CENTER for PRODUCE SAFETY

CPS 2021 RFP
FINAL PROJECT REPORT

Project Title
Validation study for the tree fruit industry: effective strategies to sanitize harvest bins and picking bags

Project Period
January 1, 2022 – December 31, 2023

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Objectives

1. *Evaluate the effectiveness of commercially available sanitizers in reducing Listeria monocytogenes, Salmonella, and Shiga-toxigenic E. coli on experimentally inoculated coupons representative of food contact surfaces commonly used in harvest bags and bins in the apple industry.*
2. *Validate the selected sanitizer strategies in representative commercial apple orchards and packinghouses.*

Funding for this project was provided partly through the CPS Campaign for Research

FINAL REPORT

Abstract

Foodborne outbreaks and recalls within the tree fruit industry are making producers re-evaluate appropriate cleaning and sanitation practices during harvesting. Without effective sanitation, bacteria can create niches and form biofilms. Validation of different sanitation methods can help producers develop strategies to reduce and mitigate risks.

This study had two main objectives: 1) evaluate the efficacy of five commercially available sanitizers to control and reduce *Listeria*, *Salmonella,* and *Escherichia coli* sessile and biofilm forms on experimentally inoculated coupons (nylon, high density polyethylene [HDPE] plastic, and wood) representative of harvest bins and picking bags; 2) select two strategies and validate their efficacy to control sessile and biofilm forms on experimentally inoculated harvesting equipment at commercial orchards within the Midwest and Pacific Northwest regions.

For the laboratory experiments (objective 1) sessile bacteria were grown on tryptic soy agar (TSA) plates, and lawns were harvested using 0.1% peptone water. Solutions were transferred into a sterile tube and each strain was combined in equal quantity to create a cocktail for each pathogen. These cocktails were used to experimentally spot-inoculate the selected coupons representing food contact surfaces. For biofilm formation, multi-strain microbial solutions were grown in a Centers for Disease Control and Prevention reactor at 25 ± 2 °C for 96 hours. Experimentally inoculated coupons (with sessile form or biofilm) were exposed to 500 ppm chlorine, 500 ppm peracetic acid, 75 psi steam, and 4% silver dihydrogen citrate for 1 or 2 minutes, and 100 ppm chlorine dioxide gas for 24 hours. The remaining cells were enumerated. Samples were serially diluted and incubated for 24 hours at 37 ± 2 °C.

Overall, for both bacterial forms a higher log reduction was observed on plastic surfaces, as compared to nylon and wood ($P<0.05$). The type of material had a significant effect on microbial recovery after treatment application ($P<0.05$). An effect of treatment time was also observed ($P< 0.05$), with longer treatments having a greater log reduction. The lowest log reduction was observed on wood ($P>0.05$).

Silver dihydrogen citrate and chlorine dioxide gas (ClO_2) were selected for the validation study (objective 2). Rifampicin-resistant *E. coli* and *Listeria* were grown in tryptic soy broth supplemented with rifampicin at 80 µg/ml for either 24 hours or 96 hours on plastic (HDPE), wood, or nylon. Surfaces were allowed to dry for 1 hour and then exposed to chlorine dioxide gas (100 ppm) for 24 hours or silver dihydrogen citrate (4%) for 2 minutes. Coupons were swabbed, and remaining populations enumerated. Temperature and relative humidity conditions were noted.

ClO_2 was the most effective treatment ($P<0.05$) in controlling sessile *E. coli* and *Listeria* on HDPE and nylon in the Midwest and Pacific Northwest region trials. A lower level of inactivation was observed for biofilms grown on wood after chlorine dioxide treatment ($P<0.05$). In both regions, all biofilm populations on HDPE were reduced after exposure to silver dihydrogen citrate ($P<0.05$). Silver dihydrogen citrate exposure did not reduce the population of sessile *E. coli* on HDPE in the Pacific Northwest region, or on any form of *E. coli* on nylon in either region ($P>0.05$).

Background

Tree fruits have been implicated in multi-state foodborne illness episodes, with the latest apple recall in October 2019 for *Listeria* concerns, serving as a prominent reminder. Several of these incidents have been associated with poor sanitation practices during harvesting and handling through contact with contaminated equipment, transport containers, knives, tools, operator hands and gloves. When harvesting tools, bins and containers are not effectively cleaned and sanitized, microbial communities with foodborne pathogens may develop, providing a reservoir for continuous produce contamination. Additionally, apples are a storage crop and will be held in harvest bins for up to 9–12 months in controlled atmosphere or cold storage. After, bins are stored outside until use in the next crop season. Both scenarios allow for contamination and growth of foodborne pathogens such as *Listeria, Salmonella* and *Escherichia coli*.

Good sanitation and postharvest handling practices are essential to ensure the safety and improve the quality of produce. The lack of effective science-based recommendations for sanitation of harvest bins and picking bags have been increasingly posing pressure for the produce industry, which needs to determine effective strategies for managing these surfaces in order to control and reduce the risk of microbial contamination. Current guidelines suggest cleaning and sanitation of harvesting tools as needed and/or on a regular basis. However, sanitizers used by the produce industry are designed to be applied on non-porous surfaces, which does not help growers and packers facing the realities of porous materials such as wood and nylon fabric regularly used during tree fruit harvest.

To date, researchers have been focusing mainly on postharvest interventions to control *Listeria* and other pathogens in the tree fruit supply chain. However, there is a critical need to support the development of science-based and effective on-farm and/or packinghouse cleaning and sanitation strategies for harvest bins and picking bags.

