CENTER for PRODUCE SAFETY

CPS 2020 Rapid Response Project
FINAL PROJECT REPORT

Project Title
Investigation of potential preharvest and postharvest treatments targeting *Salmonella* risk reduction on peach in Australia

Project Period
January 7, 2020 – May 30, 2021

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Objectives

1. *Identify optimum dose rates of Cu-EDTA, zinc sulfate heptahydrate (23% Zn), and Peroxy Treat™ applied pre-harvest to achieve potential* Salmonella *risk reduction and identify possible thresholds for phytotoxicity.*
2. *Test and optimize the efficacy of different postharvest sanitizer treatments against* Salmonella *spp. on peach surfaces.*
3. * *Determine whether interactions between postharvest sanitizers and fungicides reduce the efficacy of the sanitizers.*
4. *To evaluate the impact of the selected treatments identified in Objectives 1, 2 and 3 on peach shelf-life and quality in comparison to standard industrial practices.*

* Objectives were reviewed at the start of the project in consultation with industry. Research of sanitizer-fungicide interactions was not considered a priority and was removed as an objective.

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CPS Campaign for Research

FINAL REPORT

Abstract

This Rapid Response (RR) project was funded in the wake of the *Salmonella enterica* Enteritidis outbreak linked to peaches in the United States in 2020. The aim of this project was to assess several preharvest foliar treatments and postharvest sanitizer treatments for efficacy in reducing *Salmonella* risk in peach fruit. The purpose of the research was to take advantage of counter-season peach production in Australia to generate preliminary data that would help prioritize interventions for further research ahead of the next peach season in the United States. Several preharvest foliar treatments were investigated using bacterial inhibition assays and field trials to assess bactericidal activity and phytotoxicity. Peroxy Treat (hydrogen peroxide/peroxyacetic acid) demonstrated bactericidal activity but copper chelate and zinc sulfate did not. The treatments caused varying levels of foliar and fruit phytotoxicity when applied at high dose rates. Several postharvest sanitizer treatments were investigated in laboratory and packhouse challenge experiments. Nylate (bromo-chloro-dimethyl hydantoin) achieved more than 3.5 log reduction in *Salmonella* in the laboratory challenge, demonstrating that there are opportunities to effectively reduce risk through sanitizer optimization. The preharvest foliar treatments tested were not readily actionable interventions for peach orchards. However, we recommend that postharvest interventions be prioritized as an approach likely to yield actionable strategies in a relatively short time frame.

Background

In June–August 2020 an outbreak of *Salmonella enterica* Enteritidis infections led to 101 reported illnesses and 28 hospitalizations across 17 states in the United States. The subsequent epidemiological and traceback investigation led by the U.S. Food and Drug Administration found links to peach fruit packed or supplied by Prima Wawona or Wawona Packing Company. Multiple *Salmonella* isolates were found on fruit and trees from the peach orchards. Although none matched the outbreak strain, there were some strains that genetically resembled chicken and cattle isolates (with no known association with foodborne disease). Combined with geospatial analysis, the investigation suggested airborne transmission of contaminated dust from adjacent poultry and cattle operations to be a plausible route of contamination.

The California Fresh Fruit Association commissioned this Rapid Response (RR) research in the wake of this outbreak. The purpose of the project was to take advantage of counter-season peach production in the southern hemisphere to advance research on food safety management options ahead of the next peach season in the United States. The overarching objective of the RR project was to help prioritize preharvest and postharvest interventions that had potential to reduce the risk of *Salmonella* contamination in peaches, and that were readily available for commercial adoption.

Research Methods and Results

Preharvest Foliar Treatments

Industry requested research on the potential of copper, zinc, and OxiDate foliar sprays to reduce *Salmonella* risk in the orchard. A scenario was envisaged in which preharvest treatments could be applied to the buffer tree zone around a known or prospective source of contamination to reduce risk of foodborne illness. Fruit from the buffer trees might be marketable, diverted from fresh market (e.g., to processing), or sacrificed depending on the efficacy of treatments against *Salmonella* and severity, if any, of phytotoxicity.

Copper and zinc products are commonly applied to stone fruit as micronutrient treatments but are also reported to have bactericidal activity. OxiDate is a broad-spectrum bactericide/fungicide that contains hydrogen peroxide as the active ingredient and is registered for use in a range of crops including stone fruit. Following discussion with industry advisors, George Nikolich and Trevor Suslow, we tested copper chelate, zinc sulfate, and Peroxy Treat, the latter as an alternative product to OxiDate (which was not available in Australia) with similar chemistry (Table 1).

The hypothesis for the study was that preharvest foliar application of commercial products would directly or indirectly reduce the survival of *Salmonella* on peach leaves and fruit.

Table 1. Preharvest foliar treatments investigated in this project

Chemical	Manufacturer	Description	Rationale
Librel® Cu Copper chelate	Ciba Specialty Chemicals	Formulated as copper ethylenediamine tetra acetate disodium salt (CuEDTA Na_2) Contains 14% (w/w) Cu	Cu products are primarily used as micronutrient fertilizers in the peach industry. However, Cu also has applications as a bactericide.
Peroxy Treat Fungicide	E. E. Muir and Sons, Australia	Contains 250 g/L hydrogen peroxide and 50 g/L peroxyacetic acid	This product is registered in Australia for suppression of *Botrytis cinerea* on grapevines close to harvest. However, the combination of active ingredients is expected to have broad spectrum efficacy against bacteria, fungi, and viruses[1].
Zinc sulfate	Swancorp, Australia	Formulated as zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) Contains 23% (w/w) Zn	$ZnSO_4$ is commonly applied as a dilute spray to stone fruits during the late-dormant period as a nutritive amendment. There are a few reports of $ZnSO_4$ having antibacterial effects[2] although not in a horticultural application.

[1] Briñez WJ, Roig-Sagués AX, Herrero MMH, López-Pedemonte T, Guamis B (2006). Bactericidal efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and nonpathogenic strains of *Staphylococcus* spp., *Listeria* spp. and *Escherichia coli*. *Food Control* **17**, 516-521.
[2] Abdalkade D, Al-Saedi F (2020). Antibacterial effect of different concentrations of zinc sulfate on multidrug resistant pathogenic bacteria. *Systemic Reviews in Pharmacy* **11**, 282-288.

There were two components to the study: (a) laboratory experiments to determine optimum dose rates of CuEDTA, Peroxy Treat, and $ZnSO_4$ to achieve potential *Salmonella* risk reduction on peach fruit; and (b) two field experiments to identify possible thresholds for phytotoxicity (Field Trial 1 and 2).

The laboratory experiments and Field Trial 1 were undertaken simultaneously rather than sequentially in the interests of maximizing data collection, as there was limited time remaining for the Australian peach season. A smaller set of treatments was tested in Field Trial 2 considering their respective efficacies in the laboratory experiments.

Laboratory Experiments

Bacterial inhibition assays were used to determine the minimum inhibitory (MIC) and minimum lethal (MLC) concentrations for CuEDTA, Peroxy Treat, and $ZnSO_4$ efficacy against *S.* Enteritidis and surrogate *Escherichia coli* MG1655. The purpose of the assays was to confirm the efficacy of the active ingredients and appropriateness of concentrations used in the field.

Methods

To enable selective detection of inoculum, *S.* Enteritidis (ATCC 13076) and *E. coli* MG1655 were induced to become resistant to nalidixic acid by consecutively subculturing in fresh tryptone soy broth supplemented with 40 ppm nalidixic acid (TSBN, Merck and Sigma-Aldrich, respectively) for 5 days at 37°C, 200 rpm. The growth of nalidixic acid-resistant and wildtype colonies were compared and confirmed normal before use in subsequent experiments.

Bacterial inhibition assays were modified microbroth dilution assays. CuEDTA, Peroxy Treat, and $ZnSO_4$ working solutions were diluted in TSBN to cover a testing range of 11.7–6,000 ppm and 120 µL aliquoted into the microtiter plate wells. These were then inoculated with overnight TSBN cultures of *S*. Enteritidis and *E. coli* MG1655 (4-5 log CFU/mL). Negative controls contained TSBN only and serially diluted sanitizers in TSBN only. Positive control contained TSBN only and the bacterial inoculum. The microtiter plates were incubated at 37°C for 24 h and the bacterial inoculum were plated on TSAN to verify the cell concentration. The MIC was determined by spectrometer measurements at OD_{630}. The MLC was determined by the absence of growth from wells with no turbidity after plating on TSAN and incubating at 37°C for 48 h.

Key Results

- Peroxy Treat had a MIC of 188-375 ppm and MLC of 375-750 ppm against *S.* Enteritidis.
- Peroxy Treat had a MIC of 375 ppm and MLC of 375 ppm against *E. coli* MG1655.
- CuEDTA had neither a MIC nor MLC against either microorganism.
- $ZnSO_4$ had neither a MIC nor MLC against either microorganism.

In summary, the laboratory experiments showed that Peroxy Treat was likely to have antimicrobial activity against *Salmonella* at the concentrations (2,500-10,000 ppm) used in the field experiments (Table 2). Peroxy Treat also had good activity against surrogate *E. coli* MG1655 (used in the packhouse trial). On the other hand, CuEDTA and $ZnSO_4$ were not

effective against *Salmonella* or *E. coli* MG1655 even when applied at a high concentration (6,000 ppm).

