FINAL PROJECT REPORT

Project Title: Validation of fresh apple packing food safety interventions

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Drs. Dong Hyun Kang and Gene Kupferman also served as co-investigators in previous years of this project.

Cooperators:

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Several chemical suppliers have donated supplies, other resources and time to discuss aspects of this project and ensure relevancy to the apple packing industry.

An equipment donation from Aquapulse systems was made to WSU with estimated value of $10,000 arrived in 2011 for chlorine and chlorine dioxide experiments.

Several apple packing facilities have participated in packing plant studies or with insight on packing conditions to ensure that laboratory results relate to large scale commercial treatments.

Total Project Request: Year 1: $50,990 (awarded) **Year 2:** $53,030 (awarded) **Year 3:** $55,152 (awarded).

Other funding sources

Agency Name: Washington State USDA Specialty Crop Block Grant Program
Amt. awarded: $55,868
Notes: This project supported a literature review, initial laboratory experiments to select methodology for apple inoculation and an educational meeting with the tree fruit industry. The literature review provided information on the state of knowledge regarding antimicrobial interventions for whole, fresh apples, which was found to be relatively limited. Additionally, the review of literature indicated that methodology used in evaluating antimicrobial interventions for apples varied significantly between studies. Therefore, microbial studies were conducted to assist in selection of methods for apple inoculation, such as preparation of apples prior to inoculation, inoculation methods and media and drying time. These experiments developed a foundation for methodology that was utilized in Years 1 and 2 and the proposed work of Year 3 in order to provide the industry with scientific information using standardized methods that will allow for comparison of results. An educational food safety meeting, "Safety of Northwest Produce" was conducted with 100 participants, primarily from the Washington tree fruit industry, and provided important opportunities to discuss research needs with industry representatives.

Budget 1

Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: (509) 335-7667 **Email address: mdesros@wsu.edu**

Item	2009	2010	2011
Salaries	22,014	22,895	23,811
Benefits	1,560	1,622	1,688
Wages	5,100	5,304	5,516
Benefits	816	849	883
Equipment	0	0	0
Supplies	16,500	17,160	17,846
Travel	5,000	5,200	5,408
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	50,990	53,030	55,152

Footnotes:

[1] Additional funds are not requested

Objectives:

1) Perform laboratory validation studies to examine foodborne pathogen and indicator organism reduction by antimicrobial treatments currently used in the apple packing industry
2) Validate antimicrobial interventions under industrial packing line conditions using indicator organisms
3) Conduct appropriate food safety extension outreach with the apple packing industry

Significant Findings:

- Our study represents the most robust examination of the effectiveness of peroxyacetic acid (PAA), chlorine and chlorine dioxide on whole, fresh apples using industry-relevant concentrations and application durations as indicated by currently available scientific literature. Many food safety experts utilize a 2-3 log reduction as a benchmark when evaluating the effectiveness of a single antimicrobial intervention in a given process.

- Laboratory experiments on PAA involved treatments using water and 40, 60 and 80ppm PAA with application times typical for current industry practices (5 seconds of direct application followed by exposure times of 10, 25, 40 and 60 seconds) to represent spray bar application times and time on a conveyance system after the spray bar. Three replications for generic *E.coli* and pathogenic *E.coli* O157:H7 were completed.

    - Treatments at or near 80ppm PAA were significantly different than water and all but one treatment at 60ppm PAA were significantly different than water. PAA treatments produced a 90% microbial reduction (0.7 - 1.4 $\log_{10}$) for generic *E.coli* and pathogenic *E.coli*.

- Commercial experiments for PAA examined a single spray bar at 40, 60 or 80ppm PAA (2 seconds average direct, application time) and double spray bar at 80ppm PAA (9 seconds average direct application time). Four replications were conducted and results from two replications using generic *E. coli* are presented.

    - A single spray bar at 40, 60 or 80ppm PAA produced less than a 90% reduction in generic *E. coli* (0.3 - 0.5 $\log_{10}$ reduction), which was not different than the generic *E. coli* levels on the inoculated controls.
    - A double spray bar using 80ppm PAA (9 seconds average direct application time) produced a 0.8 $\log_{10}$ reduction in generic *E. coli* which was significantly different than the inoculated control, but not significantly different than water.

