Project Title: Understanding Food Safety Risks During Post-Harvest Cherry Production

Report Type: Final Project Report

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Cooperators: Between the 3 field objectives, 7 unique cherry packinghouses in Washington and 4 in California were cooperators across objectives 1 - 3. The identity of the operations serving in cooperator roles will be kept confidential.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $74,255
Total Project Request for Year 2 Funding: $76,231
Total Project Request for Year 3 Funding: $69,785

Other related/associated funding sources: None
Funding Duration: NA
(Type Current Funding Year) Amount: NA
Agency Name: NA
Notes: NA

WTFRC Collaborative Costs:

Item	2024	2025	2026
Salaries	$3,900.00	$4,056.00	$4,218.00
Benefits	$1,218.00	$1,267.00	$1,317.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$5,118.00	$5,323.00	$5,535.00

Footnotes: an estimate of the percent of time spent for Mendoza (4%) at a 31.2% benefit rate and 2% annual increases

Budget 1
Primary PI: Claire Murphy
Organization: Washington State University Irrigated Agriculture Research and Extension Center
Contract Administrator: Hollie Tuttle
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Item	2024	2025	2026
Salaries	$32,981.00	$34,300.00	$35,672.00
Benefits	$2,848.00	$2,848.00	$2,848.00
Wages	$9,955.00	$10,353.00	$10,767.00
Benefits	$1,353.00	$1,407.00	$1,463.00
RCA Room Rental			
Shipping			
Supplies	$20,500.00	$20,500.00	$13,000.00
Travel	$1,500.00	$1,500.00	$500.00
Plot Fees			
Miscellaneous			
Total	$69,137.00	$70,908.00	$64,250.00

Footnotes:

- Salaries: $32,981, $34,300, and $36,672 are requested in years 1, 2, and 3, respectively, for a graduate research assistantship for a Ph.D. student to work on all objectives

- Benefits: $2,848 is requested in years 1, 2, and 3 for benefits tied to the graduate research assistantship
- Wages: $9,955, $10,353, and $10,767 are requested in years 1, 2, and 3, respectively, hourly employees to assist with sample collection and conducting experiments related to all three objectives
- Benefits: $1,353, $1,407, and $1,463 are requested in years 1, 2, and 3, respectively, for benefits tied to the hourly employees
- Supplies: $20,500, $20,500, and $13,000 are requested in years 1, 2, and 3, respectively, to purchase disposal supplies such as glassware, inoculation loops, pipette tips, sponge swabs, microbiological media, Petri dishes, PCR reagents, etc.
- Travel: $1,500, $1500, and $500 are requested in years 1, 2, and 3, respectively, for mileage and travel-associated cost, adhering to all university policies

Recap Original Objectives

1. Evaluate the prevalence of *Listeria* spp., as an indicator for *L. monocytogenes*, on food contact surfaces on the packing line within commercial cherry packinghouses at three time points (beginning, middle, and end of the season) over two packing seasons.
2. Evaluate the microbiological status of postharvest water and cherries to understand the change in microbial status from receiving to packing over a packing day.
3. Evaluate the cross contamination risks associated with hydrocooling.
4. Evaluate the survival of foodborne pathogens on cherries during storage and determine changes in pathogen populations over time.

Significant Findings

Objective 1:

- *Listeria* spp. was detected in all packinghouses across both years, with an overall prevalence of 2.0% (38/1,920) in Washington and 2.4% (15/636) in California.
- Bristle flaps, solid conveyor belts, and singulators were the most frequently contaminated food contact surfaces in both years and both states.
- *Listeria* spp. prevalence was slightly higher during packing (2.19%) than pre-packing (1.96%).
- Only 2 sites (3.9%) were positive during the same sampling event, both pre- and during packing, indicating that most *Listeria* spp. detections were transient rather than persistent.

Objective 2:

- Generic *E. coli* was detected on 1.5% of cherries (4/261) and in 2.5% of water samples (5/204), while total coliforms were detected on 18.0% of cherries (47/261) and in 45.1% of water samples (92/204).
- Sanitizer concentration was not consistently related to any water quality examined, highlighting the need to directly monitor sanitizer levels during packing.
- Higher aerobic plate counts (APC) on incoming cherries were associated with higher APC in the water and on outgoing cherries. Water APC was also associated with APC on outgoing cherries, demonstrating the potential for cross-contamination.

Objective 3:

- Hydrocooling was not associated with an overall increase in microbial loads on cherries. Total coliform and HPC concentrations decreased by an average of 0.87 and 0.77 log MPN/cherry, respectively, from pre- to post-hydrocooling ($p < 0.001$ and $p = 0.001$).
- Each 1-log increase in hydrocooler water HPC was associated with a 0.59-log greater pre- to post-hydrocooling change in cherry HPC ($p < 0.001$), indicating that higher microbial loads in the water were associated with smaller reductions or increases in HPC on cherries.

Objective 4:

- *L. monocytogenes* demonstrated greater persistence on cherries during refrigerated storage, decreasing by approximately 2.2 log CFU/cherry over 14 days, compared with reductions of approximately 3.6 and 4.0 log CFU/cherry for *Salmonella* and STEC, respectively.

