FINAL PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Systems-based approach for improved packinghouse sanitation

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Total Project Request: **Year 1:** 67,369 **Year 2:** 71,399 **Year 3:** 58,209

WTFRC Budget:

Item	2018	2019	
Salaries	4,050	4,131	
Benefits	1,337	1,363	
Wages	4,500	4,703	
Benefits	1,485	1,552	
RCA Room Rental			
Shipping			
Supplies			
Travel	500	500	
Plot Fees			
Miscellaneous			
Total	11,872	12,249	

Footnotes:
Salaries/Benefits: estimate of percent of time spent for Mendoza (5%) and Hanrahan (1%), a 33% benefit rate and 2% annual increases.
Wages/Benefits: calculated based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00), approx. 350 hours
Travel: in state travel for Hanrahan (lodging in Wenatchee)

Budget 1
Organization Name: Washington State University
Contract Administrator: Samantha Bridger
Telephone: (509)786-9204 **Email address:** prosser.grants@wsu.edu

Item	2018	2019	2020
Salaries	26,274	27,509	28,807
Benefits	2,373	2,468	2,566
Wages	6,000	8,112	5,192
Benefits	600	811	519
Equipment			
Supplies	19,250	19,250	21,125
Travel	1,000	1,000	
Miscellaneous			
Plot Fees			
Total	55,497	59,150	58,209

Footnotes: Salaries: $26,274, $27,509, and $28,807 is requested in years 1, 2 and 3 , respectively, for a Graduate Research Assistantship for a MS student to work on all objectives.

Benefits: $2,373, $2,468, and $2,566 is requested in years 1, 2 and 3, respectively, for benefits tied to the Graduate Research Assistantship for a MS student to work on all objectives.

Wages: $6,000 in year 1, $8,112 in year 2 and $5,192 in year three are requested for hourly wages for student employee to conduct experiments as relating to the surface characteristics of the different types of materials used on packing lines from an engineering point of view.

Benefits: $600 in year 1, $811in year 2 and $519 in year three are requested for benefits of the student employee.

Supplies: Supply costs of $19,250 in year 1, 19,250 in year 2 and 21,125 in year 3 are requested to purchase disposable supplies such as glassware, microbiological media, Petri dishes, pipettes, and PCR reagents tied to objectives 1 and 3.

Travel: $1,000 is requested in years 1 and 2 for mileage and associated travel costs at a rate of $0.535/mi and adhering to all university policies for per diem associated with overnight travel.

Objectives

1. Identify harborage points and niches for *Listeria monocytogenes* indicator organism (*Listeria* spp.) on food contact surfaces in produce packinghouses (complete).
2. Rank surfaces based upon prevalence of indicator organisms to identify material types and design features with the greatest likelihood of harborage (complete).
3. Evaluate standard design features from a microbiological and engineering perspective to determine if alternative sanitation practices can compensate for less than ideal hygienic design.

Significant Findings

- Among 2,988 samples tested, 4.6% (n=136) were positive for *Listeria* spp.
- Wax coating was the unit operation from which *Listeria* spp. were most frequently isolated.
- The FCS that showed the greatest prevalence of *Listeria* spp. were polishing brushes, stainless steel dividers and brushes under fans/blowers, and dryer rollers
- The prevalence of *Listeria* spp. on FCS increased throughout apple storage time.
- The application of a degreaser followed by a sanitizer significantly improved the effectiveness of sanitation methods against *L. innocua.*
- Polishing brushes made of a horsehair mix were the type of surface with the lowest *Listeria innocua* reduction, amongst sanitation approaches evaluated, except when a degreaser followed by 500ppm PAA was used.
- Steam application was consistently the worst sanitation approach for inactivating *L. innocua* regardless of surface type.

Methods

Objective 1. Identify harborage points and niches for *Listeria monocytogenes* indicator organism (*Listeria* spp.) on food contact surfaces in produce packinghouses (years 1-3).

Packinghouse selection. Five packinghouses were enlisted into the study and have been sampled once quarterly during packing season for a total of eight data collection points per facility (Figure 1).