- Laboratory results using increased direct PAA application times (30, 60 or 120 seconds) demonstrated an increased, 90 - 99% (1.5 - 2 $\log_{10}$) reduction of generic *E. coli* and pathogenic *E. coli* O157:H7. These laboratory results indicate greater reductions using PAA in commercial settings are possible.

- Laboratory experiments examined chlorine dioxide, using oxidation-reduction potential (ORP) in millivolts (mV) for measurement of chlorine dioxide activity. Treatment combinations included water and concentrations of chlorine dioxide that yielded three ORP levels (665, 750, 850mV) were applied at 3 application times (2, 3.5, and 5 minutes) representing application time in dump tanks.

    - Four replications for non-pathogenic (generic) *E.coli* and pathogenic *E.coli* O157:H7 were completed. Chlorine dioxide (665, 750, and 850mV) treatments at all application times responded similarly, producing less than a 90% reduction (0.5 to 0.7 $\log_{10}$ cfu/mL) of generic *E.coli* and *E.coli* O157:H7.

- Laboratory experiments examined chlorine, using ORP in mV for measurement of chlorine activity. Treatment combinations included a water treatment, and concentrations of chlorine that yielded three ORP levels (665, 750 and 850 mV ORP), and three application times (2, 3.5 and 5 minutes) to represent time of application in dump tanks.

    - Based on industry input of common practices, an additional treatment of phosphoric acid buffer (approximate pH 3.5) was included to represent treatment of apple varieties when chlorine is not used in the dump tank.
    - Five replications for generic *E. coli* and pathogenic *E. coli* O157:H7, respectively, were completed. Chlorine (665, 750 and 850mV ORP) and phosphoric acid treatments responded similarly producing less than a 90% reduction (0.5 to 0.8 $\log_{10}$) reduction of generic *E.coli* and *E.coli* O157:H7.

- Bacterial adherence to apples was not consistent over the four year study. However, there was no difference between the response of pathogenic *E. coli* O157:H7 or generic *E. coli* to chlorine, phosphoric acid or chlorine dioxide.
    - In two years of the study, experiments indicated pathogenic *E. coli* O157:H7 had greater adherence to apples than generic *E. coli*; whereas in two years of the study, pathogenic *E. coli* O157:H7 and generic *E. coli* adhered at similar levels to apples.
    - In one experiment using only generic *E. coli*, reduced levels of adherence on apples were observed.
    - Seasonal variation and apple physiology may influence bacterial adherence to apples.

Results and Discussion:

Objective 1.

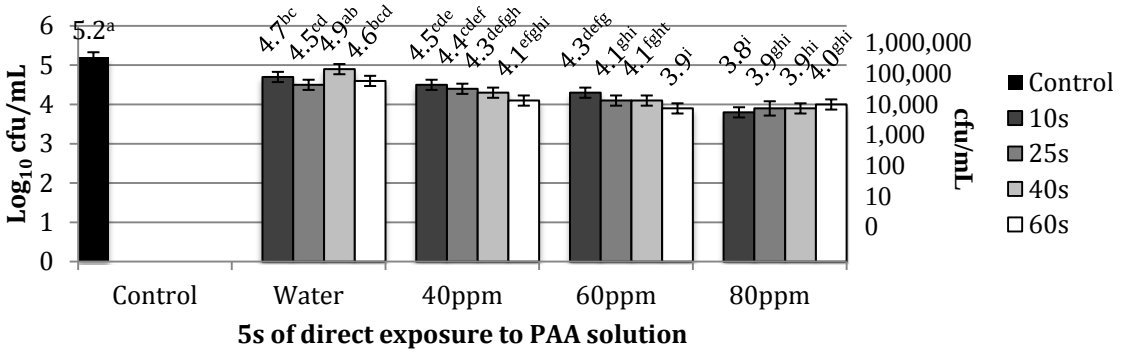
<u>Laboratory Examination of Peroxyacetic Acid (PAA) at Spray Bar Application Time and Exposure Durations.</u>

Three replications examining generic *E. coli* and pathogenic *E. coli* O157:H7 on washed, Gala apples were performed. The compounds and concentrations tested included: water and peroxyacetic acid at 3 concentrations (40, 60 and 80ppm). Apples were placed directly in water or PAA treatments for 5 seconds of application time followed by removal, and 4 exposure durations were examined (10, 25, 40 or 60 seconds) to mimic time on a conveyance system, based on industry input and plant visits. For the experiments, 5 apples were examined in each of the 16 treatment combinations. Uninoculated and inoculated control treatments were also examined.