Methods

Objective 1:

Study Design: Four commercial cherry packinghouses in Washington and four in California were recruited into this study and swabs of food contact surfaces were collected from each of the packinghouses during three separate visits (beginning, middle, and end of the season). The food contact surfaces for sampling were selected at each operation before the first sampling event. Sampling sites for each packinghouse were photographed and described in detail to ensure consistency during all sampling events. Samples were collected prior to the start of packing, as well as 5 h into packing. Sampling was conducted during the 2024 and 2025 seasons in WA, and the 2025 season in CA.

Sample Collection: Swab samples were collected from each site using sponge sticks that were premoistened with 10mL of Dey Engley neutralizing broth. Each sponge stick was aseptically removed from the bag with sterile gloves, used according to the manufacturer's specifications, returned to the bag, sealed, and placed in a cooler. All samples were processed within 24h at the Murphy Bio-Safety Level 2 Laboratory at Washington State University's Irrigated Agriculture Research and Extension Center.

Sample Processing: The isolation, detection, and confirmation of *Listeria* spp. was conducted following a modified FDA Bacteriological Analytical Manual method (FDA, 2022). Briefly, 90 mL of buffered *Listeria* enrichment broth was added to each sample bag, stomached, and incubated at 30ºC. Following a 4h incubation period, a 360µL aliquot of *Listeria* selective enrichment supplement was added to each bag and returned to the 30ºC incubator for 20h. At both 24h and 48h, the enriched sample was sub-streaked onto modified oxford agar and incubated for 48h at 30ºC. Presumptive positive colonies were re-streaked onto modified oxford agar and incubated for 48h at 30ºC before being PCR-confirmed.

Objective 2:

Experimental design and processing: Four commercial cherry packinghouses (A–D) in Washington State were sampled during the 2025 season. Packinghouses A and B operated four sequential discrete water systems, while C and D operated three. Production water was treated with calcium hypochlorite, with sanitizer levels managed using oxidation-reduction potential (ORP) targets of 800–850 mV (A and B) or >750 mV (C and D). Water pH was adjusted using acid-based treatments to approximately pH 6.0–7.5. At each sampling time point, three individual cherries were collected before entering the discrete water systems and three after exiting the discrete water systems.

Sample collection: Triplicate 100 mL water samples were collected from each discrete water system into sterile bottles containing sodium thiosulfate to neutralize residual sanitizer. Sampling was repeated throughout the production day. Samples were transported on ice and stored at 4°C for up to 48 h before microbiological analysis. Water pH, turbidity, temperature, and free chlorine concentration were monitored throughout each sampling day. Turbidity was measured using a portable turbidimeter and reported as nephelometric turbidity units (NTU). Free chlorine was measured using a titration kit with 0.5 ppm increments.

Sample processing: Individual cherries were rinsed with 10 mL of 0.1% peptone using a standardized rub-shake-rub procedure. Cherry rinsates and water samples were serially diluted as needed and analyzed for aerobic plate counts (APC), *Enterobacteriaceae* (EB), total coliforms (TC), and generic *Escherichia coli* (EC) using Petrifilm methods. Colonies were enumerated according to manufacturer instructions following incubation. Limits of detection were 0.0 log CFU/mL for water and 1.0 log CFU/cherry for cherry samples.

Structural equation model: Structural equation modeling is a statistical approach that allows multiple relationships to be evaluated simultaneously, helping identify how factors such as water quality, sanitizer concentration, water hold time, and microbial populations may be related to one another and ultimately influence microbial populations on cherries (Figure 1). Separate SEMs were developed for each DWS using the same model structure to evaluate both direct and indirect relationships among water quality, microbial populations in the water, and microbial populations on cherries over the water hold period.

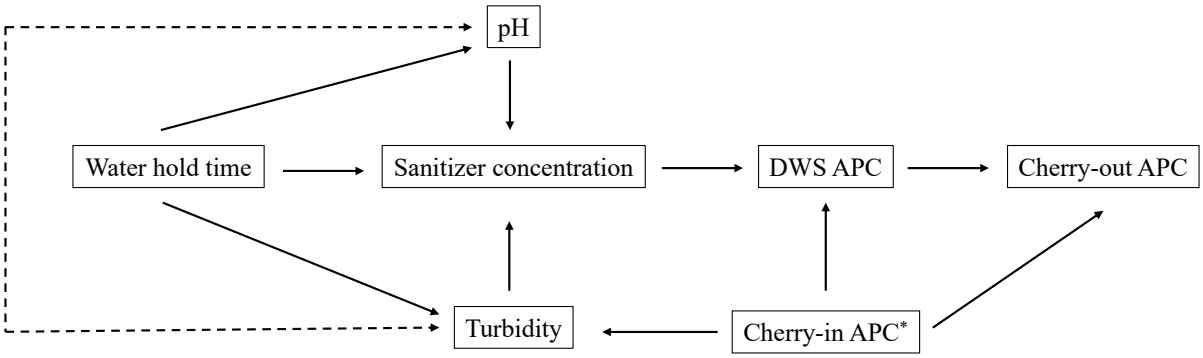


Figure 1. Graph illustrating the structural equation model structure applied to each discrete water system in the separate SEMs. The solid arrows represent direct effects, whereas the dashed arrow is representative of a covariance structure.

