APPLE CROP PROTECTION RESEARCH REVIEW
Wednesday, January 24, 2018

FINAL PROJECT REPORT **YEAR:** 3 of 3 (no-cost ext.)
WTFRC Project Number: AP14-103A

Protect title: WA 38 rootstocks and training systems

PI: Stefano Musacchi
Organization: WSU-TFREC
Telephone: (509) 663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 Western Avenue
City/State/Zip: Wenatchee, WA 98801

Co-PI (1): Matt Whiting
Organization: WSU-IAREC
Telephone: (509) 786-9260
Email: mdwhiting@wsu.edu
Address: 24106 N. Bunn Rd.
City/State/Zip: Prosser, WA 99350

Co-PI (2): Karen Lewis
Organization: WSU Regional Extension
Telephone: (509) 754-2011 x412
Email: kmlewis@wsu.edu
Address: PO Box 37 Courthouse
City/State/Zip: Ephrata, WA. 98837

Co-PI (3): Karina Gallardo
Organization: Washington State University
Telephone: (253) 445-4584
Email: karina_gallardo@wsu.edu
Address: 2606 West Pioneer
City/State/Zip: Puyallup, WA 98371

Co-PI (4): Tom Auvil
Organization: WTFRC
Telephone: (509) 665-8271
Email: auvil@treefruitresearch.com
Address: 1719 Springwater Avenue
City/State/Zip: Wenatchee, WA 98801

Cooperators: Sara Serra (WSU-TFREC)

Total Project Funding: **Year 1:** $98,903 **Year 2:** $74,523 **Year 3:** $69,093

Other funding sources: none

WTFRC Collaborative expenses

Item	2014	2015	2016
Wages[1]	6,000	7,000	9,000
Travel[2]	1,500	1,800	1,800
Total	7,500	8,800	10,800

Footnotes:
[1] Pruning, floral evaluation, harvest and fruit evaluations (second and third years).
[2]Travel to the orchards (Roza and Sunrise) from Wenatchee.

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016	2017 (NCE)
Salaries[1]	35,632	39,601	33,249	0
Benefits[2]	6,057	7,112	4,863	0
Wages[3]	4,080	4,243	4,412	0
Benefit[4]	395	411	428	0
Equipment[5]	25,000	0	0	0
Travel [6]	7,591	4,849	5,823	0
Supplies[7]	4,688	1,688	1,587	0
Miscellaneous[8]	2,760	2,819	2,931	0
Plot Fees[9]	4,000	4,000	4,000	0
Goods and Services[10]	1,200	1,000	1,000	0
Total	91,403	65,723	58,293	0

Footnotes:
[1]Salary for Ag. Research Assistant (Musacchi) and Research Associate (Gallardo).
[2]Benefits costs include increase of 4% per year.
[3]Student employee for 1.4 wks: 40/wk at $10/hr (Musacchi) and Non-Student Temporary (Whiting).
[4] Benefits at 9.7%.
[5] Ethylene reader and dry matter reader.
[6] Travel to Prosser and Sunrise Orchard (Musacchi) and Travel to Wenatchee and Yakima to facilitate focus group meetings (Gallardo).
[7] Supply costs to complete structure, pollinator trees, mineral analysis, trellis.
[8] Labor for installing trellis, planting trees and pruning,
[9] Standard annual plot fee, Sunrise Orchard and Roza Station.
[10]Fee for the venue of the focus group meetings and cost of refreshments to be served during the meetings ($50/meeting x 4 meetings = $200).

OBJECTIVES

1. Identify growth and productivity characteristics of WA 38 on M9-Nic29 and Geneva 41 trained to conventional vertical (spindle) and angled (V) systems.
2. Identify growth and productivity characteristics of WA 38 on M9-Nic29 and Geneva 41 trained to a bi-axis (fruiting wall) with and without mechanization.
3. Conduct an economic analysis of WA 38 production in the three training system scenarios.

SIGNIFICANT FINDINGS

❖ *Objective 1: Identify growth and productivity characteristics on spindle and V systems*

- The highest trunk cross-sectional area (TCSA) and annual trunk growth were reported for spindle trees in both Sunrise and Roza orchard locations for 4 consecutive years. In Sunrise, G41 had significantly higher TCSA and annual trunk growth than Nic29 and opposite trend in Roza (2014-2017).
- Hand pruning the V systems confirmed to take significantly longer to prune in hours per acre than the spindle system.
- No chemical or mechanical thinning has been applied, this variety sets generally one or 2 fruit per cluster. The variety anyway shows fluctuations year after year in number of flower buds/tree, more significant in combinations of G41-"bending". Despite the fruit self-thinning trait of 'WA 38', a minimal crop load adjustment should be considered to guarantee a constant crop year after year and avoid alternate bearing tendency.

- Spindle had higher yield per tree in both locations in the last 3 years. Yield per acre was higher in V system in Roza only. No difference in production between rootstocks in Roza in the last 3 years, while G41 confirmed to produce bigger fruit than Nic29 in Sunrise.
- In Sunrise, approx. 60% of fruit graded were considered fancy (WFCY).

❖ *Objective 2: Identify growth and productivity characteristics on bi-axis*

- The mechanical pruning of bi-axis in Roza did not have an impact on TCSA respect to hand pruning.
- In 2017 there were no differences in yield per acre between all the 4 combinations with values ranging from 64 to 74 Mton/Acre in the first 3 years of crop (2015-2017).
- Bi-axis-Nic29-Mechanical+hand reported the lowest cull incidence but also the highest percentage of poor color fruit (19%).
- For Nic29 only, the mechanical pruning seemed to delay maturity (accordingly to I_{AD} measures) and to slightly reduce the intensity of red overcolor and TA in comparison to hand pruning.

❖ *Objective 3: Conduct an economic analysis of 'WA 38' production in the three training system scenarios.*

- The aggregate pruning cost was lowest with angled, G41 and "bending" in Roza; and spindle, M9-Nic29 and "bending" in Sunrise. The total pruning cost was highest with spindle, G41 and "click" in both Roza and Sunrise.
- In general, 'WA 38' variable costs are higher than those of 'Fuji' but the estimated revenues of 'WA 38' across all treatments are much higher. Even with the inclusion of fixed costs, estimated revenues were more than enough to offset the increased production costs of 'WA 38' across all treatments.
- In both Roza and Sunrise, the highest net return is for 'WA 38' spindle, M9-Nic29 and "click" when the different prices of different fruit sizes are taken into account.

RESULTS AND DISCUSSION

Objective 1: Identify growth and productivity characteristics on spindle and V systems (Musacchi).

In Roza, a hail event during spring 2017 occurred followed by a fire blight attach.

Vegetative parameters

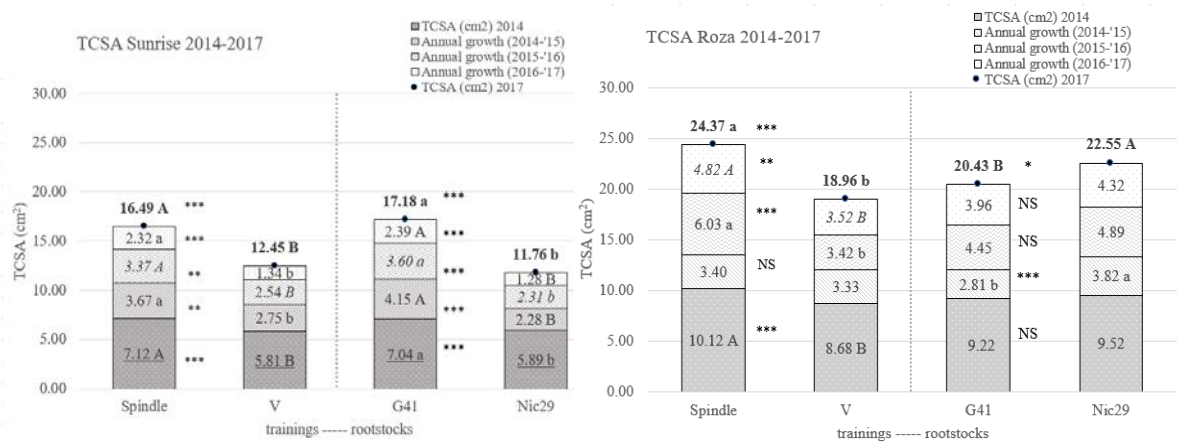


*Figure 1: Trunk cross sectional areas (TCSA) in Roza and Sunrise from 2014 to 2017, included annual growth. Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.*

The highest trunk cross-sectional area (TCSA) and annual trunk growth were reported for trees trained to spindle in both locations (for all years, Figure 1). In Sunrise, G41 rootstock had significantly higher TCSA and annual trunk growth than Nic29 (from 2014 to 2017). In Roza, Nic29 had higher TCSA than G41, while the annual growth was not significantly different except for 2014-2015 when Nic29 was higher than G41. This difference between rootstocks in Roza depended on the statistical significance for spindle, but not for the V system (data not shown). TCSA was not significantly different in the comparison between "bending" pruning technique and "click" technique in Roza and Sunrise, while in general annual trunk growth was higher for "bending" than for "click" (in Roza in 2016-2017 and in Sunrise, also 2015-2016). As combinations, spindle-G41-"bending" and "click" were the most vigorous for TCSA 2015, 2016 and 2017 in Sunrise, while spindle-Nic29-"bending" was the most vigorous in TCSA in Roza from 2014 to 2017. V-Nic29-"bending" and "click" were the least vigorous combinations in Sunrise, while in Roza V-G41-"bending" and V-Nic29-"click"reported the smallest areas in 2016, whereas in 2017 differences were less significant. The average number of rootsuckers per tree in Sunrise was higher in Nic29 than G41 (from 2014 to 2016).

Winter Pruning

Spindle hand pruning took significantly less time than V system hand pruning in both locations in 2016 (4th leaf) (26 h/A vs 20 h/A in Roza, Table 1). In 2017, pruning time was recorded only in Sunrise and this observation was confirmed with V system taking 10 hours more per Acre than spindle. The time spend in pruning, regardless the specific training system, increased of about 12 hours from 2016 to 2017 (Table 1). The material removed during winter hand pruning 2017 from spindle was significantly more than V (reaching almost double in Roza), while less difference was observed for Sunrise. In Sunrise, trees on G41 rootstock required more pruning time than Nic29, while no differences in time were reported in Roza (data confirmed for 2014-2015-2016, excluding 2017), but in 2017 more material was cut from Nic29. G41 in Sunrise reported a higher amount of wood removed in 2017 during winter pruning by hand in comparison to Nic29 (double amount) as reported in 2015 and 2016 (Table 1). The interaction of training system x rootstock in Roza revealed that within Spindle, G41 had less wood cut in comparison to Nic29, while in V system there was no

Table 1: 'WA 38' pruning material removed in 2016 and 2017 in both orchards and time of pruning 2016 only for Roza and 2016-2017 for Sunrise.

	ROZA							SUNRISE							
	2016 winter PRUNING (hours:min:sec/ Acre)#		2016 cut material (wood) in winter (kg/tree)		2017 winter PRUNING (hours:min:sec/ Acre)@	2017 cut material (wood) in winter (kg/tree)		2016 winter PRUNING (hours:min:sec/ Acre)#		2016 cut material (wood) in winter (kg/tree)		2017 winter PRUNING (hours:min:sec /Acre)#		2017 cut material (wood) in winter (kg/tree)	
Trainin systems															
spindle	19:56:10	B	0.59	A	.	1.43	A	14:41:46	B	0.32		27:18:10	B	0.63	A
V	26:08:31	A	0.28	B	.	0.74	B	25:01:47	A	0.26		37:02:09	A	0.47	B
significance	***		***			***		***		NS		**		*	
Rootstocks															
G41	22:06:22		0.40		.	0.96	B	25:31:14	A	0.39	A	38:27:16	A	0.75	A
M9 NIC29	23:44:19		0.46		.	1.18	A	14:12:19	B	0.19	B	25:53:02	B	0.36	B
significance	NS		NS			*		***		***		***		***	
Pruning treatment															
BENDING	21:38:21		0.46		.	1.10		17:19:28	B	0.30		27:58:32	B	0.58	
CLICK	24:26:20		0.41		.	1.07		22:24:06	A	0.28		36:21:47	A	0.53	
significance	NS		NS			NS		***		NS		**		NS	
significance training system* rootstock	NS		NS			NS (0.05)		***		NS		NS		NS	
significance training system* treatment	NS		NS			*		***		NS		NS		NS	
significance rootstock* treatment	NS		NS			NS		NS		NS		NS		NS	
significance training * rootstock* trt	NS		NS			NS		NS		NS		NS		NS	

calculations done referring to 1 person pruning; @ in 2017 no pruning time was recorded in Roza;
p <0.05, *; p<0.01, **; p<0.001, ***; *ns*, not significant for Type III sums of squares model significance
Student-Newman-Keuls *post hoc* test to assign letter groups to arithmetic means where model was significant

difference (data not shown). The "click" pruning technique took significantly more time (from 5 to 8 hours/Acre more) than “bending” during winter for Sunrise orchard only in 2016-2017 (Table 1). In Sunrise, the most time-consuming combination to hand prune in 2017 was V-G41-“click”(as it was in 2016 also in Roza) and the fastest to prune was spindle-NIC29-“bending” (data not shown).

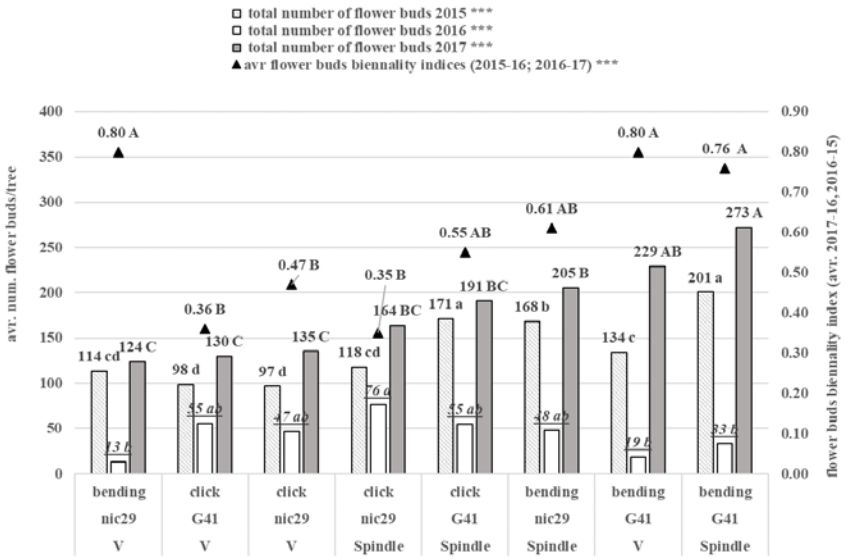


*Figure 2: Total flower buds count (as average per tree) in 2015, 2016 and 2017 by combination. Average of two years of biennality indices are reported. Biennality index based on flower buds is calculated as the absolute value of the difference in number of flower buds in 2 consecutive years divided the sum of the flower buds in those 2 years. Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.*

Flower buds data

The counting of flower buds at the end of March was done for 3 consecutive years on the same selected trees. Spindle reported to produce every year more flower buds than V system, in particular more primary flower buds (brindilla/rami misto tips+spurs) but also secondary flower buds (laterals of brindilla/ramo misto). Between rootstock, G41 presented more total flower bud/tree than Nic29 in 2015 and 2017 (not significant in 2016). Between pruning technique, “bending” showed more flower buds than “click” in 2015 and 2017, while in 2016 (year off), “click” emerged with higher value. A biennality index applied to flower buds counting (instead of yield) reported a significant difference between pruning technique in term of tendency to alternate bearing (Figure

Table 2: ‘WA 38’ yield in both orchards for 2017 and previous year 2015 and 2016 as Mton/Acre.

Training system	Rootstock	Pruning trt	total number fruit/ tree 2017	kg fruit/tree 2017	Average fruit weight (g) 2017	yield Mton/Acre 2015	yield Mton/Acre 2016	yield Mton/Acre 2017	bins#/Acre 2017
ROZA									
Spindle			110	24.69 A	229 A	16.07 B	28.73 B	37.01 B	92.72 B
V			98	19.91 B	206 B	28.43 A	40.87 A	59.68 A	149.52 A
Sign.			NS (5.0%)	***	***	***	***	***	***
	G41		102	21.77	216	22.19	36.09	46.74	117.10
	M9-Nic29		105	22.57	217	22.30	34.18	50.39	126.23
	Sign.		NS	NS	NS	NS	NS	NS	NS
		bending	105	21.87	211 B	23.16	31.64 B	47.19	118.23
		click	102	22.58	223 A	21.34	38.67 A	50.46	126.42
		Sign.	NS	NS	*	NS	**	NS	NS
Training x rootstock			NS	NS	NS	NS	NS	NS	NS
Training x pruning trt			NS	NS	NS	NS	NS	NS	NS
Rootstock x pruning trt			NS	NS	NS	NS	*	NS	NS
Training x rootstock x pruning trt			NS	NS	NS	NS	NS	NS	NS
SUNRISE									
Spindle			87 A	20.20 A	240	14.10	20.65	30.3	75.9
V			46 B	11.20 B	250	15.20	20.33	33.6	84.1
Sign.			***	***	NS	NS	NS	NS	NS
	G41		60 B	15.46	262 A	13.00	19.19	31.6	79.1
	M9-Nic29		73 A	15.94	228 B	16.50	21.78	32.3	80.9
	Sign.		**	NS	***	***	NS	NS	NS
		bending	72 A	16.54	238 B	15.00	14.86 B	33.4	83.6
		click	61 B	14.86	252 A	14.40	26.12 A	30.5	76.4
		Sign.	*	NS	*	NS	***	NS	NS
Training x rootstock			NS	NS	NS	NS	NS	NS	NS
Training x pruning trt			*	NS	NS	NS	NS (5.9%)	NS	NS
Rootstock x pruning trt			NS	NS	NS	NS	NS	NS	NS
Training x rootstock x pruning trt			NS	NS	NS	*	NS	NS	NS

1 bin = 880 lb (by Tom Auvil)

p <0.05, *; p<0.01, **; p<0.001, ***; *ns*, not significant for Type III sums of squares model significance

Student-Newman-Keuls *post hoc* test to assign letter groups to arithmetic means where model was significant

2). “Bending” in fact reported value closer to 1 (0.74) that characterizes extreme biennality tendency, while “click” showed a significantly lower average of this index (0.43, Figure 2).

