CPS CENTER for PRODUCE SAFETY

CPS 2016 RFP
FINAL PROJECT REPORT

Project Title
Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus

Project Period
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Objectives
The overall goal of the project is to provide data that the California fresh citrus packinghouse industry can use to support preventive controls that reduce or eliminate cross contamination where citrus fruits are comingled or where contact materials are reused. Specific objectives are:

1. *Determine the potential for* Salmonella *and* Listeria monocytogenes *to survive in float tanks, drench systems, and systems where the fungicides sodium bicarbonate, imazalil, and soda ash are recirculated/reused.*
2. *Determine minimum levels of compatible sanitizers or thermal treatments that control, reduce, or eliminate* Salmonella *and* L. monocytogenes *in recirculated/reused sodium bicarbonate, imazalil, and soda ash solutions.*
3. *Determine the potential for survival of* Salmonella *and* L. monocytogenes *on citrus fruit (predominantly Navel and Valencia oranges and Eureka lemons) during pre-shipping storage under typical and sub-optimal conditions.*

NOTE: Items in red were added to the original objectives.

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FINAL REPORT

Abstract
After harvest, fresh oranges and lemons are sorted, washed and packed for further distribution and sale in packinghouses. In California, the growth of *Penicillium* species that cause green and blue molds results in significant losses of citrus fruit during storage and shipping. To reduce these losses, one or more fungicides (e.g., sodium bicarbonate, imazalil, soda ash) are applied to citrus fruit during packing, often in recirculating, in-line drench applications that significantly increase efficacy when compared to low-volume spray applications. However, recirculation of fungicides that are not also bactericidal may result in increased bacterial loads and a potential for cross contamination among fruit and on equipment surfaces. While chlorine is routinely added to sodium bicarbonate (SBC) to maintain water quality in fruit immersion tanks or recirculating systems, data are lacking to support the efficacy of chlorine at the generally high pH (>8.0) of these solutions. The fungicide imazalil is widely used in the citrus industry but is incompatible with chlorine. Documented efficacy of alternative water sanitizers such as peracetic acid in combination with imazalil are lacking. Heated soda ash (40°C/104°F) in fruit immersion tanks is sometimes used in lemon packinghouses; the soda ash preparations are routinely heated to 60°C (140°F) on a daily basis to reduce microbial loads but the impact of this treatment on reducing levels of foodborne pathogens is unknown. Under laboratory conditions the minimum free chlorine concentrations at higher pH levels for SBC solutions and minimum peracetic acid concentrations in imazalil solutions were determined for reduction of *Salmonella* and *Listeria monocytogenes*. The survival of *Salmonella* and *L. monocytogenes* on the surface of oranges and lemons was determined as a function of surface location, inoculum carrier, and commercial storage conditions. The overall goal of the project was to improve the ability of the citrus industry to successfully apply validated preventive controls that prevent the spread of bacterial foodborne pathogens in recirculating fungicide applications used by the packinghouse industry.

Background
Fresh citrus has not been associated with outbreaks of foodborne illness. However, salmonellosis has been associated with citrus juices (Danyluk et al., 2012) and *Listeria monocytogenes* has been associated with recalls or cases of illness in other California tree fruits (apples, avocados, and stone fruit). In some outbreaks associated with citrus juice, there was evidence that contamination may have occurred in the packing/processing facility during washing or waxing of the fruit (Parish, 1998).

Most of the published research on foodborne pathogens in citrus packing facilities is tied to practices more common 15 years ago in Florida citrus juicing operations, and is focused on reduction of microorganisms (native and nonpathogenic *E. coli*) on the surface of oranges (Pao and Brown, 1998; Pao and Davis, 1999; Pao et al., 1999). Significantly greater microbial reductions on the orange surface were observed with inoculated (generic *E. coli*) than native populations and of those organisms at the midsection rather than the stem scar area. Fungicides relevant to California citrus were not evaluated in those studies nor was the potential for cross contamination among fruit during aqueous immersion or drenching of fruit.

In California, the growth of *Penicillium* species that cause green and blue molds results in significant losses of citrus fruit during storage and shipping. To reduce these losses, one or more fungicides (e.g., sodium bicarbonate, imazalil, pyrimethanil, fludioxonil, soda ash) are applied to citrus fruit during packing. Recirculating, in-line drench applications are commonly used with some fungicides because they significantly increase efficacy when compared to low-volume spray applications (Kanetis et al., 2008). Recirculation of fungicides that are not also bactericidal may allow for increases in bacterial loads and provide an opportunity for cross contamination among fruit.