Objective 3:

Experimental design, sample collection and processing: Four commercial cherry packinghouses in Washington State were sampled during the 2026 season. Triplicate water samples (100 mL) were collected from hydrocooler water systems, and three individual cherries were collected before entering and after exiting the hydrocooler at each sampling time point throughout the total water hold period. Water samples were collected into sterile bottles containing sodium thiosulfate to neutralize residual sanitizer. All water and cherry samples were transported on ice and stored at 4°C for up to 48 h before microbiological analysis. Individual cherries were rinsed with 100 mL of 0.1% peptone using a standardized rub-shake-rub procedure. Cherry rinsates and water samples were analyzed for heterotrophic plate counts (HPC), total coliforms, and generic *Escherichia coli* using the IDEXX Quanti-Tray system according to manufacturer instructions. The limit of detection for water samples was 0 log CFU/100 mL and 0 log CFU/cherry for cherries. Water pH, turbidity, temperature, and sanitizer concentrations were monitored throughout the water hold period. Microbial trends were evaluated to assess changes over water hold time, potential cross-contamination between cherries and water, and physicochemical and operational factors associated with microbial water quality and post-hydrocooler cherry contamination.

Objective 4:

Experimental design, sample collection and processing: Fresh cherries were evaluated for survival of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC) during refrigerated storage. Separate five strain rifampicin-resistant cocktails were prepared for each pathogen. Each pathogen cocktail was independently spot inoculated onto 10 g cherry samples at a ~ 5 log CFU/g. Inoculated cherries were dried for 1 h and stored at 4°C (40°F) for up to 14 days. Samples were collected at 0 and 4 h and on days 1, 2, 3, 5, 7, 10, and 14. At each sampling time point, 20 mL of 0.1% peptone water was added to each 10 g cherry sample, followed by a rub-shake-rub procedure. Samples were plated in duplicate on TSA supplemented with rifampicin and incubated at

35°C (95°F) for 48 h for *L. monocytogenes* and 24 h for *Salmonella* and STEC before enumeration. Survival and reductions of pathogens on fresh cherries were characterized.

Results and Discussion

Objective 1:

The prevalence of *Listeria* spp. on food contact surfaces was assessed in four Washington State cherry packinghouses over two packing seasons. *Listeria* spp. was isolated from all four packinghouses during both the 2024 and 2025 packing seasons. Among all tested samples, 2.0% (38/1920) were confirmed positive for *Listeria* spp. (Table 1). Of the 14 food contact surface types sampled, *Listeria* spp. was most frequently isolated from bristle flaps (6.3%; 9/144), followed by solid conveyor belts (4.2%; 6/144) and singulators (3.8%; 5/132; Table 1). One year of data was collected from four California packinghouses, where the overall prevalence of *Listeria* spp. was 2.4% (15/636). The three most frequently contaminated surfaces were the same as those in WA, though in a different order: solid conveyor belts (6.3%; 3/48), bristle flaps (4.6%; 3/65), and singulators (4.5%; 1/22).

Table 1: Frequency of isolation of *Listeria* spp. by food contact surface in Washington State and California

Food Contact Surface	Washington			California		
	No. of Total Samples	No. of Positive Samples	Frequency	No. of Total Samples	No. of Positive Samples	Frequency
Bristle Flap	144	9	6.3%	65	3	4.6%
Cherry Elevator	168	1	0.6%	50	0	0.0%
Cluster Cutter	84	1	1.2%	24	0	0.0%
Interlocking Conveyor Belt	228	1	0.4%	122	3	2.5%
Packing Guide	60	2	3.3%	43	0	0.0%
Plastic Flap	24	0	0.0%	30	0	0.0%
Singulator	132	5	3.8%	22	1	4.5%
Sizing Rollers	84	1	1.2%	32	1	3.1%
Solid Conveyor Belt	144	6	4.2%	48	3	6.3%
Sorter Cups	96	3	3.1%	32	0	0.0%
Sorter Flap	84	1	1.2%	56	1	1.8%
Stainless Steel Flume	312	4	1.3%	76	3	3.9%
Textured Conveyor Belt	180	0	0.0%	-	-	-
Transfer Point	180	4	2.2%	36	0	0.0%
Total	1920	38	2.0%	636	15	2.4%

When the prevalence of *Listeria* spp. was analyzed by operational step (Table 2), the receiving stage had the highest prevalence of *Listeria* spp. (3.1%; 22/720) both overall and when broken down by pre-packing (2.22%; 8/360) and during-packing (3.89%; 14/360). However, when the operational step frequency was broken down by year and location, differences were observed. For example, in Washington in year 1, the highest prevalence of *Listeria* spp. pre-packing was during the receiving step (2.2%, 1/45), during packing was the packing step (6.7%, 8/120), and overall was the packing step (3.75%; 9/240). On the other hand, in Washington in year 2, the highest prevalence overall was found to be during the sorting operation (2.4%; 15/630), while the highest number of positives in

California overall were found during the receiving stage (4.17% 2/48), all of which were collected during packing. Because *Listeria* spp. prevalence varied by step, year, and location, strong good agricultural practices (GAPs) in the field and consistent post-harvest sanitation protocols remain critical, but if time or resources are limited, efforts should focus on likely harborage sites.

