Project/Proposal Title: Evaluation of an alternative postharvest fungicide applicator

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PI: Achour Amiri
Organization: Washington State University
Telephone: 509-293-8752
Email: a.amiri@wsu.edu
Address: WSU-TFREC
Address 2: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA, 98801

Co-PI 2: Gwen Hoheisel
Organization: Washington State University
Telephone: 509-786-5609
Email: ghoheisel@wsu.edu
Address: 620 Market Street
Address 2: WSU Extension
City/State/Zip: Prosser, WA 99350

Cooperators: Jason and Jordan Matson, Matson Fruit, Faith Critzer, University of Georgia; Clark Kogan, Statscraft LLC, Pace International LLC.

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Budget 1
Primary PI: Achour Amiri
Organization Name: WSU
Contract Administrator: Kevin Rimes
Telephone: 509-786-2226
Contract administrator email address: kevin.rimes@wsu.edu

Item	2021	2022	2023
Salaries	69,064	63,989	0
Benefits	21,563	18,520	0
Wages	6,483	5,411	0
Benefits	807	541	0
Equipment	0	0	0
Supplies	29,160	21,060	4,500
Travel	5,716	1,472	0
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	132,793	110,993	4,500

OBJECTIVES

1. Optimize coverage of fruit in alternative sprayer with fluorescent tracer and water sensitive paper (Hoheisel; yr 1).

2. Comparison of efficacy against postharvest decay organisms between drench and alternative fungicide application (Amiri; yr 1and 2).

3. Quantification of indicator organisms (*E. coli* and coliforms) in water and on fruit treated with fungicides applied in drench and alternative applications (Hoheisel; yr 1 and 2).

4. Communication of findings with the apple and allied industries and engage regulatory bodies for approaches for implementation of alternative fungicide application on farm (Amiri, Hoheisel; yr 1, 2 and 3).

Significant Findings

- A novel field drencher (FD) was optimized for spray coverage.

- Coliform counts were higher in the field sprayer whereas *E. coli* recovery was higher in the commercial packinghouse drencher (CPD).

- Residue levels of thiabendazole (TBZ) were similar between the field drencher and commercial packinghouse drencher (CPD) but levels of fludioxonil (FDL) were higher on apples treated with the field drencher.

- Spores of *Penicillium* spp. (blue mold) were neither detected on apples nor in fungicide solutions of field sprayer or warehouse drencher in 2021 but increased in the CPD tank as the number of bins increased to 600 bins.

- Total microflora recovered from apples treated with fungicides through the FD was significantly reduced compared to the control and fruit treated via the CPD.

- Overall, decay incidence after 8 months of storage was lower in apples treated via the FD compared to those treated with CPD.

Results and Discussion

Objective 1. Optimize coverage of fruit in alternative sprayer with fluorescent tracer and water sensitive paper.

The overall deposition was higher in the non-recycling FD (255.4 µg/mL) but not significantly different ($P = 0.27$) from the packinghouse drencher (CPD) (175.9 µg/mL) (**Figure 1**). In the non-stacked bins drenched through the filed drencher (FD), deposition was higher on the top versus bottom sections of two out of four bins (**Figure 2**). In the stacked bins treated through the CPD, deposition was significantly ($P < 0.001$) higher on the top bin section of the 3rd high bin on the truck, whereas deposition was overall uniform among the other zones and stacked bins (**Figure 2**).

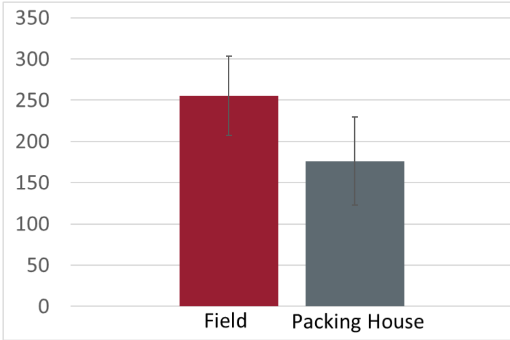
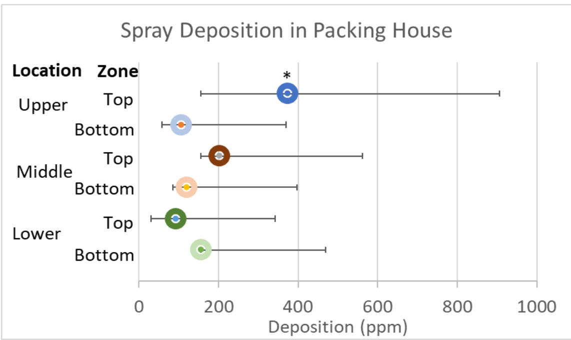