In most produce packinghouses, sanitizers are used to maintain the quality of water that might be used to transport or wash product. However, not all fungicides used in citrus packinghouses are compatible with common sanitizers (e.g., chlorine and peracetic acid [PAA]) used to maintain packinghouse water quality (Kanetis et al., 2008).

Brief immersion of citrus fruit in solutions of sodium bicarbonate (SBC; $NaHCO_3$) in a tank or recirculating flood reduces the incidence of postharvest green mold (Smilanick et al., 1999). Chlorine is routinely added to sodium bicarbonate to reduce microbial loads in the solution during routine use. Most of the microbicidal activity of chlorine is attributed to undissociated hypochlorous acid (HOCl); the dissociated hypochlorite ion (OCl^-) is less microbicidal. The balance of the two forms of hypochlorite in water changes dramatically with pH. At pH 6.5 to 7.0 the faster-acting antimicrobial form, HOCl, makes up 95 to 80% of the "free chlorine." This level drops to less than 20% at pH levels greater than 8.0. Higher levels of free chlorine are thus needed to compensate for increasing pH. A disadvantage of SBC is that carbon dioxide evolution over time results in an increase in pH to well above 9. Data are lacking to support the use of chlorine as an effective antimicrobial at these higher pH levels and with the organic loads expected during commercial operation.

The effectiveness of SBC is significantly improved when the treatment is followed by application of the fungicide imazalil (Smilanick et al., 1999). Imazalil is not compatible with chlorine but can be used with some formulations of PAA. However, packinghouses have been reluctant to use PAA, in part, due to the lack of data on the efficacy of PAA in combination with imazalil, especially under conditions of normal use. Heated soda ash (40°C/104°F) in immersion tanks is sometimes used in lemon packinghouses; the soda ash preparations are routinely heated to 60°C (140°F) to reduce microbial loads, but the impact of this treatment on reducing levels of foodborne pathogens is unknown.

With the finalization of the Produce Safety and Preventive Control Rules, many commercial California citrus packinghouses need to characterize potential public health risks of standard practices (hazard analysis) and apply appropriate science-based mitigation strategies (preventive controls) to current standard practices. The packing facility will be responsible for documenting the scientific basis for the effectiveness of preventive controls that reduce or eliminate foodborne pathogens, including those controls that prevent cross contamination of ready-to-eat foods. There was a 3-year implementation period for the preventive controls rule (2016–2018); California will begin inspections for the Produce Safety rule in 2019.

This study assessed the ability of fungicides commonly used in California citrus packinghouses to serve as reservoirs for cross contamination of oranges and lemons with foodborne pathogens, and evaluated appropriate mitigation strategies designed to decrease or eliminate this potential. The ability for foodborne pathogens to survive on citrus fruit during typical storage conditions was also evaluated.

Research Methods and Results

Throughout these studies, 16°C (60°F) and 40°C (104°F) were used as holding temperatures. These temperatures were chosen based on an informal survey among project partners, and represent minimum and maximum temperatures typically used in California packinghouse facilities.

1. Methods

Bacterial strains and inoculum preparation

Six rifampin-resistant strains of *Listeria monocytogenes* and six strains of *Salmonella enterica* isolates from produce commodities were used (Table 1). Frozen stock cultures of the strains were streaked on tryptic soy agar (TSA) supplemented with rifampin at 50 µg/ml (TSAR) and incubated at 37°C for 24 h. A loop of this culture was transferred two successive times into 10

ml of tryptic soy broth and incubated at 37°C for 24 h. An aliquot (20 µl) of the second overnight culture was plated on TSAR with a spiral plater set on the lawn mode. Plates were incubated at 37°C for 24 h, and the resulting bacterial lawn was collected in 5 ml of 0.1% peptone. The resulting cell suspensions were vortexed and then equal volumes (1 ml) of each strain were mixed to prepare the cocktail. Separate cocktails were prepared for *L. monocytogenes* and *Salmonella* and diluted in 0.1% peptone to the final concentration of ~7.0 or 8 log CFU/ml.