Table 2: Frequency of isolation of *Listeria* spp. by operation step and based on sampling time during a sampling event

Step	Percentages of positive samples		
	Pre-packing	During-packing	Total
Receiving	2.2% (8/360)	3.9% (14/360)	3.1% (22/720)
Sorting	0.9% (1/144)	1.8% (2/114)	1.3% (3/228)
Packing	2.0% (16/802)	1.5% (12/802)	1.7% (18/1604)
Total	2.0% (25/1276)	2.2% (28/1276)	2.1% (53/2552)

In addition to the food contact surface and unit of operation, the timing of sample collection had an impact on *Listeria* spp. prevalence. Of the 1,279 pre-packing samples, 1.96% (25/1276) were positive for *Listeria* spp. compared to the 2.19% (28/1276) of the samples during packing (Table 3). In addition, the timing of *Listeria* spp. isolation was evaluated among all of the positive samples based on three scenarios: (i) the sampling site testing positive pre-packing and negative during-packing, (ii) the sampling site testing negative pre-packing and positive during-packing, or (iii) the sampling site testing positive both pre and during packing (Table 4). For all years and locations combined, the largest number of positive samples fell into scenario 2 (50.98%; 26/51), followed by scenario 1 (45.10%; 23/51) and 3 (3.92%; 2/51). However, similar to the operational step, when the scenarios were broken down by year and location, differences were observed, with the majority of positive samples in WA year 1 and CA falling into scenario 2 (64.7% and 66.7%, respectively), while the majority in WA year 2 were scenario 1 (68.4%). Pre-packing sampling occurred after any cleaning and/or sanitation events but before the product was run for the day, which indicates the effectiveness of the cleaning and sanitation. The higher prevalence during packing suggests that *Listeria* spp. may be introduced or redistributed during production, including through incoming fruit or movement within the packing environment. Additionally, only 2 sampling sites tested positive both pre-packing and during packing, during the same sampling event: one singulator and one transfer point. The low prevalence of paired positive samples during both pre- and during-packing suggests that most *Listeria* spp. findings are transient (sporadic introductions that do not persist) rather than resident (persistent strains that survive and establish in the facility over time).

Table 4: Frequency of *Listeria* spp. isolation for a specific sampling site based on sampling time by location and year

	Scenario	Operational Timepoint		No. of Sites	Frequency (%)[a]
		Pre-packing	During-packing		
Washington Year 1	1[b]	Positive	Negative	5	29.4%
	2[c]	Negative	Positive	11	64.7%
	3[d]	Positive	Positive	1	5.9%
Washington Year 2	1	Positive	Negative	13	68.4%
	2	Negative	Positive	5	26.3%
	3	Positive	Positive	1	5.3%

California	1	Positive	Negative	5	33.3%
	2	Negative	Positive	10	66.7%
	3	Positive	Positive	0	0.0%
Overall	1[b]	Positive	Negative	23	45.10%
	2[c]	Negative	Positive	26	50.98%
	3[d]	Positive	Positive	2	3.92%

[a] Total number of sampling sites with at least one positive detection of *Listeria* spp. in Washington Year 1, Year 2, and California was 17, 19, and 15, respectively.
[b] Sampling sites in which *Listeria* spp. were detected in only the pre-packing sampling.
[c] Sampling sites in which *Listeria* spp. were detected in only during-packing sampling
[d] Sampling sites in which *Listeria* spp. were detected in paired pre- and during-packing samplings.

Objective 2:

The microbial quality of discrete water systems and cherries before and after each system was evaluated in four Washington State cherry packinghouses. Each packinghouse had three or four discrete water systems. In total, 261 cherry samples (90 from packinghouse A, 75 from packinghouse B, 48 from packinghouse C, and 48 from packinghouse D) and 204 water samples (72 from packinghouse A, 60 from packinghouse B, 36 from packinghouse C, and 36 from packinghouse D) were collected as part of this study.

Only 4 cherries (1.5%) and 5 water (2.5%) samples had detectable levels of generic *E. coli* (≥ 1.0 log CFU/cherry and 0.0 log CFU/mL, respectively), and 47 cherries (18.0%) and 92 (45.1%) water samples had detectable levels of generic total coliforms (≥ 1.0 log CFU/cherry and 0.0 log CFU/mL, respectively). In agricultural water quality assessments, total coliforms and generic *E. coli* are commonly employed as indicator organisms. Total coliforms represent a broad group of bacteria present in soil, vegetation, and aquatic environments, and their detection indicates general microbial contamination. In contrast, generic *E. coli* originates from the intestines of warm-blooded animals and is associated with fecal matter; thus, its presence serves as a more specific indicator of fecal contamination. However, the current study found a low prevalence of cherry and water samples that were at or above the limit of detection for generic *E. coli* and total coliforms. While generic *E. coli* is an effective fecal indicator, using it alone in a postharvest setting to assess water quality and cross-contamination risk may be insufficient across various commodities. The relatively limited detection of generic *E. coli* and total coliforms suggests that these organisms alone may have limited utility for characterizing microbial dynamics and cross-contamination in cherry postharvest water systems.