Appended Data

- Table 4. MIC and MLC results

Field Trials

Field Trial 1 was conducted in mid-January 2021 at Glenbernie Orchard, Darkes Forest, approximately 60 km southwest of Sydney, New South Wales. The main purpose of the field trial was to establish possible thresholds for phytotoxicity following preharvest foliar treatments of peach trees in a commercial orchard. The concentrations used were maximum rates recommended by the manufacturer and higher, with the expectation that this would encompass concentrations with bactericidal efficacy.

Field Trial 2 was conducted at Sunland Orchard, Barooga, approximately 680 km southwest of Sydney, New South Wales, near the border of Victoria. This field trial was designed with knowledge that CuEDTA and $ZnSO_4$ had not been effective against *Salmonella* in the bacterial inhibition assays, and these products were not re-tested. The Peroxy Treat concentrations used were the rate recommended by the manufacturer and half the recommended rate.

Methods

Chemical treatments (Table 2) were applied to peach trees 2 days before harvest. In Field Trial 1, ‘Snow King’ white peach trees were sprayed with a motorized knapsack whereas in Field Trial 2, ‘Snow Ice’ white peach trees were sprayed with a commercial mist blower (Figure 1). Each treatment was applied to four replicate trees. The treatments were allocated pragmatically rather than randomly, with buffer trees between different chemicals to minimize the potential impact of spray drift.

Leaf phytotoxicity was assessed by monitoring defoliation and leaf injury in four randomly selected shoots per tree 10 days after treatment (1 week after harvest). Defoliation was measured as the percentage of leaves (counted prior to treatment) that had abscised, while leaf injury was measured as the percentage of extant leaves with symptoms of bleaching, browning or shot holes. Leaf area index of each peach tree was measured before and 10 days after treatment using a CI Plant Canopy Imager (CID Bio-Science Inc., Camas, WA, US).

Fruit phytotoxicity was assessed by examining 50 fruit from each treatment immediately after harvest for symptoms (e.g., spotting, pitting, and discoloration). The postharvest development of phytotoxicity was assessed by observing the same fruit after 1 and 2 weeks of refrigerated storage. For each storage duration, 25 fruit were transferred to ambient temperature for 3 days as a simulated retail period before assessment.

Table 2. Preharvest foliar treatments.

Product	Field Trial 1[b]			Field Trial 2		
	%	g/L	ppm	%	g/L	ppm
CuEDTA	0.1[c]	1.0	1000			
	0.2	2.0	2000			
	0.3	3.0	3000			
Peroxy Treat[a]				1	2.5	2500
	2[c]	5.0	5000	2[c]	5.0	5000
	3	7.5	7500			
	4	10.0	10000			
$ZnSO_4$	0.1[c]	1.0	1000			
	0.2	2.0	2000			
	0.3	3.0	3000			
Controls	Wetting agent only			-		
	No treatment			No treatment		

[a] Calculated to 250 g/L hydrogen peroxide.
[b] Treatments were applied with a non-ionic wetting agent (Libsorb).
[c] Maximum rate recommended by the manufacturer.

Figure 1. Preharvest foliar treatments were applied using (A) a motorized knapsack sprayer in Field Trial 1 and (B) a commercial mist blower in Field Trial 2.

The same fruit were assessed for several quality indicators. Weight loss during storage was measured to determine if any treatments caused accelerated (atypical) weight loss. Total soluble solids (TSS) was measured as the °Brix of expressed juice using an Atago PAL-1 digital refractometer. Flesh firmness was measured as the force (N) required to penetrate 10 mm into the fruit flesh at the equator (skin removed) using an FTC TMS Touch texture analyzer fitted with an 8-mm cylindrical probe moving at 5 mm/s.

Antimicrobial activity was assessed by determining treatment effects on pre-existing microflora. Three fruit per tree were collected at the time of harvest, manually massaged in phosphate-buffered saline (PBS) for 2 min, then spread on plate count agar (PCA; Oxoid) and dichloran-rose bengal-chloramphenicol agar (DRBC; Oxoid). Field Trial 2 samples were additionally plated on MacConkey II agar (Oxoid). Total aerobic bacteria were enumerated from the PCA plates after incubation for 48 h at 35°C. Yeasts and molds were enumerated from the DRBC plates after incubation for 5 days at 22°C. Total coliforms were enumerated from the MacConkey plates after incubation for 24 h.

Data was analyzed statistically using ANOVA and Tukey's HSD tests (Genstat 18th edition, VSNI, UK).

Key Results

- CuEDTA (0.1-0.3%) caused severe defoliation (Figure 2) and leaf injury (Figure 3) in Field Trial 1, which worsened as the application rate increased. Peroxy Treat (2-4%) and $ZnSO_4$ (0.1-0.3%) appeared to cause mild defoliation and leaf injury, but levels were not statistically different ($P>0.05$) to the control trees.

- Peroxy Treat (2-4%) caused severe fruit phytotoxicity (Figure 4) in Field Trial 1, which worsened as the application rate increased. The typical symptoms were fine spots at harvest that progressed to brown lesions after 1 week of storage. CuEDTA (0.3%) appeared to cause some 'watermark' in a small proportion of fruit. Fruit treated with $ZnSO_4$ did not exhibit obvious symptoms of phytotoxicity.

- Peroxy Treat (1-2%) did not cause any obvious symptoms of phytotoxicity in peach fruit or trees in Field Trial 2 (Figure 5).

- Preharvest treatments did not affect ($P>0.05$) the quality of fruit evaluated after 1 week of storage. The postharvest weight loss and flesh firmness of fruit from the various treatments and controls did not differ significantly ($P>0.05$). The treatments had no meaningful impact on TSS, although there was a statistical difference in TSS of fruit treated with 0.2 and 0.3%. Postharvest storage for 2 weeks did not reveal any meaningful differences among treatments in fruit quality (data not shown).

- Preharvest treatments did not affect ($P>0.05$) the orchard microflora in Field Trial 1.

- Peroxy Treat (1-2%) decreased ($P<0.05$) the total aerobic bacteria count in Field Trial 2, but only by 0.3-0.4 log CFU/g compared to the control treatment. Peroxy Treat also significantly reduced ($P<0.05$) the total coliform count by 0.6-1.8 log CFU/g compared to the control but had no effect ($P>0.05$) on the yeast and mold count.

Figure 2. Typical tree condition in Field Trial 1. (A) Healthy full canopy prior to treatment and (B) defoliation five days after treatment with high-dose CuEDTA (0.3%).

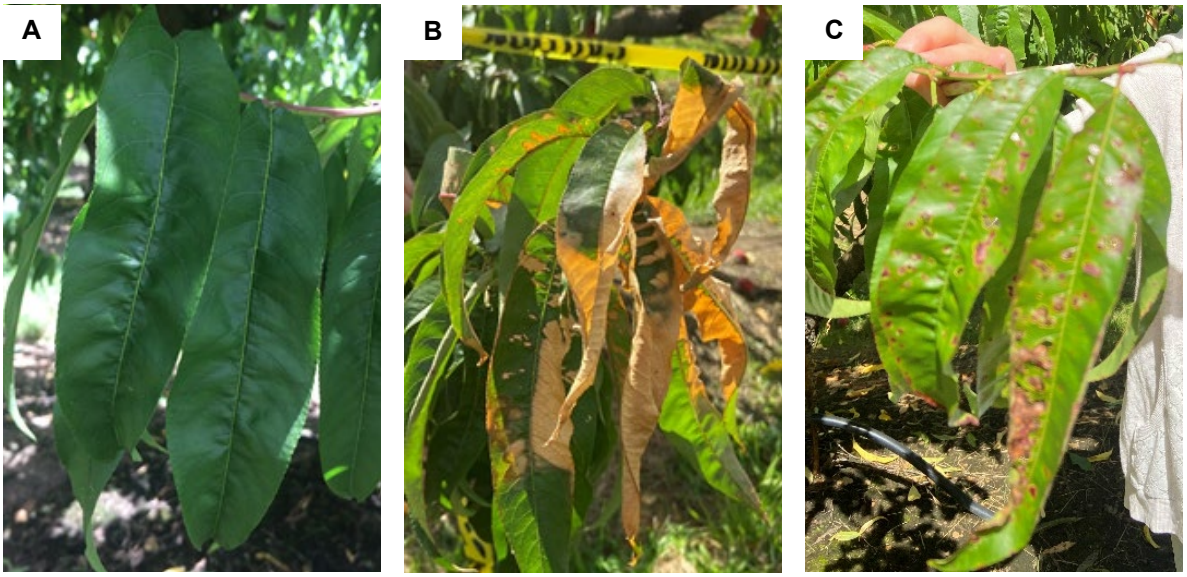

Figure 3. Typical leaf condition in Field Trial 1. (A) No symptoms in water-control; (B) bleaching in high-dose Peroxy Treat (4%); and (C) margin browning in high-dose $ZnSO_4$ (0.3%).

