EVALUATION OF OVERHEAD SPRAY AND BRUSH ROLLER TREATMENT OF TOMATOES AND ITS EFFECT ON SAFETY AND SHELF LIFE

By

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To Abue, Ethel, and Grampy

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Overhead spray and brush roller (OSBR) treatment removes significantly more *Salmonella* from tomato surfaces than flume treatment. However, little is known about whether brushing causes unseen physical damage to tomatoes. The aim of this research was to determine if OSBR treatment has a negative effect on the safety and/or shelf life of tomatoes.

Pectobacterium-inoculated tomatoes were OSBR-washed with 100 ppm NaOCl, 5 ppm ClO_2, 80 ppm PAA, and water. OSBR treatment achieved a 3 $\log_{10}$ CFU/mL reduction after a 15 s treatment for all sanitizers, including water. Survival of *Pectobacterium* and *Salmonella* on OSBR-treated and untreated tomatoes was also assessed. Neither *Pectobacterium* nor *Salmonella* survived better on OSBR treated vs. unwashed tomatoes, indicating that brushing does not damage tomato fruit to an extent that better pathogen survival results. Finally, the OSBR system was assessed for its effect on shelf life relative to unwashed and flume-washed tomatoes. OSBR treatment caused significantly more shrivel than flume or no treatment, however there were

minimal differences between OSBR, flume, or untreated tomatoes when a wax was applied post-wash.

These results show that the OSBR system is effective at removing *Pectobacterium* from tomato surfaces, does not damage fruit to the extent that *Pectobacterium* or *Salmonella* can survive at higher levels relative to unwashed tomatoes, and does not have a significant effect on tomato shelf life as long as a wax is applied to tomato surfaces post-wash.

CHAPTER 1
INTRODUCTION

Tomatoes are a commodity frequently implicated in produce-related foodborne disease outbreaks. These outbreaks place an unacceptable burden on society, and have cost the Florida tomato industry over $100 million dollars in the past five years alone (Brown 2008). As a result, members of the Florida tomato industry are constantly looking for ways to protect themselves and their consumers from foodborne disease. The industry standard for washing tomatoes is by immersion treatment in a flume of sanitizer. However, some packinghouses use an overhead spray and brush roller (OSBR) system in which tomatoes are scrubbed with rolling brushes while being sprayed with a sanitizer.

It has already been shown that the OSBR system is significantly more effective at removing *Salmonella* spp. from the surfaces of tomatoes as compared to immersion treatments (Pao and others 2009; Chang and Schneider 2012). Further, immersion treatments have previously been shown to leave tomatoes vulnerable to contamination with *Pectobacterium*, the causative agent of bacterial soft rot (Bartz and Showalter 1981). Soft rot is the most economically important bacterial cause of post-harvest losses of produce, causing an estimated $100 million in produce losses worldwide every year (Bhat and others 2010).

The purpose of this research was to answer basic questions about the OSBR system's impact on the shelf life or wholesomeness of tomatoes. By addressing gaps in the knowledge about the OSBR system, a more complete recommendation can be made to the tomato industry regarding whether installation of an OSBR system is worth the investment. If it is found that the OSBR system both reduces the number of

pathogens on tomato surfaces and does not otherwise decrease the shelf life or safety of tomatoes, its implementation at the packinghouse level could reduce the burden of foodborne illness as well as reduce the burden of post-harvest product losses on the tomato industry.

CHAPTER 2
LITERATURE REVIEW

Produce and Tomato-Related Foodborne Disease Outbreaks

In light of recent health trends, there has been a substantial increase in consumer demand for fresh produce. Contaminated produce leaves consumers vulnerable to disease. Annually, it is estimated that 9.4 million of the 36.4 million domestically acquired illnesses in the US are foodborne. Of the domestically acquired foodborne illnesses, 59% are viral, 39% are bacterial, and 2% are parasitic. The most common etiological agents of foodborne disease outbreaks include norovirus (58%), non-typhoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%) and *Campylobacter* (9%) (Scallan and others 2011).

The percentage of produce-associated foodborne disease outbreaks has increased from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam and others 2004). The pathogens of greatest concern in fresh produce include *Aeromonas hydrophila, E. coli, Salmonella* spp., *Shigella* spp., *Vibrio cholerae,* and *Listeria monocytogenes* (Brackett 1999). Of these, the pathogens most commonly implicated in produce-related foodborne disease outbreaks are *Shigella* spp. (lettuce, green onions (Brackett 1999)), *Salmonella* spp. (tomatoes, sprouts, watermelon, cantaloupe, unpasteurized orange juice (Brackett 1999)), *E. coli* O157:H7 (unpasteurized apple cider and juice (Mshar and others 1997), sprouts (CDC 2012b), lettuce and spinach (CDC 2012c)), enterotoxigenic *E. coli* (carrots, sprouts (Brackett 1999)), *V. cholera*e (coconut milk (Brackett 1999)), *L. monocytogenes* (cabbage (Conly and Johnston 2008), cantaloupe (CDC 2012a)), and *B. cereus* (sprouts and rice (Brackett 1999)).

In May of 2011, an outbreak of Shiga toxin producing *E. coli* O104:H4 associated with sprouted seeds sickened 3,816 people in Germany and resulted in 54 deaths (Frank and others 2011). This outbreak was immediately followed by a *L. monocytogenes* outbreak in cantaloupes in August 2011 that killed 32 people (FDA 2011). These are all prime examples of how dangerous fresh produce can be when food safety plans fail.

In the US, the consumption of fresh produce has been implicated in 82 outbreaks between the years 1996-2008, 17% of which were associated with tomatoes and caused 1,927 illnesses and three deaths (FDA 2009). Tomato-associated foodborne disease outbreaks are extremely costly to society, the government, and the tomato industry as a whole. In 2008, a *Salmonella* outbreak was falsely associated with raw red tomatoes from Florida and Mexico. Though the outbreak was eventually linked to jalapeño and Serrano peppers from Mexico (Palma and others 2010), the incident still cost the Florida tomato industry an estimated $100 million (Brown 2008).

There are many sources of contamination that could explain how pathogens are transmitted to fresh produce. If a field is exposed to run-off from a nearby pasture, is irrigated with contaminated water, or is fertilized with raw or inadequately composted manure, pathogens may be spread. Insects and soil have also been shown to transmit disease to plants. Once the produce has been harvested, any number of post-harvest processing practices including hydrocooling/washing with contaminated water, cutting or treating with contaminated equipment, or inappropriate storage conditions, could allow for pathogenic transmission onto produce (Lynch and others 2009; Berger and others

2010). Additional concerns include worker hygiene, animal and pest control, and the culling of dropped or obviously infected fruit (FDACS 2008).

Salmonellosis and Tomatoes

Salmonella are gram negative, facultatively anaerobic rods in the *Enterobacteriaceae* family. The genus is divided into two species: *bongori* and *enterica* (home to six subspecies and nearly 2,500 serovars) (Brenner and others 2000). In nutritive broth, *Salmonella* can grow at a pH and a_W minimum of 3.94 and 0.942 respectively, between 25-35 °C (Koutsoumanis and others 2004). *Salmonella* can survive on intact green tomato surfaces at detectable levels for at least 20-48 h, but will rapidly grow if the tomato surface is broken (Joy 2005).

Pathogenic *Salmonella* can be classified as either typhoidal (causing typhoid fever) or non-typhoidal (causing gastroenteritis). The incubation period for gastroenteritis caused by *Salmonella* is typically 6-72 h depending on level of contamination and the individual being infected. Symptoms include fever, diarrhea, and cramping. About 5% of individuals with gastroenteritis may develop bacteremia, a potentially fatal infection of *Salmonella* in the blood (Acheson and Hohmann 2001). Possible sequelae associated with salmonellosis include reactive arthritis (Arnedo-Pena and others 2010).

Non-typhoidal *Salmonella* has been implicated in dozens of multistate foodborne outbreaks in the US. These outbreaks have been traced back to a variety of food products, including chicken, ground beef, peanut butter, mangoes, cantaloupes, scrape tuna, Turkish pine nuts, ground turkey, papayas, alfalfa and spicy sprouts, and shell eggs in the past three years alone. Many of these products, however, have transmitted disease from other foodborne pathogens as well, such as *L. monocytogenes* and

various *E. coli* serotypes (CDC 2013). Tomatoes have been unique in that, with few exceptions, *Salmonella* has been the primary etiological agent of every tomato-related foodborne disease outbreak. From 1990-2009, there have been 11 confirmed *Salmonella* outbreaks in the US associated with raw tomatoes, seven of which were multistate. These outbreaks were caused by a variety of serovars including Javiana, Newport, Braenderup, Typhimurium, Montevideo, Anatum, Muenchen, Berta, and Thompson. Though no deaths were reported as a result of these outbreaks, over 1000 individuals were sickened (CDC 2011).

Fresh Tomato Treatment

Tomatoes are typically harvested at a green or breaker maturity either from open fields or greenhouses (Vigneault and others 2000). After harvesting, tomatoes are sent to packinghouses for washing and waxing (FDA 2009). In order to reduce mechanical injury to the fruit, packinghouses typically use water-filled receiving tanks and flumes in which tomatoes are floated from field bins to the packing line. Poor execution of this process leaves tomatoes vulnerable to contamination with plant and human pathogens, which can spread from tomato to tomato while in the flume. For this reason, dump tanks, flumes, and hydrocoolers usually use water that is treated with a sanitizer (most commonly calcium or sodium hypochlorite solutions) (Vigneault and others 2000). According to the Tomato Best Practices Manual (T-GAPs and T-BMPs) (an outline of practices intended to improve the safety of tomatoes produced, packed, repacked, distributed and sold in or from Florida), the following rules should be adhered to when washing tomatoes in a packinghouse. Tomatoes should be washed in flume water kept at a concentration of 150 ppm free chlorine, a pH of 6.5-7.5, and a temperature of 4-10 °C above pulp temperature, for a maximum of 2 min. Other sanitizers may be used in

place of free chlorine, including peroxyacetic acid (PAA), aqueous chlorine dioxide (ClO_2), gas-phase and aqueous ozone, or other chemicals, so long as they meet the appropriate Environmental Protection Agency (EPA), Occupation Safety and Health Administration (OSHA), and State of Florida standards and achieve at least a 3 log unit reduction of *Salmonella* or like organisms. Free chlorine may also be administered from an overhead spray, as long as the equipment is registered with the Florida Department of Agriculture and Consumer Services (FDACS) and concentration/contact times are followed closely and documented (FDACS 2008).

After washing, the tomatoes are sorted by weight, color, and quality, then packaged and distributed. Though each packinghouse may differ in terms of location, environment, product volume, type of tomatoes processed, and so on, consistent and appropriate sanitation and food safety practices can effectively reduce the risk posed by microbial hazards (FDA 2009).

Immersion Treatment vs. Overhead Spray and Brush Roller Treatment

Washing tomatoes in a flume of sanitized water (also known as immersion treatment) is a technique commonly used in the tomato industry, but it has several disadvantages. The accumulation of debris and contaminants in dump tanks leaves the fruit vulnerable to bacterial contamination (Pao and others 2009), and the system requires massive amounts of water and sanitizer. Some packing facilities use an overhead spray and brush roller (OSBR) system, which combines the overhead spraying of fruit with a sanitizer, with mechanical brushing by roller brushes. This system is thought to be more efficient at pathogen reduction than the flume system because it combines the physical removal of microbial contaminants with the chemical action of the sanitizer. Pao and others (2009) corroborated this thought by testing the

impact of both immersion and OSBR-type washing treatments, both with and without a ClO_2 sanitizer, on the removal of *Salmonella* contamination from tomatoes. Pao and others (2009) found that immersion treatment, using either water or 5 ppm ClO_2, did not significantly reduce air-dried *Salmonella* contamination from tomato surfaces. The OSBR system, however, removed 3.2 – 3.4 $\log_{10}$ CFU/mL of air-dried *Salmonella* with water alone, and removed 4.4 – 5.2 $\log_{10}$ CFU/mL when 5 ppm ClO_2 was incorporated (Pao and others 2009).

Chang and Schneider (2012) supported the findings of Pao and others (2009) confirming the efficacy of a lab-scale flume and lab-scale OSBR system head-to-head using 100 ppm NaOCl at 5, 13, 30, and 60 s treatment times. Chang and Schneider (2012) showed that at 15 s and 30 s treatment times, the OSBR system achieved a significantly greater $\log_{10}$ CFU/mL reduction of *Salmonella* on tomato surfaces than the flume (3.98 $\log_{10}$ CFU/mL vs. 1.25 $\log_{10}$ CFU/mL at 15 s, and 5.55 $\log_{10}$ CFU/mL vs. 3.17 $\log_{10}$ CFU/mL at 30 s).

A Possible Link Between Tomato Quality and Tomato Safety

Soft rot is a disease in produce caused primarily by *Pectobacterium carotovorum, Pectobacterium atrosepticum* and *Pectobacterium chrysanthemi*. Other species that cause bacterial soft rot include *Pseudomonas cichorii, P. marginalis, and P. viridiflava*. These plant pathogens cause destructive disease of the succulent, tender tissues of the storage organs of produce, including tubers, fruits, roots, bulbs, corns, and rhizomes. The symptoms of bacterial soft rot begin with water-soaked lesions at surface wounds on leaves, stems, and storage organs mentioned above, then the lesions gradually expand as the host tissues become watery and mushy. Slimy bacterial and cellular debris oozes out of lesions further dispersing the pathogen to adjacent and nearby fruit.

Decaying tissue gives off a putrid odor usually attributed to secondary bacterial infection of the lesions, and may appear opaque, white, cream-colored, gray, brown, or black. Soft rot bacteria persist in infected fleshy tissues of the plant, in the field or greenhouse, in the soil, on insects, and on contaminated tools. Bacteria typically enter the plant through wounds, but uninjured tissue may become infected at high humidity or if in contact with free water. After infection, the bacteria multiply in intercellular spaces and produce enzymes which dissolve the middle lamella, separating host cells, causing cell lysis and proceeding into a watery, mushy mass of cells (UIUC 1990).

