A RECOVERY STUDY OF *Salmonella* SPP. FROM THE SURFACES OF TOMATOES AND PACKING LINE MATERIALS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2003

To my parents, for without your love and never-ending support none of this would have been possible. Your guidance and belief in me allowed me to get this far. Dad, I thank you for always wanting something better for your children. You have sacrificed to no end for me. Mom, your faith has always made you strong in my eyes. You were always here to lean on and so many things make you great.

ACKNOWLEDGMENTS

I would like to give special thanks to my committee chair, Dr. Keith R. Schneider, for the opportunities he has given me and the guidance he has offered me throughout the past two years. I admire all he does and I am thankful to have obtained my master's degree under his direction. I would also like to thank the members of my graduate committee, Dr. Douglas Archer and Dr. Steve Sargent, for their help and assistance with this project.

I thank my family. Their love and support have inspired me to succeed. I thank my brother, for always having an encouraging word for my ear.

I want to thank my colleagues Ben Warren and Tom Ballesteros. I am blessed to have earned this degree with such wonderful friends. I also want to thank my friends, Kim Saranko, Michelle Burtch, Christen McGinnis, the Tucci's, Meg Mizell and Liz Benz.

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Abstract of Thesis Presented to the Graduate School
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Requirements for the Degree of Master of Science

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December 2003

Chair: Keith R. Schneider
Major Department: Food Science and Human Nutrition

Salmonellosis is a common gastrointestinal foodborne illness that is caused by the bacterium *Salmonella*. Every year, approximately 40,000 culture confirmed cases of salmonellosis are reported in the United States. Multi-state salmonellosis outbreaks have occurred due to the consumption of contaminated raw tomatoes.

This study was designed to evaluate the recovery of *Salmonella* spp. from tomato, stainless steel, polyvinyl chloride (PVC), sponge roller, conveyor belt and unfinished oak surfaces. Fruit and material surfaces were maintained at specific temperatures and relative humidity (RH), 30°C/80%RH, 20°C/60%RH and 20°C/90%RH for 28 days.

Different temperature and relative humidity combinations had a significant effect on the survival of *Salmonella* on tomato and packing line surfaces. An ambient temperature (20ºC) combined with 90%RH or 60%RH seemed to better facilitate the survival of *Salmonella* as compared to an elevated temperature (30ºC) combined with 80%RH.

Log_{10} values of recovered *Salmonella* from tomato surfaces decreased over time in all three simulated environments. Tomatoes stored at 20°C/60%RH and 20°C/90%RH had an approximate 4.0 log_{10} CFU/ml reduction of *Salmonella* over 28 days. A lower amount of *Salmonella* was recovered from tomatoes stored at 30°C/80%RH over 28 days (1.0 log_{10} CFU/ml by Day 14).

Salmonella was recovered from stainless steel and PVC surfaces stored at 20°C/60%RH for all sampling intervals. *Salmonella* was only recovered from stainless steel and PVC surfaces on Days 0, 1, 3 and 7 while contained at 30°C/80%RH. No *Salmonella* was recovered from conveyor belt surfaces stored at 30°C/80%RH after Day 3 or from sponge roller surfaces stored at 30°C/80%RH after Day 0. *Salmonella* was recovered from conveyor belt surfaces stored at 20°C/60%RH until Day 14 (0.60 log_{10} CFU/ml). No *Salmonella* was recovered from sponge roller surfaces held at 20°C/60%RH after Day 3. Recovery of *Salmonella* from unfinished oak surfaces was variable. *Salmonella* was recovered from oak surfaces held under 20°C/60%RH at approximately 2.0 log_{10} CFU/ml on Day 28. *Salmonella* recovery fluctuated over 28 days for oak surfaces stored at 30°C/80%RH. On Days 3 and 14 there were increases in *Salmonella* recovery (approximately 3.0 log_{10} CFU/ml and 1.0 log_{10} CFU/ml, respectively). On Days 7, 11, 21 and 28, no *Salmonella* was recovered from oak surfaces. It is suspected that oak pieces harbored and protected *Salmonella* in its matrix.

Results show the importance of a regular sanitation program for surfaces, since *Salmonella* could survive for weeks on tomato and packing line surfaces in an accommodating environment, thus increasing the risk of foodborne illness in fresh-market tomatoes.

CHAPTER 1
INTRODUCTION

During the past two decades, an increase in consumption of fresh produce has occurred in the United States (Tauxe et al. 1997). Greater distribution distances for fresh produce from new geographic sources have allowed a variety of fresh produce to be readily available to consumers year round. Increased availability of fresh produce accompanied with increased demand of fresh produce has resulted in an elevation of produce-associated foodborne illness outbreaks in the U.S. (Tauxe et al. 1997). The Centers for Disease Control and Prevention (CDC) report that the number of produce-associated outbreaks has doubled between the periods of 1973 to 1987, and 1988 to 1991, and that the number of cases associated with these outbreaks has more than doubled (Tauxe et al. 1997). In January of 1997, President Clinton announced a Food Safety Initiative in response to a report he received from the U.S. Department of Health and Human (DHHS) Services, the U.S. Department of Agriculture (USDA) and the U.S. Environmental Protection Agency (EPA). This report announced domestic produce as an area of concern for food safety in the U.S. (Rajkowski and Baldwin 2003). Later that year, a plan entitled Produce & Imported Foods Safety Initiative was announced in hopes to provide further assurance for higher health and safety standards for fruits and vegetables consumed by the American public (FDA 1999). In 1999, a survey was conducted by the Food and Drug Administration (FDA) concerning imported produce, and 40 out of 1000 samples (4%) tested positive for bacterial pathogens, of which 35 of

these samples (80%) tested positive for *Salmonella* contamination and 9 (20%) with *Shigella* (CFSAN-FDA 2001).

Fresh fruits and vegetables were traditionally considered safe to eat raw, straight from the field, but now pathogenic microorganisms may contaminate fresh commodities. Fruits and vegetables can become contaminated with pathogenic microorganisms by the way of many mechanisms. Contamination can occur in fields or orchards, through contaminated irrigation water, harvesting, postharvest handling, processing, distribution and preparation in food service or home settings (Beuchat 1995). All varieties of produce have the potential to harbor pathogenic microorganisms. If contaminated commodities enter a packinghouse facility, cross-contamination of processing equipment and other produce is likely to occur (Brackett 1999).

The survival or growth of pathogens found on or in raw produce are affected by environmental surroundings as well as pathogens' metabolic capabilities. These metabolic capabilities are greatly influenced by intrinsic and extrinsic ecological factors naturally present in the produce or imposed during production, processing, distribution and preparation at the site of consumption (Beuchat et al. 2001). Two very important environmental characteristics that can greatly affect fresh commodities are temperature and relative humidity. The impact of these two extrinsic factors will affect endogenous microflora and pathogen populations that may be present on fresh commodities (Brackett 1987).

Bean sprouts, watermelon, cantaloupe, honeydew, green grapes and tomatoes are fresh commodities that have been associated with foodborne salmonellosis (Tauxe et al. 1997). Salmonellosis is a common gastrointestinal foodborne illness that is caused by the

bacterium called *Salmonella*. The role of *Salmonella* in foodborne disease was first documented in the late 1800's, whereas the human clinical disease, typhoid fever, dates back to the beginning of that century (Cox 2000). Worldwide, *Salmonella* is the second most causative agent of foodborne illness (Cox 2000). Every year, approximately 40,000 cases of salmonellosis are reported in the United States (CDC 2001). Foodborne outbreaks of *Salmonella* spp. are most commonly linked to animal derived foods; however plant derived foods have also served as sources of illness (Cox 2000; Nguyen-The and Carlin 1994; Tauxe et al. 1997; Brackett 1999). Recent surveys of fresh produce have identified several *Salmonella* serotypes as the causative agents in human foodborne illness (CFSAN-FDA 2001).

Large outbreaks of salmonellosis have been caused by consumption of contaminated raw tomatoes. Three multi-state outbreaks of foodborne illness were caused by the consumption of raw tomatoes contaminated with *Salmonella* Javiana in 1992, *Salmonella* Montevideo in 1993 and *Salmonella* Baildon in 1999 (CFSAN-FDA 2001). These outbreaks were all traced to *Salmonella*-contaminated packinghouse facilities where the tomatoes were minimally processed. In June of 2002, *Salmonella* Javiana was the cause of an outbreak at the 2002 United States Transplant Games in Orlando, Florida. The origin of the outbreak was identified as raw, diced tomatoes (CDC 2002).

This recovery study evaluates the survival and recovery of *Salmonella* spp. from the surfaces of tomatoes and typical tomato packing line materials. Materials that were evaluated included stainless steel, conveyor belt, polyvinyl chloride (PVC), sponge rollers and unfinished oak wood. Fruit and material surfaces were inoculated with a

known amount of a rifampicin resistant five-serovar *Salmonella* cocktail. *Salmonella* recovery off the various surfaces was assessed by a vigorous rub-shake recovery method. Inoculated fruit and material surfaces were subjected to specific temperature and relative humidity combinations for 28 days. The temperature and relative humidity combinations were selected to imitate Florida fall/winter and spring tomato production season conditions and ripening room parameters for mature green tomatoes during ethylene treatment and storage.

CHAPTER 2
LITERATURE REVIEW

The United States Centers for Disease Control and Prevention (CDC) claims that more than 200 diseases are known to be transmitted through food consumption (Bryan 1982). It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths annually in the United States (Mead et al. 1999). Tauxe et al. (1997) report that due to a shift in diet toward greater consumption of fresh fruits and vegetables and farther distribution distances from new geographic sources, there are more reported illnesses involving fresh produce. The United States Food and Drug Administration (FDA) has conducted surveys on both imported and domestic produce, and a report on domestic products revealed a 1.6% contamination rate on sampled produce (Rajowski and Baldwin 2003). In the United States from 1988 to 1992, 64 outbreaks of foodborne diseases were attributed to the consumption of fresh fruits and vegetables; nine deaths resulted from the outbreaks (Bean et al. 1997).

Worldwide, many pathogens have been identified as the causative agents of foodborne disease associated with the consumption of contaminated produce. Non-typhoidal *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Yersinia* spp., *Aeromonas* spp., *Campylobacter* spp., *Staphylococcus aureus* and *Escherichia coli* O157:H7 are all bacterial pathogens that have caused foodborne infections (Nguyen-The and Carlin 1994; Beuchat 1995). The presence of pathogens on fresh produce alone can cause an outbreak; replication of pathogens on or in fresh produce does not have to occur. However, extensive research has documented that human pathogens are capable of

replication on many types of undamaged or specifically wounded produce (Beuchat 1995). The FDA states that the survival of pathogens on fresh fruits and vegetables at low infective doses can initiate foodborne disease in the elderly, children and immunocompromised, but for healthy individuals a higher infective dose would be necessary (FSIS 2001).

Temperature and relative humidity are extrinsic factors that influence the persistence and survival capacity of microorganisms on the surfaces of fruits and vegetables. Storage of healthy fruits and vegetables kept at optimum temperature, relative humidity, and atmospheric gas composition will yield maximum sensory and preservation attributes. However, optimum storage settings do not always result in minimizing the growth of microorganisms found on the produce (Beuchat 1992). Studies have shown that a variety of lettuce types, leafy greens and fruit can support postharvest multiplication of pathogenic bacteria under conditions of permissive temperature and relative humidity increasing the risk of foodborne illness (Abdul-Raouf 1993). Many types of vegetables and low-acid fruits are capable of supporting rapid multiplication of pathogens at temperatures ranging between 15 to 25°C (Suslow 2002).

Microbial quality of fresh produce is a large safety issue in the produce processing industry. A blanching or thermal kill step cannot be applied to fresh-market produce to eliminate bacteria (Hurst and Schuler 1992). Fresh produce facilities rely heavily on proper temperature control and good plant and employee sanitation to uphold quality and safety. Fresh fruits and vegetables are very nutritious and overall are categorized as safe foods (Harris et al. 2002). There is potential for fresh produce to become a risk in the food chain if postharvest techniques are abused. Produce quality can be judged from

aesthetic factors (color, texture, aroma), but presence of foodborne pathogens are not so simple to detect. Preventing contamination of fresh-market produce from pathogens is crucial in assuring wholesome foods for safe human consumption (Harris et al. 2002).

Fresh-market produce can be sold as whole entities or produce can be prepared and processed to a greater extent. Fresh-cut products have grown rapidly during the past decade (Cantwell and Suslow 2002). These fruit and vegetable products are prepared and handled to maintain freshness while offering convenience to consumers. Preparation of fresh-cut produce involves cleaning, washing, trimming, coring, slicing, shredding and other similar steps. These steps increase perishability of the produce items. Examples of fresh-cut produce are mixed salads, broccoli florets, diced onions and sliced and diced tomatoes. Fresh-cut produce items usually only have a shelf-life of 10-14 days. Higher respiration rates indicate a very active metabolism and a faster deterioration rate (Cantwell and Suslow 2002).

Foodborne Illnesses Associated with Fresh Produce

World-wide, the per capita consumption of fresh and lightly processed fruits and vegetables has increased over the last decade. With an increase in consumption of fresh produce, a heightened amount of human foodborne disease outbreaks involving fresh produce have resulted (Beuchat 1995). There are many pathogenic microorganisms that have been associated with foodborne disease resulting from contaminated produce. Pathogens of great concern are *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7 and *L. monocytogenes.*

Poultry, eggs and dairy products are most commonly associated with salmonellosis outbreaks. In recent years, *Salmonella* has been linked to many produce-associated outbreaks. Raw bean sprouts were the causative agents in salmonellosis outbreaks that

occurred in the United Kingdom and Sweden in the late 1980's (Beuchat 1995). *Salmonella* Saintpaul was identified as the epidemic serovar in many cases of foodborne infection. Melons contaminated with *Salmonella* have also been causative agents of foodborne disease. As early as 1955, *S*. Miami and *S*. Bareilly were linked to the consumption of fresh-cut watermelon (Gayler et al. 1955). *S*. Javiana and *S*. Oranienburg were identified to have been the cause of salmonellosis outbreaks associated with the consumption of watermelon (CDC 1979; Blostein 1991). Studies have demonstrated that *Salmonella* (a five-serovar cocktail of *S*. Anatum, *S*. Chester, *S*. Havana, *S*. Poona and *S*. Seftenberg) can grow on rind-free pieces of watermelon, cantaloupe and honeydew (Golden et al. 1993). Over a 24-hour period, *Salmonella* populations exhibited multiple log-unit increases on melon varieties maintained at 23ºC. Tomatoes have also been documented as vehicles of foodborne disease. The consumption of raw tomatoes contaminated with *Salmonella* led to two separate multi-state outbreaks in 1992 and 1993 (Hedburg et al. 1999). *S*. Javiana implicated the outbreak in 1992 and *S*. Montevideo implicated the outbreak in 1993.