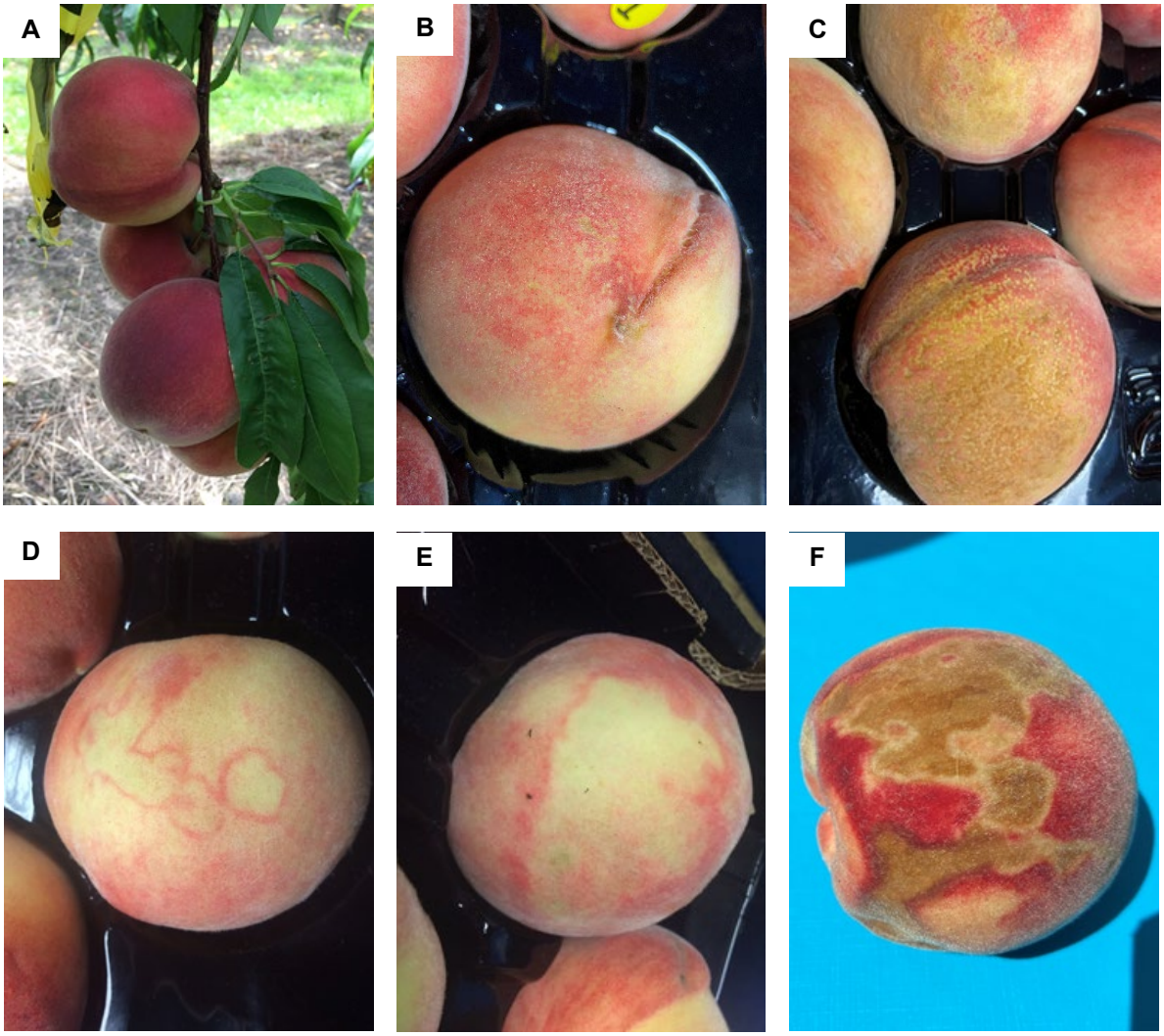

Figure 4. Typical fruit condition in Field Trial 1. (A) No symptoms prior to treatment; (B) mild and (C) severe 'spotting' after treatment with high-dose Peroxy Treat (4%); (D) mild and (E) severe 'watermarks' after treatment with high-dose CuEDTA (0.3%) after 1 week of cold storage, which developed into (F) brown lesions after 2 weeks of cold storage.

Figure 5. Typical leaf and fruit condition of trees treated with Peroxy Treat (2%) in Field Trial 2 (A and B) at harvest and (C) postharvest after one week of cold storage.

In summary, the preharvest foliar treatments did not have consistent, significant bactericidal effects in our laboratory or field experiments. Further research would be required to optimize the treatments and confirm bactericidal efficacy before these products could be recommended to industry as preharvest interventions for *Salmonella* risk reduction. If there is interest to fund further research, Peroxy Treat could be considered as a potentially useful product. Our research suggests that it has bactericidal activity and is safe for application to peach trees immediately prior to harvest at application rates recommended by the manufacturer. The risk of fruit phytotoxicity, aside from being dose-dependent, may vary with peach cultivar or other orchard factors. Peroxy Treat did not affect fruit quality in this study. We found that the preharvest sprays did not impact postharvest fruit quality. However, this requires further confirmation as the fruit in our study were not professionally harvested or graded, leading to variable initial quality, which may have obscured treatment effects.

Appended Data

Postharvest Sanitizer Treatments

The hypothesis for this study was that postharvest sanitizer treatments can be optimized to reduce the survival of *Salmonella* on peach fruit. We tested several sanitizers for efficacy against *S.* Enteritidis or a surrogate (Table 3). It was not feasible to carry out comprehensive screening with the framework of a RR project. Rather, the goal was to help direct the scope of subsequent research in the next US peach season.

There were two components to the study: (a) a laboratory challenge experiment to test a range of sanitizers against *S.* Enteritidis inoculated onto peach fruit and (b) a packhouse trial to test the most effective sanitizers against surrogate *E. coli* MG1655 in a commercial environment.

Table 3. Postharvest sanitizer treatments investigated in this project.

Sanitizer	Manufacturer	Notes	Rationale	Tested in…	
				L	P
Chlorine (as sodium hypochlorite)	Commercial bleach	Treatment: 100 ppm; total Cl: 39-99; free Cl: 2.23-8.7; pH 6	Chlorine is the most common sanitizer used in the stone fruit industry.	Y	Y
Chlorine dioxide	Natural Water Solutions, Australia	Treatment: 10 ppm; pH 5-6	Chlorine dioxide is the second-most used sanitizer in the stone fruit industry.	Y	
Lactic acid	Univar Solutions, USA	Treatment: 2% (v/v); pH 2	Lactic acid is used in wash water to reduce levels of pathogenic bacteria, mainly in the meat industry or in combination with other sanitizers.	Y	Y
Nylate® YM-FAB	Wobelea, Australia	Contains bromo-chloro-dimethyl-hydantoin Treatment: 20 ppm; pH 5	Nylate is registered in Australia for use as a disinfectant in postharvest wash water. Similar products are available in the United States but not commonly used on produce.	Y	Y
Peroxy Treat Fungicide	E. E. Muir and Sons, Australia	Contains 250 g/L hydrogen peroxide and 50 g/L peroxyacetic acid Treatment: 80 ppm H_2O_2 equivalent; pH 5	The combination of active ingredients is expected to have broad-spectrum efficacy against bacteria, fungi, and viruses.	Y	

Sanitizer	Manufacturer	Notes	Rationale	Tested in…	
				L	P
PhageGuard S	PhageGuard, USA	*Salmonella*-specific bacteriophages (applied at ~8 log CFU/g) Treatment: 1% (v/v); pH 5-6	Bacteriophages are naturally occurring microorganisms that recognize and kill specific bacterial strains. This product is used to kill *Salmonella* on food product surfaces and is approved by the FDA and USDA as a processing aid on produce.	Y	
Plasma-activated water (PAW)	Generated using University of Sydney equipment	PAW was generated using a 4-hole plasma generator (10 min treatment time; 1,000 Hz discharge frequency; 60 kHz resonance frequency; 100 µs duty cycle) Treatment: pH 5-6	PAW is produced by passing plasma-ionized gas through water, which generates a wide range reactive oxygen and nitrogen species that can inactivate bacteria. The main species generated in tap water are ozone, hydrogen peroxide, and nitrite.	Y	
Tsunami-on-Farm Peracetic Acid Biocide	Ecolab, USA	Contains 30-60% acetic acid, 10-30% peroxyacetic acid, and 10-30% hydrogen peroxide Treatment: 80 ppm; pH 5-6	Tsunami products are EPA-registered biocides.		Y

L laboratory experiments; P packhouse trial

Laboratory Experiments

The purpose of the laboratory experiment was to evaluate the effectiveness of several postharvest sanitizers to reduce *Salmonella* populations, particularly in comparison with chlorine as the standard industry treatment.

Methods

Peach fruit were inoculated with overnight TSBN cultures of *S.* Enteritidis by submersion for 10 min in the inoculum (8 log CFU/mL). The inoculated fruit were dried for 1 h at ambient temperature, then overnight at 4°C. Sanitizers were diluted in sterile tap water and made up as described in Table 3. The fruit were dipped in the sanitizer solutions for contact times of 1, 30, and 60 s, immediately transferred to sterile bags, and massaged for 2 min in 50 mL PBS. The control treatment was dipping fruit in water only. Dilutions were plated onto xylose lysine tergitol 4 (XLT4) agar supplemented with 40 ppm nalidixic acid (XLT4N) and enumerated after incubation for 48 h at 37 °C.