As a general food safety recommendation, food contact surfaces must be first cleaned and then sanitized. The cleaning step allows removal of food, soils and organic debris that could potentially react with the sanitizer, thus lowering efficacy. An ideal sanitizer should have a broad antimicrobial spectrum, be effective in the presence of organic load, be stable, have good cleaning properties, be non-toxic, odorless, easy to use, readily available, and low cost. Several strategies are currently available for the produce industry:

- **Chlorine**-based sanitizers are widely applied in the fresh produce industry. Their antimicrobial efficacy largely depends on pH and organic load. These agents are relatively low cost, effective but can present disadvantages such as corrosivity and production of carcinogenic and mutagenic compounds.
- **Chlorine dioxide** (ClO_2) is a strong oxidizing compound with a broad antimicrobial spectrum. This agent does not react with nitrogen or ammonia to form harmful by-products and it has low reactivity with organic matter, as compared to chlorine. It is also less corrosive. Aqueous and gaseous forms are both available and approved for food-contact surfaces. Usually, aqueous solutions are less effective than gas, especially on porous surfaces. Nevertheless, some disadvantages exist for the gaseous form, such as the need to be generated on-site, the dependency on pH, and degradation by light.
- **Peracetic acid** or **peracid** (PAA) is also commonly used in the fresh produce industry. This agent is a strong, fast-acting sanitizer that works on the same basis as chlorine. PAA does not react with proteins to form toxic or carcinogenic compounds and its efficacy has been studied on several commodities and against different foodborne pathogens.
- **Steam** is a quick and effective treatment to reduce pathogens and spoilage microorganisms on produce and food-contact surfaces. Nevertheless, it requires energy-efficient systems to

ensure high water temperatures. Time, temperature, and pressure relationships need to be considered for an effective use of this strategy.

- **Silver dihydrogen citrate** (SDC) is a relatively new ready-to-use sanitizer that has a broad spectrum of activity against bacteria, fungi, and viruses. This agent is a blend of electrolytically generated silver ions in citric acid.

Our study focused on: 1) validating the above-listed cleaning and sanitation practices to minimize contamination risks during harvesting and handling; and 2) encouraging growers to implement these routines through data sharing and education.

Furthermore, this research expanded a previously funded project by the Center for Produce Safety (CPS), "Assessment of sanitation techniques for tree fruit storage bins," where the effect of several cleaning and sanitation practices (chlorine, peracetic acid, pressure washing and heat treatment alone and in combination) were investigated on the background microflora present on tree fruit bins.

Two bacteria forms were investigated in this project: **sessile** and **biofilm**. The sessile form is the first state of confluent growth of bacteria on agar plates, while a biofilm is a mature and more complex form of a bacterial community living in a complex matrix of extracellular polymeric substance (EPS) and nucleic acids, in addition to the microorganisms. Both conditions are relevant and practical to understand sanitizers' effectiveness and microbial susceptibility. The sessile conditions gave us information on facilities that practice cleaning and sanitation on a frequent basis, since bacteria will be attached on surfaces for less time (24 hours, representing sanitation once a day). The mature biofilm conditions instead simulated sporadic sanitation and handling practices. Bacteria are strongly attached to the surfaces and embedded in the EPS. Biofilms are usually 100 to 1000 times more resistant to sanitizers as compared to their free-floating planktonic form.

Research Methods

Objective 1

Bacterial strains. All strains were selected because they were isolated from produce or produce-related outbreaks. A mixture of three strains of *L. monocytogenes* were used for this study: LM 390-1 (serotype 1/2a) and LM390-2 (serotype 1/2b), both environmental isolates from the 2011 cantaloupe outbreak, and LM573-035 (serotype 4b), a clinical isolate from the 2014-2015 caramel apple outbreak. The *Salmonella* strains were: *S.* Enteritidis ATCC BAA-1045 from raw almonds, *S.* Agona LJH 517 from alfalfa sprouts, and *S*. Newport ATCC 6962 from a clinical isolate. A mixture of three Shiga toxin-producing *E. coli* (STEC) was used as well: *E. coli* O157:H7 KSU 31 from an apple juice outbreak, *E. coli* O104:H4 a clinical isolate from the 2011 German sprouts outbreak, and *E. coli* O45:H2 from a clinical isolate.

Food contact surfaces (FCS) selection. Wood, plastic (HDPE), and nylon from used harvest bins, as well as used picking bags, served as surfaces for microbial attachment and growth. These materials were selected because they are commonly used during harvesting operations. Fabrics and wood were cut into coupons of 1.4 cm by 5 cm (with a total area of 7 cm^2) for the sessile form experiments, while smaller coupons of 1.27 cm diameter were fabricated to fit into the Centers for Disease Control and Prevention (CDC) Biofilm Reactor.

Sessile growth conditions and inoculation of FCS. Bacterial lawns were allowed to grow on tryptic soy agar (TSA) plates overnight at 35°C. Lawns were harvested using 0.1% peptone water and a sterile spreader to gently scrape the bacteria from the agar. Solutions were transferred into a sterile tube and each strain combined in equal quantity to create a cocktail (~10^9 CFU/ml) for each pathogen. Cocktails were used to experimentally spot-inoculate the selected FCS in order to obtain a final population of 10^6 CFU/cm^2.

Biofilm growth conditions and inoculation of FCS. A CDC Biofilm Reactor was used to grow *Listeria*, *Salmonella* and *E. coli* biofilms (**Figure 1**). The system consists of a 1-liter glass vessel with a liquid capacity of up to 500 ml. Eight independent removable rods hold three coupons each of 1.27 cm diameter that serve as the biofilm growth surface. The coupons were cleaned following the manufacturer's instructions before use (BioSurface Technologies Corp., Bozeman, MT). A protocol developed in our lab was followed to grow *Listeria* biofilms. Briefly, the reactor with sterile media (TSB at a concentration of 3 g/l) was placed on a stir plate and a constant flow of nutrients was provided. The baffle speed was set at 120 rpm and the overnight cocktails (strains combined in equal quantity to reach ~10^9 CFU/ml) used for inoculation at 1%. The reactor operated in batch for the first 24 hours to allow initial attachment. A continuous media flow was then pumped into the reactor at 11.7 ml/min for the remaining experiment time (up to 3 days). Biofilms were grown at 25 ± 2 °C. A similar procedure was used to grow *Salmonella* biofilms [14] and *E. coli* biofilms, but the media flow during the continuous phase was pumped at 6.9 ml/min [15] A population of 10^8 CFU/cm^2 was achieved on the coupons after 3 days of growth for all bacteria tested.