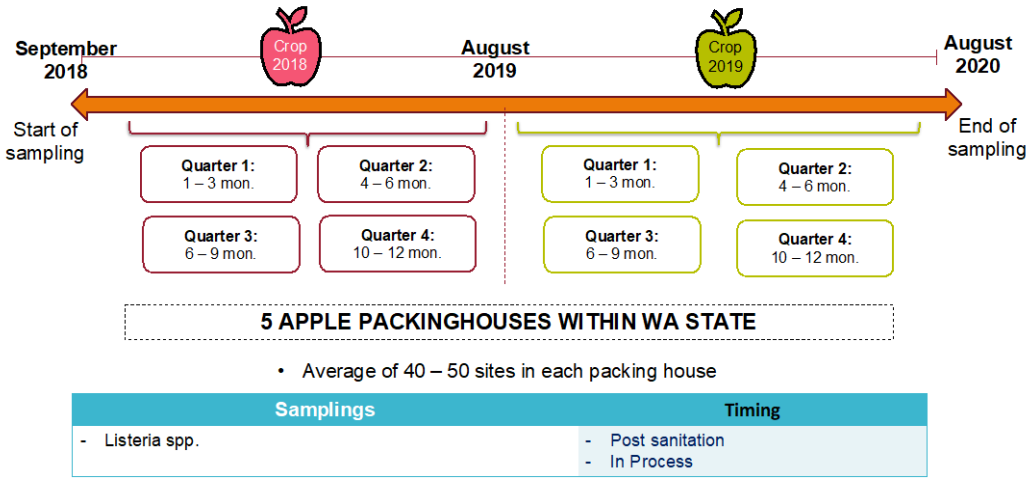

Figure 1. *Listeria* species sampling overview of apple packinghouses for the 2018 and 2019 apple crop.

Surface sampling methods. Sampling was coordinated to occur both after a sanitation (post sanitation) event and within 4 hrs of startup (in-process) to align with current FDA guidance. A pre-moistened sterile sponge is being utilized to sample a 100 cm^2-area or as large a space as is permissible for smaller surfaces.

Isolation of *Listeria* species. Bacteria are eluted in D/E neutralizing buffer, enriched in Buffered Listeria Enrichment Broth (BLEB) with antibiotic supplements, and confirmed through polymerase chain reaction (PCR) targeting the *iap* gene (Figure 2). This approach identified only *Listeria* sensu strictu as a group (*Listeria* species including: *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri,* and *L. welshimeri*) and did not identify *Listeria monocytogenes* specifically.

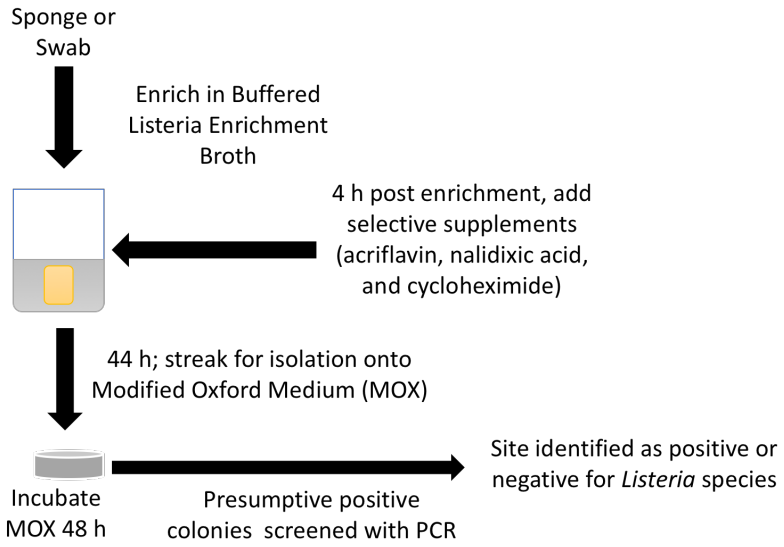

Figure 2. Sample processing to determine presence or absence of *Listeria* species (environmental indicator for *L. monocytogenes*).

Statistical analysis. A chi-square test or Fisher's exact test (when expected observations were lower than 5) was used to analyze the categorical data of the presence or absence of Listeria spp. based upon the following categorical variables: unit operations (washing, washing/sanitizing/rinsing, fan drying, wax coating, tunnel drying, sorting, and packing), timing of sampling (postsanitation and in-process), sampling periods (Q1, Q2, Q3, and Q4), and type of FCS (e.g., brushes under fans, polishing brushes, dryer rollers, bristle rollers, dump tank, and plastic flaps). A post hoc pairwise comparison was used to compare the levels of each categorical variable when a significant difference was observed.

Objective 2. Rank surfaces based upon prevalence of indicator organisms to identify material types and design features with the greatest likelihood of harborage (year 3).

Review for hygienic design features. Outcomes from the statistical analysis in objective 1, combined with pictures of sampling locations and measurements taken from surfaces within packinghouses were analyzed to evaluate hygienic design features of equipment with significantly more prevalence of *Listeria* spp. on food contact surfaces. Surfaces were ranked by type based upon likelihood of *Listeria* spp. presence (Table 4).