Enterobacteriaceae was detected in 45% (175 /387) of water samples, with populations among positive samples ranging from 0.30 to 2.15 log CFU/mL. For cherries, *Enterobacteriaceae* was detected on 18% (95/518) of samples collected, with concentrations of positive samples ranging from 1.30 to 3.80 log CFU/cherry. *Enterobacteriaceae* is a bacterial family that includes many species commonly associated with environmental and fecal contamination, making it a useful indicator of sanitation effectiveness and cross-contamination evaluation. Among the indicator organisms tested, APC had the greatest prevalence rate and was therefore used as an indicator of microbial water quality and populations on cherries for structural equation models. APC measures the total number of viable bacteria capable of growing in oxygenated conditions, providing an overall estimate of

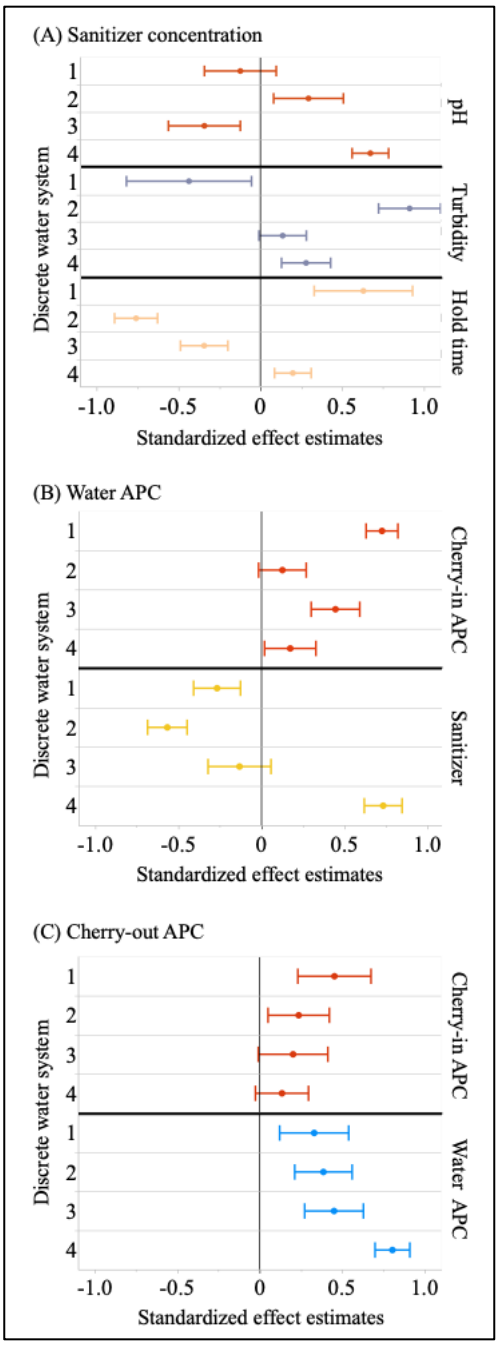


Figure 2. The association between explanatory variables and (A) sanitizer concentration, (B) water aerobic plate count (APC) populations, or (C) cherry-out APC levels. The effect estimates should be interpreted as the effect of a one standard deviation (SD) increase in the explanatory variable on the outcome variable.

microbial load. APC was detected in 88% (341/387) water and on 81% (419/518) of the cherry samples.

Four identical structural equation models were developed for each discreate water system. The relationship between pH and sanitizer concentration varied among water systems, with no consistent pattern observed across discrete water system (Figure 2a). Turbidity and sanitizer concentration also showed different relationships among systems. In discrete water system 1, higher turbidity was associated with lower sanitizer concentrations [Confidence intervals (CI) = -0.81, -0.06], while the opposite pattern was observed in discrete water system 2 (CI = 0.72, 1.10). Relationships were weaker in discrete water system 3 (CI = -0.01, 0.28) and discrete water system 4 (CI = 0.13, 0.43). Water hold time also showed different relationships with sanitizer concentration across systems, with positive associations in discrete water system 1 (CI = 0.33, 0.92) and discrete water system 4 (CI = 0.09, 0.31) and negative associations in discrete water system 2 (CI = -0.89, -0.63) and discrete water system 3 (CI = -0.49, -0.20). These results demonstrate that sanitizer concentration cannot be predicted from a single water quality factor across all packinghouse systems. The differences among water systems highlight the importance of individual packinghouses routinely monitoring sanitizer concentration and water quality during operation rather than relying on pH, turbidity, or water hold time alone to indicate whether sanitizer levels are adequate.

The cherry results further demonstrate that microbial levels can change as fruit moves through the packing process (Figure 3). The direction and magnitude of these changes varied among packinghouses and discrete water system, with some systems showing decreases in APC after entering the water system and others showing increases at later processing stages. The SEM results also showed a consistent relationship between the microbial levels on incoming cherries and in the process water (Figure 2b). Higher bacterial levels on incoming cherries were associated with higher bacterial levels in the water, indicating that incoming fruit can contribute to the microbial load in the water. Higher sanitizer concentrations were associated with lower bacterial levels in the water in discrete water system 1 (CI = -0.40, -0.13), discrete water system 2 (CI = -0.68, -0.45), and discrete water system 3 (CI = -0.32, 0.06). Higher bacterial levels on incoming cherries were also generally associated with higher bacterial levels on outgoing cherries (Figure 2c), with positive associations in discrete water system 1 (CI = 0.24, 0.66), discrete water system 2 (CI = 0.21, 0.56), discrete water system 3 (CI = -0.01, 0.41), and discrete water system 4 (CI = -0.03, 0.30). Water bacterial levels were also associated with higher bacterial levels on outgoing cherries, with associations

observed in discrete water system 1 (CI = 0.12, 0.54), discrete water system 2 (CI = 0.04, 0.43), discrete water system 3 (CI = 0.27, 0.63), and discrete water system 4 (CI = 0.70, 0.91). Together, these findings demonstrate the potential for microbial transfer from incoming fruit to process water and from process water back to fruit. The association between water microbial levels and outgoing fruit microbial levels further supports the importance of managing process water to reduce opportunities for cross-contamination. These results reinforce the value of monitoring incoming fruit, maintaining effective sanitizer concentrations, and monitoring water quality throughout the packing process.