Figure 1. There is no significant difference (P-value: 0.2696) between the mean of the tracer concentration from the field and packing house, averaging across the experiment.

Deposition within the packing house was uniform except for the upper most collection zone and location receiving more (Figure 3, left). This is obviously due to the shower-down nature of the application. Nonetheless, it is positive that the lowest collection area (Lower, bottom zone) had similar deposition to other areas and is likely due to the extremely high flow rate in the packing house. The field drencher (Figure 2, right) is not stacked but goes under the spray bar with bin 1 going in first. After the last bin is sprayed, the driver waits 30 seconds and backs out with the bin 4 being the first under the spray bar. In this analysis there was a difference in deposition with the third bin receiving slightly less. We need to inspect possible differences in driving or patterns that could explain this difference. It contrasts with the regularity of time sprayed per bin (12 sec) which showed no significant difference in spray time among bins. Additional differences can be seen between the top and bottom zone of the bin, however, the impact of this would need to be assessed with efficacy data from storage rots. Meaning, there may be adequate deposition in the lower portion to control, but if not, rate should be increased to achieve more deposition in the bottom.



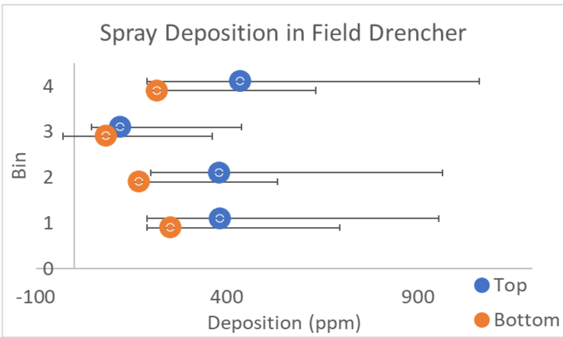


Figure 2. Spray deposition in the packing house and field drencher. In the packing house, bins are stacked (location) and there are two zones within a bin. Only the upper top collection area showed significant difference (p<0.001). In contrast, the field drencher is not stacked, but goes under the spray bar from bin 1 to 4. Significant differences were seen between the top and bottom of the bin (p>0.0197) and bin order (p>0.047).

Objective 2. Comparison of efficacy against postharvest decay organisms between drench and alternative fungicide application

2.b. Quantification of spores of fungal pathogens on fruit treated via two drench applicators

In 2021, the mixture of FDL and TBZ applied through the FD and CPD significantly (P = 0.03) reduced the total microbiota in four and three Honeycrisp lots, respectively, compared to the control (**Table 1**). The most frequent fungal pathogen was *Alternaria* spp. which was equally recovered regardless of the application method. The number of propagules of *Penicillium* spp. recovered from Honeycrisp apples at harvest ranged from 0.0 to 0.05 CFU/cm^2 and was similar between the FD and CPD. In 2022, the total microbiota was significantly lower on apples treated with PYR via the FD compared to the CPD (four lots) and the control (three lots). The density of *Alternaria* spp. was higher on treated compared to the untreated apples and increased in three lots drenched through the FD. The density of *Penicillium* spp. was significantly higher on apples drenched via the CPD in three out of four Honeycrisp lots in 2022 (**Table 1**).