Objective 3. Evaluate standard design features from a microbiological and engineering perspective to determine if alternative sanitation practices can compensate for less-than-ideal sanitary design (Year 4).

The following surfaces were associated with >5% frequency of *Listeria* spp. isolation and were evaluated: polishing brushes made of two different material types (horsehair mix and 100% nylon), dryer rollers made of stainless steel and wrapped with Teflon, brushes, and plastic interlocking belts. These surfaces were inoculated with *L. innocua* (as a surrogate of *L. monocytogenes*) and wax where appropriate, and various sanitation practices were evaluated to determine if they can mitigate less than ideal hygienic design. This is extremely beneficial given that the cost of design improvements may be prohibitive in the short-term, but alternate sanitation strategies could prove to be effective.

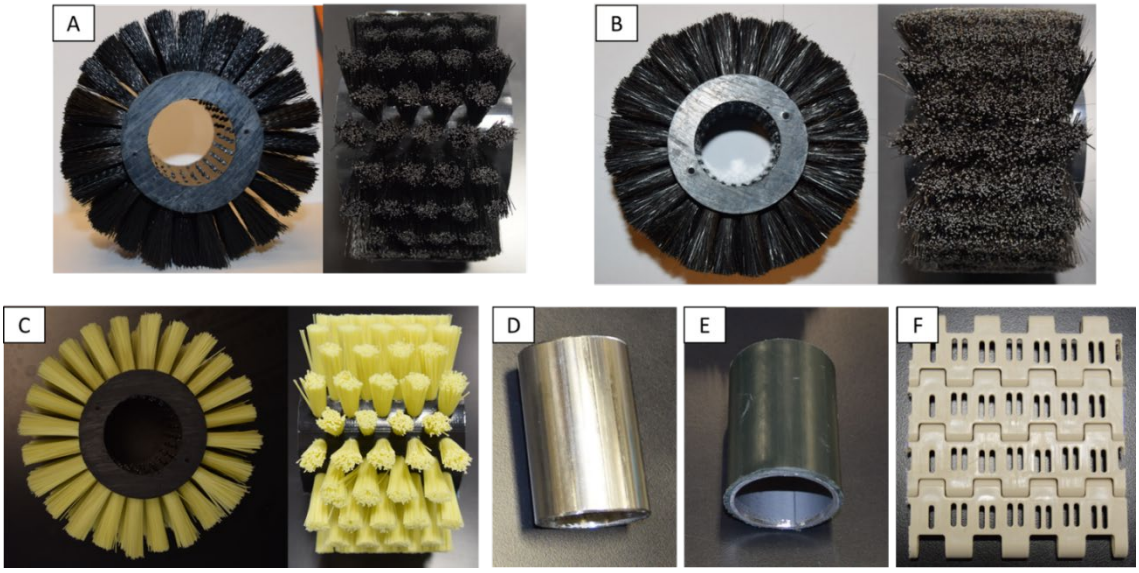

Figure 3. Selected food contact surfaces: (A) Polishing brushes-100% nylon, (B) Polishing brushes-50%horsehair/50%polyethylene, (C) Brush rollers- 100% polyethylene, (D) Stainless steel rollers, (E) Teflon wrapped rollers, (F) Interlocking belts

Preparation of surfaces. Each surface was acquired new and cut in coupons from suppliers and were sterilized to remove background microflora prior to inoculation.

Inoculation of surfaces with *Listeria innocua*. *L. innocua* was grown in Tryptic Soy Broth with Yeast Extract (TSBYE) at 35°C for 24 h with three successive transfers prior to inoculation of Tryptic Soy Agar (TSA) plates. TSA was incubated at 35°C for 24 h to achieve a lawn. The plate was flooded with 10 ml of Buffered Peptone Water (BPW) to harvest cells. Surfaces were spot inoculated with 100 µL of the *L. innocua* inoculum. Surfaces were held in a biosafety cabinet at room temperature for one hour to allow the inoculum to dry.

Application of wax on surfaces where appropriate. To simulate conditions of the apple packing process, surfaces that receive a direct wax application (through wax nozzles) or indirect wax application (through waxed apples) were treated by spraying food-grade wax after inoculation. Surfaces such as polishing brushes, which receive a constant direct application of wax, were sprayed with approximately 2 ml of wax. Dryer rollers and interlocking conveyor belts were sprayed with a lessen amount of approximately 0.5 ml of wax. Surfaces were held in a biosafety cabinet at room temperature for one hour to allow the wax to dry. Brush rollers (100% polyethylene) are typically located before the wax coating operation; thus, they were not sprayed with wax.

