Chance of non-contaminated tomato	=1-C16	% (0-1)	Calculated
C18	Choose contaminated or non-contaminated	=RiskBinomial(1,C16)	No units	Calculated
C19	Concentration on tomato	=IF(C18=1,C14,C15)	Log CFU/tomato	Calculated

Table 4: Overview of simulation variables and parameters for *Salmonella* survival on tomatoes.

Cell	Variable				Value	Units	Source
G2	**Survival On Tomato in Field**						
G3	Days in the field after contamination				=ROUND(RiskUniform(1,85),0)	Days	Expert opinion
G4	**Log Change on Tomato in Field**	**Day**	**Cumm**	**Day pick**			
G5	Starting concentration	0	=G5	=IF(C5=G3,1,0)=D5*E5	-	Log CFU/tomato	User input
G6	Log change on tomato - day 1	1	=D5+G6	=IF(C6=G3,1,0)=D6*E6	=RiskTriang(-5,1,3.5)	Log CFU/day	Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, Zhuang et al. 1995
G7	Log change on tomato – day 2	2	=D6+G7	=IF(C7=G3,1,0)=D7*E7	=RiskTriang(-1.5,0.5,1.5)	Log CFU/day	Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, Zhuang et al. 1995
G8	Log change on tomato - day 3	3	=D7+G8	=IF(C8=G3,1,0)=D8*E8	=RiskTriang(-1.5,0.5,1.5)	Log CFU/day	Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, Zhuang et al. 1995
G9	Log change on tomato - day 4 and more	4	=D8+G9	=IF(C9=G3,1,0)=D9*E9	=RiskTriang(-0.3,0,0.3)	Log CFU/day	Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, Zhuang et al. 1995
	Log change at day	=G3			=SUM(F5:F9)	Log CFU/tomato	
					=IF(F11>8,8,F11)	Log CFU/tomato	

Table 5: Overview of simulation variables and parameters for reduction of *Salmonella* in chlorine dump tank.

Cell	Variable	Value	Units	Source
C2	Concentration on incoming tomato	-	Log CFU/tomato	User input
C3	Mean log reduction on contaminated pieces	1.22	Log CFU/tomato	Iturriaga and Escartín 2010, Sapers and Jones 2006
C4	SD log reduction on contaminated pieces	0.31144823	Log CFU/tomato	Iturriaga and Escartín 2010, Sapers and Jones 2006
C5	Log reduction on contaminated pieces	=RiskNormal(C3,C4)	Log CFU/tomato	Calculated
C6	Concentration on contaminated pieces, Log CFU	=C2-C5	Log CFU/tomato	Calculated
C7	Concentration on contaminated pieces, CFU	=10^C6	CFU/tomato	Calculated

Table 6: Overview of simulation variables and parameters for reduction of *Salmonella* with peroxyacetic acid in a spray bar and roller system.

Cell	Variable	Value	Units	Source
C2	Amount on tomato	-	Log CFU/tomato	
C3	Water Flow Rate	-	ml/s	User input
C4	**Fast flow rate**			
C5	Mean log reduction on contaminated pieces - flow rate 9.3 ml/s or higher	4.725	Log CFU/tomato	Pao et al. 2009
C6	SD log reduction on contaminated pieces - flow rate 9.3 ml/s or higher	0.45	Log CFU/tomato	Pao et al. 2009
C7	Log reduction on contaminated pieces - flow rate 9.3 ml/s or higher	=RiskNormal(D5,D6)	Log CFU/tomato	Calculated
C8	**Slow flow rate**			
C9	Mean log reduction on contaminated pieces - flow rate lower than 9.3 ml/s	3.475	Log CFU/tomato	Pao et al. 2009
C10	SD log reduction on contaminated pieces - flow rate lower than 9.3 ml/s	0.525	Log CFU/tomato	Pao et al. 2009
C11	Log reduction on contaminated pieces - flow rate lower than 9.3 ml/s	=RiskNormal(D9,D10)	Log CFU/tomato	Calculated
C12	Log reduction on contaminated pieces - actual	=IF(D3<9.3,D11,D7)	Log CFU/tomato	Calculated
C13	Amount on tomato	=D2-D12	Log CFU/tomato	Calculated
C14	Amount on tomato	=10^D13	CFU/tomato	Calculated