For inoculated control treatments, the levels of generic *E. coli* and pathogenic *E. coli* O157:H7 on the apple surface after inoculation were similar. Therefore, in this experiment, bacterial adherence was similar between generic and pathogenic *E. coli* O157:H7. There was no difference in response to PAA treatments between generic and pathogenic *E. coli*. At any concentration of PAA (40, 60, or 80ppm) exposure duration did not significantly increase bacterial reduction.

At concentrations and exposure durations typically used by the industry, the laboratory study indicated PAA treatments reduced bacterial levels about 90% (0.7 - 1.4 $\log_{10}$ reduction) (Figure 1). Treatments at or near 80ppm PAA were significantly different than water and all but one treatment at 60ppm PAA were significantly different than water.

Figure 1. Average bacterial levels (generic *E. coli* and *E. coli* O157:H7) on apple surfaces after inoculation and direct application of peroxyacetic acid (40ppm, 60ppm, 80ppm) for 5s followed by air-drying exposure times (10s, 25s, 40s, 60s) in a laboratory study. Values reported in $\log_{10}$ colony forming units (cfu)/ml scale (5=100,000 cfu/ml, 3=1000cfu/ml).

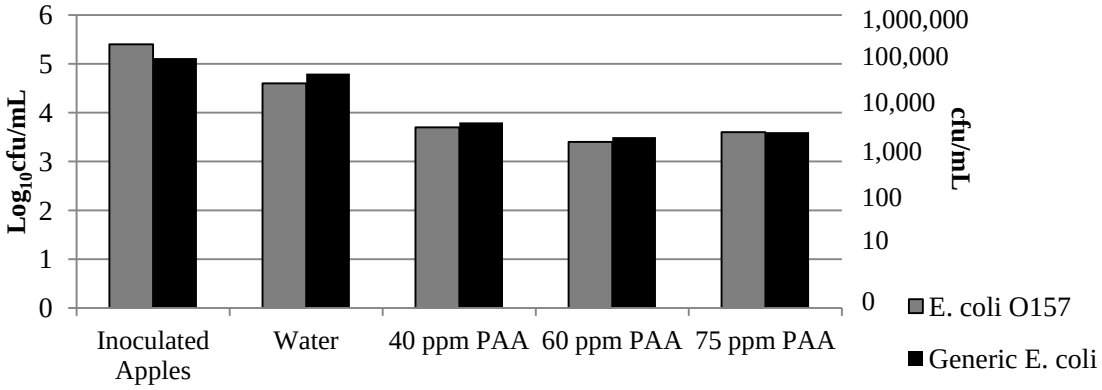


a-i For treatments not sharing a common superscript differ($p<0.05$).

Laboratory Examination of Peroxyacetic Acid (PAA) at Application Times of 30 – 120 seconds.

Three replications were performed examining generic *E. coli* and pathogenic *E. coli* O157:H7 on washed, Gala apples. The compounds and concentrations tested included: water and peroxyacetic acid at 3 concentrations (40, 60 and 75ppm). Apples were placed in water or PAA treatments for longer, direct application times (30, 60 and 120 seconds). Overall in this experiment, *E. coli* O157:H7 and generic *E. coli* appeared to have similar responses to treatments in this experiment. For some treatments, duration of application (30, 60 or 120 seconds) did not appear to affect microbial reduction. For generic *E. coli* and pathogenic *E. coli* O157:H7, when longer, direct application times (30-120 seconds) of peroxyacetic acid were used, a 90-99% reduction (1.5 – 2 $\log_{10}$ reduction) was observed (Figure 2).

Figure 2. Average *E. coli* O157:H7 and generic *E. coli* levels (log_{10} colony forming units/ml, cfu/ml) on apples after microbial inoculation and treatment with 40, 60 and 75 ppm peroxyacetic acid (PAA). Values are averaged over application times of 30, 60 and 120 seconds. Values are reported in log_{10} scale (5=100,000 cfu/ml, 3=1000cfu/ml).