Each sanitizer/time combination was applied to three 'Snow King' and three 'Snow Ice' fruit. The initial inoculum concentrations were calculated to be 7.06 ± 0.42 log CFU/g and 5.63 ± 0.43 log CFU/g on 'Snow King' and 'Snow Ice' fruit, respectively. Data of *Salmonella* recovery was converted to log reduction and analyzed statistically using ANOVA and Tukey's HSD tests (Genstat 18th edition, VSNI, UK).

Key Results

- Nylate (20 ppm) was the most effective sanitizer tested. For all contact times, it reduced the *Salmonella* count to below the limit of detection (100 CFU/g). Given an average initial inoculum concentration of 6.3 log CFU/g, this represented approximately 4 log reduction in *S.* Enteritidis.

- Lactic acid (2%) was the second-most effective sanitizer tested at all contact times. It delivered a 3-4 log reduction in *S*. Enteritidis when applied for 1-30 s, and a 4 log reduction when applied for 60 s.

- Chlorine (100 ppm), the industry standard treatment, delivered a numerically higher log reduction in *Salmonella* than the other tested sanitizers when applied for 30-60 s. These were not necessarily statistically significant differences due to high standard deviations (i.e., high fruit-to-fruit variability in bacterial recovery).

- PhageGuard S (1%) delivered a 1-2 log reduction in *S*. Enteritidis at each contact time tested, which was statistically similar (P>0.05) to chlorine. PhageGuard S is expected to provide 1-3 log reduction over a period of 6 days, according to the manufacturer. Our results may under-represent the potential application of bacteriophages as postharvest sanitizers. Fruit were sampled as for the other (contact) sanitizers, immediately after treatment, rather than a time relevant to bacteriophage activity.

- Peroxy Treat (80 ppm) applied for 30-60 s gave a 1-2 log reduction in *Salmonella*, which was statistically similar (P>0.05) to chlorine.

- Water and PAW were among the least effective treatments, though there were not necessarily statistical differences from chlorine, for the reason provided earlier.

- Chlorine dioxide (10 ppm) had highly variable efficacy at 60 s (99% relative standard deviation) and performed poorly at 1 and 30 s. The reason for this is unclear, given that this is a commonly used product in industry. The variable performance suggests that the product may have been incompletely dissolved.

Appended Data

- Figure 17. *S.* Enteritidis log reduction in laboratory challenge experiment

Packhouse Trial

A packhouse trial was carried out to test the efficacy of selected sanitizers under conditions that would be relevant to industry. An Australian peach grower (Mark Jolly) provided access and operation of his commercial grading line (GP Graders) at Canoelands Orchard, Glenorie, New South Wales, approximately 65 km north-northwest of Sydney. The setup and functionality of the grader were dated and quite different to that used in the US. The most important difference for this study was that fruit were washed and sanitized in a 3,000-L dump tank rather than a spray system. We had iterative in-depth discussions with our industry advisors, George Nikolich and Trevor Suslow, as well as with Mark Jolly to develop an experimental design that, though not ideal, would generate meaningful data in the short time that remained of the Australian peach season.

Methods

'Snow Fall' white peach fruit were spot-inoculated with overnight TSBN cultures of *E. coli* MG1655 (10 log CFU/mL). The fruit were dried for 3 h at ambient temperature then overnight at 4°C. Chlorine (100 ppm), lactic acid (2%), Nylate (20 ppm), and Tsunami-on-Farm (80 ppm) were diluted in the grader spray tank with tap water and pH adjusted as necessary.

Fruit is normally processed on the Canoelands Orchard grader as follows:

1. Washed in 3,000-L dump tank containing sanitizer solution (usually chlorine).
2. Manually sorted for defects on inline sorting table.
3. Conveyed on brush rollers under spray bars.
4. Sprayed with fungicide pumped from a recirculating tank.
5. Dried on foam donut rollers.
6. Conveyed on singulator belt for weight-sorting into rotary bins.

Grader operation was modified for our trial, and fruit were processed as follows (Figure 6):

1. Positioned on the brush roller section of the grader, under the spray bars.
2. Sprayed with sanitizer solution for 1, 15, and 30 s as they rolled in place. The run-off was collected in a large plastic tray to make this single pass instead of recirculating.
3. Sprayed with tap water for 30 s using a motorized knapsack as they rolled in place.
4. Dried on the foam donut rollers for 15 s to remove excess liquid.
5. Transferred to individual sterile bags containing 50 mL neutralizing buffer (Difco).
6. Stored in an insulated container for transport to the laboratory.

Each treatment was applied to two batches of fruit, with 15-20 fruit per batch. An additional 15-20 non-inoculated fruit were treated and evaluated for phytotoxicity after 1 week of storage at 4ºC by visual inspection for symptoms. The equipment was cleaned between treatments using a quaternary ammonium sanitizer following a 'professional protocol' (Sani-Klean). Samples were manually massaged for 2 min, serially diluted, spread on TSAN plates, and enumerated after overnight incubation at 37ºC.

Figure 6. Processing of white peach fruit in packhouse trial. (A) Sanitizer solution sprayed on fruit as they rolled in place on brush rollers; (B) tap water rinse applied using motorized knapsack sprayer; (C) fruit dried on foam donut rollers; and (D) sampling of individual fruit into sterile bags containing neutralizing buffer.

Key Results

- When comparing the 15-s treatments only, the most effective (P<0.05) sanitizers were chlorine and Tsunami-on-Farm, which delivered 2.0 and 1.7 log reductions, respectively.
- Nylate and lactic acid applied for 15 s were less effective, delivering 1.4 and 0.7 log reductions, respectively.
- There was no difference (P>0.05) among the 1-s treatments, with the sanitizers delivering an average 0.5-0.7 log reduction.
- There was no difference (P>0.05) among the 30-s treatments, with the sanitizers delivering an average 1.4-1.8 log reduction.

Appended Data

- Figure 18. *E. coli* MG1655 log reduction in packhouse trial

Outcomes and Accomplishments

The overarching purpose of this RR project was to investigate preharvest and postharvest interventions that could potentially reduce *Salmonella* risk in the peach orchard or packhouse. The research objectives and design were refined following in-depth discussion with industry advisors (George Nikolich and Trevor Suslow) to deliver results that would be relevant to industry within the time and resource constraints of the project. The proposal was revised to have three main objectives:

1. Determine optimum dose rates of CuEDTA, $ZnSO_4$, and Peroxy Treat to achieve potential *Salmonella* risk reduction on peach fruit and identify possible thresholds for phytotoxicity.

2. Determine the efficacy of a range of postharvest sanitizer treatments to achieve potential *Salmonella* risk reduction on peach fruit.

3. Evaluate the potential impacts of selected treatments (identified in Objectives 1 and 2) on peach shelf-life and quality.

Objective 1 was achieved by conducting bacterial inhibition assays in the laboratory and two field trials on commercial orchards. The preharvest foliar treatments that we tested were *not* found to be readily actionable interventions for peach orchards. Peroxy Treat had *in vitro* efficacy against *S*. Enteritidis and *E. coli* MG1655 but did not have consistent, significant bactericidal effects in the field. CuEDTA and $ZnSO_4$ products are reported to have bactericidal effects in industry and scientific literature, but neither our laboratory assays nor our field trials confirmed this. We found that preharvest foliar treatments at high application rates (i.e., higher than recommended by the manufacturer) were likely to cause varying levels of foliar or fruit phytotoxicity depending on the product. Industry should be aware of phytotoxicity as a potential trade-off when pursuing risk reduction in the orchard using similar foliar products at high concentrations.

Although the preharvest foliar treatments that we tested were not obviously effective, they should not be ruled out as potentially useful interventions. The scope of our experiments was limited not only by the timeframe of the RR project but by the duration of the New South Wales peach season, as COVID travel restrictions prevented access to fruit in other states. Consequently, we did not have the opportunity to replicate experiments, optimize treatment variables, or explore the interactions of biotic and abiotic factors (such as cultivar and weather conditions) on treatment effects. We cannot recommend the preharvest foliar treatments that we tested based on our results, but there is certainly scope for further research if industry prefers this approach.

In the absence of a preharvest intervention to inactivate *Salmonella* in the orchard, a recommended strategy to manage contamination is to separate and segregate buffer zone fruit from the rest of the harvest. The fruit could potentially be subjected to additional postharvest interventions, diverted from fresh market to processing, or destroyed depending on availability of validated processes and severity of contamination. Segregation is a common strategy for food safety management in certain industries (e.g., mycotoxin risk management in grain and peanut industries) and may be worth investigating in a horticultural context. To this end, the development of evidence-based guidelines for appropriate demarcation and management of a buffer tree zone would be useful.

Objective 2 was achieved by conducting a laboratory challenge study and a packhouse trial. We tested a variety of sanitizers that were available to us with the goal of establishing preliminary data that might help narrow the scope of subsequent research. The products we tested have commercial use in Australia apart from plasma-activated water, which is an emerging technology still in the research and development phase.

