EVALUATION OF OVERHEAD SPRAY-APPLIED SANITIZERS FOR THE REDUCTION OF *Salmonella* ON TOMATO SURFACES

By

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To MF
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Numerous foodborne disease outbreaks have been linked to fresh tomatoes. *Salmonella* is a leading cause of foodborne disease and has been implicated in all multistate fresh tomato outbreaks in the United States (US). Because the outbreaks occurred nationwide, the source of contamination likely originated early in production, possibly in a packinghouse. Effective sanitation of tomatoes post-harvest is one intervention method. Overhead spray and brush roller systems are used in commercial packing lines for sanitizing tomatoes and have not been extensively studied. Compared to flumes, the traditional tomato sanitation system, an overhead spray and brush roller system was hypothesized to achieve higher pathogen reduction on tomatoes because of increased physical removal of bacteria in conjunction with antimicrobial efficacy of sanitizers. The aim of this research was to examine the efficacy of sanitizers in the overhead spray and brush roller system for reducing *Salmonella* on unwaxed, mature green tomatoes. Sodium hypochlorite (NaOCl; 25, 50 and 100 mg/L) was tested against a water control. A sanitizer study tested NaOCl (100 mg/L), chlorine dioxide (ClO₂; 5 mg/L), peroxycetic acid (PAA; 80 mg/L) and water. Efficacy of NaOCl (100 mg/L) was also compared between the overhead spray and brush roller system
and a scale-model flume. Surface inoculated tomatoes were tested for 5, 15, 30 and 60 s per treatment. Results of the sodium hypochlorite study showed that all NaOCl concentrations were significantly more effective at removing *Salmonella* than water and achieved at least a 3-log$_{10}$ CFU/ml reduction at different treatment times (p<0.05). NaOCl (100 mg/L) in particular achieved an average reduction of 3.98 ± 1.78 log$_{10}$ CFU/ml at 15 s contact time. In the sanitizer study, all sanitizers achieved at least a 3-log$_{10}$ CFU/ml reduction of *Salmonella* at 15 s. NaOCl (100 mg/L) in the overhead spray and brush roller system significantly reduced more *Salmonella* than in the flume at 15 to 60 s. This research demonstrated the ability of the overhead spray and brush roller system to reduce *Salmonella* on tomato surfaces. Compared to flumes, an overhead spray and brush roller system can achieve higher pathogen reduction with less water and sanitizer use, thereby lowering packing costs. This research has the potential to influence current industry practices by supporting the implementation of overhead spray systems to improve safety of tomatoes and keep the tomato industry a viable part of Florida’s economy.
CHAPTER 1
INTRODUCTION

Foodborne disease outbreaks related to fresh produce have increased significantly in recent decades. Between the 1970s and 1990s, reported produce-associated outbreaks increased 5.3% and the median number of illnesses associated with the outbreaks increased by 11% (Sivapalasingam and others 2004). In 2007, there were 37 reported outbreaks attributed to vegetables that were responsible for over 800 illnesses (CDC 2010a).

While many types of produce have been linked to outbreaks, fresh, large round tomatoes have been linked to numerous multistate outbreaks in the US since 1990 (CDC 2002a, 2005, 2007, 2010c; Cummings and others 2001; Greene and others 2008; Hedberg and others 1999). Fresh tomatoes are of particular concern in Florida because the state is the number one producer of fresh tomatoes in the US, with the industry generating $630 million in 2010 (USDA 2011a). Additionally, more people are consuming fresh tomatoes. Annual per capita consumption of fresh tomatoes grew to an estimated 9.8 kg in 2011 compared to 5.8 kg in 1980 (USDA 2011b). To meet year-round demand of fresh fruits and vegetables, produce distribution has expanded nationwide, which may contribute to a higher proportion of consumers acquiring foodborne disease. The increased handling throughout the supply chain leads to an increased risk for contamination with human pathogens.

*Salmonella* is a leading cause of bacterial foodborne illness in humans, causing over one million illnesses in the US per year (Scallan and others 2011a). *Salmonella* has also been implicated in all US multistate outbreaks of fresh tomatoes. Because these outbreaks occurred throughout the US, the source of contamination likely
originated early in production, possibly on the farm or in a packinghouse. Sources of contamination in the field include manure, contaminated irrigation water, dust, animals, poor worker hygiene and dirty equipment and harvest containers. In the packinghouse, unsanitary conditions increase risk for contamination such as presence of pests, dirty packing lines and trucks, poor worker hygiene and contaminated water or ice used to cool or wash produce (FDA 1998; Beuchat 1996).

Prevention of contamination is the best method to minimize risk of a tomato foodborne disease outbreak because once tomatoes are contaminated, there are no effective treatments to totally eliminate pathogens, with the exception of cooking or irradiation. Prevention methods are a part of Good Agricultural Practices (GAPs) that began in 1998 with the US Food and Drug Administration (FDA)’s Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. Though GAPs are not yet mandatory for the entire fresh produce industry, the FDA Food Safety Modernization Act of 2011 requires food producers implement a prevention-based food safety program of which GAPs are usually a prerequisite (PL 2011). In Florida, Tomato-GAPs (T-GAPs) and Tomato Best Management Practices (T-BMPs) have been required since 2008 (FAC 2007). As part of the rule, tomatoes must be sanitized in flumes containing 150 mg/L free chlorine at pH 6.5-7.5 for a maximum of 2 min. Water temperature must also be 5°C greater than pulp temperature of tomatoes. NaOCl is a common source of the active form of chlorine, hypochlorous acid (HOCI), also referred to as free chlorine. Alternative approved sanitizers at their maximum allowed concentration are aqueous ClO₂ at 5 mg/L and PAA at 80 mg/L (CFR 2010c, d; EPA 2006). These sanitizers are strong oxidizers that disrupt cell permeability. Efficacy of NaOCl ClO₂ and PAA against
pathogens on produce has been studied in flume systems (Felkey and others 2006; Lopez-Galvez and others 2010; Pao and others 2007; Shirron and others 2009; Walter and others 2009; Yuk and others 2005, 2006). Any other sanitizer or process used in tomato sanitation must be proven in a scientific study to reduce *Salmonella* or a similar organism by at least 3-log$_{10}$ units on tomato surfaces (FDACS 2007).

An overhead spray and brush roller system, referred to as an overhead spray system for short, is not always used for tomato sanitation. It is aimed to replace part or all of flume systems traditionally used in tomato packinghouses and has been studied with ClO$_2$ with tomatoes (Pao and others 2009). Washing in a flume or water bath is effective at removing about 2 to 3 log$_{10}$ units of bacteria on produce surfaces (Gil and others 2009). Overhead spray systems are believed to achieve higher pathogen reduction on tomatoes because of increased physical removal of bacteria in conjunction with antimicrobial efficacy of sanitizers, but they have not yet been extensively studied. Overall aim of this research was to determine the efficacy of sanitizers in a laboratory model overhead spray system for the reduction of inoculated *Salmonella* on tomato surfaces. NaOCl at three concentrations, ClO$_2$, PAA and water were evaluated for their ability to achieve at least a 3-log$_{10}$ unit reduction of *Salmonella* on tomato surfaces in order to support the overhead spray system as an effective method of reducing contamination and risk of foodborne disease outbreaks.
CHAPTER 2
LITERATURE REVIEW

Fresh Produce Overview

Fresh produce is defined as fruits and vegetables that are sold to consumers unprocessed. Fresh produce harvested and sold in a whole form includes berries, melons and tomatoes whereas fresh produce that are cut during harvest but are still unprocessed include celery, lettuce and broccoli (FDA 1998). In contrast, produce that has been modified from its original, raw state through methods like thermal processing, canning, freezing or dehydrating are defined as processed.

Fresh-cut produce is a sub category of fresh produce that includes produce that has been peeled or cut and not further processed. Fresh-cut produce is often washed and packaged such as bagged salads and fresh-cut melon prepared at retail locations. All fresh-cut produce are ready-to-eat though they are sometimes re-washed or cooked by consumers (FDA 2001). Prepackaged fresh-cut produce is a growing trend because of the added value of convenience for consumers.

US Dietary Guidelines recommend individuals increase fruit and vegetable intake as part of a healthy eating pattern and to reduce the risk for chronic diseases such as coronary heart disease, stroke, cancer and diabetes (USDA and USDHHS 2010). Improving eating habits to include fresh produce is extremely important because the prevalence of overweight and obesity has expanded in recent decades to include over two-thirds of adults and a growing percentage of children in the US. Recommendations to increase consumption of fruits and vegetables have ramifications for the produce industry to provide safe produce for consumers. Advances in distribution and storage have led to the availability of fresh produce throughout the US year-round.
Unfortunately, fresh produce is a potential source of human pathogens and can cause foodborne disease (Beuchat 1996).

**Foodborne Disease**

In 1999, Mead and others estimated there to be 76 million illnesses and 5,000 deaths a year due to food. Recent data and improved methodology refined the numbers to 9.4 million illnesses and 1,351 deaths a year from known pathogens, and 38.4 million illnesses and 1,686 deaths a year from unknown agents (Scallan and others 2011a, b).

Estimates of foodborne disease varied greatly partly because diseases are often undiagnosed and are self-limiting, thus are not reported. Conversely, severe cases of foodborne disease can require hospitalization, especially if secondary complications or sequelae occur. People at higher risk of developing sequelae are individuals with weaker immune systems due to age or illness (Samuel and others 2007).

Numerous outbreaks occur worldwide because of contaminated food. A foodborne disease outbreak is defined by the Centers for Disease Control and Prevention (CDC) as the occurrence of two or more cases of a similar illness resulting from eating a common food (CDC 2010a). The CDC (2010a) reported 1,097 foodborne disease outbreaks in 2007 that caused 21,244 cases and 18 deaths. Given the scope of outbreaks, foodborne disease creates a major health and economic burden on the US. Costs to individuals and society include diagnosis, treatment, loss of work, public control efforts, pain and suffering, loss of revenue and perhaps legal actions.

Outbreaks may be limited to a household or community while other outbreaks are widespread, traced around the country over several weeks. Multistate outbreaks are more likely caused by distribution of a food contaminated at the farm or manufacturing
Bacterial pathogens associated with outbreaks include *Salmonella* spp., *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. Other causes of outbreaks include viruses like norovirus and hepatitis A, parasitic protozoa like *Cryptosporidium* and *Giardia*, and toxins produced by microorganisms. Norovirus is the most common cause of foodborne disease outbreaks, causing 39% of confirmed outbreaks attributed to a single food in 2007 (CDC 2010a). Scallan and others (2011a) estimate norovirus causes more than five million illnesses a year in the US, though the number of cases is compounded by non-foodborne transmission.

A variety of foods have caused foodborne disease. Poultry, beef and leafy vegetables were the cause of most outbreaks in 2007 (CDC 2010a). The largest outbreak in 2007 was *Salmonella* in hummus that caused 802 illnesses. The second largest outbreak was norovirus at a hotel conference linked to several foods. Other outbreaks included poultry contaminated with *C. perfringens*, leafy vegetables contaminated with norovirus, and beef contaminated with *E. coli* O157:H7 (CDC 2010a).

While many foods commonly cause foodborne illness, fruits and vegetables are especially problematic since they are often eaten raw. There are several types of fruits and vegetables with different methods of growing and harvesting. Furthermore, different physical characteristics such as size, shape and surface texture may promote or inhibit microbial attachment. Consequently, there are multiple ways produce can become contaminated in the field and/or after harvest and it is difficult to impose mandatory “one size fits all” safety and sanitation standards. Despite the wide variety of
produce, general GAPs established by the FDA are voluntarily adapted to specific operations. Proper implementation of GAPs helps reduce the risk of contamination.

Sources of Contamination

Fresh produce have the potential of becoming contaminated at any step during production. Produce normally contain nonpathogenic microorganisms as part of their natural microflora, which can become spoilage organisms and plant pathogens. These organisms include the fungi, *Botrytis, Rhizopus* and *Geotrichum*, and the bacteria *Pseudomonas, Xanthomonas* and *Erwinia carotovora* (Narayanasamy 2006). Some human pathogens can survive on the outside of produce, especially under ideal humidity and refrigeration temperatures (Harris and others 2002). Microorganisms can enter produce if there are bruises, punctures or abrasions that break open produce epidermis. In tomatoes, microorganisms usually cannot penetrate the epidermis and can only enter through wounds or at blossom-end or stem scar-end (Mahovic and others 2007). Decay organisms that enter produce can cause various rots that promote improved colonization of pathogens that can spread and infect an entire container of produce. Produce could also become contaminated pre-harvest. Guo and others (2001) inoculated tomato plant stems and flowers with *Salmonella* and found 11 of 30 tomatoes harvested from the plants contained *Salmonella*, six of which contained *Salmonella* in the pulp. Once internalized, pathogens are not affected by chemical surface treatments and more easily survive with access to nutrients and moisture (Harris and others 2002).

Fruits and vegetables are grown in fields and can be exposed to many pathogens naturally found in the environment. The goal of GAPs is to minimize exposure to pathogens by preventing produce contact with various hazards such as manure,
contaminated irrigation water, dust, wild animals, poor human handling and dirty equipment and harvest containers (FDA 1998; Beuchat 1996). One of the most basic sources of contamination is soil. Soil can contain native pathogens such as *Clostridium botulinum*, *Bacillus cereus* and *L. monocytogenes*, or become contaminated from human or animal wastes (Santamaria and Toranzos 2003). Root vegetables such as carrots grow in soil while other produce are grown close enough to soil to be splashed when raining. If manure is used as fertilizer and is not properly aged, it can inoculate produce from the close or direct contact with soil. Manure stored uphill to fields can contaminate plants and water supplies if erosion occurs. Bacteria can also build up on harvest knives and containers if they are not washed and sanitized often (FDA 1998).

Certain materials such as wood cannot be as easily cleaned as plastic and are also more likely to injure produce (Thompson and others 2002).

After harvest, produce can be transported to a facility for sorting and packing before being shipped to customers. Unsanitary conditions in a packinghouse such as presence of pests and dirty packing lines and trucks can contaminate produce (FDA 1998). Other sources of contamination include contaminated ice used to cool produce, wash water and dust (Beuchat 1996).

Another important contributor to contamination is poor personal hygiene of workers who handle fresh produce. Infected employees can transfer pathogens through feces, blood and by skin anytime produce is handled, from harvest to the preparation of fresh-cut produce in retail establishments (Todd and others 2008). Fresh-cut produce requires more stringent safety standards because in contrast to whole produce, they are
stripped of their skins that provide natural protection against microorganisms (FDA 2001).

**Fresh Produce Outbreaks**

Outbreaks attributed to fresh produce are on the rise compared to foods that traditionally have caused foodborne disease. Sivapalasingam and others (2004) reported that from 1973 to 1997 there were 190 produce-associated outbreaks associated with 16,058 illness, 598 hospitalizations and eight deaths. In the 1990s, 6% of reported outbreaks were caused by produce compared to only 0.7% in the 1970s. Most outbreaks during this time period were caused by multiple produce items such as salads, mixed fruit and mixed vegetables. Single produce items implicated in outbreaks included lettuce, melons, sprouts, juice, berries and tomatoes (Sivapalasingam and others 2004). Bacteria commonly isolated from produce include *Aeromonas, Shigella, Salmonella, E. coli* O157:H7, *Campylobacter, Yersinia enterocolitica* and *L. monocytogenes* (Beuchat 1996).