Antimicrobial Treatments. The selected FCS were experimentally inoculated with the sessile or form biofilm. Experimentally inoculated coupons were then exposed to 500 ppm chlorine, 500 ppm peracid, 75 psi steam, and 4% SDC for 1 or 2 minutes, and 100 ppm chlorine dioxide gas for 24 hours. FCS inoculated, but not treated, were considered as positive controls (for each pathogen cocktail).

Analysis of remaining population. After each treatment, coupons were neutralized with Dey/Engley broth (D/E) and vortexed for 30 sec. For biofilm enumeration, a sonication step (of 30 sec) was also performed to assure complete microbial detachment. Serial dilutions were made in 0.1% peptone water and spread-plated on selected media: Modified Oxford Media (MOX) agar for *Listeria*, Xylose-Lysine-Deoxycholate (XLD) agar for *Salmonella*, and MacConkey (MAC) agar for *E. coli*. Plates were incubated at 35 ± 2 °C for 24 h, colonies counted, and results recorded and expressed in log CFU/coupon.

Objective 2

Locations selected for validation study. Small-scale handling and processing apple facilities were represented by two facilities in Kansas and Iowa. Both operations (family-owned) included a cold storage and packing facility, and a cider pressing line. Two large-scale growers and packers located in Washington State were also identified for the validation study. These facilities were vertically integrated operations that pack over 7 million boxes of apples, cherries and pears each year.

Bacterial surrogate strains. The following rifampicin-resistant surrogates were chosen based on our previous studies and knowledge: *L. innocua* ATCC 51742 and *E. coli* TVS 353.

Antimicrobial efficacy of the selected sanitizer treatments. Based on the results obtained from objective 1, SDC and chlorine dioxide gas were selected for the validation study. Rifampicin-resistant *E. coli* and *Listeria* were grown in tryptic soy broth (TSB) supplemented with rifampicin at 80 µg/ml for either 24 hours or 96 hours at 25 ± 2 °C on plastic (HDPE), wood, or nylon coupons. Surfaces were allowed to dry for 1 hour and then exposed to SDC for 2 minutes or chlorine dioxide gas (100 ppm) for 24 hours. Coupons were swabbed, and remaining populations enumerated by spread-plating on TSA containing rifampicin. Temperature and relative humidity conditions were noted. Three independent replicates were conducted for each surrogate and sanitizer treatment combination. Negative and positive controls for each combination were also included.

Statistical analysis. All experiments will be performed in triplicate. Effects of parameters and interactions were evaluated. All models were analyzed using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC).

Results

LISTERIA

Results for *Listeria* sessile form are available in **Figure 2**. All the interventions evaluated showed a significant log reduction on plastic as compared to wood and nylon ($P<0.05$). These observations are probably due to the topography of the different surfaces. Microbial cells most likely harbor in cracks and crevices on wood coupons, while bacteria are completely absorbed in the texture of the nylon bags. Overall peracid, chlorine and SDC had the highest log reduction on experimentally inoculated plastic coupons with *Listeria*, as compared to wood and nylon. Peracid and SDC had also a significant log reduction on nylon (~5 log CFU/coupon). Nevertheless, almost 3 log reductions were observed when wood was treated with peracid and SDC. Steam seems to be the sanitizer with the lowest antimicrobial efficacy for *Listeria* sessile form at the times tested and no significant difference ($P>0.05$) was observed among treatment times (30 sec, 1 min or 2 min).

Results for *Listeria* biofilm form are available in **Figure 3**. All the interventions evaluated showed a greater log reduction on plastic as compared to wood and nylon ($P<0.05$). Chlorine dioxide gas achieved below the detectable limit during laboratory experiments independently of the surface analyzed ($P<0.05$). Both SDC and steam did not achieve a significant reduction ($P>0.05$) on experimentally inoculated and treated wood coupons, while significant log redutions were obtained on plastic and nylon, as compared to the untreated coupons ($P<0.05$). A significant time effect for all the sanitizers used was observed on plastic and nylon ($P<0.05$).

SALMONELLA

The results observed for *Salmonella* sessile form are available in **Figure 4**. Also for *Salmonella* all the sanitizers tested showed greater activites on plastic as compared to wood and nylon (between 5 and 6 log CFU/coupon reduction were reported, respectively). Peracid and steam seem to work effectively on nylon as well: 3 and 4 log CFU/coupon reduction were recorded. Peracid reached a 4 log CFU/coupon reduction for *Salmonella* on wood.

Completed results for *Salmonella* biofilm form are in **Figure 5**. All the sanitizers tested showed greater activities on plastic as compared to wood and nylon (between 5 and 6 log CFU/coupon reductions were reported). Peracid and steam seem to work effectively on nylon. Chlorine dioxide applied for 24 hours was able to completely reduce microbial populations under detectable limits on all three surfaces.

ESCHERICHIA COLI

E. coli sessile form results are illustrated in **Figure 6**. Also for this bacteria we observed a consistent trend where the best performace for sanitizers was observed on plastic. Time in this case did not seem to alter the efficacy of the treatment.

The results for *E. coli* biofilm form are available in **Figure 7.** Overall no significant log reductions were observed when SDC was used on wood, plastic and nylon coupons experimentally inoculated with *E. coli.* Chlorine and steam on plastic had more than 5 log CFU/coupon reduction, while less reduction was observed on wood and nylon. A significant effect was observed with peracid: a greater log reduction was reported for nylon and plastic when the intervention was applied for 2 minutes. No significant effect was observed for wood when longer retention time were applied. Chlorine dioxide gas for 24 hours completely reduced microbial populations under detectable limits on all three surfaces.