Table 1. Rifampin-resistant strains used in the study, with parent strain ID indicated.

Pathogen	ID	Isolate origin
L. monocytogenes	LIS0234	Raw diced yellow onions
	LIS0235	Diced yellow onions
	LIS0133	Environmental fresh cut celery
	LIS0110	Whole cantaloupe
	LIS0087	Environmental cantaloupe packinghouse
	LIS0077	Environmental cantaloupe packinghouse
Salmonella Gaminara	F2712	Orange juice outbreak 1995
Salmonella Rubislow	F2833	Orange juice outbreak 1999
Salmonella Muenchen	LJH1337	Orange juice outbreak 1999
Salmonella Montevideo	G4639	Tomato outbreak 1993
Salmonella Enteritidis PT30	LJH636	Raw almond outbreak 2001

Chemicals and test solutions

The fungicide imazalil (Deccozil® EC-289 [22.2% imazalil]; Decco, Monrovia, CA) and the sanitizer peracetic acid (PAA) (Perasan A [5.6% peracetic acid and 26.5% hydrogen peroxide]; Enviro Tech Chemical Services, Inc., Modesto, CA) were kindly supplied by Decco US Postharvest. Imazalil (IMZ) solutions with a target concentration of 300 ppm were prepared in the laboratory by diluting the concentrated imazalil product with water obtained from a California citrus packing facility; IMZ concentrations of the prepared solutions were determined by Decco US Postharvest. PAA concentrations were determined by using a reflectometer (RQflex plus 10 Reflectoquant; Merck, Darmstadt, Germany) following the manufacturer's instructions. 3% SBC (pH 8.11) and 3% soda ash solution were prepared just before the inoculation experiment. The pH of the 3% SBC solution was raised to 9.6 by leaving the solution stirring for 24 h at ambient temperature.

Inoculation of solution, recovery, and enumeration

The cocktail inoculum of *L. monocytogenes* (6 strains) or *Salmonella* (first three strains in Table 1) (100 µl), at a concentration of ~7.0 log CFU/ml, was added to sterile 15-ml conical Falcon tubes containing 9.9 ml of test solution and the temperature was adjusted to 16°C (60°F) or 40°C (104°F) using an Eppendorf Thermomixer C (Eppendorf, Germany). The test solution was prepared with packinghouse water alone (control) or with 3% SBC–pH 8.11 or 3% SBC–pH 9.6 or 300 ppm IMZ. PAA was added at target concentrations from 0 to 60 ppm to the IMZ test solution. Chlorine was added at target concentrations from 10 to 20 ppm to the SBC test solution. Each tube was mixed manually by inverting the tube five times before each sampling

time point. A 1-ml aliquot of the inoculated control or inoculated fungicide solution with or without sanitizers was placed into 9 ml of Dey-Engley (D/E) broth (Difco, BD) to neutralize the PAA or chlorine. To neutralize soda ash, D/E broth was modified to include 0.1 M phosphate buffer (pH 6.7). The surviving population was determined by plate count on TSAR; the remaining sample was incubated at 37°C for 48 h, and the presence of the pathogens in the enrichment broth was confirmed by plating on selective agar (CHROMagar Listeria and CHROMagar Salmonella) after incubation at 37°C for 24 h.

Inoculation of lemons or oranges, recovery and enumeration
Unwashed lemons (Eureka) or oranges (Navel), obtained from a lemon or orange packinghouse in the Central Valley, CA, were marked with circles (about 2.5-cm in diameter) at the midsection for both citrus and at the blossom end for oranges. Lemons or oranges were inoculated by spotting 10 µl (in 10 1-µl drops) of the *L. monocytogenes* cocktail (6 strains) (~8 log CFU/ml) or *Salmonella* cocktail (6 strains) within the marked circular area with a repeater pipettor (M4, Eppendorf). Different carriers were used for the cocktail: ultrapure water, 0.1% peptone, 300 ppm IMZ, 3% SBC at pH 8.2 or 9.6. Inoculated lemons or oranges were allowed to dry for 1 h in a biosafety cabinet resulting in a final *L. monocytogenes* concentration of ~6 log CFU per circle. At each sampling time point, the circles were excised with a sterile scalpel and then placed in a 0.7-oz filter bag by using sterile tweezers. Samples were processed by adding 10 ml D/E broth and shaking for 30 s, rubbing for 15 s, and shaking again for 30 s. The bacterial population levels on the inoculated lemons were confirmed by plating on TSAR or by enrichment, as described in the previous paragraph, when *L. monocytogenes* was not recovered by plating.