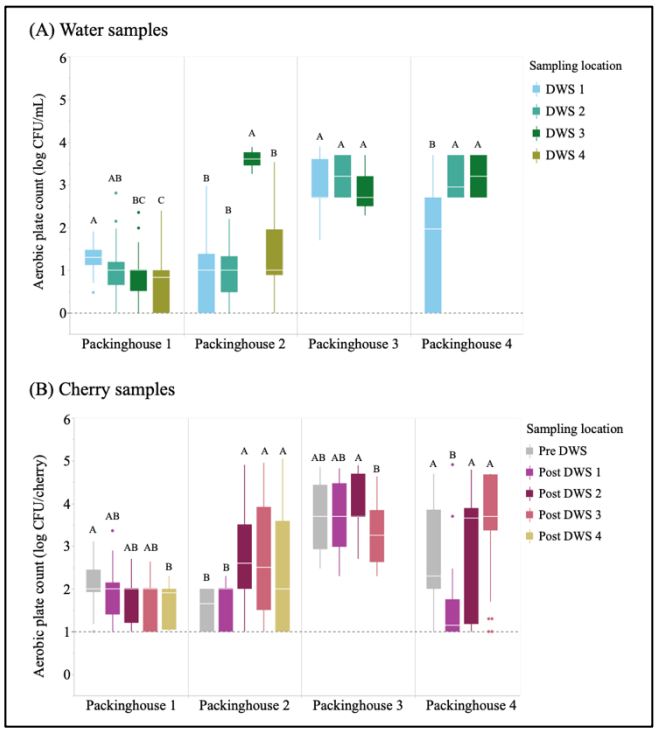


Figure 3. Aerobic plate count populations of (A) water, and (B) cherry samples by packinghouse and discrete water source (DWS). Different uppercase letters above box plots represent a significant difference in population means among sampling locations within individual packinghouses ($P < 0.05$).

Objective 3:

Generic *E. coli* was detected in 33% (30/90) of water samples, with concentrations among positive samples ranging from 1.16 to 3.38 log MPN/100 mL. For cherries, generic *E. coli* was detected on 8% (14/165) of samples collected, with concentrations among positive samples ranging from 0.30 to 2.81 log MPN/cherry. Total coliforms were detected in 61% (55/90) of water samples, with concentrations among positive samples ranging from 0.30 to 4.66 log MPN/100 mL. For cherries, total coliforms were detected on 80% (132/165) of samples collected, with concentrations among positive samples ranging from 0.30 to 5.19 log MPN/cherry. Heterotrophic plate counts (HPC) were detected in 98% (87/89) of water samples, with concentrations among positive samples ranging from 0.30 to 5.38 log MPN/100 mL. For cherries, HPC were detected on 100% (165/165) of samples collected, with concentrations ranging from 0.30 to 5.38 log MPN/cherry.

Across sampling events with paired pre- and post-hydrocooling samples (n = 27), total coliform concentrations decreased by an average of 0.87 log MPN/cherry (95% CI: 0.47, 1.27 log MPN/cherry; $p < 0.001$), while HPC decreased by an average of 0.77 log MPN/cherry (95% CI: 0.34, 1.21 log MPN/cherry; $p = 0.001$). Total coliform concentrations increased following hydrocooling during 6 of 27 sampling events, while HPC concentrations increased during 7 of 27 sampling events. Although HPC generally decreased following hydrocooling, the magnitude of the pre- to post-hydrocooling change was associated with HPC concentrations in the hydrocooler water. After accounting for packinghouse and sampling time, each 1 log increase in water HPC was associated with a 0.59 log greater change in HPC on cherries from pre- to post-hydrocooling (95% CI: 0.26, 0.92; $p < 0.001$; Figure 4). In contrast, water TC concentration was not associated with the pre- to post-hydrocooling change in TC on cherries ($\beta = 0.08$; 95% CI: −0.20, 0.37; $p = 0.568$). Among the

physicochemical water-quality parameters evaluated, turbidity was positively associated with total coliform concentrations in hydrocooler water (β = 0.020, $p < 0.001$). However, turbidity was not associated with post-hydrocooling total coliform concentrations on cherries ($P = 0.108$). Sanitizer concentration, temperature, and pH were not associated with total coliform concentrations in the water or on post-hydrocooling cherries ($p > 0.05$). None of the physicochemical water-quality parameters evaluated were significantly associated with HPC concentrations in the hydrocooler water or on post-hydrocooling cherries ($p > 0.05$).