Fresh produce outbreaks can be large and widespread. Leafy green vegetables such as lettuce and spinach have been linked to several multistate *E. coli* O157:H7 outbreaks and recalls (Grant and others 2008; Rangel and others 2005). Between 1982 and 2002, while outbreaks associated with *E. coli* O157:H7 included 38 in produce and 75 in ground beef, the median number of cases per outbreak was 20 and 8, respectively (Rangel and others 2005). *Salmonella* has been also associated with numerous large fresh produce outbreaks. In 2008, *S. Saintpaul* caused an outbreak in jalapeno and serrano peppers that involved 43 states and Canada and caused 1,500 illnesses, 308 hospitalizations and perhaps two deaths (CDC 2008a; Barton Behravesh and others 2011).
The increase in fresh produce outbreaks may be due to the availability of fresh produce for purchase year round. Demand for off-season produce requires nationwide distribution, which increases risk for contamination because of increased handling after harvest. More people consuming produce could explain the increased incidence of illness. Conversely, improved epidemiological data and collection methods may increase reported numbers and may not reflect an actual significant increase in outbreaks.

**Tomatoes**

Fresh tomatoes are an important US commodity but have been linked to numerous outbreaks (Table 2-1). There are several varieties of tomatoes grown in the US, either in the field or in greenhouses. Field-grown tomatoes include round, Roma (plum), cherry and grape tomatoes. Greenhouse tomatoes may be grown hydroponically and sold on the vine.

Tomatoes are a large industry for the US, second in the world after China (USDA 2010a). In the US, more than 100,000 acres of tomatoes were harvested in 2010, with a value of almost $1.4 billion (USDA 2011a). Florida was ranked number one in the nation in market share of fresh tomatoes, with 29,000 acres harvested in 2010. The tomato plays a vital role in Florida’s economy as it generated $630 million in 2010. Comparatively, California, the second major producer of fresh tomatoes in the US, harvested 38,000 acres of tomatoes with a value of $396 million in the same year. Other states that produce fresh tomatoes are Tennessee, Virginia, Ohio, New Jersey, Georgia, North Carolina and Michigan. In contrast to fresh tomatoes, California is the leader of tomatoes for processing, with 270,000 acres harvested with a value of $878 million in 2010 (USDA 2011a).
Though the US produced over 27 billion pounds of tomatoes in 2008, it still has imported at least two billion pounds of tomatoes a year for the past decade (USDA 2010a, b). Florida’s tomato season runs from October to June and imports supplement the Florida tomato supply during winter. The majority of tomatoes are imported from Mexico (USDA 2010b). Prevention of foodborne outbreaks caused by tomato contamination is important to protect the tomato’s role in the nation’s food supply.

Tomatoes are fruit vegetables typically harvested at a mature-green stage and further ripened post-harvest. Tomatoes are harvested by hand into small buckets. They are then transferred to large bins or a gondola attached to a trailer for transport to a packinghouse (Thompson and others 2002). Potential contamination during harvest can occur from contaminated irrigation water, animals, unhygienic handling practices, unsanitary harvest bins or transport (FDA 1998; Beuchat 1996).

At the packinghouse, tomatoes may undergo an initial cooling and storage period before processing. Processing begins with the unloading of tomatoes from the large bins. In large operations, the unloading is mechanized for controlled speed of tomato flow into a chlorinated water dump tank. The dump tank functions as a cushion to prevent tomato injury as well as remove debris and dirt. Tomatoes travel in a flume through pre-sizing, which removes obviously defective fruit, and often a second, clean water tank or rinse for further washing. The flume then carries tomatoes through hand sorting and electronic, belt or weight sizing. Tomatoes are packed by weight into 25-lb corrugated boxes or may be place-packed if already ripe. Tomatoes are cooled by room or forced-air cooling and stored at about 13°C. Temperatures below approximately 10°C will cause chilling injury. Postharvest ripening occurs in degreening
rooms by exposure to 100 mg/L ethylene for 3 to 4 d before being shipped to
distribution and retail centers (Cantwell and others 2002). In packing facilities, tomatoes
can become contaminated through dirty equipment in processing lines, overhead drips,
wash water, pests and poor worker hygiene (FDA 1998; Beuchat 1996).

**Salmonella**

Most tomato outbreaks have been linked to *Salmonella*. *Salmonella* has been
associated with disease since it was first isolated in 1885. It is now divided into two
species, *Salmonella enterica* and *Salmonella bongori* and over 2,500 serovars.
*Salmonella* are facultative anaerobes, gram-negative and straight rods of the family
*Enterobacteriaceae*. Most strains are motile through flagella and grow optimally at
37°C, though growth can occur between 2 and 54°C. *Salmonella* is also able to survive
in a large range of pH, from 4.5 to 9.5 (D’Aoust and Maurer 2007).

**Salmonellosis**

Most strains of *Salmonella* are nontyphiodal and can cause salmonellosis, a
gastrointestinal infection. The strain of *Salmonella* that causes typhoid fever is *S. Typhi*.
Salmonellosis symptoms typically appear 8 to 72 hours after ingestion and include non-
bloody diarrhea and abdominal pain. The infection is usually self-limiting. Sequelae of
salmonellosis are reactive arthritis and ankylosing spondylitis (D’Aoust and Maurer 2007).

*Salmonella* causes disease by invading intestinal cells via a type three secretion
system and causing an influx of Ca$^{2+}$ into the intestinal tract. *Salmonella* can produce
an enterotoxin and cytotoxin that can induce apoptosis and cause diarrhea. Other
virulence factors include the ability to acquire iron and the ability to avoid the
complement system of innate immunity and antibacterial substances. Since ingesting
fewer than 100 *Salmonella* cells and perhaps as few as 1 to 10 cells could cause illness, it is critical to prevent any *Salmonella* contamination in food (D’Aoust and Maurer 2007).

**Salmonella Outbreaks**

*Salmonella* is one of the leading causes of foodborne disease in humans. It is estimated that *Salmonella* causes over one million foodborne diseases, 19,336 hospitalizations and 378 deaths in the US per year (Scallan and others 2011a). After norovirus, *Salmonella* caused the most foodborne disease outbreaks in 2007 and caused the majority of confirmed outbreaks attributed to bacteria. *S. enterica* serovar Enteritidis caused the most *Salmonella* outbreaks (CDC 2010a). *Salmonella* is ubiquitous and has been linked to every category of food. Products that have caused outbreaks include egg salad, fish, cheese, chocolate, milk, ice cream, spices, pork, cooked chicken, peanuts, orange juice and a variety of fruits and vegetables (D’Aoust and Maurer 2007). Food associated with *Salmonella* outbreaks in 2007 were frozen pot pies, processed vegetable snacks, eggs, spinach, tomatoes, tuna, ground beef, cheese, alfalfa sprouts and fresh basil (CDC 2010a).

Poultry is the main reservoir of *Salmonella*. Studies that examined the prevalence of *Salmonella* in chicken have found up to 100% of samples testing positive (CAST 1994). *Salmonella* has also been associated with shell eggs. In 2010, a multistate outbreak of *S. Enteritidis* in shell eggs caused 1,939 illnesses (CDC 2010b). Egg contamination results from the transmission of bacteria from the hen ovary to the interior of the egg prior to shell formation.

Though traditionally associated with animals, in the period 1973 to 1997, *Salmonella* was the most common bacteria agent that caused foodborne disease
outbreaks in produce. While there were 20 different serotypes involved, most common ones were Typhimurium, Montevideo, Javiana, Anatum, Enteritidis, Infantis, Newport and Stanley (Sivapalasingam and others 2004). *Salmonella* outbreaks have been associated with imported produce. An outbreak of *S.* Saphra was reported in 1997 from cantaloupes imported from Mexico (Mohle-Boetani and others 1999). In 1999, mangos from a Brazilian farm caused an outbreak of *S.* Newport (Sivapalasingam and others 2003). In each spring from 2000 to 2002, a multistate outbreak of *S.* Poona occurred from eating fresh cantaloupe imported from Mexico (CDC 2002b). More recently, imported cantaloupe was associated with an outbreak of *S.* Litchfield in 2008 and an outbreak of *S.* Panama in 2011 (CDC 2008b, 2011). Because of the link between *Salmonella* and imported produce, the prevalence of *Salmonella* in produce grown in Mexico was studied for 17 different vegetables. In total, 98 samples of 1,700 tested positive, including 12% of parsley samples, 11% of cilantro samples, 9% of broccoli samples and 9% of cauliflower samples (Quiroz-Santiago and others 2009).

**Salmonella and Tomatoes**

Notably, all fresh tomato multistate outbreaks in the US have been caused by *Salmonella* (Table 2-1). *S.* Baildon caused an outbreak of raw tomatoes in 1999 (Cummings and others 2001). In 2004, *S.* Braenderup caused an outbreak associated with Roma tomatoes (Gupta and others 2007). Of the four multistate outbreaks in 2006 attributed to *Salmonella*, two were transmitted by tomatoes (CDC 2010). The fact that many outbreaks reached multiple states suggests that contamination often occurs early in production. Known sources of contamination occur at the farm or packinghouse level, often from contaminated water. Smaller tomato outbreaks have occurred at
restaurants and households and were more likely to be caused by worker or consumer handling and temperature abuse.

Outbreaks associated with a particular commodity can disrupt the entire industry. The tomato industry suffered major financial losses in 2008 when tomatoes were linked to *S. Saintpaul*, an outbreak that was later attributed to peppers and caused 1,500 illnesses (Barton Behravesh and others 2011). The FDA issued a consumer advisory against eating Roma and round tomatoes for over one month. As a result, it was estimated that the Florida tomato industry lost $100 million (Taylor 2010). This outbreak illustrates the importance of safe imported tomatoes because the contamination occurred on a farm in Mexico that grew the peppers as well as Roma tomatoes. Samples of peppers and irrigation water were found positive for *S. Saintpaul* (CDC 2008a).

Because of the frequent association of *Salmonella* with fresh tomatoes, it has been suggested that specific *Salmonella* serovars may be highly evolved or adapted to survival on and inside tomatoes. Zhuang and others (1995) found *S. Montevideo* grew on tomato surfaces at 20° and 30°C and survived at 10° to 30°C at 45 to 60% relative humidity for at least 18 d. *S. Montevideo* also survived at its initial 4.5 log_{10} CFU/g inoculum level in chopped tomatoes for at least 9 d at 5°C. The pH of the ripe, chopped tomatoes was reported to be 4.1 ± 0.1 (Zhuang and others 1995). The ability of *Salmonella* to survive in acidic tomatoes and at refrigeration temperatures shows how difficult eliminating *Salmonella* can be and the importance of preventing the initial contamination.
**Prevention Methods**

After produce is contaminated, it is nearly impossible to eliminate pathogens other than thorough cooking or irradiation. Prevention of contamination is the most efficient way to ensure food safety and to prevent foodborne disease. Prevention methods are a part of the GAPs program that began in 1998 with the FDA’s *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. Though GAPs are not yet mandatory for the entire fresh produce industry, the FDA Food Safety Modernization Act of 2011 requires food producers to implement a prevention-based food safety program of which GAPs are usually a prerequisite (PL 2011). Voluntary prevention measures by the produce industry include implementing GAPs, hazard analysis critical control points (HACCP), microbiological testing and worker training programs (CAST 1994). In several tomato outbreaks, contaminated tomatoes were distributed from farms to slicing facilities, and finally to restaurants, where consumers ultimately contracted salmonellosis. Using microbiological criteria in supplier contracts could help prevent pathogens from reaching the consumer. Additionally, implementing optional guidelines specific to certain commodities such as the FDA’s draft guide to minimize contamination of tomatoes may be helpful (FDA 2009).

Interventions against field contamination have been developed for produce production. Examples include treated effluents from field irrigation, worker education, hygienic handling and washing produce with a sanitizer. Proper sanitation can reduce risk of contamination. Sanitation standards are part of GAPs and are also found in the Code of Federal Regulations (FDA 1998; CFR 2010a).
Washing and Sanitizing

Washing and sanitizing are designed to enhance the safety of raw fruits and vegetables and involves using a sanitizer with potable water. Washing is the removal of soil from produce surfaces. Washing can remove harmful microorganisms, dirt and pesticide residues as well as pre-cool produce after harvest (Zagory 1999). Sanitizing is the use of a sanitizer to reduce the number of microorganisms on produce to a safe level. Reduction of microorganisms is usually measured on a logarithmic scale. A sanitizer, or a biocide, is a chemical agent that inactivates most microorganisms when it comes into contact with food (McDonnell and others 1999). Factors that should be considered in any washing operation include water quality, contact time, application method, targeted microorganisms, microbial load and type of produce (Gil and others 2009).

One focus of GAPs is wash water quality. General water GAPs include performing water sampling and microbial testing, developing standard operating procedures for changing water, cleaning and sanitizing water contact surfaces, installing backflow devices and performing regular inspections. Since wash water is reused throughout the day, sanitizer efficacy must be maintained by monitoring variables like concentration and water temperature (FDA 1998).

While some producers may have waited for the FDA to impose mandatory regulations, the Florida tomato industry, faced with a history of large outbreaks, decided to self-regulate. In 1998, T-GAPs and T-BMPs became rule with the goal of enhancing the safety of fresh tomatoes and preventing or minimizing contamination of tomatoes (FAC 2007). T-GAPs and T-BMPs created leadership and initiative within the industry and formalized safety practices.
Much of T-GAPs and T-BMPs are similar to general GAPs. The rule, however, requires mandatory flume sanitation of tomatoes with 150 mg/L free chlorine at pH 6.5-7.5 or another approved sanitizer for a maximum of 2 min (FDACS 2007). Florida is currently the only state that requires tomatoes be sanitized and no longer allows field packing unless tomatoes are first sanitized with the approved fluming method. Typically after harvest, tomatoes are transported in large bins to a packinghouse. The tomatoes are unloaded into a chlorinated water dump tank to reduce injury and remove debris. The flume system then conveys tomatoes through sorting and packing (Cantwell and others 2002). Water quality is therefore a crucial factor in postharvest tomato handling. Flume washing is considered a critical control point of HACCP because it is a point where microbial hazards can be prevented or reduced via proper sanitation. If sanitizer efficacy is not controlled, there is a potential for pathogens to spread from one contaminated tomato to an entire lot because of commingling and cross contamination in the flume.

**Sanitizers**

While sanitizers inactivate microorganisms, the efficacy is usually limited to a 2 to 3 log\textsubscript{10} unit reduction (Gil and others 2009). Several studies show simply washing with potable water removes 1 to 2 log\textsubscript{10} units of microorganisms (Beuchat 1998). In a flume operation, the purpose of a sanitizer is meant to maintain wash water safety and prevent cross contamination among produce that commingle in the flume. Wash water without a sanitizer can cause a build-up of bacteria and subsequent biofilms (Gil and others 2009). While the flume may act primarily to prevent injury of produce during unloading as well as cool and carry produce through to packing, sanitizers can also be applied via spray or dip methods (Adaskaveg and others 2002). HOCl, ClO\textsubscript{2} and PAA
are a few sanitizers used for produce and are the only ones currently approved for use with Florida tomatoes (FDACS 2007).

**Sodium hypochlorite**

Chlorine has been used to disinfect water since the mid 1800s (Clair and others 2003). Chlorine gas (Cl\(_2\)) can react with water (H\(_2\)O) to form HOCl and hydrochloric acid (HCl) at pH < 3. HOCl, also known as free chlorine, is the active form of chlorine solutions. Since chlorine gas is toxic, hypochlorites (ClO\(^-\)), specifically NaOCl, are more commonly used in large-scale operations and also produce HOCl with water (Clair and others 2003). Traditionally, NaOCl is used to sanitize tomatoes as it is effective, inexpensive and acts rapidly and nonspecifically (Asaskaveg and others 2002).