Enumeration of bacteria on lemons collected in the packinghouse washing line
Twenty lemons were collected with sterile gloves at receiving, from the soda ash tank, and at hand grading after application of storage wax, for a total of 60 lemons. Individual lemons were placed in 24-oz sterile bags with 20 ml of 0.1 M phosphate buffer (pH 6.7) and 0.02% tween at 15 min after being collected from the soda ash tank and 6 h after being collected for all other lemons. Lemons were shaken for 30 s, rubbed for 60 s and shaken again for 30 s. Soda ash was collected in sterile bottles and neutralized by adding 0.1 M phosphate buffer (pH 6.7, 0.8% sodium thiosulfate [final concentration]) within 5 min of sampling. The total bacterial population level on the lemons and in the soda ash was enumerated by plating on plate count agar (PCA) with a spiral plater and incubating at ambient temperature for 48 h. Coliforms and *E. coli* were enumerated by plating on CHROMagar ECC and incubating at 37°C.

Water analysis
Packinghouse water and end-of-use imazalil solutions were evaluated for various quality parameters. Turbidity and total suspended solids (TSS) were determined with a portable colorimeter (DR/850; Hach, Loveland, CO) according to the manufacturer's absorptometric method 8237 and photometric method 8006, respectively. Chemical oxygen demand (COD) was determined by the reactor digestion method using a Hach DR/870 colorimeter; the DRB200 reactor (Hach) was used for sample digestion, with a digestion time of 120 min at 150°C. Measurement of pH was performed with an S220 SevenCompact pH/Ion meter (Mettler-Toledo, Columbus, OH), and conductivity was determined with an S230 SevenCompact conductivity meter (Mettler-Toledo). Analysis for total iron, zinc, and copper was performed at the UC Davis Analytical Laboratory (Davis, CA) by a nitric acid/hydrogen peroxide microwave digestion and atomic absorption spectrometry method.

Bacterial cross contamination in a lab-based system, bacterial recovery and enumeration
The transfer of *L. monocytogenes* from inoculated to uninoculated citrus, in a 350 ppm IMZ solution containing 0 or 20 ppm PAA for oranges or in 0 or 3% soda ash for lemons, was

evaluated under laboratory conditions. Unwashed oranges or lemons, obtained directly from citrus packinghouses in California, were inoculated as described above. Inoculated oranges or lemons were allowed to dry for 2 h in a biosafety cabinet, resulting in a final *L. monocytogenes* concentration of ~6 log CFU per orange or lemon. To determine the transfer of *L. monocytogenes* from inoculated to uninoculated citrus, two inoculated and two uninoculated oranges were placed into a plastic container containing 2 liters of imazalil solution (350 ppm) adjusted to 40°C; or two inoculated and two uninoculated lemons were placed into a plastic container containing 2 liters of water or 3% soda ash. Each container was continuously shaken at 100 rpm on a shaker (MaxQ 2508; Thermo Scientific, Waltham, MA). At specific time points (1 and 3 min) immediately after circulation of oranges or lemons in the solutions, one inoculated and one uninoculated orange or lemon were placed into separate 700-ml Whirl-Pak filter bags (Nasco, Modesto, CA) containing 50 ml of D/E broth (oranges) or D/E broth supplemented with 0.1 M sodium phosphate buffer (pH 6.7). Each sample was shaken (0.5 min), rubbed (1 min), and shaken again (0.5 min) by hand to dislodge *L. monocytogenes* cells. The surviving population was determined by plate count on TSAR and reported as CFU per orange or lemon. To enumerate the population of *L. monocytogenes* in the IMZ solution or in the soda ash solution within the container, three 1-ml samples were immediately transferred into separate 15-ml Falcon test tubes containing 9 ml of D/E broth or D/E broth supplemented with 0.1M phosphate buffer (pH 6.7), and 1 ml of each mixture was plated onto four TSAR plates (250 µl/plate) and incubated at 37°C for 24 h.