Penicillium spp. was not isolated from the fungicide solutions of FDL + TBZ applied through the FD or the CPD in 2021, whereas other fungi, i.e., *Alternaria* spp. and *Mucor* spp. were detected at ≤ 1 CFU/mL regardless of the drencher type (**Table 1**). In 2022, the density of *Penicillium* spp. ranged from 0.5 CFU/mL in Hc1918 lot to 16.8 CFU/mL in Hc1156 in the PYR solutions applied through the FD (**Table 1**). The population of other fungi, i.e., *Alternaria* spp. and *Mucor* spp., ranged from 4.8 to 23.3 CFU/mL among the four Honeycrisp lots. The fungal population in the PYR solution drenched through the CPD in 2022 was positively correlated with the number of bins drenched through and increased up to 60.8 CFU/mL for *Penicillium* spp. (R^2 = 0.94) and 40.2 CFU/mL for other fungi (R^2 = 0.62), after 600 bins had been drenched (**Table 2**).

Table 1. Number of colonies of *Penicillium* spp. and other fungi recovered from the surface of the fruit treated through field (FD) and warehouse (WH) drenchers in September 2022.

		Penicillium			Other fungi		
Cultivar	**Lot**	**Control**	**FD**	**WD**	**Control**	**FD**	**WD**
Honeycrisp	1136	0.04	0.3	0.4	17.2	14.8	31.8
Gala	901	0.2	0.2	0.2	5.4	12.1	9.3
Gala	1124	0.04	0	0.3	2.4	6	4.4
Gala	1113	0.08	0	1.2	27.7	42	13

2.c. Fungicide residue levels

The overall residue levels of TBZ, FDL and PYR on Honeycrisp apples were not significantly different between the FD and CPD (**Figure 3A**). There were no significant differences between the four bins drenched through the FD across lots, therefore, the residue level values were averaged. In 2021, the residue levels of TBZ and FDL on Honeycrisp apples were not significantly different (P = 0.66) between the top, middle, and bottom sections of the bins regardless of the application method (**Figure 3B**). In 2022, the overall residue levels of PYR were significantly higher (P = 0.04) at the top bin section compared to the middle and top sections of the bins treated via the FD (**Figure 3B**). Similar to the spray deposition patterns observed with the CPD, residue levels were significantly higher at the top of the 3rd high bin in the stack on the semi-truck for TBZ (**Figure 3C**) and the top of the 3rd and 2nd high bins for PYR (**Figure 3D**), but not for FDL (**Figure 3C**).

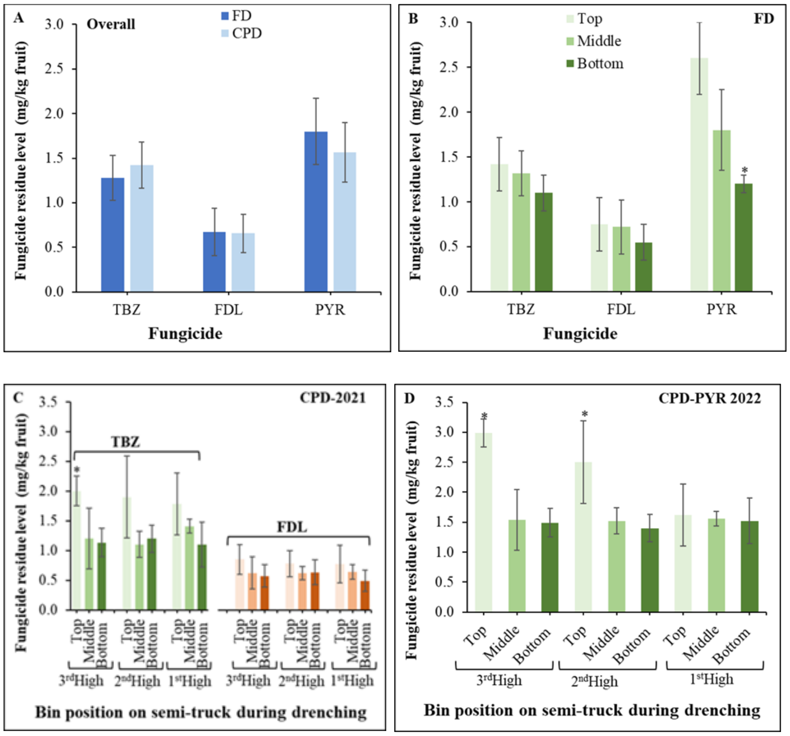


Figure 3. Residue levels of thiabendazole and fludioxonil (A) and pyrimethanil (B) on Honeycrisp apples collected from the top, middle, and bottom sections of bins treated through the field drencher or commercial packinghouse drencher in 2021 and 2022, respectively. An asterisk indicates significant difference based on Tukey's test at $P \leq 0.05$.