HOCl is a weak acid with a pKa of 7.5. Therefore, at pH > 7.5, HOCl can dissociate into its ions, OCl\(^-\) and H\(^+\), the inactive forms of chlorine. At pH < 7.5, the undissociated form predominates. The exact ratio of HOCl to OCl\(^-\) and H\(^+\) depends on the pH. A pH of approximately 7.5 is ideal to maintain about a 50/50 ratio of HOCl and its ions. The mechanism of HOCl’s antibacterial action is not completely understood but HOCl is believed to oxidize thiol groups (-SH) to disulfides (S-S), sulfoxides (S-O), or disulfoxides; destroy cellular activity of proteins; inhibit DNA synthesis by forming chlorinated derivatives of nucleotide bases; and disrupt cell membrane activity (McDonnell and others 1999).

Besides pH, the efficacy of HOCl is dependent on temperature. At higher temperature, there is shorter contact time necessary between produce and wash water, but HOCl becomes more volatile (Asaskaveg and others 2002). For immersion treatments, when HOCl is at a much lower temperature than produce, the resulting decrease in internal gas pressure may pull wash water inside produce, increasing risk of
contamination (Harris and others 2002). In one study, 10 tomatoes were immersed for 10 min in a suspension of *E. carotovora* where water was 20°C lower than tomato temperature (Bartz and Showalter 1981). Tomatoes were then rinsed in 50 mg/L chlorine and stored for 2 d. Tomatoes gained an average of 44% in weight and all tomatoes developed soft rot. When temperature differential between water and tomatoes was zero or positive, no decay occurred, even if slight water infiltration occurred (Bartz and Showalter 1981). Ideal temperature of the water is now recommended to be 5.5°C greater than the pulp temperature of produce to be washed (Mahovic and others 2007). Accordingly, Florida T-BMPs requires water temperature to be 5°C higher than pulp temperature when using chlorine (FDACS 2007).

HOCl is also affected by the presence of other compounds in water. Organic matter and other compounds interfere with oxidation reactions by reacting with chlorine and preventing its disinfection reactions. This is called chlorine demand. Enough HOCl must be added to water to reach breakpoint chlorination, the point at which all extraneous reactions are completed and chlorine can be used for disinfection (Clair and others 2003). The maximum concentration of HOCl allowed for food contact is 200 mg/L, though it is often used between 50 to 200 mg/L and must be monitored regularly (CFR 2010b; Thompson and others 2002). If chlorine reacts with organic matter, trihalomethanes (THMs) and haloacetic acids can form and vaporize. These compounds are believed to be human carcinogens (Clair and others 2003). At pH 3-6, free chlorine can also combine with nitrogenous compounds to form chloramines that have lower antimicrobial activity and are eye irritants (Asaskaveg and others 2002).
The effectiveness of HOCl in flume water has been studied with tomatoes. Felkey and others (2006) inoculated tomato surfaces with *Salmonella* and placed them in a scale-model flume at 25°C with 150 mg/L free chlorine for 0 to 120 s. *Salmonella* concentration before treatment was 6.52 \( \log_{10} \) CFU/ml compared to 3.49, 3.29 and 0.16 \( \log_{10} \) CFU/ml after 30, 60 and 120 s, respectively. All treatment times were significantly different than the control. Another simulated flume study found a 5-\( \log_{10} \) unit reduction of *Salmonella* on tomato surfaces at 200 mg/L HOCl after 60 s at 35°C (Yuk and others 2005). Other studies also have shown that HOCl is effective against *Salmonella* on tomato surfaces in water bath systems compared to controls, though populations were never eliminated (Wei and others 1995; Zhuang and others 1995).

HOCl in flumes can also be effective against decay organisms. Vigneault and others (2000) immersed 20 tomatoes in water contaminated with *E. carotovora* and *Rhizopus stolonifer* for 10 min at 20°C. Seventeen tomatoes developed decay during storage for 7 d at 26°C. When contaminated water was chlorinated at 200 mg/L however, only 1 tomato developed decay. At 400 mg/L HOCl, no decay occurred in 14 d. Researchers also hydrocooled tomatoes to 15°C in a flume or shower (1,000 L/min*m² flow rate) containing water contaminated with 6 \( \log_{10} \) CFU/ml *E. carotovora* or *R. stolonifer* and between 50 and 200 mg/L chlorine. After 10 d storage at 20°C, decay caused by *E. carotovora* only occurred with tomatoes exposed to nonchlorinated water. Decay caused by *R. stolonifer* primarily occurred with nonchlorinated water, but sporadic cases was also observed with chlorinated water in both hydrocooling methods (Vigneault and others 2000).
Chlorine dioxide

Because of some limitations of HOCl, other sanitizing solutions have recently been studied and used. ClO₂ is less affected by pH and organic matter than HOCl, thus will not form THMs (Clair and others 2003). ClO₂ works against microorganisms by disrupting cell permeability through oxidation reactions. It has a strong oxidizing capacity, though the oxidation reactions occur at a slower rate than with HOCl (Gomez-Lopez and others 2009). ClO₂ can be used as a gas or be dissolved in water and used as an aqueous sanitizing solution (Gomez-Lopez and others 2009; Fatica and Schneider 2009). Gas must be used in a closed chamber and is reported to have more penetrability than liquid sanitizers (Gomez-Lopez and others 2009). An aqueous ClO₂ solution is generated by reacting sodium chloride (NaCl) with Cl₂ gas, or reacting an acid, such as HCl with sodium chlorite (NaClO₂) and diluting with water (Mari and others 2003). Disadvantages of aqueous ClO₂ include that is must be generated on site, produces byproducts and is toxic at high concentrations (Adaskaveg and others 2002). It is also more expensive than NaOCl (Clair and others 2003). Currently, fresh produce can only be treated with a concentration of ClO₂ that does not exceed 3 mg/L residual chlorine (CFR 2010c). A maximum of 5 mg/L ClO₂ in wash water is allowed for fresh produce wash water and rinses and must be followed by a cooking process (EPA 2006).

ClO₂ has been shown to be as effective as NaOCl in sanitizing iceberg lettuce against natural microflora. Though there were no significant differences between the chlorine sanitizers and a water control, populations of Pseudomonas, Enterobacteriaceae, and yeasts and molds were reduced by an average of $1.2 \pm 0.1 \log_{10} \text{CFU/g}$ when washed in 20 L of each solution under constant agitation (Lopez-Galvez and others 2010).
Pao and others (2009) studied the efficacy of 5 mg/L ClO$_2$ and an overhead spray system. Overhead sprays continuously spray water above tomatoes on revolving brushes and the used wash water is collected. Inoculated tomatoes were dried for 24 h and then subjected to ClO$_2$ or water via a spray wash over brushes or immersion in 2 L for 10 to 60 s. It was found that spray washing with just water significantly reduced *Salmonella* on tomato surfaces after 10 s, at 3.2 ± 0.3 log$_{10}$ units. Spray washing with ClO$_2$ at 10 s increased efficacy to 4.4 ± 0.5 log$_{10}$ units. No significant reduction was observed for immersion treatment with water or ClO$_2$. While ClO$_2$ might not remove pathogens on produce surfaces in a water bath system, it is an effective sanitizer against pathogens in water that may cross contaminate produce. When sterile tap water was inoculated with *Salmonella*, Pao and others (2007) found a 5-log$_{10}$ unit reduction with 5 mg/L ClO$_2$ after 6 s. Similar results were seen with *E. carotovora*.

ClO$_2$ was found to be effective against contamination from inoculated brushes to uninoculated tomatoes in the overhead spray system. When tomatoes were placed on brushes that were previously inoculated with about 6.9 log$_{10}$ CFU/cm$^3$ *Salmonella*, 5.7 log$_{10}$ CFU/cm$^2$ was transferred to tomato surfaces without any spray. ClO$_2$ at 5 mg/L for 10 to 60 s reduced cross contamination by 4.5 ± 0.3 to 5.0 ± 0.3 log$_{10}$ units (Pao and others 2009).

**Peroxyacetic acid**

PAA, also known as peracetic acid (CH$_3$COOOH) is a non-chlorine based potent biocide that is not affected by organic matter or pH. PAA is an organic acid that works faster than ClO$_2$ by denaturing proteins and disrupting cell permeability through a drop in intracellular pH (McDonnell and others 1999; Mari and others 2003). A maximum concentration of 80 mg/L PAA is permitted for produce contact and is made by reacting
acetic acid (CH$_3$COOH) with hydrogen peroxide (H$_2$O$_2$) and diluting with water (CFR 2010d).

When tomato surfaces were inoculated with *Salmonella*, Yuk and others (2005) found a 4-log$_{10}$ unit reduction after treating with 87 mg/L PAA in a circulating water bath at 35°C for 60 s. PAA was less effective against *Salmonella* inoculated in stem scars and puncture wounds, with an average reduction of 2.12 log$_{10}$ units and 1.17 log$_{10}$ units, respectively after 60 s. In a different flume study, PAA at 75 mg/L for 60 and 120 s achieved about a 4-log$_{10}$ unit reduction of *Salmonella* on bell pepper and cucumber surfaces. Comparatively, 200 mg/L NaOCl achieved a 4-log$_{10}$ unit reduction in cucumber but only a 2-log$_{10}$ unit reduction in bell pepper (Yuk and others 2006).

The efficacy of PAA against *L. monocytogenes* has also been studied in ripened green coconuts from Brazil. Coconuts were inoculated and dried for 24 h at 36°C at 81% relative humidity. Coconuts were immersed for 2 min in 80 mg/L PAA, 200 mg/L NaOCl or sterile distilled water, which resulted in average reductions of 4.7, 2.7 and 1.7 log$_{10}$ CFU/coconut, respectively. PAA was determined to be significantly more effective than NaOCl in this study (Walter and others 2009).

In a study of natural mesophilic flora, 80 mg/L PAA was found to only achieve an additional 2.7 ± 0.2 log$_{10}$ CFU/g reduction on cut cucumber and a 0.5 ± 0.4 log$_{10}$ CFU/g reduction on parsley compared to a water rinse for 3 min (Shirron and others 2009). PAA reduced inoculated *Salmonella* by an additional 0.4 ± 0.3 and 0.2 ± 0.1 log$_{10}$ CFU/g on cucumber and parsley, respectively, compared to water. In this study, the low antimicrobial effect of PAA compared to water may be because parsley had large
surface area, leaves and crevices where bacteria could hide, and the cucumber was cut, exposing a large wound that is easily colonized by bacteria.

**Research Hypothesis and Objectives**

Implementing safety measures can help minimize risk of outbreaks. The goal of this research was to study if a non-immersion tomato sanitation method would be as effective as traditional flume methods. If so, use of the non-immersion method would likely decrease volume of water and sanitizer required for sanitation, thereby save on packing costs. An overhead spray system is not always used for tomato sanitation but has been shown to be more effective than flumes against *Salmonella* inoculated on tomato surfaces (Pao and others 2009). Numerous studies have examined the efficacy of different sanitizers on reducing *Salmonella* from tomatoes in flume systems. It is believed that no studies have compared efficacy of NaOCl, ClO₂ and PAA with tomatoes in an overhead spray system.

An overhead spray system is believed to achieve higher pathogen reduction on tomatoes because of the increased physical removal of bacteria from the mechanical action of the brushes and pressure of the spray, in conjunction with antimicrobial efficacy of sanitizers. It was therefore hypothesized that lower concentrations of NaOCl than typically used in flumes would be able to achieve a 3-log₁₀ reduction of *Salmonella* on tomato surfaces. Therefore, 25, 50 and 100 mg/L NaOCl were tested. It was also hypothesized that all sanitizers tested would achieve at least a 3-log₁₀ CFU/ml reduction of *Salmonella* on tomato surfaces, though treatment time could vary. Water could achieve up to a 3-log₁₀ CFU/ml reduction of *Salmonella* from physical removal. Overall, the overhead spray system would be more effective at removing *Salmonella* from
tomato surfaces than a scale-model flume system, but perhaps not be as effective at removing natural tomato microflora.

This research was meant to determine optimum operating parameters of the overhead spray system including type of sanitizer, concentration of sanitizer and contact time, and develop them into useful recommendations for the tomato industry. Doing so would potentially benefit consumers and the tomato industry by providing a scientific basis for using an overhead spray sanitation system.

The objectives of this research were to:

1. Establish growth curves for rifampicin resistant *Salmonella* strains, *S. Typhimurium*, *S. Braenderup*, *S. Enteritidis*, *S. Newport*, and *S. Javiana*.
2. Determine *Salmonella* recovery from inoculated tomatoes.
3. Determine the extent of cross contamination from inoculated tomatoes to uninoculated tomatoes via brush rollers in the overhead spray system.
4. Examine the efficacy of NaOCl in the overhead spray system at different concentrations and treatment times against *Salmonella* on tomato surfaces.
5. Examine the efficacy of other sanitizers in the overhead spray system against *Salmonella* on tomato surfaces.
6. Compare a scale-model flume against the overhead spray system for the reduction of *Salmonella* from tomato surfaces.
7. Examine the efficacy of sanitizers in the overhead spray system against natural microflora on tomato surfaces.
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$^a$Tomatoes were all domestically grown or of unconfirmed/unspecified source.
CHAPTER 3
MATERIALS AND METHODS

Bacterial Strains

Salmonella enterica serovars used in this study were S. Typhimurium (ATCC 13311), S. Braenderup (ATCC BAA-664), S. Enteritidis (ATCC 4931), S. Newport (ATCC 6962) and S. Javiana (ATCC BAA-1593). Salmonella strains were selected based on their association with fresh tomato outbreaks. Bacterial strains were adapted to be 200 mg/L rifampicin (Fisher, Fair Lawn, NJ) resistant. A 10,000 mg/L rifampicin stock was made by dissolving 0.4 g rifampicin in 40 ml methanol. The stock solution was filter sterilized (0.20 µm pore size, Fisher) and stored at 2°C until used. Cultures were stored at -80°C in CryoCare beads (Key Scientific, Round Rock, TX) and on tryptic soy agar (TSA) (Difco, Sparks, MD) slants at 2°C.

To prepare broth cultures, each strain was streaked for isolation on TSA supplemented with 200 mg/L rifampicin (denoted TSA/rif200) and incubated at 37°C. After 24 h, an individual colony was transferred to 10 ml tryptic soy broth (TSB) (Difco) supplemented with 200 mg/L rifampicin (TSB/rif200) and incubated at 37°C. After 24 h, 10 µl of culture was transferred to fresh TSB/rif200 and incubated at 37°C. Cultures were subsequently transferred to fresh TSB/rif200 at least once every 48 h.

Inoculum Preparation

To prepare the inoculum, cultures underwent a successive three-day transfer, the first two in 10 ml TSB/rif200 as described above. The final transfer was into 20 ml TSB/rif200 to ensure a 9.0 log_{10} CFU/ml or higher inoculum level. The five 20-ml cultures were combined as a 100-ml cocktail and centrifuged at 4000 x g for 10 min. The culture was then washed by pouring off the supernatant and suspending the pellet.
in 10 ml buffered peptone water (BPW) (Difco). The culture was centrifuged and washed two more times before adding a final 10 ml of BPW to complete the inoculum. TSB and TSA used in experiments were supplemented with 80 mg/L of rifampicin (TSB/rif80 or TSA/rif80) to reduce stress on organisms yet still select for resistant strains. Inoculum was serially diluted and plated with TSA/rif80 to determine initial concentration. All serial dilutions in experiments were performed 1:10 in BPW.