FIELD TRIALS

Given that the industry is already using peracid and chlorine-based sanitizers, the efficacy of chlorine dioxide and SDC was evaluated with packinghouse cooperators in Washington, Iowa, and Kansas. These sanitizer solutions are newer to the industry and showed efficacy during controlled lab setting experiments (objective 1). The results observed for the field trials are summarized in **Table 1.**

Chlorine dioxide gas was the most effective treatment ($P<0.05$) in controlling sessile *E. coli* and *Listeria* on HDPE and nylon in both the Midwest and Pacific Northwest regions, with a complete population reduction to the limit of detection (1.95 log CFU/coupon) for *Listeria* in both forms and sessile *E. coli* on both surfaces tested. A final count near the limit of detection for biofilm *E. coli* was observed: 2.28 log CFU/coupon on HDPE and 2.03 log CFU/coupon on nylon. A lower level of inactivation was recorded ($P>0.05$) after chlorine dioxide gas exposure in the Pacific Northwest region for all surfaces except for sessile bacteria grown on HDPE (1.95 log CFU/coupon for both), and *E. coli* biofilms (2.19 log CFU/coupon). Both sessile bacteria on nylon showed lower populations than control (5. 96 log CFU/coupon for *E. coli,* 6.25 log CFU/coupon for *Listeria*), as well as the biofilm form (3.55 log CFU/coupon for *E. coli*, 2.34 log CFU/coupon for *Listeria*). Sessile bacteria grown on wood surfaces did have lower populations after exposure to chlorine dioxide gas (5.75 log CFU/coupon for *E. coli*, 4.51 log CFU/coupon for *Listeria*), but biofilms grown on wood had much higher populations (8.7 log CFU/coupon for *E. coli*, 7.84 log CFU/coupon for *Listeria*).

Exposure to SDC was able to decrease biofilm populations on HDPE for both regions (Midwest: 5.80 log CFU/coupon *E. coli*, 5.7 log CFU/coupon *Listeria*; Pacific Northwest: 6.37 log CFU/coupon *E. coli*, 5.12 log CFU/coupon *Listeria*). Nevertheless, this treatment did not reduce populations of sessile *E. coli* on HDPE in the Pacific Northwest region (9.04 log CFU/coupon), or any form of *E. coli* on nylon in either region. A significant population reduction after SDC exposure ($P<0.05$) was found on nylon surfaces inoculated with *Listeria* except for biofilms in the Pacific Northwest region.

Outcomes and Accomplishments

The efficacy of free chlorine, peracetic acid (PAA), chlorine dioxide gas, silver dihydrogen citrate (SDC), and steam was determined for the three most common causes of bacterial foodborne illness in sensitive (sessile) and resistant (biofilm) forms on three commonly encountered food contact surfaces (wood, plastic [HDPE], and nylon) encountered in harvest of pome fruit, which completes the scope of work for objective 1. Findings indicate best efficacy on hard plastic, with nylon and wood proving to be the hardest to sanitize. Sanitizers used by the industry (chlorine and PAA) currently for the management of these surfaces were effective against sessile organisms, resulting in at least a 90% reduction of target organisms under the treatment conditions. The only instance that a 90% reduction was not observed for biofilms treated with chlorine and PAA was against *L. monocytogenes* on wood surfaces. This information will help the industry make informed decisions when selecting sanitizers for porous (wood) and multi-component (nylon picking bag) surfaces that they need to manage during harvest and storage.

Additionally, novel (chlorine dioxide, SDC, and steam) sanitation approaches were also evaluated in the lab setting. Efficacy was very limited with steam treatment of sessile cells on surfaces, but greater efficacy was observed against biofilms. Conversely, SDC was more active against the sessile form compared to the biofilm form. Chlorine dioxide performed very well against biofilms and sessile cells, with the exception of *L. monocytogenes* (sessile) on nylon.

Field trials in Washington, Kansas, and Iowa allowed for greater exploration of chlorine dioxide and SDC with industry partners to assess if either of these treatments may serve as a suitable sanitizer in the future, achieving the scope of objective 2. SDC performed better inactivating non-pathogenic *Listeria innocua*, with limited efficacy on non-pathogenic *E. coli* when treating picking bags and bins. The ready-to-use nature of SDC makes it appealing for atypical situations or field settings where mixing of concentrated sanitizers may be difficult, but the field trials demonstrated limited antimicrobial activity. Gaseous chlorine dioxide demonstrated efficacy in the field setting on all surfaces tested in both regions. Differences in the field settings were observed, with the arid Northwest proving to have harder conditions given lower relative humidity, a known influence for chlorine dioxide.

With respect to treating harvest bins, many industry members indicated they would ideally treat in a storage room. Many segments of the food industry utilize gaseous chlorine dioxide as a sanitizer, especially with equipment prone to biofilm production (e.g., cooling tunnels, spiral freezers). If considering adoption, the industry will need to work with supplier technical service experts to optimize treatment conditions in order to achieve adequate distribution of the gas. Different treatment SOPs will need to be developed based upon storage room bin capacity and fans will be necessary to disperse chlorine dioxide, which is denser than air. At the concentrations that will be generated, chlorine dioxide is hazardous to human health and treatments must be conducted in sealed containers that allow for the capture of generated chlorine dioxide gas and subsequent dissipation. Given the long treatment time, gaseous chlorine dioxide will not be appropriate for situations in that require <24 h between sanitizing and use.

Findings from this work have already been shared with growers at the Pace International's Postharvest Academy in Chelan, WA, in May 2023, with 165 industry members in attendance. Additionally, an article that describes project outcomes has been submitted to the *Good Fruit Grower* for an upcoming issue. The *Good Fruit Grower* has a readership over 10,000 growers and businesses, with a strong representation from the pome tree fruit industry. Through these efforts, we have surpassed our initial goal of 150 stakeholders that have increased their understanding of the preharvest and postharvest process impacts on microbial and chemical threats as a result of this project.

An unexpected outcome observed by the team was the difficultly in sanitizing multi-component nylon surfaces commonly used in the body of apple-picking bags. Upon further reflections and microscopic observation, it was apparent that the weave of the surface allows for adsorption of cells, so they may be protected from treatment. This emphasizes the importance of cleaning and sanitizing events at appropriate frequency so the microbial load can be appropriately managed. On some occasions, variability in efficacy of SDC was observed over time once a container was opened (in objective 1). To evaluate the best activity, a new container was used for each field study. Clearly it would be important for a compound to maintain activity throughout its shelf life. Additionally, in areas where SDC aggregated, a white chalky residue formed. SDC never aggregated on the surfaces treated, but the ground (i.e., concrete, rock) was impacted.