Our laboratory challenge experiment demonstrated that more than 3.5 log reduction in *Salmonella* is achievable through optimization of a sanitizer treatment. The most promising product we tested was Nylate, which is a registered processing aid in Australia and commonly used in some industries (e.g., apples and pears). However, there is little publicly available information the regulatory status of Nylate in the United States. It apparently has FDA approval for use in process water, but there does not seem to be a registered product for use on fresh produce. Further research of Nylate to optimize the treatment and support regulatory approval in the United States may be worthwhile. Having said this, our experiments did not constitute comprehensive screening. More extensive testing of alternative sanitizers, optimization of treatment variables, and evaluation of sequential sanitizer treatments are recommended.

The results from our packhouse trial were not as persuasive as the laboratory challenge. Lactic acid, Nylate, and Tsunami-on-Farm achieved similar or lower log reductions in *E. coli* MG1655 to a standard chlorine treatment. The goal of our packhouse trial was to test selected sanitizers using industry-relevant spray equipment, and we modified the operation of a commercial grader to better reflect industry practice in the United States. We achieved good coverage of the fruit surface with sanitizer solution, but experimentation in the packhouse environment introduced other variables that may have impacted microbial survival and that were difficult to control. For example, additional transport and handling under non-refrigerated conditions, contact with a range of surfaces on the grading line, variability in spray pressure and coverage, and variability in rinse pressure and coverage.

Our use of *E. coli* MG1655 as a *Salmonella* surrogate was pragmatic as it was available in our strain collection. Although we did not have time to validate its suitability for our food system, our choice of surrogate was reasonable as *E. coli* K-12 strains (including MG1655) have been validated as surrogates for *S*. Enteritidis (microwave, thermal, and high pressure) pasteurization and *S*. Poona (electron beam irradiation)[3]. *Enterococcus faecium* NRRL B-2354 may be a more commonly used surrogate for *S*. Enteritidis; however, published validation studies are predominantly for thermal processing interventions. The use of surrogate organisms is necessary for conduct of challenge experiments in commercial premises, but results need to be interpreted cautiously and in conjunction with laboratory experiments.

Another complicating factor in our packhouse trial was that we had reached the end of the New South Wales peach season. The quality of peach fruit that we were able to access was variable, with a substantial proportion of immature, overripe, and bruised fruit. This heterogeneity in fruit quality is likely to have contributed to variability in microbial survival on the peach surface as well as to variability in the extraction of bacteria for enumeration. We did not have the opportunity to replicate the laboratory or packhouse experiments, optimize treatment parameters, or explore interactions other than a few contact times.

Despite these limitations, our research has confirmed that there are opportunities to optimize postharvest sanitizer treatments to achieve effective *Salmonella* risk reduction in peach fruit. We

[3] Hu M, Gurtler JB (2017) Selection of surrogate bacteria for use in food safety challenge studies: a review. *Journal of Food Protection* **80**, 1506-1536.

recommend that further research of postharvest interventions be prioritized as an approach likely to yield actionable strategies in a relatively short time frame.

Objective 3 was delivered by assessing peach quality indices (i.e., weight loss, total soluble solids, and flesh firmness) after postharvest cold storage. The preharvest foliar treatments did not impact postharvest fruit quality in our experiments. The quality of fruit used in our postharvest sanitizer experiments was generally poor and highly variable. This heterogeneity was likely to obscure any treatment effects, making results unreliable, so we did not evaluate postharvest fruit quality following sanitizer treatments apart from visual inspection for the development of obvious defects. None were observed. While we did not find evidence of the treatments adversely impacting fruit quality, this should be monitored in future experiments.

Summary of Findings and Recommendations

Preharvest foliar treatments (CuEDTA, $ZnSO_4$, and Peroxy Treat) were *not* found to be readily applicable interventions for effective *Salmonella* risk reduction in this project.

Peroxy Treat demonstrated efficacy as a bactericide. It had a MIC of 188-375 ppm and MLC of 375-750 ppm against *S*. Enteritidis in bacterial inhibition assays. Peroxy Treat (1-2%) decreased the total aerobic bacteria (0.3-0.4 log CFU/g) and total coliforms (0.6-1.8 log CFU/g) on fruit in Field Trial 2 but not in Field Trial 1.

CuEDTA and $ZnSO_4$ did not demonstrate bactericidal activity in the bacterial inhibition assays and treatments did not significantly affect pre-existing microflora in the orchard.

Varying levels of foliar and fruit phytotoxicity were found to be a risk when products were sprayed at high application rates.

Our recommendations regarding preharvest interventions include:

- Separation and segregation of fruit from the buffer zone around a known or presumptive source of contamination.
- Research to develop of evidence-based guidelines for appropriate demarcation and management of the buffer tree zone.

Postharvest sanitizer treatments were found to offer opportunities for effective *Salmonella* risk reduction in peach fruit. We demonstrated that it is possible to achieve more than 3.5 log reduction in *S.* Enteritidis through selection of the postharvest sanitizer.

Nylate was the most effective sanitizer tested in the laboratory challenge study. For all contact times (1, 30, and 60 s), it reduced the *Salmonella* count to below the limit of detection (100 CFU/g). Given an average initial inoculum concentration of 6.3 log CFU/g, this represented approximately 4 log reduction in *S*. Enteritidis.

Lactic acid (2%) was the second-most effective sanitizer tested in the laboratory challenge study. It delivered a 3-4 log reduction in *S*. Enteritidis when applied for 1-30 s, and a 4 log reduction when applied for 60 s.

In the packhouse trial, chlorine, lactic acid, Nylate, and Tsunami-on-Farm produced similar decreases in bacterial count when applied for 1 s (0.5-0.7 log reduction) and 30 s (1.4-1.8 log reduction).

When applied for 15 s, the most effective sanitizers were chlorine and Tsunami-on-Farm, which delivered 2.0 and 1.7 log reductions, respectively. Nylate and lactic acid were less effective, delivering 1.4 and 0.7 log reductions, respectively.

Our testing was constrained by time and resources, and we have several recommendations for further research of postharvest interventions, including:

- More extensive screening of alternative sanitizer treatments including optimization of relevant treatment variables (e.g. concentration, contact time).
- Evaluation of potential additive, if not synergistic, effects on *Salmonella* risk reduction from sequential sanitizer treatments.
- Characterization of biotic and abiotic interactions with sanitizers to ensure that conditions for reliable and effective performance are well defined.
- Optimization of Nylate treatment specifically, given its promising performance in our challenge study, depending on its regulatory status and likelihood of registered products with approved use on fresh produce becoming available.

APPENDICES

Publications and Presentations

The Principal Investigator presented final results (in webinar format, due to COVID-19 restrictions) in July 2021 at the CPS Research Symposium Webinar Series, Session IV.

The intention is to submit the research for publication in a peer-reviewed scientific journal.

Budget Summary

The grant funds ($81k) were spent on technical staff salaries, fieldwork and laboratory consumables, and travel to conduct the field and packhouse trials. An approximate breakdown of the expenditure follows:

- 72% on technical personnel salaries
- 10% on peach fruit, fieldwork consumables, and laboratory consumables
- 5% on travel expenses
- 3% on hire of the packhouse including operational assistance
- 4% on indirect costs
- 6% not spent

At the time of final report submission, some expenses were yet to be processed by the University of Sydney.

Acknowledgements

We would like to thank the following people for their support of this project:

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- George Nikolich (George Nikolich Consulting, Fresno CA) provided insights into industry practices and perspectives that also helped to guide the research and ensure its relevance to industry.
- Bonnie Fernandez-Fenaroli (CPS) for supporting and facilitating this international collaboration.
- Glenn and Jo Fahey (Glenbernie Orchard, Darkes Forest NSW, Australia) provided access to their orchard and facilitated Field Trial 1.
- Ashley Napolitano (Sunland Orchard, Barooga NSW, Australia) provided access to his orchard and facilitated Field Trial 2.
- Mark and Linda Jolly (Canoelands Orchard, Glenorie NSW, Australia) provided access to their packhouse and operational assistance to carry out the postharvest sanitizer trial.
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- CPS and California Fresh Fruit Association for funding the project.

Appended Data: Table 4 and Figures 7–18

Preharvest Foliar Treatments

Fruit phytotoxicity and quality data collected after 2 weeks of storage are not included, as the data does not provide further information to the data collected after 1 week of storage.

Table 4. Minimum inhibitory (MIC) and minimum lethal (MLC) concentrations of preharvest foliar treatments against a *S.* Enteritidis and *E. coli* MG1655 according to bacterial inhibition assays. The tested range was 11.7-6,000 ppm.