Productive data

Spindle trees had more fruit per tree and higher yield per tree (like in 2015 and 2016) in both locations but not significant in Roza (Table 2). Yield per acre was higher in V system than spindle in Roza only (60 Mton/A vs 37 Mton/A). There was no statistical difference between rootstock behaviors in Roza in term of productions like in 2015 and 2016, while in Sunrise G41 produced bigger fruit than Nic29 (262 g vs 228 g, respectively). “click” technique induced higher production than “bending” in terms of kg per tree but also Mtons/A in both orchards in 2016 but not significant differences were reported in 2017 (Table 2). “Bending” pruning seems to penalize the average fruit weight in 2017 in both locations. The combinations that resulted in the most production in terms of bins/A in Roza was V-Nic29-“click”and “bending” and V-G41-”bending”, while the least productive were all combinations of spindle. In Sunrise, in 2017 there was no significant difference to discriminate the yield by combination. Highest average fruit weight in 2017 in Sunrise was reported in Spindle-G41-“click”(Figure 3).

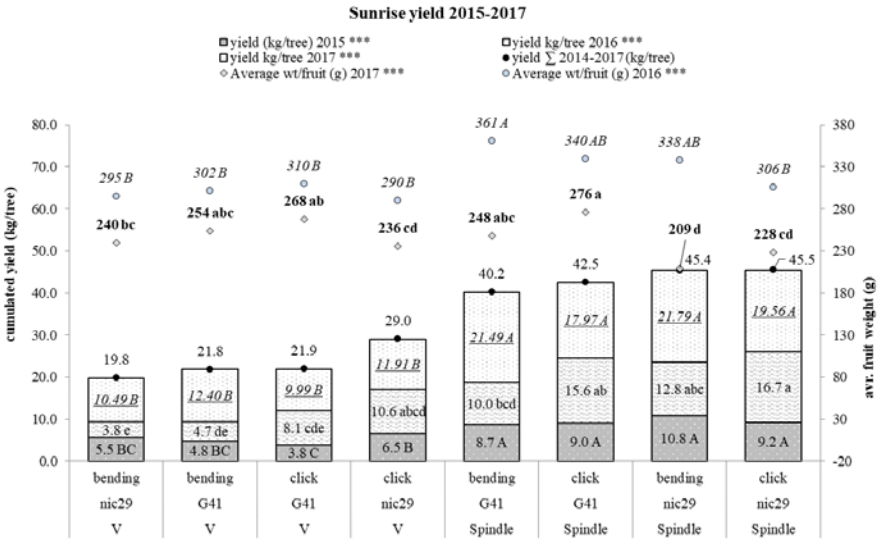


*Figure 3: ‘WA 38’ yield from 2015 to 2017 as kg per tree by combinations and average fruit weight for 2016 (year off) and 2017 (year on). Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.*

Fruit grading 2017

In Roza 5328 fruit from Spindle and V system were sized and graded for defects and judged on the basis of a hypothetical future commercial standard.

From fruit size distribution, Spindle-G41 and Nic29-“click”emerged as the combinations with the highest percentage of fruit in the fruit classes ≥ 85 mm (36% and 28% respectively), while both combinations of V-G41 as the ones with highest percentage of smaller fruit (≤75 mm, 62-64%, data not shown).

In Roza, the green spot incidence was less 13% (19% in 2016), and limb rub/bruises was 30% (31% in 2016), while sunburn was 2% (9% in 2016), similar to Sunrise [2% in 2017 with overcooling (OC) system and 8% in 2016 without OC]. Russet was 8.5% in Roza, while 21% in Sunrise. Insect damage was higher in Roza (11% versus 6% in Sunrise). The average of fruit affected by poor color in Roza is 13% (like in 2016), but V-Nic29-“bending” showed 37% of poor color apples (not shown).

The percentage of cull on all the block was on average 35% (31% in 2016) of all harvested fruit. This distribution was similar to Sunrise where the cull was 36% slightly higher than in Roza.

In 2017 we were able to register all reasons why each apple was culled (Figure 4). In Roza, the percentage of cull for size (≤ 65 mm) was 34% of the total cull while in Sunrise only 17%. Cull for poor color was also higher in Roza with 7% (Figure 4) versus <1% in Sunrise, probably due to the high vigour of the tree and the shading effect of canopy on lower fruit (data not shown). Cull for insect was more severe in Roza (8%) than n Sunrise (3%). In both orchards, V-G41-"bending" was one the combinations that produced the highest percentage of cull fruit in 2017, respectively 40% and 43% in Sunrise and Roza. Whereas, Spindle-Nic29-"click"the one in both locations with a quite low percentage of cull respect to the general average, respectively 28% and 23% in Sunrise and Roza (data not shown). In Roza, among the 8 combinations under comparison, the ones that reported highest percentage of cull were V-G41-"bending" (43%) and V-Nic29-"bending" (43%), while the combinations showing the lowest percentage of cull were Spindle-Nic29-"click"(23%) and V-Nic29-"click"(29%). The worst performing combinations in terms of cull in Roza presented high percentage of cull for size (43% of total cull for V-G41-"bending"), green spot (9% of total cull for V-G41-"bending") and poor color (13% of total cull for V-Nic29-"bending"). On the other hand, Spindle-Nic29-"click"reported higher incidence of mechanical and insect damages causing cull (data not shown).

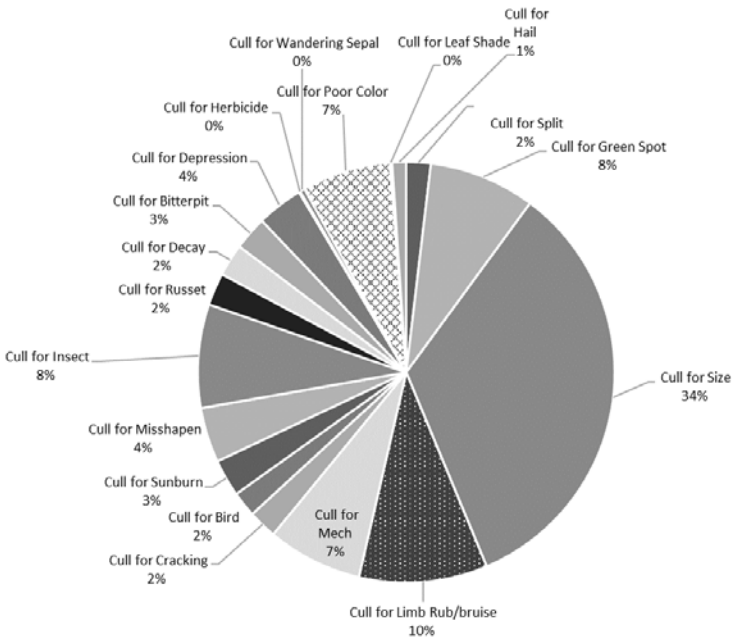


Figure 4: Defects determining cull for 'WA 38' in 2017 in Roza. Pie chart represent the reasons why 1847 out of 5328 apples were culled (35% of the total harvested), each sector represents the percentage of each defect on the total culled apples.

In Sunrise, 4,666 fruit graded and sized for Spindle and V system and the most represented fruit sizes in 2017 were 80 and 85 mm (Figure 5). From fruit size distribution, Spindle-G41-"click"emerged as the combination with the highest percentage of fruit in the fruit classes ≥ 85 mm while Spindle-Nic29-"bending" as the one with highest percentage of smaller fruit (≤75 mm, Figure 5).

The "green spot" disorder had an incidence of 26% (32% in 2016) on the graded fruit at harvest followed by limb rub and bruises (26%), while sunburn hit only 2% of fruit graded. "Leaf shade" breaking the red color coverage was impacting the 26% of apples. Split and insect damage were present respectively on 7 and 6 % of the fruit graded. The percentage of fruit affected by poor color ranged between 0 to 1.3% in 2017 (data not shown). Other defects were not present at noticeably high frequencies.

Of the total fruit graded in Sunrise, 60% were fancy (WFCY) apple, 4% extra fancy (WXF) apples and 36% cull. Fruit with minor and acceptable defects not impacting the flesh were considered fancy while perfect fruit in terms of color (<5% green/yellow background) in addition to acceptable defects were classified as extra-fancy (criteria for extra-fancy were strict). Both combinations of G41 in V reported the lowest percentage of extra-fancy fruit (0-1% approx.), while both combinations of Nic29 in V had 8-10% of extra-fancy fruit.

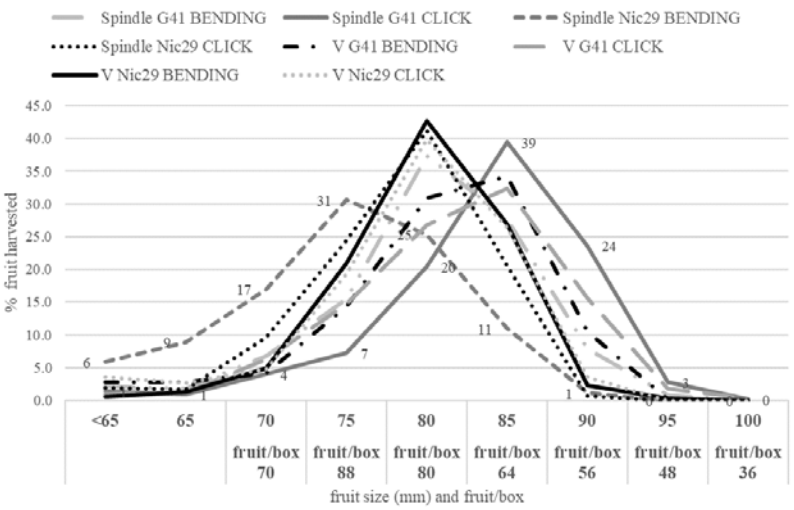


Figure 5: 'WA38' fruit size distribution in Sunrise harvest 2017: comparison between combination of training system-rootstock and pruning technique. In the X axis sizes are reported in mm classes with correspondences with US fruit/box.

Among the 8 combinations under comparison, the ones that reported highest percentage of cull were spindle-G41-"bending" (45%, like in 2016) and V-G41-"click"(44%) while the combinations showing the lowest percentage of cull were V-Nic29-"bending" (23%) and Spindle-Nic29-"click"(28%).

The worst performing combinations in terms of cull in Sunrise presented high percentage of cull for split (25% of total cull for Spindle-G41-"bending"), green spot (22% of total cull for Spindle-G41-"bending") and limb rub (30% of total cull for V-G41-"click"). On the other hand, V-Nic29-"bending" reported higher incidence of mechanical and sunburn damages causing cull (respectively 11% and 8%, data not shown), but also one the highest percentage of Extra-fancy fruit (8%) only lower to the 11% reported for V-Nic29-"click".

Fruit quality harvest 2017 T0 (=1 month after harvest)

Fruit quality sampling at harvest 2017 was done only in Sunrise for Spindle and V system and not in Roza where quality had been assessed for 3 consecutive years (2014-2016). V system fruits in Sunrise experienced more advanced maturity by I_{AD} and lower firmness than spindle (0.48 vs. 0.61 I_{AD}). After 1 month from harvest 2017, there were no significant differences between training systems in terms of color (except higher red Chroma for spindle), TA, ethylene, dry matter (approx. 14%), starch and soluble solids content (SSC; data not shown). The comparison between rootstocks confirmed G41 tends to produce larger 'WA 38' than Nic29 despite the fact that the selection of sampling fruit was standardized in the 80-85 mm range. 'WA 38'/Nic29 apples showed a higher blush overcolor %, higher average and maximum red intensities (respectively 4.00 and 4.74 in a 1 to 5 scale, where 5 is the darkest red for the variety) and lower starch than G41. Whereas, G41 conferred higher firmness, pH and TA than Nic29. SSC, DM%, ethylene did not differ between rootstocks one month after harvest (data not shown).

In 2016, spindle-Nic29-"click"fruits had the highest soluble solids content at T0 over all other combinations and among the highest after 6 months of regular air storage in Sunrise. However, in 2017 the highest red overcolor coverage and maximum red intensity (4.92) was observed for V-Nic29-"click", but at the same time this combination was significantly penalized in terms of DM %

respect to all the others. No significant differences reported between 8 combinations for SSC, traditional DM%, ethylene.

Objective 2: Identify growth and productivity characteristics on bi-axis (Musacchi S., Lewis K.).

In Roza a hail event during spring 2017 occurred followed by a fire blight attach. In Roza, TCSA in 2017 did not differ between hand and mechanical+hand pruning as reported for the two previous years, but annual trunk growth (2016-2017) was higher for the hand treatment as for 2015-2016 (data not shown).

Comparing the 4 combinations in trial there were no differences in the TCSA for the last 3 years. Winter pruning time in 2017 was not recorded but the amount of wood removed did not show any differences between mechanical and hand pruned trees with 0.60 kg/tree of material removes as average per tree (data not shown). Mechanical+hand pruning in biaxis reported for 2017 (no mechanical pruning has been performed in Roza during 2017 due to the fire blight infection during blossom) a higher number of fruit per tree and a significant difference in the production per tree, while the hand pruning showed higher average fruit weight than the mechanical ones, only due to bi-axis-Nic29-hand (ns between treatments in G41).

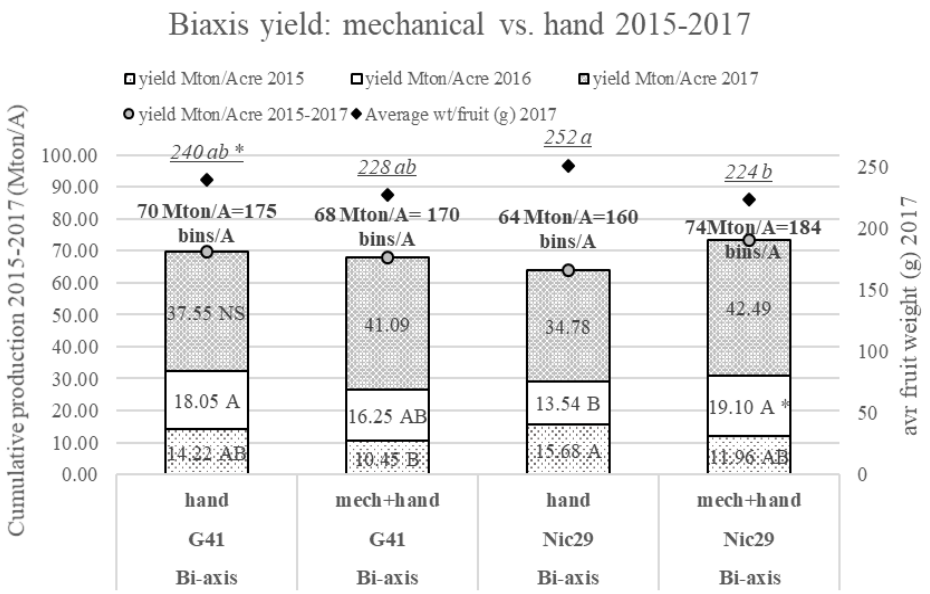


*Figure 6: 'WA 38' biaxis in Roza: comparison between mechanical +hand pruning versus just hand pruning productions from 2015 to 2017 for the rootstocks G41 and Nic29. Yield expressed ad Mton/Acre by year and on the secondary axis the average fruit weight for 2017 fruit by combinations. Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant for Type III sums of squares model significance. Student-Newman-Keuls post hoc test to assign letter groups to arithmetic means where model was significant.*

No significant difference in 2017 for yield in Mton per Acre but biaxis-Nic29-mechanical+hand reported the highest yield efficiency (1.81 kg/cm^2) while biaxis-Nic29-hand the lowest (1.29 kg/cm^2) and respectively the lowest and the highest average fruit weight (Figure 6).
From fruit size distribution, biaxis-Nic29-hand emerged as the combination with the highest percentage of fruit in the fruit classes ≥ 85 mm (37%), while biaxis-Nic29-mechanical+hand as the one with highest percentage of smaller fruit (≤75 mm, 54%, data not shown). The highest percentages of cull in 2017 were reported for both the combinations of bi-axis and hand pruning (opposite of 2016) with up to 45% for G41, while bi-axis-Nic29-Mech+hand reported the lowest cull incidence but also the highest percentage of poor color fruit (19%). Defects tendencies showed a higher incidence of cull for size (15%) and also cull for poor color (14%) in the mechanical+hand respect to hand pruning (6% and 1 % respectively), while 22% of culled hand pruning fruit were due to limb rub damage and 21% for green spot (both disorders were lower in mechanical +hand, data not shown).

Objective 3: Conduct an economic analysis of WA 38 production (Gallardo K.).

We followed two approaches. First, we calculated the mean pruning time across three treatments: training system (spindle and angled=V), rootstock (M9-Nic29 and G41), and pruning technique ("bending" and "click"). During Years 2 and 3 of establishment, pruning was done during the winter and summer, and during Years 4 and 5 of establishment, pruning was done during the winter only. Pruning is expressed as hour per tree and labor cost was estimated considering a wage of $12.00 per hour. Note that pruning data were collected in Sunrise through Year 5 but only up to Year 4 in Roza. Second, we compared costs and returns for the 'WA 38' experimental trial with commercially produced 'Fuji' apples. Based on experts' opinion, the horticultural management for 'WA 38' is quite comparable to 'Fuji'. This enables an approximation of the likely 'WA 38' production costs at a commercial scale. In this report, we present results for the second through the fifth year of establishment for both 'Fuji' and 'WA 38'. The returns for 'WA 38' were estimated using a 3-year average price of 'Honeycrisp' for different fruit sizes, estimated from price data for 2014-15, 2015-16 and 2016-17 marketing seasons. To enable comparisons, the pruning costs per acre for each 'WA 38' treatment were estimated given the density of trees in commercial 'Fuji' orchards — 1,089 trees per acre under a spindle system, and 1,452 trees per acre under an angled system. In addition, we included a royalty fee per box for 'WA 38'. Since prices considered were higher than $50 per 40-lb box, the royalty fee was at $3 per 40-lb box. Results from the field trial were compared to pruning time and costs for commercial 'Fuji'. In both Roza and Sunrise, majority of the treatments exhibited more pruning time per tree, thus higher cost, in the third year compared to second year, then a decreasing pruning time and cost in the fourth year (and fifth year in Sunrise); compared to 'Fuji' where pruning costs increase every year (data not shown). Also, pruning costs appear to be higher with 'WA 38' treatments compared to 'Fuji' between Year 2 and Year 4, and the costs are drastically lower in Year 5 relative to that of Fuji. Considering Year 2 up to Year 4 of establishment in Roza, and up to Year 5 in Sunrise, the aggregate pruning cost was lowest with angled, G41 and "bending" in Roza; and spindle, M9-Nic29 and "bending" in Sunrise. The total pruning cost was highest with spindle, G41 and "click" in both Roza and Sunrise.