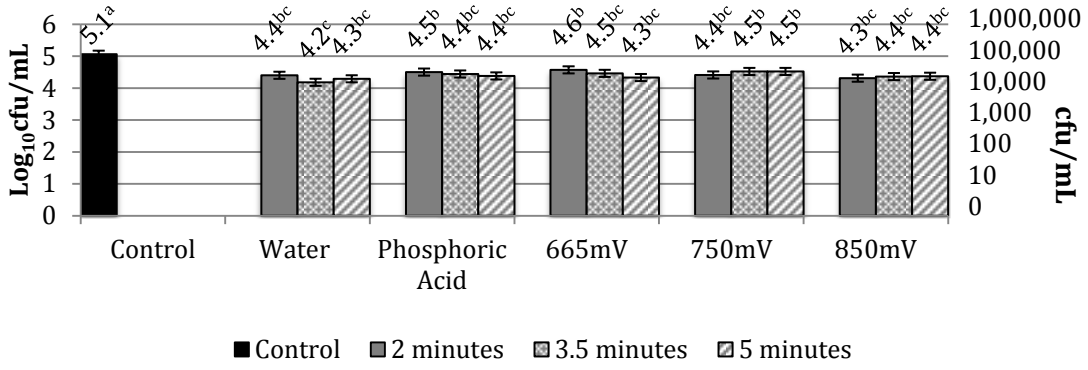
Laboratory Examination of Chlorine and Phosphoric Acid Treatments at Dump Tank Application Times.

Five replications examining generic *E. coli* and pathogenic *E. coli* O157:H7 on washed, Gala apples were performed. Industry input indicated that a treatment of phosphoric acid alone should be included to mimic dump tank treatment of apple varieties that do not involve the use of chlorine. The compounds and concentrations tested included: water, phosphoric acid (pH 3.5) and three concentrations of chlorine as measured by oxidation-reduction potential (ORP) (665, 750 and 850 mV). To mimic dump tank application, three application times were examined (2, 3.5 and 5 minutes).

For inoculated control treatments, levels of pathogenic *E. coli* on apples were higher than levels of generic *E. coli*. Therefore, in this experiment, adherence of pathogenic *E. coli* O157:H7 was higher after initial inoculation compared to non-pathogenic, generic *E. coli*. However, there was no difference in the response of pathogenic *E. coli* O157:H7 and generic *E. coli* to the chlorine treatments. Also, application time (2, 3.5 and 5 minutes) did not appear to significantly affect bacterial reduction.

For generic *E. coli* and pathogenic *E.coli* O157:H7, all treatments including water had significantly lower bacterial levels than the inoculated controls (Figure 3). All chlorine treatments (665, 750 and 850mV ORP) produced bacterial reductions similar to water, less than a 90% reduction (0.5 - 0.9 log_{10} reduction). Similarly, phosphoric acid produced results that were similar to water for generic *E. coli* and *E. coli* O157:H7, less than a 90% reduction (0.6 - 0.9 log_{10} reduction). While the laboratory data indicated that microbial levels were not reduced on apple surfaces when compared to water, it is likely that chlorine controls cross-contamination risk by controlling microbial levels in the treatment solution.

Figure 3. Average bacterial levels (generic *E. coli* and *E. coli* O157:H7) on apple surfaces after inoculation and direct application of chlorine (target ORP levels 665mV, 750mV, 850mV) and phosphoric acid (pH 3.5) for 2, 3.5 and 5 minutes in a laboratory study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1000cfu/ml).



a-c treatments that do not share a common superscript differ ($p<0.05$).

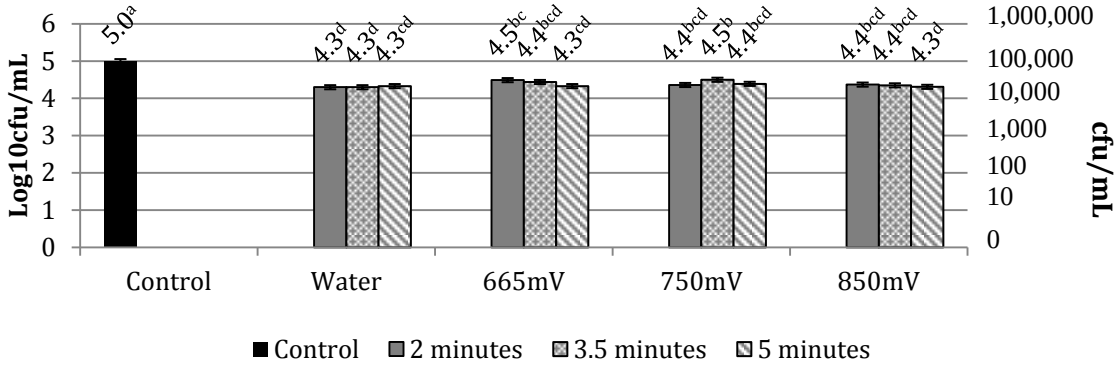
Laboratory Examination of Chlorine Dioxide Treatments at Dump Tank Application Times.