**Growth Curves**

Growth of the five *Salmonella* strains was individually measured each hour over 12 h. Triplicate broth cultures were prepared individually and underwent a successive three-day transfer in TSB/rif200. To begin the experiment, cultures were serially diluted to approximately $6 \log_{10} \text{CFU/ml}$. One milliliter of culture was transferred to 99 ml TSB/rif200. The new cultures were incubated at 37°C for 12 h. At each hour including hour 0, cultures were serially diluted and dilutions near the countable range (25-250 CFU/ml) were plated by pour plate with TSA/rif80. Original cultures were also plated. Plates were incubated at 37°C for 48 h and then counted. Average $\log_{10}$ count at each hour was calculated for the three cultures per *Salmonella* strain. Analysis of variance (ANOVA) and Duncan’s Multiple Range test were performed using Statistica (Statsoft, Tulsa, OK) to determine differences among strain concentration at each hour. Significant difference was determined at $p<0.05$.

**Tomato Inoculation and Plating**

Unwashed, unwaxed, mature green tomatoes were obtained from Pacific Tomato Growers (Palmetto, FL and Tracy, CA) and DiMare Fresh (Riverview, FL). New inoculum was prepared for every experiment. Inoculum was approximately
9.5 log_{10} CFU/ml. Inoculum was plated to determine the exact concentration. For each experiment, tomatoes were spot inoculated in a circle around the blossom scar with 10 spots of 10 µl inoculum each, for a total of 100 µl inoculum per tomato, or about 8.5 log_{10} CFU/tomato. Tomatoes were dried for 2 h in a fume hood.

To obtain bacterial counts, tomatoes were placed in individual sterile Stomacher bags (Fisher) containing 100 ml BPW supplemented with 0.1% sodium thiosulfate (Na_{2}S_{2}O_{3}) (Fisher) to inactivate chlorine (Kemp and Schneider 2000). Chlorine was inactivated to be able to determine the effect of chlorine treatment at specific time points and not any residual effects. Bags were shaken and tomato surfaces were rubbed for a total of 1 min per bag using a rub-shake method (Zhuang and others 1995). Tomatoes were serially diluted (1:10) and pour plated with TSA/rif80. Plates were incubated at 37°C and counted after 48 h. Negative controls of all media were pour plated. For the cross contamination and efficacy studies, five inoculated tomatoes (the positive controls), were plated to determine recovery of inoculum. The positive controls were inoculated and dried for 2 h but not treated. For each experiment, an uninoculated tomato was used as a negative control and was plated to ensure no rifampicin resistant organisms were found on tomatoes.

**Recovery Study**

A recovery study was conducted to determine how much *Salmonella* would be recovered from tomato surfaces after inoculation and drying for 2 h. Three inoculum cultures were prepared. One tomato was inoculated per culture. Tomatoes were dried for 2 h in a biological hood and then plated. Plates were incubated at 37°C and counted after 48 h. The experiment was performed in triplicate and log_{10} colony counts
averaged. ANOVA and Duncan’s Multiple Range test were performed using Statistica to determine differences between replicates. The amount of *Salmonella* inoculated on tomatoes was compared to amount of *Salmonella* recovered after 2 h drying.

**Sanitizer Solution Preparation**

**Sodium Hypochlorite (NaOCl)**

NaOCl solution was prepared at room temperature. NaOCl was prepared by adding 22 ml 5.65 to 6% NaOCl (Fisher) to 10 L deionized (DI) water (University of Florida) for a final concentration of 100 mg/L. Other concentrations were calculated from this ratio. For experiments using the overhead spray system, additional NaOCl was added to adjust for the aerosolization of NaOCl from the spray nozzles that lowered concentration. The NaOCl solution was adjusted to pH 6.5 with HCl (Fisher).

A Hach DR/890 colorimeter (Hach Co., Loveland, CO) was used to verify hypochlorous acid/chlorite ion (free chlorine) concentration using Hach method 8091. The DPD method uses AccuVac free chlorine ampoules (Hach Co.) that contain the indicator, N, N-diethyl-p-phenylenediamine sulfate, which forms a pink color when reacted with chlorine. The range for the ampoules is 0 to 2.00 mg/L Cl\(^+\). To measure concentration, NaOCl solution was diluted 1:100 in chlorine-demand-free-water, or 18 MOhm water, from Barnstead Nanopure Diamond Lab Water System (Barnstead, Dubuque, IA). An ampoule was filled with the dilution and was measured by the colorimeter.

**Chlorine Dioxide (ClO\(_2\))**

Aqueous ClO\(_2\) was generated with Selectrocide 2L500 (Selective Micro Technologies, Canal Winchester, OH). Selectrocide 2L500 is a pouch containing sodium chlorite and other ingredients. Following the manufacturer’s directions, when
2 L of water was poured into the pouch, ClO$_2$ was activated at 500 mg/L. ClO$_2$ solution was only used in experiments in the overhead spray system. For each experiment, 180 ml ClO$_2$ was added to 10 L DI water to make a solution that delivered 5 mg/L ClO$_2$ from the spray nozzles.

Concentration of ClO$_2$ was measured with a Hach DR/890 colorimeter and Hach method 10126 using AccuVac free chlorine ampoules (Hach Co.). The range of the ampoules in this test is 0 to 5.00 mg/L ClO$_2$, though the colorimeter displays concentrations up to 5.50 mg/L.

**Peroxyacetic Acid (PAA)**

PAA was made by diluting a commercially available concentrate, Tsunami 100 solution (Ecolab Inc., St. Paul, MN), with DI water to obtain a working concentration of 80 mg/L. Tsunami 100 contains 15% PAA and 11% H$_2$O$_2$. PAA solution was only used in experiments in the overhead spray system. For each experiment, 5.24 ml Tsunami 100 was added to 10 L DI water to make a solution that delivered 80 mg/L PAA from the spray nozzles.

PAA concentration was measured with an RQflex 10 meter and Reflectoquant test strips (EMD Chemicals, Gibbstown, NJ) with a range of 75 to 400 mg/L. The meter measures PAA concentration spectrophotometrically by the color change of the strip when exposed to PAA.

**Water Control**

Deionized water (University of Florida) was used for water sprays in the studies testing the overhead spray system. As a control, water was tested in addition to the other sanitizers to see if reductions of *Salmonella* were due to the sanitizer or the mechanical action of the spray and brush rollers. Water was also used as a control in
the flume study to see if *Salmonella* reductions were due to the sanitizer or the mechanical action of the circulating water. Water was supplemented with 0.1% Na$_2$S$_2$O$_3$ to inactivate any chlorine that remained in the sanitation system.

**Overhead Spray System**

A custom built overhead spray and brush roller system was used in the cross contamination study and efficacy studies (Figure 3-1). The overhead spray system was located in a biological fume hood. Two nylon rollers (46 cm long and 12 cm dia) sat alongside in a box measuring 46 cm by 34 cm. The rollers rotated in the same direction at 180 rpm. The tomato to be tested sat in the valley between the two rollers and revolved on its axis in a direction that depended on its shape. The distance between the top of the brush rollers and spray nozzles was 13 cm. Three spray nozzles (Spraying Systems, Co., Wheaton IL) released a cone shaped spray at a pressure of 12 psi and flow rate of 21.4 ml/s. A 20-L bucket with a spigot fed sanitizer through piping to the nozzles. At least 6 L of solution had to be in the bucket at all times in order for the spigot to be submerged.

**Cross Contamination Study**

The cross contamination study was conducted to determine if there was cross contamination of *Salmonella* from inoculated tomatoes to uninoculated tomatoes via brush rollers in the overhead spray system. NaOCl (100 mg/L) was tested for its ability to prevent cross contamination. Ten liters of 100 mg/L NaOCl was prepared and its concentration as released from the spray nozzles was verified. The experiment had four stages characterized by what was sprayed in the overhead spray system. Stage 1 was an initial 100 mg/L NaOCl spray for 15 s to wet the rollers. After the spray, each roller was swabbed in a 10 cm by 10 cm square area to determine initial contamination,
The swabs were each placed in 10 ml BPW with 0.1% Na$_2$S$_2$O$_3$. In stage 2, five inoculated tomatoes were placed on the brush rollers and sprayed with 100 mg/L NaOCl for 15 s. Tomatoes were removed and placed in Stomacher bags containing 100 ml BPW with 0.1% Na$_2$S$_2$O$_3$. Each roller was also swabbed in the same way and area as in stage 1 to determine transfer of inoculum to brush rollers. In stage 3, five uninoculated tomatoes were placed on rollers and sprayed with 100 mg/L NaOCl for 15 s. Tomatoes were removed and placed in Stomacher bags containing 100 ml BPW with 0.1% Na$_2$S$_2$O$_3$. In stage 4, water with 0.1% Na$_2$S$_2$O$_3$ was sprayed for 15 s to neutralize residual chlorine. Each stage was performed continuously. Five positive control tomatoes were run to determine initial inoculum level and calculate reduction of _Salmonella_ from inoculated tomatoes. Experiments were performed in triplicate with different areas of the rollers swabbed each time.

Triplicate experiments were also conducted with water as a control. The same procedure was followed as above except water with 0.1% Na$_2$S$_2$O$_3$ was sprayed instead of NaOCl. In stage 4, 100 mg/L NaOCl was sprayed instead of water with 0.1% Na$_2$S$_2$O$_3$ to sanitize rollers between experiments. All tomatoes were plated with TSA/rif80, incubated at 37°C and counted after 48 h. Average log$_{10}$ recovery of _Salmonella_ on uninoculated tomato surfaces and on brush rollers was calculated for 100 mg/L NaOCl and the water control. ANOVA and Duncan’s Multiple Range test were performed with Statistica to determine the difference between the ability of NaOCl and water at preventing contamination of brush rollers, which then would prevent cross contamination of uninoculated tomatoes.
Sodium Hypochlorite Efficacy Study

The efficacy of NaOCl for reducing *Salmonella* on tomato surfaces in the overhead spray system was determined at 25, 50 and 100 mg/L NaOCl. Inoculated tomatoes were placed on brush rollers and NaOCl was released through the overhead spray. Water was run as a control. NaOCl solution was prepared in 10 L batches and concentration released from the spray nozzles was verified. For each NaOCl concentration and the water control, five tomatoes were tested for each of four treatment times of 5, 15, 30 and 60 s. After treatment, tomatoes were placed in Stomacher bags containing 100 ml BPW with 0.1% Na$_2$S$_2$O$_3$. Tomatoes were plated with TSA/rif80, incubated at 37°C and counted after 48 h. Experiments were run in triplicate. Five positive controls were also run to calculate average log$_{10}$ reduction of *Salmonella* from tomato surfaces. ANOVA and Duncan’s Multiple Range test were performed with Statistica to determine differences among treatment time and NaOCl concentration.

Sanitizer Efficacy Study

The efficacy of 100 mg/L NaOCl, 5 mg/L ClO$_2$, 80 mg/L PAA and a water control for reducing *Salmonella* on tomato surfaces in the overhead spray system was determined for treatment times of 5, 15, 30 and 60 s. Five positive controls were run as time 0. Inoculated tomatoes were placed on brush rollers and sanitizer was released through the overhead spray. For each sanitizer and the water control, five tomatoes were tested per treatment time. After treatment, tomatoes were placed in Stomacher bags containing 100 ml BPW with 0.1% Na$_2$S$_2$O$_3$. Tomatoes were plated with TSA/rif80, incubated at 37°C and counted after 48 h. Experiments were run in triplicate and average log$_{10}$ reduction of *Salmonella* from tomato surfaces was calculated.
ANOVA and Duncan’s Multiple Range test were performed with Statistica to determine differences among treatment time and sanitizers.

**Flume vs. Overhead Spray Comparison Study**

A study examining the efficacy of a scale-model flume for reducing *Salmonella* from tomato surfaces was conducted to compare to the overhead spray system. A Precision circulating water bath (Jouan, Inc., Winchester, VA) measuring 38.7 cm by 30.5 cm and 19.0 cm deep was used as the scale-model flume. Inoculated tomatoes were tested in 10 L of NaOCl (100 mg/L) and 10 L water at 25°C. Five tomatoes were tested per sanitizer and treatment time of 5, 15, 30 and 60 s. Five positive controls were also run as time 0. For a given treatment time, all five tomatoes were placed in the flume at once with the blossom scar-end down so that the inoculum was submerged. After the specific time, tomatoes were removed with sanitized metal tongs and placed in Stomacher bags containing 100 ml BPW with 0.1% Na₂S₂O₃. Each treatment time was tested sequentially with no change of NaOCl or water between times. Flume water was tested at time 0 and after the 15 and 60 s treatment times to quantify the buildup of *Salmonella* that could cross contaminate subsequent tomatoes. Tomatoes and sanitizer samples were plated with TSA/rif80, incubated at 37°C and counted after 48 h. Experiments were run in triplicate and average log₁₀ reduction of *Salmonella* from tomato surfaces was calculated. ANOVA and Duncan’s Multiple Range test were performed with Statistica to determine differences among treatment time and sanitizer. Flume data was also compared statistically with 100 mg/L NaOCl overhead spray data from the sodium hypochlorite efficacy study.
Natural Tomato Microflora Study

The efficacy of sanitizers in the overhead spray system against natural tomato surface microflora was examined. Sanitizers tested were NaOCl (100 mg/L), ClO₂ (5 mg/L), PAA (80 mg/L) and a water control. Five positive controls were run as time 0. Uninoculated tomatoes were placed on brush rollers and sprayed for 5, 15, 30 and 60 s. For each sanitizer and the water control, five tomatoes were tested per treatment time. After treatment, tomatoes were placed in Stomacher bags containing 100 ml BPW with 0.1% Na₂S₂O₃. Tomatoes were plated with TSA, incubated at 37°C and counted after 48 h. Experiments were run in triplicate and average log₁₀ reduction of bacteria from tomato surfaces was calculated. ANOVA and Duncan's Multiple Range test were performed with Statistica to determine differences among treatment time and sanitizers.
Figure 3-1. Overhead spray system. A) Entire overhead spray system, B) Brush rollers and spray nozzles.
CHAPTER 4

RESULTS

Growth Curves

The growth of five rifampicin resistant *Salmonella* strains, *S*. Typhimurium, S. Braenderup, S. Enteritidis, S. Newport and S. Javiana was measured over 12 h at 37°C in TSB/rif200. Average log\(_{10}\) concentration of each strain at each hour was calculated from triplicate cultures (Table 4-1 and Figure 4-1). Results show that all five *Salmonella* strains had an initial average concentration of approximately 4 log\(_{10}\) CFU/ml at hour 0 and grew to approximately 9 log\(_{10}\) CFU/ml by hour 12. There was no statistically significant difference in average log\(_{10}\) concentration between strains at hour 0 or after 1 h of growth (p>0.05). Significant differences among strains began at hour 2 (p<0.05). Differences in average concentration were observed in at least one strain from hour 2 through hour 9, though the difference between the most concentrated and least concentrated strain was always less than 1.0 log\(_{10}\) CFU/ml. Average difference between the most concentrated and least concentrated strain was 0.70 ± 0.15 CFU/ml. Between hour 2 and 9, *S*. Enteritidis and *S*. Javiana each exhibited the highest average log\(_{10}\) concentrations for 4 of the 8 h. Conversely, *S*. Typhimurium exhibited the lowest average log\(_{10}\) concentration for 6 of the same 8 h. At each of the last 3 h of the measured growth, there were no significant differences in average log\(_{10}\) concentration among strains (p>0.05). Additionally, the concentration of each strain was not significantly different from each other during at least the last 3 h of growth but was significantly different from the previous hours of growth, indicating growth reached a plateau or stationary phase (not shown in Table 4-1).
Recovery Study

Surface inoculated tomatoes were tested for recovery of *Salmonella* after drying for 2 h at room temperature. Average log$_{10}$ concentration of inoculum, *Salmonella* recovery from tomatoes and *Salmonella* loss was calculated from triplicate experiments (Table 4-2). Average concentration of inoculum cultures was $9.67 \pm 0.11$ log$_{10}$ CFU/ml. Since tomatoes were inoculated with 100 μl inoculum, each tomato was inoculated with an average of $8.67 \pm 0.11$ log$_{10}$ CFU/tomato. Average concentration of *Salmonella* inoculated on tomatoes for each experiment replicate was 8.83, 8.60 and 8.57 log$_{10}$ CFU/ml. There was no significant difference in inoculum concentration between replicates (p>0.05).