The estimated variable costs and total revenues of the different 'WA 38' treatments are presented in Figure 7 (Roza) and Figure 8 (Sunrise). The different prices depending on the fruit sizes of 'WA 38', and yields and pack-outs recorded during the study period were considered in estimating the revenues for each treatment. More detailed information about yield and revenues by treatment are provided in appendix tables 3-6. The costs of harvest activities, packing charges and royalty are tied with the gross and net yields of WA 38 given a particular treatment. The greater the yields, the greater are these costs.

In Roza and Sunrise, the total variable costs of most 'WA 38' treatments were higher than those of 'Fuji', but the total revenues of all 'WA 38' treatments were much higher during the crop-producing years (i.e., starting Year 3). For 'Fuji', the estimated total costs of production are not offset by revenues during Year 3 and Year 4 of establishment in a spindle system and during Year 3 of establishment in an angled system. Net returns for 'Fuji' become positive in Year 5 in a spindle system, and in Years 4-5 in an angled system (see figure 8). For 'WA 38' on the other hand, results across all treatments imply that despite the higher variable costs, and even with the inclusion of the fixed costs, the estimated revenues were more than enough to cover the production costs of 'WA 38'. When comparing individual 'WA 38' treatments in each of the two sites, net returns are highest under the spindle system, M9-Nic29 and "click" in Roza during the fourth year of establishment, as well as in Sunrise during the fifth year of establishment.

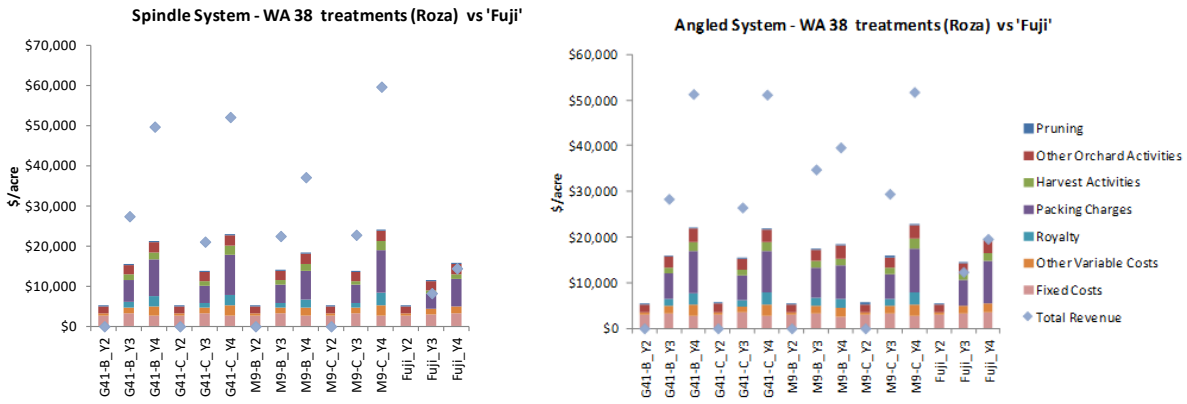


Figure 7. Estimated annual revenues and variable costs ($/acre) by treatment in Roza compared to 'Fuji', Year 2 to Year 4 of establishment. Notes: B – "bending"; C-"click"; Other orchard activities - training, thinning, irrigation, etc.; Other variable costs – R&M, fuel and lube, crop insurance, interest, overhead.

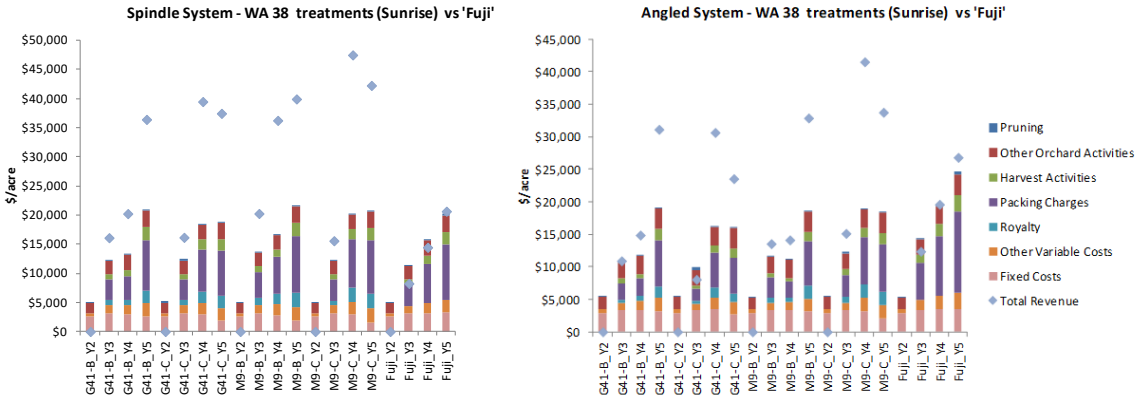


Figure 8: Estimated annual revenues and variable costs ($/acre) by treatment in Sunrise compared to 'Fuji', Year 2 to Year 5 of establishment.

EXECUTIVE SUMMARY

The 'WA 38' tree has been characterized by an upright and spreading growth habit with medium vigor (like Granny Smith is a Type IV). It is a tip bearing cultivar, with a natural tendency to produce on one-year old fruit. To enhance spurs development on two-year-old wood and to minimize blind wood near the trunk a "click" pruning is recommended. The fruit is crisp, firm and juicy with non-browning flesh and consistently showing excellent long-term storability. This project has been carried out in two different sites: WSU Wenatchee Sunrise Orchard and WSU Prosser Roza Orchard. The two-site trial was planted in 2013 to assess training system (spindle and V-system with densities of 1,499 trees/acre and 2,997 trees/acre, respectively), pruning style ("click" and "bending") and rootstocks (M9-Nic29 and Geneva 41). In terms of bins/acre in 2017 V-system result more productive than spindle in both locations. No difference in pruning has been noticed in 2017 while in 2016 click pruned trees showed higher level of yield.

Bi-axis tree can be considered one-year younger compared to the spindle and v-system due to the head back cut done in 2013. In Roza, all the biaxis trees were trained with the goal to obtaining a 'Fruit Wall'. Half of the tree were mechanically pruned in 2015 and 2016 in comparison with traditional hand pruning. The mechanical winter pruning of bi-axis in Roza showed a 93% reduction in time of pruning with 1h:26 min/A versus 21h:47 min/A for the hand pruned in 2016.

Economic analysis revealed that 'WA 38' variable costs are higher than those of 'Fuji' but the estimated revenues of 'WA 38' across all treatments are much higher. Even with the inclusion of fixed costs, estimated revenues were more than enough to offset the increased production costs of 'WA 38' across all treatments. In both Roza and Sunrise, the highest net return is for 'WA 38' spindle, M9-Nic29 and click when the different prices of different fruit sizes are taken into account.

Projects leveraging funds obtained from this grant:

USDA (United State Department of Agriculture) - SCRI (Specialty Crop Research Initiative) - Accelerating the Development, Evaluation and Adoption of New Apple Rootstock Tech to Improve Apple Grower Profit and Sustainability. PI Lailiang Cheng Cornell University, Co-PI Stefano Musacchi. Total budget: $412,061.

WSDA (Washington State Department of Agriculture) -Specialty Crop Block Grant Program. Cosmic Crisp: Training system and orchard management to optimize vigor control and quality. PI Stefano Musacchi, Co-PIs Lee Kalcsits, Desmond Layne, Sara Serra and Karina Gallardo. Total budget: $249,191.

Project outcomes:

Field days

'WA 38' Fall Field Visit –Rock Island - September 27, 2017 (Stefano Musacchi, Ines Hanrahan, Kate Evans, Sara Serra, Karen Lewis and Tianna Dupont). 102 people attended the field day.

'WA 38' pruning days – December 13 and 14, 2017 (Musacchi S.)

Web articles

Living online documents about how to grow Cosmic Crisp; http://treefruit.wsu.edu/wa-38-characteristics-and-horticulture/; http://treefruit.wsu.edu/wa38-faqs/

Professional presentations/conferences

Washington State Tree Fruit Association 112th annual meeting – Wenatchee, WA- December 5 to December 7, 2016. Musacchi S. presented "WA 38 Horticulture: Planting, Growing, Harvest and Storage."

American Society for Horticultural Science annual meeting – Hawaii – September 18 to September 22, 2017. Musacchi S. presented "Cosmic Crisp® optimization of rootstock, training system and pruning technique."

American Society for Horticultural Science annual meeting – Hawaii – September 18 to September 22, 2017. Anthony B. (graduate student) presented the poster "Crop Load Effect on Fruit Quality in 'Cosmic Crisp'".

Future direction:

- Optimization of crop load management for 'WA 38'
- Consumer preference regarding 'WA 38' of different size and predicted dry matter content.
- Comparison of productive performances of 'WA38' trained at 1, 2 o 3 axis system after top graft of an old Granny Smith orchard.
- Optimization of pollination for 'WA 38' with new source of compatible pollen.

FINAL PROJECT REPORT

WTFRC Project Number: AP-16-108A

Project Title: Validation of the Red Delicious pollen tube growth model

PI:	Keith Yoder	**Co-PI :**	Sherif Sherif
Organization:	Virginia Tech	**Organization:**	Virginia Tech
Telephone:	(540)-869-2560 X21	**Telephone:**	(540)-869-2560 X19
Email:	ksyoder@vt.edu	**Email:**	ssherif@vt.edu
Address:	595 Laurel Grove Rd.	**Address**:	595 Laurel Grove Rd.
Address 2:	Va. Tech AHS-AREC	**Address 2:**	Va. Tech AHS-AREC
City:	Winchester	**City:**	Winchester
State/Zip:	VA 22602	**State/Zip:**	VA 22602

Cooperators: Leon Combs, Research Specialist, Virginia Tech AHS-AREC; Winchester, VA
E-mail: lecombs@vt.edu
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Other funding sources: None

Total Project Funding: $33,280

Budget History:

WTFRC Collaborative expenses:

Item	2016	2017
Salaries	2000	2000
Benefits	600	600
Wages	1000	1000
Benefits	250	250
Shipping	50	50
Supplies	50	50
Travel	250	250
Total	**$4,200**	**$4,200**

Budget:
Organization Name: Virginia Polytechnic Institute and State University (Virginia Tech)
Contract Administrator: Eric James Dinwiddie, Pre-Award Administrator
Telephone: 540-231-9368 Email address: EricJD@VT.edu

Item	2016	2017
Salaries*	8000	8000
Benefits	4080	4080
Supplies	360	360
Total	**$12,440**	**$12,440**

***Note: Salary for Research Specialist Leon Combs.**

OBJECTIVES

1. **Validation Testing of Red Delicious Pollen Tube Growth Model in Washington Orchards. (Virginia Tech & WTFRC)**
   Pollen tube growth model validation includes criteria from three tests in 2016 and 2017:

   Test 1: Commercial use of the pollen tube growth models. In this test, grower-participants use the models made available to them through the AgWeatherNet website. These growers (beta-testers) trained in the use of the models then monitor the blocks start times and bloom thinning application timings. At the end of harvest, the beta-test participants rate their actual crop relative to their ideal expected yield. Comparing the desired yield with the actual harvested yield would demonstrate whether the beta-test participants understand the principles of the model and if it is working to their satisfaction. This harvest data will be cross-referenced with application timings as done with other models in previous years.

   Test 2: Validation test 2 includes flower samples collected in Washington orchards after thinning chemicals were applied, by comparing model-predicted pollen tube growth versus actual growth in flowers. Flower samples from beta-test blocks will be evaluated microscopically to determine if fertilization occurred on the segment of the flower population that was intended to be the harvested crop. Bloom thinning applications can then be re-applied to reduce unwanted additional cropping.

   Test 3: Harvest data from selected Washington orchard blocks that will be bloom thinned using the pollen tube growth model in the 2016-17 growing seasons for validating the Red Delicious model, will come from selected contributing beta-testers who had access to the Red Delicious beta test model for the 2016-17 growing season.

Objective 2:

WTFRC:

WTFRC staff will work with commercial growers to select several orchard blocks for these tests. In each block, they will randomly flag four trees (border and unhealthy trees will be avoided). On the flagged trees, they will tag or flag six flower clusters (with the king bloom open) that represent part of target crop load. In other words, these are typical of the flowers that should become fertilized before the first chemical thinning application. Forty-eight hours after first bloom thinning spray, whole flagged clusters will be removed from the tree. Petals will then be removed, and the king bloom marked with a permanent marker to distinguish it from the lateral blooms. The whole cluster will then be placed into a plastic bottle containing a 5% sodium sulfite (5 g/100 ml distilled water) solution. After all samples are collected, the samples will be shipped to Virginia Tech AHS-AREC for histological evaluation.

Virginia Tech

Upon receipt at the Virginia Tech facility, samples will be refrigerated at 38°F until processing. The flowers will be prepared and examined as described for Objective 1. Collected data will be the same as described for Objective 1.

SIGNIFICANT FINDINGS

- More than 100 Washington State Red Delicious beta-test blocks were bloom-thinned using the pollen tube growth model as a tool in 2016-17. From about 30 of those sites we received spray timing, yield data, and/or evaluated flower samples for evidence of fertilization.

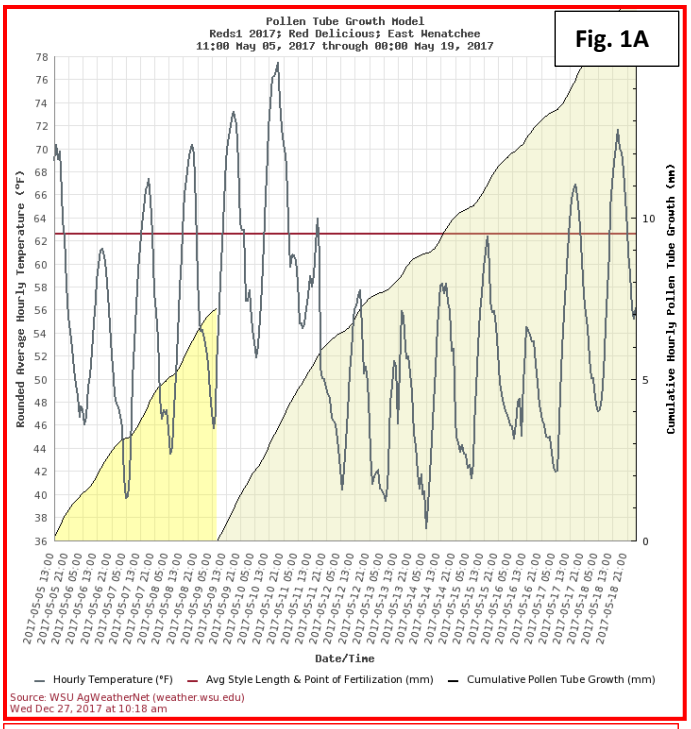
- Microscopic evaluation of sampled flowers in the laboratory to determine the percentage of flowers that had been fertilized showed predictive effectiveness of Red Delicious model in the field.
- Reported harvest data showed that the pollen tube growth model is helping growers to achieve their targeted crop loads.
- Style length data acquired from 2016-17 tests showed the importance of properly measured field style length when compared with style measurements in laboratory.
- There was some unexplained discrepancy in the style-length measurements taken in some test blocks compared to those measured in the samples submitted to the laboratory. Flowers with shorter styles would have been fertilized more quickly, resulting in higher than desired yield.
- Data showing pollen tube growth in styles confirms that proper timing of second and sometimes third follow-up applications is critical in reducing crop load.
- Of any cultivar we have tested, pollen tube growth is the slowest in Red Delicious styles, but with some appropriate cautions, this model will be released to the industry for 2018.

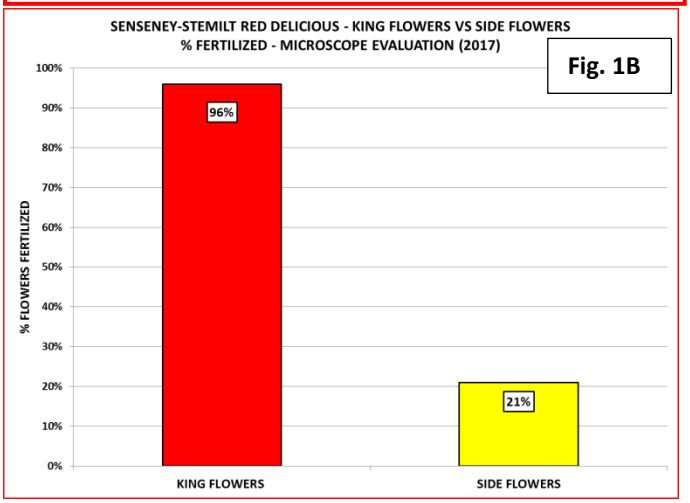
RESULTS & DISCUSSION

As stated in previous reports on models presently being used by growers (Table 1), tracking actual bloom thinning application timing and harvest totals versus desired cropping is needed to verify the models' effectiveness. These growers (beta-testers) are trained in the use of the models and monitor the blocks start times and bloom thinning application timings (Fig. 1A, 2A, 3A) as predicted by the AgWeatherNet website from inputs by model users. As in 2016, evaluation of the model in 2017 included sampling flowers from the field (Figs. 1B, 2B, 3B) to determine the percentage of flowers that have been fertilized, which further validates model predictions. Comparing desired crop load with actual harvest data (Fig. 1C, 2C, 3C) demonstrates either understanding of beta-testers in model implementation or the need for further training in initiation of the modeling program at the proper time. Results from field evaluations of desired bins/acre vs actual bins/acre harvested shows if the model helps beta-testers/growers achieve their targeted crop. Comparing average style length determined in the field and in the laboratory (Fig. 1D, 2D, 3D) is an integral part of evaluating and refining the model to actual field conditions as well.

Table 1. Chronology of beta-testing and release of the pollen tube growth models.