The concentrations of TBZ and FDL in 2021 and PYR in 2022 in the solutions of the FD tanks was similar between lots and ranged from 547 to 610 µg/mL for TBZ, 277 to 303 µg/mL for FDL, and 320 to 360 µg/mL for PYR (**Figure 4A,B**). The concentrations of the three fungicides in the CPD tanks decreased gradually as more bins were drenched resulting in a positive correlation between the number bins treated with the recycled solution and the concentration of TBZ ($R^2 = 0.92$), FDL ($R^2 = 0.84$), and PYR ($R^2 = 0.92$) in 2021 (**Figure 4C)** and 2022 (**Figure 4D**).

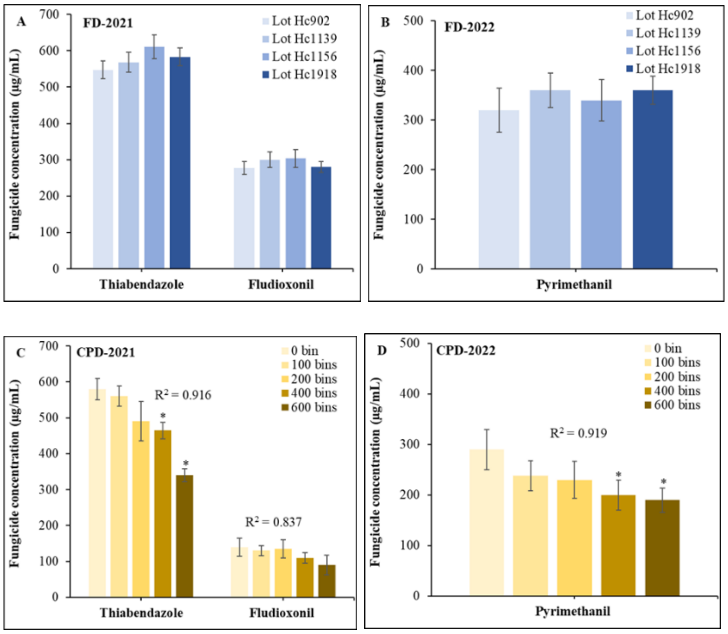


Figure 4. Residue levels of thiabendazole and fludioxonil (**A**) and pyrimethanil (**B**) in fungicide solutions applied through the field drencher (FD); residue levels of TBZ and FDL (**C**) and PYR (**D**) applied through the commercial packinghouse drencher (CPD) after several (0-600) bins had been drenched in 2021 and 2022, respectively. An asterisk indicates significant difference based on Tukey's test at $P \leq 0.05$.

<u>2.d. Determination of decay incidence and decay types in cold storage on fruit treated at harvest with fungicides through alternative and drench approaches</u>

Three hundred apples (100 apples/treatment) were collected from each lot and stored at 55°F for 2 weeks, then at 37°F in RA. Overall decay varied between lots and was either lower in field drencher after 9 months or equal to incidence recorded in warehouse drenched-fruit except in lot 1139 (Figure 5).

During the 2022-23 season, five lots of Honeycrisp and three lots of Gala that were not treated with any fungicide preharvest were treated at harvest with Penbotec. Four bins of each lot were treated using the field sprayer and 4 other bins from each lot were treated with the warehouse sprayer. Bins were stored at the collaborating were house in CA. The overall decay incidence after 8 months for Honeycrisp lots was significantly lower in two lots (1162 and 1918) and was numerically lower in the 3 other lots when treated with the field drencher (Table 2). For the Gala lots after 9 months of storage, incidence was equal in two lots and was significantly higher in apples treated with the warehouse drencher for lot 1124 (Table 2).