After 2 h drying, tomatoes were placed in 100 ml BPW and plated to determine *Salmonella* recovery. Because tomatoes were diluted in 100 ml BPW, the limit of detection was 2 log$_{10}$ CFU/ml. Recovery data was adjusted 2 log$_{10}$ CFU/ml because colony counts were 2 log$_{10}$ CFU/ml lower than what was actually on the tomato surface. For example, average concentration recovered from tomatoes in experiment 1 was $6.09 \pm 0.79$ log$_{10}$ CFU/ml but was reported as $8.09 \pm 0.79$ log$_{10}$ CFU/ml. Average recovery of *Salmonella* from tomato surfaces in each of the three experiment replicates was 8.09, 7.86 and 7.90 log$_{10}$ CFU/ml, with an overall average recovery of $7.95 \pm 0.30$ log$_{10}$ CFU/ml. There was no significant difference in recovery between experiment replicates (p>0.05).

Statistical significance between inoculum and recovery varied among replicates. There was no significant difference between inoculum and recovery in experiment 1 but there were differences in the last two experiments (p<0.05). Overall average log$_{10}$ inoculum and recovery was significantly different with $p = 0.001$, therefore there was a
statistically significant loss of *Salmonella* after the 2 h drying. Average loss of *Salmonella* in the three replicates was 0.79, 0.75 and 0.67 log$_{10}$ CFU/ml, respectively. There was no significant difference in average loss between replicates (p>0.05). Overall average loss of *Salmonella* was 0.73 ± 0.24 log$_{10}$ CFU/ml.

**Cross Contamination Study**

The cross contamination study tested whether *Salmonella* would be transferred from inoculated tomatoes to uninoculated tomatoes via brush rollers in the overhead spray system during 100 mg/L NaOCl or water sanitation. A 10-L 100 mg/L NaOCl solution was prepared by adding 22 ml of 5.65% NaOCl to 10 L water. Aerosolization of chlorine in the overhead spray nozzles required an addition of 3 to 5 ml NaOCl to the 10 L solution to deliver 100 mg/L NaOCl from the nozzles. Concentration of free chlorine measured 95 mg/L before the experiment and 85 mg/L after. Ten liters of water supplemented with 0.1% sodium thiosulfate was also prepared. Five inoculated tomatoes and five uninoculated tomatoes were tested per sanitizer and each sanitizer was tested in triplicate. Inoculated tomatoes were sanitized for 15 s followed by 15 s sanitation of uninoculated tomatoes. Average reduction of *Salmonella* from inoculated tomatoes after the 15 s spray was 3.40 ± 1.02 log$_{10}$ CFU/ml for NaOCl and 2.86 ± 0.43 log$_{10}$ CFU/ml for water. There was no significant difference in average log$_{10}$ reduction of *Salmonella* from inoculated tomatoes between NaOCl and water (p>0.05).

Spraying uninoculated tomatoes with water immediately after spraying inoculated tomatoes resulted in a transfer of 4.88 ± 0.41 log$_{10}$ CFU/ml *Salmonella* (Table 4-3). Using 100 mg/L NaOCl in the spray significantly reduced cross contamination by 2.25 log$_{10}$ CFU/ml (p=0.0001). Average recovery of *Salmonella* from tomatoes sprayed
with NaOCl was $2.63 \pm 0.28 \log_{10} \text{CFU/ml}$. These values were adjusted $2 \log_{10} \text{CFU/ml}$ to because tomatoes were rinsed in 100 ml BPW, causing a dilution effect.

Contamination of brush rollers with *Salmonella* from inoculated tomatoes was also determined (Table 4-3). Each of the two brush rollers was swabbed before and after contact with inoculated tomatoes. Any initial contamination on brush rollers before contact with inoculated tomatoes was subtracted from colony counts after contact. Colony counts were averaged for the two rollers. Average recovery of *Salmonella* from brush rollers was not statistically significant between NaOCl and water at 1.24 and 1.95 $\log_{10} \text{CFU/cm}^2$ recovered, respectively ($p>0.05$).

**Sodium Hypochlorite Efficacy Study**

The efficacy of NaOCl at reducing *Salmonella* on tomato surfaces in the overhead spray system was tested at concentrations of 25, 50 and 100 mg/L NaOCl and a water control. NaOCl solutions were prepared in 10-L buckets and used at room temperature. Five tomatoes were tested per treatment time of 5, 15, 30 and 60 s. Average $\log_{10}$ reduction of *Salmonella* on tomato surfaces was calculated from triplicate experiments per NaOCl concentration and water by comparing recovery of *Salmonella* on treated tomatoes to five positive control tomatoes.

Statistically significant differences in efficacy of NaOCl and water were observed depending on concentration and treatment time (Table 4-4 and Figure 4-2). There was no significant difference between NaOCl concentrations and water at 5 s, with an average reduction of $1.37 \pm 0.24 \log_{10} \text{CFU/ml}$ ($p>0.05$). At 15 s, 100 mg/L NaOCl was significantly different from 25 and 50 mg/L NaOCl and water by achieving a $3.98 \pm 1.78 \log_{10} \text{CFU/ml}$ reduction of *Salmonella*. A 3-log$_{10}$ unit reduction was also achieved by 50 mg/L NaOCl at 30 s and 25 mg/L NaOCl at 60 s. A 3-log$_{10}$ unit reduction was not
observed for the water control, though it did achieve a $2.95 \pm 0.44 \log_{10}$ CFU/ml reduction at 60 s. At 30 s, a $5.55 \pm 0.37 \log_{10}$ CFU/ml reduction was observed for 100 mg/L NaOCl. Increasing treatment time to 60 s did not significantly increase efficacy for 100 or 50 mg/L, suggesting there is a limit in sanitizer efficacy. NaOCl at 25 mg/L was not more effective than water at 30 s and shorter treatment times, but it achieved a $4.23 \pm 1.11 \log_{10}$ CFU/ml reduction at 60 s. Efficacy of water did not increase much as treatment time increased. Highest reduction by water was seen at 60 s at $2.95 \pm 0.44 \log_{10}$ CFU/ml.

**Sanitizer Efficacy Study**

The sanitizer efficacy study examined the efficacy of NaOCl (100 mg/L), ClO$_2$ (5 mg/L), PAA (80 mg/L) and a water control in the overhead spray system for reducing *Salmonella* inoculated on tomato surfaces. All sanitizers were prepared in 10-L batches and held in a bucket to feed into the overhead spray system. All sanitizers required a higher concentration in the bucket to offset the aerosolization and loss of concentration as it was released from the spray nozzles. Average pH and concentration of each sanitizer before and after triplicate experiments was verified (Table 4-5). For each sanitizer, five tomatoes were tested per treatment time of 5, 15, 30 and 60 s. Average $\log_{10}$ reduction was calculated from triplicate experiments by comparing recovery of *Salmonella* from treated tomato surfaces and five positive controls.

Statistically significant differences were observed in at least one sanitizer at all treatment times (Table 4-6 and Figure 4-3). After only 5 s, PAA nearly reached a 3-$\log_{10}$ unit reduction at $2.79 \pm 0.94 \log_{10}$ CFU/ml. Conversely, NaOCl, ClO$_2$ and water had a 1.94, 1.87 and 1.86 $\log_{10}$ CFU/ml reduction, respectively. PAA consistently achieved about a 1-$\log_{10}$ unit higher reduction than the other sanitizers for 5, 15 and
30 s treatment. At 15 s, all sanitizers reached at least a 3-log$_{10}$ reduction of *Salmonella*, including water. Increasing treatment time to 30 s did not significantly increase reduction by ClO$_2$ or NaOCl but did for PAA to $5.50 \pm 0.12 \log_{10}$ CFU/ml. At 60 s, average log$_{10}$ reductions by NaOCl, ClO$_2$ and PAA were all significantly higher than the water control. NaOCl, ClO$_2$ and PAA had a 5.51, 4.85 and 5.52 log$_{10}$ CFU/ml reduction, respectively, and were not significantly different from each other (p<0.05). Compared to their efficacy at 30 s, efficacy of ClO$_2$ significantly increased at 60 s whereas PAA did not. Water only had a 3.75 log$_{10}$ CFU/ml reduction at 60 s.

**Flume vs. Overhead Spray Comparison Study**

The efficacy of NaOCl (100 mg/L) and water at 25°C for reducing *Salmonella* on tomato surfaces was compared between a scale-model flume and the overhead spray system. Ten liters each of NaOCl and water supplemented with 0.1% sodium thiosulfate were prepared. Average measured starting pH for triplicate experiments was 6.50 for NaOCl and 7.16 for water. Average measured concentration of NaOCl before and after experiments was 102 and 100 mg/L, respectively. Five inoculated tomatoes were tested per treatment time of 5, 15, 30 and 60 s. Average log$_{10}$ reduction of *Salmonella* from tomato surfaces was calculated and compared to overhead spray 100 mg/L NaOCl data from the sodium hypochlorite efficacy study.

The flume water control did not produce significantly different reductions in *Salmonella* among treatment times. Average reduction by the flume water control was $1.03 \pm 0.74 \log_{10}$ CFU/ml no matter how long tomatoes were treated. At 5 s, no significant differences were found among any sanitizer or sanitation system (p>0.05). Average reduction of *Salmonella* at 5 s was $0.97 \pm 0.56 \log_{10}$ CFU/ml.
Statistically significant differences were found between NaOCl and water, and overhead spray and flume starting at 15 s (Table 4-7 and Figure 4-4). Overhead spray NaOCl treatments of at least 15 s significantly reduced more *Salmonella* from tomatoes compared to flume treatments (p<0.05). At 15 s, average reduction by NaOCl in the overhead spray was $3.98 \pm 1.78 \log_{10} \text{CFU/ml}$. Conversely, NaOCl and water in the flume had an average reduction of 1.25 and $0.98 \log_{10} \text{CFU/ml}$, respectively. Reduction by NaOCl was enhanced to $5.55 \pm 0.37 \log_{10} \text{CFU/ml}$ at 30 s when treated with the overhead spray. NaOCl in the flume achieved a $3.17 \pm 2.59 \log_{10} \text{CFU/ml}$ reduction at 30 s. Increasing spray time to 60 s did not result in a significantly higher reduction in either the overhead spray or flume.

Concentration of *Salmonella* in the flume was tested at time 0 and after treating contaminated tomatoes for 15 and 60 s. At time 0, *Salmonella* was undetectable in flume water. *Salmonella* was recovered from flume water at an average of $4.54 \pm 0.37 \log_{10} \text{CFU/ml}$ after the 15 s treatment and $5.05 \pm 0.16 \log_{10} \text{CFU/ml}$ after the 60 s treatment, though these populations were not significantly different from each other (p>0.05). NaOCl (100 mg/L) effectively eliminated *Salmonella* in the flume as populations were undetectable throughout the study.

**Natural Tomato Microflora Study**

NaOCl (100 mg/L), ClO$_2$ (5 mg/L), PAA (80 mg/L) and a water control were tested for their ability to remove natural microflora from tomato surfaces in the overhead spray system. Sanitizers were prepared in 10-L batches and required a higher concentration feeding into the spray system to offset aerosolization as it was released from the spray nozzles. Starting pH and concentration of sanitizers before and after use were verified (Table 4-8). For each sanitizer, five tomatoes were tested per treatment time of 5, 15,
30 and 60 s. Five control tomatoes were also run to determine initial concentration of natural microflora. Average log_{10} reduction was found from triplicate experiments by comparing recovery of bacteria from treated tomatoes and the five control tomatoes.

Average initial population of natural microflora on tomato surfaces was 5.31 ± 0.57 log_{10} CFU/ml. No difference was observed among sanitizers at the 5 s treatment (p>0.05) (Table 4-9 and Figure 4-5). At 15 s, only NaOCl significantly reduced more natural microflora than water with a 0.81 ± 0.60 log_{10} CFU/ml reduction (p<0.05). Similar efficacy was observed at 30 s of treatment. NaOCl reduced microflora by an average of 1.41 ± 0.90 log_{10} CFU/ml, which was significantly higher than all other sanitizers (p<0.05). ClO₂, PAA and water had a 0.55, 0.84 and 0.56 log_{10} CFU/ml reduction, respectively at 30 s. Increasing treatment time to 60 s did not significantly affect efficacy.