Pollen Model	Began field beta-testing using Excel spreadsheet models (Year)	Began field beta-testing using AgWeatherNet website models (Year)	Released for public use (Year)
Gala	2007	2012	2014
Golden Delicious	2007	2012	2014
Fuji	2009	2012	2014
Pink Lady	2011	2012	2014
Honeycrisp	2013	2013	2017
Granny Smith	2014	2014	2017
Red Delicious	**2014**	**2015**	**2018**





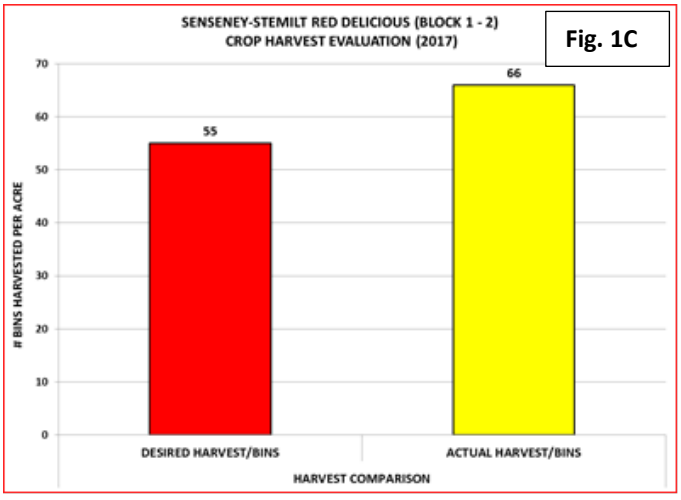


Figure 1. Beta-testing at Senseney-Stemilt Red Delicious (Block 1), East Wenatchee, WA.

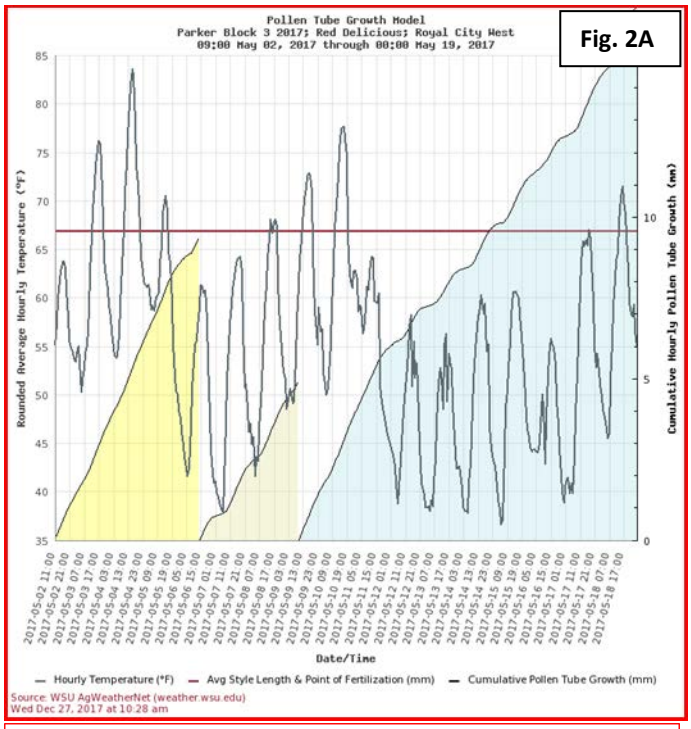
1-A: An image of the pollen tube growth model from WSU AgWeatherNet, showing progress of the pollen tube growth in this block, as related to temperatures and measured Red Delicious style length, and timing of a single bloom-thinning application.

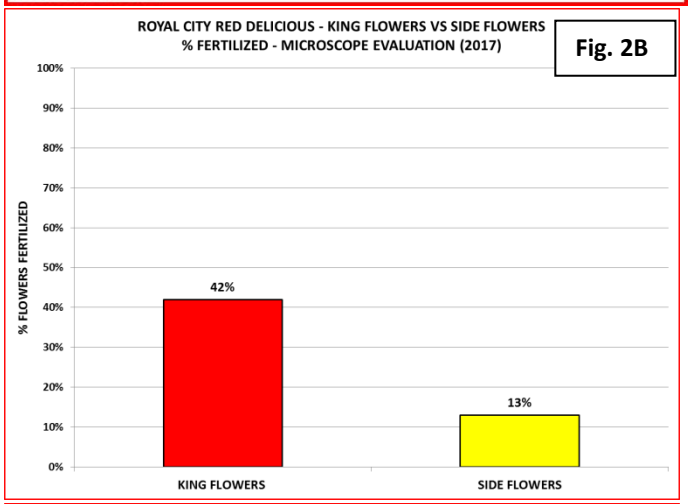
In this block, the first application was made early to compensate for a delay in starting the model until 75% king bloom open instead of the suggested 10-25% king bloom. The early application resulted in a high percent of king bloom fertilized and a relatively low percent of side bloom fertilized; however, the delay probably allowed too much set of king bloom, resulting in more bins per acre than was targeted. Also, not having a second application allowed all later bloom to set, further increasing the number of bins per block.

1-B: Laboratory assessment of percent fertilization of king bloom vs. side bloom of sampled flower clusters.

1-C: Comparison of targeted crop to actual harvested crop.

Ideally, comparison of targeted crop to actual harvested crop would demonstrate the model effectiveness in predicting thinning applications and the understanding of model concepts by end users.





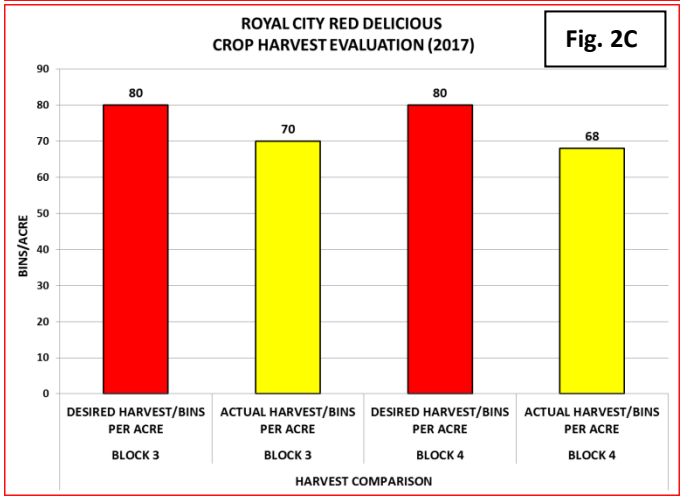


Figure 2. Beta-testing at Royal City West Red Delicious (Parker Blocks 3 & 4), Royal City, WA.

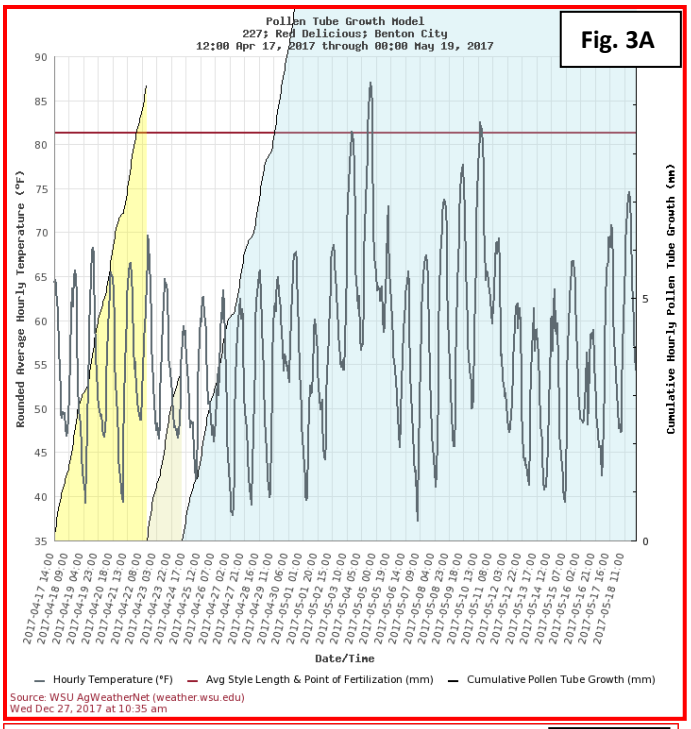
2-A: An image of the pollen tube growth model from WSU AgWeatherNet, showing progress of the pollen tube growth, as related to temperatures and measured Red Delicious style length, and timing of two bloom-thinning applications.

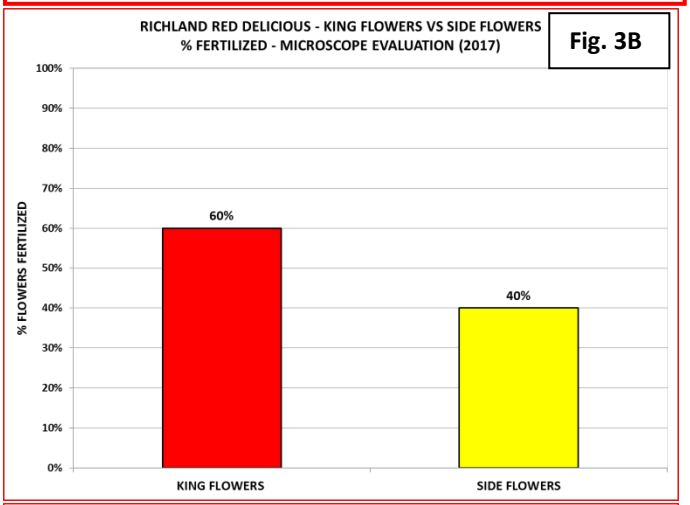
In these blocks, the first application was closely timed by the model, resulting in a good comparison of king bloom vs. side bloom fertilized. The well-timed follow-up application reduced further fruit set, resulting in fewer bins than the targeted yield in both blocks

2-B: Laboratory assessment of percent fertilization of king bloom vs. side bloom of sampled flower clusters.

2-C: Comparison of targeted crop to actual harvested crop.

Here, comparison of targeted crop to actual harvested crop suggests that there may have been more thinning than desired. Whether this is true, and its economic significance would need to be confirmed by looking at fruit sizes in the packout and checking return bloom in 2018.





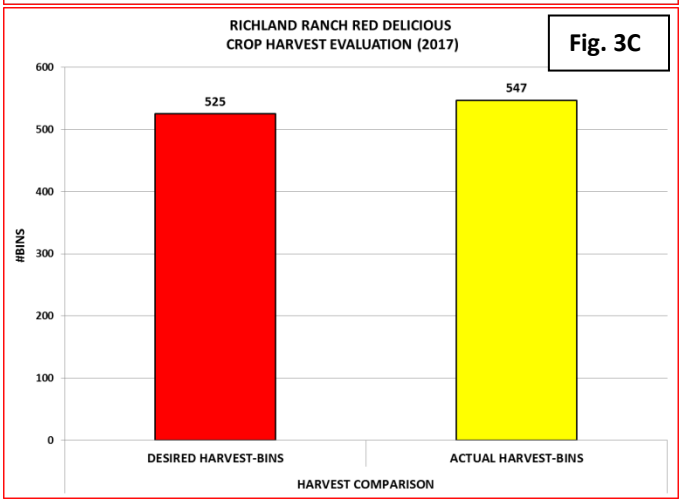


Figure 3. Beta-testing at 227, Red Delicious, Benton City, WA.

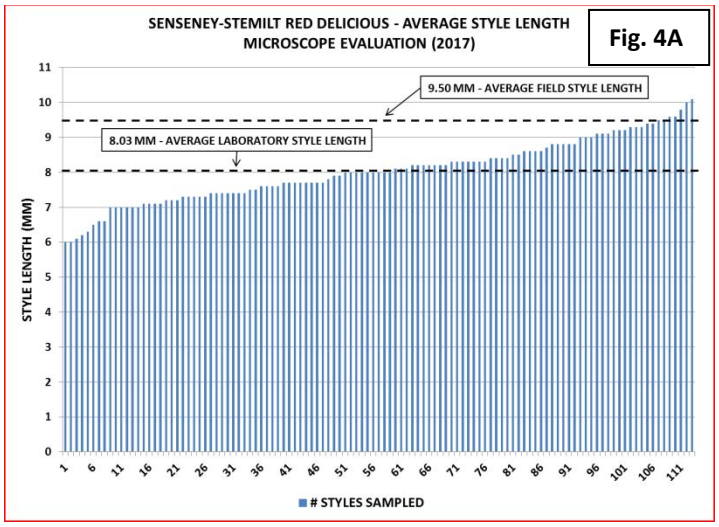
3-A: An image of the pollen tube growth model from WSU AgWeatherNet, showing progress of the pollen tube growth, as related to temperatures and measured Red Delicious style length, and timing of two bloom-thinning applications.

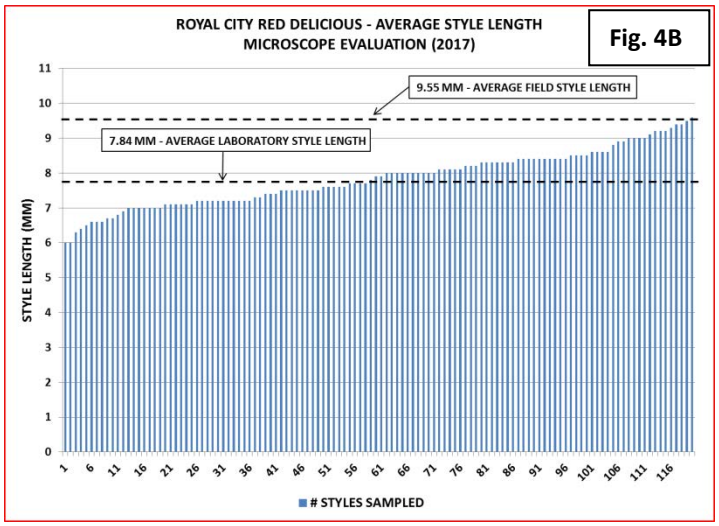
In this block, the first application was well-timed to allow adequate fruit set, the follow-up application two days later resulted in a reduced percentage of side bloom setting fruit, and yield was relatively close to the targeted amount.

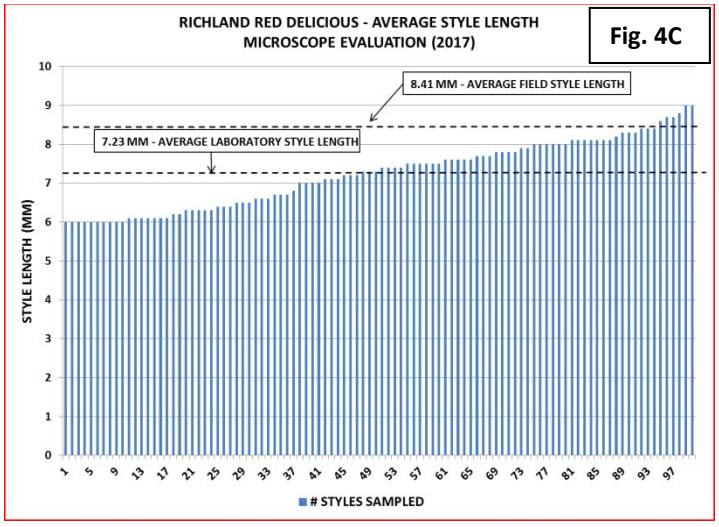
3-B: Laboratory assessment of percent fertilization of king bloom vs. side bloom of sampled flower clusters.

3-C: Comparison of targeted crop to actual harvested crop.

Again, comparison of targeted crop to actual harvested crop offers a good assessment of the effectiveness of the model in predicting thinning applications and understanding of model concept by users.







Figures 4A-C show the lengths of styles measured in sampled in the beta-test blocks. The average field style length lines are of the measurements that were recorded on site; the average laboratory style length lines were recorded from 100 or more styles on sampled flowers that were sent to our laboratory from the test sites. It is surprising that the measurements taken in the orchard average consistently longer than those in the lab, by 1.2-1.7 mm. We do not have an explanation for this, but it could have made a significant difference in the amount of thinning. Thinning applications were applied at each location based on measurements in the orchard. But, if the styles were indeed shorter than those indicated in Figures 1A, 2A and 3A, more thinning would have resulted because fewer flowers would have been fertilized by the time of the first application, and this would reduce the number of bins per block.

As was stated in our proposal, an in-orchard study at Winchester, VA in 2007 showed that there might be as much as a three-fold difference in Snowdrift pollen tube growth rates among cultivars, with 'Red Delicious' standing out as having the slowest pollen tube growth of seven cultivar models available to the public for use as a bloom thinning tool. This delayed pollen tube growth in Red Delicious could lead to serious over-thinning if one ignored this difference and based the timing of Red Delicious bloom-thinning applications on the models for other cultivars available through AgWeatherNet rather than on a fully validated Red Delicious model. In all models, the user will need to implement the models according to thinning factors, whether thinning for conventional crops or organic production. The options for thinning are more restricted in organic crops so those growers need to test the Red Delicious model rigorously in their own orchard conditions. As shown below in Figure 5, crop load results for 2016 at beta-test sites in Washington showed that the model helped in crop load management in all but one test site.