Figures

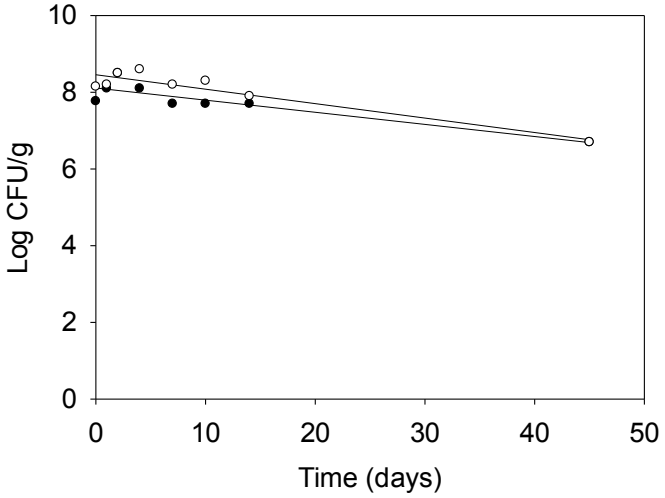

Figure 1: Survival of *Salmonella* in soil recovered on BHI/Amp agar (●) and bismuth sulfite agar (○) extracted from Guo et al. 2002.

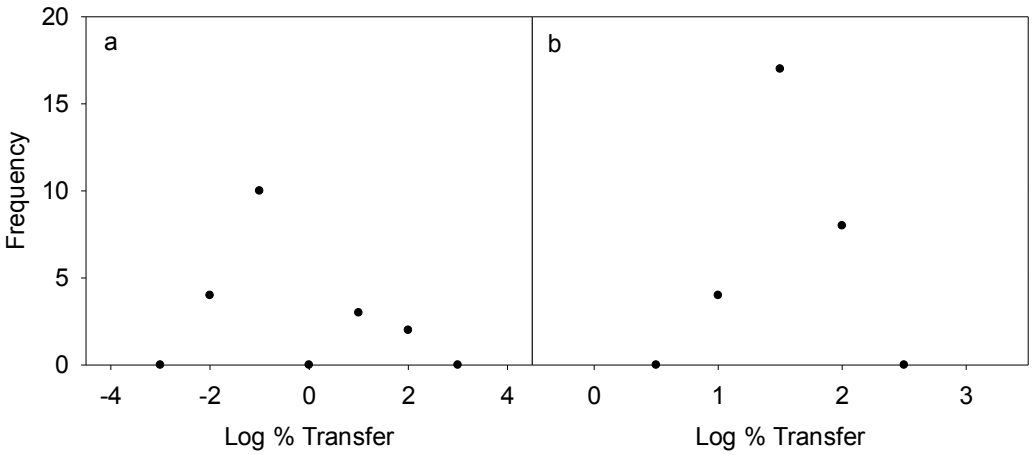

Figure 2: Frequency distribution for log % transfer of *Salmonella* to tomatoes in the field derived from Guo et al. 2002 and Todd-Searle et al. Panel A shows transfer of *Salmonella* from soil to tomato. Panel B shows transfer of *Salmonella* from plastic mulch to tomato.