Product	*S.* Enteritidis				*E. coli* MG1655			
	MIC (ppm)		MLC (ppm)		MIC (ppm)		MLC (ppm)	
	Geomean	Range	Geomean	Range	Geomean	Range	Geomean	Range
CuEDTA	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000
Peroxy Treat	298	188-375	473	375-750	375	375	375	375
$ZnSO_4$	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000	>6,000

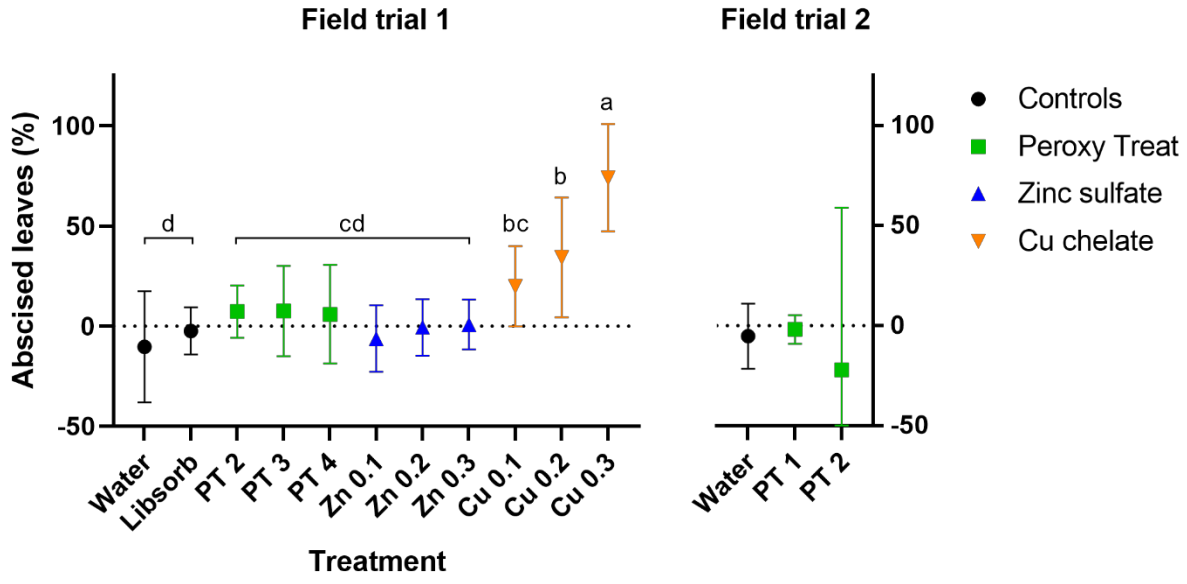

Figure 7. Defoliation (mean ± standard deviation) as the percentage of leaves abscised 10 days after preharvest foliar treatments were applied (N = 16 shoots). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no significant differences (P>0.05) among treatments in Field Trial 2.

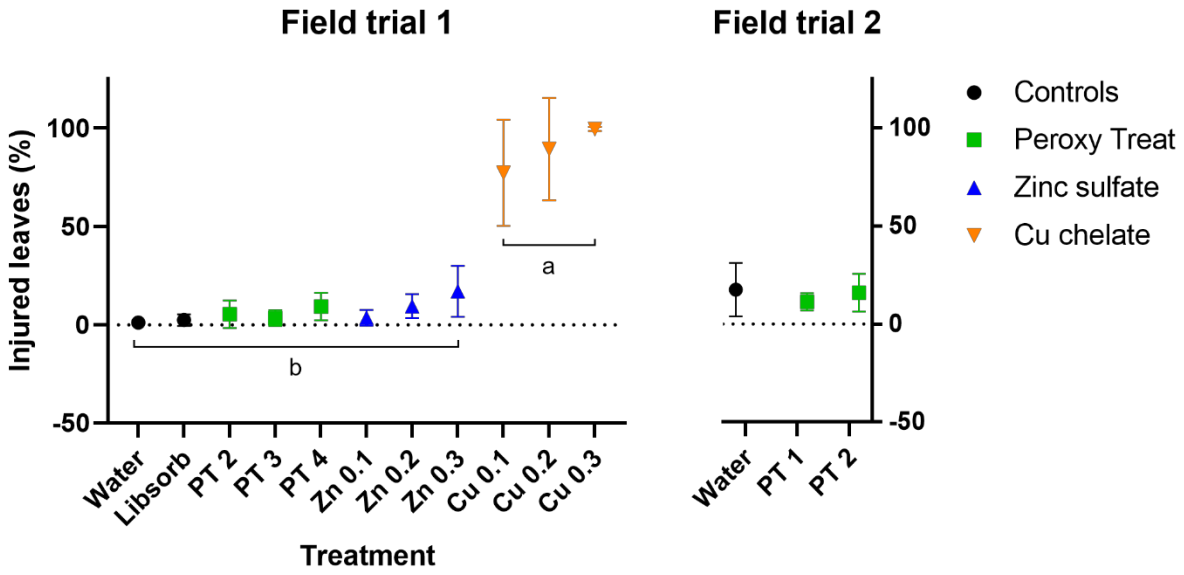

Figure 8. Leaf injury (mean ± standard deviation) as the percentage of extant leaves with symptoms (e.g., bleaching and browning) 10 days after preharvest foliar treatments were applied (N = 16 shoots). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no significant differences (P>0.05) among treatments in Field Trial 2.

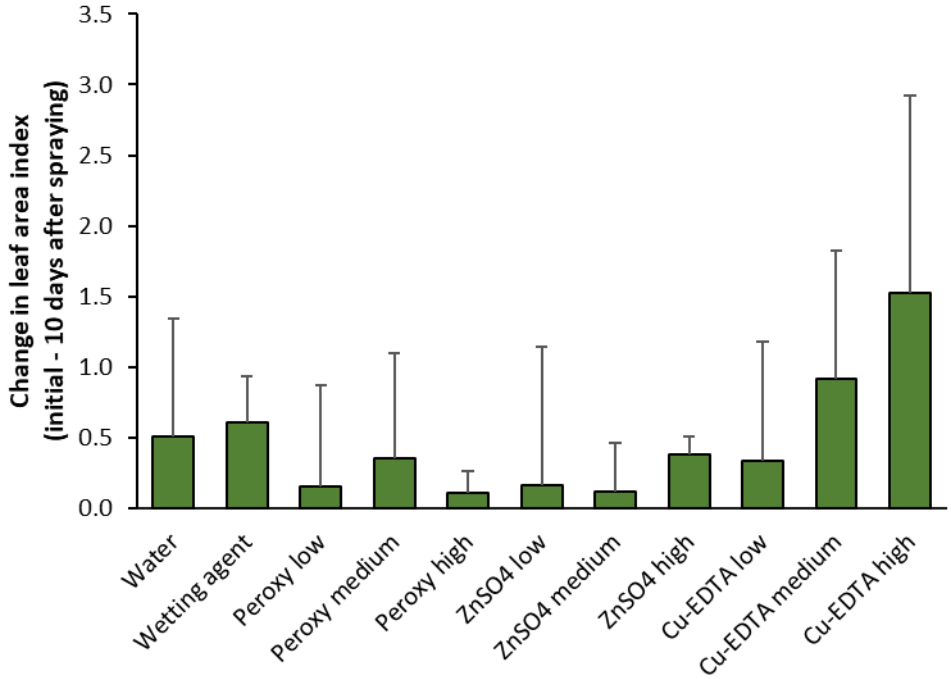

Figure 9. Change in leaf area index (mean ± standard deviation) 10 days after preharvest foliar treatments were applied (N = 4 trees) compared to initial measurements taken before treatments.

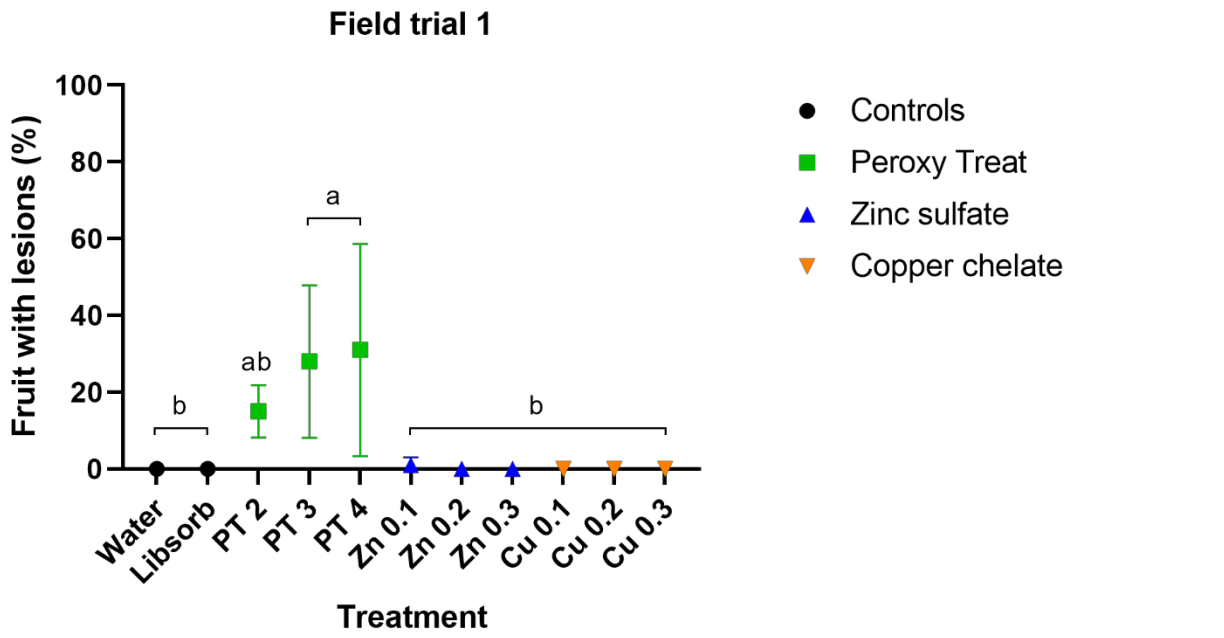

Figure 10. Fruit phytotoxicity (mean ± standard deviation) as percentage of fruit with symptoms after 1 week at 4°C and 3 days at ambient temperature (N = 25 fruit). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no symptoms of phytotoxicity in fruit from Field Trial 2.