Generally, the efficacy of the sanitizers increased significantly with increased treatment time. The exception is ClO₂, whose efficacy was not affected by time of exposure. As mentioned, NaOCl achieved a significantly greater reduction of microflora after 30 s treatment compared to 5 and 15 s. While PAA did not significantly reduce more microflora compared to the water control throughout the study, it did achieve a significantly higher reduction at 60 s of treatment compared to 5 s. The average reduction of microflora by PAA was 1.19 ± 0.28 log_{10} CFU/ml at 60 s and 0.48 ± 0.20 log_{10} CFU/ml at 5 s. Similarly, water achieved a significantly higher reduction after 60 s of treatment compared to 15 s. Reduction of microflora by water was 0.88 ± 0.12 log_{10} CFU/ml at 60 s and 0.26 ± 0.27 log_{10} CFU/ml at 15 s.
Table 4-1. Average log$_{10}$ concentration of rifampicin-resistant *Salmonella* strains over 12 h at 37°C

<table>
<thead>
<tr>
<th>Hour</th>
<th>Typhimurium</th>
<th>Braenderup</th>
<th>Enteritidis</th>
<th>Newport</th>
<th>Javiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.91 ± 0.04 a</td>
<td>4.09 ± 0.04 a</td>
<td>4.09 ± 0.02 a</td>
<td>3.98 ± 0.11 a</td>
<td>4.18 ± 0.10 a</td>
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<td>1</td>
<td>4.01 ± 0.09 a</td>
<td>4.08 ± 0.04 a</td>
<td>4.10 ± 0.15 a</td>
<td>4.04 ± 0.13 a</td>
<td>4.14 ± 0.05 a</td>
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<tr>
<td>2</td>
<td>4.28 ± 0.00 b</td>
<td>4.29 ± 0.03 b</td>
<td>4.65 ± 0.08 a</td>
<td>4.22 ± 0.13 b</td>
<td>4.06 ± 0.43 b</td>
</tr>
<tr>
<td>3</td>
<td>4.93 ± 0.16 b</td>
<td>4.98 ± 0.15 ab</td>
<td>5.24 ± 0.06 a</td>
<td>4.71 ± 0.23 b</td>
<td>4.97 ± 0.16 ab</td>
</tr>
<tr>
<td>4</td>
<td>5.34 ± 0.17 b</td>
<td>5.79 ± 0.05 a</td>
<td>5.97 ± 0.18 a</td>
<td>5.36 ± 0.34 b</td>
<td>5.72 ± 0.27 a</td>
</tr>
<tr>
<td>5</td>
<td>5.94 ± 0.20 c</td>
<td>6.39 ± 0.01 ab</td>
<td>6.63 ± 0.03 a</td>
<td>6.23 ± 0.27 b</td>
<td>6.64 ± 0.11 a</td>
</tr>
<tr>
<td>6</td>
<td>6.69 ± 0.17 b</td>
<td>7.08 ± 0.04 a</td>
<td>7.12 ± 0.07 a</td>
<td>7.08 ± 0.37 a</td>
<td>7.29 ± 0.29 a</td>
</tr>
<tr>
<td>7</td>
<td>7.21 ± 0.07 c</td>
<td>7.85 ± 0.11 b</td>
<td>7.89 ± 0.07 b</td>
<td>7.68 ± 0.38 b</td>
<td>8.18 ± 0.12 a</td>
</tr>
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<td>8.53 ± 0.07 a</td>
<td>8.68 ± 0.13 a</td>
<td>8.25 ± 0.39 b</td>
<td>8.71 ± 0.06 a</td>
</tr>
<tr>
<td>9</td>
<td>8.37 ± 0.11 c</td>
<td>9.01 ± 0.02 ab</td>
<td>8.72 ± 0.17 b</td>
<td>8.91 ± 0.29 ab</td>
<td>9.14 ± 0.08 a</td>
</tr>
<tr>
<td>10</td>
<td>8.90 ± 0.07 a</td>
<td>9.08 ± 0.00 a</td>
<td>8.93 ± 0.06 a</td>
<td>9.11 ± 0.07 a</td>
<td>9.22 ± 0.09 a</td>
</tr>
<tr>
<td>11</td>
<td>8.91 ± 0.08 a</td>
<td>8.93 ± 0.02 a</td>
<td>9.14 ± 0.02 a</td>
<td>9.04 ± 0.09 a</td>
<td>9.03 ± 0.09 a</td>
</tr>
<tr>
<td>12</td>
<td>9.08 ± 0.15 a</td>
<td>8.91 ± 0.05 a</td>
<td>9.15 ± 0.03 a</td>
<td>9.06 ± 0.07 a</td>
<td>9.17 ± 0.26 a</td>
</tr>
</tbody>
</table>

*a*Values are mean ± standard deviation of triplicate strains grown in tryptic soy broth supplemented with 200 mg/L rifampicin (n=3).

Means with same letter in the same row (abc) are not statistically different (p<0.05).
Figure 4-1. Average $\log_{10}$ concentration of rifampicin-resistant *Salmonella* strains over 12 h at 37°C

Error bars represent standard deviation of triplicate strains
Table 4-2. Average log\textsubscript{10} recovery and loss of \textit{Salmonella} from tomato surfaces after 2 h drying at room temperature

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inoculation\textsuperscript{a}</th>
<th>Recovery\textsuperscript{b}</th>
<th>Loss\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.83 ± 0.17 a, x</td>
<td>8.09 ± 0.79 a, x</td>
<td>0.79 ± 0.70 x</td>
</tr>
<tr>
<td>2</td>
<td>8.60 ± 0.30 a, x</td>
<td>7.86 ± 0.26 b, x</td>
<td>0.75 ± 0.26 x</td>
</tr>
<tr>
<td>3</td>
<td>8.57 ± 0.08 a, x</td>
<td>7.90 ± 0.30 b, x</td>
<td>0.67 ± 0.30 x</td>
</tr>
<tr>
<td>Average</td>
<td>8.67 ± 0.11 a</td>
<td>7.95 ± 0.30 b</td>
<td>0.73 ± 0.24</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values are mean ± standard deviation of 3 inoculum cultures inoculated on tomato surfaces.

\textsuperscript{b}Values are mean ± standard deviation of \textit{Salmonella} recovered from 3 tomatoes, adjusted for the 2 log\textsubscript{10} CFU/ml loss from rinsing tomatoes in 100 ml BPW.

\textsuperscript{c}Values are mean ± standard deviation of difference between inoculum and recovery of \textit{Salmonella} from tomatoes.

Means with same letter in the same row (ab) or in the same column (x) are not statistically different (p<0.05).

Table 4-3. Cross contamination of \textit{Salmonella} from inoculated tomatoes to uninoculated tomatoes and brush rollers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uninoculated tomatoes\textsuperscript{a}</th>
<th>Brush rollers\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl 100 mg/L</td>
<td>2.63 ± 0.28 y</td>
<td>1.24 ± 1.12 x</td>
</tr>
<tr>
<td>Water control</td>
<td>4.88 ± 0.41 x</td>
<td>1.95 ± 0.27 x</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values are mean ± standard deviation (log\textsubscript{10} CFU/ml) of triplicate experiments with 5 tomatoes each (n=15) adjusted for the 2 log\textsubscript{10} CFU/ml loss from rinsing tomatoes in 100 ml BPW.

\textsuperscript{b}Values are mean ± standard deviation (log\textsubscript{10} CFU/cm\textsuperscript{2}) of triplicate experiments of 4 swabs each, adjusted to 1 swab each for the subtraction of initial contamination and average between 2 rollers (n=3).

Means with same letter in the same column (xy) are not statistically different (p<0.05).
Table 4-4. Average log_{10} reduction of *Salmonella* after overhead spray treatment of 25, 50 and 100 mg/L sodium hypochlorite and water control

<table>
<thead>
<tr>
<th>Treatment (s)</th>
<th>100 mg/L</th>
<th>50 mg/L</th>
<th>25 mg/L</th>
<th>Water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.66 ± 0.51 a, z</td>
<td>1.43 ± 0.79 a, z</td>
<td>1.02 ± 0.24 a, z</td>
<td>1.36 ± 0.36 a, y</td>
</tr>
<tr>
<td>15</td>
<td>3.98 ± 1.78 a, y</td>
<td>2.84 ± 1.10 b, y</td>
<td>1.96 ± 0.06 b, yz</td>
<td>2.29 ± 0.36 b, xy</td>
</tr>
<tr>
<td>30</td>
<td>5.55 ± 0.37 a, x</td>
<td>4.24 ± 0.75 b, x</td>
<td>2.50 ± 0.66 c, y</td>
<td>2.52 ± 0.23 c, x</td>
</tr>
<tr>
<td>60</td>
<td>5.51 ± 0.96 a, x</td>
<td>4.96 ± 1.19 ab, x</td>
<td>4.23 ± 1.11 b, x</td>
<td>2.95 ± 0.44 c, x</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of triplicate experiments of 5 tomatoes each (n=15).

Means with same letter in the same row (abc) or in the same column (xyz) are not statistically different (p<0.05).

Figure 4-2. Average log_{10} reduction of *Salmonella* after 25, 50 and 100 mg/L sodium hypochlorite and water overhead spray treatment

Error bars represent standard deviation of triplicate experiments
Table 4-5. Average starting pH and measured concentration of sanitizers before and after sanitizer efficacy study experiments

<table>
<thead>
<tr>
<th>Sanitizer</th>
<th>pH</th>
<th>Before (mg/L)</th>
<th>After (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>6.48</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>ClO₂</td>
<td>7.50</td>
<td>4.48</td>
<td>4.82</td>
</tr>
<tr>
<td>PAA</td>
<td>3.62</td>
<td>77</td>
<td>76</td>
</tr>
<tr>
<td>Water</td>
<td>6.61</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

*aNot tested*
Table 4-6. Average log$_{10}$ reduction of *Salmonella* after overhead spray treatment of sanitizers and water control

<table>
<thead>
<tr>
<th>Treatment (s)</th>
<th>NaOCl 100 mg/L</th>
<th>ClO$_2$ 5 mg/L</th>
<th>PAA 80 mg/L</th>
<th>Water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.94 ± 0.59 b, z</td>
<td>1.87 ± 0.73 b, z</td>
<td>2.79 ± 0.94 a, y</td>
<td>1.86 ± 0.68 b, y</td>
</tr>
<tr>
<td>15</td>
<td>3.49 ± 1.06 b, y</td>
<td>3.54 ± 0.74 b, y</td>
<td>4.73 ± 0.53 a, x</td>
<td>3.17 ± 0.75 b, x</td>
</tr>
<tr>
<td>30</td>
<td>4.07 ± 1.22 b, y</td>
<td>3.93 ± 0.26 b, y</td>
<td>5.50 ± 0.12 a, x</td>
<td>3.41 ± 1.07 b, x</td>
</tr>
<tr>
<td>60</td>
<td>5.51 ± 0.17 a, x</td>
<td>4.85 ± 0.26 a, x</td>
<td>5.52 ± 0.12 a, x</td>
<td>3.75 ± 0.74 b, x</td>
</tr>
</tbody>
</table>

*Values are mean ± standard deviation of triplicate experiments of 5 tomatoes each (n=15). Means with same letter in the same row (ab) or in the same column (xyz) are not statistically different (p<0.05).

Figure 4-3. Average log$_{10}$ reduction of *Salmonella* after sanitizer and water control overhead spray treatment

Error bars represent standard deviation of triplicate experiments
Table 4-7. Average log_{10} reduction of *Salmonella* after sodium hypochlorite and water control flume treatment, compared to sodium hypochlorite overhead spray treatment

<table>
<thead>
<tr>
<th>Treatment (s)</th>
<th>Overhead spray 100 mg/L NaOCl</th>
<th>Flume 100 mg/L NaOCl</th>
<th>Flume water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.66 ± 0.51 a, z</td>
<td>0.79 ± 0.91 a, y</td>
<td>0.46 ± 0.27 a, x</td>
</tr>
<tr>
<td>15</td>
<td>3.98 ± 1.78 a, y</td>
<td>1.25 ± 1.14 b, y</td>
<td>0.96 ± 0.84 b, x</td>
</tr>
<tr>
<td>30</td>
<td>5.55 ± 0.37 a, x</td>
<td>3.17 ± 2.59 b, x</td>
<td>1.39 ± 0.76 c, x</td>
</tr>
<tr>
<td>60</td>
<td>5.51 ± 0.96 a, x</td>
<td>3.34 ± 2.56 b, x</td>
<td>1.30 ± 1.09 c, x</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of triplicate experiments of 5 tomatoes each (n=15). Means with same letter in the same row (abc) or in the same column (xyz) are not statistically different (p<0.05).

Figure 4-4. Average log_{10} reduction of *Salmonella* after 100 mg/L sodium hypochlorite and water flume treatment compared to 100 mg/L sodium hypochlorite overhead spray treatment

Error bars represent standard deviation of triplicate experiments
Table 4-8. Average starting pH and measured concentration of sanitizers before and after natural tomato microflora study experiments

<table>
<thead>
<tr>
<th>Sanitizer</th>
<th>pH</th>
<th>Before (mg/L)</th>
<th>After (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>6.50</td>
<td>100</td>
<td>102</td>
</tr>
<tr>
<td>ClO₂</td>
<td>7.55</td>
<td>5.09</td>
<td>4.80</td>
</tr>
<tr>
<td>PAA</td>
<td>3.58</td>
<td>81</td>
<td>84</td>
</tr>
<tr>
<td>Water</td>
<td>6.59</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are concentration of measured free chlorine
Table 4-9. Average log$_{10}$ reduction of natural microflora after overhead spray treatment of sanitizers and water control

<table>
<thead>
<tr>
<th>Treatment (s)</th>
<th>NaOCl 100 mg/L</th>
<th>ClO$_2$ 5 mg/L</th>
<th>PAA 80 mg/L</th>
<th>Water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.50 ± 0.36 a, y</td>
<td>0.58 ± 0.26 a, x</td>
<td>0.48 ± 0.20 a, y</td>
<td>0.56 ± 0.38 a, xy</td>
</tr>
<tr>
<td>15</td>
<td>0.81 ± 0.60 a, y</td>
<td>0.75 ± 0.40 ab, x</td>
<td>0.74 ± 0.72 ab, xy</td>
<td>0.26 ± 0.27 b, y</td>
</tr>
<tr>
<td>30</td>
<td>1.41 ± 0.90 a, x</td>
<td>0.55 ± 0.20 b, x</td>
<td>0.84 ± 0.72 b, xy</td>
<td>0.56 ± 0.38 b, xy</td>
</tr>
<tr>
<td>60</td>
<td>1.58 ± 0.46 a, x</td>
<td>1.06 ± 0.46 b, x</td>
<td>1.19 ± 0.28 ab, x</td>
<td>0.88 ± 0.12 b, x</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of triplicate experiments of 5 tomatoes each (n=15). Means with same letter in the same row (ab) or in the same column (xy) are not statistically different (p<0.05).

Figure 4-5. Average log$_{10}$ reduction of natural tomato microflora after sanitizer and water control overhead spray treatment

Error bars represent standard deviation of triplicate experiments
Numerous multistate outbreaks of *Salmonella* in tomatoes in past decades illustrate the need for effective food safety measures. Risk of foodborne disease outbreaks can be prevented or minimized on the farm and in the packinghouse by implementing GAPs and other intervention methods. Flume washing in fresh tomato postharvest handling was targeted as a critical step in production where improper procedures could amplify potential contamination. Proper sanitizer use and monitoring of efficacy may reduce pathogen populations on tomatoes but more importantly, should prevent cross contamination from one tomato to another. While many studies have shown chlorinated flumes to achieve the $3\log_{10}$ unit reduction of *Salmonella* required by Florida T-GAPs and T-BMPs, use of a non-immersion sanitation system would decrease volume of water and sanitizer needed and reduce costs. The goal of this research was to determine if a laboratory model overhead spray system would be as effective as a flume. Efficacy of sanitizers in the overhead spray system was evaluated for the reduction of inoculated *Salmonella* from tomato surfaces.

**Growth Curves**

A five-strain cocktail of rifampicin resistant *Salmonella* was used as the inoculum. The five serotypes used were *S. Typhimurium*, *S. Braenderup*, *S. Enteritidis*, *S. Newport* and *S. Javiana*. *Salmonella* serotypes were selected based on their association with multistate fresh tomato outbreaks and adapted to be rifampicin resistant in order to select for only the bacteria that was inoculated (Table 2-1). Though *S. Enteritidis* has not been the cause of a multistate fresh tomato outbreak in the US, it is frequently the cause of outbreaks associated with other foods (CDC 2010a).
growth curve study was meant to confirm that the five strains reached a stationary phase of about 9 log₁₀ CFU/ml at the same time. Therefore, all strains in a combined cocktail would initially be in equal concentrations and potentially be recovered after tomato inoculation. The five-strain cocktail represented a worst-case scenario of *Salmonella* contamination that eliminated differences in overall *Salmonella* recovery due to differences between strain survival during sanitizer treatment.

Growth of each strain was measured over 12 h (Table 4-1 and Figure 4-1). In a typical bacterial growth curve, stationary phase follows a lag and logarithmic phase where growth stops, or rate cell division equals rate of cell death and concentration reaches a plateau. Within each strain, average concentrations during at least the last 3 h of growth were not statistically significant from each other but were significantly different from the previous hours of growth, indicating that all strains reached stationary phase. Additionally, the same significance pattern was observed between strains, indicating all strains reached stationary phase at the same time. Average concentration of the five strains at stationary phase (hour 12) was 9.07 ± 0.11 log₁₀ CFU/ml. Because the selected strains exhibited very similar growth curves, they were used as an inoculum cocktail in subsequent studies.