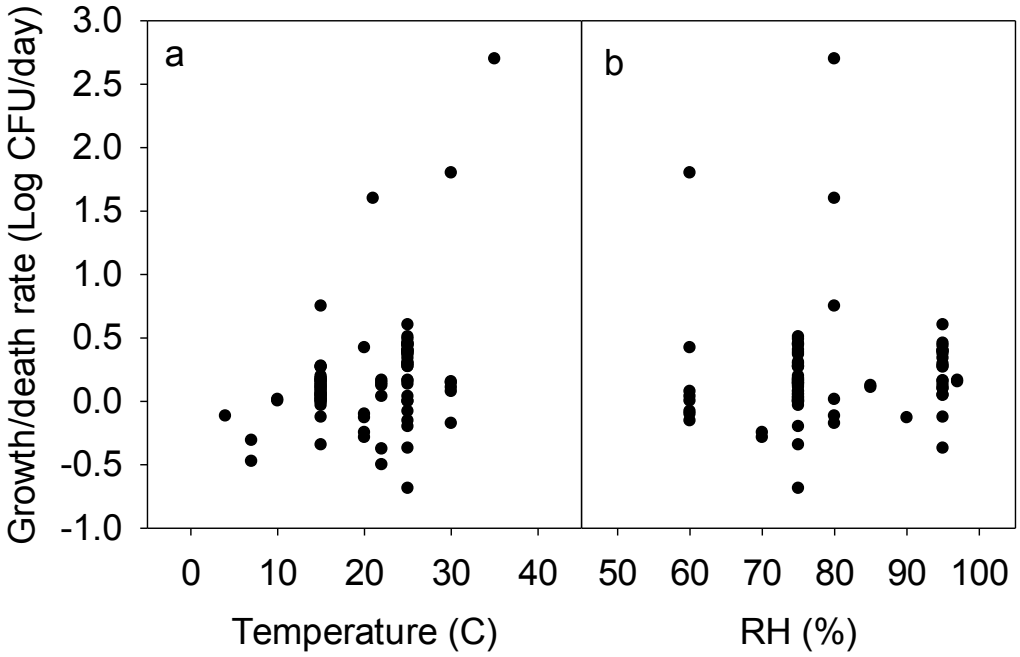

Figure 3: Published data on survival of *Salmonella* on tomatoes based on temperature and RH. Panel A shows the growth or death rates of *Salmonella* based strictly on temperature (all other factors lumped together. Panel b shows the growth or death rates of *Salmonella* based strictly on RH (all other factors lumped together). Data was extracted from Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, and Zhuang et al. 1995.

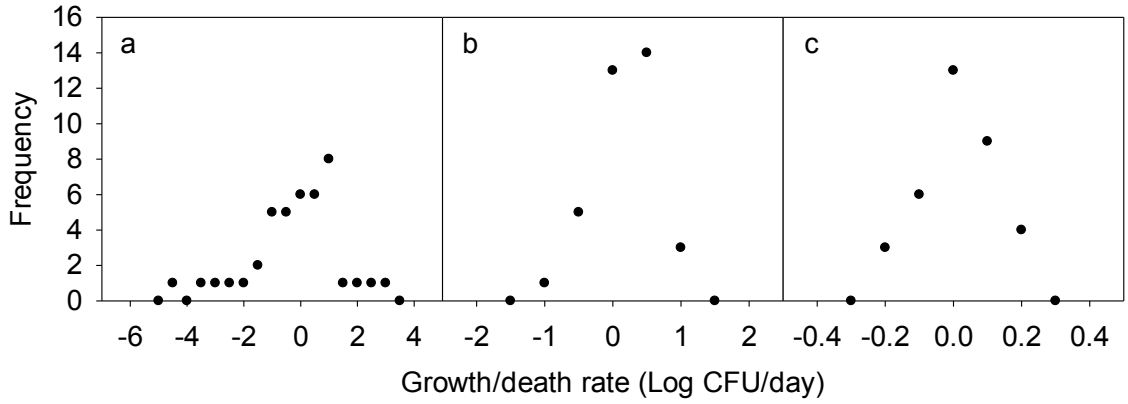

Figure 4: Frequency distributions for survival of *Salmonella* on tomatoes in the first day after inoculation (A), in days 2 and 3 after inoculation (B) and for 4 or more days after inoculation (C). Growth and death rates are based on data was extracted from Allen et al. 2005, Das et al. 2006, Guo et al. 2002, Iturriaga et al. 2007, Pao et al. 2012, Shi et al. 2007, Todd-Searle et al., Yuk et al. 2005, and Zhuang et al. 1995.