A link between tomato quality and tomato safety has been suggested by several studies. Wells and Butterfield (1997) reported that, among 48 different types of healthy and soft rotted fruits and vegetables sampled from Northeastern US supermarkets, 33% of the healthy samples were presumptive positive for *Salmonella*, whereas 59% of soft-rotted samples were presumptive positive for *Salmonella*. Further, Wells and Butterfield (1997) noted that disks of potato, carrot, and pepper tissues inoculated with *S.* Typhimurium contained10-fold or 5-fold higher populations of this bacterium if co-inoculated with *Pectobacterium* or pectolytic *Pseudomonas*, respectively, versus controls by 16 h post-inoculation (Wells and Butterfield 1997). A similar study by Barak and others (2004) found that the presence of soft rot disease promotes *Salmonella* growth on plants. It was shown that an increase in *Salmonella* population size strongly correlates with an increase in the population size of *P. chrysanthemi* on cilantro leaves. Further, *Salmonella* Thompson's ability to colonize cilantro leaves increases when co-inoculated with *Enterobacter agglomerans* (formerly *Erwinia herbicola*) (Barak and others 2004).

The Effect of Packinghouse Treatment on the Quality of Fresh Tomatoes

There are at least two ways that *Pectobacterium* can internalize in tomatoes: through wounds in the fruit, and through stem end tissues or open blossom pores. Infiltrated fruit usually becomes rapidly symptomatic of soft rot, whereas with wounded fruit, lesion development may be delayed until after the fruit becomes fully red, leaving it vulnerable to being marketed and consumed without prior evidence of disease (Bartz and Showalter 1981). The latent period of the disease can last from 24 h to 3 weeks, meaning that infected but asymptomatic tomatoes may be shipped but must be discarded later. Bacterial soft rot causes increasing losses in fresh market tomatoes after rainy periods (Smith and others 2007), which leads to some question as to the relationship between water absorption and disease in tomato fruit.

Stem attachment regions on fruit can absorb volumes of water on the order of milliliters, which may seep into intercellular spaces at the center of the fruit (Bartz 1991). Fruit subjected to high-pressure streams of water, or submerged too deeply in water, are likely to absorb more water (Bartz and Showalter 1981). If the water in question is contaminated with a pathogen, fruit may internalize that pathogen, leading to eventual disease (Smith and others 2008). For these reasons, it is recommended that packinghouses keep treatment water at a higher temperature than the pulp temperature (Bartz and Showalter 1981), allow fruit to float in a single layer, and avoid direct contact of the fruit with water streams during unloading (Bartz 1999).

The absorption of water during packinghouse treatment may lead to postharvest disease in tomato fruit. However, the amount of absorption is also dependent on factors intrinsic to the fruits themselves. For example, larger fruit can absorb more water than smaller fruit due to the larger size of their stem scars as well as the greater amount of

connective tissue beneath the stem scars. Another variable is fruit ripeness; the Bartz (1991) study showed that pink tomatoes absorbed less water than green, even though the pink tomatoes were larger in size. The resistance of the stem scar to water uptake is also an important factor, but can vary greatly between cultivars (Bartz 1991). Additionally, environment, growing season, and genotype were found to significantly affect water absorption (Smith and others 2008). Theoretically, old stem scars are less likely to allow water uptake because they are more congested with air than younger stem scars. The Smith and others (2007) study corroborated this by showing that tomatoes absorbed less water the longer they were held after harvest, with a 2 h holding time allowing more water uptake than 8-26 h holding times. This suggests that tomatoes should be held for several hours before immersion into a dump tank (Smith and others 2007).

Vigneault (2000) looked at the post-harvest disease risk associated with hydrocooling tomatoes in chlorinated water inoculated with *P. carotovorum* and *R. stolonifer*, two of the most prominent tomato pathogens. Two hydrocooling methods were used: a shower or drench type and a flume or submersion type. Results showed that tomatoes treated with shower hydrocooling gained more water weight after processing, suggesting an increased potential for bacterial infiltration. However, the only tomatoes that developed soft rot disease were those hydrocooled in the inoculated suspension without any chlorine. With 50-200 ppm chlorine solutions, decay from *P. carotovorum* was completely prevented, however disease from *R. stolonifer* developed sporadically (Vigneault and others 2000). These results were in conflict with those of the Bartz (1988) study in which *P. carotovorum* invaded tomatoes even in the presence

of chlorinated water. However, in the latter report tomatoes were rapidly infiltrated with chlorinated water due to hydrostatic forces, whereas Vigneault and others (2000) reported infiltration was caused by the cooling of the wet fruit. Treatment with sodium chlorite, however, had no effect, showing a range of disease incidences from 45-58% with 100-1,000 ppm $NaClO_2$ treatment. Thus, treatment with NaOCl at levels more than six times the recommended concentration significantly reduced, but did not prevent, disease incidence if infiltration occurred rapidly. Additionally, adding NaOCl to the water increased water infiltration into the tomato, which was a critical factor in disease incidence (Bartz 1988).

Sanitizers

There is an extensive variety of sanitizers used to treat fresh produce and associated processing facilities. Some of the most commonly used include sodium hypochlorite (NaOCl), chlorine dioxide (ClO_2), and peroxyacetic acid (PAA), which have a very similar mode of action and toxicity, but differ in terms of efficacy and environmental fate (as shown below).

Sodium Hypochlorite

Sodium hypochlorite is marketed as an aqueous, clear, pale yellow solution formed by treating alkali with chlorine gas. It can be used on all food contact surfaces as well as in clean in place equipment. It is stable during storage/shipping for about 6 months as long as it is not exposed to high temperatures or too much light. Addition of NaOCl to water forms hypochlorous acid (HOCl) and sodium salts. Hypochlorous acid is the active sanitizing ingredient, and is also called “free chlorine.” The addition of NaOCl to water causes an increase in pH, meaning that acid must be added in order to stabilize the free chlorine that is formed. The rate at which sodium hypochlorite kills

bacteria is directly related to the amount of free chlorine, or HOCl, in the water (SNIC 2007). The mode of action of NaOCl can be divided into three steps. First, a saponification reaction takes place in which NaOCl acts as an organic solvent to break down fatty acids, which reduces surface tension. Next, sodium hydroxide neutralizes amino acids. Finally, HOCl acid forms chloramines when in contact with organic matter. Chloramines further interfere in cell metabolism by causing irreversible denaturation of bacterial enzymes (Estrela and others 2002). Concentrated NaOCl produces toxic gas when exposed to acid. It is extremely corrosive to the skin and eyes, and can cause chemical burns. Inhalation can cause respiratory irritation and fluid build-up in the lungs. Sodium hypochlorite is biodegradable, but deadly to aquatic organisms, so it is crucial to avoid contaminating waterways (Orica Chemicals 2010). Because NaOCl freely reacts with organic materials, the formation of chloramines and other chlorinated organic compounds are of concern. Chloramine and its byproducts are substantially more difficult to dissipate, surviving even boiling processes. These chemicals can be toxic at high levels, but are typically not present in high amounts in water systems (EPA 2011).

Chlorine Dioxide

Chlorine dioxide is a gas that must be dissolved into water before use as a sanitizer. It is mainly applied to food contact surfaces as well as directly onto food, especially in processes with very high organic loads. Chlorine dioxide is formed by reacting chlorine gas or hydrochloric acid with sodium chlorite ($NaClO_2$) (SNIC 2007). Unlike NaOCl, ClO_2 does not hydrolyze into HOCl. However, HOCl may be produced as a generation byproduct. Dissolved ClO_2 is very unstable, so it must be generated on-site (EPA 1999). Chlorine dioxide differs from NaOCl in several important ways. It is

uniformly active across a wide range of pH, and remains effective up to a pH of 10. It is also less likely to form chlorinated organic compounds, so it is more effective than HOCl in systems with a higher organic load. Chlorine dioxide is considered to be as effective, or more so, than HOCl (Benarde and others 1965).

At neutral pH, ClO_2 is reduced to the chlorite ion when it reacts. Its specific mode of action is not known, however it has been shown to react readily with some amino acids and to inactivate viral capsid proteins. It is thought that reactions with both peripheral structures and nucleic acids play a role in inactivation of bacterial cells. Chlorine dioxide has also been found to increase membrane permeability and inhibit protein synthesis, and is more effective at inactivating *Bacillus* spores than HOCl (EPA 1999). At elevated temperatures, dissolved ClO_2 will release chlorine and ClO_2 gases. Chlorine gas creates hydrochloric acid when reacted with water or steam. This can cause irritation upon contact with skin and eyes. Chlorine dioxide is harmful when inhaled, and can cause coughing, headaches, nausea, shortness of breath, and pulmonary edema. Chlorine dioxide is not a known mutagen or teratogen, however there is limited data on its biodegradability (Halox Technologies Inc. 2004). On exposure to air and sunlight, ClO_2 breaks down into oxygen and chlorine gases. Its byproducts include chlorite, chlorate, and some organic disinfection byproducts (EPA 1999).

Peroxyacetic Acid

Peroxyacetic (PAA) acid is a mixture of acetic acid with hydrogen peroxide. It is effective against all microorganisms, including bacterial spores, and shows reactivity over wide temperature and pH ranges. It is a colorless liquid with a pungent vinegar-like odor, and must be used with proper ventilation (SNIC 2007). It is stable unless

exposed to heat, ignition sources, or incompatible materials such as reducing agents, metals, acids, alkalis). In addition, PAA is highly corrosive to many metals, including stainless steel 304, aluminum, and copper (Organics 2009). PAA is excellent for use on food and food contact surfaces. In addition, it shows efficacy against biofilms. The mode of action of PAA is unknown, but thought to be similar to other oxidizing agents, meaning it likely denatures proteins and disrupts cell wall permeability. PAA can inactivate gram positive and negative bacteria, fungi, and yeasts in less than 5 min at 100 ppm or less, although more is required in the presence of organic material. Bacterial spores can be inactivated in 15 s to 30 min by 500-10,000 ppm of PAA (CDC 2008).

Peroxyacetic acid is a skin and eye irritant, is hazardous when ingested, and is a lung sensitizer. Over exposure can result in death, skin burns and ulcerations. In addition, some evidence has shown that PAA may be mutagenic and carcinogenic. In its use as a sanitizer, PAA is highly reactive and short lived due to the instability of the peroxide bond. Ultimately, PAA degrades into oxygen, water, and acetic acid. Hazardous short term degradation products are unlikely, but more problematic long term products may also arise (Acros Organics 2009). The carcinogenic and mutagenic effects of PAA are thought to be due to species formed during spontaneous decomposition or enzymatic conversion, but those products only impart an effect at high chronic doses (EPA 2000).

Determining Tomato Quality

In determining the OSBR system's effect on tomato quality and susceptibility to disease, it is first important to characterize the normal development of tomatoes throughout their storage life. The organoleptic properties of food encompass the quality

of that food's taste, texture, appearance, and aroma. As a fruit ripens, different metabolic pathways influence its levels of pigments, sugars, acids, and aromatic compounds. These changes within the fruit, which are mostly controlled in tomatoes by the plant hormone ethylene, function to make it more appealing while concurrently promoting tissue softening (Oms-Oliu and others 2011).

There are many ways that sanitizing treatments can be assessed for their effect on product shelf life. A study by Trinetta and others (2010) investigated the effect of ClO_2 gas treatment on the levels of natural microflora and shelf life of tomatoes. For all samples, the levels of microflora directly after treatment on the treated tomatoes were significantly lower than on the untreated tomatoes, however, microbial counts increased throughout storage for both the treated and untreated tomatoes. For up to 14 days, no significant difference in spoilage was observed between treated and untreated samples. On day 21, however, the untreated samples exhibited considerable mold growth, the samples treated with 8 ppm ClO_2 showed reduced mold growth, and the samples treated with 10 ppm ClO_2 showed no mold growth. At 10 ppm ClO_2 gas and a 180 s treatment time, however, the tomatoes became wrinkled in appearance, suggesting possible degradation of fruit components by the ClO_2 gas (Trinetta and others 2010). A similar test was performed by comparing the shelf life of tomatoes immersion treated in chlorine with tomatoes not treated with chlorine (Nasrin and others 2008). In the Nasrin and others (2008) study, the effect on shelf life was determined by visual observation for rotting, decay, or disease throughout storage after treatment. Storage at ambient temperature after treatment increased the chlorine-treated tomato shelf life by 5 days relative to the untreated tomato (Nasrin and others 2008).

CHAPTER 3
METHODS

Bacterial Cultures and Maintenance

These studies utilized a five-strain cocktail of the following *Salmonella* serovars: *S.* Typhimurium (ATCC 13311), *S.* Braenderup (ATCC BAA-664), *S.* Enteritidis (ATCC 4931), *S.* Newport (ATCC 6962), and *S.* Javiana (ATCC BAA-1593). One strain of *Pectobacterium carotovorum* (*Pc* SR38) was also used. All strains were made resistant to 200 ppm rifampicin (rif) (Fisher, Fair Lawn, NJ) so they could be distinguished from the background microflora of tomatoes.

All strains were kept for long-term storage as frozen 35% glycerol stocks at -80 °C. As needed, the strains were revived by streaking for isolation onto 80-200 ppm TSA+rif (Difco™, Sparks, MD) and incubated at 37 °C for *Salmonella*, and 30 °C for *Pectobacterium*.

For inoculum preparation, isolated colonies no more than 48 h old from each strain were transferred to 10 mL 200 ppm TSB+rif tubes and successively transferred three times within six days before use in experimentation. For studies in which *Salmonella* spot-inoculation was required, the final culture transfer was into 20 mL 200 ppm TSB+rif (Difco™, Sparks, MD) and incubated overnight at 37 °C. All five strains were then combined to make the cocktail, then centrifuged at 4,000 x g, followed by two washes with 10 mL BPW (Difco™, Sparks, MD). One final resuspension into 10 mL BPW attained a 1.0 x 10^{10} CFU/mL inoculum. For the *Pc* inoculum, the same washing and resuspending steps were followed, but the SR38 strain was not combined with any other strains.

Growth Curves

The purpose of this study was to assess how quickly, and to what $\log_{10}$ CFU/mL level, the *Pc and Salmonella* strains grew. This data helped to reveal any major growth differences between the *Salmonella* strains, and show when all of the strains reached stationary phase. For each of the following, rif-resistant *Pc*, *S.* Typhimurium, *S.* Braenderup, *S.* Enteritidis, *S.* Javiana, and *S.* Newport, three successive culture transfers into 10 mL 200 ppm TSB+rif tubes were performed within six days. *Pc* was grown at 30 °C and 140 rpm, and all others at 37 °C stationary. After the third transfer, the *Salmonella* strains were serially diluted three times using 9 mL BPW tubes. From the third dilution, 10 µL were transferred into a bottle with 200 ppm TSB+rif and stored in a static incubator at 37 °C. Every hour for 14 h starting with hour 0, 1 mL was taken from the bottle, serially diluted, and pour plated into 80 ppm TSA+rif. The plates were stored for two days before counting.