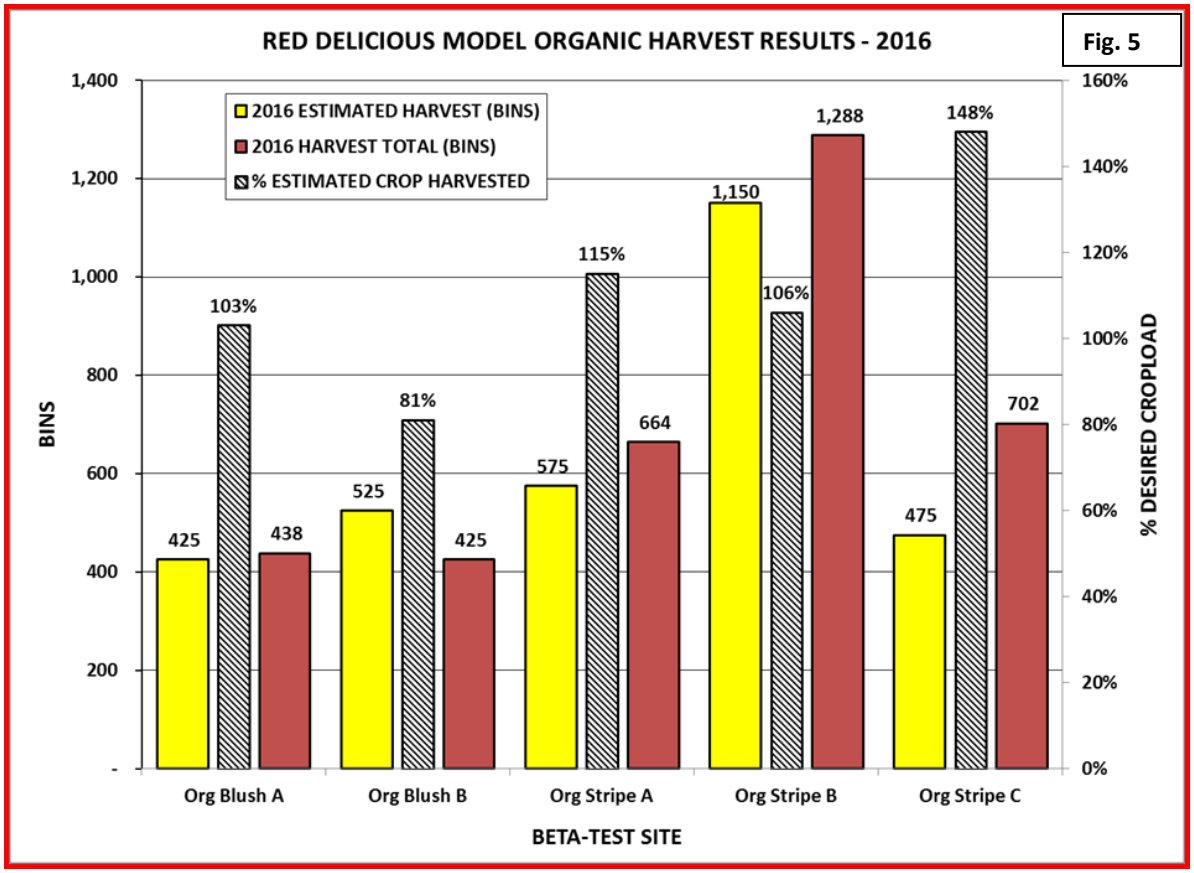


Figure 5: Targeted yields versus actual yields in five organic Red Delicious orchard blocks, 2016.

Our final report: We see this as more than just the final report for the current Red Delicious project. Release of the Red Delicious model completes our assigned series of related projects with WTFRC. The Red Delicious model will join Gala, Golden Delicious, Fuji, Cripps Pink, Granny Smith and Honeycrisp for public use on AgWeatherNet. In apple production, properly timed bloom-thinning gives the grower the optimum advantage for producing the best quality fruit. Understanding the progression of pollen tube growth after pollination is critical in applying bloom thinners at the proper time. In addition to optimal sizing benefits, crop loads not sufficiently thinned could result in trees being thrown into biennial bearing with little or no crop in the 'off' year. Previously, the application timing for this spray was often subjective, usually based upon the percent of full bloom open (e.g., applications at 20 and 80% full bloom). While this approach became a standard practice in

some growing regions, more precise application timing can be achieved through modeling the fertilization of the desired percent of king bloom needed to achieve a full crop at the desired fruit size.

From the earliest efforts in these projects, we had pursued this as a work in progress, but our main goal was to provide a better basis of fine-tuning the timing of bloom thinning applications, from the old “20 and 80% bloom” timing to a more refined method that would pinpoint narrow timing windows. Also, we recognized that, ideally, the method could and should be predictive, based on the local weather forecast, so that growers could set priorities and optimize timing for multiple blocks. The early, surprising, result with Red Delicious in 2007 forewarned us that each model must be cultivar-specific, not “one size fits all”.

Looking back, and forward: Over several project cycles since 2004 we have addressed the challenges of technical aspects of how to most efficiently conduct this research. With feedback from Commission members and beta-testers, we have continually considered priorities among cultivars and temperature ranges to be tested. We settled on Snowdrift as the model pollinizer, although we recognize that there are differences in growth rates due to different pollen sources. There have been several spin-offs technically and academically, with kindled interest in development of different products and methods for bloom thinning, and at least three graduate student projects have expanded our understanding of bloom-thinning related issues. We know that some of the techniques developed in this research are also applicable to related problems with cherries, pears and peaches.

There remains work to be done in the area of crop load management at bloom: There will always be new cultivars with different thinning needs. We know that specific pollinizers can affect the speed and rate of fertilization, apart from the common compatibility aspects. We know that style length is an important factor that must be recognized in rate of fertilization, and we also know that “average” style length can vary considerably in a cultivar, from block to block, and from year to year, but we really don’t know what factors cause this variation. Finally, there is more to be known about how different thinning materials act, and what can be done to maximize their thinning effectiveness while minimizing their injury to foliage and fruit. There are also benefits to be gained, especially in organic production, by better integrating the disease management capabilities of thinning products.

On a more personal note in this final report, we must recognize the diligent effort that Leon Combs, Research Specialist, Virginia Tech, has provided to all aspects these projects since 2004. Much of the success of these projects in the development and implementation of pollen tube growth models for crop load management in apples is due to his innovative and diligent research and development efforts. Leon has announced his retirement as of January 1, 2018.

Above, Leon Combs (standing) and Tory Schmidt (left) conduct one of the in-depth training sessions for beta-testers using the first AgWeatherNet website models in 2014.

ACKNOWLEDGEMENTS:
We thank the Washington Tree Fruit Research Commission for their ongoing support throughout the development of these models. We would particularly like to thank Tory Schmidt and support staff, whose help on the project has been essential to the project's success. We thank Sean Hill (WSU-AgWeatherNet) for rapidly providing and adjusting the AgWeatherNet interface for the models. We would like to thank the many beta-testers, growers, and others who have provided valuable feedback and guidance on many aspects of the pollen tube growth models over the years since 2004:
Jim McFerson and Tory Schmidt and support staff, Washington Tree Fruit Research Commission; Tom Butler, Dan Plath and Adam Zediker (Washington Fruit & Produce Co); Harold Ostenson (Stemilt Growers); Kevin Larson (Roche Fruit); Darin Case (Dovex Fruit Company); Harold Schell (Chelan Fruit Company); Mike Robinson (Double Diamond Fruit); Dan Flick (Wilbur-Ellis); Gary Snyder (C & O Nursery); Dena Ybarra (Columbia Basin Nursery). Sean Hill, (WSU-AgWeatherNet); Firman Pollen Co., AgriMACS; We also recognize the input of the CO-PIs in these projects, the late Dr. Rongcai Yuan (Virginia Tech), Dr. Greg Peck (Virginia Tech, now with Cornell University), and Drs. Gerrit Hoogenboom and Melba Salazar (WSU-AgWeatherNet), and Dr. Vince Jones (WSU-TFREC).

EXECUTIVE SUMMARY

The overall goal of this project was validation of the Red Delicious pollen tube growth model in Washington orchards. This involved two years of commercial use of the model by grower-participants (beta-testers) trained in the use of the model made available to them through the AgWeatherNet website. These beta-test participants monitored the blocks start times and bloom thinning application timings and at harvest they compared their actual crop relative to their ideal expected yield to demonstrate whether the beta-test participants understood the principles of the model and if it was working to their satisfaction.

To check predicted pollen tube growth versus actual growth in flowers, flower samples were collected by WTFRC staff in Washington orchards after thinning chemicals were applied, and these samples from beta-test blocks were evaluated microscopically in the laboratory at Virginia Tech to determine if fertilization had occurred on the segment of the flower population that was intended to be the harvested crop. The sampled flowers were to come from clusters, with the king bloom open, that represented part of target crop load. In other words, these were typical of the flowers that should have been fertilized before the first chemical thinning application.

Under this limited program, more than 100 Washington State Red Delicious beta-test blocks were bloom-thinned using the pollen tube growth model as a tool in 2016-17. From about 30 of those sites we received spray timing, yield data, and/or evaluated flower samples for evidence of fertilization. Microscopic evaluation of sampled flowers in the laboratory to determine the percentage of flowers that had been fertilized showed predictive effectiveness of Red Delicious model in the field. Reported harvest data showed that the pollen tube growth model is helping growers to achieve their targeted crop loads.

Style length data acquired from 2016-17 tests showed the importance of properly measured field style length when compared with style measurements in laboratory. There was some unexplained discrepancy in the style-length measurements taken in some test blocks compared to those measured in the samples submitted to the laboratory. Flowers with shorter styles would have been fertilized more quickly, resulting in higher than desired yield. Data showing pollen tube growth in styles confirms that proper timing of second and sometimes third follow-up applications is critical in reducing crop load.

Of all the cultivars we have tested, pollen tube growth remains the slowest in Red Delicious styles. Because of this unusual characteristic, we urge considerable early caution in grower use of the Red Delicious model, but with some appropriate cautions, this model will be released to the industry for use in 2018.

Release of the Red Delicious model completes our assigned series of related projects with WTFRC. The Red Delicious model will join Gala, Golden Delicious, Fuji, Cripps Pink, Granny Smith and Honeycrisp for public use on AgWeatherNet. In apple production, properly timed bloom-thinning gives the grower the optimum advantage for producing the best quality fruit. Understanding the progression of pollen tube growth after pollination is critical in applying bloom thinners at the proper time. In addition to optimal sizing benefits, crop loads not sufficiently thinned could result in trees being thrown into biennial bearing with little or no crop in the ‘off’ year. Previously, the application timing for this spray was often subjective, usually based upon the percent of full bloom open (e.g., applications at 20 and 80% full bloom). While this approach became a standard practice in some growing regions, more precise application timing can be achieved through modeling the fertilization of the desired percent of king bloom needed to achieve a full crop at the desired fruit size.

We thank the Washington Tree Fruit Research Commission for their ongoing support in the development of these models. We would particularly like to thank Tory Schmidt, whose help on the project has been essential to the project’s success. Lastly, we would like to thank the beta-testers, growers, and others who have provided critical feedback on all of the pollen tube growth models.

FINAL REPORT
WTFRC Job Number: 1087 (internal account, general food safety)

Project Title: WTFRC internal program – food safety efforts

PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509 669 0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S.18th St., Suite 100
City/State/Zip: Union Gap, WA, 98903

Cooperators: Jacqui Gordon (WSTFA), Laura Grunenfelder (formerly NHC), Kate Woods (NHC), Manoella Mendoza and Mackenzie Perrault (WTFRC), Rob Atwill, Missy Partyka, and Ronny Bond (UC Davis), Bonnie Fernandez-Fenaroli (CPS)

Acknowledgement: WTFRC seasonal crew efforts are acknowledged and appreciated.

Other funding sources

Agency Name: WA SCBGP
Amt. requested/awarded: $ 216,682 Title: Enhanced food safety education and training for tree fruit producers (awarded to WSTFA with Jacqui Gordon as PI)
Notes: In 2017 a total of four workshops (two topic areas) were organized for tree fruit producers, with WTFRC participation, two videos were produced, and one video has been started

Agency Name: FDA
Amt. requested/awarded: $243,651 for FY17 awarded to WCFSS (Atwill et al.) Title: Facilitating implementation of FSMA regulations for agricultural water quality
Notes: This budget covers sampling in both California and Washington and includes staff salaries. The budget for Washington alone is estimated at ~$140K. WTFRC participated in site selection, experimental design, planning and execution for 2017.

Agency Name: CPS
Amt. requested/awarded: $290,000 to Zhu and Suslow; Title: Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention
Notes: WTFRC supplied and delivered fruit, project logistics, and arranged industry collaborators

WTFRC internal program expenses:

Item	2016	2017 projected	2017 actual[3]
Salaries	27,146	27,689	4,975
Benefits	5,322	5,428	1,642
Wages	2,257	2,584	11,644
Benefits	855	979	3,843
RCA Room Rental	---	---	
Shipping	---	---	
Supplies[1]	177	200	1,505
Travel[2]	1,622	5,000	
Plot Fees			
Miscellaneous			
Total	**37,379**	**41,880**	**23,609**

Footnotes:

[1]Supplies include three posters (2 for IAFP, 1 for ASHS)

[2]Travel includes: CPS in Seattle, University of BC in Vancouver, trips to WSU in Pullman, in state day travel to attend trainings, IAFP in St. Louis, Annual NW Food Safety and Sanitation Conference in Portland, PSA Train the Trainer in Aurora, PCFSA training in Pullman

[3]Wages and salaries have been calculated as follows: fiscal year (July 1-June 30, 2017), costs for remainder of 2017 are not included; salaries = 9% of Mendoza, not included in salaries: 31% of Hanrahan time (from July 1-December 31, 2017 Hanrahan portion of time spent has been 8%)

NOTE: This is a final report. All internal program research projects will require new proposals to the WTFRC board in March 2018

OBJECTIVES

1. Enhance collaboration in all areas of food safety (research, policy, FSMA implementation)
    a. Participate in development of training for industry
    b. Develop effective food safety outreach program

SIGNIFICANT ACCOMPLISHMENTS IN 2017

Research:

We participated in a number of on-going collaborative projects, funded by WTFRC, CPS, and FDA (see Table 1).

The WTFRC, under leadership of Ines Hanrahan, has served as a partner in research for the Center for Produce Safety (CPS). Tree fruit specific research priorities are developed and integrated into the annual CPS call for proposals, with the help of the NHC Food Safety Committee. During the proposal process Ines frequently serves as specialist to answer questions asked by scientists preparing to propose new research projects. Currently one project has been funded by CPS: 'Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention' (Zhu/Suslow; $290,000). WTFRC has developed and executed a packing line survey for this project to determine the current industry practices related to spray manifold interventions. The team has also helped source fruit for the experiments. In 2017 Hanrahan has also participated in two site visits with CPS staff and board members: 1) Sept. 5th Vancouver, BC (Delaquis, Lu); 2) Sept. 7th Pullman, WA (Zhu lab).

Table 1: Summary of WTFRC collaborations* in food safety research in 2017 and pending research for 2018

Keyword	PI's	Affiliation(s)	Funding Source	Amount
Continuing/finishing in 2017				
Listeria storage	Zhu/Amiri/Hanrahan	WSU, WTFRC	WTFRC	195,414
Imp. Dryer	Ganjyal/Zhu	WSU	WTFRC	57,000
Water sampling	Partyka/Bond	UC Davis, WTFRC	FDA	243,651
Food Safety Training	Gordon	WSTFA	SCBG	216,682
New in 2017				
List. cleaning	Zhu/Hanrahan	WSU, WTFRC	WTFRC	306,285
FMSA PCHF	Ganjyal	WSU, WTFRC	WTFRC	98,971
Brush bed sanitation	Blakey et al.	WSU, WTFRC	WTFRC	51,967
Listeria monitoring	Kovacevic et al.	OSU	ODA SCBG	174,540
Pending for 2018				
Packing sanitation	Critzer et al.	WSU, WTFRC	WTFRC	203,000
Rapid detection tools	Critzer	WSU, WTFRC	WTFRC	112,000
Ozone in storage	Zhu	WSU, WTFRC	WTFRC	300,000
Dump tank disinfection	Zhu	WSU	CPS	240,000

*collaborations may involve a WTFRC internal budget or utilize Dr. Hanrahan as a consultant/co-PI or collaborator

FSMA implementation: In order to best serve the needs for timely information distribution related to new laws pertaining to food safety, WTFRC staff (Hanrahan) lead an effort to coordinate all outreach activities by the various industry organizations. Specifically, NHC (policy), WSTFA (education) and

WTFRC (research) efforts were combined and talking points coordinated to prevent further confusion, when learning how to implement the already complicated laws. The entire team (Grunenfelder (left in mid-2017), Woods, Gordon, and Hanrahan) has developed a uniform slide set to be used by each group member when addressing groups. This is a living document and has been updated numerous times. Further, the WSTFA has been holding numerous FSA sessions in 2017. WTFRC staff have assisted in meeting logistics and Hanrahan has served as expert to help field questions.
Hanrahan has also been obtaining FSMA certifications for PSA, FSPCA, and attended a train-the-trainer class. She is planning to apply to become a lead PSA trainer in 2018.
In March, WTFRC helped host a group of WSDA inspectors likely to take over responsibility for FSMA inspections to inform them of current industry practices and to field questions. A group of FDA officials visited Yakima in June 2017. Laura Grunenfelder (FDA) requested assistance in setting up field tours and informational sessions.

Development of industry training modules: In collaboration with WSTFA, NHC, and WSU, we repeated the existing workshop module "Putting Cleaning and Sanitation Programs into Practice" during two sessions with a total of 75 participants in 2017. These workshops provided a combination of classroom and hands-on activities and took place in collaborating packing facilities (Table 2). Dr. Hanrahan's contributions to these workshops included: leading of general curriculum development, being a trainer, delivering talks, and helping with logistical support (including staff). Secondly, the same group, in collaboration with UC Davis held two workshops named: FSMA water quality testing. This module was also a repeat of a curriculum developed in 2016. It is the first of its kind in the nation to address practical considerations for water testing under FSMA. Workshops were designed to give participants theoretical background in combination with outdoor activities geared towards learning based on examples coupled with hands on training (Table 2). For 2018, we plan on delivering the workshop "Putting Cleaning and Sanitation Programs into Practice" in Spanish, and repeat the "Verification of cleaning and sanitation programs for tree fruit packinghouses: a hands-on environmental monitoring workshop". In addition, WTFRC is collaborating with the WSFTA to develop a series of food safety videos. In 2017 we finished and the WSTFA distributed two videos: Hand Washing Training, and Cross Contamination vs. Cross Contact. These videos are available in both English and Spanish upon request from Jacqui Gordon (jacqui@wstfa.org). For a 2018 release, we have started a video on Good Agricultural Practices. WTFRC personnel contributed to content development, video shooting, voice over, and development of a training module to teach growers how to best use these materials when training their crews.

Table 2: WTFRC staff involvement in WSTFA sponsored food safety trainings in 2016

Name of Workshop/Training	**Date**
2016 FSMA Water Quality Testing Workshop Wenatchee	May 11
2016 FSMA Water Quality Testing Workshop Yakima	May 9
Putting cleaning and sanitation programs into practice - Yakima	June 2
Putting cleaning and sanitation programs into practice - Wenatchee	May 31

Food Safety outreach: Ines served as the co-session manager for the food safety session during the WSTFA 113th Annual Meeting (HortShow) in December 2017.

Based on industry feedback, Dr. Hanrahan developed a series in collaboration with the Good Fruit Grower to answer frequently asked questions related to food safety. In 2017, a total of 5 pieces were published, and for 2018 another article is planned for the February 15th issue.