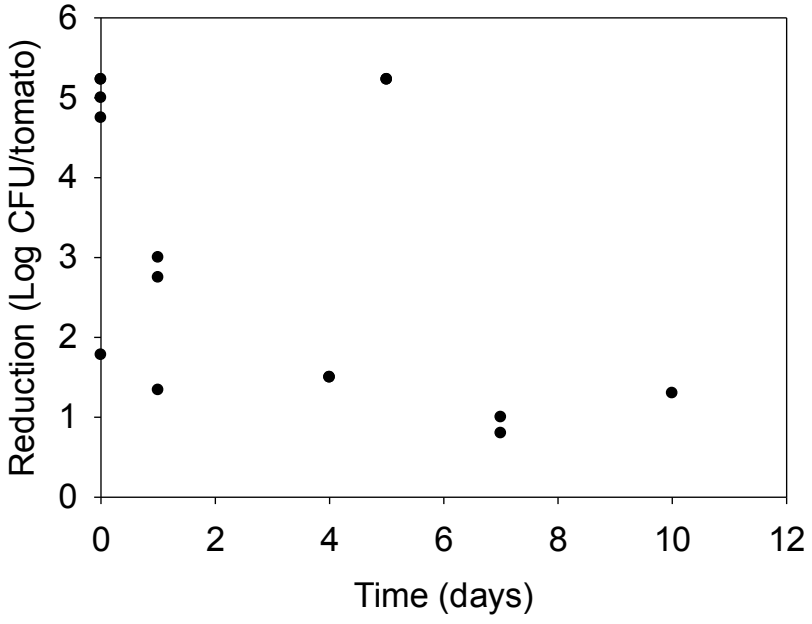

Figure 5: Literature data on *Salmonella* reduction on tomatoes treated with chlorine via dump tank extracted from Iturriaga and Escartín 2009, Sapers and Jones 2006, and Yuk et al. 2005.

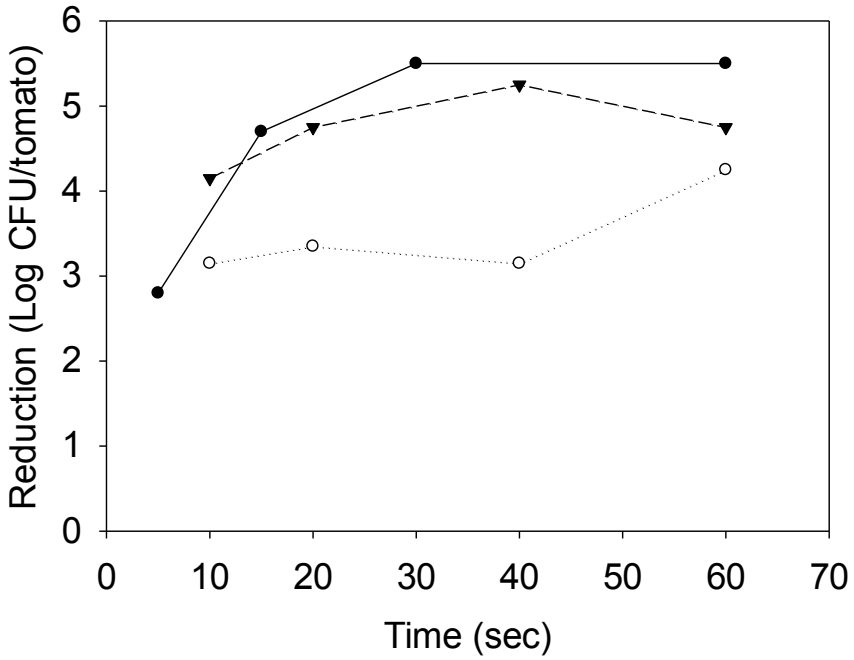

Figure 6: Reduction of *Salmonella* on tomatoes using spray bar and roller sanitation system for peroxyacetic acid based on data was extracted from Pao et al. (2009) and Chang and Schneider (2012). The three treatments are 80 ppm PAA (—●—), water alone at a high flow rate (– ▼ –), and water alone at a low flow rate (···○···).

Chapter VII: Conclusions

Tomatoes have been linked to many salmonellosis outbreaks traced back to farms and packinghouses. The findings of this dissertation can help reduce the chance of such outbreaks. Growers should aim to harvest produce dry since *Salmonella* transfers more readily from wet surfaces to tomatoes as compared to dry surfaces. Plastic mulch more readily transfers *Salmonella* to tomatoes compared to soil, given similar levels of contamination, and *Salmonella* can survive on plastic mulch for at least 14 days. Transfer of *Salmonella* from plastic mulch or soil to tomatoes can occur even after just a brief contact. Taken together all these finding suggest that growers should carefully consider the risk posed by using plastic mulch and by harvesting tomatoes that have fallen off the vine or which have contacted soil or mulch.