Four replications examining generic *E. coli* and pathogenic *E. coli* O157:H7 on washed, Gala apples were performed. The compounds and concentrations tested included: water and three concentrations of chlorine dioxide measured by ORP (665, 750 and 850mV). To mimic dump tank application times, three application times were examined (2, 3.5 and 5 minutes).

For inoculated control treatments, levels of pathogenic *E. coli* on apples were higher than generic *E. coli*. Therefore after initial inoculation in this experiment, adherence of pathogenic *E. coli* O157:H7 was higher compared to non-pathogenic, generic *E. coli*. However, there was no difference in the response of pathogenic *E. coli* O157:H7 and generic *E. coli* to the chlorine dioxide treatments. Application time (2, 3.5 and 5 minutes) did not appear to significantly affect bacterial reduction.

For generic *E. coli* and pathogenic *E.coli* O157:H7, all treatments including water had significantly lower bacterial levels than the inoculated controls (Figure 4). All chlorine dioxide treatments (665, 750 and 850mV ORP) for generic *E. coli* and pathogenic *E.coli* O157:H7 were similar to water treatments and produced less than a 90% reduction (0.5 to 0.7 $\log_{10}$ reduction). While the laboratory data indicated that microbial levels were not reduced on apple surfaces when compared to water, it is likely that chlorine dioxide controls cross-contamination risk by controlling microbial levels in the treatment solution.

Figure 4. Average bacteria levels (generic *E. coli* and *E. coli* O157:H7) on apple surfaces after inoculation and direct application of chlorine dioxide (target ORP levels 665mV, 750mV, 850mV) for 2, 3.5 and 5 minutes in a laboratory study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1000cfu/ml).



a-d treatments that do not share a common superscript differ ($p<0.05$).

Objective 2.

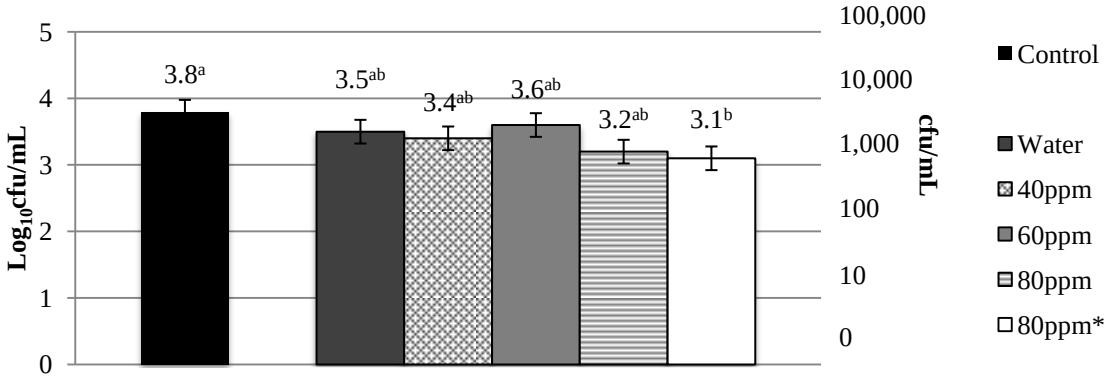
Commercial Examination of Peroxyacetic Acid Spray Bar Applications.

Four replications at a commercial packing facility were performed to assess PAA spray bar applications. Data from the first two replications indicated that generic *E. coli* adherence was reduced in 2011 compared to 2009 and 2010 using the same methods; this observation coincided with an observation from an industry partner that fungicide residue adherence on apples was also reduced in the same year. Therefore, for replications 3 and 4, the concentration of the inoculum was doubled.

The use of different nozzles was identified as a potential influencing factor. Experiments focused on examining 40, 60 and 80ppm PAA using high pressure nozzles (the more common industry practice) in all four replications. A low pressure nozzle treatment at 40ppm PAA was included in the first two replications. Data suggested that the high and low pressure nozzles did not affect reduction of generic *E. coli* levels, so this treatment was removed in order to examine increased application time using a double spray bar at 80ppm PAA application in two replications.