All four species of the genus *Shigella* are pathogenic to humans. *Shigella* spp. has been responsible for many outbreaks involving contaminated raw vegetables. Lettuce and leafy greens have been documented vehicles of *Shigella*-contaminated produce. *S. sonnei* was responsible for an outbreak involving contaminated lettuce in Texas and shredded lettuce was responsible for another outbreak involving 347 cases of *S. sonnei* gastroenteritis (Davis et al. 1988). Two U.S. midwestern outbreaks of *S. flexneri* infections where linked to green onions that were harvested from a single farm in Mexico (Cook et al. 1995). Melons and tropical fruits have also been reported to harbor *Shigella*.

Escatrin et al. (1989) reports that *Shigella* spp. can grow and survive on the surfaces of fresh-cut pieces of watermelon, papaya and jicama in slightly acidic condition (less than pH 6.0).

E. coli O157:H7 is considered an emerging foodborne pathogen (Beuchat 1995). Cattle are primary reservoirs of this pathogen and an extensive amount of foodborne disease outbreaks have been linked with undercooked beef and dairy products. Fresh produce can also harbor this microorganism. *E. coli* O157:H7 has repeatedly been connected with unpasteurized apple cider, salad bars and melons. It has been documented that *E. coli* O157:H7 rapidly multiplies in watermelon and cantaloupe cubes at 8°C (Del Rosario and Beuchat 1995). In 1994, culture confirmed *E. coli* O157:H7 infections were traced back to raw broccoli served on a salad bar. It was concluded that the broccoli was cross-contaminated with raw ground beef during the preparation of the vegetable (Beuchat 1995).

L. monocytogenes can grow on fresh produce stored at refrigeration temperatures (4ºC). Controlled atmosphere storage does not seem to affect or influence the growth of the microorganism (Beuchat 1995). *L. monocytogenes* is prevalent on plant vegetation (Beuchat et al. 1990). In 1981, a large listeriosis outbreak was attributed to the consumption of contaminated coleslaw. The outbreak was traced back to a cabbage farmer who used a combination of composted and fresh sheep manure to fertilize cabbage fields (Schlech et al. 1983). *L. monocytogenes* has been detected in bean sprouts, leafy vegetables and cut cucumbers (Arumugaswamy et al. 1994). It has also been reported that this pathogen can survive on the surface of tomatoes held at 21°C (Beuchat and Brackett 1991).

The epidemiology of foodborne diseases is constantly changing. Reoccurrence of well-recognized pathogens are observed in outbreaks and newly recognized foodborne pathogens also emerge. Fresh produce has been extensively documented as potential vehicles for foodborne disease. Worldwide, fresh fruits and vegetables are an essential part of diets and minimizing the occurrence of foodborne disease associated with contaminated produce is essential.

Salmonella Species

Documentation of the human clinical disease caused by *Salmonella*, typhoid fever, dates back to the early 1800's (Cox 2000). Historically, *Salmonella* has been documented as causing foodborne disease since the late 1800's. The bacteria were discovered by an American veterinary pathologist, Dr. Daniel E. Salmon, who isolated the microorganism from hog cholera infected swine. In the 1900's, the genus *Salmonella* was created in Dr. Salmon's honor after similar organisms were isolated from outbreaks of foodborne disease (Cox 2000). The bacterium is widely associated with food animals and their production environment.

The genus *Salmonella* exists within the family of Enterobacteriaceae. According to the Encyclopedia of Food Microbiology (Cox 2000), the genus consists of one species; *Salmonella enterica.* *Salmonella* are Gram-negative facultative, oxidase-negative, catalase-positive, anaerobic rod-shaped bacilli (Bergey's Manual of Determinative Bacteriology 1994). Most strains are motile and ferment glucose. Biochemical tests can further characterize the genus into specific serogroups and serovars. These tests characterize two antigens, the O or somatic antigen, and the H antigen or flagellin antigen. The O antigen designates differences in epitopes of lipopolysaccharide (LPS), which is the major component of the outer membrane of Gram-negative bacteria. The H

antigen differentiates strains into serovars that are based on the variation in flagellins or subunit proteins in the flagella (Bergey's Manual of Determinative Bacteriology 1994).

Salmonellosis is the illness that *Salmonellae* induce in humans, usually by ingestion the bacteria through contaminated food products. According to the Centers for Disease Control and Prevention (CDC), salmonellosis has been a reportable disease in the United States since 1943. Physicians must report cases of infection to local health departments that report to state health departments that ultimately report annual totals to the CDC (Tauxe et al. 1997). Salmonellosis is one of the most frequently reported causes of foodborne gastroenteritis and is estimated to cause 1.4 million cases each year in the United States, of which 40,000 cases are culture confirmed (CDC 2000). The CDC has estimated that 95% of *Salmonella* infections originate from foodborne sources (Frenzen et al. 1999). Two serotypes of *Salmonella* cause over half of the reported salmonellosis cases: *Salmonella Enteritidis* and *Salmonella Typhimurium* (CDC 2000). Infection is initiated by the ingestion of a dose of *Salmonella* effective enough to surpass primary host defenses. The bacteria proceed to colonize the gastrointestinal tract. Infectious doses are determined by physiological characteristics of the ingested strain and the physiological state of the host. Typical infectious doses usually range between 10^6-10^8CFU and epidemiological evidence has demonstrated that an infectious dose may be as little as a few (10) cells (Cox 2000).

A range of environmental conditions affect the survival, growth or death of *Salmonella*. The optimum growth temperature for this microorganism is 37°C, but it has been observed to grow between 2-54°C (Cox 2000). Generally, *Salmonellae* are heat labile, but exposure of *Salmonella* to adverse conditions generally increases the resistance

of the microorganism to heat. Optimum pH levels range from 6.5-7.5 and as temperature increases the sensitivity to low pH increases. *Salmonella* can grow at water activity (a_w) values between 0.999 and 0.945 in laboratory media and at a low a_w value of 0.93 in foods (Cox 2000). Growth of the microorganism has not been documented at a_w lower than 0.93, but survival time of the microorganism has been noted to increase as a_w decreases (Cox 2000). In low-moisture foods, survival of *Salmonella* can be measured in months.

Salmonellosis Outbreaks Involving Fresh Tomatoes

Tomatoes have been the sources of several foodborne illness outbreaks. The microorganism responsible for these outbreaks is usually identified as *Salmonella*. A large multi-state outbreak occurred in 1990 that resulted in 176 cases of *S*. Javiana infections. A restaurant and child care center reported illnesses associated with consuming raw tomatoes. The outbreak was traced to a repacking facility. The tomatoes were distributed to various restaurants and grocery stores. No other potential sources were associated with this outbreak (Hedburg et al. 1999).

Another large outbreak occurred in 1993. There were 100 reported cases of foodborne illness that resulted from this multi-state outbreak. The causative agent of the outbreak was *S*. Montevideo. The outbreak was traced back to the same repacking facility from which the 1990 outbreak was traced. Mature-green tomatoes were picked by hand, and transported in field bins which contained approximately 1,500 pounds of tomatoes each. The lots of tomatoes were dumped into a common water bath ("dumptank") where they were contamination most likely occurred (Hedburg et al. 1999).

In 1999, another multi-state outbreak of salmonellosis was attributed to the consumption of raw tomatoes. The origin of contamination was traced to two tomato

grower/packer cooperatives. The lots of tomatoes were handpicked and transported to the facility in covered plastic bins (Cummings et al. 2001).

Most recently in June of 2002, in Orlando, Florida there were two reported cases of *S*. Javiana infections. The illnesses were contracted from contaminated pre-packaged diced Roma tomatoes. Efforts are underway to identify the routes of contamination (CDC 2002).

Tomatoes and Salmonellae

The increased frequency of salmonellosis outbreaks involving fresh tomatoes has prompted researchers to investigate *Salmonella* in and on tomatoes. Many researchers and food scientists have conducted experiments focusing on recovery of the pathogen from tomato surfaces and tomato matrices, survival of the pathogen on and in tomatoes, and the effectiveness of sanitizers on the pathogen.

In a study conducted by Guo et al. (2001), the survival of salmonellae brushed onto tomato plants was investigated. Flowers and stems on tomato plants were inoculated with a five serovar *Salmonella* cocktail before and after fruit set. Twenty-one to 49 days elapsed between the date of inoculation and sampling. Forty-three sound, red, ripe tomatoes were harvested from inoculated plants and plants that were not inoculated. All plants were evaluated for the presence of *Salmonella*. Plants that were not inoculated produced tomatoes that were not contaminated with *Salmonella*. However, 11 of 30 tomatoes (37%) harvested from inoculated plants were positive for *Salmonella* (confirmed by polymerase chain reaction (PCR) assay). Stem-inoculated plants were positive for *Salmonella* before and after flower set at 43% and 40%, respectively. Twenty-five percent of *Salmonella*-positive tomatoes were harvested from plants that were inoculated on the flower. The surface of the tomatoes and the stem scars tissues of

the tomatoes harbored higher percentages of the pathogen compared to the pulp of the tomatoes. PCR fingerprinting patterns revealed that *S*. Montevideo was the most persistent and dominant serotype detected on positive tomatoes. The serovar was isolated 49 days after inoculation of the tomato plants.

In a study by Luasik et al. (2001), the elution, detection and quantification of seeded viruses and bacteria (*Salmonella* Montevideo) were investigated from the surfaces of strawberries and tomatoes. Mature, red Roma tomatoes were inoculated with *Salmonella* Montevideo on artificial surface scars, stem and blossom scars, and intact tomato surfaces. Results indicated a higher recovery of the pathogen from the stem, surface and blossom scars than pathogen recovery from smooth intact surfaces of tomatoes. It was also observed that when the tomatoes were immersed in *Salmonella* Montevideo-contaminated water, more attachment of the pathogen occurred in the stem scar area, followed by the blossom scar area, surface scars, and the intact tomato surface. It was hypothesized that the surface area and hydrophilicity of the rough areas evaluated (surface, stem and blossom scars) may affect microbial attachment. Tomatoes do not have lenticels, or pores, on their surface like many fruits, thus restricting gas exchange between the internal tissues of the fruit and the atmosphere. Lenticels also allow the infiltration of liquids. Pores do exist in the corky tissue of the stem scar area of tomatoes. Bacteria are more likely to attach and infiltrate into the interior of this rough portion of the fruit than the smooth epidermal surface.

In a study conducted by Zhuang et al. (1995), survival patterns of *S*. Montevideo on and in raw tomatoes were evaluated as affected by temperature and chlorine treatment. Mature green tomatoes were dip inoculated with *S*. Montevideo and inoculated tomatoes

were stored up to 18 days at different temperatures in combination with 45-60% relative humidity (RH). The stem scar tissues and core tissues of the tomatoes were analyzed for *Salmonella* populations and dipped in various chlorine concentrations. The survival and growth pattern of *S*. Montevideo was also examined in chopped, ripe tomatoes stored at various temperatures. Results of this study suggested that the persistence and viability of *S*. Montevideo on the surfaces and cores of tomatoes stored at 10°C parallel the potential for *Salmonella* survival on and in tomato fruits during transport and storage. The populations of *Salmonella* inoculated on the surfaces of tomatoes held at 10°C did not significantly change over the 18-day period. *S*. Montevideo was also observed to grow well in chopped ripe tomatoes stored at 20 or 30°C. Chlorine concentration studies revealed that *S*. Montevideo was not totally eliminated from tomatoes when subjected to a disinfection treatment at 320ppm. This study clearly indicates that *Salmonella* serotypes contaminating fresh tomatoes pose a risk for potential foodborne salmonellosis outbreaks.

A study conducted by Guo et al. (2002) demonstrated that water and soil serve as reservoirs of *Salmonella* that can potentially contaminate mature green tomatoes. *Salmonella* was observed to survive at high numbers in moist soil for at least 45 days. It was also observed that cells of *Salmonella* were able to infiltrate fruits via stem scars and enter the tomato pulp upon contact with moist, contaminated soils. Survival patterns of *Salmonella* on tomato surfaces were also investigated. Spot-inoculated tomatoes evaluated over a 14-day storage period (20°C) showed a decrease in *Salmonella* populations over time. Populations decreased by approximately 4 logs over the entire storage period. Results obtained from this study differ from survival patterns of

Salmonella reported by Zhuang et al. (1995). Zhuang et al. (1995) reported an increased amount of *Salmonella* on whole, intact tomatoes over time. Differences could be attributed to the different inoculation procedures used. A dip inoculation, as used by Zhuang et al. (1995), could result in cells becoming lodged in tissue areas that could enhance the survival and growth of cells during a prolonged storage period.

Tomato Industry

Two tomato industries exist in the United States. The fresh-market and processing tomato industries are separate markets and each possesses distinguishing characteristics. Tomato varieties are specifically bred to meet requirements of either the fresh or processing markets. All fresh-market tomatoes are picked by hand whereas, tomatoes bound for processing can be mechanically harvested (ERS 2000). Fresh-market tomatoes are widely produced and sold on the open market with higher and more variable prices than processing tomatoes (ERS 2000).

According to the Economic Research Service (ERS), California and Florida comprise two-thirds of the acres used to grow fresh tomatoes in the United States. In the United States, this industry estimates that fresh-market tomato retail value exceeds $4 billion (ERS 2000). Florida leads the domestic market in the production of fresh-market tomatoes. Florida produced 42% of the fresh-market tomatoes in the United States during 1997-1999 and brought in $5.4 million of the state's total farm value of vegetables (ERS 2000). Florida's tomato season extends from October to June. Most tomato production occurs during the months of April to May and again from November to January. Fresh-market tomatoes are available year-round in the United Stated because of imports and Florida's winter crops. Imported commodities are usually shipped to

markets in the western states and Florida's winter crops are shipped to the eastern half of the nation (ERS 2000).

The ERS (2000) reported that Americans consumed 4.8 billion pounds, or 17.8 pounds per person, of fresh-market tomatoes in 1999. Tomatoes rank third in consumer preference vegetables at the retail level and are only surpassed by potatoes and lettuce (Florida Tomato Committee 2002). Consumption of fresh-market tomatoes in the United States has most likely increased due to the increasing popularity of salads, salad bars and sandwiches dressed with tomatoes (Lucier et al. 2000). Tomatoes are very nutritious fruits and contain approximately half of the recommended daily allowance of vitamin C and 20% of the recommended daily allowance of vitamin A. Tomatoes also contain the compound lycopene which has been shown to reduce prostate cancer in men who consume at least 10 servings of tomatoes or tomato-based foods per week (Florida Tomato Committee 2002).