For the *Pc* strain, the third culture transfer was serially diluted three times into 9 mL BPW tubes. From the third dilution, 100 µL were transferred into a bottle with 100 mL of 200 ppm TSB+rif and stored in a shaking incubator at 140 rpm and 30 °C. Every hour for 18 h, starting with hour 0, 1 mL was taken from the bottle, serially diluted, and pour plated into 80 ppm TSA+rif. The plates were stored for two days before counting. For all growth curves, analysis of variance (ANOVA) and mean separation using Tukey's HSD with $p<0.05$ were performed using SAS 9.3 (SAS Institute Inc., Carey, NC) to determine differences between strain population by hour.

Pectobacterium Removal Studies

This study assessed the ability of the OSBR system to remove *Pc* from the surfaces of tomatoes. The purpose of this study was to show whether the OSBR

system is effective at removing pre-processing *Pc* contamination at the packinghouse level.

The *Pc* inoculum was prepared and then spot-inoculated with ten 10 µL drops (100 µL total) onto green tomatoes around the blossom end. An uninoculated negative control was also included for testing. After drying for 1 h in a biosafety hood, five tomatoes at a time were taken for OSBR treatment. A deionized water control, 100 ppm NaOCl, and 80 ppm PAA treatment were performed at 0, 15 and 60 s intervals.

After treatment, the tomatoes were placed into 100 mL BPW Stomacher® bags. For any tomato that had been exposed to NaOCl, ClO_2 or PAA, the BPW also included 0.1% sodium thiosulfate (Fisher, Fair Lawn, NJ), to inactivate any residual sanitizer. The bags were vigorously rubbed and shaken for 1 min, then 1 mL of liquid from each bag was serially diluted into 9 mL BPW, from which 1 mL was pour plated into 80 ppm TSA+rif. The plates were incubated at 30 °C for 2 days before counting.

ANOVAs were performed for sanitizer and for time, with a randomized block effect by rep. Mean separation was performed using Tukey's HSD ($p<0.05$). All analyses were performed using SAS 9.3 (SAS Institute Inc., Carey, NC).

Pectobacterium and *Salmonella* Survival on OSBR Treated Tomatoes

This study assessed whether *Pc or Salmonella* can survive at higher levels or for longer on OSBR treated tomatoes, as compared to untreated tomatoes. The goal of this study was to ascertain whether OSBR treatment leaves tomatoes more vulnerable to harboring post-processing contamination of human or plant pathogens.

Twenty green, unprocessed tomatoes were treated with the OSBR system for each combination of the following variables: water treatment or 100 ppm NaOCl treatment, and 15 or 60 s treatment times. NaOCl treated tomatoes were sprayed with

0.1% sodium thiosulfate immediately after treatment to halt sanitizer activity. After treatment, the tomatoes were placed onto aluminum rings on top of a fiberglass tray, blossom side up. Twenty untreated tomatoes were also included in the study as a treatment control. All tomatoes were placed under a hood to dry for at least 1 h. After drying, the tomatoes were either spot inoculated with ten 10 µL drops of *Salmonella* cocktail inoculum and dried for 2 h, or spot inoculated with ten 10 µL drops of *Pc* and dried for 1 h. Tomatoes were stored for a maximum of 7 days at 25 °C and 75-85 %RH. Starting with day 0, five tomatoes from each treatment were taken into five separate 100 mL BPW Stomacher® bags, vigorously rubbed and shaken for 1 min, then serially diluted out into 9 mL BPW tubes and plated onto 80 ppm TSA+rif. Five tomatoes for each treatment were also plated on days 1, 3, and 7, to assess the survival of the bacteria on the tomato surfaces at those time points. Analysis of variance (ANOVA) and mean separation using Tukey's HSD with $p<0.05$ were performed using SAS 9.3 (SAS Institute Inc., Carey, NC) to determine differences between growth for each treatment by day. The model included a randomized block effect for each rep, and the *Salmonella* cocktail and *Pc* studies were analyzed separately.

Wound Inoculation Studies

This study tested the survival of both the *Salmonella* cocktail and *Pc* cultures inoculated at high (9 $\log_{10}$ CFU/tomato) and low (2 $\log_{10}$ CFU/tomato) levels into wounded tomatoes. The purpose was to compare the survival rates in lightly and heavily contaminated wounded fruits against the survival rates found in OSBR-treated fruits, to show that the cultures did not behave the same in wounded fruit as in OSBR-treated fruit.

For the *Salmonella* cocktail and the *Pc* culture each, a 10 $\log_{10}$ CFU/mL inoculum was prepared as described before. That inoculum was then either used as-is for the high level inoculation study, or serially diluted seven times in 9 mL BPW to achieve a 3 log inoculum for the low level inoculation study. Twenty green, mature tomatoes were sanitized by wiping the surface with a 70% isopropyl alcohol-soaked Kimwipe™ (Kimberly-Clark Corp., Neenah, WI). The tomatoes were then placed stem scar down onto sanitized aluminum rings on sanitized fiberglass trays. A standard paper clip with a wire diameter of slightly less than 1 mm was un-coiled, flame sterilized, and used to puncture holes in the fruit at ten points radially around the blossom scar. Enough pressure was applied just to break through the skin of the tomato, then the wire was removed. One sanitized, punctured, but un-inoculated tomato was included per inoculum type as a negative control. Ten µL of the 10 or 3 $\log_{10}$ CFU/mL inoculum were pipetted directly onto each of the ten puncture wounds of each fruit, amounting to a 100 µL inoculum level per fruit.

After inoculation, the *Salmonella* cocktail-inoculated tomatoes were dried under a hood for 2 h, and the *Pc* inoculated tomatoes were dried under a hood for 1 h, to replicate the conditions of the OSBR-treated survival studies. Immediately following drying, the tomatoes were placed in 25 °C, 75-85 %RH storage for up to 7 days. Five tomatoes were sampled on days 0, 1, 3, and 7 for each of the inoculum types. The negative control tomato was also sampled on the 7th day of storage.

To sample, each tomato was taken with sanitized tongs into sterile 100 mL BPW Stomacher® bags, then sealed. Each bag was rubbed and shaken vigorously for 1 min, then a 1 mL aliquot was taken from each bag, serially diluted into 9 mL BPW tubes and

pour plated into 80 ppm TSA+rif. *Salmonella* plates were stored at 37 °C, and the *Pc* plates at 30 °C, for 2 days before counting.

The results of the wound-inoculated tomatoes treated with 9 $\log_{10}$ CFU/mL of *Pc and Salmonella* were compared to the 0 s control treatments of the *Pc and Salmonella* survival studies using a three-way ANOVA with factors bacteria, treatment, and time. A randomized block effect was included for rep with autoregressive correlation AR(1). Mean separation was performed using Tukey's HSD with $p<0.05$. Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Carey, NC).

Shelf Life Studies

Flume vs. OSBR Treated Tomatoes (30 vs. 60 s Treatment Time)

The purpose of this study was to test whether treatment with the OSBR system increases or decreases the shelf life of tomatoes compared to no treatment at all. Uninoculated, green mature tomatoes were treated with either the OSBR or flume system. For the flume system, tomatoes were treated in 10 gal of deionized water at 100 ppm NaOCl, pH 6.5, and 25 °C, each for a typical usage time (30 s) and an abuse time (60 s). Tomatoes were treated in batches of 5 with their blossom scar facing down. For the OSBR system, at 100 ppm NaOCl, pH 6.5, and 20 °C, 25 tomatoes were each treated for typical a usage time (30 s), and an abuse time (60 s).

The treated tomatoes, along with the treatment negative controls (0 s treatment), were placed onto sanitized aluminum rings on sanitized fiberglass trays. Tomatoes were stored with their stem scar facing up for 21 days at (22 °C, and 40-50 %RH, ambient retail conditions for the Fall). Tomatoes were observed every odd day for the development of soft rot, shrivel, softness, ripeness, or other spoilage. Spoiled tomatoes were immediately removed to prevent the spread of disease.

The outputs for ripeness level 6 (USDA 1997), shrivel, and softness were analyzed statistically. Data was treated with a binary regression model with first order autoregressive correlation, AR(1), to account for repeated measures. The model included a randomized block effect by replication. Mean separation was performed by day using Tukey's HSD ($p<0.05$). All analyses were performed using SAS 9.3 (SAS Institute Inc., Carey, NC).

Flume vs. OSBR Treated Tomatoes (Waxed vs. Unwaxed)

The purpose of this study was to test whether treatment with the OSBR system increases or decreases the shelf life of tomatoes compared to flume treatment or no treatment at all. Uninoculated, green mature tomatoes were treated with either the OSBR or flume system. For the flume system, tomatoes were treated in 10 gal of deionized water at 100 ppm NaOCl, pH 6.5, and 25 °C, each for 60 s. Tomatoes were treated in batches of five with their blossom scar facing down. For the OSBR system, at 100 ppm NaOCl, pH 6.5, and 20 °C, 25 tomatoes were each treated for 60 s.

The treated tomatoes along with the no treatment negative controls were placed onto sanitized aluminum rings on sanitized fiberglass trays. Half of the tomatoes for each treatment were waxed by buffing on Tri-Wax coating (Tri-Pak Machinery, Inc. Harlingen, TX) with a paper towel according to the manufacturer's instructions, while the other half were left unwaxed. Tomatoes were stored with their stem scar facing up for 27 days at typical retail conditions (22 °C, and 40-50 %RH, ambient for the Spring season). Tomatoes were observed every odd day for the development of soft rot, shrivel, softness, ripeness, or other spoilage. Spoiled tomatoes were immediately removed to prevent the spread of disease.

The outputs for ripeness level 6, shrivel, and softness were analyzed statistically. Data was treated with a binary regression model with first order autoregressive correlation, AR(1), to account for repeated measures. The model included a randomized block effect by rep. Mean separation was performed by day using Tukey's HSD ($p<0.05$). All analyses were performed using SAS 9.3 (SAS Institute Inc., Carey, NC).

OSBR Treated Tomato Shelf Life (Fall vs. Spring, Waxed vs. Unwaxed)

The OSBR 60 s data from the 30 s vs. 60 s study was compared to the waxed and unwaxed OSBR data from the waxed vs. unwaxed study. The outputs for ripeness level 6, shrivel, and softness were analyzed statistically. Data was treated with a binary regression model with first order autoregressive correlation, AR(1), to account for repeated measures. The model included a randomized block effect by rep. Mean separation was performed by day using Tukey's HSD ($p<0.05$). All analyses were performed using SAS 9.3 (SAS Institute Inc., Carey, NC).

Overhead Spray and Brush Roller System (OSBR)

The OSBR system used for all OSBR studies was a custom lab-scale system housed in a biological safety fume hood. The system consisted of two nylon brush rollers measuring 46 cm long x 12 cm in diameter with a concurrent rotation of 180 rpm. Tomatoes were placed in the groove between the two rollers for brush treatment. Three spray nozzles placed 14 cm apart (Spraying Systems, Co., Wheaton, IL) were located 13 cm above the brush rollers and delivered a cone-shaped spray at 12 psi and 21.4 mL/s. The brush system was flushed with 10 L of deionized water and the rollers were doused with 70% isopropanol following each experiment. Rollers were swabbed along their length using a sterile swab dipped in sterile BPW, then streaked onto 80 ppm

TSA+rif and checked for growth before and after experiments to ensure rollers were properly sanitized.

Flume System

The lab-scale flume used for all flume studies was a Precision circulating water bath (Jouan, Inc., Winchester, VA) of dimensions 38.7 cm x 30.5 cm x 19 cm. The water bath was sanitized with sodium hypochlorite and flushed three times with deionized water following each run.

Sanitizer Preparation

Sodium Hypochlorite

The NaOCl solutions were prepared by diluting 5.65-6.0% NaOCl (Fisher, Fair Lawn, NJ), into deionized water, then adjusting the pH to approximately 6.5 with 1N HCl. For the flume studies, the flume water was adjusted to 100 ppm free chlorine. For the OSBR studies, concentration was adjusted to 100 ppm free chlorine measured from the spray nozzles. Free chlorine concentration was confirmed using a Hach DR/890 colorimeter with Accuvac® DPD free chlorine ampoules (Hach Co., Loveland, CO).

Chlorine Dioxide

The ClO_2 was generated using Selectrocide™ 2L500 (Selective Micro Technologies, Canal Winchester, OH). The pH of the sanitizing solution was measured but not adjusted. The Selectrocide™ concentrate was diluted to 5 ppm as measured from the spray nozzles. ClO_2 concentration was confirmed using a Hach DR/890 colorimeter with Accuvac® DPD free chlorine ampoules (Hach Co., Loveland, CO).

Peroxyacetic Acid

The PAA was made using Tsunami® 100 concentrate (Ecolab Inc., St. Paul, MN) combined with DI water to an approximate 80 ppm concentration. The concentration

was verified using the LaMotte Hydrogen Peroxide and Peracetic Acid titration kit (LaMotte Company, Chestertown, MD).

Water Control

All water controls were derived from deionized water (University of Florida, AFPL building). The pH of water controls was not adjusted.

CHAPTER 4
RESULTS

Growth Curves

Salmonella

Five serovars of *Salmonella* (Typhimurium, Braenderup, Enteritidis, Newport, and Javiana) were grown at 37 °C in 200 ppm TSB+rif for 14 h with hourly sampling. The average starting concentration of the strains was approximately 1.8 $\log_{10}$ CFU/mL, and the average ending concentration at 14 h was approximately 9.2 $\log_{10}$ CFU/mL. All serovars grew to statistically similar levels between hours 0-11; differed at hours 12-13, then reached statistically similar levels again at hour 14 (Table 4-1). Throughout the growth curve, *S*. Javiana tended to maintain a higher population level than the other serovars whereas *S*. Typhimurium tended to maintain a lower population than the others, though these differences were only significant at hours 12-13.

All five serovars exhibited typical bacteria growth. For all serovars, the lag phase lasted until hour 2, followed by log growth phase between hours 2-12. Growth of all serovars reached a plateau around hour 12 and continued to level off until hour 14, indicating entry into stationary phase.