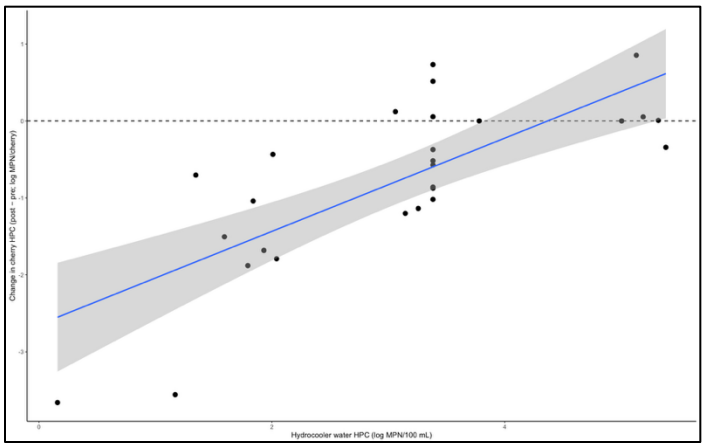


Figure 4. Association between HPC in hydrocooler water and the change in HPC on cherries following hydrocooling

These results suggest that hydrocooling under the conditions observed in the four packinghouses studied did not generally result in increased microbial loads on cherries, with both total coliform and HPC concentrations decreasing on average following hydrocooling. However, these decreases should not be interpreted as evidence that hydrocooling is a microbial reduction step. The relationship between HPC concentrations in hydrocooler water and the pre- to post-hydrocooling change on cherries indicates that the microbiological quality of recirculated water may influence microbial persistence on fruit. Importantly, increases in HPC following hydrocooling were observed during some individual sampling events despite the overall decrease, emphasizing that average reductions do not eliminate the potential for transfer under specific operating conditions. The association between turbidity and total coliform concentrations in water further suggests that accumulation of organic material or other suspended solids may coincide with declining microbiological water quality, although this relationship did not translate to increased total coliform concentrations on post-hydrocooling cherries. Maintaining water quality through appropriate sanitizer management, removal of accumulated organic material and debris, and routine monitoring throughout operation may help limit conditions that favor microbial persistence and transfer.

Objective 4:

Populations of all three foodborne pathogens decreased during refrigerated storage. *L. monocytogenes* showed the greatest persistence, decreasing from approximately 5.1 ± 0.5 log CFU/cherry at day 0 to 2.9 ± 0.1 log at day 14, a reduction of approximately 2.2 log. *Salmonella* decreased from approximately 5.3 ± 0.4 to 1.7 ± 0.5 log CFU/cherry, while STEC decreased from approximately 5.4 ± 0.3 to 1.4 ± 0.3 log CFU/cherry by day 14, corresponding to reductions of approximately 3.6 and 4.0 log CFU/cherry, respectively. These results demonstrate that none of the three foodborne pathogens grew under refrigeration and populations decreased over 14 days of storage. The greater persistence of *L. monocytogenes* highlights its ability to survive under refrigerated conditions and its continued importance in refrigerated produce and postharvest environments. Although substantial

reductions were observed for *Salmonella* and STEC, viable populations remained detectable after 14 days. These findings reinforce the importance of preventing contamination and maintaining appropriate sanitation and handling practices throughout postharvest operations.

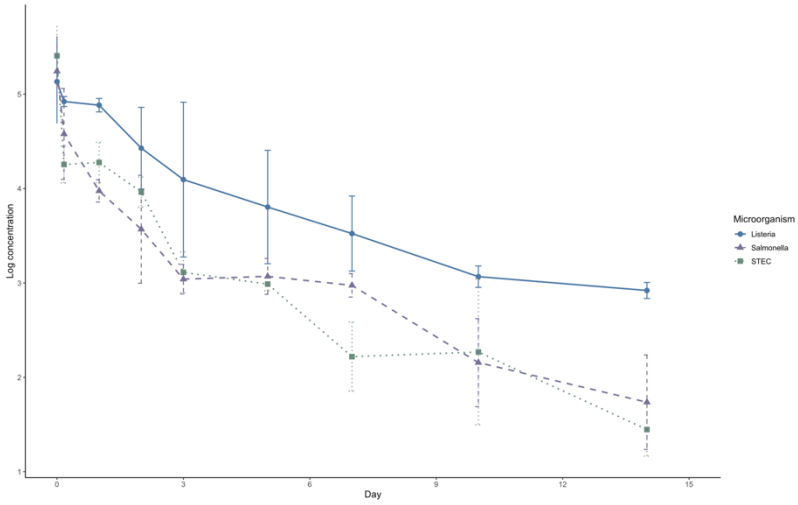


Figure 5. Change in *L. monocytogenes*, *Salmonella*, and STEC populations during 14 days of refrigerated storage

Literature Review

Limited research has evaluated food safety risks specifically associated with the postharvest handling of fresh cherries. However, studies of other fresh produce commodities demonstrate that packinghouse environments and food contact surfaces can provide opportunities for microbial introduction, persistence, and cross-contamination. *Listeria* spp., in particular, have been recovered from food contact surfaces in fresh produce packinghouses. Hamilton (2018) reported *Listeria* spp. on 10.9% (62/565) of food contact surfaces sampled from three tomato packinghouses in Tennessee, while Ruiz-Llacsahuanga et al. (2021) detected *Listeria* spp. on 4.6% (136/2,988) of food contact surfaces in Washington apple packinghouses. In apple packinghouses, polishing brushes and other surfaces containing bristle materials were among the sites with the greatest prevalence. Similarly, bristle flaps were among the most frequently contaminated food contact surfaces in the current cherry study, with *Listeria* spp. detected on 6.3% of samples in Washington and 4.6% in California. Given the potential for bristle materials to retain debris and moisture and serve as harborage sites for *Listeria* spp., the cherry industry should prioritize replacing these materials with more hygienic alternatives when feasible and, when replacement is not possible, implement routine cleaning, sanitization, and replacement when signs of wear are observed.