Data examining inoculated apples prior to and after the spray bar will be discussed from the last two replications as this data was the most consistent due to fluctuations in microbial levels as noted above (Figure 5). All treatments achieved less than a 90% reduction (<0.1 log cfu/ml). Only the double spray bar treatment was significantly different than the inoculated control but was not significantly different than the water treatment. The water treatment produced a 0.3 log reduction and treatment with 40ppm PAA and 60 ppm PAA produced a 0.4 and 0.2 log reductions, respectively. However, 80ppm PAA achieved a 0.6 log reduction. Average direct application time under the single spray bar treatment was 2 seconds (range 1-4 seconds). The double spray bar treatment with 80ppm PAA achieved a 0.7 log reduction with an average direct application time under the double spray bar of 9 seconds (range 6-13 seconds). Equivalent treatments examined in the laboratory studies achieved greater bacterial reductions (between 90-99%); therefore, greater reductions using PAA in commercial settings are possible.

Figure 5. Average generic *E. coli* levels on apple surfaces after inoculation and direct application of peroxyacetic acid (40ppm, 60ppm, 80ppm, 80ppm* double spray bar) for in a commercial study. Values reported in $\log_{10}$ colony forming units (cfu)/mL scale (5=100,000 cfu/ml, 3=1000cfu/ml).



a-c For treatments not sharing a common superscript differ($p<0.05$).

*Double spray bar application

Objectives 1 and 2.

Levels of bacterial indicator organisms.

All of the experiments above involved measuring background bacterial levels. Almost all experiments involved Gala apples to reflect average wax levels among typical varieties grown in the Pacific Northwest; when Galas were not available Braeburn apples were used. Information on apple handling varied. In all experiments, unwaxed apples were used. In a few experiments, organic apples were used. In most experiments, pre-sized apples were used; these apples received a chlorine antimicrobial treatment.

In total, 200 apples were examined for total coliforms and generic *E. coli*, and 170 apples were examined for aerobic plate counts. Aerobic bacterial counts could not be recovered from organic apples due to mold growth on the tryptic soy agar. It is acknowledged that these results represent a relatively small sample size.

Indicators of fecal contamination (total coliforms and generic *E. coli*) were not detected on 86.5 – 98.5% of the apples, respectively. Higher levels (100-1000 cfu/ml) of total coliforms were detected on 4% of the apples and higher levels (100-1000 cfu/ml) of generic *E. coli* were detected on 0.5% of the apples. These levels are similar to other published literature. For overall bacterial levels (aerobic plate counts), 71.7% averaged 100 or fewer cfu/ml, 20% averaged 1,000 cfu/ml and 8.2% averaged 10,000-100,000 cfu/ml. These values align with or are lower than other published literature. The data indicate that fecal contamination levels and overall bacterial levels on apples are not consistent, and the risk of pathogen contamination exists.

Table 1. Average values for background microflora and percent of total apples examined for aerobic bacteria, total coliforms and generic *E. coli* on the surface of whole fresh apples. Values reported in $\log_{10}$ colony forming units (cfu)/mL and equivalent value of colony forming units provided.

Microbial level	Total Coliform (n=200)	Generic *E.coli* *(n=200)*	Aerobic Bacteria (n=170)
Not Detected	173 (86.5%)	197 (98.5%)	0 (0.0%)
<1 Log *<10 colonies*	8 (4.0%)	2 (1.0%)	15 (8.8%)
1 Log *10 colonies*	10 (5.0%)	0 (0.0%)	58 (34.1%)
2 Log *100 colonies*	7 (3.5%)	1 (0.5%)	49 (28.8%)
3 Log *1,000 colonies*	2 (1.0%)	0 (0.0%)	34 (20.0%)
4 Log *10,000 colonies*	0 (0.0%)	0 (0.0%)	8 (4.7%)
5 Log *100,000 colonies*	0 (0.0%)	0 (0.0%)	6 (3.5%)
6 Log *1,000,000 colonies*	0 (0.0%)	0 (0.0%)	0 (0.0%)

Objective 3.

Food Safety Outreach and Education. Two workshops were conducted and attended by several representatives from the tree fruit industry. The workshop "Farm Production Practices for Food Safety" featured 4 national speakers, 3 regional speakers and 4 WSU faculty. Over 70 participants attended the workshop. Presentations were also delivered at the Washington State Horticultural Association meetings in 2010, 2011 and 2012.