This study was similar to one conducted by Felkey (2002) in which growth of five rifampicin resistant *Salmonella* strains were measured after 20 h. It was found that all strains grew to approximately the same concentration between 8.32 to 9.20 log₁₀ CFU/ml. The difference in concentration between studies could be because the incubation time was longer and different *Salmonella* strains were used.
Recovery Study

A recovery study compared the concentration of inoculum and the concentration of *Salmonella* recovered from inoculated tomatoes. Tomatoes were inoculated and dried for 2 h in a fume hood at room temperature. The 2 h drying period was meant to allow time for *Salmonella* to attach to tomato surfaces. Results revealed an average difference of $0.73 \pm 0.24 \log_{10} \text{CFU/ml}$ *Salmonella* between inoculum and inoculated tomatoes (Table 4-2). The loss may have been due to the 2 h drying period. After 2 h, inoculum was visually dry and appeared like residue spots. Freshly inoculated tomatoes had wet spots. Though freshly inoculated tomatoes were not tested or statistically analyzed, a few single tomatoes were inoculated and immediately plated. These tomatoes produced similar colony counts to theoretical inoculation levels. Additionally, the fume hood could have increased inoculum loss because the airflow increased evaporation. Another possible explanation is that some *Salmonella* may have remained attached to the tomato surface during the rub and shake step of plating.

Because this research aimed to determine the efficacy of various sanitizers in an overhead spray system, inoculated tomatoes needed to have a high enough initial level of *Salmonella* to show that sanitizers could achieve at least a $3\log_{10}$ unit reduction or even up to a $5\log_{10}$ unit reduction. The $3\log_{10}$ unit reduction was a selected threshold of risk based on Florida T-GAPs and T-BMPs and represents an estimated amount of contamination reasonably likely to occur (FDACS 2007). Any alternative sanitizer or sanitation system must be validated for its ability to reduce *Salmonella* or like pathogens by a minimum of $3 \log_{10}$ units. The initial *Salmonella* level on tomatoes should also be high enough considering the limit of detection in the plating method was $2 \log_{10} \text{CFU/ml}$. Therefore, the purpose of the recovery study was to verify that any loss that occurred
was not too high to prevent the detection of *Salmonella* on treated tomatoes. From the
results, the less than 1-log<sub>10</sub> unit loss was not considered too high because tomatoes
still retained about 8 log<sub>10</sub> CFU/ml. For that reason, all inoculated tomatoes were dried
for 2 h in the fume hood and underwent the same plating procedure in subsequent
studies.

**Cross Contamination Study**

The cross contamination, sodium hypochlorite efficacy, sanitizer efficacy, flume vs.
overhead spray comparison and natural tomato microflora studies all examined the
efficacy of a treatment for removing bacteria from tomato surfaces. Efficacy was
measured by average log<sub>10</sub> reduction of bacteria from tomato surfaces. Any difference
in average recovery between positive controls and treated tomatoes was considered an
average reduction of bacteria attributed to the specific treatment applied.

Accumulation of microorganisms can occur in flume systems because tomatoes
commingle and water is recirculated (Mahovic and others 2007). Cross contamination
also presents a concern in an overhead spray system because tomatoes directly
contact the brush rollers as a common surface. In the cross contamination study,
NaOCl (100 mg/L) and water were tested for their ability to prevent cross contamination
between inoculated tomatoes and uninoculated tomatoes via brush rollers in the
overhead spray system. Inoculated tomatoes were treated for 15 s and removed,
followed by 15 s treatment of uninoculated tomatoes. It was found that average log<sub>10</sub>
reduction from inoculated tomatoes in the initial 15 s spray was not significantly different
between NaOCl and water (p>0.05). Both NaOCl and water reduced *Salmonella* by
about 3 log<sub>10</sub> units. As also seen in the sanitizer efficacy study discussed later, water at
times achieved similar efficacy as NaOCl and other sanitizers in the overhead spray
system depending on the treatment time. Generally, efficacy of water was comparable
to other sanitizers at shorter treatment times less than 30 s when sanitizers may not
have enough contact time on tomatoes. It was proposed that the mechanical action of
the brushes and pressure of the spray were able to physically remove *Salmonella* from
tomato surfaces to a certain extent. After a specific treatment time, the combined
mechanical action of the overhead spray and antimicrobial power of sanitizers
surpassed mechanical action alone, resulting in higher efficacy of sanitizers over water.

Brush rollers were contaminated with an average of 1.60 ± 0.70 log\(_{10}\) CFU/cm\(^2\)
*Salmonella* after spraying inoculated tomatoes for 15 s (Table 4-3). There was no
significant difference in recovery of *Salmonella* on brush rollers between NaOCl and
water (p>0.05). Cross contamination occurred at an average of 4.88 ± 0.41 log\(_{10}\)
CFU/ml on uninoculated tomatoes when sprayed with water. Spraying with NaOCl
significantly reduced cross contamination to 2.63 ± 0.28 log\(_{10}\) CFU/ml (p<0.05). NaOCl
was better able than water to prevent cross contamination from inoculated tomatoes to
uninoculated tomatoes, but still resulted in greater than 2-log\(_{10}\) unit transfer of
*Salmonella*. Uninoculated tomatoes had 15 s of contact time with rollers and contacted
a large surface area that likely contributed in the transfer of *Salmonella*.

It is believed than only one scientific study has examined contamination of brush
rollers in an overhead spray system. Pao and others (2009) found that when tomatoes
were placed on brushes that were previously inoculated with about 6.9 log\(_{10}\) CFU/cm\(^3\)
*Salmonella*, 5.7 log\(_{10}\) CFU/cm\(^2\) was transferred to tomato surfaces without any spray. A
5 mg/L ClO\(_2\) spray significantly reduced contamination by 4.5 log\(_{10}\) units after 10 s.
Water reduced contamination by 2.1 log\(_{10}\) units only after 40 s. Even though ClO\(_2\) was
significantly more effective than water at 10 s, there were still 1.2 log$_{10}$ units *Salmonella* recovered from tomato surfaces, somewhat comparable to the 2.63 log$_{10}$ CFU/ml recovered in the cross contamination study after 15 s NaOCl spray. Increasing the ClO$_2$ spray to 60 s increased reduction by only 0.5 log$_{10}$ units, resulting in 0.7 log$_{10}$ units *Salmonella* remaining on tomatoes. Additional treatment times could be tested with the experimental protocol used to measure extent of cross contamination at longer spray times. Level of contamination from contaminated rollers to uninoculated tomatoes without any spray could also be tested.

Though the methods were different, these results are similar to the Pao and others (2009) study. The researchers did not examine cross contamination but rather directly inoculated rollers with a known theoretical volume. In this study, the 3 log$_{10}$ CFU/ml of *Salmonella* removed from each inoculated tomato during the 15 s spray was hypothesized to have transferred to brush rollers. The overhead spray systems were also constructed differently with different sized brushes, rotation speed and spray flow rate. The overall conclusions are valuable, however. Using a sanitizer instead of water in an overhead spray system effectively reduces contamination of *Salmonella* from brush rollers to tomatoes. Conversely, the overhead spray may not be more effective than chlorinated flumes at preventing cross contamination. Chlorinated water is known to be very effective at destroying microorganisms freely suspended in water. At 2 min exposure, lowest lethal dose of chlorine shown to kill 7 log$_{10}$ units of *E. carotovora* was 0.4 to 0.5 mg/L at pH 6 to 7 (Robbs and others 1995). Less than 1 mg/L free chlorine has been shown to kill vegetative bacteria within 30 s (Dychdala 2001). *Geotrichum candidum* mold has been inactivated in 20 to 25 mg/L free chlorine in 30 s (Bartz and
others 2001). While bacteria on tomato surfaces may only be reduced by a few log_{10} units in a flume, as seen in the flume vs. overhead spray comparison study discussed later, the bacteria become freely suspended and should be inactivated by properly sanitized water. Pao and others (2007) supported this conclusion when they found that 5 mg/L ClO₂ effectively prevented cross contamination from inoculated tomatoes to uninoculated tomatoes placed in the same flume.

Though about 2 log_{10} units Salmonella was transferred to uninoculated tomatoes in the overhead spray system, the extent of contamination that would likely occur outside a laboratory may be much lower. First, tomatoes, or brushes in the Pao and others (2009) study, were inoculated with a high concentration of Salmonella in order to be able to show a high log_{10} reduction by specific treatments. The high concentration of inoculum also represents a worst-case scenario of contamination and survival of at least one serovar of Salmonella after treatment. Such a high level of contamination would likely only occur if GAPs were not properly followed. Implementation and adherence to GAPs should limit the extent of contamination that could occur in the field or packinghouse. GAPs include using clean irrigation water, storing manure away and downhill of crops, practicing good worker hygiene when handling fruit, using clean harvest bins and maintaining a validated, effective concentration of sanitizer in wash water (FDA 1998).

**Sodium Hypochlorite Efficacy Study**

The sodium hypochlorite efficacy study examined efficacy of NaOCl (25, 50 and 100 mg/L) and water at reducing Salmonella on tomato surfaces in the overhead spray system. Significant reduction of Salmonella occurred at 15 s with NaOCl (100 mg/L) (Table 4-4 and Figure 4-2). This time point had the highest relative standard deviation,
however. Average reduction by NaOCl (100 mg/L) at 15 s from 3 experiments ranged from 2.47 to 5.94 log\textsubscript{10} CFU/ml. Standard deviation was high likely because differences in morphology of individual tomatoes as illustrated by the fact that reduction on individual tomatoes ranged from 0.59 to 6.08 log\textsubscript{10} CFU/ml. Differences in reduction were likely due to treatment and not initial inoculum levels because positive control tomatoes were not significantly different. All tomatoes were placed in the same orientation on rollers with the inoculum area around the blossom scar adjacent to where tomatoes would initially contact the spray. If tomatoes remained in this orientation, they would spin on their axis in a way that the inoculated area would directly contact rollers and the spray. Throughout the overhead spray studies, however, a few tomatoes were observed to roll on their axis in a direction that did not result in contact between the inoculated area and the brush rollers and/or direct contact with spray due to differences in tomato morphology, including surface characteristics, size and force from the characteristics of neighboring tomatoes. The inoculum on these tomatoes would not be subjected to as much physical removal or exposure to sanitizer and thus achieve a lower reduction. Including more replicates should lower standard deviation, but since statistical significance was observed, the n=15 sample size was thought to be sufficient.

Little research examining efficacy of NaOCl in an overhead spray system is available. Vigneault and others (2000) examined a chlorinated overhead spray shower system as a tomato hydrocooler. Because flume systems result in immersion of tomatoes, infiltration of water and pathogens into tomatoes is a concern, especially via the stem scar (Bartz and Showalter 1981). Potential for infiltration in the hydrocooler shower system was investigated. Tomatoes were placed stem scar up or down and
sprayed with water contaminated with \textit{R. stolonifer} and up to 200 mg/L chlorine. Because tomatoes were heated to 35°C and water was 10°C, all tomatoes increased in weight by the uptake of water, but tomatoes in the stem scar-up orientation gained significantly more weight. Despite the water infiltration, chlorine prevented decay of all tomatoes after 10 d storage at 20°C (Vigneault and others 2000). This paper showed that there is likely little risk of water infiltration via tomato stem scars in overhead spray systems. The hydrocooler water flow rate was 1,000 L/min\(\cdot\)m\(^2\) and the exposure time was over 13 min, which are both much higher than the treatment parameters of the overhead spray system of this research. Therefore, lower flow rates and shorter contact times may present an even smaller risk of infiltration.

In summary, this study showed that all NaOCl concentrations achieved a 3-log\(_{10}\) CFU/ml reduction or more depending on treatment time. The data of this study will be able to support the use of the specific concentration and treatment time combinations in an overhead spray system in accordance with T-GAPs and T-BMPs. T-BMPs require sanitation of tomatoes with an approved sanitizer or process. If not pre-approved, the sanitizer or process must be shown in a reproducible scientific study to achieve at least a 3-log\(_{10}\) unit reduction of \textit{Salmonella} or like organisms (FDACS 2007). Beyond this requirement, a 5-log\(_{10}\) CFU/ml reduction was achieved by 100 mg/L NaOCl at 30 s. Because T-BMPs require flumes to have at least 150 mg/L free chlorine and flumes may be chlorinated up to 350 mg/L in practice to maintain sufficient free chlorine, implementing an overhead spray system with just 100 mg/L NaOCl would reduce the amount of NaOCl currently used (FDACS 2007; Suslow 1997). A lower NaOCl concentration would reduce packinghouse operating costs.
Sanitizer Efficacy Study

The sanitizer efficacy study examined efficacy of NaOCl (100 mg/L), ClO₂ (5 mg/L), PAA (80 mg/L) and water at reducing *Salmonella* on tomato surfaces in the overhead spray system. The overall conclusion gathered from results was that all sanitizers achieved a 3-log₁₀ reduction at 15 s (Table 4-6 and Figure 4-3). Similar to the sodium hypochlorite efficacy study, NaOCl (100 mg/L) achieved a 3.49 ± 1.06 log₁₀ CFU/ml reduction of *Salmonella* at 15 s. Efficacy of NaOCl at 30 s was 4.07 ± 1.22 log₁₀ CFU/ml compared to 5.55 ± 0.37 log₁₀ CFU/ml in the sodium hypochlorite efficacy study. Difference in efficacy may be explained by different tomato spins or unaccountable differences between experiments since average reduction at 30 s per experiment ranged from 2.72 to 5.10 log₁₀ CFU/ml. Conducting additional experiment replicates should decrease standard deviation values. Still, this data can support the use of 100 mg/L NaOCl in overhead spray systems.

Pao and others (2009) examined ClO₂ in an overhead spray system with inoculated tomatoes dried for 24 h at 40 to 50% relative humidity. Tomatoes were sprayed with 5 mg/L ClO₂ or water at a flow rate of 5.0 or 9.3 ml/s. It was found that ClO₂ achieved a 4.4 to 5.2 log₁₀ unit reduction of *Salmonella* from 10 to 60 s exposure, which was significantly better than water (p<0.01). The reductions were comparable to the 3.54 to 4.85 log₁₀ CFU/ml reductions from 15 to 60 s exposure seen in this study. Flow rate did not significantly change efficacy. While PAA has not been previously studied in an overhead spray system, it has been studied in flumes. PAA has been shown to achieve a 4-log₁₀ unit reduction of *Salmonella* on surfaces of various produce including tomatoes when treated for at least 60 s (Yuk and others 2005, 2006). PAA in this study achieved greater than 4 log₁₀ CFU/ml reductions in just 15 s in the overhead
spray system. While ClO$_2$ and PAA are already approved in Florida for use in flumes, the results of this study can support their use in an overhead spray system.

As also seen in the cross contamination study discussed earlier, water sometimes achieved similar efficacy to the other sanitizers in the overhead spray system. The physical removal of *Salmonella* from tomatoes by brush rollers and spray pressure must play an important role in efficacy in an overhead spray system. The efficacy of the brushes or water spray alone could be tested as a control in a future study. Brushing is known to aid sanitation efforts because of physical removal of microbes. For example, Parnell and others (2005) inoculated rinds of whole melons with *S. Typhimurium*. Melons were treated with a 200 mg/L total chlorine or distilled water soak, or wet with one of the above solutions and then scrubbed with a sterile brush for 60 s. Soaking resulted in about 1 and 2 log$_{10}$ CFU/sample reductions of *Salmonella* on melons in water and chlorine, respectively. Scrubbing with a brush significantly reduced *Salmonella* by about an additional 1 log$_{10}$ CFU/sample. Overall, scrubbing in chlorine provided the highest reduction (Parnell and others 2005).