The WTFRC board, WTFRC manager Mike Willet, project manager Ines Hanrahan, guest Johnny Gebbers and WSU scientists Meijun Zhu and Girish Ganjyal went on a 2 day intensive study tour to California in March 2017. The overarching goals of the trip were: learn about the various activities related to food safety research at UC Davis and the Center for Produce Safety (CPS) and observe practical implications in a sprout facility.

Dr. Hanrahan also served for two years on the search committee for the WSU Food Safety Extension position. The committee had to perform the search twice, after the first attempt failed and reviewed over 60 applications in the process. To date, Dr. Faith Critzer has been hired and started employment at WSU in Prosser in January of 2018.

In addition, Ines has served as an adjunct faculty member for the WSU School of Food Science. She is currently serving as a committee member on two Ph.D. committees in the Food Science Department. Committee meetings were held in December, to approve the course of study and the thesis research topics. Both students will work in the general area of food safety on very industry relevant topics and are interested in a career in tree fruit upon graduation

Ewa Pietrysiak (former WTFRC intern, Girish Ganjyal serves as major advisor)
Title: Strategies to reduce microbial loads on apples in the packing process

Alice Shen (Meijun Zhu serves as major advisor)
Title: Understanding sanitizer and fruit surface interactions of fresh apples to reduce attachment and proliferation of Listeria monocytogenes on apple surfaces

Further, Dr. Hanrahan is serving on the stirring committee of the PNW Food Safety and Sanitation Conference, a regional conference with 450 attendees annually. Other outreach activities included: 2 posters at national/international meetings, and nine invited talks. The press covered WTFRC food safety activities in four Good Fruit Grower articles and three videos. A complete list of publications has been compiled below.

Publication record for food safety efforts 2017

Peer reviewed publications:

Sheng, L., Hanrahan, I., Sun, X., Taylor, M., Mendoza, M., Zhu, M. 2017. Survival of *Listeria innocua* on Fuji apples under commercial cold storage and ozone. Food Micribiology (accepted)

Sheng, L., Edwards, K., Tsai, H.-C, Hanrahan, I., Zhu, M. 2017. Fate of Listeria Monocytogenes on Fresh Apples under Different Storage Temperatures. Front. Microbiol. 8: 1396.

Other publications:
Jan. 20: Moving ahead with FSMA: A Good to Know (www.goodfruit.com/hanrahan-food-safety-questions-and-answers/)

Feb. 23: Woods: Food safety answers (http://www.goodfruit.com/woods-food-safety-answers/) (written by Kate Woods)

March 9: FSMA answers: preparing your facility (www.goodfruit.com/fsma-answers-preparing-your-facility/) (written by Laura Grunenfelder)

May 10: Food safety answers: What's in your water? (http://www.goodfruit.com/food-safety-answers-whats-in-your-water-videos/) (written by Hanrahan, Woods, Partyka)

March 29: Where can I get training to be prepared for FSMA? (http://www.goodfruit.com/where-can-i-get-training-to-be-prepared-for-fsma/) (written by Jacqui Gordon)

Ines Hanrahan: WTFRC Board Study Tour 2017: Food Safety. Fruit Matters Tree Fruit News March 27, 2017 and www.treefruitresearch.com

Mendoza, M., Hanrahan, I., Gordon, J., Grunenfelder, L. Improving apple packinghouse food safety in Washington state with tailored workshop modules. ASHS Annual meeting in Hawaii (abstract)

Sheng, L., Hanrahan, I., Sun, S., Xue, Y., Taylor, M., Brosi, G., Zhu, M. Survival of Listeria innocua on Fuji apples under commercial cold storage with or without ozone gaseous. IAFP, St. Louis (poster)

Sheng, L., Edwards, K., Tsai, H., Bibil, S., Hanrahan, I., Zhu, M. Fate of Listeria monocytogenes on fresh apples under different storage temperatures. IAFP, St. Louis (poster)

Talks:
Colorado Fruit and Vegetable Association: 'Washington Tree Fruit Industry Response to the Listeria monocytogenes Caramel Apple Outbreak'

North Carolina Tree Fruit: 'Orchard Management for Food Safety'

Empire State Growers Expo: 'On-Farm and Packinghouse Management to restrict foodborne Pathogen contamination'

Empire State Growers Expo: 'Orchard Management to restrict foodborne Pathogen Contamination & Proliferation'

NHC Food Safety Committee Annual Meeting: 'Food safety Research: 2016 Update'

WSU Pullman, FS 220, guest lecturer: 'Food Safety in the Tree Fruit Industry: Interventions and challenges' (75 mins)

Hanrahan, I., Mendoza, M., Sheng, L., Zhu, M.: Antimicrobial efficacy of gaseous ozone during commercial cold storage of Fuji apples (CaMa, Poland)

Mendoza, M., Hanrahan, I., Gordon, J., Grunenfelder, L.: Improving apple packinghouse food safety in Washington state with tailored workshop modules. ASHS Annual meeting in Hawaii (presented as oral presentation by Gordon)

Mendoza, M., Hanrahan, I., Zhu, M., Jeong, K. and Killinger, K.: Survival of Generic E. coli on Fuji Apples with the Applications of Overhead Evaporative Cooling Water near Harvest (presented as oral presentation at ASHS in Hawaii by Mendoza)

Media coverage:
Jan. 14: Food safety research focuses on packing (www.goodfruit.com/food-safety-research-focuses-on-packing/)

Jan. 26: Targeting bacterial die-off in cold storage with ozone (www.goodfruit.com/targeting-bacterial-pathogens-in-cold-storage-with- ozone/)

Feb. 23: Is it really clean: aggressive cleaning makes a big difference (www.goodfruit.com/is-it-really-clean-aggressive-cleaning-makes-big- difference/)

March 10: Study: Overhead cooling does not appear to impact the survival of E. coli on apples (www.goodfruit.com/study-overhead-cooling-does-not-appear-to-impact-the- survival-of-e-coli-on-apples/

Colorado Fruit and Vegetable Association Annual meeting, video recording of talk: "Listeria lessons learned"; https://livestream.com/BarnMedia/CFVGA2017/videos/150264922

Other:
WSTFA Food safety training videos: Hand washing training and cross contamination vs. cross contact

Pacific Northwest Food Safety and Sanitation Conference, Portland (lead panel discussion)

Meijun lab visit in Yakima Valley in October (1 day: arranged schedule and hosted)

FINAL PROJECT PROPOSAL **(First year report -extended)**

Project Title: Understanding and managing the food safety risk of packline brushbeds

PI: Rob Blakey
Organization: WSU
Telephone: 509-608-9394
Email: rob.blakey@wsu.edu
Address: WSU IAREC
Address 2: 24106 N Bunn Rd
City/State/Zip: Prosser, WA 99350

Co-PI (2): Ines Hanrahan
Organization: WTFRC
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: Yakima County Extension Office
Address 2: 2403 S 18th St Suite 100
City/State/Zip: Union Gap, WA 98903-1637

Co-PI (3): Faith Critzer*
Organization: WSU
Telephone: 509-786-2226 x203
Email: faithc@utk.edu
Address: WSU IAREC
Address 2: 24106 N Bunn Rd
City/State/Zip: Prosser, WA, 99350
*Faith Critzer will assume PI in January 2018.

Cooperators: Six packing facilities in Washington

Total Project Request: **Year 1:** $51,966

WTFRC Collaborative expenses:

Item	2017
Salaries & Benefits	$2,736
Wages	$3,472
Benefits	$729
Travel	$4,180
Total	**$11,117**

Footnotes: Salary and benefits for Ines Hanrahan; Wages and benefits for intern.

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2017
Wages	$6,256
Benefits	$1,314
Equipment	$11,000
Supplies	$16,707
Travel	$5,574
Total	**$40,850**

Footnotes: Wages and benefits for technical assistant; Equipment is incubator ovens, plate counter and pipettes; Supplies for microbial test plates and sponges.

Objectives

1. Compare current brush cleaning and sanitation procedures in five (expanded to six) apple packing houses in Washington to determine the effectiveness of these procedures.
2. Determine if fruit sanitation practices are adequate to reduce the risk of cross-contamination from wax brushes during a production shift.
3. Determine if wax brushes are a commercially significant source of spoilage organisms (yeasts and molds)
4. Determine if there is a difference between the packing organic and conventional fruit in the above objectives.
5. Conduct appropriate food safety extension outreach with the apple packing industry

Significant Findings

- Newer lines generally had lower microbial counts than older lines, because of higher counts on the wax brushes of older lines. In this regard, ATP monitoring can be used to indicate when these brushes should be cleaned more intensively or even replaced.
- Lines with higher aerobic colony counts tended to have higher counts of coliforms, *E.coli*, yeasts and molds, and *Listeria* species.
- Clean out of place (COP) steam cleaning was very effective in reducing microbial counts on packing line brushes – aerobic colony counts were 1700 times lower than the average of the other five packing facilities.

Methods

Six representative apple packing facilities in Washington were selected for this project, partly based on responses from a project pre-survey. Packing facilities are numbered to maintain confidentiality (Table 1). Brushes and other packing line surfaces (oven rollers, drying oven walls, belts, curtains and transfer rubbers) were swabbed (3M™ Quick Swab) both before and after a production shift. The focus of the study was drying and wax brushes, but also included other brushes and surfaces in the wet area of the packing line.

Fruit samples were taken off the line before and after the brushbed at the start and end of the production shift; 10 fruit were taken at each location. Swabs and fruit were stored in a cooler box with ice packs during transportation from Yakima or Wenatchee, stored in a refrigerator overnight, and plated the following morning at WSU IAREC in Prosser. Fruit were placed in buffered peptone water incubation pouches for 1 h before plating.

The following microbial tests were conducted on swabs using 3M Petrifilm™ plates: aerobic colony count, coliform/*E.coli*, environmental *Listeria*, and yeasts and molds following the 3M Petrifilm methods for each test. The same tests were conducted on fruit samples, except that environmental *Listeria* and coliforms/*E.coli* testing were omitted. Enumeration was done using a 3M Petrifilm Plate Reader. Samples were diluted 1:10 using Butterfields solution for ACC and yeast and molds if high microbial loads were anticipated.

Table 3: Packing facility numbers and description

	Packing Facility Number					
	1	**2**	**3**	**4**	**5**	**6**
Relative Age of Line	Newer	Older	Newer	Newer	Older	Newer
Wet/Dry Separation	Yes	No	Yes	No	No	Yes
Hygiene Monitoring	Yes	No	Yes	Yes	Yes	Yes
Brush CIP/COP	CIP & COP	CIP	CIP & COP	CIP	CIP	COP
Brush Cleaning Method	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Steam
Sanitizer during Production	Ozone, PAA, ClO_2	PAA	Ozone, PAA, ClO_2	PAA	Ozone	PAA

CIP, Clean in Place; COP, Clean out of Place; PAA, Peracetic acid.
Newer lines < 5 years old; Older lines >15 years old

Results & Discussion

Environmental Listeria

Only facility 5 (older line) had environmental *Listeria* detections in 2017. These detections were on:

- a transfer rubber at the end of shift (10/2),
- soap brushes and a felt fabric transfer curtain at the start of shift (10/30), and
- a wax brush at end of shift on 10/30.

This facility had high aerobic colony counts at the start and end of shifts (Figure 2).

Coliforms & *E.coli*

Coliforms were detected at least once at all packing facilities at the start of the production shift (Figure 5). Areas that regularly tested positive for coliforms at the start of the production shift were:

- Wax brushes
- Transfer brushes after the drying oven
- Bin filler brushes
- Transfer brushes in general

There were four *E.coli* detections:

- Facility 1 on a wax brush under the wax applicator at the start of the shift.
- Facility 2 on repair tape on a spacer bar.
- Facility 5 on a transfer rubber – the same date (10/2) and location where environmental *Listeria* was detected (see above) – and one fruit sample at the start of shift after going over the brushbed.

These three facilities had the highest average aerobic colony counts (Figure 2).

Aerobic Bacteria

Aerobic colony counts (ACCs) provide a general indicator of adequate cleaning and sanitation (although not food safety because food borne human pathogens can be present at low counts and can

provide some means to rank the packing facilities. Facility 6 was by far the best performing packing facility in this project. Facility 6 had aerobic colony counts 3 orders of magnitude lower at the start of shift and 2 orders of magnitude lower at the end of shift than the other five facilities – the ACCs at the end of the production shift at facility 6 were often lower than the ACCs at the start of the shift at other facilities. Their success demonstrates that it is possible to clean a packing line to very low counts, and reduce these by 2-3 $\log_{10}$ values with COP steam cleaning and a multi-hurdle approach during a production shift.

Facilities 3 and 6 had the lowest average aerobic colony counts on the packing line (Figure 2), and also had the lowest aerobic colony counts on fruit (Figure 4). Facility 5 had high counts throughout the line and consequently the fruit from that facility had the highest counts. General comments regarding specific areas on packing lines are given below in Table 2.

Table 2: Comments on cleaning and sanitation procedures for zones 1 and 2 areas on apple packing facilities.

Area	General Comments
Soap and sanitizer brushes	Need attention during cleaning
Drying brushes	ATP swab first brushes; highest ACC there, decreasing down bed
Wax brushes	All CIP lines have high ACCs, especially under the wax applicator. ATP swab brushes under the wax applicator.
Oven rollers	Lower concern, but high residues indicate higher ACCs
Post-oven transfer brushes	Can have high loads, need more attention during cleaning and sanitation
Alignment brushes	Lower concern
Bin filler brushes	Some concern, need more attention during cleaning and sanitation
Transfer brushes	Some concern, need more attention during cleaning and sanitation
Other surfaces	Other surfaces, like fruit pushers, oven walls, etc. require attention during cleaning and sanitation in the worse performing packing facilities. Surfaces like tape, foam, cloth and rubber should ideally be removed from the line because they are potential harborage sites for food pathogens.

ACC, aerobic colony count; CIP, Clean in Place

Molds

The mold counts on the packing lines generally increased during the production shift and correlated with the aerobic colony counts. Facilities 3 and 6 having lower mold counts and facilities 1, 2, and 5 having higher loads (**Figure 9** and **Figure 10**). This will vary by lot and storage duration, however, and requires longer term monitoring. Facility 1 did not have good mold control over the brushbed and consequently through the shift, with both mold and aerobic counts increasing over time on fruit (**Figure 10**). Yeast counts followed a similar trend to molds so data were not presented for brevity. Good cleaning and sanitation practices not only reduce food safety risks, but may improve returns by reducing rejections of packed fruit with an extended storage period – such as exports or in a high production season.

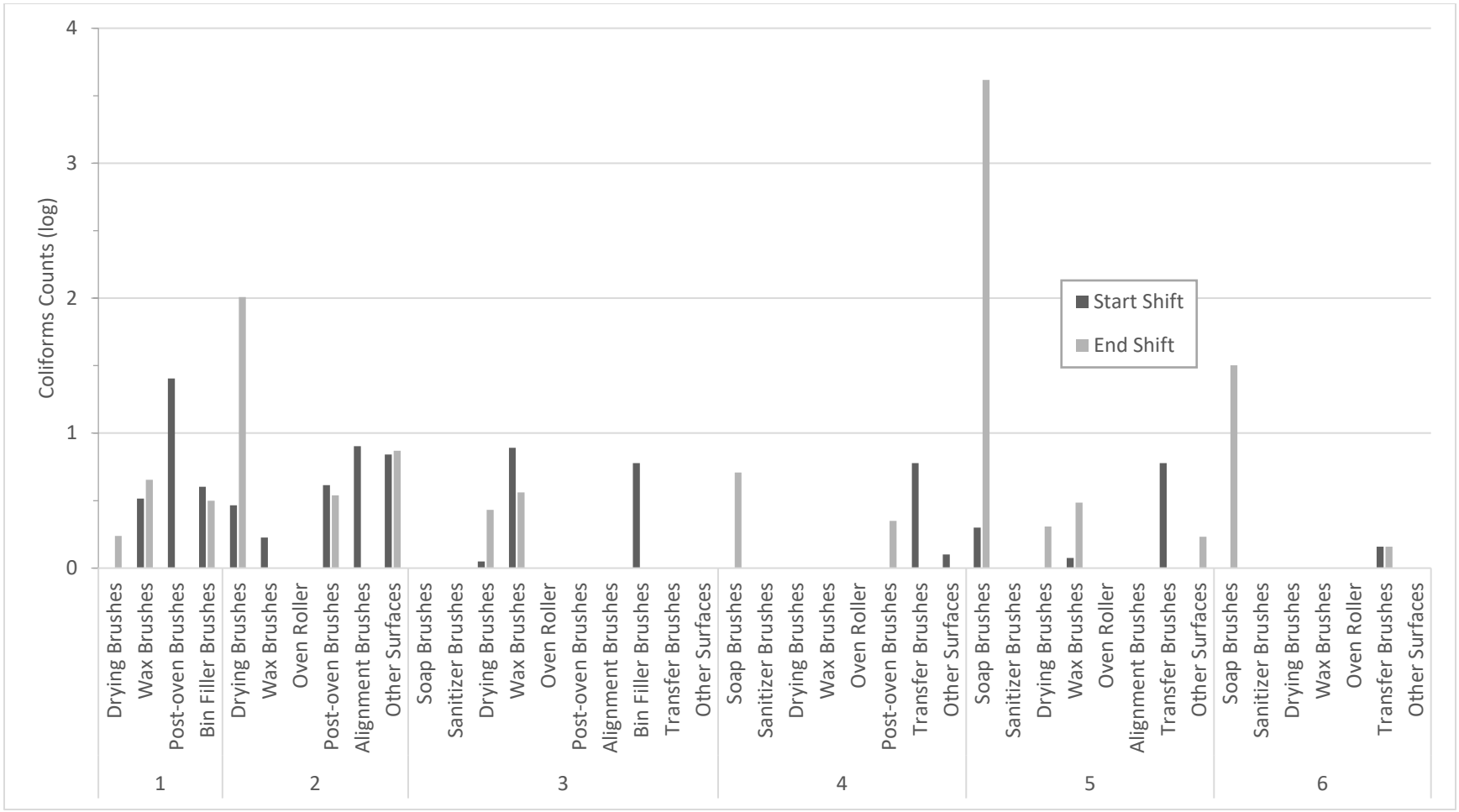


Figure 5: Coliform counts on the packing lines of six particpating packing facilities.