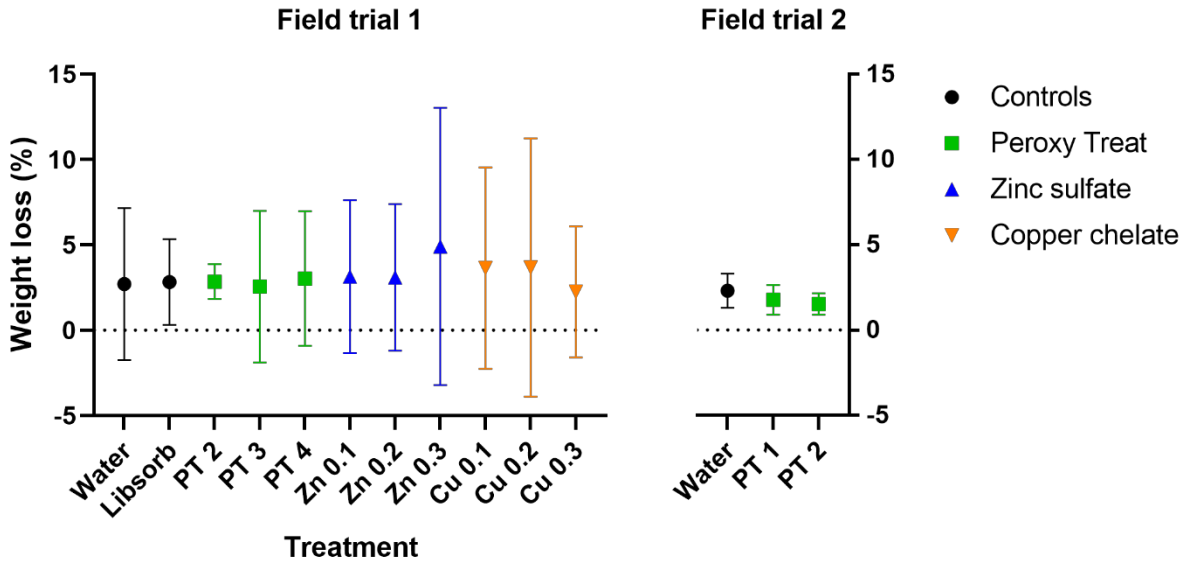

Figure 11. Weight loss (%) in peach fruit (mean ± standard deviation) after 1 week at 4°C and 3 days at ambient temperature (N = 25 fruit). There were no significant differences (P>0.05) among treatments according to Tukey's HSD test.

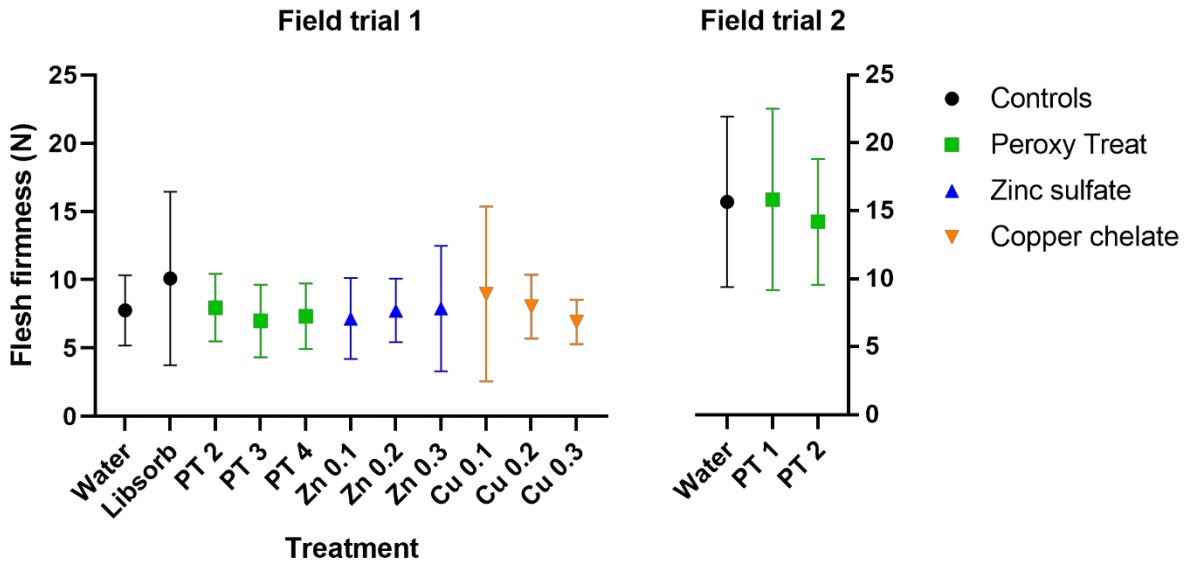

Figure 12. Flesh firmness (N) of peach fruit (mean ± standard deviation) after 1 week at 4°C and 3 days at ambient temperature (N = 25 fruit). There were no significant differences (P>0.05) among treatments according to Tukey's HSD test.

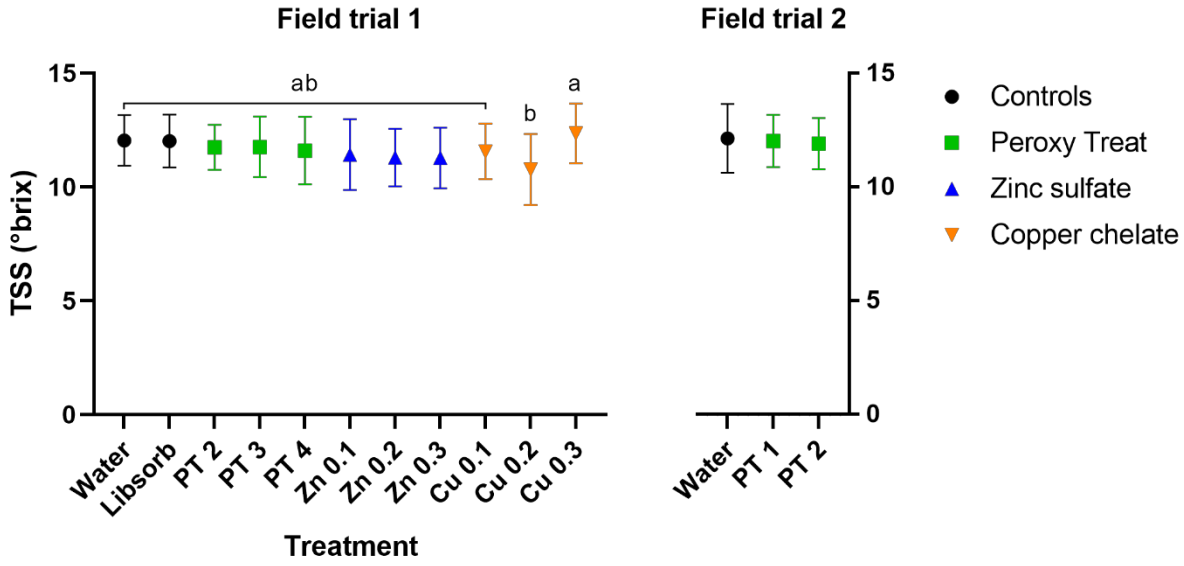

Figure 13. Total soluble solids (TSS; °brix) in expressed peach juice (mean ± standard deviation) after 1 week at 4°C and 3 days at ambient temperature (N = 25 fruit). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no significant differences (P>0.05) among treatments in Field Trial 2.

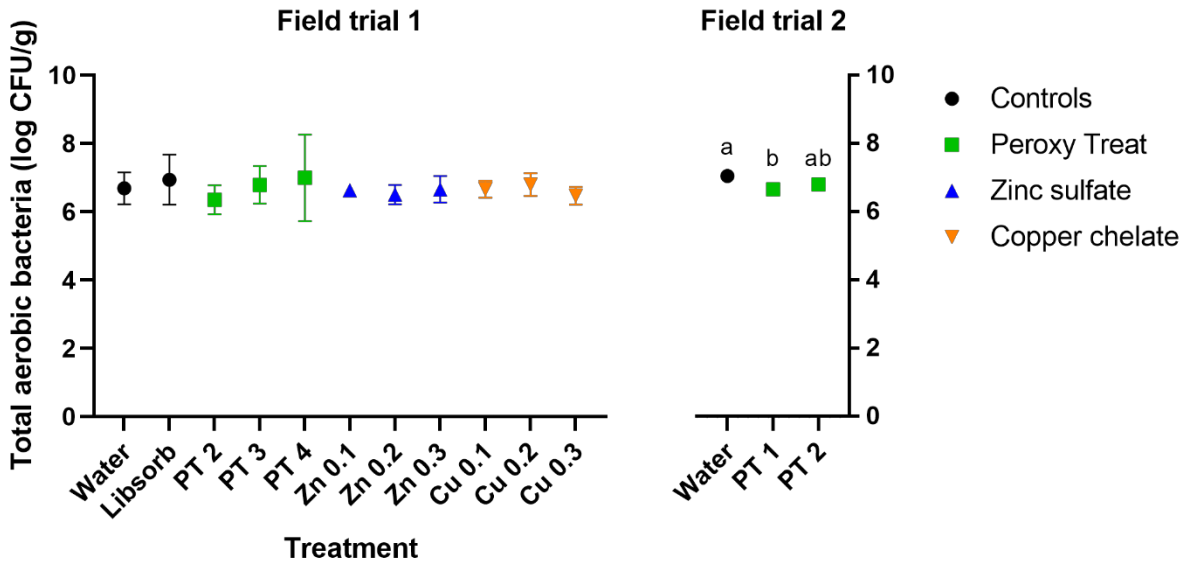

Figure 14. Total aerobic bacteria count (mean ± standard deviation) on peach fruit sampled at harvest, or 2 days after foliar treatments were applied (N = 4 trees). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no significant differences (P>0.05) among treatments in Field Trial 1 according to Tukey's HSD test.