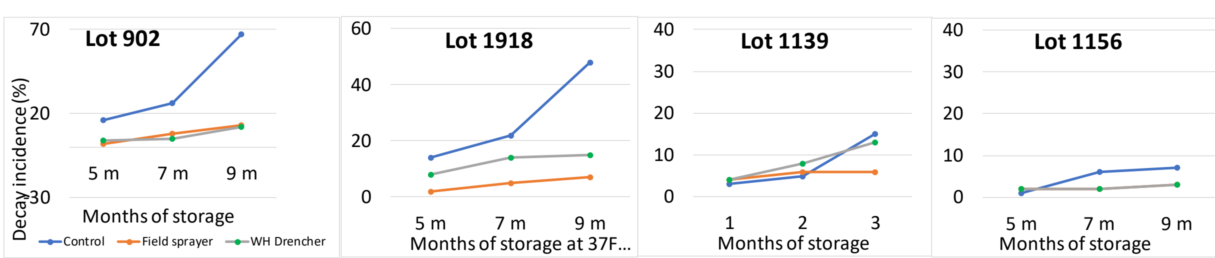


Figure 5. Overall decay incidence in four Honeycrisp lots untreated (control) or treated via field or warehouse drenchers in 2021-22 season and stored in regular atmosphere at 37°F.

Table 2. Overall decay incidence on Honeycrisp and Gala apples stored at the commercial storage cold room during the 2022-23 season under controlled atmosphere for 8 months.

		Decay incidence (%)	
Cultivar	Lot	Field sprayer	Warehouse Drencher
Honeycrisp	903	1.5	1.83
	1156	2.5	2.5
	1162	0.9	1.4
	1918	7.1	15.2
	1136	3.2	3.4
Gala			
	901	0.3	0.24
	1113	0.8	0.6
	1124	0.4	2.1

Objective 3. Quantification of indicator organisms (*E. coli* and coliforms) in water and on fruit treated with fungicides applied in truck and alternative applications

Water samples were collected in the harvest of 2021 and 2022, while apple samples were collected in 2022. Approximately 94% (85-98%) of the apple samples in the packing house and 84% (70-93%) in the field are coliform free before any drench treatment (Figure 7a). However, post drench treatment, 6% (2-17%) of the apple samples in the packing house and 94% (84-98%) of the apple samples in the field are coliform free after treatment. This is a significant ($p>0.001$) decrease for the packing house with an 87% (75-94%) decrease. Although there is a 9% difference (0.8- 20.5%) for the field drencher, pre and post treatments are not significantly different to each other.

***Apple samples**:* Of the samples that tested positive for Coliform, some also showed *E. coli* populations. Nearly 100% (96-100%) of the packing house apple samples and 96% (86-99%) of the field apple samples were *E. coli* free on arrival. After the drench treatment, an estimated 93% (79-98%) of the packing house apple samples and 98% (92-99%) of the field apple samples were *E. coli* free. There was no significant different between pre- and post-spray application for either Drencher.

For the subset of apples that did have contamination, the colony forming units (CFU) were compared pre and post spray applications. The mean CFUs for Coliform contaminated post application apple samples for field and packing house drenchers was 548 (127-2371) and 23899 (8255-69190), respectively (Fig 8a). For the field drencher, there is a non-significant 0.9-fold decrease in the CFUs for contaminated apples. In contrast, there is a 36.9-fold increase in the coliform CFUs for apples that tested positive for coliform. The mean CFUs for *E. coli* contaminated post application apple samples for field and packing house was 254 (51-1278) and 2288 (706-7417), respectively (Fig 8b). For apples from the field drencher, that is only 1.0 fold non-significant change in *E. coli* CFUs. Whereas apples from the packing house were nearly 100% free of coliform before treatment, the drench application introduces on average 2288 *E. coli* CFUs.