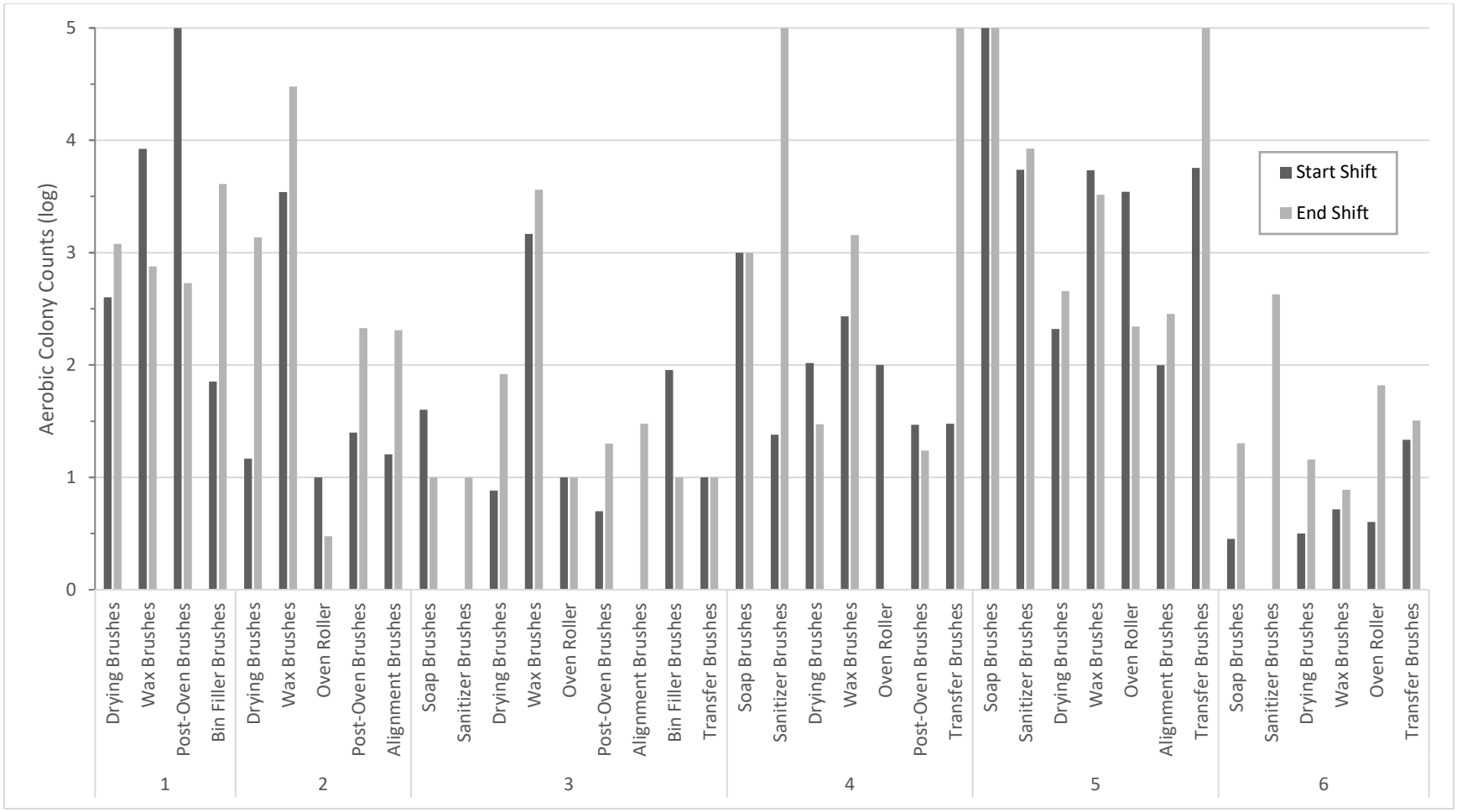


Figure 6: Aerobic colony counts on the packing lines of six participating packing facilities

Figure 7: Aerobic colony counts on selected packing line surfaces at four of the participating packing facilities.

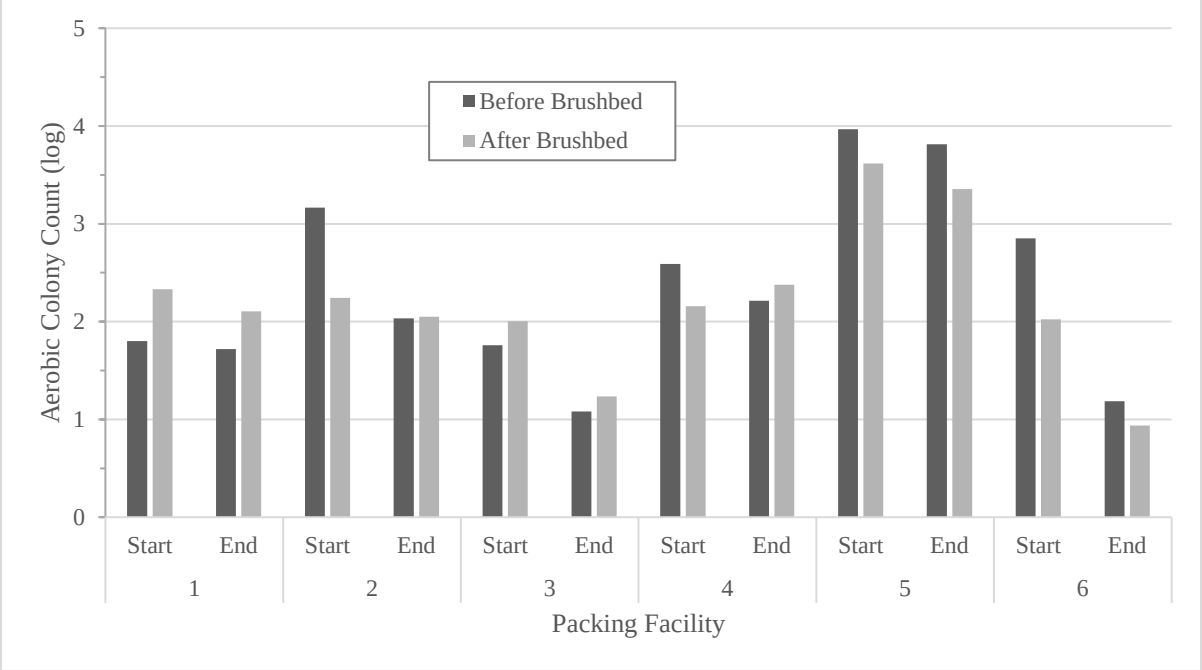


Figure 8: Aerobic colony counts on apple fruit at the start and end of shift, sampled before and after the brushbeds of the six participating packing facilities.

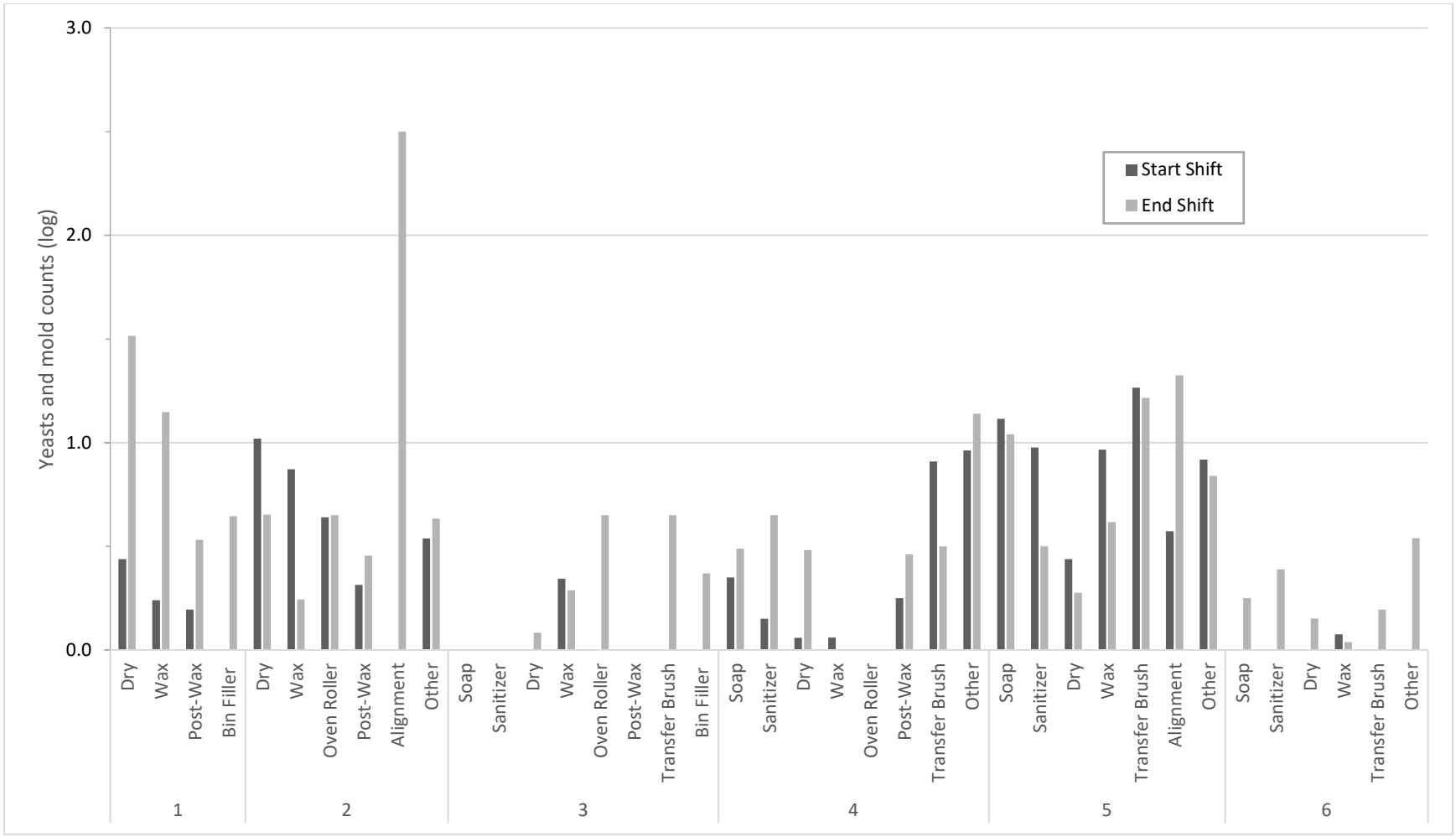


Figure 9: Mold counts on the brushes and other surfaces of packing lines of six participating packing facilities.

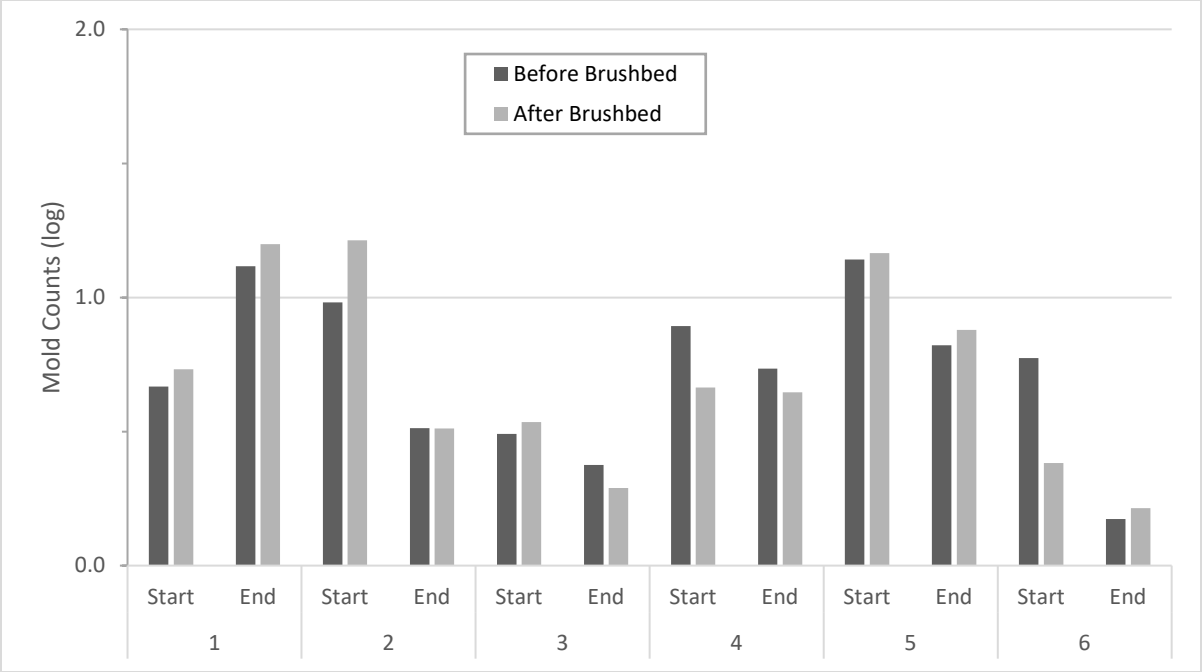


Figure 10: Mold counts on fruit at the start and end of shift, taken before and after the brushbeds of the six participating packing facilities.

Conclusion

Newer facilities generally had lower aerobic colony counts, but this was mostly caused by high microbial counts just on the wax brushes of older lines - attention needs to be paid to cleaning these brushes, and using ATP monitoring to determine when to replace these. That being said, newer facilities can have unacceptably high microbial counts, without daily attention to cleaning and sanitation. COP steam cleaning of brushes resulted in a significant reduction in microbial counts, but has been noted to reduce the life of brushes. The other five facilities did not have appreciable differences in their sanitation SOPs, so we believe that the differences are often in the execution of these SOPs. Some key points to improving hygiene levels in packing facility, and reducing food safety risk are: a motivated, properly equipped sanitation crew with attention to detail and sufficient time to clean and sanitize the packing facility, a validated hygiene monitoring system, an appropriate sanitizer monitoring system and protocol, and leadership from management to continually improve hygiene levels in a facility.

These results only provide a snap shot at each packing facility. To be effective, a food safety program requires daily attention, and long-term planning for continual improvement. These assays, excluding the environmental Listeria test, can be done easily at a packing facility and the results used to improve cleaning and sanitation procedures at the facility.

Executive Summary

Aerobic colony counts (ACCs), coliforms and *E.coli*, environmental *Listeria*, and yeasts and molds samples were taken at six apple packing facilities in Yakima and Wenatchee between August and October 2017. These facilities were representative of the types of packing lines currently in Washington. The brushbed was swabbed before and after a production shift. Fruit samples were also taken at the same times, before and after going over the brushbed. Microbial tests were performed using 3M Petrifilm™ plates.

In general, **ACCs** at the start of production were lower in the three newest lines, but results show that it is possible to clean older facilities to levels comparable to those of newer lines. This may require extra attention to remove dirt and wax residues, and may require more frequent brush replacement but this should be considered against the risk and cost of a food safety recall. **Coliforms** were detected on all the packing lines, with detections associated with high ACCs. ***E.coli*** was sporadically detected at the three facilities with the highest ACCs. **Environmental *Listeria*** was only detected on one older line with high ACCs. **Yeasts and molds** also correlated with ACCs, suggesting that beyond food safety, cleaner lines may have reduced post-packing decay – particularly on fruit with an extended post-packing storage duration.

One packing facility stands out amongst the six, having aerobic colony counts (ACCs) 3.2 orders of magnitude lower than the average of the other five facilities. Brushes at this facility were cleaner at the end of the production shift than most facilities' brushes at the start of their production shift. This facility uses a multi-hurdle approach with multiple sanitizers during production and a clean out of place (COP) steam sanitation system for brushes. Five of the six (old and new) facilities used foaming chlorine cleaner and a quaternary ammonium compound or PAA sanitizer, so differences are not in these products but in the execution. In our opinion, this comes down to: a motivated, properly equipped sanitation crew with attention to detail and sufficient time to clean and sanitize the packing facility, a validated hygiene monitoring system, an appropriate sanitizer monitoring system and protocol, and leadership from management to continually improve hygiene levels in a facility. Monitoring will continue in 2018.

FINAL PROJECT REPORT **YEAR:** 2017
WTFRC Project Number: N/A

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S 18th St. Suite 100
City/State/Zip: Union Gap, WA, 98903

Cooperators:

- WTFRC internal program: Manoella Mendoza, Mackenzie Perrault, Felix Schuhmann
- Others: Rob Blakey (former WSU), Grower collaborators, WTFRC seasonal crew and interns, Glade Brosi/Hannah Walters (Stemilt), Garrett Bishop (GS Long)
- WA 38 starch scale review panel: Lauren Gonzalez (Kershaw), Suzanne Bishop (Allan Bros.), Jim Mattheis (USDA-ARS), Kate Evans + team (WSU-Wenatchee), Bill Wolk (BC)

Other funding sources
Supplies and fruit were donated by industry cooperators (approx. value: $2,500); GS Long covered the cost for mineral analysis for WA 38

Europe trip: WTFRC internal travel ($ 2,500); Chile trip: sponsored by Dr. Torres program (valued at $3,000)

Budget 1
Organization Name: WTFRC **Contract Administrator: Kathy Coffey**
Telephone: 509 665 8271 **Email address: Kathy@treefruitresearch.com**

Item	2016	2017 (projected)	2017 (actual)*
Salaries	6,785	6,785	2,247
Salary benefits	3,401	4,301	742
Wages	15,734	7,800	9,548
Wage benefits	5,156	2,643	4,703
RCA rental	1,800	1,800	0
Equipment + supplies	268	300	210
Travel	167	500	0
Reimbursements	---	---	0
Total net costs	**33,311**	**24,129**	**19,466**

Footnotes:
2017 actual budget is based on the calendar year 2017 (July 1, 2016-June 30, 2017); July 1- Dec. 2017 (not shown)
Salaries*: incl. proportional time spent on outlined projects for Mendoza (4%); NOT included 6% of Hanrahan's time
Supplies: experimental fruit, storage boxes and trays donated
RCA rental: numbers based on fiscal year (@ approx. $6,300/room/year)
Reimbursements: monetary contributions by chemical suppliers, if applicable

NOTE: This is a final report. All internal program research projects will require new proposals to the WTFRC board in March 2018.

OBJECTIVES[A]

1. Document Honeycrisp fruit quality in local store displays.
2. Test new tools to determine fruit quality parameters (no work in 2017).
3. Serve on WSU Tree Fruit Extension team as postharvest specialist (2017: DISORDER GUIDE, WA 38, POSTHARVEST FRUIT SCHOOL).
4. Field test methods to induce bitter pit in Honeycrisp.
5. Compare and document fruit quality of Premier Honeycrisp and Honeycrisp strains (NEW 2017).
6. Expand collaborative efforts with other research programs working on fruit quality management.