Pectobacterium

One strain of *Pectobacterium carotovorum (Pc)* SR38 was grown at 30 °C in 200 ppm TSB+rif in a shaking incubator at 140 rpm. The initial starting concentration of *Pc* was purposefully set higher (about 3.5 $\log_{10}$ CFU/mL on average) than that of *Salmonella* due to variability of *Pc* at lower inoculation levels. A shaking incubator was utilized to shorten the time of the stationary phase and also to promote growth consistency. Three repetitions showed that *Pc* grew to approximately 9 $\log_{10}$ CFU/mL in

18 h (Table 4-2). The growth of *Pc* was generally slower than that of *Salmonella*, with a shorter lag phase (0-1 h) and longer log phase (1-15 h). *Pc* growth began to plateau after hour 15 and continued to level off until hour 18, indicating entry into stationary phase.

Pectobacterium Removal Studies

The OSBR system was assessed for its ability to remove dried-on *Pc* from green tomato surfaces at different treatment times with different sanitizers. The treatment times used were 5, 15, and 30 s, and the sanitizers tested were 100 ppm NaOCl, 5 ppm ClO_2, 80 ppm PAA, and a water control. The reduction of *Pc* on tomato surfaces increased significantly with each increase in time for ClO_2, PAA, and water. For ClO_2, PAA, and water, removal at 30 s was significantly higher than 15 s, and removal at 15 s was significantly higher than 5 s. In the case of NaOCl, there was a significant increase in removal between times 5 and 15 s, but not between 15 and 30 s (Table 4-3). The type of sanitizer used only significantly affected *Pc* reduction at 30 s. At 5 and 15 s, there was no significant difference in removal between sanitizers. At 30 s, PAA showed significantly higher removal than water or NaOCl, and water showed significantly lower removal than ClO_2 or PAA. The removal achieved by NaOCl and ClO_2 were statistically the same (Table 4-3).

All sanitizers achieved an average *Pc* reduction of over 3 $\log_{10}$ CFU/mL by 15 s. Peroxyacetic acid was unique in that it was the only sanitizer to achieve complete reduction of *Pc* to below the detection limit (1 CFU/mL) in all samples at 30 s, corresponding to at least a 5.71 $\log_{10}$ CFU/mL reduction. By contrast, the maximum reduction achieved by ClO_2, NaOCl and water was 5.19 ± 0.63, 5.09 ± 1.65, 4.44 ± 1.61 $\log_{10}$ CFU/mL, respectively (Table 4-3).

Pectobacterium and *Salmonella* Survival on OSBR Treated Tomatoes

The survival of *Pc* and *Salmonella* on tomatoes over a 7-day period on OSBR-treated tomatoes was compared for 100 ppm NaOCl versus water treatments at 0, 15, and 60 s treatment times. The purpose of this study was to investigate whether OSBR treatment could damage tomato surfaces to the extent that *Pc* or *Salmonella* could persist or grow to a greater extent than on untreated tomatoes.

Pectobacterium

The *Pc* populations began at an average level of 5.16 $\log_{10}$ CFU/mL across all treatments, dropped to an average level of 2.50 $\log_{10}$ CFU/mL at day 1, then continued to drop to below the statistical limit of detection of 1.40 $\log_{10}$ CFU/mL across all treatments at day 3 and 7. There were no significant differences found between any of the treatments at any time point (Table 4-4).

Salmonella

The *Salmonella* populations began at an average level of 5.56 $\log_{10}$ CFU/mL across all treatments on day 0, then dropped below the statistical limit of detection at day 1 and remained there for days 3 and 7. Some statistical differences were found between treatments at day 1 (primarily that the NaOCl-treated tomatoes had significantly lower recovery than the other treatments), however that result fell below the statistical limit of detection (Table 4-5).

Wound Inoculated Studies

The survival of *Pc and Salmonella* on both wounded and intact tomatoes at a 9 $\log_{10}$ CFU/tomato inoculation level were compared over a period of 7 days. There were significant differences found between treatments for each day. On day 0, the recovery of *Pc* and *Salmonella* from wounded tomatoes was statistically the same at 6.03 ± 0.53

and 6.14 ± 0.22 $\log_{10}$ CFU/mL, respectively. Significantly less *Salmonella* was recovered from intact samples on day 0 at 5.52 ± 0.22 $\log_{10}$ CFU/mL. The *Pc* recovery from intact samples was significantly lower than all other samples (including *Pc*-inoculated wounded and *Salmonella*-inoculated intact and wounded) at day 0 at 5.12 ± 0.26 $\log_{10}$ CFU/mL (Table 4-6).

For days 1 and 3, *Pc* recovery from wounded tomatoes was significantly higher than any other wounded or intact treatment, which corresponded to the development of soft rot disease in the samples. Due to the rapid progression of soft rot, the day 7 *Pc*-inoculated wounded tomatoes were not sampled. By contrast, the *Pc* recovery from intact samples continued to decline over the 7-day period, dropping to 2.09 ± 0.89 $\log_{10}$ CFU/mL on day 1 and continuing to fall below the statistical detection limit of 1.40 $\log_{10}$ CFU/mL on days 3 and 7 (Table 4-6).

On days 1, 3, and 7, *Salmonella* recovery from wounded tomatoes was significantly lower than *Pc* recovery from wounded tomatoes, but significantly higher than the recovery from intact tomatoes. On wounded surfaces, *Salmonella* recovery remained stable at approximately 6 $\log_{10}$ CFU/mL for the entire 7 days. The *Salmonella* recovery from intact tomatoes dropped below the statistical detection limit of 1.40 $\log_{10}$ CFU/mL at day 1 and remained there for days 3 and 7 (Table 4-6).

Wounded recovery studies were also conducted at a 2 $\log_{10}$ CFU/tomato inoculation level for both *Pc* and *Salmonella*. When inoculated at 2 $\log_{10}$ CFU/tomato levels, both *Pc* and *Salmonella* grew to approximately the same level each day. At day 0, both *Pc* and *Salmonella* were below the detection limit of 1 CFU/mL. At day 1, *Pc* and *Salmonella* grew to 3.38 ± 0.57 and 3.73 ± 0.42 $\log_{10}$ CFU/mL respectively, raised

slightly to 3.86 ± 0.38 and 4.18 ± 0.37 $\log_{10}$ CFU/mL respectively at day 3, then ended at 3.86 ± 0.35 and 3.74 ± 0.53 $\log_{10}$ CFU/mL respectively on day 7. When wounded tomatoes were inoculated with *Salmonella* at 9 $\log_{10}$ CFU/tomato, the initial recovery was approximately 6 $\log_{10}$ CFU/mL and remained near that level for the 7-day period. When inoculated with *Salmonella* at 2 $\log_{10}$ CFU/tomato, rapid growth was seen between days 0 and 1 that then leveled off between days 1 and 7, never reaching a level higher than ~4 $\log_{10}$ CFU/mL (Table 4-7).

Shelf Life Studies

Flume vs. OSBR Treated Tomatoes (30 vs. 60 s Treatment Time)

The shelf life of tomatoes treated with 100 ppm NaOCl in either a model flume or OSBR system was assessed at 0, 30, and 60 s treatment times. Shelf life was studied in terms of ripeness (when tomatoes reached a ripeness level of 6), incidence of shrivel, and incidence of softness. Tomatoes were examined every 2 days for each of those three attributes as well as development of disease. Only one tomato (flume 60 s, day 15) developed soft rot during the entire period of observation.

The treatments had minimal significant effects on tomato ripening. For all days with the exception of day 9, there was no significant difference in development of ripeness level 6. On day 9, the OSBR 60 s treatment had significantly more ripe fruit on average than the flume 30 s treatment. None of the other treatments were significantly different from any other treatment on day 9. All treatments achieved 93.3-100% level 6 ripeness by day 21 (Table 4-8).

Treatment type had a significant effect on development of shrivel. The OSBR 60 s treatment showed significantly more shrivel than the flume 30 s treatment between days 13 and 21. For that same time period, OSBR 30 s never significantly differed from

flume 60 s, and flume 60 s never differed significantly from the 0 s control. During days 19 and 21, both OSBR treatments showed significantly more shrivel than the 0 s or flume 30 s treatments, however there was no significant difference between the OSBR 30 s and flume 60 s treatments. The OSBR treatment samples showed 56.7-63.3% shrivel on average, while the flume and control samples showed 23.3-33.3% shrivel on average by day 21 (Table 4-9).

Softness did not develop in the samples from any treatment until day 17. By day 21, there was a 26.7-36.7% incidence of softness across all treatments. There were no significant differences between any of the treatments in terms of softness at any time during the observational period (Table 4-10).

Flume vs. OSBR Treated Tomatoes (Waxed vs. Unwaxed)

Tomato shelf life when treated with 100 ppm NaOCl utilizing either a model flume or OSBR system for 60 s followed by a wax or no-wax treatment was assessed over a 27-day observation period. Shelf life was studied in terms of ripeness (when tomatoes reached a ripeness level of 6), incidence of shrivel, and incidence of softness. Tomatoes were examined every 2 days for each of those three attributes as well as development of disease. Only one tomato developed disease, a 0 s control waxed sample at day 25 of storage.

All treatments had begun to reach ripeness level 6 by day 9. By day 15, the ripeness of all of the unwaxed treatments significantly exceeded the ripeness of the waxed, with the exception of the waxed 0 s control. For all of the observations past day 15, the unwaxed treatments tended to have higher levels of ripeness than the waxed, though these differences were not always significant. By day 27, none of the treatments were significantly different from each other, however the average incidence of ripeness

level 6 in unwaxed samples (96.7-100%) exceeded that of the waxed samples (73.3-83.3%). Within the unwaxed and waxed subsets, none of the treatments (0 s control, flume, or OSBR) significantly differed from each other in terms of ripeness for any of the observations (Table 4-11).

The unwaxed treatments had all begun to exhibit shrivel by day 9, whereas the waxed 0 s control did not begin until day 11, the waxed flume samples did not begin until day 17, and the waxed OSBR samples did not begin until day 21. From days 11-27, the unwaxed subset developed significantly more shrivel than the waxed for every observation. Within the waxed subset, there was never a significant difference between treatments in terms of shrivel. In the unwaxed subset, the OSBR treated samples tended to have a higher incidence of shrivel then the other treatments between days 11-21, though this difference was not always significant. There was no significant difference between treatments within the unwaxed subset between days 23-27. By day 27, the unwaxed subset showed a shrivel incidence of 93.3-100%, whereas the waxed subset showed an incidence of only 3.3-16.7% (Table 4-12).

The unwaxed subset began developing softness by days 11-13, whereas the waxed subset did not develop softness until days 21-23. Within the unwaxed subset, the OSBR treated samples tended to have a higher incidence of softness than the 0 s control or flume treatments, though this difference was not always significant. The 0 s control and flume treated samples in the unwaxed subset never differed significantly in terms of softness for any observation. Within the waxed subset, both the OSBR and 0 s control samples showed higher levels of softness than the flume treated samples between days 23-27, but not always to a significant extent. By day 27, the unwaxed

subset had a softness incidence of 86.7-96.7% on average, whereas the waxed subset had 10.0-26.7% (Table 4-13).

OSBR Treated Tomato Shelf Life (Fall vs. Spring, Waxed vs. Unwaxed)

Tomatoes treated with 100 ppm NaOCl using the OSBR system were compared over fall (22 °C, 50-60 %RH) and spring (22 °C, 40-50 %RH) storage. These comparisons included two unwaxed subsets (one under fall conditions, the other under spring) and one waxed subset (under spring conditions). Shelf life was studied in terms of ripeness (when tomatoes reached a ripeness level of 6), incidence of shrivel, and incidence of softness. Tomatoes were examined every other day for each of those three attributes as well as development of disease.

The unwaxed fall samples began reaching ripeness level 6 first at day 7, followed by the unwaxed spring and waxed spring at day 9. From day 11 onward, both of the unwaxed treatments developed significantly more ripeness than the waxed treatment at every observation point. There was no significant difference between the two unwaxed treatments at any of the observation points. The unwaxed fall samples showed 100% ripeness by the last observation day (day 21) for that treatment, while the unwaxed spring samples showed 96.7% ripeness by the last observation day (day 27) for that treatment. The waxed spring sample only developed 76.7% ripeness by day 27 (Table 4-14).

The unwaxed spring sample began exhibiting shrivel first at day 9, followed by unwaxed fall at day 13, and finally waxed spring at day 21. From day 13 onward, there was a significant difference in shrivel development between all treatments for every observation. The unwaxed spring samples exhibited a higher incidence of shrivel than the unwaxed fall, and both unwaxed treatments exhibited a higher incidence of shrivel

than the waxed spring samples from day 15 onward. The incidence of shrivel of the unwaxed fall samples had only reached 63.3% by the last day of observation for that treatment (day 21). By contrast, the unwaxed spring samples reached that same incidence of shrivel between days 13-15, then continued on to develop 100% shrivel incidence by day 21 and onward. The waxed spring samples only developed a 3.3% incidence of shrivel by day 21, with no change between days 21-27 (Table 4-15).

The unwaxed spring samples began developing softness first at day 11, followed by unwaxed fall at day 17, and waxed spring at day 21. From day 13 onward, the unwaxed spring samples exhibited a significantly higher incidence of softness than the other two treatments, neither of which differed significantly from each other. The unwaxed fall samples developed a maximum incidence of softness of 26.7% by day 21, a level that was reached by the unwaxed spring samples by day 15. The unwaxed spring samples developed a maximum incidence of softness of 96.7% by day 25, whereas the maximum incidence of softness in the waxed spring samples was 23.3% by day 27 (Table 4-16).