Postharvest Handling of Tomatoes

Tomatoes bound for the fresh-market are harvested by hand at a mature-green stage. Internally, a mature-green tomato will have a jellylike matrix in all locules, but maturity is difficult to determine from external examination. At a mature stage, tomato seeds will be sufficiently developed when a knife slices the fruit and the seeds are not penetrated by the cut (UF/IFAS 1998). When tomatoes are harvested, pickers place the fruits into plastic buckets or wooden field bins that usually hold up to 40-50 pounds of tomatoes. The buckets are carried to field trucks and emptied into pallet bins or gondolas. Next, tomatoes are transported to the packinghouse and dumped into a chlorinated dump tank. Dump tanks contain heated, chlorinated water to wash the fruits. Wash water should be maintained at a pH of 7 (neutral) and contain a recommended level

of chlorine range of 100 to 150 parts per million (ppm) of chlorine (Sargent et al. 2001). Water temperature of dump tanks should be elevated 10 degrees above the pulp temperature of the tomato. Bartz and Showalter (1981) demonstrated that warm tomatoes (26°C to 40°C) immersed in cold water (approximately 18 degrees colder than incoming fruit) for 10 minutes or longer infiltrated water and any bacteria present in the water. Infiltration through the stem scar is associated with a negative temperature differential between the water and the tomato therefore; warm water is used in dump tanks to reduce the extent of infiltration of water into the tomato. Failure to maintain adequate chlorine levels in dump tanks can lead to increased microbial populations. It has been reported that Enterobacteriaceae populations increased on tomatoes washed in water containing 114 ppm chlorine and populations decreased once tomatoes were subjected to water containing 226 ppm (Beuchat 1992).

Tomatoes exit the dump tank and travel over a series of perforated conveyor belts. Conveyor belts play an integral part in the functions of a packinghouse. Belts are fabricated from rubber compounds and they transport tomatoes at several points during handling. Conveyor belts are used to pre-size, cull, sort and size tomato fruits. Undersized tomatoes will fall through holes in the belts and travel to a cull chute. Sponge rollers also serve a very important role in tomato packinghouse operations. Tomato fruits are susceptible to injury and bruising. Sponge rollers buff and cushion the fruits as they proceed along the processing lines. Tomatoes will also contact sponge rollers after washing and absorb water off the fruit surface; as a result the sponges are constantly moist. Many Florida tomatoes are waxed with a food grade wax that increases

the shine of the tomato and reduces water loss during marketing. Contamination of tomatoes has been known to occur during waxing procedures (Beuchat 1992).

Sorting and grading of tomatoes is a laborious process. Color sorting occurs first, which separates tomatoes possessing any red color from fruits that are completely green in color. The fruits are then separated into grades that meet specific requirements for the U.S. No. 1, U.S. Combination, U.S. No. 2 or U.S. No. 3 of the U.S. Standards for Grades of Fresh Tomatoes (Florida Tomato Committee 2002). Following sorting and grading, tomatoes are mechanically sized by passing over continuous conveyor belts containing increasingly larger round holes that sort tomatoes by maximum allowable diameter for each designated size. In February of 1998, the Florida Tomato Committee (2002) ordered the following sizing classifications: 6x7 (formerly medium), 6x6 (formerly large) and 5x6 (formerly extra large). The sizing dimensions (diameter of fruit is measured in inches) are categorized by a minimum and maximum range for each size class.

Graded and sized tomatoes are transported via conveyor belts to automatic fillers where the fruits are jumble-packed into corrugated fiberboard containers to a designated weight (UF/IFAS 1998). Boxes of tomatoes are then palletized and moved by units. Wooden pallets are used to transport unitized loads of tomato boxes and are used in many packinghouse facilities. Pallets are usually constructed from unfinished oak wood.

Usually, mature-green tomatoes are immediately subjected to a ripening treatment. Ethylene is a natural ripening hormone that is released in ripening rooms. Ripening rooms are capable of holding many pallets of tomatoes at one time, and are maintained at very specific parameters. Precise optimum conditions of a typical ripening room are kept at 20°C, 85-95%RH with a concentration of up to 150 ppm ethylene (UF/IFAS 1998).

Tomatoes are susceptible to extensive water loss through the stem scar so a high relative humidity is necessary (UF/IFAS 1998). Constant air exchange is provided in ripening rooms to supply tomatoes with a continuous ripening-effective blend of ethylene and air to avoid the accumulation of carbon dioxide. Mature-green tomatoes are usually subjected to ethylene for 3 days. Once tomatoes are at a minimal color stage of "breaker", the first sign of external yellow or pink color at the blossom end of the fruit, ethylene will not further accelerate the ripening process since the fruits are producing their own ethylene (Cantwell and Kasmire 2002). A constant supply of air also prevents carbon dioxide buildup when tomatoes respire. Carbon dioxide inhibits the ripening process and is an unwanted byproduct (Reid 2002).

Ripe tomatoes are susceptible to chilling injury at temperatures below 10°C (Cantwell and Kasmire 2002). However, ripening tomatoes develop chilling injury below 13°C (Maul et al. 2000). Low temperatures inhibit development of full color and flavor in green mature fruit and the fruits are more susceptible to *Alternaria* decay (UF/IFAS 1998). Tomatoes are tropical commodities and must be maintained at warm temperatures. If tomatoes are held above 30°C (85-86°F) the fruits will develop more orange pigments than the desirable red pigments (UF/IFAS 1998).

In 1994 and 1995, Rushing et al. (1996) tested tomatoes bound for the fresh-market for the presence of *Salmonella* spp. and verified that a proposed Hazard Analysis Critical Control Point (HACCP) program was effective in controlling the risk of contamination in the packinghouse. This study revealed that contamination seemed more likely to occur at the packinghouse where minimally processed fruits were dumped into a water bath, transported across conveyor belts and hand sorted prior to being packed into cartons.

Packinghouse operations are designed to preserve and package fresh produce in a timely manner. Packinghouse facilities are currently included under the Good Agricultural Practices (GAP) guidelines and are exempt from Good Manufacturing Practices (GMP) regulations. GAP guidelines are generic and do not contain specific testing and monitoring guidelines (CFSAN-FDA 2001). The potential risk of contamination can be controlled by employee training and traceback plans. The *Guide To Minimize Microbial Food Safety Hazards For Fresh Fruits and Vegetables* (FDA 1998) has become a valuable tool for focusing on crucial areas of presumptive risk potential for fresh produce handling (CFSAN-FDA 2001).

Extrinsic Factors Influencing Microbial Viability

Growth and survival of microorganisms on fresh produce are influenced by the characteristics of the surrounding environment (Tauxe et al. 1997). Foodborne diseases that occur from contaminated produce often involve fruits and vegetables that have been subjected to nonthermal, minimal processing prior to time/temperature combinations permitting pathogens to survive and grow (Tauxe et al. 1997). The exteriors of produce act as physical barriers to protect from internalization of bacteria present on a commodity's surface. Temperature and relative humidity are two environmental factors that can affect microbial populations on produce.

For minimally processed fruits and vegetables, two factors should be considered when evaluating the effect of temperature and growth rates of bacteria. First, storage temperature determines respiration rates of a commodity and the behavior of microorganisms may be influenced by changes in the gaseous atmosphere. Secondly, temperature can also influence the rate of senescence of a commodity therefore modifying the environment for microorganisms (Nguyen-The and Carlin 1994).

Improper refrigeration during storage and preparation and poor product quality can enhance the survival of pathogens. Growth of pathogens on fresh, minimally processed fruits and vegetables has been reported in many studies. It was observed by Maxcy (1978), that *E. coli, S. Typhimurium* and *Staphyloccocus aureus* grew on shredded lettuce at room temperature (22-24°C). Yu et al. (2001) reported the growth of *E. coli* O157:H7 on both externally and internally inoculated strawberries. A study by Zhuang et al. (1995) revealed that *S*. Montevideo populations significantly increased on tomato tissues after storage at 20 and 30°C. Refrigeration temperatures limit the growth of most foodborne pathogens, but some pathogenic microorganisms will survive at lower temperatures. *S*. Typhimurium declined rapidly in apple juices stored at 4°C, but managed to survive in the juices for a significant amount of time (Goverd et al. 1979). In the study by Yu et al. (2001), it was observed that *E. coli* O157:H7 populations were also recovered from externally and internally inoculated strawberries. However, there was a significant reduction in the populations recovered from the outside of the strawberry fruits than from the inside of the fruits at 5°C.

Another key environmental factor in determining the survival of bacteria is relative humidity. Traditionally, human pathogens are considered poor survivors in the natural plant surface environment (Suslow 2002). Beattie and Lindow (1994) state that the death of cells subjected to low relative humidity conditions is rather fast and viability of cells can decrease very close to first-order kinetics. In a study conducted by Guo et al. (2001), tomatoes inoculated with *Salmonella* were stored at 20°C for one day with at a relative humidity of 70%. It was reported that a reduction of approximately one $\log_{10}$ CFU per tomato occurred and the population slowly decreased by an additional 3 logs

between days 1 and 14. In the same study, tomatoes were stored in contact with moist *Salmonella* inoculated soil for 14 days. An increase of approximately 2.5 $\log_{10}$ CFU per tomato occurred during the first 4 days of storage. Similar counts remained constant for days 4 though 10 and the incidence of decay on tomatoes stored 10 days or more could not be analyzed for populations of *Salmonella*.

There have been studies focusing on the incidence of *Salmonella* associated with bacterial soft rots and/or physical injury. Bacterial soft rot is the leading cause of postharvest losses of potatoes, tomatoes along with other types of fresh produce. Bacterial soft rot caused by group of plant pathogens which are harmless to humans, that includes *Erwinia carotovora* (subspecies *carotovora* and *atroseptica*), pectolytic *Pseudomonas fluorescens* and *Pseudomonas viridiflava* (Lund 1983). Infected tissues are broken down resulting in the softening and liquefaction of the internal fruit tissues and spreads bacteria over other commodities and food-handling equipment (Wei et al. 1995). *E. carotovora* is the most common of the soft rotting bacterial complex and is a member of Enterobacteriaceae of which *Salmonella* is also a member (Wells and Butterfield 1997). A study by Wells and Butterfield (1997) involving over 500 samples of healthy and soft rotted commodities collected from retail markets showed the incidence of suspected Salmonella was twice that on soft rotted samples than of healthy samples. Another study conducted by Wells and Butterfield (1999) showed that unlike bacterial soft rotted commodities, fungal rotted commodities (*Alternaria tenuis, Bortrytis cinerea, Geotrichum candidum* or *Rhizopus stolonifer*) showed no greater risk of elevated *Salmonella* populations.

Attachment of Microorganisms to Various Surfaces

Bacteria can be introduced to fresh commodities in the field through irrigation water, sewage, or contaminated soil and introduced into packinghouse environments. Attachment of bacteria to food processing surfaces is possible and can easily lead to product contamination (Zottola 1994).

Some typical food contact surfaces found in processing facilities include stainless steel, rubber (conveyor belts), wood and plastic. Microorganisms on contaminated produce can easily attach to a variety of these surfaces in short contact times. It was observed by Mafu et al. (1990) that *L. monocytogenes* attached to stainless steel, glass, polypropylene and rubber surfaces after a brief contact time. Contact times ranged from 20 minutes to 1 hour. Attachment of the pathogen was reported at both 20°C and 4°C for all surfaces. Sanitizers were applied to each of the surfaces after attachment of *L. monocytogenes* and it was observed that porous surfaces (rubber surfaces in this study) seemed to protect the bacteria whereas sanitizers were more effective on nonporous surfaces. Wood is another porous material that is used in the form of field bins, pallets or containers to hold fresh produce. A study conducted by Boucher et al. (1998) observed the enhanced survival of *Campylobacter jejuni* cells when incubated at 30°C in nutrient broth. The physical structure of wooden cubes acted as a protective environment for the bacteria. Plastic cubes were evaluated in the same manner as the wooden cubes, but enhanced survival of *Campylobacter jejuni* cells was not observed.

Microorganisms can irreversibly attach themselves to surfaces and form biofilms. Biofilm-associated cells produce an extracellular polymeric substance (EPS) and have a defined architecture. Microbial biofilms have been known form on food processing surfaces. Pathogenic microorganisms such as *Campylobacter, Salmonella* and *E. coli*

have been known to form strong biofilms on various surfaces (Somers et al. 1994). A study conducted by Joseph et al. (2001) was also in agreement reporting that *Salmonella* strains will form biofilms on plastic, stainless steel and cement. Biofilms are much more resistant to sanitizers as compared to planktonic cells and serve as a source of contamination for foods (Somers et al. 1994). Hood and Zottola (1997) inoculated stainless steel surfaces with *S*. Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 and reported that all pathogens adhered to the surface when grown in media, but adherence levels often did not increase after 1 hour. In a study conducted by Ronner and Wong (1993), it was found that the behaviors of biofilm cells were greatly influenced by surface type. Buna-n rubber (nitrile rubber), a gasket material commonly used in the food industry, had a bacteriostatic effect on *S*. Typhimurium and *L. monocytogenes*. The bacteriostatic effect of the rubber was most pronounced under lower nutrient conditions. *S*. Typhimurium was less affected by the bacteriostatic component than *L. monocytogenes.*

It is easier to prevent the formation of biofilms and microbial contamination than to eliminate a biofilm from a surface after establishment. Attachment of bacteria to food processing equipment and contact surfaces can easily lead to contamination of product. Sanitation procedures and environmental awareness in food processing facilities can reduce the incidence of foodborne illness.

Equipment parts and food contact surfaces such as stainless steel, PVC, conveyor belts, sponge rollers and wood surfaces are widely used in tomato processing facilities. Limited studies on these types of surfaces have been reported. Microorganisms attached to surfaces are a hazardous source of potential contamination for any material coming in

contact with the surfaces. Factors such as temperature, relative humidity, nutrient level of the growth medium, type of attachment surface and species or strain of bacteria can influence the amount of adherence to surfaces.

Microbiological Recovery Methods Involving Fresh Produce

The increase in foodborne illnesses associated with fresh produce in the past decade has resulted in the increase of testing commodities for the presence and enumeration of pathogens (Burnett and Beuchat 2001). Conventional methods of detection, enumeration, identification and characterization of microorganisms are described in such reference books as *Compendium of Methods for the Microbiological Examination of Foods* (CMMEF), *FDA Bacteriological Analytical Manual* (BAM), *Official Methods of Analysis of the AOAC*, and *Standard Methods for the Examination of Dairy Products* (Fung 2001). Methods for analyzing foods of animal origin and thermally processed food of plant origin for both spoilage and pathogenic microorganisms have been clearly defined in such reference manuals. Methods for selecting and preparing samples of raw fruits and vegetables for analysis of microorganisms are less defined (Burnett and Beuchat 2001). Procedures for preparing and isolating *Salmonella* for 18 food groups are outlined in the FDA BAM (Andrews et al. 1998). Currently, a specific protocol for preparing samples of raw produce is not defined. Microbiologists and food scientists are currently utilizing a wide variety of procedures to prepare whole and fresh-cut fruit and vegetables to enumerate pathogens. Sample weight/diluent volume ratios, diluent composition, type of processing and time used to process samples all greatly vary. Some types of processing include blending, stomaching, homogenizing, macerating, rubbing and shaking (Burnett and Beuchat 2001).