Postharvest water represents another potential route for microbial cross-contamination during fresh produce handling. Recirculated water can allow microorganisms introduced by incoming fruit to accumulate in the water and potentially transfer to subsequently handled fruit. The primary role of sanitizers in postharvest water is to control microorganisms in the water and limit cross-contamination. Luo et al. (2012) found that *E. coli* O157 cross-contamination during leafy green washing occurred when free chlorine dropped below 1 ppm but was not detected when ≥1 ppm was maintained. Similarly, Pabst et al. (2024) observed widespread *Salmonella* cross-contamination of tomatoes in the absence of PAA, while maintaining 10–80 ppm PAA prevented detectable transfer. These findings reinforce the importance of directly monitoring sanitizer concentrations and water quality during operation.

Additionally, traditional fecal indicator organisms may have limitations for characterizing microbial dynamics in packinghouses. Schneider et al. (2017) reported that 93.8% (1,498/1,597) of pre- and post-processed tomato samples had generic *E. coli* populations below the detection limit, while Strawn et al. (2026) detected generic *E. coli* in only 1.6% of pre- and post-flume apple samples and 1.3% of postharvest water samples. Similarly, generic *E. coli* was detected in only 1.5% of cherry

samples and 2.5% of water samples in data presented. In contrast, APC was widely detected and demonstrated relationships among incoming cherries, process water, and outgoing cherries.

Finally, few studies have evaluated the survival of foodborne pathogens directly on fresh cherries. Previous research demonstrated that *L. monocytogenes* did not grow on cherries during refrigerated storage, consistent with the current findings (Girbal, et al., 2021). This research here demonstrated that none of the three pathogens evaluated grew during 14 days of storage at 4°C, however, all remained detectable at the end of storage.

Collectively, these findings demonstrate that food safety efforts during cherry packing should focus on preventing contamination and limiting microbial transfer through effective equipment sanitation and process-water management, as neither hydrocooling nor refrigerated storage should be relied upon to eliminate contamination once it occurs.

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Executive Summary

Project Title: Understanding Food Safety Risks During Post-Harvest Cherry Production

Key Words: cherries, food safety, *Listeria*, postharvest water, hydrocooling, cross-contamination, sanitation

Abstract: This three-year project evaluated food safety risks associated with commercial postharvest cherry handling, with emphasis on *Listeria* spp. on food contact surfaces, microbial quality and cross-contamination in postharvest water systems, hydrocooling, and survival of foodborne pathogens on cherries during refrigerated storage. Commercial cherry packinghouses in Washington and California participated across the study objectives.

Listeria spp. was detected in all packinghouses evaluated, with an overall prevalence of 2.0% (38/1,920) on food contact surfaces in Washington and 2.4% (15/636) in California. Bristle flaps, solid conveyor belts, and singulators were among the most frequently contaminated surfaces. Only 3.9% of positive sampling sites were positive both before and during packing within the same sampling event, suggesting that most detections represented transient contamination rather than persistent harborage.

Evaluation of commercial postharvest water systems demonstrated that microbial populations on incoming cherries can influence microbial quality throughout the packing process. Generic *E. coli* was detected in 1.5% of cherries and 2.5% of water samples, while total coliforms were detected in 18.0% and 45.1%, respectively. Higher APC populations on incoming cherries were associated with higher APC populations in process water and on outgoing cherries, and higher water APC was also associated with higher APC on outgoing cherries. Relationships between sanitizer concentration and individual water-quality parameters varied among water systems, emphasizing the importance of directly monitoring sanitizer concentrations rather than relying on individual physicochemical parameters as indicators of sanitizer status.

Hydrocooling did not generally increase microbial populations on cherries under the commercial conditions evaluated. Total coliform and HPC populations decreased by an average of 0.87 and 0.77 log MPN/cherry, respectively, following hydrocooling. However, increases occurred during some individual sampling events, and higher HPC populations in hydrocooler water were associated with less favorable changes in HPC on cherries. These findings indicate that hydrocooling should not be considered a microbial reduction step and reinforce the importance of maintaining the microbiological quality of recirculated water.

Finally, *L. monocytogenes*, *Salmonella*, and STEC did not grow on cherries during 14 days of refrigerated storage, but viable populations of all three pathogens remained detectable. *L. monocytogenes* demonstrated the greatest persistence, decreasing by approximately 2.2 log CFU/cherry compared with reductions of approximately 3.6 and 4.0 log CFU/cherry for *Salmonella* and STEC, *respectively*.

Overall, the results demonstrate that effective cherry food safety management should focus on preventing contamination and limiting microbial transfer throughout postharvest handling. Priority practices include hygienic design and sanitation of difficult-to-clean food contact surfaces, particularly bristle materials; routine monitoring and management of process-water quality and sanitizer concentrations; control of organic material and debris accumulation; and recognition that incoming fruit can contribute microbial loads to recirculated water systems.