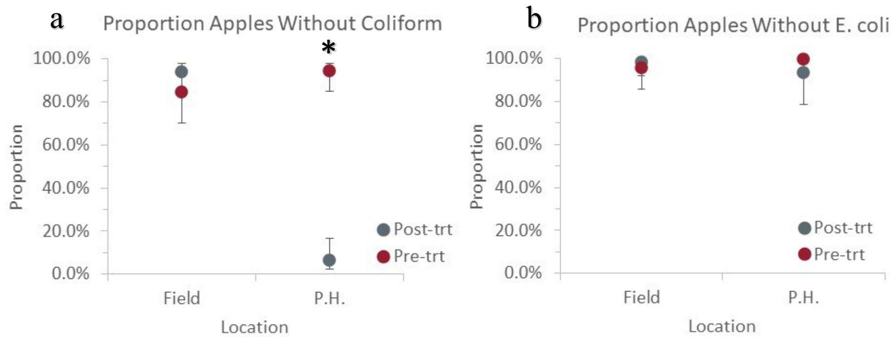


Figure 5. Proportion of apples without coliform (a) and *E. coli* (b) populations for apples pre- and post-drench treatment for Field and Packing House (P.H.) drenchers. There is a significant difference in apples with coliform (*=P-value>0.001) between the pre and post treatments in the packing house. While the field drencher showed no significant differences. And there was not a significant increase in apples with *E. coli* (b) pre or post drench for either treatment

Water samples*:* There was an estimated mean of 17 (95% CI: 9-33) thousand coliform CFU in the typical field drencher water sample and a mean of 0.6 (95% CI: 0.3-1.3) million coliform CFU in the typical packing house drencher water sample. This is an estimated 35 (95% CI: 13-93) times the number of coliform CFU in the packing house compared to the field (Table 3). There was an estimated mean of 111 (95% CI: 24-523) *E. coli* CFU in the typical field drencher water sample and a mean of 2 (0.5-7.1) thousand coliform CFU in the typical packing house drencher water sample. This is an estimated 17 (95% CI: 2-131) times the number of E. coli CFU in the packinghouse compared to the field (Table 3).

Table 3. Average concentrations of total coliforms and generic *Escherichia coli* in the fungicide solutions of the field and packinghouse drenchers and on apple surfaces before and after drenching.

Type of sample	Method of application	Sampling point	Indicator Organism	
			Total Coliforms	Generic *E. coli*[4]
Fungicide solutions	CPD	During Treatment	5.71 ± 0.51 c	3.03 ± 1.36 b
	FD	During Treatment	3.59 ± 1.38 b	1.26 ± 1.07 a
Apple surfaces	CPD	Pre-treatment	2.02 ± 0.17 a	2.00 ± 0.00 a
		Post-treatment	3.96 ± 1.18 b	2.08 ± 0.36 a
	FD	Pre-treatment	2.08 ± 0.28 a	2.01 ± 0.08 a
		Post-treatment	2.40 ± 0.23 a	2.00 ± 0.08 a

Discussion

The field drencher (FD) was optimized for spray coverage and carries approximately five times less fungicide solution than the traditional commercial packinghouse drencher (CPD). As used in this study, the FD applies approximately 1.5 gal of the fungicide solutions per bin, 50% less than the estimated 3.17 L through the CPD. Despite this difference, deposition patterns were equal or better through the FD likely because bins are not stacked and that 90% of the fungicide solution is retained on the fruits and bins during FD drenching. Comparatively, the concentration of the active ingredient strongly correlated with the number of bins drenched via the CPD and fungicide (a.i.) loss was estimated to be 40, 36 and 35% for TBZ, FDL and PYR, respectively, between 0 and 600 bins. Apples treated through the CPD were collected after approximately 200 bins had been treated, and it is possible that residue levels may be lower on CPD-treated fruit at the end of the lifespan of the tank. As expected from the "shower-down" nozzle, more deposition on the top of bins occurred on some occasions, but it was not always significantly different from the middle and bottom of the bins.