The workshop "Safety of Northwest Produce" featured 1 national speaker, 3 regional speakers and 2 WSU faculty. Over 100 participants attended, including growers and packers, processors, suppliers, sales and marketing representatives, laboratory technical staff, trade association representatives, consultants and extension personnel. Participants reported increased knowledge in 7 food safety topics. Half to two-thirds of participants agreed that the information gained at the workshop would be useful in communicating to others in their operation about food safety, working with other groups on food safety and conducting food safety training in their operations. Some participants indicated that as a result of the workshop they would do the following: refine or implement food safety practices, share food safety information with employees, communicate the importance of food safety and share food safety information with management.

Executive Summary:

The objectives of the study were to conduct laboratory and commercial experiments to examine the ability of commonly used antimicrobial compounds in the apple packing industry to reduce foodborne pathogen contamination risk and conduct appropriate extension outreach. Selected antimicrobials were: peroxyacetic acid (PAA), chlorine, phosphoric acid and chlorine dioxide at concentrations and application times relevant to the industry. Laboratory studies examined the response of pathogenic *E. coli* O157:H7 and compared the response of generic *E. coli*. Only generic *E. coli* (non-pathogenic) were examined in commercial studies. Therefore, the laboratory comparison was important to understand differences between pathogenic and generic *E. coli* so that accurate estimation of pathogen reduction with commercial scale interventions could be performed.

Laboratory studies examining peroxyacetic acid (PAA) simulating typical industry practices, concentrations of 40, 60 and 80 ppm and 5 seconds of direct application resulted in up to a 90% (0.7 - 1.4 $\log_{10}$) reduction of pathogenic *E. coli* O157:H7 and generic *E. coli*. A commercial study examining typical industry practices with PAA, a single spray bar of 40, 60 or 80ppm PAA and 2 seconds average direct, application time produced less than a 90% reduction in generic *E. coli* (0.3 - 0.5 $\log_{10}$ reduction). A double spray bar at 80ppm PAA (9 seconds average direct, application time) produced a significant, but less than 90% reduction (0.8 $\log_{10}$ reduction) in generic *E. coli*. Laboratory studies using longer, direct application times (30, 60 and 120 seconds) of PAA at 40, 60 and 80ppm PAA produced a greater reduction, up to 99% (1.5-2 $\log_{10}$) for pathogenic *E. coli* O157:H7 and generic *E. coli*. Therefore, enhanced reductions using PAA in commercial settings are possible. A 99% (2 log) bacterial reduction for a single intervention would likely be viewed more favorably as an antimicrobial intervention by regulatory officials.

Laboratory studies examined chlorine and chlorine dioxide using typical industry practices, concentrations yielding 665, 750 and 850mV oxidation reduction potential (ORP) and direct application times of 2, 3.5 and 5 minutes as well phosphoric acid (pH 3.5) at direct application times of 2, 3.5 and 5 minutes. All treatments resulted in a less than 90% reduction (0.5 – 0.8 $\log_{10}$). While the laboratory data indicated that microbial levels were not reduced on apple surfaces when compared to water, it is likely that chlorine and chlorine dioxide control cross-contamination risk by controlling microbial levels in the treatment solution.

An important finding of the study was that the adherence of pathogenic *E. coli* O157:H7 and generic *E. coli* are not always similar, with pathogenic *E. coli* O157:H7 having greater adherence to apples in some cases. An increased understanding of the response of pathogenic *E. coli* on apples and the potential influence of apple physiology on bacterial interactions would provide the industry with advanced knowledge to enhance food safety efforts.

A limited examination of initial microbial levels on apples was performed. Information on treatment of the apples prior to receiving was not always available; many of the apples had been pre-sized and treated with a chlorine antimicrobial. Indicators of fecal contamination (total coliforms and generic *E. coli*) were not detected on 86.5 – 98.5% of the apples, respectively. Higher levels (100-1000 cfu/ml) of total coliforms were detected on 4% of the apples and higher levels (100 cfu/ml) of generic *E. coli* were detected on 0.5% of the apples.

Although the presence of fecal contamination was not often detected, there is a need to ensure that when higher levels of fecal contamination are encountered, the contamination risk is controlled by preventing cross-contamination and reduced through the use of effective antimicrobial interventions. Based on the data in this study, current apple packing interventions likely reduce pathogen risk less than 90%; therefore, there is a need to enhance current antimicrobial interventions to more effectively reduce pathogen risk.