The research on NJ packinghouses presented in this dissertation show widely varying sanitizer concentrations and treatment times. Better control of these parameter should aid in achieving more consistent bacterial reductions. Monitoring sanitizer concentration, performing microbiological tests on water samples, regularly cleaning equipment, and maintaining a sanitation program likely contributed to a consistent reduction in the percentage of *E. coli* positive tomatoes in those NJ packinghouses that followed these best practices. The practices observed in NJ packinghouses indicate that additional laboratory research should be conducted using more realistic (i.e. shorter) treatment times, combination dump/spray application with different sanitizers, as well as use of other sanitizers in spray bar type applications. This would create a scientific basis for recommendations to growers that would assist in reducing *Salmonella* concentrations on tomatoes and preventing cross-contamination.

Average pulp temperature almost always exceeded wash water temperatures at NJ packinghouses, which is not a best practice. Wash water temperature differentials are believe to contribute to *Salmonella* internalization in tomatoes if it is present in wash waters. Internalized *Salmonella* is much less vulnerable to sanitizers and may even grow inside tomato flesh. Packinghouses should carefully consider the risks posed by wash water temperatures lower than average pulp temperatures.

The survival model presented in the dissertation demonstrates how unpredictable *Salmonella* survival on tomatoes can be. While *Salmonella* survival on tomatoes seems to be influenced by many factors, it is clear that a higher RH environment typically leads to better survival. Storage facilities for tomatoes are commonly kept at a higher RH (~90%) to improve tomato shelf life, but which also appears to promote *Salmonella* survival and potential growth on tomatoes. Research should be conducted to find an optimum RH environment that ensures adequate shelf life, but that reduces *Salmonella* survival on tomatoes. Only one tomato outbreak strain was used in the survival experiments in this dissertation. Further evaluation of the survival of outbreak strains may lead to improved understanding and control measures.

When pathogen growth is dependent on temperature, such as *Salmonella* on bagged leafy greens, the short transportation period from retail to home is unlikely to contribute significantly to pathogen growth. Growth would be more likely to occur at longer periods of temperature abuse, which may occur during transportation to the store or during long storage periods throughout the distribution chain.

Control of *Salmonella* on tomatoes can occur at many points along the farm-to-fork continuum. Growers can lower the risk by harvesting dry tomatoes and reducing

contact with plastic mulch or soil. Control of wash water sanitizer levels and other best practices in packinghouses can reduce pathogen prevalence and lower risk of cross-contamination to other tomatoes. Storage of tomatoes at lower RH values could potentially reduce the survival of *Salmonella* with further research needed to help identify ways to better control *Salmonella* survival on tomatoes.

Chapter VIII: Appendix

Questions for NJ Tomato Packinghouses on Food Safety and Sanitation

Date: _____

State/county: _____

Packinghouse code: _____

1. What sanitizer is used? If used, what is the target concentration?

2. Is the pH adjusted? If so, what type of acid is used?

3. How is the sanitizer level monitored?

4. How often is the sanitizer level monitored?

5. How many monitoring points?

6. Where are points located?

7. What is the procedure if sanitizer level is too low?

8. How often is the wash water completely replaced?

9. How was the desired concentration level for the sanitizer decided upon?

10. Is any microbiological testing done on the tomatoes, equipment, or wash waters?

11. If yes, what tests? Who does the testing? Is this a test and hold program? Any presumptive testing (e.g., LAF assay)? Is PCR used rather than traditional plating? Are tomatoes tested after packaging/prior to shipping?

12. What is the cleaning procedure for equipment? (How often, type of cleaner, etc.). Is an outside contractor used to sanitize the equipment?

13. How are the tomatoes held before and after dump tank? (Type of container, temperature held at, location, etc.)

14. Do you use a temperature differential between tomatoes and the dump tank water?

15. If yes, how is the tomato pulp temperature monitored? How frequently?

16. If yes, is the water temperature monitored?

17. If yes, is there a consequence if it is out of compliance?

18. If yes, what are the consequences?

19. What kinds of safety protocols are in place to prevent microbial, chemical, or physical contaminants?

20. Do you only pack fruit you grow, or from other growers as well?

21. What is the approximate total acreage you pack tomatoes from for the season?

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