2. Results

Objectives 1 and 2:

Sodium bicarbonate (SBC) and chlorine. The survival of a cocktail of *L. monocytogenes* and *Salmonella* was evaluated in 3% SBC at pH ~8.0 and ~9.5 at 16°C (60°F). No significant reduction of either pathogen was observed in 1 min in the absence of chlorine (0 ppm). A ≥5-log reduction of *L. monocytogenes* could be achieved in 20 s at 10 or 15 ppm free chlorine at pH ~8.0 and ~9.5, respectively. To evaluate the efficacy of chlorine in presence of organic material, SBC samples were collected at the end of use at two different orange packinghouses (pH 9.0 and COD 1400 or 1800), and the survival of *L. monocytogenes* and *Salmonella* was evaluated alone or in the presence of 10 and 15 ppm of free chlorine at 16°C. Free chlorine (10 and 15 ppm) resulted in a ≥5-log reduction of *L. monocytogenes* and *Salmonella* within 20 sec in both end-of-use SBC samples.

Imazalil and peracetic acid. Peracetic acid (PAA; 20 to 60 ppm) was added to a target ~300 ppm imazalil (prepared in the laboratory with citrus packinghouse water) to determine a concentration of PAA that would achieve a ≥5-log reduction of a six-strain cocktail of *Listeria monocytogenes* and a three-strain cocktail of *Salmonella* in ≥2 min at 16 or 40°C (60 or 104°F). In addition, survival of the *L. monocytogenes* cocktail in the presence of PAA was evaluated in three different “used” imazalil solutions that were collected from three different orange packinghouses. The *Salmonella* cocktail was more sensitive to PAA than the *L. monocytogenes* cocktail, and both pathogens were significantly ($P < 0.05$) more sensitive to PAA at the higher temperature. At 16°C, ≥5-log reductions of *L. monocytogenes* and *Salmonella* were observed in ≥2 min in the presence of 30 and 20 ppm PAA, respectively. At 40°C, ≥5-log reductions of *L. monocytogenes* and *Salmonella* were observed in ≥1 min in the presence of 20 ppm of PAA. Cross contamination from *L. monocytogenes*–inoculated to uninoculated oranges was prevented at 20 ppm in imazalil at 16°C. No significant difference was observed in the reduction of *L. monocytogenes* in the lab-prepared and used imazalil solutions from two packinghouses.

However, the initial reductions of *L. monocytogenes* were significantly greater in one used imazalil solution of one packinghouse.

Soda ash. Based on early discussions with the citrus industry, evaluation of 3% soda ash was added to Objectives 1 and 2. Observations at lemon packinghouse facilities and discussions with operators suggested that higher temperatures are more commercially relevant when using soda ash. In addition, soda ash preparations are routinely heated at night to reduce microbial populations. No significant reduction of *L. monocytogenes* was observed in 3% soda ash at 16 or 40°C over 5 min. At 16°C no reduction of *L. monocytogenes* was observed after 8 h, whereas at 40°C decreases of 2.5 log CFU/ml were observed after 30 min. At 16°C, 3.72 and ≥5 log reductions of *Salmonella* were achieved after 2 h and 8 h, respectively. At 40°C, 1.36 and ≥5 log reductions were observed after 30 sec and 5 min, respectively. To evaluate the effect of higher temperatures, *L. monocytogenes* survival in soda ash was tested at 45, 50, 55, and 60°C; heating at 60°C (140°F) but not 55°C (131°F) reduced *L. monocytogenes* populations by >5 log in 1 min. Cross-contamination from *L. monocytogenes*-inoculated to uninoculated lemons could be demonstrated in lab-based experiments in soda ash at both 16 and 40°C (60 and 104°F).

In preliminary experiments, lemons that had been harvested from one orchard and held for 2 days under de-greening storage conditions were collected at receiving, after the soda ash tank, and at hand grading after application of storage wax but prior to storage. Total bacterial counts of 5.03 ± 0.35, 2.88, and 4.13 log CFU/lemon were measured at the receiving step, after the soda ash tank, and at hand grading, respectively. However, the neutralization buffer was only added 15 min after sampling lemons at the soda ash tank; reduction of microbial populations during this time could not be ruled out. Coliforms and *E. coli* were under the limit of detection by plating (80 CFU or 1.9 log CFU/lemon). The bacterial load in the soda ash was 1.53 log CFU/ml.