It is essential for standard methods to be defined in order to accurately determine the presence and populations of pathogenic microorganisms on fresh fruits and vegetables. Development and validation of standard methods can be applied to determine survival and growth characteristics in challenge studies and the efficacy of antimicrobial treatments in eliminating pathogens on fresh produce (Beuchat et al. 2001). One single protocol would be ideal, but is not feasible for all produce types. An optimum protocol for produce depends upon the site of retrieval of pathogens. Analysis from a surface, tissue or both will vary in methodology, but a basic analytical method for each procedure would form standard guidelines to optimize the recovery of pathogens (Beuchat et al. 2001). An acceptable method for evaluating whole fruits and vegetables is a process in which the whole intact produce is vigorously hand massaged or hand rubbed for a period of time which can ranges from 40 seconds to two minutes (Beuchat et al. 2001; Burnett and Beuchat 2001; Harris et al. 2001; Zhuang et al. 1995).

Inoculation procedures for fruits and vegetables usually occur by either spot inoculation and dipping or spraying. The major problem with inoculation via dipping or spraying is that the number of cells applied to the produce is unknown. Spot inoculation allows a known volume of inoculum and a known cell density that is applied to the produce. Spot inoculation is superior to dip or spray inoculation and this type of inoculation imitates contamination of the produce from a source such as contact with soil, workers' hands, or equipment surfaces (Beuchat et al. 2001). In studies determining the efficiency for retrieval of cells, the applied inoculum should be dried at a standard temperature and relative humidity for a specific amount of time before recovery of cells or treatment is administered (Beuchat et al. 2001).

Due to the increased frequency of documented outbreaks of foodborne disease attributed to fresh produce, many researchers are focused upon pathogenic microorganisms on raw fruits and vegetables. Standard methods that accurately determine the presence and numbers for a wide variety of pathogenic microorganisms associated with fresh produce are needed so studies conducted on this subject can be compared without controversy.

The following objectives were explored in this recovery study.

- Establish growth characteristics for five rifampicin-resistant Salmonella serovars.
- Recover inoculated Salmonella from surfaces of tomatoes and packinghouse materials.
- Determine if a specific temperature and relative humidity combination affect the survival of Salmonella spp. on the surfaces of tomatoes and packing line materials.

CHAPTER 3
MATERIALS AND METHODS

Three separate temperature and relative humidity environments were simulated using an environmental humidity chamber. Tomato surfaces and packing line material surfaces were inoculated with a *Salmonella* cocktail comprised of five rifampicin-resistant serovars. *Salmonella*-inoculated fruit and material surfaces were subjected to specific environmental conditions inside the chamber for 28 days. Simulated environments mimicked standard tomato ripening room parameters and Florida fall/winter and spring tomato production seasons. Recovery of *Salmonella* from tomato surfaces and packing line material surfaces for each simulated environment was monitored on Days 0, 1, 3, 7, 11, 14, 21 and 28. Tomato ripening room parameters were simulated to evaluate only *Salmonella*-inoculated tomato fruits for 28 days. Both *Salmonella*-inoculated tomatoes and *Salmonella*-inoculated packing line materials were evaluated in environments paralleling typical Florida fall/winter and spring tomato production environments.

Selection of Temperature and Relative Humidity Combinations

The selected temperature and relative humidity settings for Florida fall/winter and spring tomato production seasons were based upon weather archives obtained from the Florida Automated Weather Network (FAWN) (University of Florida Institute of Food and Agricultural Sciences 2003) (Table 3-1). The average documented temperature and relative humidity were accumulated for the 2001 and 2002 fall/winter and spring tomato

production seasons in Quincy, FL. The chosen parameters for each production season were used to simulate an open-air packinghouse environment.

Table 3-1. Temperature and relative humidity combinations selected to simulate a ripening room environment (20ºC/90%RH) and a fall/winter (20ºC/60%RH) and spring (30ºC/80%RH) tomato production conditions.

Simulated Environment	Temperature (°C)	Relative Humidity (%)
Standard tomato ripening room	90	20
Florida spring tomato production season	80	30
Florida fall/winter tomato production season	60	20

Acquisition and Maintenance of *Salmonella* Cultures

Salmonella serovars were obtained through Dr. Linda J. Harris at the University of California, Davis, Department of Food Science and Technology. The five *Salmonella enteritidis* serovars used in this study were Agona, Gaminara, Michigan, Montevideo, and Poona (Table 3-2). The serovars obtained were adapted to the antibiotic rifampicin at the University of California, Davis. The serovars were adapted to rifampicin (rif+) by methods described in a study conducted by Lindeman and Suslow (1987). The five *Salmonella* serovars (rif+) were transferred to PROTECT ™ Bacterial Preservers (Scientific Device Laboratories, Des Plaines, IL) upon arrival to the laboratory (summer of 2002) and stored at -70ºC.

Rifampin is synonymous with rifampicin. This antibiotic inhibits protein synthesis of mammalian cells and it is freely soluble in methanol (Merck Index 2001). A 10,000 ppm (1%) stock solution of rifampicin was utilized throughout this study. The stock solution was prepared by dissolving 0.1 g of rifampin (Fisher #BP267925, Fisher Scientific, Pittsburg, PA) dissolved in 10 ml of high performance liquid chromatography

(HPLC) grade methanol (Fisher, Fair Lawn, NJ). The stock solution was filter sterilized. Rifampicin is light-sensitive therefore, the stock solution was protected from light and was stored at room temperature. The media used to recover *Salmonella* off inoculated surfaces, Tryptic Soy Agar (TSA) (Difco™, Sparks, MD), was supplemented with 80µg/ml rifampin (rif+) antibiotic. The antibiotic-resistant serovars allowed differentiation from natural micoflora or non-rifampicin resistant bacteria that may have been present on the matrices evaluated; enabling the sole isolation of *Salmonella* serovars (rif+) (Beuchat et al. 2001; Lukasik et al. 2001).

Table 3-2. *Salmonella enteritidis* serovars obtained from Dr. Linda J. Harris at the University of California, Davis: wild types* and rifampicin-resistant serovars listed with source.

Serovar Designation	Serovar Name	Origin
LJH517* LJH618	Agona	Alfalfa sprouts
LJH518* LJH616	Gaminara	Orange juice
LJH521* LJH615	Michigan	Cantaloupe
LJH519* LJH614	Montevideo	Human isolate from tomato outbreak
LJH630* LJH631	Poona	Human isolate from tomato outbreak

Growth Levels of *Salmonella* Serovars after a 20-Hour Incubation

Growth studies were conducted to determine the rate of growth for each of the five serovars after a 20-hour incubation period. Growth rates were determined so the *Salmonella* cocktail would consist of equivalent quantities (CFU/ml) of each serovar, as one or more serovars would not dominate the inoculum suspension. The five *Salmonella* serovars (rif+) were revived off PROTECT ™ Bacterial Preservers by aseptically transferring one bacterial preserver into 10 ml of Tryptic Soy Broth (TSB) (Difco™, Sparks, MD) supplemented with 80 µl of rifampin. The cultures were then incubated in a

shaking incubator (Queue Systems, Asheville, NC) at 30 rotations per minute at 37°C for 24 hours. The cultures were successively transferred for three days in TSB (rif+) to obtain uniform cell type (Beuchat et al. 2001). Each of the five serovars were transferred into 10 ml of fresh TSB (rif+) and incubated at 37°C for 20 hours. Following the incubation period, three replicates of each serovar was serially (1:10) diluted in 9 ml tubes of sterile Phosphate Buffered Saline (PBS) (ICN Biomedicals Inc., Aurora, OH). Appropriate dilutions were plated out by pour plate technique using TSA (rif+). Plates were statically incubated at 37°C for 48 hours. Colony forming units (CFU) were counted and recorded. Serovars were taken off PROTECT™ Bacterial Preservers at the beginning of each 28-day experiment. Two 20-hour growth studies were conducted to ensure growth rates for all five serovars were successively similar upon revival off bacterial preservers.

Preparation of Inoculum

Three days prior to each experiment, the five *Salmonella* serovars were revived from bacterial preservers. Overnight transfers were performed using 10 ml tubes of TSB (rif+) each day. On the day of the experiment, an 18-hour culture of each serovar was harvested via centrifugation (2,000 x g, 15 minutes at 22°C). Cells were washed twice with PBS. Equivalent aliquots of the five serovars at approximately 1.0×10^8 CFU/ml were combined as a *Salmonella* cocktail. The cocktail was maintained at room temperature for one hour. If the time between preparation of the inoculum and inoculation of the surfaces exceeded one hour the inoculum was stored at 4°C until the surfaces could be inoculated that day. The inoculum was serially diluted using 9 ml tubes of PBS to confirm cell concentration. The dilutions were plated in triplicate via pour plate technique using TSA (rif+).

Inoculation Procedures

Inoculation of Tomatoes

Domestic-market mature green tomatoes (Florida 47) were supplied by DiMare (Tampa, Inc., Tampa, FL) for all experimental studies. Tomato samples were extracted from the processing lines prior to the waxing process. Size classification of the tomatoes, according to the Florida Tomato Committee, was 6x7 (formerly medium) (Florida Tomato Committee 2002). For fruit inoculation, tomatoes were aseptically placed onto sterile fiberglass trays with the stem scars facing down. Ten 10 µl drops of inoculum suspension, for a total of 100 µl of inoculum suspension per whole tomato, were placed around the blossom scar area using a Repeater® Plus pipette (Eppendorf AG, Germany). The inoculum suspension was not placed directly onto the blossom scar. Immediately after inoculation, the tomatoes were placed under a hood (LABCONCO Corporation, Kansas City, MO) at room temperature (approximately 22°C) and the inoculated surfaces were allowed to completely dry for a maximum of 2 hours. Dried samples were placed in a Caron 6030 (Caron, Marietta, OH) environmental humidity chamber. The Caron humidity chamber was equipped with a Caron CRS 101 (Caron, Marietta, OH) water supply system to deliver distilled water to the humidify the chamber. A Whatlow Series 96 temperature and relative humidity controller (Whatlow, Winona, MN) installed in the environmental chamber continuously monitored, displayed and controlled the temperature and relative humidity output inside the chamber.

Periodically, a calibrated humidity meter (Control Company, Friendswood, TX) was placed inside the chamber to verify the relative humidity reading on the output panel. A magnetic thermometer (Fisherbrand by ERTCO™, West Paterson, NJ) was placed on the inside wall of the chamber to verify the temperature output on the panel.

Inoculation of Packing Line Materials

Recovery studies included the following packinghouse materials: stainless steel (type 304, no.4 finish), conveyor belt, polyvinyl chloride (PVC) rollers, sponge rollers, and wood (unfinished oak). The packinghouse materials were obtained from Tri-Pak Machinery, Inc. (Harlingen, TX). Tri-Pak Machinery, Inc. is a Texas-based retailer and manufacturer of materials and equipment used in tomato packinghouses. The unfinished oak pieces were supplied by Lowe's® Home Improvement Warehouse (Gainesville, FL).

The materials were chosen based upon contact surfaces that fresh-market tomatoes encounter from harvest (into wooden field bins) to various other surfaces encountered by tomatoes on a typical packing line. Stainless steel surfaces and conveyor belt surfaces were cut into coupons by Tri-Pak Machinery, Inc. (Table 3-3). Polyvinyl chloride (PVC) cylindrical rollers and sponge rollers were received as whole entities from the manufacturer (Table 3-3). The PVC cylinders were cut into equivalent pieces by the Mechanical Engineering Department at the University of Florida (Table 3-3). The sponge rollers were cut into equivalent sections by laboratory personnel (Table 3-2). The wood pieces were cut into cubes of equivalent dimensions by Lowe's® Home Improvement Warehouse (Table 3-3).

Table 3-3. Surface area dimensions of each type of packing line material that was inoculated with a five serovar rifampicin-resistant *Salmonella* cocktail.

Packing Line Material	Dimensions of each Inoculated Surface
Stainless Steel	2.5cm x 2.5cm
Conveyor Belt	2.5cm x 2.5cm
PVC	2.5cm x 2.5cm
Wood	2.5cm x 2.5cm
Sponge roller	2.5cm x 2.5cm

The pre-cut stainless steel coupons were immersed in methanol (Fisher, Fair Lawn, NJ) overnight to remove any oil residue. The next day, stainless steel coupons were thoroughly rinsed with deionized water (University of Florida). All packinghouse material pieces were autoclaved for 20 minutes at 121°C, 15 psi (Consolidated Stills and Sterilizers, Boston, MA) to achieve sterility. Autoclaved material pieces were aseptically placed onto sterile fiberglass trays. Sponge roller pieces were dampened with sterile deionized water prior to inoculation due to the wet nature of sponge rollers found along the processing lines in tomato packinghouses. Each type of material was marked with a single dot made by a Sharpie® Permanent Marker (Sanford, Bellwood, IL) on the area where a tomato would most likely be encountered.

All materials were inoculated with ten 10-µl spots of inoculum suspension near the marked area of each piece. The inoculated materials were placed under a hood until completely dry. The trays containing the dried inoculated materials were placed in the Caron 6030 environmental chamber.

Salmonella Recovery off Tomato Surfaces and Packing Line Surfaces

Tomatoes and packinghouse materials were extracted at pre-determined time intervals from the environmental chamber and recovery studies were performed. Each recovery study involved sampling at 0, 1, 3, 7, 11, 14, 21 and 28 days for each surface. Each sampling period consisted of three single-fruit or single-packinghouse material replicates. On Day 0, the samples were aseptically removed from the fiberglass tray prior to being placed into the environmental chamber and placed into sterile Stomacher™ (Fisherbrand, Fair Lawn, NJ)) bags containing 100 ml of sterile PBS. For all other sampling days (1 through 28), the tomatoes or packinghouse material pieces were aseptically removed from the environmental chamber and individually placed into sterile

Stomacher™ bags containing 100 ml of sterile PBS on the appropriate days. Tomato samples were constantly rubbed and shaken for one minute (Burnett and Beuchat 2001; Harris et al. 2001; Zhuang et al. 1995). Rubbing action was concentrated around the inoculated blossom scar area of the tomatoes to loosen any reversibly attached bacteria. The packinghouse materials were also rubbed and vigorously shaken for one minute in 100 ml of PBS. Vigorous rubbing was specifically applied to the dotted area on each piece of material. The PBS diluent was squeezed in and out of the sponge as well as rubbed and shaken to try and recover any *Salmonella* that may have migrated into the sponge matrix. The sample diluent from each Stomacher™ bag was then serially (1:10) diluted using sterile PBS dilution tubes. The serial dilutions were then pour plated using TSA (rif+). A negative control for the TSA (rif+) was poured to make certain the media was not contaminated. The plates were statically incubated at 37°C for 48 hours.

Tomato fruits and pieces of each of the materials that were not inoculated with the *Salmonella* cocktail were sampled for control purposes. The control samples were rubbed and shaken for one minute in 100 ml of PBS and serially (1:10) diluted as previously described for the inoculated samples. The serial dilutions were pour plated using TSA (rif+) and statically incubated at 37°C for 48 hours.

Statistical Analysis

Results from the 20-hour growth studies were evaluated using a Students *t* test with an α level of 0.05. All results from recovery studies were averaged counts (CFU/ml) of recovered *Salmonella*. Statistical analyses were performed using the Statistical Analysis System (SAS; SAS Institute, Cary, NC). The GLM procedure in SAS was used to analyze changes of bacterial populations between replications in each experiment. Multiple comparisons were performed using the Least Squares Mean adjusted by the

Bonferroni method for the tomato and material data. Results that yielded *P* values of < 0.05 were considered significant in this recovery study.