The team is very appreciative of the collaboration and support of the tree fruit industry in Washington, Kansas, and Iowa that allowed for us to evaluate efficacy in operations of varying sizes. We also appreciated the conversations with industry collaborators to better understand how they would utilize different novel treatments, in addition to the timing of cleaning and sanitizing bins as well as picking bags.

Summary of Findings and Recommendations

In this research, we evaluated nylon, plastic (high density polyethylene), and wood surfaces with the following sanitizer treatments: chlorine, peracetic acid, silver dihydrogen citrate, chlorine dioxide gas, and steam. Wood proved to be one of the most challenging surfaces among the three evaluated. Surprisingly, nylon was also equally hard to sanitize in many cases. The hard plastic was consistently the easiest surface to sanitize, but it can easily transfer microorganisms if not regularly cleaned and sanitized. In the lab-based experiments, additional exposure time did not significantly improve efficacy within the time evaluated. When pathogens were inoculated on food contact surfaces and treated, they were successfully reduced by at least 99% in multiple treatments for all contact surfaces. Far less inactivation was observed when cells were allowed to grow into a biofilm. This is why cleaning and sanitizing at regular frequency is important to control the formation of biofilms on surfaces. Chlorine dioxide was the most effective treatment against biofilms, while steam was the least effective.

Chlorine dioxide and silver dihydrogen citrate were selected for the field trials in Washington, Iowa, and Kansas. These strategies are newer to the industry and showed efficacy in the lab setting (objective 1). Chlorine dioxide treatments resulted in 90 to >99.99% inactivation of target organisms, with the greatest reductions on plastic and least on wood. In the Midwest where relative humidity in the treatment room was higher, greater inactivation was observed, highlighting the potential need to increase relative humidity if utilizing gaseous chlorine dioxide in arid regions (e.g., Washington). Silver dihydrogen citrate performed better at inactivating *Listeria*, with limited efficacy on *E. coli*.

When thinking about which sanitation strategies to apply in produce operations, the interactions between material, sanitizer, and application times should be taken into account when implementing the intervention.

APPENDICES

Publications and Presentations

Presentations 2023

Chalamalasetti, H., B. Ruiz-Llacsahuanga, V. Trinetta, and F. Critzer. Effective strategies to sanitize harvesting bins and picking bags concerning *Listeria monocytogenes* and *Salmonella*. IAFP Annual Meeting, July 2023 (Technical Oral Presentation).

Critzer, F. Food safety from postharvest washing to picking bags, effective application of sanitizers. Postharvest Academy, Pace International; Chelan, WA, May 2023 (Invited Oral Presentation).

Critzer, F., U. Yucel, M. Bhullar, L. Nwadike, and V. Trinetta. Validation study for the tree fruit industry: effective strategies to sanitize harvest bins and picking bags. CPS Research Symposium, Buckhead, GA, June 2023 (Poster Presentation).

Ivers, C., M. Bhullar, F. Critzer, L. Nwadike, V. Trinetta, and U. Yucel. Efficacy of commercially available sanitizers to control *Salmonella* biofilms on harvesting bins and picking bags. IAFP Annual Meeting, July 2023 (Technical Poster Presentation).

Stewart, S., M. Bhullar, F. Critzer, L. Nwadike, V. Trinetta, and U. Yucel. Efficacy of chlorine, chlorine dioxide, peroxyacetic acid, steam and silver-dihydrogen citrate in controlling *Escherichia coli* biofilms on harvesting bins and picking bags. IAFP Annual Meeting, July 2023 (Technical Poster Presentation).

Trinetta, V. Selecting the right sanitizer… what does the science say? IAFP Annual Meeting. July 2023 (Invited Oral Presentation).

Presentations 2022

Trinetta, V., U. Yucel, F. Critzer, M. Bhullar, and L. Nwadike. Validation study for the tree fruit industry: effective strategies to sanitize harvest bins and picking bags. CPS Research Symposium, La Jolla, CA, June 2022 (Poster Presentation).

Trinetta, V. Understanding *Listeria monocytogene*s adhesion and biofilm formation on different surfaces and the effect of interventions on biofilm survivability. IAFP – Florida Section Annual Meeting, April 2022. (Invited Oral Presentation).

Budget Summary

This project was awarded a total of $251,381 in funds. The majority of the funding was spent to support graduate students, both at KSU and UGA, for the duration of the project (~$100,000). Supplies, media, and consumables to grow biofilms and evaluate sanitation approaches in laboratory settings also covered a good part of the budget, both in Year 1 and Year 2 ($70,000). Some funds ($10,000) were used to support travels for the field trials.

Figures 1–7 and Table 1 (see below)

Figures and Table

Figure 1: Schematic representation of the completely assembled reactor system.