Objective 3:
Preliminary experiments were conducted to compare the survival of *L. monocytogenes* and *Salmonella* on orange surfaces using different inoculum carriers at ambient temperature 23°C (73°F) and 45% relative humidity. After 24 h, *L. monocytogenes* and *Salmonella* populations declined by 1 to 1.5 log CFU with a 0.1% peptone carrier. A >3.65-log reduction was observed when water, IMZ (300 ppm), 3% SBC (pH 8.2), or 3% SBC (pH 9.6) was used as the carrier. Water and 0.1 % peptone, the least and most supportive carriers, respectively, were selected to inoculate unwashed lemons. After harvest and before washing, lemons and oranges can be stored up to 7 days for de-greening. *L. monocytogenes* and *Salmonella* survival on lemons and oranges was evaluated under de-greening conditions at 19°C (66°F) and 80% (lemons) or 90% (oranges) relative humidity. *L. monocytogenes* survived significantly better than *Salmonella* on both lemons and oranges. Both pathogens survived significantly better on lemons than on oranges. Oranges were inoculated at an initial ~6 log CFU with 0.1% peptone carrier at the midsection and the blossom end. There were no significant differences in the survival of both pathogens at the midsection and the blossom end; therefore, data were combined. After 7 days in storage, *L. monocytogenes* populations were 5.02 ± 0.24 and 4.43 ± 0.55 CFU on lemons and oranges, respectively; *Salmonella* populations were 4.59 ± 0.14 and 2.91 ± 0.74 log CFU/ disk on lemons and oranges, respectively. Over 7 days, *L. monocytogenes* and *Salmonella* populations declined by 1.26 and 2.1 log CFU on lemons, respectively, and by 1.6 and 3.12 log CFU on oranges, respectively. The survival of *L. monocytogenes* was also evaluated at 12°C (54°F) and 80% relative humidity, conditions that simulate commercial storage in a packinghouse. Populations of *L. monocytogenes* in both water and 0.1% peptone carriers decreased by 3.41, 4.22, and 4.85 log after 7, 13, and 19 days of storage, respectively, when inoculated at an initial population of 6.5 log CFU. After 26 days, a >5-log reduction was observed and samples were negative upon enrichment.

Outcomes and Accomplishments

- *L. monocytogenes* and *Salmonella* survival was evaluated in sodium bicarbonate, imazalil, and soda ash.
- Concentrations of chlorine and PAA were identified that result in rapid reduction of *L. monocytogenes* and *Salmonella* in both freshly prepared and end-of-use sodium bicarbonate and imazalil solutions, respectively.
- Temperatures that rapidly reduce *L. monocytogenes* in heated soda ash were determined.
- Survival of *L. monocytogenes* and *Salmonella* on oranges (Navel) and lemons (Eureka) were determined under de-greening conditions and weeks of simulated commercial storage.
- Results were communicated to the citrus packinghouse industry via presentations and fungicide-specific fact sheet (imazalil) and through several meetings and conference calls.

Summary of Findings and Recommendations

This project has generated significant new findings, relative to factors affecting pathogen reduction in citrus fungicides. The key findings and recommendations are as follows:

- In 3% SBC, 18 ppm free chlorine resulted in a >5-log reduction of *L. monocytogenes* and *Salmonella* within 20 s at both pH ~8.0 and ~9.5. Similar results were achieved in end-of-use SBC solutions.
- In 300 ppm imazalil at 16°C (60°F), >5-log reductions of *Salmonella* and *L. monocytogenes* were achieved with 30 and 60 ppm of PAA, respectively, within 1 min; 10 and 20 ppm of PAA, respectively, were sufficient at 40°C (104°F). Cross contamination from inoculated to uninoculated fruit was prevented at these PAA levels.
- No significant difference was observed in the reduction of *L. monocytogenes* in lab-prepared and end-of-use imazalil solutions from two packinghouses, and initial reductions of *L. monocytogenes* were significantly greater in one end-of-use imazalil solution.
- In 3% soda ash, no significant reduction of *L. monocytogenes* was observed at 16°C for 8 h, but a 2.5-log reduction was obtained at 40°C in 30 min. Reduction of *Salmonella* was more rapid in soda ash; a >5-log reduction of *Salmonella* was observed in 3 min at 40°C and in 8 h at 16°C. A >5-log reduction in *L. monocytogenes* was achieved in 1 min when the soda ash was heated to 60°C.
- Under de-greening conditions over 7 days, *L. monocytogenes* and *Salmonella* declined by 1.26 and 2.1 log CFU on lemons, respectively, and by 1.6 and 3.12 log CFU on oranges, respectively. *L. monocytogenes* populations declined by >5 log on lemon surfaces after 26 days at 12°C (54°F) and 80% relative humidity, conditions that simulate commercial storage in a packinghouse.