Table 4-1. Average $\log_{10}$ CFU/mL concentration of *Salmonella* strains in 200 ppm TSB+rif over 14 h at 37 °C

	Salmonella Concentration ($\log_{10}$ CFU/mL)[a]				
Hour	Typhimurium	Braenderup	Enteritidis	Newport	Javiana
0	1.54 ± 0.62 a	1.99 ± 0.67 a	1.68 ± 0.53 a	1.81 ± 0.73 a	2.07 ± 0.48 a
1	1.89 ± 0.41 a	1.96 ± 0.49 a	1.78 ± 0.53 a	1.79 ± 0.43 a	1.86 ± 0.60 a
2	2.10 ± 0.57 a	1.67 ± 1.45 a	2.37 ± 0.36 a	2.15 ± 0.49 a	2.12 ± 0.77 a
3	2.61 ± 0.58 a	2.49 ± 0.69 a	2.97 ± 0.52 a	2.65 ± 0.46 a	2.92 ± 0.77 a
4	3.22 ± 0.51 a	3.16 ± 0.79 a	3.66 ± 0.56 a	3.50 ± 0.57 a	3.72 ± 0.80 a
5	3.83 ± 0.48 a	3.98 ± 0.67 a	4.42 ± 0.51 a	4.06 ± 0.41 a	4.46 ± 0.74 a
6	4.55 ± 0.31 a	4.56 ± 0.77 a	4.98 ± 0.58 a	4.83 ± 0.49 a	5.29 ± 0.64 a
7	5.05 ± 0.41 a	5.38 ± 0.66 a	5.74 ± 0.53 a	5.68 ± 0.42 a	6.03 ± 0.81 a
8	5.75 ± 0.44 a	6.28 ± 0.78 a	6.16 ± 1.07 a	6.34 ± 0.54 a	6.88 ± 0.88 a
9	6.39 ± 0.25 a	6.90 ± 0.56 a	7.18 ± 0.40 a	7.13 ± 0.39 a	7.66 ± 0.74 a
10	7.13 ± 0.45 a	7.66 ± 0.86 a	7.80 ± 0.49 a	7.81 ± 0.63 a	8.32 ± 0.92 a
11	7.72 ± 0.34 a	8.21 ± 0.59 a	8.39 ± 0.37 a	8.42 ± 0.33 a	8.83 ± 0.58 a
12	8.32 ± 0.37 b	8.83 ± 0.52 ab	8.84 ± 0.11 ab	9.06 ± 0.28ab	9.36 ± 0.33 a
13	8.67 ± 0.21 b	9.11 ± 0.33 ab	9.02 ± 0.21 ab	9.12 ± 0.02ab	9.31 ± 0.02 a
14	8.91 ± 0.31 a	9.44 ± 0.24 a	8.95 ± 0.15 a	9.15 ± 0.04 a	9.36 ± 0.12 a

[a]Values are mean ± standard deviation of triplicate experiments (n=3).

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

Table 4-2. Average $\log_{10}$ CFU/mL concentration[a] of *Pectobacterium* SR38 strains in 200 ppm TSB+rif over 18 h at 30 °C and 140 rpm

Hour	*Pc* SR38 Concentration ($\log_{10}$ CFU/mL)
0	3.56 ± 0.08
1	3.68 ± 0.13
2	3.93 ± 0.13
3	4.20 ± 0.12
4	4.69 ± 0.07
5	5.00 ± 0.12
6	5.31 ± 0.27
7	5.89 ± 0.10
8	6.12 ± 0.15
9	6.43 ± 0.27
10	6.90 ± 0.16
11	7.16 ± 0.24
12	7.41 ± 0.22
13	7.88 ± 0.23
14	8.17 ± 0.16
15	8.47 ± 0.30
16	8.71 ± 0.21
17	8.87 ± 0.13
18	8.95 ± 0.01

[a]Values are mean ± standard deviation of triplicate experiments (n=3).

Table 4-3. Average log_{10} CFU/mL reduction of *Pectobacterium* SR38[a] after OSBR treatment of sanitizers and water control

	Pectobacterium SR38 log reduction (log_{10} CFU/ml) from tomatoes[a]			
Treatment (s)	NaOCl 100 mg/L	ClO_2 5 mg/L	PAA 80 mg/L	Water control
5	1.37 ± 1.24 a, x	1.56 ± 1.00 a, x	1.77 ± 1.43 a, x	1.33 ± 0.79 a, x
15	3.84 ± 1.92 a, y	3.78 ± 1.36 a, y	3.71 ± 1.94 a, y	3.19 ± 1.47 a, y
30	5.09 ± 1.65 ab, y	5.19 ± 0.63 bc, z	5.71 ± 0.00 c, z	4.44 ± 1.61 a, z

[a]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 ($p<0.05$).

Table 4-4. Survival of *Pectobacterium*[a] on OSBR treated tomatoes at 25 °C, 75-85 %RH

	Control	Water		100 ppm NaOCl	
Day	0 s	15 s	60 s	15 s	60 s
0	5.12 ± 0.26 a	5.24 ± 0.38 a	4.96 ± 1.23 a	5.32 ± 0.55 a	5.16 ± 0.59 a
1	2.09 ± 0.89 a	2.70 ± 1.30 a	2.62 ± 1.07 a	2.37 ± 0.95 a	2.70 ± 0.91 a
3	0.78 ± 0.88 a	0.70 ± 0.67 a	1.17 ± 0.87 a	1.18 ± 0.84 a	1.12 ± 1.02 a
7	0.66 ± 1.04 a	0.72 ± 0.79 a	1.08 ± 1.25 a	0.51 ± 0.82 a	0.93 ± 1.28 a

[a]Values are mean ± standard deviation of quadruplicate experiments of 3-5 tomatoes each (n=15-20).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-5. Survival of *Salmonella*[a] on OSBR treated tomatoes at 25 °C, 75-85 %RH

	Control	Water		100 ppm NaOCl	
Day	0 s	15 s	60 s	15 s	60 s
0	5.52 ± 0.16 b	5.58 ± 0.30 b	5.80 ± 0.39 a	5.43 ± 0.20 b	5.45 ± 0.20 b
1	1.06 ± 0.74 a	0.65 ± 0.62 abc	0.83 ± 0.70 ab	0.35 ± 0.43 bc	0.32 ± 0.49 c
3	0.51 ± 0.64 a	0.73 ± 0.97 a	0.61 ± 0.77 a	0.84 ± 0.84 a	0.86 ± 1.22 a
7	0.72 ± 0.99 a	0.81 ± 1.00 a	1.05 ± 1.15 a	0.61 ± 0.81 a	0.68 ± 0.81 a

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

Table 4-6. Average survival[a] of *Salmonella* and *Pectobacterium* SR38 on wounded and intact green tomato surfaces at 25 °C, 75-85 %RH

	Salmonella		*Pectobacterium*	
Day	Wounded	Intact	Wounded	Intact
0	6.14 ± 0.22 a	5.52 ± 0.22 b	6.03 ± 0.53 a	5.12 ± 0.26 c
1	6.37 ± 0.21 b	1.06 ± 0.21 d	7.90 ± 0.27 a	2.09 ± 0.89 c
3	6.32 ± 0.39 b	0.51 ± 0.39 c	9.43 ± 0.22 a	0.78 ± 0.88 c
7	5.70 ± 0.33 a	0.72 ± 0.33 b	n/a	0.66 ± 1.04 b

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

Table 4-7. Average survival[a] of *Salmonella* and *Pectobacterium* SR38 on wounded green tomato surfaces with 2 and 9 log10 CFU/tomato inoculation levels at 25 °C, 75-85 %RH

	Salmonella		*Pectobacterium*	
Day	9 $\log_{10}$ CFU/tomato	2 $\log_{10}$ CFU/tomato	9 $\log_{10}$ CFU/tomato	2 $\log_{10}$ CFU/tomato
0	6.14 ± 0.22	0.00 ± 0.00	6.03 ± 0.53	0.00 ± 0.00
1	6.37 ± 0.21	3.73 ± 0.42	7.90 ± 0.27	3.37 ± 0.57
3	6.32 ± 0.39	4.18 ± 0.37	9.43 ± 0.22	3.85 ± 0.38
7	5.70 ± 0.33	3.74 ± 0.53	n/a	3.86 ± 0.35

[a]Values are mean $\log_{10}$ CFU/mL ± standard deviation of triplicate experiments of 5 tomatoes each (n=15).

Table 4-8. Incidence (%) of full ripeness[a] in OSBR and flume treated tomatoes at 22 °C, 50-60 %RH

Day	0 s control	Flume 30 s	Flume 60 s	OSBR 30 s	OSBR 60 s
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	26.7 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	6.7 ± 11.5 a	13.3 ± 15.3 a
9	46.7 ± 30.5 ab	0.0 ± 0.0 b	10.0 ± 17.3 ab	46.7 ± 50.3 ab	56.7 ± 49.3 a
11	83.3 ± 41.6 a	36.6 ± 32.1 a	50.0 ± 43.6 a	50.0 ± 50.0 a	56.7 ± 49.3 a
13	90.0 ± 20.8 a	73.3 ± 37.8 a	70.0 ± 20 a	66.6 ± 30.6 a	76.7 ± 25.2 a
15	96.7 ± 10 a	83.3 ± 28.8 a	76.7 ± 11.5 a	80.0 ± 20.0 a	90.0 ± 10.0 a
17	100 ± 5.77 a	93.3 ± 11.5 a	90.0 ± 17.3 a	96.7 ± 5.6 a	100 ± 0.0 a
19	100 ± 0.0 a	93.3 ± 11.5 a	90.0 ± 17.3 a	100 ± 0.0 a	100 ± 0.0 a
21	100 ± 0.0 a	93.3 ± 11.5 a	93.3 ± 11.5 a	100 ± 0.0 a	100 ± 0.0 a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-9. Incidence (%) of shrivel[a] in OSBR and flume treated tomatoes at 22 °C, 50-60 %RH

Day	0 s control	Flume 30 s	Flume 60 s	OSBR 30 s	OSBR 60 s
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	3.3 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
11	10.0 ± 5.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
13	10.0 ± 0.0 ab	0.0 ± 0.0 b	6.7 ± 5.8 ab	10.0 ± 10.0 ab	20.0 ± 10.0 a
15	20.0 ± 0.0 ab	0.0 ± 0.0 b	13.3 ± 5.8 ab	10.0 ± 10.0 ab	23.3 ± 11.5 a
17	20.0 ± 10.0 bc	6.7 ± 11.5 c	20.0 ± 10.0 bc	30.0 ± 17.3 ab	43.3 ± 11.5 a
19	26.7 ± 10.0 c	16.7 ± 15.2 c	30.0 ± 20.0 bc	46.7 ± 32.1 ab	63.3 ± 5.8 a
21	26.7 ± 11.5 c	23.3 ± 5.8 c	33.3 ± 20.8 bc	56.7 ± 41.6 ab	63.3 ± 5.8 a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-10. Incidence (%) of softness[a] in OSBR and flume treated tomatoes at 22 °C, 50-60 %RH

Day	0 s control	Flume 30 s	Flume 60 s	OSBR 30 s	OSBR 60 s
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
11	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
13	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
15	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
17	20.0 ± 23.1 a	0.0 ± 0.0 a	6.7 ± 11.5 a	16.7 ± 28.9 a	10.0 ± 17.3 a
19	36.7 ± 26.5 a	3.3 ± 5.8 a	10.0 ± 10.0 a	16.7 ± 28.9 a	10.0 ± 17.3 a
21	36.7 ± 23.1 a	30.0 ± 26.5 a	33.3 ± 5.8 a	30.0 ± 26.5 a	26.7 ± 25.2 a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).

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

Table 4-11. Incidence (%) of full ripeness[a] in waxed and unwaxed tomatoes at 22°C, 40-50 %RH

Da	0 s Unwaxed	0 s Waxed	Flume 60 s Unwaxed	Flume 60 s Waxed	OSBR 60 s Unwaxed	OSBR 60 s Waxed
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	20.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	30.0 ± 17.3 ab	16.7 ± 5.8 b	53.3 ± 32.1 a	23.3 ± 15.3 ab	26.7 ± 25.2 a	10.0 ± 10.0 b
11	70.0 ± 26.5 ab	36.7 ± 5.8 ab	56.7 ± 28.9 a	26.7 ± 20.8 ab	60.0 ± 26.5 a	20.0 ± 10.0 b
13	90.0 ± 17.3 ab	53.3 ± 11.5 ac	60.0 ± 34.6 ac	26.7 ± 20.81 c	80.0 ± 10 a	46.7 ± 30.6 bc
15	100 ± 10.0 a	63.3 ± 5.8 ab	93.3 ± 5.8 a	30.0 ± 20 b	90.0 ± 10 a	46.7 ± 30.6 b
17	100 ± 0.0 a	63.3 ± 5.8 c	100 ± 0.0 a	66.7 ± 28.9 bc	96.7 ± 5.8 a	50.0 ± 26.5 c
19	100 ± 0.0 a	63.3 ± 5.8 b	100 ± 0.0 a	73.3 ± 25.2 ab	96.7 ± 5.8 a	63.3 ± 15.3 b
21	100 ± 0.0 a	63.3 ± 5.8 b	100 ± 0.0 a	73.3 ± 25.2 ab	96.7 ± 5.8 a	63.3 ± 15.3 b
23	100 ± 0.0 a	66.7 ± 5.8 bc	100 ± 0.0 a	73.3 ± 25.2 ac	96.7 ± 5.8 a	63.3 ± 15.3 c
25	100 ± 0.0 a	73.3 ± 15.3 ab	100 ± 0.0 a	73.3 ± 25.2 ab	96.7 ± 5.8 a	63.3 ± 15.3 b
27	100 ± 0.0 a	83.3 ± 20.8 a	100 ± 0.0 a	73.3 ± 25.2 a	96.7 ± 5.8 a	76.7 ± 15.3 a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-12. Incidence (%) of shrivel[a] in waxed and unwaxed tomatoes at 22 °C, 40-50 %RH

Day	0 unwaxed	0 waxed	Flume 60 unwaxed	Flume 60 waxed	OSBR 60 s unwaxed	OSBR 60 s waxed
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	3.3 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	30.0 ± 5.8 ab	0.0 ± 0.0 b	10.0 ± 17.3 ab	0.0 ± 0.0 b	20.0 ± 20.0 a	0.0 ± 0.0 b
11	50.0 ± 10.0 ab	3.3 ± 5.8 c	23.3 ± 11.5 b	0.0 ± 0.0 c	46.7 ± 15.3 a	0.0 ± 0.0 c
13	63.3 ± 10.0 ab	3.3 ± 5.8 c	36.7 ± 15.3 b	0.0 ± 0.0 c	60.0 ± 17.3 a	0.0 ± 0.0 c
15	66.7 ± 5.8 ab	3.3 ± 5.8 c	43.3 ± 15.3 b	0.0 ± 0.0 c	76.7 ± 15.3 a	0.0 ± 0.0 c
17	83.3 ± 5.8 b	3.3 ± 5.8 c	53.3 ± 15.3 b	3.3 ± 5.8 c	86.7 ± 5.8 a	0.0 ± 0.0 c
19	83.3 ± 15.3 a	3.3 ± 5.8 c	56.7 ± 20.8 b	3.3 ± 5.8 c	90.0 ± 10.0 a	0.0 ± 0.0 c
21	83.3 ± 15.3 b	3.3 ± 5.8 c	83.3 ± 5.8 b	3.3 ± 5.8 c	100 ± 0.0 a	3.3 ± 5.8 c
23	100 ± 0.0 a	16.7 ± 15.3 b	90.0 ± 10.0 a	3.3 ± 5.8 b	100 ± 0.0 a	3.3 ± 5.8 b
25	100 ± 0.0 a	16.7 ± 15.3 b	90.0 ± 10.0 a	3.3 ± 5.8 b	100 ± 0.0 a	3.3 ± 5.8 b
27	100 ± 0.0 a	16.7 ± 15.3 b	93.3 ± 11.5 a	3.3 ± 5.8 b	100 ± 0.0 a	3.3 ± 5.8 b