CHAPTER 4
RESULTS

Recovery studies were designed to assess the recovery of *Salmonella* from the surfaces of tomato fruits and packing line materials. Inoculated fruit and material surfaces were subjected to three separate temperature/relative humidity environments for 28 days. The simulated environments were traditional tomato ripening room parameters, Florida fall/winter tomato production parameters and Florida spring tomato production parameters. *Salmonella*-inoculated fruit and material samples were periodically extracted from the simulated environments and evaluated for the survival of *Salmonella.* The recovery of *Salmonella* off fruit and material surfaces were assessed to determine if a specific temperature and relative humidity combination would affect *Salmonellae* survival over a prolonged period of time.

Each type of surface was sampled in triplicate for all observation intervals; Day 0, 1, 3, 7, 11, 14, 21 and 28. The recovered *Salmonella* from each replicate was averaged and data was compiled into graphs. Graphs depict the relationship between $\log_{10}$ CFU/ml *Salmonella* survivors and time (days) for all surfaces in each simulated environment.

Growth Levels of *Salmonella* Serovars after a 20-Hour Incubation

Two 20-hour growth studies were conducted for each of the five rifampicin-resistant serovars. No significant differences in growth rates were found to exist for any of the five serovar's growth rates observed between the two studies ($P < 0.05$). Results from these preliminary studies ensured that serovar growth rates were equivalent to one another and a consistent inoculum could be created (Figure 4-1). Prior to each

experiment, the cell concentration of each *Salmonella* cocktail was estimated. This was accomplished by pour plating appropriate dilutions for each cocktail in triplicate using TSA (rif+). No significant differences (P <0.05) were found to exist between any inocula suspensions used for any recovery studies (data not shown).

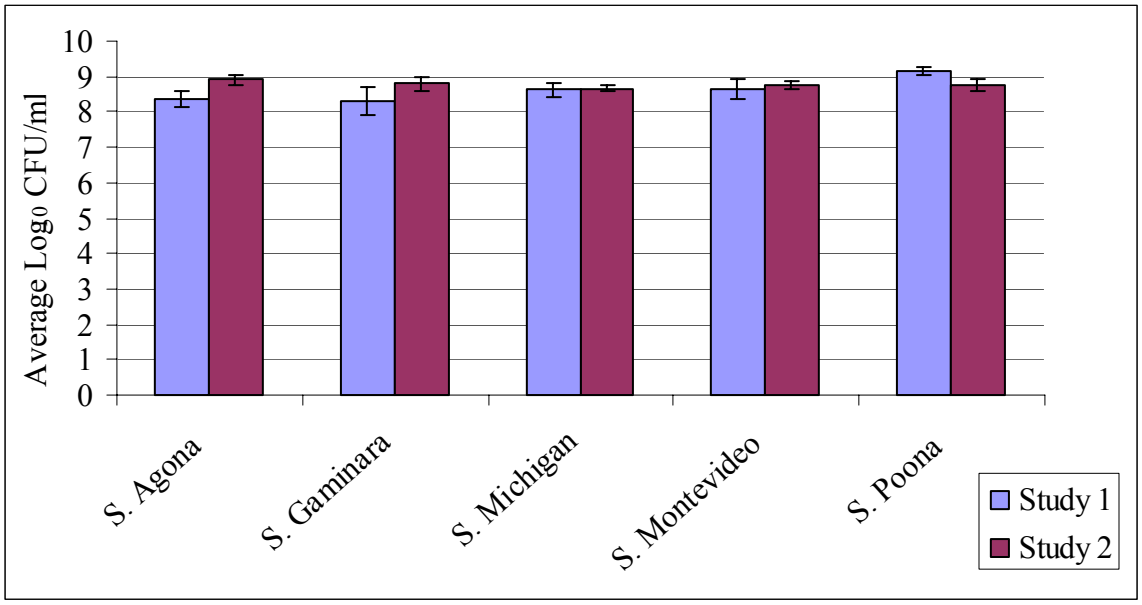

Figure 4-1. Average $\log_{10}$ counts of five *Salmonella* serovars (rif+) after a 20-hour incubation.

Recovery of *Salmonella* off Tomato Surfaces

Mature green tomatoes (Florida 47) were inoculated with a five serovar *Salmonella* cocktail and stored separately for 28 days in all simulated environments. It should be noted that the simulated ripening room parameters did not include the addition of ethylene. Commercially, ethylene is typically applied during the ripening process of mature green tomatoes. Tomatoes not inoculated with the *Salmonella* cocktail were sampled at the beginning of each experiment to ensure the rifampicin-supplemented TSA eliminated all background microflora present on the fruits. All controls were found to be negative.

Tomatoes Subjected to Spring Parameters

Tomatoes subjected to spring production parameters, 30°C and 80%RH, showed an overall decrease in $\log_{10}$ values of *Salmonella* for Day 0 to Day 21, but a slight increase in *Salmonella* recovery was observed between Day 21 and Day 28 (Figure 4-2). The inoculum applied to tomato surfaces was estimated at 8.26 $\log_{10}$ CFU/ml. On Day 0, 5.08 $\log_{10}$ CFU/ml of the applied inoculum was recovered from tomato surfaces. At Day 21, no *Salmonella* was recovered from tomato surfaces. The greatest average reduction of recovered *Salmonella* was observed between Day 3 and Day 7 at 2.55 $\log_{10}$ CFU/ml. Unexpectedly, a 1.17 $\log_{10}$ CFU/ml increase was then observed on Day 28. This was unexpected because no *Salmonella* was recovered on Day 21. This increase was found to be significant (P <0.05). As the experiment progressed and tomatoes ripened, fruits held in this regime (30°C/80%RH) appeared more orange in color than the tomatoes held at a lower temperature (20°C).

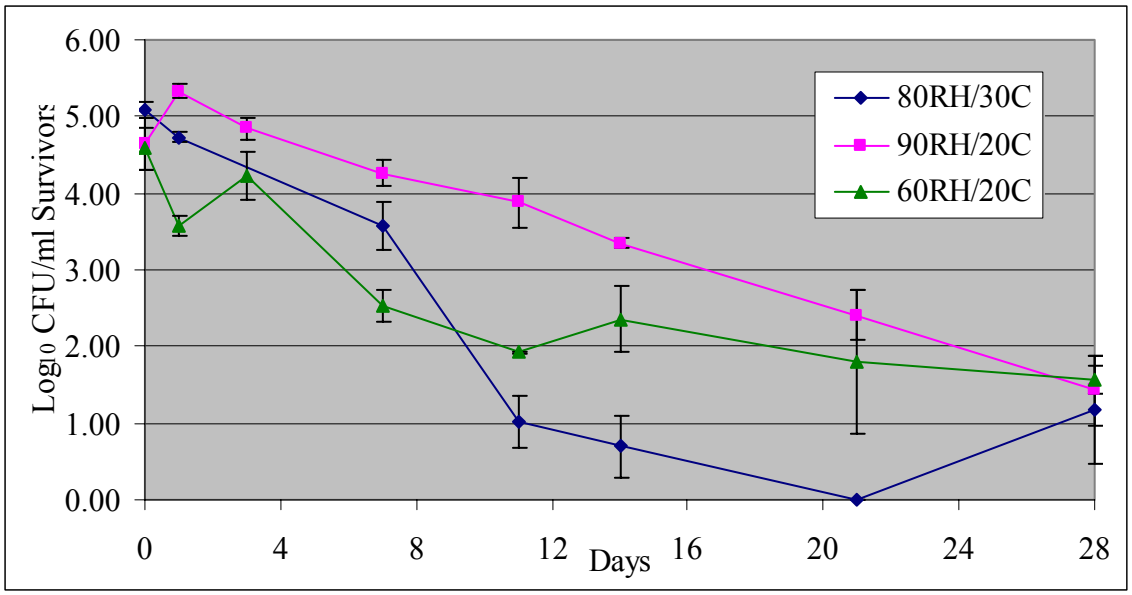

Figure 4-2. *Salmonella* recovery ($\log_{10}$ CFU/ml) from tomato surfaces in ripening room parameters (20°C/90%RH) and spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Tomatoes Subjected to Fall/Winter Parameters

Tomatoes subjected to Florida fall/winter tomato season parameters, 20°C and 60%RH, also showed an overall $\log_{10}$ reduction of *Salmonella* on the surfaces of tomatoes over 28 days (Figure 4-2). The inoculum applied to tomato surfaces was estimated at 8.59 $\log_{10}$ CFU/ml. On Day 0, 4.01 $\log_{10}$ CFU/ml of *Salmonella* cocktail was recovered. A 1.00 $\log_{10}$ CFU/ml reduction was observed between Day 0 and Day 1, but a 0.66 $\log_{10}$ CFU/ml increase of recovered *Salmonella* was observed from Day 1 to Day 3. This slight increase was found to be insignificant (P <0.05). Again, a significant decrease in $\log_{10}$ CFU/ml was observed from Day 3 to Day 11 at 2.31 $\log_{10}$ CFU/ml. From Day 11 to Day 14, another insignificant increase of *Salmonella* was observed at 0.44 $\log_{10}$ CFU/ml. For the remainder of the 28-day period (Day 14 to Day 28) a slight reduction in CFU/ml recovery was observed. This minimal reduction in *Salmonella* over these days was not significant (P <0.05).

Tomatoes Subjected to Ripening Room Parameters

Tomatoes subjected to ripening room parameters, 20°C and 90%RH, exhibited an overall reduction of *Salmonella* on the surfaces of tomatoes for a 28-day period (Figure 4-2). The inoculum suspension applied to the fruits was estimated at an average value of 8.15 $\log_{10}$ CFU/ml. After the applied inoculum was allowed to completely dry, three tomatoes were sampled for Day 0. An average value of 4.64 $\log_{10}$ CFU/ml of recovered *Salmonella* was observed on Day 0. The average $\log_{10}$ value for Day 1 exhibited a slight increase of 0.7 $\log_{10}$ CFU/ml in recovered *Salmonella* from Day 0. This increase was found to be insignificant (P <0.05). From Day 1 to Day 28, the average recovered *Salmonella* off tomato fruits exhibited a significant decrease in value over time. On Day 28, 1.42 $\log_{10}$ CFU/ml of *Salmonella* was recovered from tomato surfaces.

Comparison of Tomato Recovery Studies

Recovery observations showed that *Salmonella* was recovered on final sampling interval (Day 28) in all three simulated environments. The levels of recovered *Salmonella* at the end of the three experiments were not significantly different from one another ($P < 0.05$). The largest $\log_{10}$ value reduction of *Salmonella* was observed for tomatoes held at 30°C and 80%RH for 28 days. Tomatoes that were held at 20°C and 60%RH had variable recovery that exhibited two separate increases in $\log_{10}$ values for between sampling periods of Day 1 and 3, and Day 11 and 14. The increases were found to be insignificant; nonetheless it was unexpected that a slightly greater amount of *Salmonella* was recovered on Day 14 than Day 11. Tomatoes held at 20°C and 90%RH exhibited a very linear pattern of reduction for $\log_{10}$ values between Day 1 and Day 28 ($R^2 = 0.9965$). *Salmonella* was able to survive in all simulated environments, but survival patterns were very different. Day 21 in spring parameters (30°C/80%RH) was the only sampling interval for any environment where no *Salmonella* was recovered.

Recovery of *Salmonella* off Packing Line Surfaces

Fresh-market tomato packinghouses are typically open-air facilities. The environments simulated for all materials paralleled spring and fall/winter parameters for tomato production seasons in Florida. Each type of material was subjected to both simulated environments for 28 days.

Stainless Steel Surfaces Subjected to Spring Parameters

Stainless steel surfaces held at 30°C and 80%RH showed a total $\log_{10}$ reduction at Day 11 (Figure 4-3). The inoculum applied to stainless steel surfaces was estimated at 8.01 $\log_{10}$ CFU/ml. A value of 4.39 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. No significant $\log_{10}$ reduction was observed between Day 0 and Day 1. A significant log

reduction of 4.34 $\log_{10}$ CFU/ml was observed from Day 1 to Day 11. On Day 11, no *Salmonella* was recovered. The reduction in *Salmonella* followed a linear pattern (R^2 = 0.9875). On Days 11, 14, 21 and 28 no *Salmonella* was recovered. Day 7 was the last sampling interval where *Salmonella* was recovered (1.29 $\log_{10}$ CFU/ml) from the surfaces of stainless steel.

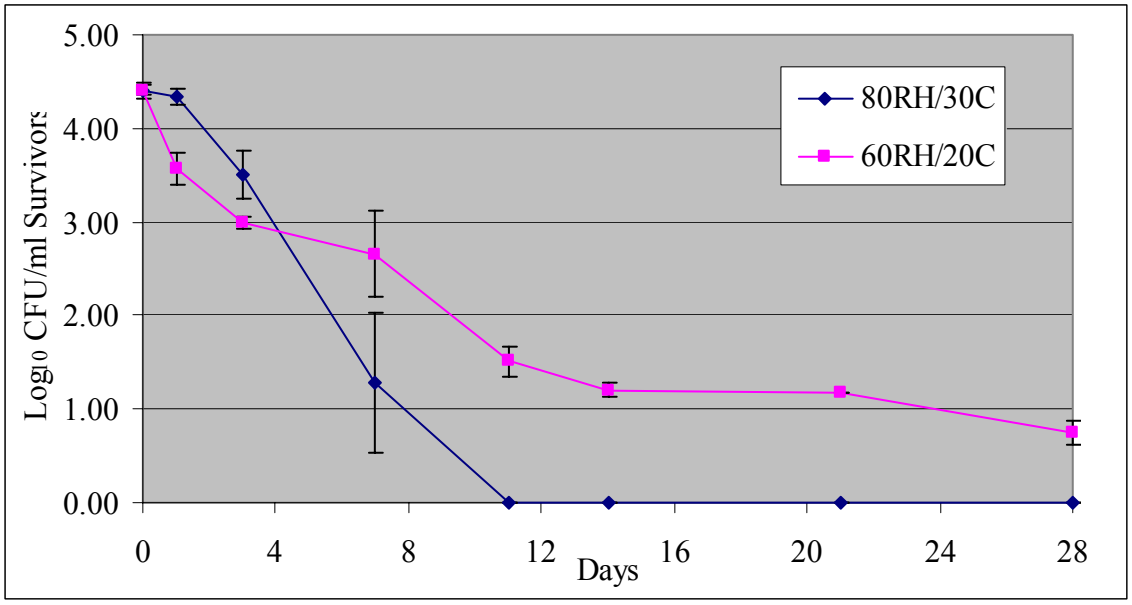

Figure 4-3. *Salmonella* recovery ($\log_{10}$ CFU/ml) from stainless steel surfaces in spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Stainless Steel Surfaces Subjected to Fall/Winter Parameters

Stainless steel surfaces held at 20°C and 60%RH did not exhibit a total $\log_{10}$ value reduction of *Salmonella* at the conclusion of the sampling period (Figure 4-3). For the entire 28-day period, an overall log value reduction of 3.67 $\log_{10}$ CFU/ml was observed. The inoculum applied to stainless steel surfaces was estimated at 8.59 $\log_{10}$ CFU/ml. A value of 4.41 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. From Day 0 to Day 11, a 2.96 $\log_{10}$ CFU/ml reduction of *Salmonella* was observed. On Day 14, the amount of recovered *Salmonella* was similar to the $\log_{10}$ value recovered on Day 11. A 0.46 $\log_{10}$

CFU/ml reduction was observed between Day 14 and Day 28. This reduction was not significant (P <0.05). On Day 28, 0.74 $\log_{10}$ CFU/ml of *Salmonella* was recovered.