Treatment of surfaces. Surfaces were exposed to seven treatments in addition to a no treatment control based on knowledge of current industry practices. Brush rollers (100% polyethylene) did not get the application of T_6 and T_7. All experiments were replicated two times with three samples evaluated per replicate (n=6).

Treatment 1 (T_1: "Cl 200 ppm"): Approximately 3 ml of chlorine at 200 ppm was applied onto the surfaces using a pressurized sprayer for a contact time of 15 min. No water rinsing was needed.

Treatment 2 (T_2: "PAA 500 ppm"): Approximately 3 ml of PAA at 500 ppm was applied onto the surfaces using a pressurized sprayer for a contact time of 15 min. No water rinsing was needed.

Treatment 3 (T_3: "Scrub with detergent + Cl 200 ppm"): An alkaline detergent at a concentration of 15.6 ml/1000 ml water, was applied onto the surfaces using a pressurized sprayer. The surface was scrubbed by hand for 30 seconds using a brush, followed by a rinsing step with water. A sanitation step was carried out using chlorine at 200 ppm as described in T_1.

Treatment 4 (T_4: "Scrub with detergent + PAA 500 ppm"): The application of an alkaline detergent, scrubbing and rinsing step was performed as described in T_3, followed by a sanitation step using PAA at 500 ppm as described in T_2.

Treatment 5 (T_5: "Steam"): Surfaces were exposed to dry steam at 95 °C for 15 seconds generated by a dry steam cleaner. The temperature was monitored using a 4-input thermocouple meter. The maximum steam contact time of 15 seconds was determined based on the surfaces' material resistance to heat damage.

Treatment 6 (T_6: "WaxStrip + Cl 200 ppm"): Surfaces were sprayed with WaxStrip Plus cleaner at full strength. Based upon the type of surface, polishing brushes were sprayed with a greater amount of the cleaner. Surfaces were soaked for 15 min, and residues were flushed with warm water (50 ºC) as indicated by the manufacturer's instructions. Then, a sanitation step was carried out using chlorine at 200 ppm as described in T_1.

Treatment 7 (T_7: "WaxStrip + PAA 500 ppm"): The application of WaxStrip Plus cleaner was followed as described in T_6. A sanitation step using PAA at 500 ppm was followed as described in T_2.

Treatment 8 (T_8: "Control treatment"). For the brush rollers (100% polyethylene), the control treatment consisted of surfaces inoculated with the *L. innocua* inoculum, and air dried in a biosafety cabinet for one hour. For all the other surfaces, the control treatment consisted of surfaces inoculated with the *L. innocua* inoculum, air dried in a biosafety cabinet for one hour, and sprayed with the respective amount of wax as previously described.

Enumeration of *Listeria innocua*. After treatment, surfaces were hand massaged for 30 s in a solution of 100 mL BPW containing 1% Tween 20 to remove attached *L. innocua*. The rinsate was serially diluted and 100 µl was direct plated in duplicate on MOX plates and incubated at 35°C for 48 h. *L. innocua* colonies were enumerated based on characteristic esculin hydrolysis (black halo formation). For instances where the population of attached *L. innocua* was below the limit of detection (3.0 Log CFU/surface), the remaining rinse solution was vacuum-filtered through a sterile 0.45-µm S-Pak Membrane Filter, using a Pall manifold filtration system. The membrane filters were placed on MOX plates and incubated at 35°C for 48 h. The rinse solution from polishing brushes was not vacuum-filtered due to a greater presence of wax residues which caused clogging of the filter paper. When below the limit of detection, bacterial counts were reported as 2.9 Log CFU/surface.

Statistical analysis. Data was reported as Log reduction (CFU/surface) of *L. innocua* population between the control and evaluated treatment. Data were subjected to a one-way analysis of variance (ANOVA), followed by a Tukey-Kramer highest significant difference (HSD) test to perform multiple mean comparisons of log reduction of *L. innocua* of each treatment by type of surface.

Results and Discussion

Prevalence of *Listeria* spp. in apple packinghouses. *Listeria* spp. were isolated from all five packinghouses during both packing seasons. Among all tested samples (n=2,988), 136 (4.6%) were confirmed positive for *Listeria* spp. The prevalence of *Listeria* spp. was compared neither between packinghouses nor across packing seasons.