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-13. Incidence (%) of softness[a] in waxed and unwaxed tomatoes at 22 °C, 40-50 %RH

Day	0 unwaxed	0 waxed	Flume 60 unwaxed	Flume 60 waxed	OSBR 60 s unwaxed	OSBR 60 s waxed
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 00 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
11	6.7 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	10.0 ± 11.5 a	0.0 ± 0.0 a
13	23.3 ± 5.8 ab	0.0 ± 0.0 b	10.0 ± 0.0 ab	0.0 ± 0.0 b	16.7 ± 11.5 a	0.0 ± 0.0 b
15	33.3 ± 5.8 a	0.0 ± 0.0 b	16.7 ± 5.8 a	0.0 ± 0.0 b	30.0 ± 10.0 a	0.0 ± 0.0 b
17	33.3 ± 5.8 a	0.0 ± 0.0 b	26.7 ± 5.8 a	0.0 ± 0.0 b	43.3 ± 5.8 a	0.0 ± 0.0 b
19	40.0 ± 5.8 b	0.0 ± 0.0 c	26.7 ± 5.8 b	0.0 ± 0.0 c	60.0 ± 17.3 a	0.0 ± 0.0 c
21	40.0 ± 10.0 b	6.7 ± 5.8 c	36.7 ± 11.5 b	0.0 ± 0.0 c	76.7 ± 25.2 a	10.0 ± 17.3 c
23	56.7 ± 5.8 b	20.0 ± 0.0 c	50.0 ± 10.0 b	3.3 ± 5.8 d	80.0 ± 20.0 a	13.3 ± 15.3 cd
25	70.0 ± 17.3 b	23.3 ± 5.8 c	83.3 ± 11.5 ab	3.3 ± 5.8 d	96.7 ± 5.8 a	20.0 ± 10.0 c
27	86.7 ± 5.8 a	26.7 ± 5.8 b	96.7 ± 5.8 a	10.0 ± 10.0 c	96.7 ± 5.8 a	23.3 ± 15.3 bc

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-14. Incidence (%) of full ripeness[a] in waxed and unwaxed OSBR treated tomatoes during fall (50-60 %RH, 22 °C) and spring (40-50 %RH, 22 °C)

Day	Unwaxed Spring	Waxed Spring	Unwaxed Fall
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	13.3 ± 15.3 a
9	26.7 ± 25.2 ab	10.0 ± 10.0 b	56.6 ± 49.3 a
11	60.0 ± 26.5 a	20.0 ± 10.0 b	56.6 ± 49.3 a
13	80.0 ± 10.0 a	46.7 ± 30.6 b	76.6 ± 25.2 a
15	90.0 ± 10.0 a	46.7 ± 30.6 b	90.0 ± 10.0 a
17	96.7 ± 5.8 a	50.0 ± 26.5 b	100 ± 0.0 a
19	96.7 ± 5.8 a	63.3 ± 15.3 b	100 ± 0.0 a
21	96.7 ± 5.8 a	63.3 ± 15.3 b	100 ± 0.0 a
23	96.7 ± 5.8 a	63.3 ± 15.3 b	n/a
25	96.7 ± 5.8 a	63.3 ± 15.3 b	n/a
27	96.7 ± 5.8 a	76.7 ± 15.3 a	n/a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-15. Incidence (%) of Shrivel[a] in waxed and unwaxed OSBR treated tomatoes during fall (50-60 %RH, 22 °C) and spring (40-50 %RH, 22 °C)

Day	Unwaxed Spring	Waxed Spring	Unwaxed Fall
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	20.0 ± 20.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
11	46.7 ± 15.3 a	0.0 ± 0.0 b	0.0 ± 0.0 b
13	60.0 ± 17.3 a	0.0 ± 0.0 c	20.0 ± 10.0 b
15	76.7 ± 15.3 a	0.0 ± 0.0 c	23.3 ± 11.5 b
17	86.7 ± 5.8 a	0.0 ± 0.0 c	43.3 ± 11.5 b
19	90.0 ± 10.0 a	0.0 ± 0.0 c	63.3 ± 5.8 b
21	100 ± 0.0 a	3.3 ± 5.8 c	63.3 ± 5.8 b
23	100 ± 0.0 a	3.3 ± 5.8 b	n/a
25	100 ± 0.0 a	3.3 ± 5.8 b	n/a
27	100 ± 0.0 a	3.3 ± 5.8 b	n/a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

Table 4-16. Incidence (%) of softness[a] in waxed and unwaxed OSBR treated tomatoes during fall (50-60 %RH, 22 °C) and spring (40-50 %RH, 22 °C)

Day	Unwaxed Spring	Waxed Spring	Unwaxed Fall
0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
9	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
11	10.0 ± 11.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a
13	16.7 ± 11.5 a	0.0 ± 0.0 b	0.0 ± 0.0 b
15	30.0 ± 10.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
17	43.3 ± 5.8 a	0.0 ± 0.0 b	10.0 ± 17.3 b
19	60.0 ± 17.3 a	0.0 ± 0.0 b	10.0 ± 17.3 b
21	76.7 ± 25.2 a	10.0 ± 17.3 b	26.7 ± 25.2 b
23	80.0 ± 20.0 a	13.3 ± 15.3 b	n/a
25	96.7 ± 5.8 a	20.0 ± 10.0 b	n/a
27	96.7 ± 5.8 a	23.3 ± 15.3 b	n/a

[a]Values are mean ± standard deviation of quadruplicate experiments of 10 tomatoes each (n=30).
Means with same letter in the same row (ab) are not statistically different ($p<0.05$).

CHAPTER 5
DISCUSSION

Several *Salmonella* outbreaks in past decades have been associated with tomatoes (CDC 2011), an association which has been extraordinarily costly to the Florida tomato industry (Brown 2008). The washing of tomatoes in a sanitized flume has been an important practice used by Florida packinghouses. However, flume treatment of tomatoes is not as effective at removing *Salmonella* from tomato surfaces as an overhead spray brush roller (OSBR) system (Chang 2011). OSBR systems have many advantages over flumes: 1) they combine physical brushing with chemical sanitizing to better wash tomato surfaces, 2) fresh sanitizer is continuously sprayed on tomatoes, meaning more chemistry is used for sanitizing tomato surfaces rather than just preventing cross-contamination of flume water, and 3) though yet untested, OSBR systems could theoretically require less water and chemistry than flumes, saving money on chemicals, water, water treatment, and packinghouse space, while reducing the impact on the environment. While OSBR systems have many advantages, the purpose of this research was to assess whether this treatment negatively affects the safety or quality of tomatoes to the point where the costs might outweigh the benefits. Three objectives of this research were to test the OSBR system for: 1) its ability to remove *Pectobacterium carotovorum* (*Pc*), an organism which causes substantial product loss due to spoilage and appears to have somewhat synergistic growth with *Salmonella*, 2) its potential to cause greater post-wash survival of *Pc or Salmonella* due to mechanical damage from brushing, and 3) its effect on tomato shelf life compared to flume treatment or field packed (no treatment).

Growth Curves

Growth curves were performed for five rifampicin-resistant strains of *Salmonella* (Table 4-1) and for one rifampicin resistant strain of *Pc* (SR38) (Table 4-2). All cultures used were made rifampicin resistant in order to easily distinguish experimental strains from tomato natural microflora on TSA+rif. The purpose of these tests was to ensure that all cultures could grow to a consistent level in a consistent period of time in both TSA+rif and TSB+rif.

Salmonella

Five serovars of *Salmonella enterica* were chosen for experimentation to reduce variation associated with differences in susceptibility of serovars to sanitizing treatments, as recommended by the FDA (Beuchat and others 2001). Four of the serovars were chosen due to their association with tomato-related outbreaks (*S.* Typhimurium, *S.* Braenderup, *S.* Newport (CDC 2011), and *S.* Javiana (Hedberg and others 1999) and the fifth (*S.* Enteritidis) was chosen due to its association with other food-related outbreaks, including several where tomatoes were suspected vehicles (CDC 2011).

Growth curves were performed to ensure that the five serovars all reach stationary phase at similar times and grow to similar concentrations. As Table 4-1 shows, by hour 14, all serovars reached stationary phase and grew to statistically similar $\log_{10}$ CFU/mL concentrations (9.2 $\log_{10}$ CFU/mL on average). As seen in a similar study (Chang 2011), *S.* Javiana tended to have a higher population than *S.* Typhimurium throughout the duration of the growth curves. This difference tapered off by the end of the observational period, however, showing that no matter the differences

in growth rate during log phase, all serovars reach a statistically similar level in stationary phase after 14 h.

Pectobacterium

Pc SR38 was chosen for testing due to its association with Florida tomatoes (Bender and others 1992) and presence in the literature (Brandl and others 2013; Zheng and others 2013; Danyluk and others 2010; Joy 2005). *Pc* growth curves were performed differently from the *Salmonella* growth curves in order to promote consistency of growth. Initial testing (data not shown) revealed that the *Pc* SR38 culture would not grow reliably in 200 ppm TSB+rif when inoculated at a 2 $\log_{10}$ CFU/mL level. It was noted that beginning with a slightly higher initial inoculum (3.5 $\log_{10}$ CFU/mL) level promoted more consistent growth. In addition, it was found that multiple strains of *Pc* SR38 would grow at unpredictably different rates when kept under static conditions. By growing *Pc* cultures under shaking conditions (140 rpm), they would reach stationary phase much faster and at nearly identical rates, as seen by the low levels of variation shown in Table 4-2. As seen in Table 4-2, *Pc* cultures reached stationary phase by hour 18 of the study, reaching maximum growth approximately 9 $\log_{10}$ CFU/mL.

Pectobacterium Removal Studies

T-GAPs regulations (FDA 2009) require that any sanitizing treatment used for processing Florida tomatoes must achieve at least a 3-log unit reduction in *Salmonella* or like organisms. Previous research (Chang and Schneider 2012) has shown that the OSBR system can achieve at least a 3 $\log_{10}$ CFU/mL reduction in *Salmonella* on tomato surfaces after 15 s treatment with 100 ppm NaOCl, 5 ppm ClO_2, 80 ppm PAA, or water. This study replicated the conditions of the Chang and Schneider (2012) experiment to discover whether similar reductions in the plant pathogen *Pc* could be achieved.

This research showed that OSBR treatment for 15 s using 100 ppm NaOCl, 5 ppm ClO_2, 80 ppm PAA, and water achieved at least a 3 $\log_{10}$ CFU/mL reduction in *Pc* on tomato surfaces (Table 4-3). This result shows that a 3 $\log_{10}$ CFU/mL reduction in *Pc* can be achieved in at least the same amount of time as seen in *Salmonella* (Chang 2011). The efficacy of the OSBR system in this study dispels any concern that *Pc* might be more resistant to OSBR treatment of tomatoes due to its status as a plant pathogen. In short, OSBR treatment is analogously effective at removing both *Pc and Salmonella* from tomato surfaces.

A study by Pao and others (2009) investigated the removal of dried on *Salmonella* from tomato surfaces using OSBR treatment with 5 ppm ClO_2. Pao and others (2009) showed that OSBR treatment with 5 ppm ClO_2 removed more than 4 $\log_{10}$ CFU/cm^2 after only 10 s, whereas in this study, a 4 $\log_{10}$ CFU/mL reduction was not seen even at 15 s. Pao and others (2009) also found that OSBR with water treatment could achieve a 3 $\log_{10}$ CFU/cm^2 reduction of *Salmonella* at 10 s. This study showed a 3.19 ± 1.47 $\log_{10}$ CFU/mL reduction in *Pc* using water at 15 s, thus it is possible that at least 3 $\log_{10}$ CFU/mL may have been removed by 10 s. Discrepancies between this study and the findings of Pao and others (2009) could be explained by differences in the OSBR system used (which would greatly affect physical removal of bacteria), differences in bacterial enumeration (Pao and others (2009) sampled a particular surface area of tomatoes while this study sampled the entire surface area), or differences in organism studied (*Pc* vs. *Salmonella*).

In this study, treatment time tended to have more of an impact on removal than type of sanitizer used. For all treatments, significantly higher removal was achieved at

each increasing time point with the exception of the increase from 15 s to 30 s for NaOCl. In contrast, removal was not significantly different between sanitizers used for either 5 s or 15 s. The only significant difference in removal between sanitizers was seen at 30 s, where PAA showed significantly higher removal than NaOCl or water, but the removal achieved by ClO_2 was statistically the same as both PAA and NaOCl (Table 4-4). These results suggest that removal is more a function of the physical action of the brush rollers (at least at 5-15 s treatment times) rather than the chemical action of the sanitizers. The similarity in efficacy between water and 100 ppm NaOCl across all treatment times also speaks to this point.

There was appreciable variation seen in this study, with standard deviations sometimes approaching as high as a ± 2 $\log_{10}$ CFU/mL. There are two possible explanations for this. First, the inoculated tomatoes used in this study were allowed to freely roll along the brushes of the OSBR system. The axis at which each tomato rolled was largely a function of the shape of the tomato. Due to the irregularity of the shape of round tomatoes, some samples would continue to spin on the axis on which they were placed while others would spin on different axes. Since the samples were spot inoculated around their blossom scars, it is possible that better or worse physical removal was achieved depending on the alignment of the axis of rotation with the inoculation points. Second, for all studies, five tomatoes were treated at once in an OSBR system with only three spray nozzles, thus some samples might have inadvertently been exposed to higher levels of sanitizer than others, as well as to more physical action from the impact of the spray itself depending on their position on the brush bed.