APPENDICES

Publications and Presentations:

1. Dissemination of research findings to stakeholders

June 20–21, 2017. PI attended the CPS Annual Symposium, and gave a 5-min presentation and attended a poster session describing the work to date.

August 23, 2017. The PI and postdoctoral fellow attended a citrus industry meeting arranged by the California Citrus Quality Council (CCQC) in Lindcove, CA, at the University of California citrus research facility, and gave the following presentation to approximately 40 attendees: "Fresh citrus food safety fungicide application" and "Research update: Characterization and mitigation of *Salmonella* and *Listeria* risks." Results were discussed and feedback on the upcoming research was sought. A better understanding of citrus industry challenges was gained including information on the types of scientific data needed to respond to audits regarding cross contamination risks in their recirculating or tank systems.

September 5, 2017. A 1.5-h phone conference call was held with eight invited participants (CCQC), some of whom were unable to attend the Lindcove meeting. Research results and the strategies for next steps were discussed.

September 12, 2017. A call was made with a citrus postharvest disease specialist to continue the discussions on research strategies, especially with respect to standardization of "organic load" in laboratory experiments.

November 15, 2017. The PI and postdoctoral fellow attended a 1.5-h conference call meeting with service companies arranged by Sunkist, Inc. Research results and the strategies, in particular on the soda ash tanks in lemon packinghouses, were discussed. Some industry concerns regarding the preventive control rule of FSMA were also discussed, and the PI clarified some FSMA concepts.

March 8, 2018. PI attended a CPS video conference meeting, and gave a presentation on the update of the project progress.

March 13, 2018. The PI and postdoctoral fellow visited a lemon packinghouse (Kink Citrus, Ivanhoe, CA) and had a meeting with representatives of the citrus industry (food safety and technical service managers at Sunkist Growers and Decco Postharvest, Inc.). Some discussions and decisions were made for the next step research strategies on the soda ash experiments in laboratory and in the packinghouse facility.

April 16, 2018. The PI visited two lemon packinghouses in Oxnard, CA, and met with representatives of the citrus industry (food safety and technical service managers at Sunkist Growers and Decco Postharvest, Inc.).

2. Presentations

April 17, 2018. The PI presented some project results to ~60 members of the citrus industry. "*Fate of* Salmonella *and* Listeria monocytogenes *in imazalil with and without added peracetic acid and in soda ash*", 39th Annual Citrus Postharvest Pest Control Conference, Oxnard, CA.

June 19–20, 2018. PI and postdoctoral fellow attended the 2018 CPS Research Symposium. The PI gave a presentation in the Research Session Part 1 – Packinghouse and Processing Plant Sanitation and Wash Water Control.

July 9, 2018. The postdoctoral fellow presented a poster at the 2018 IAFP Annual meeting (funding to attend the meeting provided from other sources). Shiroodi, S. G., and L. J. Harris. 2018. Survival of *Listeria* in imazalil with added peracetic acid and in soda ash fresh citrus fungicide solutions, (Abstract P1-184), Annual IAFP Meeting 2018, Salt Lake City, UT, July 8–11.

3. Publications

Fact sheets are in preparation to support the California fresh citrus packinghouse industry by providing our scientific lab data and information to prevent cross contamination in their tanks or recirculating fungicide systems. One completed fact sheet (on imazalil/PAA – see below) has been shared with the industry and one on sodium bicarbonate is in preparation.

Shiroodi, S. G., and L. J. Harris. 2018. Efficacy of peracetic acid (PAA) in reducing *Listeria monocytogenes* and *Salmonella* in imazalil preparations.

Two manuscripts for publication in a scientific journal are in preparation.

Budget Summary

Total funds awarded were $171,393. Overall, the project expenditures have followed the proposed budget closely. The only notable difference was that less was spent on travel than expected because the proposed packinghouse trials were replaced by an evaluation of soda ash use in lemon packinghouses.

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