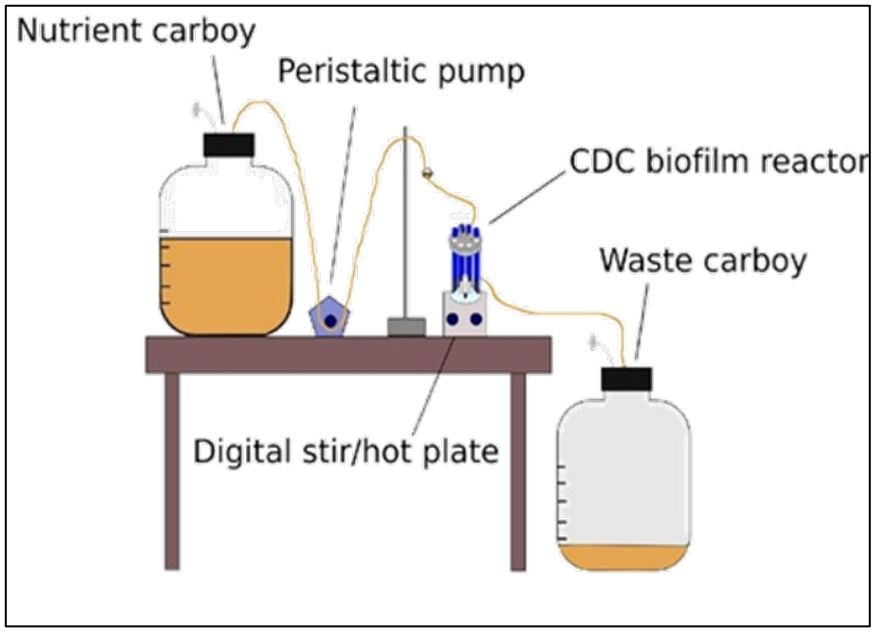
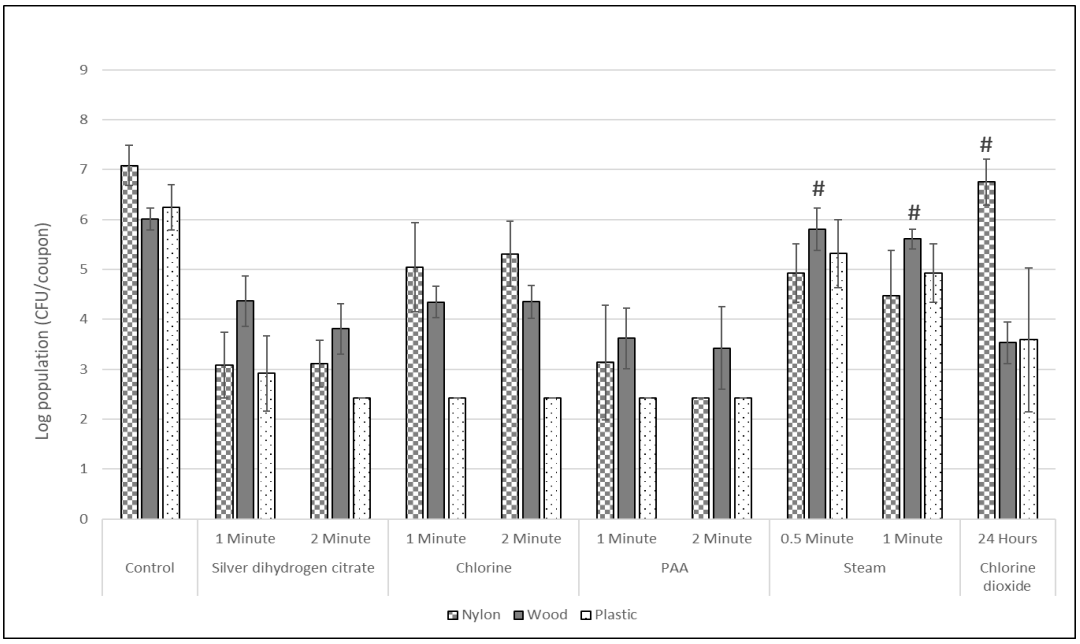
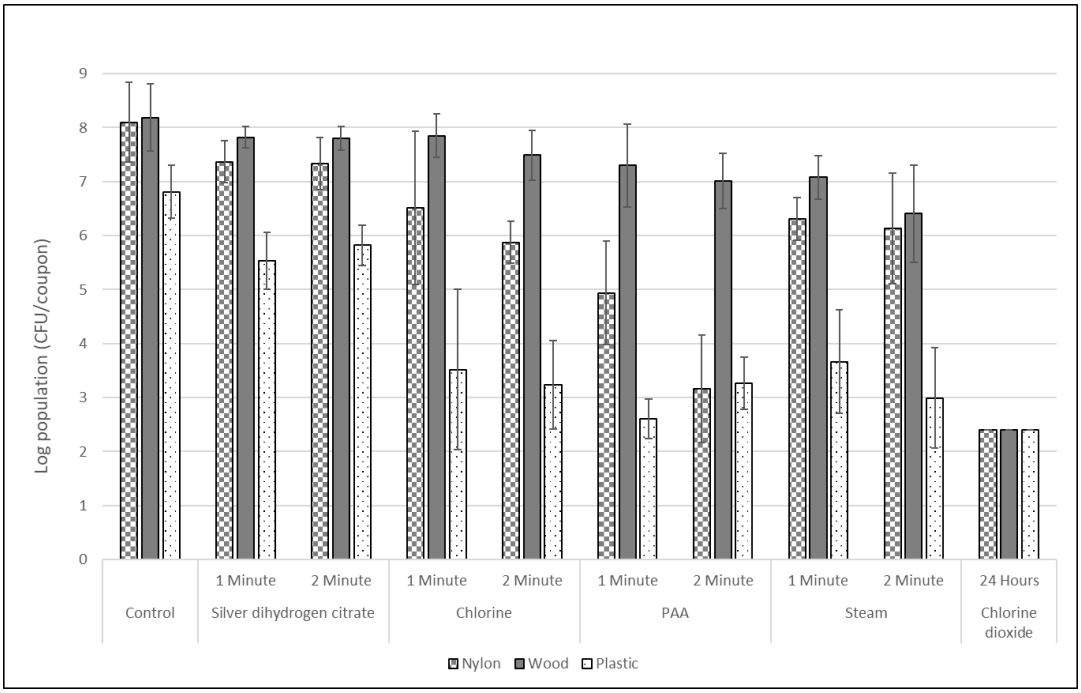


Figure 2. Remaining *L. monocytogenes* population (sessile) on common food contact surfaces when treated with sanitizers at different contact times