Spray coverage results were further supported by the fungicide residue levels detected on apples. Thus, FDL and TBZ levels were not significantly different between bin sections in 2021, whereas apples at the bottom received less PYR in 2022. Residue levels of FDL, TBZ, and PYR were all below the maximum residue levels of 5, 10, and 15 ppm, respectively, for both FD and CPD. The lower fungicide residue levels at the bottom of the bins treated through the FD are unlikely to reduce their efficacy as the minimum residue levels required for appropriate control are met for all three fungicides. The FD is practical as it can be used to treat fruit immediately after harvest at the vicinity of orchards and therefore may protect fruit from infections that start on fresh wounds caused during harvest, transportation and handling at the storage facility. The FD is a portable system that can be transported between orchards but can also be used at vicinity of packinghouses. The spray turnout is only slightly higher through the CPD, which treats approximately 192 bins/h, when three bins are stacked, versus approximately 160 unstacked bins/h for the FD. Besides the mentioned benefits, future economic analyses and risk analysis accounting for changes in labor, waste management costs, decay, and food safety management are needed to accurately assess the economic benefits of the FD. It is a complex analysis in that operation of the FD requires more operational hours to move bins from the orchards to the drencher then to a semi-truck, however, the risk of potential introduction of fungal and food-borne pathogens must be assessed in the return on investment.

The two major postharvest pathogens known to spread through water recirculation are *Mucor* and *Penicillium* spp., the causal agents of blue mold and mucor rot, respectively. *Mucor* spp. was not isolated from the surface of apples treated with either drencher in this study, and the frequency of *Penicillium* spp. on fruit was relatively low at harvest confirming that infection by this pathogen occur mainly after harvest. However, there was evidence of increased fruit contamination with *Penicillium* spp. spores via the recycling CPD in 2022 as their density increased 7.5 to 15-fold compared to the control in three Honeycrisp lots. Meanwhile, there were significantly less spores of *Penicillium* spp. on apples of 75% of lots drenched with the FD compared to the CPD and the control. This may indicate that the combination of TBZ and FDL in 2021 had a better efficacy against *Penicillium* spp. that may be resistant to either fungicide or that spores that are PYR-resistant have accumulated in the CPD at the time the apples were drenched in 2022. The FD and CPD equally reduced the carpoplane population of *Alternaria* spp. on apples treated with TBZ+FDL in 2021, whereas in 2022, *Alternaria* spp. increased in three and two lots on FD- and CPD-treated apples, respectively, post-drenching. Since spores of *Alternaria* spp. originate from the orchard, it is unlikely that spores were spread though the FD tank solution but rather due to different apples within the bins carrying different spore loads. Moreover, the large volume applied through the CPD may detach more spores from the apple surface than the FD.

After eight months of storage in RA and ~78% relative humidity (RH), the FD provided a greater efficacy in reducing the overall disease incidence in 50% of apple lots compared to the CPD, whereas equal efficacy was seen in the other two Honeycrisp lots, a cultivar highly susceptible to postharvest diseases. The efficacy of the FD in mitigating postharvest diseases was particularly evident in 2022, when the disease pressure was higher, as significant reductions were observed compared to the CPD in all but one lot. In the larger commercial trial including Honeycrisps and Gala apples from seven lots stored in CA at RH > 90%, the overall disease incidence was lower in 57% of the lots treated with PYR through the FD, albeit not always significantly compared to the CPD. The most prevalent postharvest diseases encountered in 2021 and 2022 were blue mold and gray mold. While the incidence of gray mold was either equivalent between the FD and the CPD or significantly lower in fruit treated through the CPD, the incidence of blue mold was significantly reduced by the non-recycling FD in 75% of the lots treated in 2021 and 2022. In the controlled atmosphere (CA) commercial trial, the incidences of blue mold and Mucor rot were reduced in 71% of Honeycrisp and Gala lots treated through the FD at harvest. Like in RA conditions, the incidences of gray mold, Alternaria rot, and bull's eye rot, caused by the preharvest pathogens *Botrytis* spp., *Alternaria* spp., and *Neofabraea* spp., respectively, were higher in 50% of the fruit lots treated through the FD compared to the CPD after 10 months in CA.