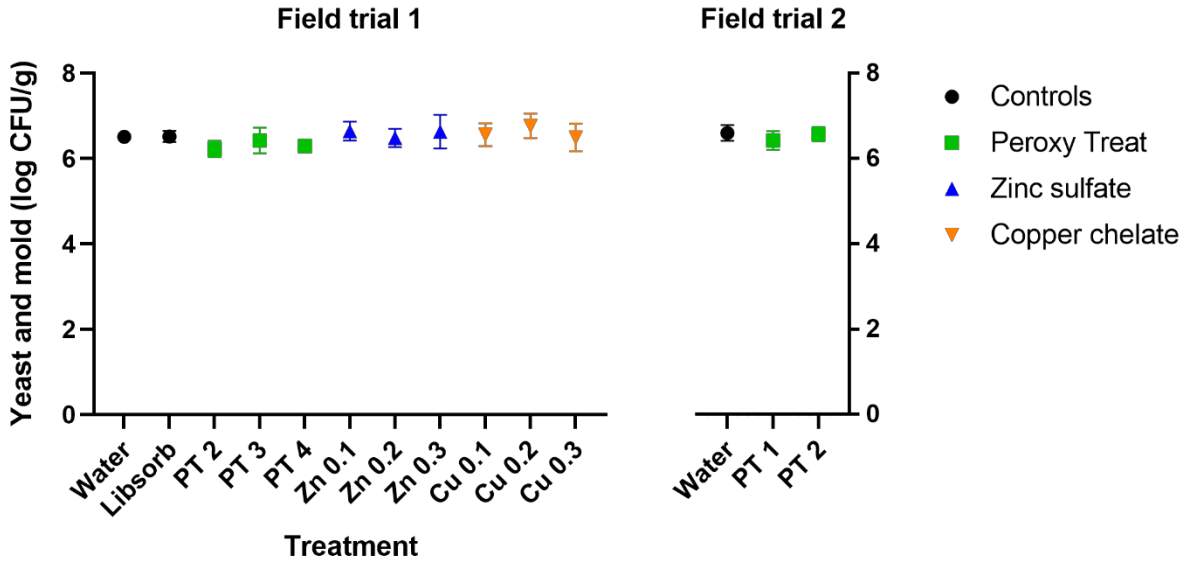

Figure 15. Yeast and mold count (mean ± standard deviation) on peach fruit sampled at harvest, or 2 days after foliar treatments were applied (N = 4 trees). There were no significant differences (P>0.05) among treatments according to Tukey's HSD test.

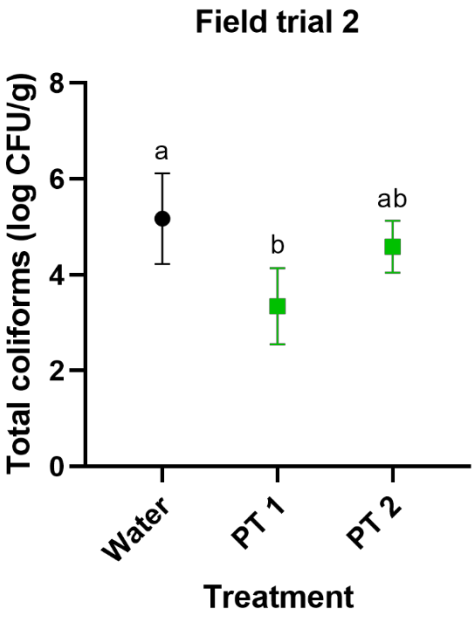

Figure 16. Total coliform count (mean ± standard deviation) on peach fruit sampled at harvest, or 2 days after foliar treatments were applied (N = 4 trees). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. Total coliforms were not enumerated in Field Trial 1.

Postharvest Sanitizer Treatments

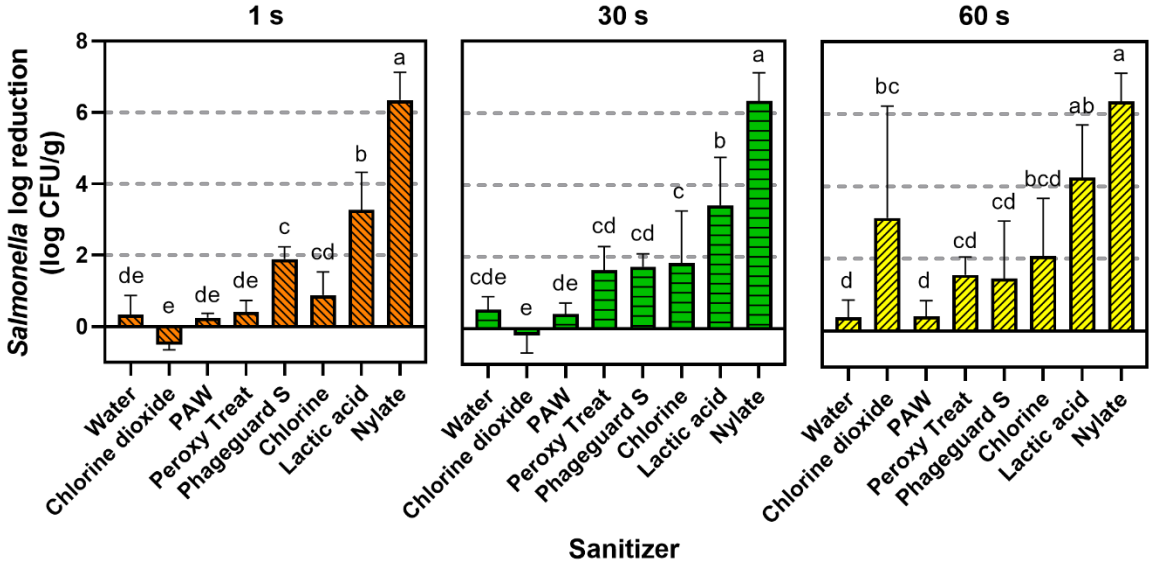

Figure 17. Log reduction (mean ± standard deviation) in *Salmonella* Enteritidis (log CFU/g) on white peach fruit after washing with sanitizers for 1, 30 and 60 s (N = 6 fruit). Treatments annotated with different lowercase letters (within contact time) were significantly different (P<0.05) according to Tukey's HSD test. Limit of detection was 100 CFU/g.

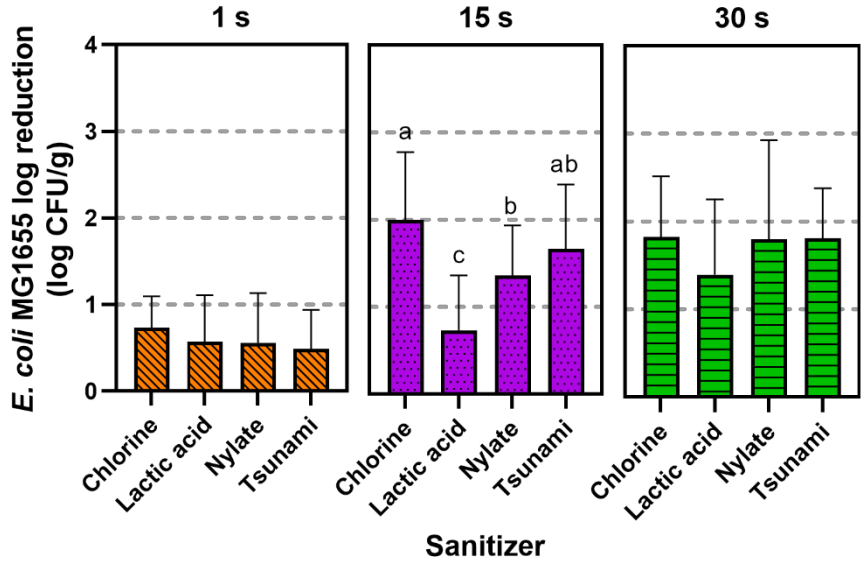

Figure 18. Log reduction (mean ± standard deviation) in *Escherichia coli* MG1655 (log CFU/g) on white peach fruit after washing with sanitizers for 1, 15 and 30 s (N = 24-39 fruit). Treatments annotated with different lowercase letters were significantly different (P<0.05) according to Tukey's HSD test. There were no significant differences (P>0.05) among 1-s and 30-s treatments. Limit of detection was 10 CFU/g.