Comparison of Stainless Steel Recovery Studies

The average $\log_{10}$ values of *Salmonella* recovered on Day 0 for each experiment were not significantly different from one another (P <0.05). In the simulated spring environment, it was observed that *Salmonella* did not survive past Day 11 on stainless steel surfaces. For fall/winter environments, it was observed that *Salmonella* survived on stainless steel surfaces for the entire 28-day period. Recovered *Salmonella* survival off the stainless steel was significantly higher at 20°C and 60%RH than recovered *Salmonella* at 30°C and 80%RH (P <0.05).

PVC Surfaces Subjected to Spring Parameters

The inoculum applied to PVC surfaces was estimated at 8.01 $\log_{10}$ CFU/ml. A value of 5.13 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. PVC surfaces subjected to spring production parameters showed a total $\log_{10}$ reduction (5.13 $\log_{10}$ CFU/ml) by Day 11 (Figure 4-4). A linear pattern of total *Salmonella* reduction was observed from Day 0 to Day 11 (R^2 = 0.9636). The last detection of *Salmonella* on PVC surfaces occurred on Day 7 with an average $\log_{10}$ value of 1.00 $\log_{10}$ CFU/ml.

PVC Surfaces Subjected to Fall/Winter Parameters

Salmonella was recovered from the surfaces of PVC surfaces for every sampling interval over a 28-day period (Figure 4-4). The inoculum applied to stainless steel surfaces was estimated at 8.59 $\log_{10}$ CFU/ml. A value of 5.14 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. A significant decrease in *Salmonella* reduction observed over the 28-day period occurred from Day 0 to Day 1 with an average $\log_{10}$ reduction of 1.19 $\log_{10}$ CFU/ml. Overall, there was an average 4.57 $\log_{10}$ CFU/ml

reduction observed from Day 1 to Day 28. On Day 28, an average of 0.573 log10 CFU/ml of *Salmonella* was recovered from the surfaces of PVC.

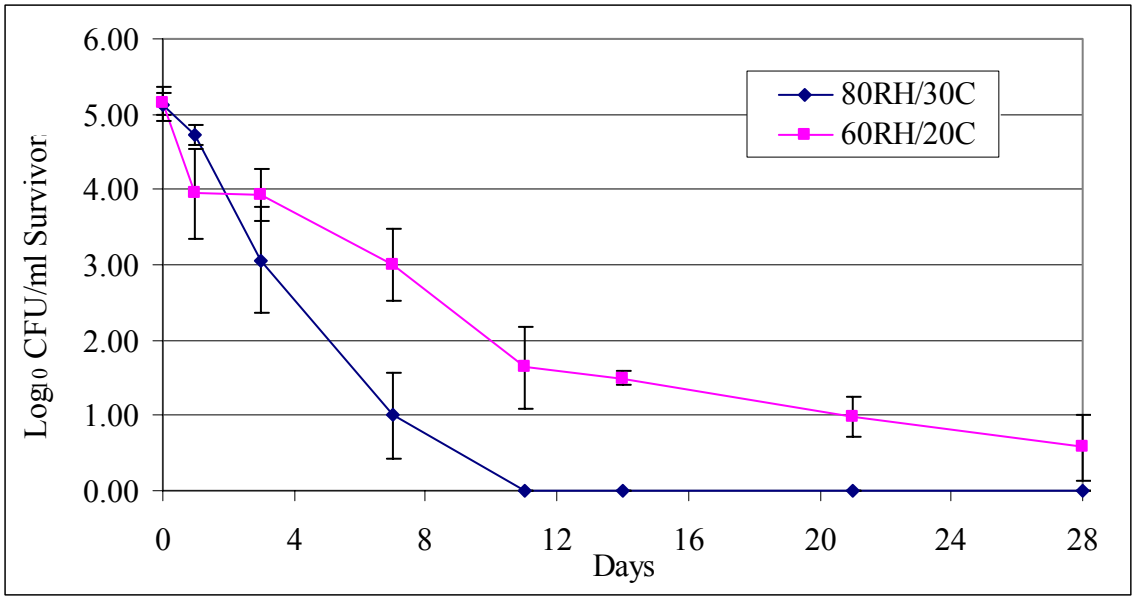

Figure 4-4. *Salmonella* recovery (log_{10} CFU/ml) from PVC surfaces in spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Comparison of PVC Recovery Studies

The average log_{10} values recovered on Day 0 for each experiment were not significantly different from one another (P <0.05). The most significant decrease in *Salmonella* recovery in fall/winter parameters was observed in Days 0 through Day 11 with a 3.50 log_{10} CFU/ml reduction. For the final three sampling periods (Day 14, 21 and 28), *Salmonella* only exhibited a 1.07 log_{10} CFU/ml reduction. *Salmonella* was recovered off PVC surfaces held in spring parameters for the first four sampling intervals (Day 0-Day 7). *Salmonella* was recovered for a longer period of days from PVC surfaces at a lower temperature/relative humidity combination than at a temperature/higher relative humidity combination. *Salmonella* was not recovered from PVC surfaces held in spring parameters after Day 7. However, *Salmonella* was recovered off PVC surfaces held in fall/winter parameters for the entire 28-day period. The survival of *Salmonella* on PVC

surfaces held in 20°C and 60%RH was significantly higher than *Salmonella* on PVC surfaces at 30°C and 80%RH (P <0.05).

Sponge Rollers Subjected to Spring Parameters

The inoculum applied to sponge roller surfaces was estimated at 8.01 $\log_{10}$ CFU/ml. A value of 4.97 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0 (Figure 4-5). Sponge rollers held at spring parameters exhibited a complete $\log_{10}$ value reduction of 4.97 $\log_{10}$ CFU/ml by Day 1. *Salmonella* was only recovered on Day 0. *Salmonella* was not able to be recovered from sponge rollers once they had entered the simulated environment at 30°C and 80%RH.

Sponge Rollers Subjected to Fall/Winter Parameters

The inoculum applied to sponge roller surfaces was estimated at 8.59 $\log_{10}$ CFU/ml. A value of 4.06 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. Sponge rollers held at fall/winter parameters exhibited a complete $\log_{10}$ value reduction of 4.06 $\log_{10}$ CFU/ml by Day 7 (Figure 4-5). *Salmonella* was only recovered on Days 0, 1 and 3. On Day 3, the average $\log_{10}$ value of *Salmonella* recovered was 0.30 $\log_{10}$ CFU/ml. A very linear and significant $\log_{10}$ value reduction of *Salmonella* was observed between Day 0 and Day 3 (R^2 = 0.9999).

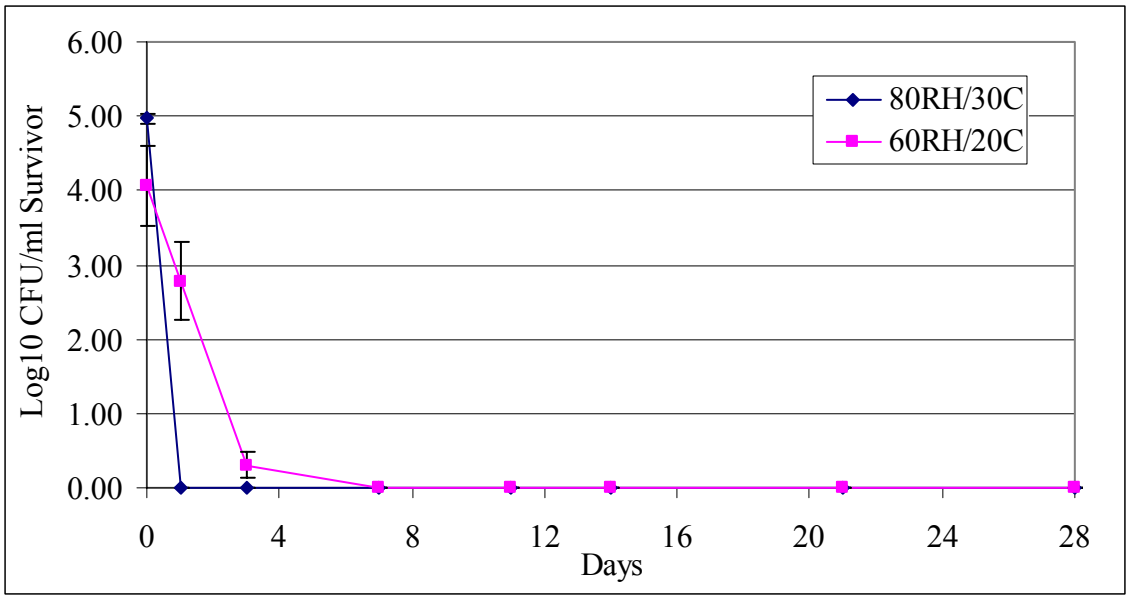

Figure 4-5. *Salmonella* recovery (log_{10} CFU/ml) from sponge roller surfaces in spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Comparison of Sponge Roller Recovery Studies

The log_{10} values recovered on Day 0 for each experiment were not significantly different from one another (P <0.05). Significant reduction of *Salmonella* was observed from Day 1 to Day 3 off sponge rollers held in fall/winter parameters. Significant reduction of *Salmonella* was observed from Day 0 to Day 1 from sponge rollers held in spring parameters. *Salmonella* was recovered in one more sampling interval (Day 3) in the simulated fall/winter parameters than in spring parameters. *Salmonella* was recovered only at Day 0 from sponge rollers held in spring parameters. No *Salmonella* was recovered from any of the two environments at Days 7, 11, 14, 21 and 28. On Day 3, *Salmonella* recovery from surfaces of sponge rollers held at 20°C and 60%RH was significantly higher than the *Salmonella* recovery on sponge rollers held at 30°C and 80%RH (P <0.05). Sponge rollers were dampened with sterile, distilled water when inoculated. Sponge surfaces did not remain moist over the 28 day sampling intervals.

Conveyor Belt Surfaces Subjected to Spring Parameters

Salmonella was only recovered off conveyor belt surfaces held at spring parameters (30°C/80%RH)on Days 0, 1 and 3. The inoculum applied to conveyor belt surfaces was estimated at 8.01 $\log_{10}$ CFU/ml. A value of 4.10 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0 (Figure 4-6). A linear and significant reduction in the recovery of *Salmonella* was observed between Day 0 and Day 3 ($R^2 = 0.9803$). *Salmonella* was last recovered on Day 1 at 2.26 $\log_{10}$ CFU/ml from conveyor belt surfaces.

Conveyor Belt Surfaces Subjected Fall/Winter Parameters

The inoculum applied to conveyor belt surfaces was estimated at 8.59 $\log_{10}$ CFU/ml. A value of 4.25 $\log_{10}$ CFU/ml of *Salmonella* was recovered on Day 0. Conveyor belt surfaces stored at 60%RH and 20°C showed a $\log_{10}$ value reduction of 1.4 $\log_{10}$ CFU/ml between Day 0 and Day 1 (Figure 4-6). Between Day 1 and Day 21, a 2.85 $\log_{10}$ CFU/ml reduction was observed. *Salmonella* was last recovered from conveyor belt surfaces on Day 14 at an average $\log_{10}$ value of 0.60 $\log_{10}$ CFU/ml.

Comparison of Conveyor Belt Recovery Studies

The average $\log_{10}$ values recovered on Day 0 for each experiment were not significantly different from one another ($P < 0.05$). *Salmonella* was recovered for a longer period of days from conveyor belt surfaces in 20°C and 60%RH than at 30°C and 80%RH. *Salmonella* was only recovered for Day 0 and Day 1 in the simulated spring environment, whereas *Salmonella* recovery was observed until Day 14 in the simulated fall/winter environment. *Salmonella* recovery from conveyor belt surfaces in 20°C and 60%RH was significantly higher than *Salmonella* recovery from conveyor belt surfaces in 30°C and 80%RH ($P < 0.05$).

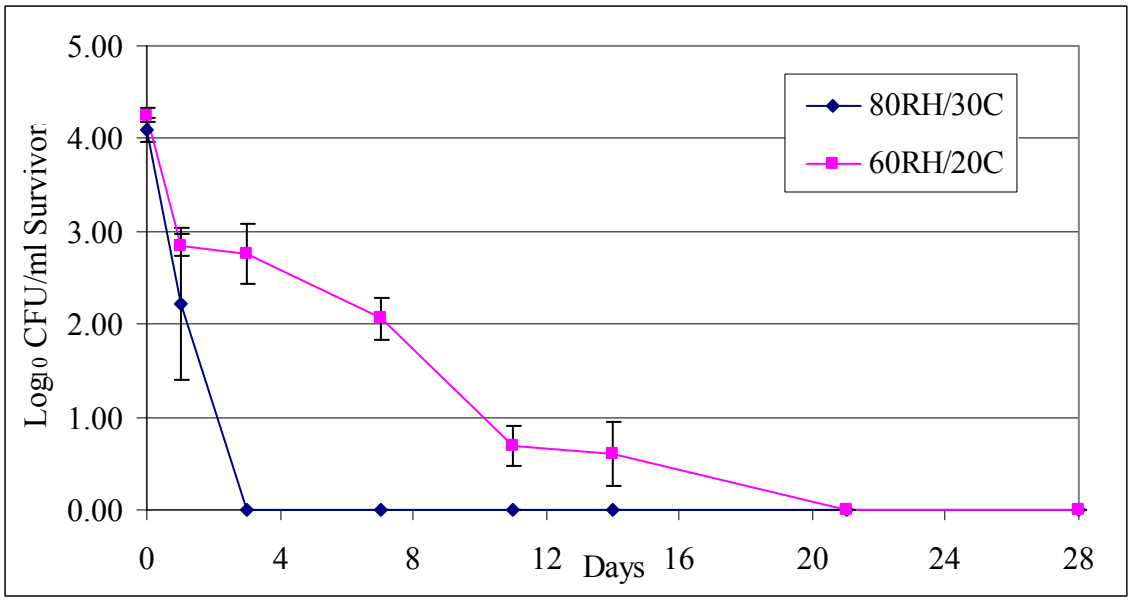

Figure 4-6. *Salmonella* recovery (log_{10} CFU/ml) from conveyor belt surfaces in spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Unfinished Oak Surfaces Subjected Spring Parameters

The inoculum applied to unfinished oak surfaces was estimated at 8.01 log_{10} CFU/ml. A value of 4.73 log_{10} CFU/ml of *Salmonella* was recovered on Day 0. Unfinished oak surfaces held in spring parameters exhibited a total log_{10} value reduction by Day 21 of the 28-day sampling period (Figure 4-7). A significant decrease of 2.62 log_{10} CFU/ml was observed from Day 0 to Day 1. From Day 1 to Day 3, a 0.89 log_{10} CFU/ml increase was observed. This increase was found to be insignificant (P <0.05). On Day 3, no *Salmonella* was recovered and this trend continued until Day 14. On Day 14, *Salmonella* was recovered from oak surfaces at 1.00 log_{10} CFU/ml. *Salmonella* was not recovered from unfinished oak surfaces for the final two sampling periods, Day 21 and Day 28.