Occurrence of *Listeria* spp. in different unit operations. The prevalence of *Listeria* spp. in each unit operation is displayed in Table 1. *Listeria* spp. were most frequently isolated from the wax coating unit operation (17.3%; n=110), followed by both the first drying (fan/blower) (9.4%; n=394, and the second drying (tunnel dryer) (8.2%; n=304) unit operations. The lowest prevalence of *Listeria* spp. was obtained from the washing, washing/sanitizing/rinsing, and packing unit operations (<1.2%).

In the wax coating unit operation, polishing brushes were the FCS most commonly implicated. These findings suggested a deficiency of routine sanitation procedures at this sampling site, and the ability of these FCS to trap wax residues and *Listeria* cells within polishing brush bristles. In the 2014 caramel apple listeriosis outbreak, polishing brushes were one of the FCS that *L. monocytogenes* was isolated from. Studies that support our results have reported a greater long-term survival of *L. monocytogenes* on waxed apples than unwaxed apples due to moisture retention over time, ultimately theorizing that entrapment of *L. monocytogenes* cells and moisture within a wax coating was conducive for forming a microenvironment that enhances the survival of *Listeria* in apples, and E. coli O157:H7 cells that were found embedded to wax platelets on apples. Another factor that could explain our results is the pH level of commercial waxes (6.7 to 8.6). The optimal pH level for *Listeria* to grow is 7.0; therefore, if sufficient water activity, nutrients, and temperature are maintained, wax residues on FCS and NFCS may support the growth of *Listeria* spp. if not otherwise removed.

In the first drying unit operation, dividers and brush rollers located underneath fans/air blowers (NFCS) were the FCS that showed the greatest prevalence of *Listeria* spp. Migration of pathogens from zones 2 or 3 (NFCS) to zone 1 (FCS) has been previously reported. As fans and air blowers circulate air, they also spread pathogens contained on the blades, motor, and cover of the fan, leading to cross-contamination of the dividers and brush rollers. Moreover, repeated isolation of *Listeria* spp. has been shown on fans over brush beds in produce packinghouses, and on freezer fans in meat facilities. These devices represent potential niches for *L. monocytogenes* and are recommended to be scheduled into daily cleaning and sanitation programs.

In the second drying unit operation, dryer rollers were the FCS that were most implicated. Tunnel dryer operating temperatures of 30-50 ºC may create opportunities for *Listeria* growth in niches if other growth conditions are met. The optimal growth temperature of *L. monocytogenes* is 30-37 ºC, and it can also grow at temperatures up to 50 ºC. Packinghouses in this study often operated within the range of the optimal growth temperatures, thus increasing the potential proliferation of *Listeria* over time.

Table 1. Prevalence of *Listeria* spp. (%) by unit operation and timing of sampling.

Unit operation	Examples of surfaces tested	N[a]	Timing of sampling		Total prevalence
			Post- sanitation (n=1,497)	In-process (n=1,491)	
Washing (Dump tank/flume)	Dump tank, flumes, PVC[b] rollers, traction belting.	285	0 (a)[c]	1.4 (a)	0.7 (a)
Washing/Sanitizing/Rinsing (Spray bars)	Brush rollers, plastic flaps, side edges.	331	0.6 (a)	1.8 (a)	1.2 (a)
First drying (Fan and/or blower)	Brush rollers, dividers.	394	4.6 (b)	14.2 (cd)	9.4 (c)
Wax coating	Polishing brushes, plastic flaps, transfer points.	110	10.9 (b)	23.6 (d)	17.3 (d)
Second drying (Tunnel dryer)	Dryer rollers, bristle rollers, transfer points.	304	4.6 (b)	11.8 (bc)	8.2 (c)
Sorting	Sorter cups, interlocking conveyor belts, solid conveyor belts, plastic guide rails, side edges, Teflon tape, transfer points.	1,254	0.8 (a)	6.9 (b)	3.8 (b)
Packing	Packing tables, solid conveyor belts, plastic crates, plastic flaps.	310	0 (a)	0.7 (a)	0.3 (a)
Total		2,988	1.9	7.2	4.6

[a] Number of samples tested.
[b] Polyvinylchloride
[c] Values within a column that are not followed by the same letter are significantly different ($p \leq 0.05$).