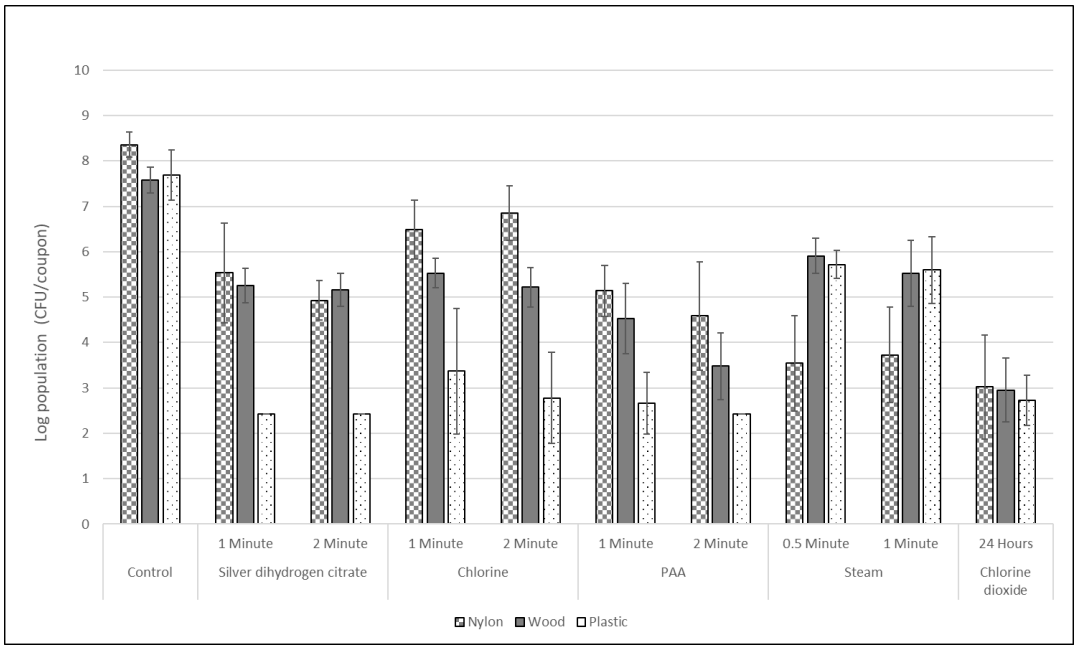
#: treatment not different than control; all other treatments at all timepoints were statistically significant as compared to the control ($P < 0.05$).

Figure 3. Remaining *L. monocytogenes* biofilms on common food contact surfaces when treated with sanitizers at different contact times



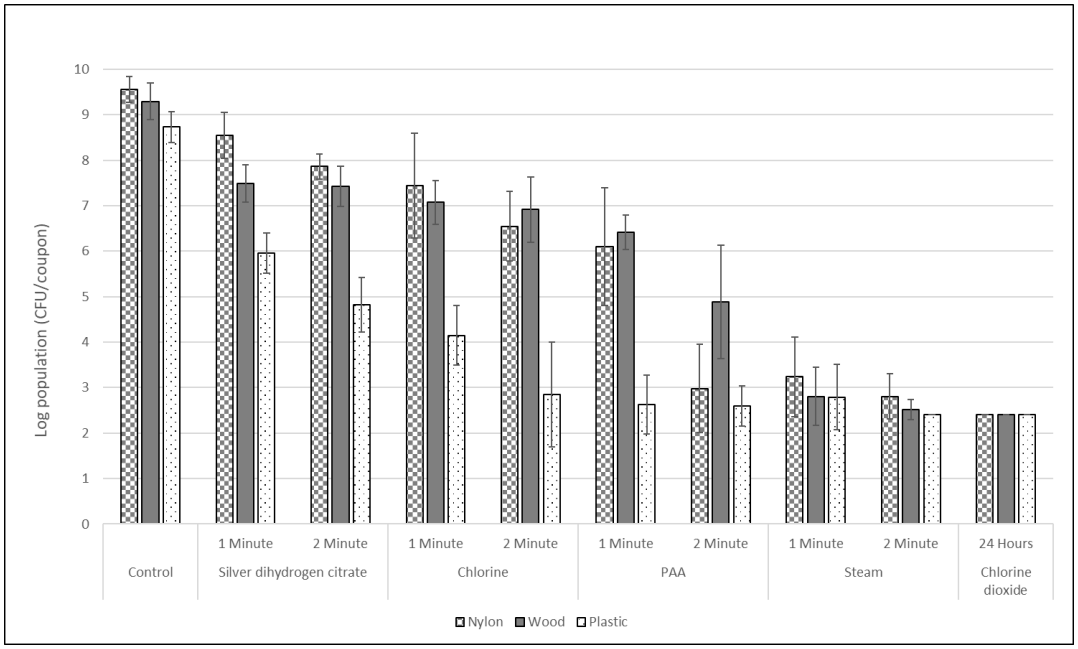
All treatments at all timepoints were statistically significant as compared to the control ($P < 0.05$).

Figure 4. Remaining *Salmonella* population (sessile) on common food contact surfaces when treated with sanitizers at different contact times



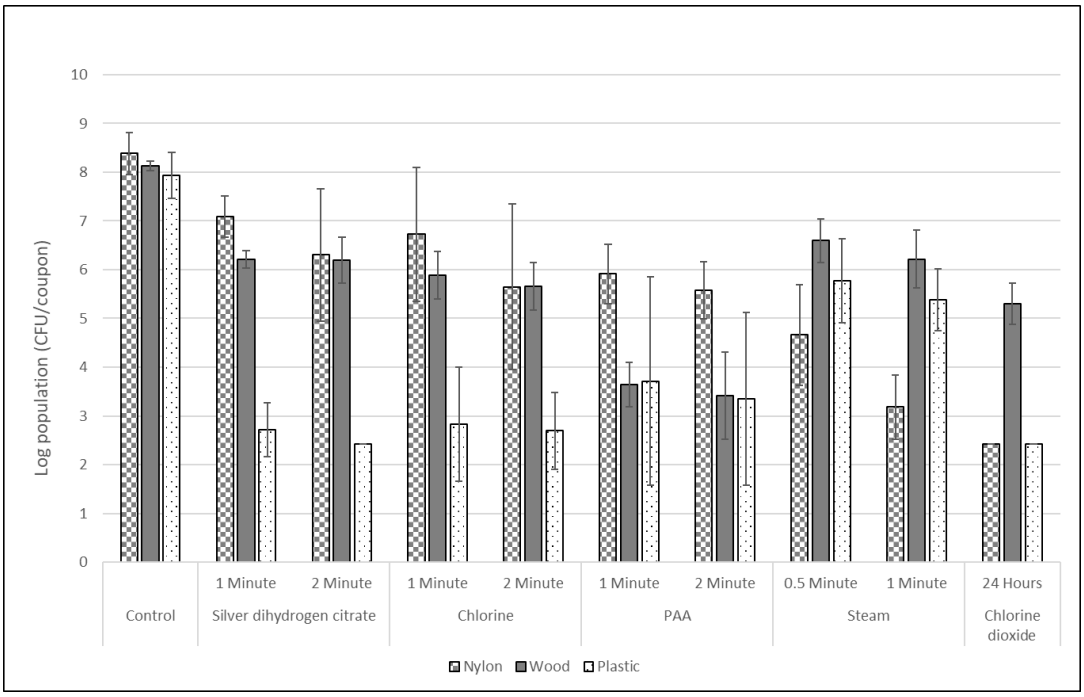
All treatments at all timepoints were statistically significant as compared to the control ($P< 0.05$).