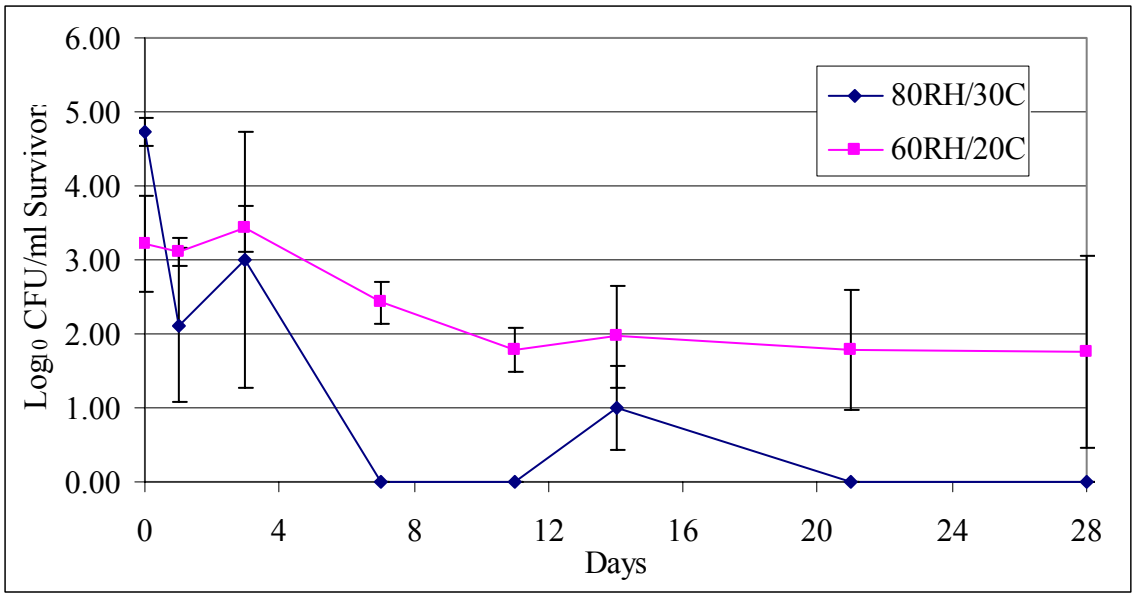

Figure 4-7. *Salmonella* recovery (log_{10} CFU/ml) from unfinished oak surfaces in spring (30°C/80%RH) and fall/winter (20°C/60%RH) regimes over 28 days.

Unfinished Oak Surfaces Subjected to Fall/Winter Parameters

The inoculum applied to unfinished oak surfaces was estimated at 8.59 log_{10} CFU/ml. A value of 3.22 log_{10} CFU/ml of *Salmonella* was recovered on Day 0. *Salmonella* was recovered off the surfaces of unfinished oak at every sampling interval (Figure 4-7). An initial 0.12 log_{10} CFU/ml reduction of recovered *Salmonella* was observed from Day 0 to Day 1. From Day 1 to Day 3, a 0.33 log_{10} CFU/ml increase in recovered *Salmonella* was observed. This slight increase was determined to be insignificant (P <0.05). A 1.65 log_{10} CFU/ml reduction was observed from Day 3 to Day 11. Unexpectedly, *Salmonella* was recovered at a log_{10} value on Day 14 at 0.18 log_{10} CFU/ml. The decrease log_{10} values observed from Day 14 to Day 21 was not significant (P <0.05). A 0.20 log_{10} CFU/ml reduction of *Salmonella* was observed during the final two sampling periods.

Comparison of Unfinished Oak Recovery Studies

The $\log_{10}$ values recovered on Day 0 for each experiment were not significantly different from one another (P <0.05). Viable *Salmonella* recovered from unfinished oak surfaces was recovered in greater amounts and for a longer period of days in spring parameters than at fall/winter parameters. Survival of *Salmonella* on oak surfaces stored at fall/winter parameters was significantly higher (P <0.05) than the survival of *Salmonella* on oak surfaces held in spring parameters. *Salmonella* recovery off of unfinished oak surfaces was variable for both simulated environments.

CHAPTER 5
DISCUSSION

Consumption of fresh fruits and vegetables has significantly increased over the past ten years. The industry is constantly challenged with the concern of microbial food safety hazards. Many steps are taken to harvest, process and distribute fresh produce and with each step the opportunity for potential pathogenic contamination increases. Environmental factors such as temperature and relative humidity have a large impact on the quality of fruits and vegetables along with the survival capacity of present pathogens. Effective intervention strategies have been implemented in packinghouses, such as chlorinated dump tanks, but these strategies cannot totally eliminate all microbiological dangers associated with the consumption of raw produce. It is also necessary that packinghouse equipment receive regular cleaning and disinfecting. In recent years, multiple foodborne illnesses associated with consumption of *Salmonella*-contaminated tomatoes have been traced to packinghouse facilities. In this study, a five serovar rifampicin-resistant *Salmonella* cocktail was administered to tomato and packing line surfaces. The various surfaces were subjected to different temperature and relative humidity combinations that simulated conditions encountered during tomato growing, packing and ripening. Recovery of *Salmonella* from the surfaces was performed by placing the surfaces into 100 ml of PBS and applying a rub-shake method as previously described.

It has been recommended that a minimum of five strains at approximately equal populations be selected for the inoculum (CFSAN-FDA 2001). The five *Salmonella*

enterica serovars selected for this study were *S.* Agona, *S.* Gaminara, *S.* Michigan, *S.* Montevideo and *S.* Poona. These serovars were obtained from Dr. Linda J. Harris, University of California Davis, and were marked with 80µg/ml rifampicin. Rifampicin was selected because it is a stable marker and is particularly effective for isolating pathogens from inoculated fruits that have significant natural background microflora and adhering soil. *S.* Agona, Gaminara and Michigan serovars were isolated from fresh produce or produce products (orange juice). *S.* Montevideo and Poona serovars were human isolates linked to fresh produce outbreaks.

Growth characteristics for all five serovars were evaluated by conducting 20-hour growth studies. Two studies were conducted on each of the five serovars. The population of each serovar at the end of a 20-hour incubation period was found to be insignificantly different (Student's *t* test, $\alpha = 0.05$) from one another (Figure 4-1). *S.* Poona was observed to have the highest population at the end the 20-hour incubation period (37^{o}C), but there was less than a 0.5 $\log_{10}$ CFU/ml difference between *S.* Poona's population and the serovar with the lowest growth level. This was the case in both growth studies. It was determined that all five serovars achieved counts of at least 1.0×10^{8} CFU/ml after 20 hours of incubation. It was then concluded that acceptable inocula could be prepared from the five rifampicin-resistant *Salmonella* serovars. Prior to each recovery study, appropriate serial dilutions of the inoculum were pour-plated to determine the viable population of *Salmonella*. These counts for each prepared inoculum for all experiments conducted also showed little variation between one another. All inocula were determined to contain viable *Salmonellae* populations at 1.08×10^{8} CFU/ml.

Recovery of *Salmonella* off Tomato Surfaces

Tomato surfaces were subject to three simulated environments: ripening room parameters (20°C/90% RH), fall/winter tomato production season parameters (20°C/60% RH) and spring tomato production season parameters (30°C/80%RH). Surface recovery was assessed by applying a rub-shake method, as previously described. Tomatoes have a fairly firm surface that can withstand moderate rubbing and agitation. This rub-shake method of recovery was chosen because presently, it seems to be the most effective protocol for removing microorganisms from the surfaces of whole fruits and vegetables like tomatoes (CFSAN-FDA 2001). Whole, unblemished tomatoes were specifically chosen for inoculation studies. It has been researched that microbial cells that contact the surface of produce and interact with organic acids or other antimicrobials that are naturally found in plant tissue fluid or ruptured cells as a result of mold or insect invasion, cellular death may occur (Sofos et al. 1998). The rub-shake method is a simple surface wash that recovers surface bacteria without rupturing any plant cells that might interact with the inoculated pathogen. Spot inoculation was utilized because it enables the measurement of a known number of cells adhering to the produce. Dip or spray inoculation procedures do not allow the measurement of a known amount of inoculum.

Results from this study were in agreement with Guo et al. (2002) in that *Salmonella* populations decreased over time on tomato surfaces in all simulated environments. The lowest quantities of *Salmonella* were recovered from tomatoes held in the spring season parameters (30°C/80%RH). Viable *Salmonella* populations seemed to die-off or enter a nonculturable state by Day 21 of the recovery study. On Day 28, an unexpected increase in $\log_{10}$ value was observed. *Salmonella* was recovered from two of the three tomato replicates sampled on Day 28, but $\log_{10}$ values were significantly higher

than Day 21 where no recovery of *Salmonella* was observed. Between Day 7 and Day 11, a $\log_{10}$ value decrease of 2.55 $\log_{10}$ CFU/ml was observed. This was the largest $\log_{10}$ value decrease of *Salmonella* seen between any two sampling periods for all simulated tomato environments.

Tomatoes held in ripening room (20°C/90%RH) and fall/winter production parameters (20°C/60%RH) seemed to exhibit similar patterns of *Salmonella* recovery. The highest $\log_{10}$ values of *Salmonella* populations were recovered from tomato surfaces held in ripening room parameters. Slightly more *Salmonella* was recovered on Day 1 than on Day 0. This increase was insignificant, but a possible explanation for this phenomenon could be that some cells of *Salmonella* did not survive the drying process while others could have survived, but were shocked and could not be recovered by conventional culture methods (CFSAN-FDA 2001). For Days 1 through Day 28, a linear reduction of *Salmonella* was observed with the largest decrease in recovered $\log_{10}$ values seen between Day 21 and Day 28 at 1.0 $\log_{10}$ CFU/ml.

Tomato surfaces held in fall/winter parameters showed the most *Salmonella* reduction between Day 0 and Day 1 when compared to the other environments. On Day 3, an increase in S*almonella* recovery was observed. The same phenomenon for injured cells could have occurred as previously mentioned. The lower amount of moisture (60%RH) in the environment could explain the delayed recovery of injured cells on Day 3 instead of Day 1 as seen in ripening room parameters (90%RH). The availability of more moisture could have possibly allowed *Salmonella* to recover at a faster rate. An approximate 2.0 $\log_{10}$ CFU/ml reduction in recovered *Salmonella* was observed from Day

3 to Day 11 and again, on Day 14 a slight increase in recovered *Salmonella* was observed.

Very similar levels of *Salmonella* were recovered from tomato surfaces for the last three sampling intervals. All three simulated environments exhibited *Salmonella* recovery on Day 28 at very equivalent $\log_{10}$ values (approximately 1.5 $\log_{10}$ CFU/ml). *Salmonella* survival patterns were different for every simulated environment, but the final sampling interval yielded similar $\log_{10}$ values of recovered *Salmonella.* Overall, *Salmonella* was recovered more in an environment where the temperature was maintained at 20°C and the relative humidity was at a high level. It has been documented by many researchers that bacterial populations have a greater chance of survival and growth in the presence of free moisture on leaves, from precipitation, dew or irrigation. Essentially, a higher level of humidity enhances the survival of bacterial cells (Beattie and Lindow 1999). *Salmonella* was still observed to survive very well at 20°C in conjunction with a slightly lower relative humidity. *Salmonella* seemed less likely to survive in an elevated temperature (30°C).

Salmonella was consistently recovered from tomato surfaces at a greater $\log_{10}$ value than any of the packinghouse surfaces while utilizing the rub-shake method of recovery. Tomatoes are organic surfaces that respire and participate in gas exchange with the surrounding atmosphere. The tomatoes may have experienced different rates of respiration at 20°C than at 30°C and this could have had some effect on the *Salmonella*. Viable *Salmonella* could have aggregated in the stem scar of the fruit or have become irreversibly attached to the fruit's surface and was not recovered. Over time, *Salmonella*

could have entered a nonculturable state do to nutrient depletion, injury or environmental stress.

After harvest, pathogens seem to survive but not proliferate on the outer surface of tomato fruits, especially in a high humidity and an ambient temperature (20^{o}C). Pathogen levels were observed to decline on the outer surface of tomatoes over time and the rate of reduction seemed to be strongly related to temperature. Growth on intact surfaces was not observed. Foodborne pathogens do not produce the necessary enzymes to destroy the protective outer barriers on most produce, thus restricting the availability of nutrients. *Salmonella* was recovered from tomato surfaces in all three simulated environments and this indicates that the pathogen can survive on tomato surfaces for a significant amount of time and should be a concern in the fresh produce industry. If contaminated fruits enter a packinghouse facility it is very probable that cross-contamination is likely upon the contact of processing equipment.

Recovery of *Salmonella* off Packinghouse Surfaces

Five types of packing line materials were inoculated with a five serovar rifampicin-resistant *Salmonella* cocktail and subjected to fall/winter and spring tomato production season parameters. Typically, Florida packinghouse facilities are open-sided, shed-like buildings that shelter the minimal processing of fresh produce harvested in near by fields. The five materials that were evaluated in recovery studies were stainless steel (type 304, no. 4 finish), polyvinyl chloride (PVC), sponge rollers, conveyor belts and unfinished oak surfaces.

Stainless steel is commonly found in most food processing facilities. It is a smooth, easily sanitized surface that is widely recognized as an excellent material for the food industry (Midelet and Carpentier 2002). Dump tanks, processing lanes and much

equipment in tomato packinghouses are made of stainless steel. Overall, *Salmonella* was recovered at greater $\log_{10}$ values and over a longer period of days off stainless steel surfaces held in fall/winter parameters than stainless steel surfaces held in spring parameters. *Salmonella* was only recovered from stainless steel surfaces held at 80%RH and 30ºC for the first four sampling intervals (Days 0, 1, 3 and 7). *Salmonella* was last recovered at a $\log_{10}$ value of 1.29 $\log_{10}$ CFU/ml on Day 7. On Days 11, 14, 21 and 28 no *Salmonella* was recovered. Significantly more *Salmonella* was recovered from stainless steel surfaces held at 20ºC and 60%RH when compared to surfaces held at 30°C and 80%RH. The most reduction of *Salmonella* for both environments was observed from Day 0 to Day 11 off surfaces held in spring parameters. After Day 11 for surfaces held in spring parameters, the recovered populations of *Salmonella* did not significantly decrease. On Day 28, *Salmonella* was recovered at 0.74 $\log_{10}$ CFU/ml from surfaces held in spring parameters.