Prevalence of *Listeria* spp. by timing of sampling (Post-sanitation, in-process). Of the 1,497 post-sanitation samples, 1.9% were positive for *Listeria* spp., compared to 7.2% of the 1,491 in-process samples (Table 1). Among all the positive *Listeria* spp. samples 21% (n=28) were detected during the post-sanitation sampling, whereas 79% (n=108) were detected during the in-process sampling. In addition, timing of *Listeria* spp. isolation was also evaluated for each site amongst the cohort which were positive during a sampling event based upon three scenarios, 1) the location testing positive post-sanitation and negative in-process, 2) negative post-sanitation and positive in-process, or 3) positive during both post-sanitation and in-process, to determine the frequency of each (Table 2). The outcomes of each scenario were significantly different from each other ($p \leq 0.05$), with *Listeria* spp. positive sites most frequently positive only for the in-process sample (75.9%), and 17.2% of sites positive for both.

Table 2. Frequency of *Listeria* spp. isolation for a specific sampling location based on timing of sampling during a sampling event

	Timing of sampling		**Frequency (%) (n=136)**
	Post-sanitation	**In-process**	
Scenario 1	Positive	Negative	6.9 (a)[a]
Scenario 2	Negative	Positive	75.9 (c)
Scenario 3	Positive	Positive	17.2 (b)

[a] Values within a column that are not followed by the same letter are significantly different ($p \leq 0.05$).

Prevalence of *Listeria* spp. by FCS type. The FCS that showed the greatest prevalence of *Listeria* spp. were polishing brushes (19.6%), dividers under fans/blowers (17.4%), dryer rollers (10.5%), and brushes under fans/blowers (9.7%) (Table 3). Sites which were exposed to sanitizers throughout production [brushes under spray bars (0.9%), dump tank/flume (0.9%)], as well as side edges (3.3%), sorter cups (2.6%), solid conveyor belts (1.6%), sorting guide rails (2.1%), traction belting (1.5%), PVC rollers (0.8%), packing tables and plastic crates (0.0%), sorting brushes (0.0%), and cup droppers (0.0%) had the lowest occurrence of *Listeria* spp (Table 3).

Prevalence of *Listeria* spp. by sampling periods (quarters). The highest prevalence of *Listeria* spp. was obtained during the last quarter of sampling (Q_4) in the in-process sampling (38.2%; $p \leq 0.05$). The prevalence of *Listeria* spp. increased throughout crop storage time (quarters) but differed by unit-operation. The only unit-operation where the prevalence of *Listeria* spp. increased during the post-sanitation sampling was the tunnel drying (from Q_1=0% to Q_3=13.9%; $p \leq 0.05$). The three unit operations that accounted for the increase of the in-process prevalence of *Listeria* spp. over storage time were fan drying, tunnel drying, and sorting. These unit-operations showed significantly higher frequencies of isolation after the first quarter of sampling. The increase in the prevalence of *Listeria* spp. during the in-process sampling was principally attributed to cross-contamination between apples and FCS. Throughout storage, some of the most common apple post-harvest decay diseases caused by the fungus *Botrytis cinerea*, *Penicillium expansum*, and *Mucor piriformis* can increase microbial pathogen growth. After harvest, apple bins go through a fungicide drenching step before being stored for up to 12 months, with no culling step (to eliminate bruised or damaged apples) before the storage. Punctures, wounds, or damaged skin caused during harvest and transportation facilitate the spread and growth of bacteria and fungus. Fungal growth surrounding bruised tissues degrade the protective epidermal layer and produce a pH gradient neutralizing the apple flesh, and leading to the potential for survival and growth of *Listeria*. Thus, it has been hypothesized that as the storage time increases so does the fungal growth and internal fruit pH, and when combined, these two factors lead to an increase of the *Listeria* microbial load. However, further investigation regarding the relationship between the survival of *Listeria* and fungal post-harvest disease is required in a longer-term storage setting.

Table 3. Frequency of *Listeria* spp. by food contact surface

Food contact surfaces	N[a]	Frequency (%)
Polishing brushes (e.g., polyethylene, polypropylene, nylon, horsehair mix)	92	19.6 (a) [b]
Stainless steel dividers under fan/blowers	46	17.4 (ab)
Dryer rollers (e.g., stainless steel roller wrapped with vinyl or Teflon)	143	10.5 (abc)
Brushes under fan/blower (e.g., polyethylene, polypropylene)	206	9.7 (abc)
Bristle rollers (e.g., polyethylene, polypropylene)	160	8.8 (bcd)
Plastic interlocking chain conveyor belts (e.g., polypropylene, polyethylene)	256	5.1 (cde)
Teflon transfer points and tape	304	4.6 (cde)
Plastic flaps and transfer points (e.g., polyvinylchloride (PVC), polyurethane)	427	4.2 (de)
Side edges (e.g., Painted-steel or high-density polyethylene)	123	3.3 (cdef)
Sorter cups	76	2.6 (cdef)
Solid conveyor belts (e.g., PVC, polyurethane, polyester nylon)	186	1.6 (ef)
Sorting plastic guide rails	128	1.6 (ef)
Traction belting (e.g., polyurethane, polyester nylon)	66	1.5 (cdef)
Brushes under spray bars (e.g., polyethylene, polypropylene)	227	0.9 (f)
Stainless steel dump tank and flume	108	0.9 (ef)
PVC rollers	123	0.8 (ef)
Packing tables and plastic crates	64	0.0 (ef)
Cup droppers (e.g., painted steel)	60	0.0 (ef)
Sorting brushes (e.g., polyethylene, polypropylene)	193	0.0 (f)