Although the residue levels are above the minimum levels (0.5 to 2 ppm) needed to control sensitive isolates of the above pathogens, it is plausible that the relatively lower levels observed in the middle and lower sections of the bins treated through the FD may have not provided the anticipated efficacy against some of these preharvest pathogens, as opposed to a higher efficacy observed against wound pathogens like *Penicillium* spp. and *Mucor* spp. Furthermore, all lots used in this study were not treated with preharvest fungicides, which may be highly recommended to further enhance the efficacy of the FD against the preharvest pathogens. The FD has already been used by packers in the PNW in the past three years and feedback was positive in terms of reducing postharvest losses. Additional commercial trials testing different cultivars and fungicides are necessary to verify these observations, which may warrant additional adjustments in the volume of fungicide applied and the nozzle types utilized in the FD.

Differences observed in populations of *E. coli* and total coliforms on apples before and after FD and CPD drenching suggest that the CPD has a greater risk of cross-contamination compared to the FD, similar to the risk of spreading spore of plant pathogens. While pathogenic strains could not be employed in the present study, results support the notion that the FD reduces the risk of cross-contamination, including from foodborne pathogens, thereby enhancing overall food safety. Further evaluating the cross-contamination risk by modeling the transfer of inoculated surrogate organisms with phenotypic markers in both systems would be beneficial to help inform risk assessments tied to food safety. Additionally, postharvest water that comes into contact with crops must have no detectable *E. coli*/100 mL based upon water quality criterion in the U.S. Produce Safety Rule. While both drenchers had populations of *E. coli* recovered that are contributed from fruit and bins, the FD fungicide solution is not be recirculated, contrary of the CPD water, which is recycled until it reaches the end of life based on the number of bins treated. With a population of 3.03 ± 1.36 MPN/100 mL *E. coli* recovered in CPD fungicide solutions; it is obvious that water will not meet the water quality criteria specified in the PSR as the indicator concentration increases with each subsequent pass through the system. These findings have significant implications for regulatory compliance, as continued use of recirculated fungicide solutions in CPD systems could lead to failure to meet the PSR's microbial water quality standards.

Since total coliform and generic *E. coli* accumulated in both drenchers, the addition of antimicrobial agents compatible with fungicides without affecting their efficacy, could effectively reduce bacterial levels in both systems. PAA as used in this study may have further reduced total coliform and generic *E. coli* better in the FD. Future work to optimize sanitizer use and examine compatibility with fungicides within a single use and recirculated drencher is needed.

Executive Summary

Project title: Evaluation of an alternative postharvest fungicide applicator

Key words: Fungicides, non-recycling drencher, postharvest decay, food safety.

Abstract: Recycling drenchers used to apply postharvest fungicides in pome fruit may spread microorganisms, i.e., plant and foodborne pathogens, that increase fruit loss and impact food safety. A non-recycling field drencher (FD), which drenches unstacked bins of fruit, was compared to a commercial recycling packinghouse drencher (CPD) for fruit coverage, fungicide residues, postharvest diseases control and spread of plant pathogens, total coliforms and generic *Escherichia. coli*. A mixture of fludioxonil (FDL) and thiabendazole (TBZ) was used in 2021, while pyrimethanil (PYR) was applied in 2022 to alternate fungicides. The overall spray coverage assessed with pyranine was not significantly different between the FD and CPD. The residue levels of FDL and TBZ were similar between the two methods on Honeycrisp apples at the top, middle, and the bottom of the bins, whereas the residue levels of PYR were significantly lower at the bottom of the bins treated through the FD. The density of plant pathogens and overall disease incidence were similar on apples drenched through both systems in 2021 and significantly lower in FD-treated apples in 2022. The incidence of blue mold, the most important postharvest disease caused by *Penicillium* spp., was significantly lower in apples treated through the FD in both years. The levels of total coliforms and generic *E. coli* were significantly higher in fungicide solutions collected from the CPD compared to the FD. Total coliforms increased significantly on apples treated via the CPD but not on apples treated through the FD. Findings from this study suggest that the new non-recycling drencher has potential as an alternative to recycling packinghouse drenchers in reducing the spread of plant and foodborne pathogens.