Polyvinyl chloride (PVC) is another commonly used material in the food industry. It is a polymer that is typically used to cover roller bars that move tomato fruits along processing lines. PVC rollers allow tomatoes to be smoothly transported so extensive bruising and injury is minimal. Recovery patterns of *Salmonella* from PVC surfaces for both simulated environments were very similar to recovery patterns from stainless steel surfaces. *Salmonella* was only recovered on Days 0, 1, 3 and 7 from PVC surfaces held in spring parameters. The most significant amount of *Salmonella* reduction (2.0 $\log_{10}$ CFU/ml) observed in spring parameters was seen between Days 3 and 7. Similar to recovery patterns from stainless steel surfaces, more $\log_{10}$ values of *Salmonella* were recovered from PVC surfaces at 60%RH and 20°C than from surfaces held at 30°C and

80%RH. *Salmonella* was recovered for every sampling period from PVC surfaces held in fall/winter parameters. It was very clear that viable *Salmonella* cells were able to survive on PVC surfaces for a prolonged period of days at 60%RH and 20°C.

Stainless steel and PVC are both hydrophobic surfaces (Midelet and Carpentier 2002). Stainless steel is a nonporous surface but is often marked by grooves and crevices. This was clearly seen in scanning electron photomicrographs taken of stainless steel type 304, no. 4 finish (Mafu et al. 1990). Stainless steel is also very resistant to wear. PVC is a dense polymer with smooth surface, but contains microscopic holes and crevices. PVC is also resistant to wear, but is more likely to bend or accumulate cracks or holes than stainless steel surfaces. Of all the surfaces tested in this recovery study, stainless steel and PVC were the least porous materials. The most amounts of *Salmonella* were recovered from these two surfaces when compared to the other surfaces that were tested. It is most likely that very few salmonellae infiltrated into the matrix of these two surface types. Hydrophobic qualities accompanied with the dense nature of the materials most likely prevented bacteria from migrating very far from the point of inoculation. It was evident that more *Salmonella* was recovered off both of these surface types at 20°C than at 30°C. *Salmonella* was not recovered from surfaces held at 30°C for a prolonged period of time. Viable cells of *Salmonella* were not recovered from either surface past Day 7 at 30°C and 80%RH. It is possible that *Salmonella*, at 30°C, had entered a nonculturable state due to environmental stresses. Biofilm formation is yet another possibility, although this is not likely because no planktonic cells were recovered from either surface during the final four sampling intervals. It has been documented that bacteria can readily and irreversibly attach to many surface types upon very short contact

times, even within one minute (Mafu et al. 1990). Surface types that have been exposed to bacteria over short contact times and have been observed to form biofilms are glass, rubber, stainless steel, and many types of plastics (Ronner and Wong 1993; Mafu et al. 1990).

Conveyor belts play an integral part in the functions of a packinghouse. Belts are rubber compounds (composition not disclosed by manufacturer) that transport tomatoes to all areas in the facility. Conveyor belts are used to pre-size, cull, sort and size tomato fruits. Rubber surfaces are smooth to the touch, but scanning electron pictographs show that particles, crevices and holes appear on the surface (Mafu et al. 1990).

Salmonella was recovered from conveyor belt surfaces held at 30°C and 80%RH on Days 0 and 1. No *Salmonella* was recovered for any other sampling intervals for spring parameters. However, *Salmonella* was recovered at a significantly higher amount from conveyor belt surfaces held at 20°C and 60%RH. Recovery was observed on Days 0 through 14. A sharp reduction of *Salmonella* was seen between Day 0 and Day 1. Between Days 1 and 11, an approximate 2.0 $\log_{10}$ CFU/ml reduction of *Salmonella* was observed. The reported $\log_{10}$ values of recovered *Salmonella* on Days 11 and 14 were very similar, almost no reduction was seen. For the final two sampling intervals, no *Salmonella* was recovered from conveyor belt surfaces held at 20°C and 60%RH.

The patterns of recovered *Salmonella* from conveyor belt surfaces differed from those of stainless steel and PVC because no *Salmonella* was recovered from conveyor surfaces held at 20°C and 60%RH after Day 14. *Salmonella* was recovered from stainless steel and PVC surfaces until Day 28 for surfaces held at 20°C and 60%RH. It was still evident that *Salmonella* survived for a longer period of days on conveyor

surfaces held at 20°C than surfaces held at 30°C. It is possible that the composition of the conveyor belts had a slight bacteriostatic affect on *Salmonella.* The pathogen was recovered at very low amounts on Days 11 and 14 and no survival was observed to occur at Days 21 and 28. An extreme reduction was also recorded between Day 0 and Day 1. It has been documented that some types of rubber surfaces have a strong bacteristatic effect on pathogens. Buna-n rubber (nitrile rubber) is a gasket material typically used in food processing environments. It has been documented that material has a slight bacteriostatic effect on *Salmonella* Typhimurium and a strong bacteristatic effect on *Listeria monocytogenes* under low nutrient conditions. It also inhibited the growth of several other pathogens to varying degrees (Ronner and Wong 1993).

Sponge rollers also serve a very important role in tomato packinghouse operations. Tomato fruits are susceptible to injury and bruising. Sponge rollers buff and cushion the fruits as they proceed along the processing lines. Sponge rollers absorb dumptank water off the fruit surface and the sponges are constantly moist. Thus, sponge roller samples were dampened with sterile, distilled water prior to inoculation. The surface and matrix of the rollers were extremely porous and small holes were clearly visible. Sponge roller surfaces were hydrophilic and absorbed the inocula whereas previous surfaces were hydrophobic.

Very little *Salmonella* was recovered from sponge rollers held in fall/winter or spring parameters. Approximately 5.0 $\log_{10}$ CFU/ml of *Salmonella* was recovered from sponge rollers held at 30°C and 80%RH on Day 0. Day 0 was the only sampling interval in which *Salmonella* was recovered for spring parameters. Fall/winter parameters allowed *Salmonella* to be recovered from sponge rollers for a longer period of days than

spring parameters. Recovery was observed on Days 0, 1 and 3 for rollers held in fall/winter parameters. Day 1 was the only sampling interval that was found to be significantly different between the two simulated environments. On Day 1, a significantly higher $\log_{10}$ value of *Salmonella* was recovered from sponge rollers held at 20°C and 60%RH. On Day 3, a very low $\log_{10}$ value of *Salmonella* was recovered from surfaces held in fall/winter parameters and the difference was determined to be insignificant when compared to the recovery on Day 3 for sponges in spring parameters. Overall, the two recovery patterns for the two simulated environments were very similar. The composition of the sponge rollers (not disclosed by the manufacturer) seemed to have a strong bacteriostatic effect on *Salmonella.* Even with the extreme reduction of *Salmonella* seen in both environments, the pathogen was recovered for a longer time period at 20°C and 60%RH.

Wooden pallets are used to transport unitized loads of tomato boxes and are used in many packinghouse facilities. Wooden field bins are sometimes used to collect harvested tomatoes, although plastic field bins are more common. Pallets are usually constructed from unfinished oak wood. Wood surfaces are very rough and porous. The oak surfaces were hydrophilic and inocula quickly soaked into the surfaces.

Salmonella recovery was extremely variable off unfinished oak surfaces. Overall, recovery of the pathogen did follow the pattern of recovery from the other surfaces. It was evident that *Salmonella* was recovered more from wooden surfaces held at 20°C and 60%RH. Surfaces held in fall/winter parameters seemed to facilitate the survival of *Salmonella* over the entire 28 day period. An approximate 1.0 $\log_{10}$ CFU/ml reduction of *Salmonella* was observed over the entire experiment. This amount of *Salmonella*

reduction was much lower than any reduction observed for other surface types. Recovery values did fluctuate between sampling intervals in both environments. On two separate sampling intervals, an increase in *Salmonella* recovery was observed rather than an anticipated decrease. For the final sampling interval (Day 28) in fall/winter parameters, *Salmonella* recovered from oak surfaces was significantly higher than the recovered *Salmonella* in spring parameters. The most variable recovery pattern observed throughout the entire recovery study was unfinished oak surfaces held at 30°C and 80%RH. From Day 0 to Day 1 a large reduction in *Salmonella* was observed. On Day 3, *Salmonella* recovery increased by 1.0 $\log_{10}$ CFU/ml. For the next two sampling periods, Days 7 and 11, no *Salmonella* was recovered. For all other materials, recovery patterns followed a trend. When no *Salmonella* had been recovered during one sampling interval, no other sampling intervals yielded the recovery of *Salmonella.* Unfinished oak surfaces did not follow this trend. On Day 14, *Salmonella* was recovered from wood surfaces at 1.0 $\log_{10}$ CFU/ml. On Days 21 and 28, the pathogen was not recovered.

Unfinished oak surfaces are known to be very coarse and irregular. It is suspected that the inocula seeped into the matrix of the wood samples. When the samples were rubbed and shaken for recovery purposes, it was evident that the recovery of *Salmonella* was extremely variable. *Salmonella* was most likely harbored by the matrix of the wood. Once the pathogen had migrated into the wood matrix recovery methods utilized were not able to extract the pathogen very easily. These results were in agreement with Boucher et al. (1998). *Campylobacter jejuni* was observed to exhibit enhanced survival on cubes of wood when compared to survival on cubes of plastic. Bacteria were observed to be sealed inside the porous membrane of the wood cubes. The physical structure was

necessary for the protection of *Campylobacter jejuni* and soluble free-radical scavengers from the wood were not responsible for the observed protection. Deeply scored plastic cubes did not offer enhanced survival in aerated broths. Scanning electron microscopy was utilized to determine the size of the openings within the wood in relation to the bacterial cells. Holes and crevices in the wood were noted to be larger than the bacterial cells allowing the cells to enter the wood matrix. It was established that the physical structure of the wood, rather than its chemistry was responsible for the wood's protective effect. It is postulated that the unfinished oak surfaces behaved similarly to the wood cubes examined in the previously described experiment. It is very likely that viable *Salmonella* was harbored inside the oak pieces and were not recovered.

Proliferation of *Salmonella* was not observed on any surface type. As previously stated, growth of pathogens on intact surfaces of fruit is not common because foodborne pathogens do not produce the enzymes necessary to breakdown the outer barriers that protect the produce (CFSAN-FDA 2001). The availability of nutrients and moisture is therefore limited. However, after harvest, pathogens are able to survive on the outer surfaces of fresh fruits and vegetables, especially if the humidity is high. This indicates that temperature was an important variable for the survival of *Salmonella* on the various surfaces evaluated in this study. High relative humidity was present in ripening room parameters and spring parameters, but it was seen that *Salmonella* was recovered in greater quantities at a lower temperature (20°C). It should be noted that bacterial soft rot microorganisms commonly infect tomatoes and the incidence of *Salmonella* increases in infected fruits (this was not a factor in this study).

The two temperatures selected for this recovery study were 20°C and 30°C. The ambient temperature (20°C) seemed to allow *Salmonella* to survive for a longer period of days. The warmer temperature (30°C) seemed to inhibit the ability of *Salmonella* to survive as well on various surfaces. *Salmonella* most likely exhausted all resources very quickly at 30ºC. The microorganism grows very well at 37ºC, but nutrient depletion encountered by *Salmonella* over the 28 days most likely inhibited survival over time. This trend was observed for every recovery study performed. Lower temperatures seem to facilitate the survival of *Salmonella* rather than higher temperatures. It has also been reported that certain strains of salmonellae can survive for longer periods of time under refrigeration temperatures than at room temperature (Parish 1997; Zhao et al. 1993). A further study might explore the recovery of *Salmonella* off various surfaces under refrigeration temperatures (4°C) to see if survival of the pathogen is enhanced.

Recovery of *Salmonella* at low levels is still an important concern. Lower levels of the pathogen were still recovered as time increased (up to 28 days for tomatoes). The infectious dose of salmonellae ranges from 10 to 100,000 cells (CFSAN-FDA 2001). This indicates that even low levels of *Salmonella* in favorable conditions can facilitate a foodborne disease outbreak. This is a chief concern for the fresh produce industry due to the fact that edible horticultural crops are consumed without a treatment to help eliminate any pathogenic microorganisms that may be present.

CHAPTER 6
CONCLUSION

All objectives of this study were accomplished. Growth rates of five rifampicin resistant *Salmonella* serovars were established and it was determined that an appropriate cocktail could be made. *Salmonella* was successfully recovered from tomatoes and all material surfaces. It was observed that the pathogen survived longer on all surface types in the simulated fall/winter regime (20°C/60%).

Salmonella has the capability to survive over a prolonged period of time in certain temperature and relative humidity combinations on tomato fruits and various equipment surfaces. Results showed that *Salmonella* populations on tomato surfaces held at 20°C were observed to decline over time (approximately a 4.0 $\log_{10}$ CFU/ml reduction over 28 days). Of all simulated environments, spring tomato production parameters (30°C/80%RH) yielded the lowest recovery of *Salmonella* over 28 days. Stainless steel and PVC surfaces had similar recovery patterns of *Salmonella* at 20°C and 60%RH over 28 days. No *Salmonella* was recovered after Day 7 at 30°C and 80%RH off these surfaces. No *Salmonella* was recovered from conveyor belt and sponge roller surfaces after 21 and 7 days, respectively. *Salmonella* recovery off wood surfaces exhibited the most variability. Wood surfaces maintained at 60%RH and 20°C exhibited the most *Salmonella* recovery of any surface type at the end of 28 days. An ambient temperature (20°C) combined with a higher (90%RH) and moderate (60%RH) relative humidity seemed to facilitate *Salmonella* survival better than an elevated temperature (30°C)

combined with a high (80%RH) relative humidity. Temperature seemed to be an important factor affecting the survival of *Salmonella* on various surface types.

Surface types of the materials also seemed to affect *Salmonella* recovery and survival over time. More *Salmonella* was recovered from the smooth and nonporous surfaces like stainless steel and PVC. Rough and porous surfaces, like the wood surfaces, seemed to harbor *Salmonella* in this matrix better than smoother surfaces. Sponge rolls and conveyor belt surfaces also showed a possible bacteriostatic effect on *Salmonella* over time. Sponge rollers did not allow *Salmonella* survival for longer than Day 1 in the spring regime and *Salmonella* was not recovered from the rollers after Day 3 in the fall/winter regime. *Salmonella* was not recovered after Day1 in the spring regime from conveyor belt surfaces. *Salmonella* did survive on conveyor surfaces until Day 14 in the fall/winter regime, but it was recovered at very low levels.

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BIOGRAPHICAL SKETCH

Raina Leneve Allen was born in Tampa, FL, on July 28, 1979. In 2001, she received her Bachelor of Science from the University of Florida in food science and human nutrition. Upon graduation, she was accepted into the University of Florida's food science master's program. In this program, her specialization focused on food microbiology with a special interest in microbial safety of fresh-market produce.

Upon receiving her master's degree, Raina plans to pursue a career in the food industry and continue working in the area of food safety.