Figure 5. Remaining *Salmonella* biofilms on common food contact surfaces when treated with sanitizers at different contact times



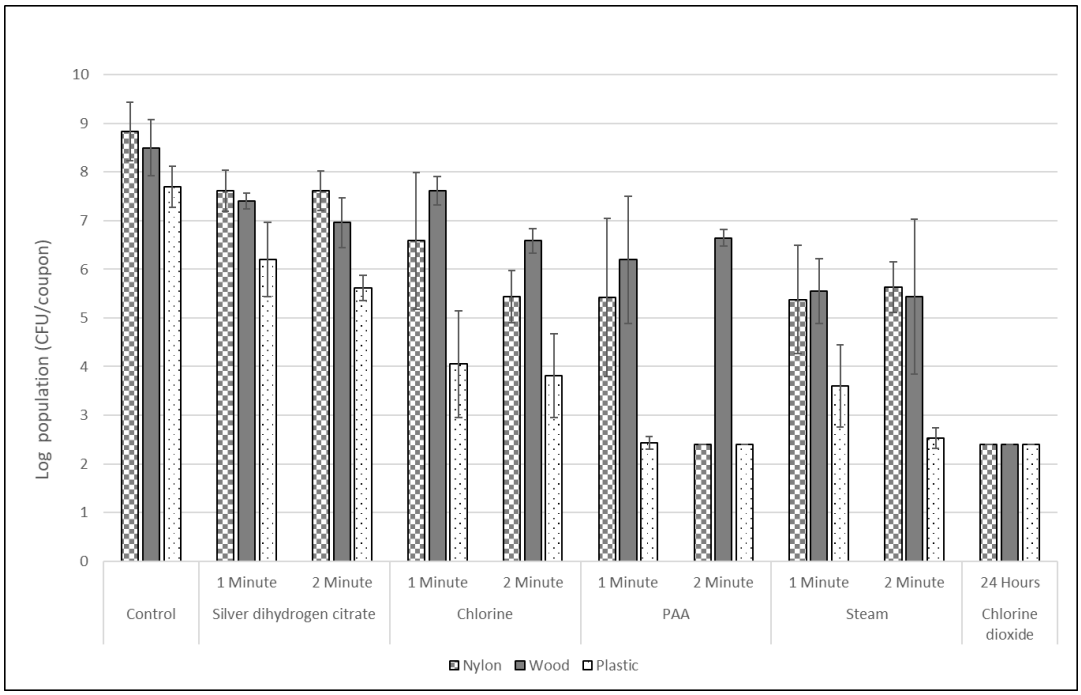
All treatments at all timepoints were statistically significant as compared to the control ($P< 0.05$).

Figure 6. Remaining *E. coli* population (sessile) on common food contact surfaces when treated with sanitizers at different contact times



All treatments at all timepoints were statistically significant as compared to the control (P< 0.05).

Figure 7. Remaining *E. coli* biofilms on common food contact surfaces when treated with sanitizers at different contact times



All treatments at all timepoints were statistically significant as compared to the control (P< 0.05).

Table 1. Remaining populations on plastic (HDPE), wood, and nylon surfaces treated with silver dihydrogen citrate (SDC) or chlorine dioxide gas (ClO_2) during field trials (results are compiled based on locations and materials).

	Log CFU/coupon *E. coli*						Log CFU/coupon *Listeria*					
	Sessile			**Biofilm**			**Sessile**			**Biofilm**		
Midwest	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*
HDPE	7.76 ± 0.40	4.60 ± 0.34	1.95 ± 0.34	7.99 ± 0.43	5.80 ± 0.41	2.28 ± 0.41	8.54 ± 0.22	4.39 ± 0.18	1.95 ± 0.18	8.45 ± 0.20	5.70 ± 0.18	1.95 ± 0.18
Nylon	8.17 ± 0.40	7.38 ± 0.34	1.95 ± 0.34	8.38 ± 0.43	7.66 ± 0.41	2.03 ± 0.41	9.03 ± 0.22	6.61 ± 0.18	1.95 ± 0.18	9.31 ± 0.20	7.37 ± 0.18	1.95 ± 0.18

	Log CFU/coupon *E. coli*						Log CFU/coupon *Listeria*					
	Sessile			**Biofilm**			**Sessile**			**Biofilm**		
Washington	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*	*Control*	*SDC*	*ClO_2*
HDPE	10.02 ± 0.39	9.04 ± 0.39	1.95 ± 0.39	8.75 ± 0.28	6.37 ± 0.28	2.19 ± 0.28	9.03 ± 0.60	6.59 ± 0.60	1.95 ± 0.60	8.60 ± 0.36	5.12 ± 0.36	3.64 ± 0.36
Nylon	10.09 ± 0.39	9.39 ± 0.39	5.96 ± 0.39	9.80 ± 0.28	9.28 ± 0.28	3.55 ± 0.28	10.59 ± 0.60	7.91 ± 0.60	6.25 ± 0.60	10.05 ± 0.36	9.54 ± 0.36	2.34 ± 0.36
Wood	9.94 ± 0.39	9.49 ± 0.39	5.75 ± 0.39	9.90 ± 0.28	9.30 ± 0.28	8.70 ± 0.28	7.91 ± 0.60	7.36 ± 0.60	4.51 ± 0.60	9.06 ± 0.36	8.08 ± 0.36	7.84 ± 0.36