Pectobacterium and *Salmonella* Survival on OSBR Treated Tomatoes

These studies were performed to determine whether OSBR treatment of tomatoes increases their ability to harbor post-processing *Pc or Salmonella* contamination. The concern with brush washing is that it is a more physical process than flume washing, and as such may cause microabrasions on tomato surfaces that might allow *Pc or Salmonella* to survive at higher levels. To investigate this concern, green tomatoes were treated with water and 100 ppm NaOCl in the OSBR system for 0, 15, or 60 s, then inoculated with *Pc or Salmonella* and sampled on days 0, 1, 3, and 7.

Pectobacterium

Pc levels on tomato surfaces were approximately 5.16 $\log_{10}$ CFU/mL on day 0, dropped to 2.50 $\log_{10}$ CFU/mL by day 1, then dropped to below the statistical limit of detection of 1.40 $\log_{10}$ CFU/mL for both day 3 and 7 (Table 4-4). For each day, there were no significant differences between any of the treatments. Since populations of *Pc* did not increase or plateau at any point for any of the treatments (Table 4-4), it does not appear that OSBR treatment affected the growth behavior of *Pc* on tomato surfaces. Other researchers have investigated the growth behavior of *Pc* on tomato surfaces with similar results. In a study by Joy (2005), the growth of *Pc* on intact tomato surfaces was investigated under standard ripening room conditions (90 %RH, 20 °C), fall/winter conditions (60 %RH, 27 °C), and optimal conditions for *Pc* growth (90 %RH, 27 °C). It was found that at 60 %RH and 27 °C, *Pc* was unrecoverable by day 1. At 90 %RH and 20 ºC, the population dropped to just under 2 $\log_{10}$ CFU/mL by day 1, down to near 1.5 $\log_{10}$ CFU/mL by day 2, and was unrecoverable by day 3. The 90 %RH and 27 °C achieved the best survivability with a log reduction of only 1.36 $\log_{10}$ CFU/mL by day 3. Joy's (2005) results demonstrated that survivability of *Pc* is impaired at lower

temperatures and humidities. This survival study was performed at a slightly lower temperature (25 °C) and humidity (75-85 %RH) than Joy's (2005) work, and the observed survival of *Pc* fell appropriately between the 90 %RH 27 °C and 90 %RH 20 °C results of Joy's (2005) study.

Salmonella

Initial levels of *Salmonella* on tomato surfaces were 5.56 $\log_{10}$ CFU/mL on average on day 0. These levels dropped to below the statistical limit of detection of 1.40 $\log_{10}$ CFU/mL by day 1 and remained there for days 3 and 7 (Table 4-5). It was found that the NaOCl-treated tomatoes had significantly less *Salmonella* recovery than the other treatments on day 1, however this was determined from data that fell below the statistical limit of detection and can be discounted. As seen with the *Pc* survival studies (Table 4-4), there was no population growth or maintenance throughout the observational period for any treatment (Table 4-5), suggesting that the OSBR treatment of tomatoes does not promote better harborage of *Salmonella* under the storage conditions tested.

The Joy (2005) study also investigated the survival of *Salmonella* on intact tomato surfaces. At 90 %RH and 27 °C, the population decreased by only 0.64 $\log_{10}$ CFU/mL by day 3. The population reduction was 4.42 $\log_{10}$ CFU/mL by day 3 for 90 %RH and 20 °C, and 3.59 $\log_{10}$ CFU/mL for 60 %RH and 27 °C. The only treatment that fell below the statistical limit of detection on day 3 was the 90 %RH and 20 °C treatment, and at no point was the *Salmonella* undetectable (Joy 2005). A similar downward trend in population was seen in the Castro-Rosas and others (2011) study, in which *Salmonella* populations decreased throughout 6 days of storage on jalapeño and Serrano peppers stored at 25 °C and 90 %RH. *Salmonella* populations decreased from

near 5 $\log_{10}$ CFU/Serrano chili at day 0 to about 1.5 $\log_{10}$ CFU/Serrano chili by day 6 (a 3.6 $\log_{10}$ CFU/Serrano chili reduction total). *Salmonella* survived slightly better on jalapeños, decreasing only 2.4 $\log_{10}$ CFU/jalapeño pepper after 6 days (Castro-Rosas and others 2011). Both the Joy (2005) and Castro-Rosas (2011) study showed the same downward trend in *Salmonella* population as this study during storage near 25 °C and at high humidity, however neither showed a population drop off quite as extreme as was seen in this study (Table 4-4). As with the *Pc* results, the slightly lower %RH used may help explain the difference in rate of population decline seen in this study versus others.

A study by Pao and others (2012) investigated the survival of *Salmonella* on spray and brush washed tomatoes and found completely contrary results to those found in this study. Even on intact fruit, Pao and others (2012) found that *Salmonella* populations increased by as much as 3 $\log_{10}$ CFU/cm^2 over 12 days when tomatoes were stored at 90 %RH and 10-35 °C. After either rinsing or rinsing and brushing, *Salmonella* counts on inoculated tomatoes were decreased from 3.7 to less than 1 $\log_{10}$ CFU/cm^2. The rinse and brush treated tomatoes stored at 21 °C showed a rebound of *Salmonella* to levels not significantly different than the initial inoculation levels after 1 day (Pao and others 2012). The primary difference between the Pao and others (2012) study and this study is that tomatoes were inoculated pre-wash in the Pao and others (2012) study and post-wash in this study. The difference in order of inoculation and washing may explain the difference in findings between the two studies.

Wound Inoculation Studies

Wound inoculation studies were performed to model *Pc and Salmonella* growth behavior in grossly damaged fruit. First, wounded tomatoes were compared to intact

tomatoes each at a 9 $\log_{10}$ CFU/tomato inoculation level, then they were stored under identical conditions as the OSBR-treated survival studies (25 °C, 75-85 %RH) and sampled on days 0, 1, 3, and 7. On day 0, the initial levels of *Pc and Salmonella* in the wounded fruit were statistically the same, but significantly higher than the intact *Pc* or *Salmonella* levels. This is because the puncture wounds helped to prevent desiccation loss during the initial drying step. The initial *Salmonella* inoculation level for the intact tomatoes was 5.52 ± 0.26 $\log_{10}$ CFU/mL, showing an average loss of 0.62 $\log_{10}$ CFU/mL from desiccation. The initial *Pc* inoculation level on intact tomatoes was significantly lower than all others at 5.12 ± 0.26 $\log_{10}$ CFU/mL, showing an average loss of 0.91 $\log_{10}$ CFU/mL due to drying (Table 4-6). This loss was typical of *Pc* for all studies performed, which is why the drying time for *Pc* was lowered from the 2 h used for *Salmonella* inoculated tomatoes to 1 h, to prevent further loss of inoculum from desiccation while allowing enough time to dry.

As seen in the OSBR-treated survival studies, both *Pc and Salmonella* populations decreased to below statistical detection limits within 1-3 days. On wounded tomatoes inoculated with *Salmonella*, the population increased from 6.14 ± 0.22 to 6.32 ± 0.39 $\log_{10}$ CFU/mL between days 0 and 3, then declined to 5.70 ± 0.33 $\log_{10}$ CFU/mL by day 7 (Table 4-6). This result shows that *Salmonella* can at least maintain a population near the inoculation level of 6 $\log_{10}$ CFU/mL when the fruit is wounded. *Pc* growth on wounded fruit deviated even more drastically from its intact counterpart, increasing 3.4 $\log_{10}$ CFU/mL by day 3 in the wounded samples versus decreasing 4.34 $\log_{10}$ CFU/mL in the intact. This rapid growth in population in the wounded fruit was a result of the development of soft rot caused by the *Pc* inoculum. The development of

soft rot in the samples was obvious by the appearance of watery lesions at the inoculation sites after less than one day. The disease then developed so extensively that tomatoes were completely liquefied before they could be sampled at day 7.

The trend in population growth in wounded fruit versus population decline in intact fruit seen in this study (Table 4-6) was also seen in the Joy (2005) study of shave-wounded tomatoes and the Castro-Rosas (2011) study of sliced peppers. In the Joy (2005) study, *Salmonella* populations increased 4 $\log_{10}$ CFU/mL with 90 %RH and 20 °C storage, 3.35 $\log_{10}$ CFU/mL with 90 %RH and 27 °C storage, and 1.26 $\log_{10}$ CFU/mL with 60 %RH and 27 °C storage. *Pc* populations on shave wounded tomatoes similarly increased by 6.59 $\log_{10}$ CFU/mL at 90 %RH and 27 °C, 5.93 $\log_{10}$ CFU/mL at 90 %RH and 20 °C, and 2.59 $\log_{10}$ CFU/mL at 60 %RH and 20 °C (Joy 2005). Similarly, the Castro-Rosas and others (2011) study found that *Salmonella* populations increased from 1.5 $\log_{10}$ CFU/pepper slice to nearly 5 $\log_{10}$ CFU/pepper slice after 2 days of storage at 25 °C and 90 %RH for both jalapeño and Serrano peppers.

In this study, an additional wound inoculation test was done with lower inoculation levels of both *Pc* and *Salmonella* (2 $\log_{10}$ CFU/tomato vs 9 $\log_{10}$ CFU/tomato). The purpose of this study was to determine whether undetectable levels of *Pc* or *Salmonella* could grow to detectable levels in wounded fruit. This was to show that, even if population levels of *Pc and Salmonella* were reduced to below the statistical detection limit in the OSBR-treated survival studies, if the OSBR treatment had indeed wounded the fruit, a rebound should have been seen within the observational period.

There were two primary differences in population behavior inoculated at low versus high levels onto wounded fruit. First, the fruit inoculated with *Pc* at a ~2 $\log_{10}$ CFU/tomato level never developed soft rot during the 7 day storage, while the 9 $\log_{10}$ CFU/tomato inoculated levels developed soft rot within the first day. *Pc* pathogenicity has previously been found to be concentration dependent. In a study by Yahiaoui-Zaidi (2010), it was found that potato tubers inoculated with 2.10×10^7 CFU/mL developed soft rot more frequently and extensively than tubers inoculated with only 2.10×10^5 CFU/mL (Yahiaoui-Zaidi and others 2010). Further, Bartz and others (1975) showed that if soft rot lesions do not develop within 72 h of wound inoculation, *Pc* populations plateau until the fruit turns red, after which proliferation resumes and lesions form. Second, both *Pc* and *Salmonella* cultures grew rapidly from undetectable levels on day 0 to near 3.5 $\log_{10}$ CFU/mL on day 1, after which growth plateaued (Table 4-7). This in particular is in contrast to the behavior of the 9 $\log_{10}$ CFU/tomato *Salmonella* inoculated samples, which held a consistent population level of near 6 $\log_{10}$ CFU/mL. Though there was rapid initial growth in the 2 $\log_{10}$ CFU/tomato samples (Table 4-7), neither *Pc* nor *Salmonella* populations ever reached a level greater than 4.18 $\log_{10}$ CFU/mL. This growth plateau of *Salmonella* has been seen by other researchers as well. In a study by Ibarra-Sanchez and others (2004), tomatoes were injected into their cores with a suspension of *S.* Typhimurium resulting in an initial microbial load of 1.8 ± 0.3 $\log_{10}$ CFU/mL on hour 0. By hour 48 of storage at 25-28 °C, the population had increased to 4.4 ± 0.3 $\log_{10}$ CFU/mL, but subsequently decreased to 3.2 ± 1.1 $\log_{10}$ CFU/mL by hour 60, then dropped to undetectable levels by hour 72 (Ibarra-Sanchez and others 2004). The population decline in the Ibarra-Sanchez (2004) was much more rapid than in this

one, reaching undetectable levels by day 3 while this study's population was just reaching its maximum at day 3. The same general trend is apparent, however: rapid initial growth, population growth plateau near 4 $\log_{10}$ CFU/mL, then population decline. These results suggest that *Salmonella* can maintain a larger population if heavy contamination into wounded tissue is present to begin with, however lower levels of contamination into wounded tissue cannot grow to or maintain those same higher population levels.

Shelf Life Studies

These studies were performed to investigate the effect that OSBR treatment has on the shelf life of tomatoes relative to flume treatment or no treatment at all. Consumer acceptance of fresh marketed tomatoes depends primarily on appearance (ripeness, shrivel, and spoilage) and texture (softness) (Kader 1986). The four characteristics of ripeness level 6, shrivel, softness, and presence of microbial spoilage were therefore chosen as observational parameters for their bearing on tomato marketability.

Flume vs. OSBR Treated Tomatoes (30 vs. 60 s Treatment Time)

This study was designed to determine the shelf life of tomatoes washed with 100 ppm NaOCl in either a flume or OSBR system for 0 s (control), 30 s (excessive use for OSBR), or 60 s (abuse time for OSBR). Tomatoes were stored for 21 days at 22 °C (ambient retail conditions) and ambient humidity (50-60% for the fall season).

Nearly all samples had reached ripeness level 6 (93.3-100%) by day 21 and there was no significant difference between treatments in terms of ripeness for any observation except day 9, in which the OSBR 60 s samples were significantly more ripe than the flume 30 s samples (Table 4-8). This result shows that neither OSBR nor flume treatment has an effect on how quickly tomatoes ripen relative to no treatment at

all. Similarly, there was no treatment effect on the softness of tomatoes; all treatments resulted in softness levels of 26.7-36.7% by day 21 (Table 4-10). Previous research by Jordan and others (1986) has shown that there is no significant difference in tomato firmness before or after packinghouse treatment (Jordan and others 1986), so no differences in softness were expected between wash and no-wash treatments during the early stages of storage. However, in the Jordan and others (1986) study, firmness was shown to decrease from 40 N to 27 N after 7 days of retail storage (Jordan and others 1986), so firmness was expected to continue to decrease with increasing time. At no point was there a significant difference between treatments in terms of tomato softness. This suggests that even excessive treatment (60 s) with an OSBR system will not significantly impact the softness of tomatoes stored for 21 days under ambient fall season retail conditions.