[a] Number of samples tested.
[b] Values within a column that are not followed by the same letter are significantly different ($p \leq 0.05$

Effectiveness of cleaning and sanitation methods evaluated. As reported in Figure 4, after the applied cleaning and sanitation methods, the *L. innocua* log reduction varied based upon the type of surface and treatments ($p < 0.05$). According to the U. S. Food and Drug Administration & U.S Department of Health and Human Services (2017), an effective cleaning and sanitation procedure should cause at least a 5 Log reduction of the evaluated target organism. Stainless steel and Teflon-wrapped rollers, and interlocking belts were the surfaces in which a greater than 5 log reduction was obtained when treatments T_3, T_4, T_6, and T_7 were applied. For brushes made of 100% polyethylene, T_2 and T_4 were the most effective treatments. Polishing brushes made of 50%horsehair and 50% polyethylene were the type of surface that showed the least *L. innocua* log reduction, regardless of treatment. This suggests that polishing brushes made of 100% nylon could potentially offer a better hygienic design for polishing apples in the wax coating unit operation. These results could be attributed to the fact that horsehair mix polishing brushes were particularly harder to clean and sanitize due to the more stuffed configuration of the bristles they have (Figure 3). Also, unlike nylon material, the horsehair mix bristles absorbed a greater amount of cleaners, thus requiring a more water to complete the rinsing step.

T_3, T_4, T_6, and T_7 were the treatments that caused the greatest log reduction in stainless steel and Teflon-wrapped rollers, and interlocking belts surfaces. As expected, these treatments were based on either the mechanical or chemical removal of wax residues prior to applying the sanitizer, and unlike brushes, the rinsing step was easy to verify. It is important to highlight that on those surfaces the application of wax was minimal, and wax residues were imperceptible to the human eye. This situation underscores that wax removal must still be performed on surfaces that get wax carryover to further equipment in apple packinghouses.

Also, our results showed no significant differences between both sanitizers (PAA and Cl) when applied to 100% nylon polishing brushes, stainless steel rollers, and interlocking belts as long as the wax residues were effectively removed ($p<0.05$). Only in horsehair mix polishing brushes, PAA at 500 ppm was significantly more effective than chlorine after the wax strip cleaner was applied.

The application of steam caused around three *L. innocua* log reduction in all surfaces but polishing brushes. In the latter ones, < 2 log reduction was obtained, most likely due to the amount of wax residues present.