In contrast to the sodium hypochlorite efficacy study where water could not reach a 3-log$_{10}$ CFU/ml reduction at 60 s, water achieved a 3-log$_{10}$ CFU/ml reduction at only 15 s in the sanitizer efficacy study. The discrepancy could be due to natural variation of tomatoes that caused changes in spin direction because standard deviation is relatively high, especially for 30 s. Though sanitizer treatments were run sequentially on the same day with water controls run last, it is unlikely that residual sanitizer remaining on rollers, if any, contributed to water efficacy because water was supplemented with sodium thiosulfate, which inactivates chlorine. Water was also sprayed for about 1 min
to rinse lines and rollers after the PAA tests. Pao and others (2009) also observed a 3-log$_{10}$ unit reduction of *Salmonella* from tomato surfaces from a 5.0 ml/s water spray for 10 to 40 s. Reduction increased to 4.4 log$_{10}$ units at 60 s. Increasing flow rate to 9.3 ml/s generally increased reduction by water. This could be explained by the increased pressure of the spray being able to physically force more *Salmonella* from tomato surfaces.

**Flume vs. Overhead Spray Comparison Study**

Efficacy of NaOCl (100 mg/L) and water against *Salmonella* on tomatoes were compared in a scale-model flume. NaOCl flume data was also compared to NaOCl (100 mg/L) data from the sodium hypochlorite efficacy study. Results showed that the overhead spray system was significantly more effective than the chlorinated flume between 15 to 60 s contact time (Table 4-7 and Figure 4-4). Increasing treatment time from 30 s to 60 s did not significantly affect reduction by NaOCl in either system.

The chlorinated scale-model flume (100 mg/L) did achieve a 3-log$_{10}$ unit reduction at 30 s, which supports the current use of flumes as a sanitation system in tomato packinghouses. Additionally, the chlorinated flume was effective compared to the unchlorinated flume. The flume water control achieved a maximum reduction of 1.30 ± 1.09 log$_{10}$ CFU/ml at 60 s. The water control data shows that water was able to remove some *Salmonella* from tomato surfaces, perhaps from the mechanical action of the circulating water that forced tomatoes to flow, bump and rub surrounding tomatoes and sometimes roll over. Reduction by mechanical action was limited to an average of 1.03 ± 0.74 log$_{10}$ CFU/ml, however. Rolling over so that the inoculated area on tomatoes was no longer submerged may have lowered overall reduction. This phenomenon caused by chance or natural variation in tomatoes would help explain the
relatively high standard deviation of average reduction values throughout the comparison study.

Flume water can cross contaminate commingled produce if water is not properly chlorinated. In this study, the addition of 100 mg/L NaOCl in the flume not only significantly increased reduction of Salmonella compared to a plain water flume, but could also prevent cross contamination by eliminating freely suspended Salmonella. Salmonella was recovered in unchlorinated water at $4.54 \pm 0.37$ and $5.05 \pm 0.16 \log_{10}$ CFU/ml after inoculated tomatoes were tested for 15 and 60 s, respectively. The freely suspended Salmonella could contaminate subsequent tomatoes placed in the flume. In contrast, Salmonella was undetected in the 100 mg/L NaOCl flume.

Studies that examined the efficacy of chlorinated flumes report good levels of efficacy. Felkey and others (2006) observed about a 3-log$_{10}$ CFU/ml reduction of Salmonella in a 150 mg/L free chlorine flume at 25°C for 30 to 60 s, which is very similar to these results despite the higher chlorine concentration. Salmonella populations were also reduced to $0.16 \log_{10}$ CFU/ml after treatment for 120 s, which was essentially a greater than 6-log$_{10}$ CFU/ml reduction. The maximum treatment time tested in this study was 60 s, thus it is unknown whether similar results would be seen in the flume. In a study by Zhuang and others (1995), entire surfaces of tomatoes were inoculated with S. Montevideo to about $4.81 \log_{10}$ CFU/cm$^2$ and were dried for 5 h. After submerging tomatoes in 60 and 110 mg/L NaOCl at 25°C for 2 min, populations were reduced to $4.17$ and $3.59 \log_{10}$ CFU/cm$^2$, respectively. These levels were significantly different from the control, equal to a 0.64 to 1.22 log$_{10}$ CFU/cm$^2$ reduction. Yuk and others (2005) found a 5-log$_{10}$ unit reduction of Salmonella on tomato surfaces at
200 mg/L NaOCl after 60 s at 35°C. The higher concentration of NaOCl used and longer treatment times could explain the higher log_{10} reductions achieved in these studies. Additionally, the different Salmonella serovars tested may have varying levels of survival during treatments. Felkey and others (2006) and Yuk and others (2005) both used a 5-strain cocktail of S. Agona, S. Gaminara, S. Michigan, S. Montevideo and S. Poona.

In conclusion, while the chlorinated flume was able to achieve a 3-log_{10} CFU/ml reduction in 30 s, the overhead spray system achieved a greater reduction in half the time. Maximum reduction by the NaOCl flume was about 3 log_{10} CFU/ml at 60 s whereas the overhead spray system reached a greater than 5-log_{10} CFU/ml reduction in 30 s. The longer immersion times needed by the flume to achieve similar efficacy as the overhead spray system may increase risk of water infiltration. This study demonstrates the superior ability of the overhead spray system to reduce Salmonella from tomato surfaces under laboratory conditions.

**Natural Tomato Microflora Study**

Efficacy of sanitizers was evaluated in the overhead spray system for the reduction of natural tomato microflora. The goal of this study was to determine how the overhead spray system would perform against non-artificially inoculated bacteria. Many naturally occurring organisms are actually, once internalized, pathogens to the tomato itself (Narayanasamy 2006). The uninoculated microflora represented both indigenous, innocuous organisms and potential spoilage organisms including fungi and bacteria that can affect tomato quality.

Generally, average log_{10} reductions of natural microflora were lower than those seen in the efficacy studies with inoculated Salmonella (Table 4-9 and Figure 4-5). The
low reductions of natural microflora by the sanitizers may be explained by the strong attachment of these organisms to the tomato surface that occurred over time. In contrast, in the other efficacy studies, Salmonella was inoculated and allowed to dry for only 2 h before testing. The mechanical action of the brush rollers and pressure of the spray may not have been forceful enough to physically remove the microflora from tomatoes. Because bacteria remained attached, perhaps in a protective biofilm, they were less susceptible to the antimicrobial effects of the sanitizers. This would explain why ClO$_2$ and PAA were not more effective than water in this study. A future study could examine the effect of adjusting the engineering of the overhead spray system to achieve more physical force to remove microflora yet still not injure the tomatoes. Another study could examine the fate of non-mesophilic bacteria in the overhead spray system, though they may be less likely to cause human disease. Because natural tomato microflora usually do not affect safety of tomatoes, efficacy the overhead spray system as it currently stands should be measured by pathogen reduction.

A quality issue may arise if tomatoes are mechanically injured so that natural microflora can be internalized and spread disease throughout an entire box. Opportunistic tomato spoilage fungi include Fusarium spp., Geotrichum spp. and yeasts (Narayanasamy 2006). Naturally occurring bacteria found on and inside tomatoes include Bacillus spp., Cyanobacterium spp., Erwinia spp., Enterobacter spp., Pantoea spp. and Pseudomonas putida (Shi and others 2009). While some of these genera contain human pathogenic species, most are nonpathogenic. Furthermore, some studies have shown Enterobacter and Bacillus to inhibit the growth of Salmonella in tomatoes and other plants and animals through competitive exclusion (Shi and others
If natural microflora remain attached to tomato surfaces, *Salmonella* may not have available space or nutrients to survive. The presence of natural microflora of tomato surfaces could be a prevention factor against *Salmonella* contamination.

**Conclusions and Future Work**

The overall objective of this research was to determine if a non-immersion sanitation system could be as effective as a flume system and therefore decrease water and sanitizer requirements. A laboratory model overhead spray system was evaluated for the reduction of inoculated *Salmonella* on tomato surfaces. Because sanitation of tomatoes is required in Florida as part of T-GAPs and T-BMPs, novel sanitation systems are researched and developed to improve sanitation and to reduce operating costs. Sanitation systems must be evaluated for benefits and disadvantages. Flumes and overhead spray systems can be compared in terms of sanitation ability, sanitizer use, water use, ability to prevent cross contamination, required space, non-sanitation uses and overall cost.

Several studies were performed in this research to evaluate the sanitizer and treatment time combinations needed in the overhead spray system to achieve at least a 3-log$_{10}$ unit reduction of *Salmonella*. Sanitizers in the overhead spray system achieved higher log$_{10}$ reductions of *Salmonella* compared to the scale-model flume at treatment times of at least 15 s. Three-log$_{10}$ unit reductions were achieved by all sanitizers and concentrations of NaOCl in the overhead spray system. The data collected can support the implementation of an overhead spray system because of the scientifically observed pathogen reduction.

Few studies have examined efficacy of sanitizers in an overhead spray system. One study compared a commercial overhead spray and dump tank method with an
alternative spray wash method in a cantaloupe packing facility in Mexico (Alvarado-Casillas and others 2010). The typical sanitation method was a potable water spray over polyvinyl chloride rollers followed by a chlorinated dump tank soak for 1 min. An alternative treatment was a 90-s manual water wash with a backpack sprayer followed by a 2% lactic acid spray for 15 s, both at about 17 m/s flow rate. Results showed that the alternative spray method was significantly more effective against aerobic plate counts and coliforms than the dump tank (Alvarado-Casillas and others 2010). Researchers suggest spray washing can help reduce cross contamination, though it is unlikely that tomatoes can be manually spray washed given the large volumes entering packinghouses.

NaOCl was the main sanitizer tested because of its established use in the produce industry. NaOCl concentrations tested were 25, 50 and 100 mg/L, which were substantially lower than the minimum required and typically measured free chlorine levels in packinghouse flumes (FDACS 2007; Suslow 1997). Results of the sodium hypochlorite study could support the use of less NaOCl to achieve a similar or higher level of Salmonella reduction on tomato surfaces. The ability of 100 mg/L NaOCl to achieve a greater than 3-log$_{10}$ reduction of Salmonella in 15 s in the overhead spray system shows that NaOCl usage could be drastically reduced with use of overhead spray systems. NaOCl volume would also be reduced with the decreased amount of water needed for sanitation. Flumes hold tens of thousands of gallons of water that cannot be reused from day to day. Water must be hauled away by tanker trucks. Though water still cannot be recycled, overhead spray systems could possibly achieve a higher level of pathogen reduction with less water. Exact volume of water needed
would depend on each operation such as production speed and quantity of tomatoes produced a day. Because wash water is not reused in overhead spray systems, there is limited contact between chlorine and nitrogenous and organic compounds. Therefore, there would be reduced formation of chloramines and carcinogenic trihalomethanes. The use of less water is both important in reducing costs and environment impact. While there may be no drawbacks in saving water, using a lower concentration of NaOCl has implications for the survival of other bacteria besides *Salmonella*. While *Salmonella* is an important human pathogen associated with tomatoes, optimization of the overhead spray system would require investigating its efficacy against decay organisms. For example, mold spores have been shown to require 135 to 500 mg/L NaOCl for inactivation (Dychdala 2001).

The small amount of cross contamination that occurred in the overhead spray system is its potential disadvantage compared to flumes. Additional experiments should be conducted to further examine the extent of cross contamination in the overhead spray system. In this research, inoculated tomatoes were sprayed on rollers for 15 s followed by a 15 s spray on uninoculated tomatoes. Though 100 mg/L NaOCl significantly reduced cross contamination to uninoculated tomatoes compared to water, it did not prevent the transfer of $2.63 \pm 0.28 \log_{10} \text{CFU/ml} \ Salmonella$ onto tomatoes. This level of contamination could represent the worst extent of contamination per tomato. A future study could examine the extent of cross contamination to sets of subsequent tomatoes treated by overhead spray. As replicates of uninoculated tomato sets contact rollers and are removed, the level of contamination per tomato set is hypothesized to decrease. This study would measure how long cross contamination
would persist in the overhead spray system in a worst-case scenario of initial *Salmonella* contamination. Cross contamination of bacterial and fungal fruit pathogens are also of concern in terms of tomato quality. The same method used to examine cross contamination of *Salmonella* could be used to examine the fate of inoculated postharvest decay organisms like *E. carotovora* and *Geotrichum* spp. on tomatoes in the overhead spray system.

In terms of required space inside a packinghouse, a brush roller bed engineered to achieve a 15 s tomato contact time may still be more compact than a flume. Flumes have other uses besides sanitation, however, which would likely hinder the complete removal of flumes in packinghouses. The initial section of a flume is referred to as a dump tank because tomatoes from the field are first dumped into the water to cushion their fall and prevent injury. The flume acts as a conveyor through the processing line. An overhead spray system could potentially be implemented after the initial dump tank and still conserve water. The water used in the overhead spray could be routed to the dump tank and save even more water.

Scale-up inevitably presents a challenge in terms of engineering and transfer of laboratory findings to commercial operations. Validation studies could be performed in individual operations that implement a full-scale overhead spray system to ensure efficacy is reached. Because overhead spray systems can be constructed differently, it may be necessary to standardize systems to reproduce similar sanitizer efficacy results. Overhead spray systems may need to be engineered in a way to optimize average pathogen reduction on tomato surfaces. Parameters that may affect efficacy include spray flow rate, water pressure, height of spray nozzles, number of nozzles, material of
brush rollers and brush roller speed. A problem encountered in this research was the natural variation of tomatoes that caused them to spin in different directions. It was observed that if the inoculated spot on the tomato did not directly contact the brush rollers and/or spray, it sometimes achieved a lower $\log_{10}$ reduction. Outside a laboratory, tomatoes could become contaminated at a single spot or on their entire surface. Overhead spray systems may need to be engineered to maximize the surface area of tomatoes that directly contact brush rollers and spray by forcing tomatoes to continuously spin in multiple directions so that any potential contamination is exposed. Similar efficacy studies could be performed with dip-inoculated tomatoes.

In conclusion, collected data showed that the overhead spray system achieved a 3 to 5 $\log_{10}$ unit reduction of Salmonella from tomato surfaces under specific sanitizer and treatment times. An overhead spray system could provide benefits over conventional flumes including higher pathogen reduction, less sanitizer and less water, all of which help to decrease tomato packing costs and keep the tomato industry a viable part of Florida’s economy. Additionally, because an overhead spray system does not require immersion of tomatoes in water, there may be no need to heat water to at least 5°C greater than pulp temperature of tomatoes. Using ambient temperature water would save on heating and energy costs.

Improving sanitation as part of an effective food safety program can minimize risk of contamination and potential of causing a foodborne disease outbreak. This research is only one of a few studies that have examined efficacy of sanitizers in an overhead spray system and has the potential to directly influence current industry practices by supporting the implementation of overhead spray systems for tomatoes.
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BIOGRAPHICAL SKETCH

Alexandra S. Chang was born in Fresno, California and grew up in Rockville, Maryland. In May 2009, she earned Bachelor of Science in biology from the University of North Carolina at Chapel Hill. She joined Dr. Keith Schneider’s lab at the University of Florida in June 2009 as a lab assistant and then began the food science master’s program in January 2010. She graduated in August 2011 and continued her professional interests in food safety and microbiology.