There appeared to be a treatment effect on incidence of shrivel in the samples during the latter days of storage. Previous research has shown that physical damage to fruit can affect decay rates. In a study by Wright and others (1931), frost-injured tomatoes were shown to decay (a process immediately preceded by shrivel, as noted by the authors) more quickly than non-frost-injured tomatoes (25% decay in injured vs. 8% decay in non-injured over 43 days of storage) (Wright and others 1931). If OSBR treatment causes physical damage, then an increase in shrivel would be expected with each increase in treatment time. From day 0-11, no significant differences were seen between treatments; from day 13-15, OSBR 60 s showed significantly more shrivel than flume 30 s, and from days 17-21, OSBR 60 s showed significantly more shrivel than either flume treatments or the 0 s control, whereas the OSBR 30 s treatment only

showed significantly higher shrivel than the flume 30 s treatment (Table 4-9). For all observations, the flume treatments never significantly differed from each other or the 0 s control. These results show that excessive OSBR treatment (30 s) can cause significantly more shrivel in tomatoes than flume treatment when tomatoes are stored longer than 15 days under ambient fall season retail conditions. Additionally, abusive OSBR treatment (60 s) can cause significantly more shrivel than no treatment, 30 s flume treatment, or 60 s flume treatment of tomatoes when stored for longer than 15 days under ambient fall season retail conditions. This indicates that OSBR treatment either causes some physical damage to tomato surfaces, or removes enough of the waxy coating of the tomatoes that greater moisture loss results.

Flume vs. OSBR Treated Tomatoes (Waxed vs. Unwaxed)

This study was performed to determine whether OSBR treated tomatoes would still develop significantly more shrivel if waxed. Since the increase in shrivel seen in the previous study was likely due to moisture loss after brushing removed the protective waxy coating of the tomatoes, applying a food grade wax after brushing was expected to reduce the incidence of shrivel in OSBR treated tomatoes.

Between days 17-23, the 0 s control waxed sample developed significantly less ripeness than its unwaxed counterpart. The waxed flume samples showed significantly less ripeness than unwaxed on days 15 and 17. The waxed OSBR samples showed significantly less ripeness than their unwaxed counterparts between days 9-25. By day 27, however, all treatments exhibited the same level of ripeness. It is important to note that wax treated samples displayed a noticeably slower and less consistent degree of ripening than the unwaxed samples. By day 27, the waxed samples were only 73.3-83.3% ripe, whereas the unwaxed samples were 96.7-100% ripe (Table 4-10). The wax

used in this study was Tri-Wax (Tri-Pak Machinery, Inc. Harlingen, TX), a petroleum and mineral oil blend used in some Florida tomato packinghouses. The manufacturer recommends applying the wax at 0.0025% of the weight of the tomatoes, an amount that was applied in this study as closely as possible. However, the difference in application method between a packinghouse and a laboratory (bulk application with large brush rollers vs. individual application with a paper towel) may have resulted in the presence of more wax on the experimental samples than would be seen on a typical marketed waxed tomato. It is possible that the individualized application of the wax onto the tomatoes in this experiment congested the stem scar and therefore inhibited (and in some cases, prevented) normal ripening. A study by Clendenning (1941) showed that when tomato stem scars are covered with hot paraffin wax, the fruit ripens more slowly, though the fruit can resume normal ripening once the wax is removed (Clendenning 1941). Another study by Dilmacunal and others (2011) showed that waxed tomatoes have significantly lower respiration rates than unwaxed, which results in delayed ripening (Dilmacunal and others 2011). If the waxed tomatoes in this study had a similar reduction in respiration rate, that could explain the observed differences in ripening.

A significant treatment effect on shrivel development was seen. The unwaxed OSBR treated tomatoes developed shrivel significantly more than the flume unwaxed samples (and sometimes more than the 0s control unwaxed samples) between days 11-21 of storage. Eventually all unwaxed treatments became statistically the same as shrivel increased to a 93.3-100% incidence by day 27 (Table 4-12). This result shows that OSBR-treated unwaxed tomatoes tended to develop significantly more shrivel than

the flume treated unwaxed tomatoes. The flume treated unwaxed samples tended to develop more shrivel than the 0 s control, however this difference was rarely significant. The rapid development of shrivel in the unwaxed samples was not seen in the waxed samples, which had a lower incidence of shrivel than their unwaxed counterparts from day 9 onward. This result supports the hypothesis that the increased incidence of shrivel in OSBR treated tomatoes is due to moisture loss from removal of the tomatoes' natural waxy coating by the brushes. Replacement of that coating with a food grade wax significantly slowed and/or prevented the development of shrivel in the OSBR treated samples for the 27 day period. Adding wax to tomato surfaces has previously been shown to decrease moisture loss and therefore shrivel relative to unwaxed tomatoes. In a study by Dilmacunal and others (2011), the addition of a waxy coating significantly lowered the weight loss of tomatoes compared to an unwaxed control when both were stored at 20 °C and 90 %RH throughout a 16 day storage period (Dilmacunal and others 2011).

As in the results for shrivel, unwaxed tomatoes developed softness significantly more than their waxed counterparts from day 15 onward. The waxed treatments tended not to significantly differ from one another. The unwaxed OSBR samples developed more softness than the unwaxed flume samples between days 19-23, and the unwaxed flume and 0 s control samples did not differ from one another at any observation point (Table 4-13). These results show that waxed fruit tend to develop less softness than unwaxed fruit during 27 days of storage at spring retail conditions, and that the effect of different methods of washing on increased softness is greatly mitigated by applying a wax coating post-wash. It is no surprise that softness and shrivel seemed to develop

concurrently in the unwaxed samples. The more severe the shrivel in the samples, the greater a structural deficit they were at, and thus the more quickly they showed loss of firmness. The Dilmacunal and others (2011) study also showed that by day 16 of storage at 20 °C and 90 %RH, waxed tomatoes exhibited a significantly higher amount of firmness than unwaxed tomatoes. It is thought that higher levels of moisture corresponding to greater turgor and therefore greater firmness, thus explaining the negative correlation between weight loss and firmness (Dilmacunal and others 2011).

OSBR Treated Tomato Shelf Life (Fall vs. Spring, Waxed vs. Unwaxed)

This analysis was performed to compare the effect of OSBR treatment on waxed vs. waxed tomatoes stored under different seasonal conditions. The treatments compared were unwaxed and waxed tomatoes stored under spring conditions, and unwaxed tomatoes stored under fall conditions.

Throughout the observational period (21-27 days), there was never a statistically significant difference in ripeness between the unwaxed fall and unwaxed spring samples. However, the waxed spring samples showed significantly less ripeness than the two sets of unwaxed samples throughout days 11-25 (Table 4-14). As discussed previously, this difference may have been due to stem scar congestion during wax application (Clendenning 1941). In contrast to fruit ripeness, the incidence of shrivel was significantly different according to seasonality and wax treatment. The unwaxed spring samples showed significantly higher incidences of shrivel than the waxed spring or unwaxed fall treatments from day 9 onward, while the unwaxed fall treatments showed significantly more shrivel than the waxed spring samples from day 13 onward. This difference in shrivel incidence between the unwaxed fall and unwaxed spring samples is likely due to the seasonal difference in relative humidity. The relative

humidity in spring was between 40-50%, whereas the relative humidity in fall was 50-60%. Under conditions of lower humidity, it stands to reason that more moisture would be pulled out of the fruit, thus resulting in more shrivel, which is exactly what was seen in the unwaxed spring samples. The waxed spring samples showed significantly less shrivel due to the addition of a protective waxy coated which helped prevent moisture loss, and thus helped prevent shrivel, even under spring storage conditions.

In terms of softness, the unwaxed spring samples exhibited higher incidences of softness from day 13 onward than either the waxed spring or unwaxed fall samples (Table 4-15). Seasonality has previously been shown to play a role in fruit firmness. A study by Jordan and others (1986) found that ethylene-ripened tomatoes harvested in the winter were less firm (with an average firmness of 42 N across all sizes) than those harvested in the spring (which had an average firmness of 54 N across all sizes) (Jordan and others 1986). The waxed spring and unwaxed fall samples were never significantly different from one another in terms of softness. As mentioned before, softness in the samples tended to develop more rapidly in shriveled tomatoes. This relationship was seen again in that the unwaxed spring samples had significantly more shrivel and softness than the unwaxed fall samples. The unwaxed fall samples did not have significantly more softness than the waxed spring samples, however, due to high variation and lack of day 23-27 data for the unwaxed fall samples. As seen from the 17-21 day period, softness was beginning to develop rapidly in the unwaxed fall samples, reaching a level of 26% incidence, which is higher than the waxed spring samples reached even by day 27 (Table 4-16). Had the unwaxed fall observational period been extended to 27 days, it is quite possible a significant difference between the waxed

spring and unwaxed fall samples would have eventually been seen. These results suggest that post-wash waxing or storage in higher humidity environments can significantly reduce the development of softness in abusively OSBR-treated tomatoes.

Conclusions and Future Work

The overall aim of this research was to determine if OSBR treatment of tomatoes has any negative affect on their safety or quality. Since OSBR treatment has previously been shown to be superior at *Salmonella* removal from tomato surfaces than flume systems (Chang 2011, Pao and others 2009), the replacement of some if not all flume systems in tomato packinghouses is an idea worth investigating. However, it would be irresponsible to recommend such a drastic change to tomato packing lines before the OSBR system is shown not to negatively affect the shelf life or safety of tomatoes.

Two genera were investigated in this research: 1) *Salmonella*, in the form of a five strain cocktail, and 2) *Pc* SR38, a strain of soft rot *Pectobacterium* that is widely used in research. Growth curves were performed on the five strains of *Salmonella* (all isolates associated with foodborne disease outbreaks) to ensure that all strains grow consistently and evenly to stationary phase. The growth curve confirmed that all serovars used reach a statistically similar level in stationary phase at the same time, meaning that the cocktail is suitable for use in research. A growth curve was also performed in *Pc* to ensure consistent growth to stationary phase. Results showed that *Pc* SR38 can consistently reach stationary phase in 18 h when grown with agitation, even in 200 ppm TSB+rif, thus supporting its use in this research.

Although the OSBR system has previously been shown to effectively remove *Salmonella* from tomato surfaces, this research investigated its ability to remove *Pc* using different sanitizers commonly used in produce processing (NaOCl, ClO_2, and

PAA). The OSBR system was able to achieve a 3 $\log_{10}$ CFU/mL reduction after 15 s for all sanitizers used, including the water control. The only significant difference between sanitizers seen was at time 30, in which PAA and ClO_2 achieved a significantly higher removal of *Pc* than water, but NaOCl did not. This result demonstrated the importance of the physical action of brushing in the OSBR system, which had more of an impact on *Pc* removal than the sanitizers did. More importantly, this result showed that the OSBR system is equally as effective at removing *Pc* as it is at removing *Salmonella*, which should improve both the safety and quality of tomatoes. Future research should be done to optimize brush roller systems to achieve the greatest pathogen reduction in the shortest amount of time and smallest space. Variables that could be adjusted would include brush fiber materials, brush rotational speed, spray nozzle distance and placement, spray pressure and flow rate, and optimal sanitizer concentration, among others.

A major concern associated with the OSBR system is that the physical action of the brushes might cause physical damage to the surfaces of the tomatoes. Physical damage poses the risk of allowing greater survival of *Pc* or *Salmonella* on tomato surfaces if any contamination remains or is introduced after washing. Thus, studies were performed to assess the ability of *Pc* and *Salmonella* to survive on OSBR-treated tomatoes relative to unwashed tomatoes. The results of these studies revealed no significant difference in survival of *Pc* and *Salmonella* in OSBR treated tomatoes vs. unwashed tomatoes, indicating that brushing does not damage tomato fruit to the extent that better pathogen survival is a major concern. Additional investigation along these lines could include 1) performing the test using dip inoculation to give pathogens greater

access to potential microabrasions on fruit surfaces, and 2) extending the observational period and using enrichment/MPN testing to improve the time and limit of detection of the study.

To ensure that the lack of significant result in the OSBR-treated pathogen survival study was not due to a fault in cultures used or storage conditions, the studies were repeated with wounded fruit. Puncture wounded fruit were used to better define how the two inocula would have behaved had the OSBR treated fruit been grossly damaged as a result of brushing. Further, these studies were done at high inoculum levels (to replicate the standard inoculum concentration used in the OSBR-treated survival studies) and low inoculum levels (to show behavior of pathogens on wounded fruit with an undetectable starting inoculum). The result was that both *Pc and Salmonella* maintained or exceeded their starting inoculum level no matter if there was a high (9 $\log_{10}$ CFU/mL) or low (2 $\log_{10}$ CFU/mL) initial load on the tomatoes. This result indicates that the cultures used would not have shown such a drastic die-off in OSBR-treated fruit had the OSBR-treated fruit been grossly damaged by brushing. Again, this provides more evidence that OSBR treatment does not physically compromise tomatoes in such a way that would allow enhanced pathogen survival during 7 days of storage at 25 °C and 75-85 %RH.

Finally, the OSBR system was assessed for its effect on tomato shelf life, using the attributes of full ripeness, incidence of shrivel, incidence of softness, and presence of disease as observational parameters. Presence of disease was rare and sporadic, not consistently associated with any one treatment. Under fall season retail storage conditions (40-50 %RH and 22 °C), OSBR-treated tomatoes were found to exhibit

significantly more shrivel without significant effect on ripeness or softness. To test whether waxing the fruit after washing would alleviate some of the shrivel, the study was repeated in the spring with a waxing step added. Waxing tended to hinder fruit ripening, though differences in ripeness between treatments were not significant by the last day of observation. In unwaxed samples, the OSBR treated samples tended to develop more shrivel and softness than the flume treated samples. However, there was little to no difference in terms of shrivel or softness between untreated, OSBR treated, and flume treated tomatoes when wax was applied. Additionally, a difference in seasonality was seen in that unwaxed tomatoes stored under spring retail conditions (40-50 %RH 22 °C) tended to develop significantly more shrivel and softness than those stored under fall retail conditions (50-60 %RH and 22 °C), likely due to differences in moisture loss as a function of ambient relative humidity. These results suggest that OSBR treatment may cause physical damage to tomato surfaces (or otherwise buff off the natural waxy coating of the tomatoes), which would explain the greater incidences of shrivel and softness compared to flume or no treatment. However, when a waxy coating is applied following washing, there appears to be little to no difference between washing treatments in terms of shelf life. Further studies could be done in which soft rot disease development is monitored during storage after tomatoes are flume or OSBR treated in the presence of *Pc*.

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BIOGRAPHICAL SKETCH

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