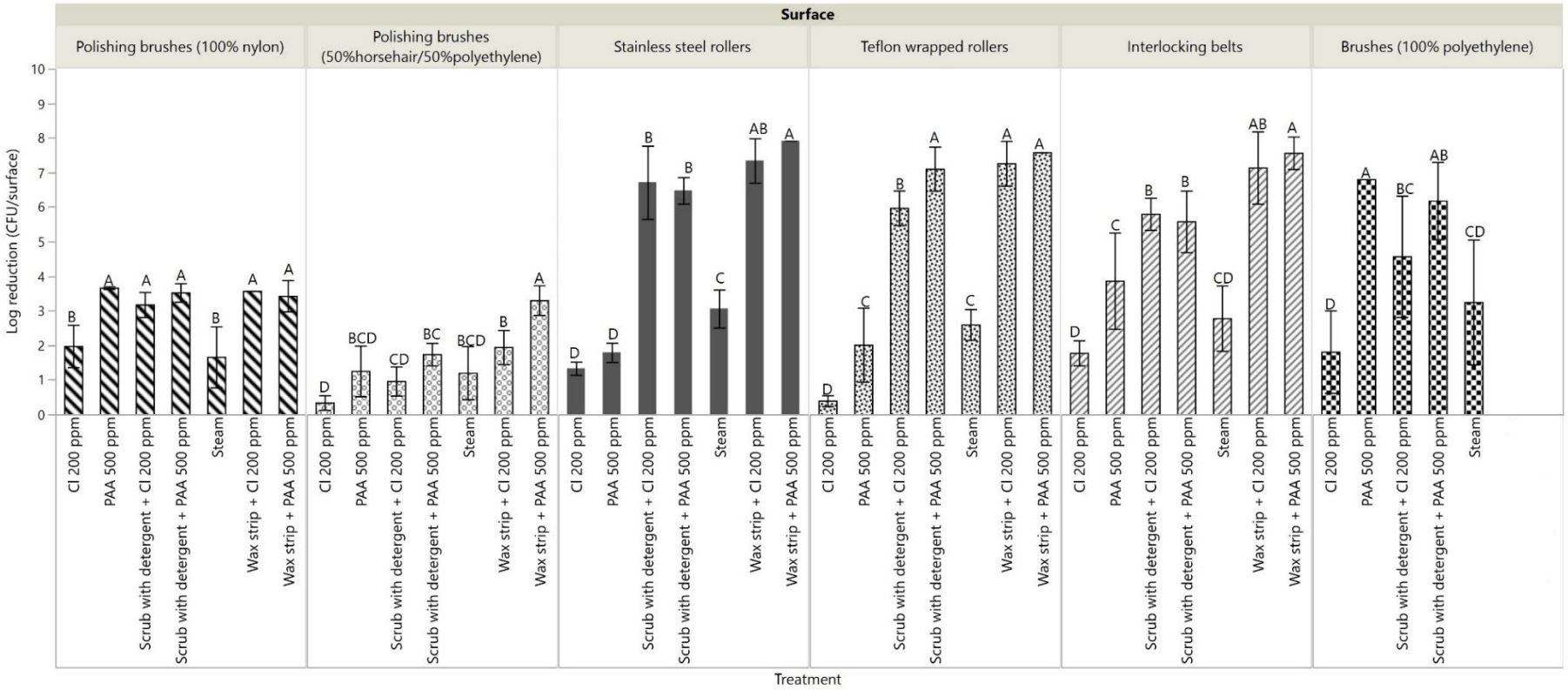

Figure 4. Log reduction of *L. innocua* after cleaning and sanitation treatments. Data shown are the means ± standard deviation. Different uppercase letters mean significant difference among treatments within each type of surface ($p < 0.05$).

EXECUTIVE SUMMARY

Project Title: Systems-based approach for improved packinghouse sanitation

Key words: *Listeria*, surface, sanitation

Abstract:

The 2014 caramel apple listeriosis outbreak was traced back to cross-contamination between food contact surfaces (FCS) of equipment used for packing and fresh apples. For Washington state, the leading apple producer in the United States with 79% of its total production directed to the fresh market, managing the risk of apple contamination with *Listeria monocytogenes* within the packing environment is crucial. The objectives of this study were to determine the prevalence of *Listeria* spp. on FCS in Washington state apple packinghouses over two packing seasons, to identify those FCS types with the greatest likelihood to harbor *Listeria* spp., and to evaluate the efficacy of different cleaning and sanitation treatments on FCS that have been found to have a higher prevalence of *Listeria* spp. harborage. Five commercial apple packinghouses were visited quarterly over two consecutive year-long packing seasons. A range of 27 to 50 FCS were swabbed at each facility to detect *Listeria* spp. at two sample times, (i) post-sanitation and (ii) in-process (3h of packinghouse operation), following a modified protocol of the FDA's Bacteriological Analytical Manual method. Among 2,988 samples tested, 4.6% (n= 136) were positive for *Listeria* spp. Wax coating was the unit operation from which *Listeria* spp. were most frequently isolated. The FCS that showed the greatest prevalence of *Listeria* spp. were polishing brushes, stainless steel dividers and brushes under fans/blowers, and dryer rollers. The prevalence of *Listeria* spp. on FCS increased throughout apple storage time. In regard to the different cleaning and sanitation methods applied, an effective wax removal using a degreaser or detergent followed by the application of a sanitizer caused the greatest *L. innocua* log reduction. The application of steam did not show significant *L. innocua* log reduction regardless of type of surface. Polishing brushes made of a horsehair mix were the type of surface with the lowest log *L. innocua* reduction ($p < 0.05$).

The results of this study will aid apple packers in controlling for contamination and harborage of *L. monocytogenes* and improving cleaning and practices for sanitation of the FCS on which *Listeria* spp. are the most prevalent. Such findings are essential for the apple-packing industry striving to further understand and exhaustively mitigate the risk of contamination with *L. monocytogenes* to prevent future listeriosis outbreaks and recalls.