[A]seasonal adjustment of objectives based on industry feedback and Hanrahan program capacity in 2017.

SIGNIFICANT FINDINGS

Objective 1: With Honeycrisp currently sourced from several countries, consistently good eating quality in the second part of the storage season (February to August) remains of concern.

Objective 3: WA38 develops two predominant starch patterns, which appear in a 6:4 ratio. WA38 converts starch very slow back to sugar. The reaction time for the iodine solution to color the starch crystals is significantly longer compared to other industry specific varieties.
A total of 7,833 photographs of 106 defects were created during 2017. The WSU CAHNRS Video & Photography Department will help to produce the final publications. These will be: (i) an online guide with rotatable photographs with annotations and descriptions, and (ii) hard copy with photographs and defect description.
A Postharvest fruit school was developed by the Extension team to be held in two locations (Prosser, Wenatchee) in March of 2018.

Objective 4: The Ethephon method delivered the fastest and best results in 2017. All methods predict best when the percentage of bitter pit affected fruit is below 10% before harvest, with no more than 25% of the remaining fruit developing symptoms in subsequent storage.

Objective 5: Premier Honeycrisp displays harvest maturity, storage life, and eating quality after storage similar to other common Honeycrisp strains/selections. Based on preliminary results, the fruit was less prone to chilling injury, but bitter pit and stem bowl splits may occur.

Objective 6: We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre and postharvest fruit quality management.

METHODS

Supermarket survey: Eight Yakima supermarkets were visited monthly from June until August 2017. Visual quality and supply of fruit was determined.

Induced bitterpit: Five orchards were selected (3 orchards = all methods, 2 orchards = PennState method [https://extension.psu.edu/fruit-disorders-new-tool-to-assess-the-potential-for-bitter-pit-in-honeycrisp]). Before the apples were harvested, the amount of Bitter Pit (BP) was assessed per tree. All apples were harvested between the 2nd and 5th wire on the west or south side of the tree. The apples were picked a few days prior to first commercial harvest. For the PennState method five typical terminal shoot measurements were taken. In the first three orchards, a total of 270 apples per orchard (passive method: 40, Ethephon method: 40, hot water method: 40, PennState method: 20, untreated control (UTC): 120 and maturity sample: 10) were harvested. In the other two orchards, 150

apples per orchard were sampled (PennState method: 20, control: 120 and maturity sample: 10). 20 representative trees were selected in each orchard.
Within 2 hours of harvest, fruit was prepared as follows:

1. All fruit were washed in 77°F (25°C) tab water.
2. The fruit were left to air dry.
3. Hot Water Method:
   a. A cooler was filled with hot water and adjusted to ~120°F (49°C).
   b. The apples were submerged into the hot water and held under water with warmed up hard plastic ice packs. If the temperature dropped below 115°F (46°C), hot water (~125°F) was added.
   c. After 30 minutes, the samples were taken out of the water and laid to dry on trays layered with paper towels.
4. Ethephon Method:
   a. A plastic box was filled with 2 gal (7.6 l) of water (temperature: 77°F) and 0.5 oz (15 ml) of Ethephon (1 gal= 0.25 oz) was mixed in.
   b. Every apple was dipped (2 sec) in the prepared solution.
   c. The samples were laid to dry on trays layered with paper towels.
5. Passive Method:
   a. No further steps were added to this method.
6. All apples from these three methods were placed in apple boxes and stored at room temperature for 3 weeks.
7. The fruit was evaluated after one, three, five, eight, eleven, fourteen and twenty-one days.
8. PennState Method:
   a. A commercial fruit dryer was preheated to 160°F (71°C).
   b. A fruit peeler was used to remove a 3/8" wide (1 cm) strip of peel from around the circumference at the calyx end of the fruit.
   c. Samples were put on a drying tray and dried for 9 hours at 160°F.
   d. After 9 hours the samples were stored in Ziploc® bags and sent to a laboratory for a nutrition analysis.

The UTC fruit was stored following the Honeycrisp storage recommendations [http://treefruit.wsu.edu/article/honeycrisp-storage-recommendations-revisited/] and apples were evaluated after 2, 4, 6, 8 and 12 weeks of cold storage.

WSU Tree Fruit Extension team:

WA 38 starch scale:

WA38 apples were picked in four locations (Roza, Prosser, Quincy, Sunrise). Before the harvest in week 10/09 - 10/12/2017 sample fruit were picked in the orchard. After harvest, the fruit was stored at 33°F. In mid-November, a sample of 40 fruit was stored at room temperature, to advance maturity. Apples were prepared and pictures taken between 9/25/2017 and 11/29/2017.
To prepare the apples:

1. The apples were cut in half through the equator.
2. The freshly cut apple surface was dipped in iodine solution to cover flesh.
3. After 30 mins the pattern was fully developed and the surface was then dried with a paper towel.
4. A picture was taken under consistent light environment (i.e. a photo box).
5. The pictures were evaluated and grouped.

We have used an industry focus group (field men, QC personnel, R&D, scientists) to gather feedback on various stages of the actual starch scale and on potential best layout options.

Apple Disorder guide:
During the 2016-17 storage season samples were collected from local packers. Fruit was then grouped by variety, disorder, and severity of symptoms. TJ Mullinax of the Good Fruit Grower photographed samples of fruit including views of external and internal symptoms, and additional photographs on a rotating platform.

Quality of HC strains and early maturating selection:
Eight orchards of Honeycrisp were selected including three different strains (Standard, Royal Red, and Firestorm) and Premier Honeycrisp (early maturing selection). All fruit was harvested at commercial maturity. A sample of 40 fruit each of Premier HC was stored for three months in a 33°F room or fruit was stored following the Honeycrisp storage protocol (see http://treefruit.wsu.edu/news/honeycrisp-storage-recommendations-revisited/). Fruit was inspected weekly for four weeks to determine changes in maturity and defects

The remainder seven orchards were all stored as untreated control (UTC) at 33°F from one month to three months depending on harvest date. After storage, fruit quality of all samples was determined.

All apples in each sample were evaluated externally for disorders rendering them culls in a commercial setting. Twenty-five apples were cut in order to determine any internal disorders. Taste was rated 1-5 using a scale specific to this experiment on four apples from each sample (1=immature flavor, 2=mature flavor, 3=over mature, 4=bland (no flavor, no detectable off flavor), and 5=off flavor (a-typical taste profile)).

RESULTS & DISCUSSION

In 2017, the fruit quality program has continued to focus part of its effort on Honeycrisp fruit quality. Based on the membership of WTFRC staff in the WSU Tree Fruit Extension team, Hanrahan added WA38 and leadership in Postharvest Fruit School organizing team.

Supermarket survey

The increasing production of Honeycrisp apples has resulted in the variety being available to consumers more months of the year, but those apples may not always be of the highest quality. In 2012 the Washington Tree Fruit Research Commission (WTFRC) began a survey to assess how long Honeycrisp apples remain on the retail shelf locally throughout the year and, more importantly, monitor the eating quality of those apples.
Last season, eight Yakima area supermarkets were visited once a month from June until August 2017. In the months of June and July, all locations continued to carry fruit from the previous year's harvest, with one store offering Chilean and New Zealand Honeycrisp apples. By August, the supply of Honeycrisp fruit was 75% foreign market supply (Chile, New Zealand). In July of 2017 we observed the first supply of fruit grown in New Zealand (Table 1).
As the current harvest season approached, the external quality of the fruit started to vary within the supermarkets. They ranged from good color and no defects in most locations (until June 2017) to half the locations supplying fruit of inferior visual appearance in August (not shown). In summary, the 2016-17 storage season proved to be the second time we documented a year-round Honeycrisp supply in local supermarkets, but during the summer visual appearance of fruit started to drop.

With Honeycrisp currently sourced from several countries (Table 1), consistently good eating quality in the second part of the storage season (February to August) remains of concern (Table 2). If consumers are having inconsistent eating experiences from good to off flavor, repeat sales could be

impacted negatively. It appears that this issue has remained a constant struggle over the past six storage seasons.

Table 1: Supply of Honeycrisp in local Yakima supermarkets by country of origin.

Sources of fruit												
Year	January	February	March	April	May	June	July	August				
2012	US	US	US	-	-	-	-	-				
2013	US	US	US	US	US	-	-	-				
2014	US	US	US	US	US	C	US	C	-		-	
2015	US	US	US	US	US	US	C	US	C	-		
2016	US	US	US	US	US	US		US		US	C	
2017	US	US	US	US	US	US	C	US	C	N	US	C	N

Note: US = USA, C = Chile, N = New Zealand

Table 2: Summary of Honeycrisp apples flavor of eight Yakima supermarkets from 2012-2016.

Percentage good flavor								
Year	January	February	March	April	May	June	July	August
2012	-	-	0	-	-	-	-	-
2013	-	-	14	-	-	-	-	-
2014	-	29	43	67	43	0	-	-
2015	-	14	14	57	33	44	20	-
2016	-	42	25	45	29	33	44	42

Induced bitter pit

All UTC fruit showed a similar pattern in developing bitter pit. After 4-8 weeks of exponential rise in bitter pit incidence, the expression of additional bitter pit in remaining fruit slowed down and eventually approached zero (see Figure 1). After a 12-week storage period, Honeycrisp apples from orchard 1 showed in average 10 apples with symptoms (25%), from orchard 2, 26 apples (64%) and from orchard 3, 19 apples (48%) had developed bitter pit.

In Orchard 1, all methods predicted the right amount of bitter pit, considering the standard deviation (SD). The Ethephon method was most accurate. In orchard 2 none of the methods predicted the right amount of bitter pit. The method with the closest prediction was Ethephon (37% off). In orchard 3, none of the methods were correct in predicting the amount of bitter pit after 12 weeks in storage. Nevertheless, the Ethephon method was within a prediction range of 33% (see Figure 2).

One aspect of bitter pit prediction that is not considered in any of the current methods involves the amount of fruit left in the orchard due to bitter pit at the time of harvest. The average in-field loss from bitter pit was between 7% and 22%. In both, Orchard 1 and 2, we determined that 7% of fruit was left in the field due to bitter pit. Orchard 3 had more in-field bitter pit with an average of 22% of the crop with discernable bitter pit symptoms before harvest. After 12 weeks in storage, between 25% and 64% of the apples harvested symptom free had developed bitter pit. Orchard 1 developed 25%, Orchard 2 64% and Orchard 3 48% bitter pit in storage. This means that one cannot predict storage bitter pit potential based on field symptom expression alone. For example: we had two orchards with low bitter pit incidence in the field (7%). However, one orchard developed 25% bitter pit in storage, while 64% of fruit from the second orchard developed bitter pit. The orchard with the highest preharvest loss due to bitter pit, was not the worst orchard for bitter pit development in storage.

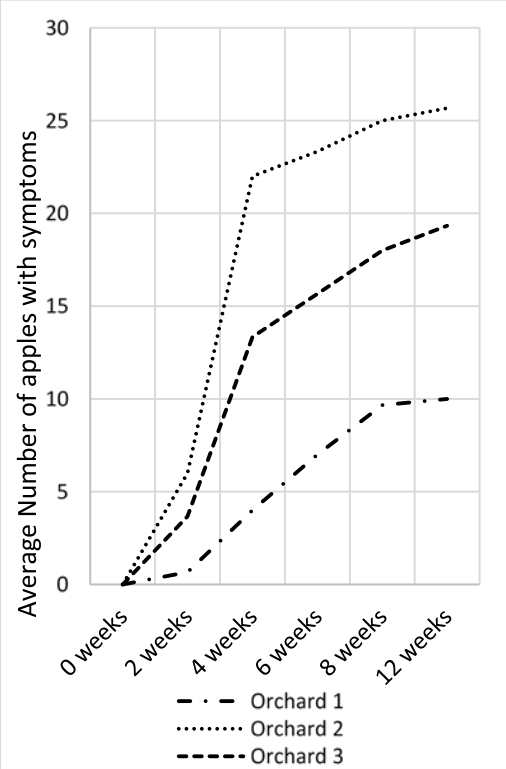


Figure 1: Average amount of untreated control fruit developing bitter pit during 12 weeks of storage (1 week at 50°F followed by 11 weeks at 36°F). n=40

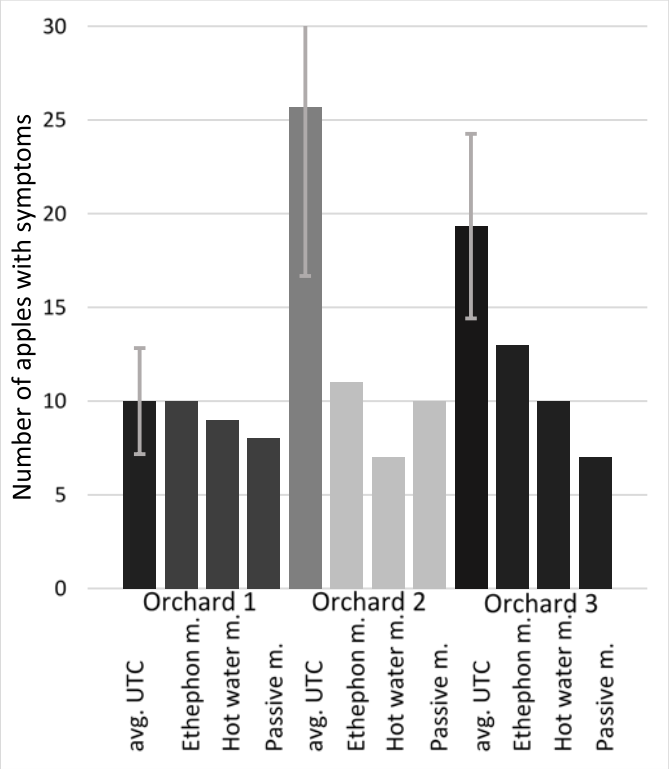


Figure 2: Comparison of three methods to predict bitter pit in Honeycrisp apples, n=40, error bars = ±SD. (UTC = untreated control)

WSU Tree Fruit Extension team

WA 38 starch scale: For the new starch scale, 638 apple samples were cut; 81 in September, 371 in October and 186 in November.

To achieve a surface reflecting the middle of the core, WA38 apple fruit were cut, unlike other industry specific varieties, right through the equator. During the experiment, two predominant patterns were observed. The commonly known “flower” pattern (see Figure 3), which is characterized by 5 “flower petals” growing with advancing maturity. The other pattern was named “radial” (see Figure 4), and can be compared with a “sunrise”. It clears larger parts of the cut surface in a radial fashion with advanced maturity. The patterns appeared in a 6:4 ratio.

Another observation was the slow disappearance (aka slow fruit ripening) of dark color and consequently starch. This was like observations from previous years with cv. WA38 apples at the WTFRC. In the new starch scale this fact was considered and a scale with half point increments was developed.

The experiment also showed that the starch patterns took longer to develop, compared to other industry specific varieties. Depending on fruit temperature and maturity, the development of a complete starch staining took up to one hour. In most cases the pattern developed fully after 30 minutes. If the fruit is warm, starch readings are possible in less than 5 minutes.

2018 plans: we will incorporate feedback received from our industry focus group and will ask for additional input from the Apple Horticulture and Postharvest Committee during the January 24, 2018 Research Review. The scale will then be refined in 2018 by incorporating: the exact time and conditions needed for accurate starch readings, a comparison of starch movement on and off the tree, and a vertical starch pattern to predict the likelihood of splits in the stem bowl.

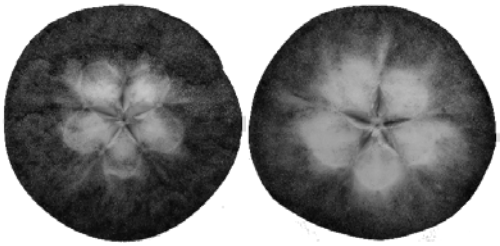


Figure 3: WA38 two different 'flower starch' patterns

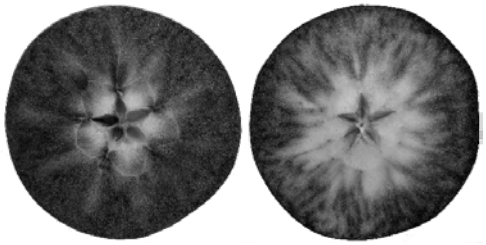


Figure 4: WA38 two different 'radial starch' patterns

Apple Disorder guide:

A total of 7,833 photographs of 106 defects (many with multiple cultivars [158 defect x cultivar "entries"]) were created during 2017. Based on those results, we developed a list of missing items to be collected during harvest and storage in late 2017. Rob Blakey has worked with Darrell Kilgore and Matt Ziegler from the WSU CAHNRS Video & Photography Department to produce the final publications. These will be: (i) an online guide with rotatable photographs with annotations and descriptions, and (ii) a hard copy with photographs and defect description. Depending on resources, a Spanish version could be produced at a later stage.

Currently Blakey and Hanrahan are creating (i) descriptive text for each disorder, (ii) annotated call outs to appear on points of interest on the rotating photographs, (iii) a sorting system including cross linking, common misidentification, and links to additional sources.

The project is being funded internally by CAHNRS Extension Publications with costs to be recovered from selling the hard copy version. It was agreed that any profits would accrue to the Tree Fruit Postharvest Extension Specialist. Karen Lewis has taken over as PI since Blakey left WSU employment.

Postharvest fruit school:

A Postharvest fruit school was developed by the WSU Extension Tree Fruit Team to be held in two locations (Prosser, Wenatchee) March 20-22 of 2018. Fruit Schools are designed to delve deep into topics important to the industry using a combination of presentation, discussion, hands-on activities, and demonstration. Regional, national, and international speakers (12 invited speakers) will be equally located at both Wenatchee and Prosser to facilitate interaction with attendees. To make the meeting affordable, we have secured 13 sponsors to date. The first day will focus on the principles of postharvest science and management. The second day will address crop specific problems with sessions for apples and berries (morning), and cherries and pears (afternoon). The third day will be warehouse tours. Registration has been open since November 2017, and to date we have 66 participants signed up, for a total of 250 available seats. More information and online registration is available under: http://treefruit.wsu.edu/postharvest-fruit-school-2018/

Quality of HC strains and early maturating selection

Our main focus of the experiment was to determine how Premier Honeycrisp performed in storage compared to other common strains of Honeycrisp. The results reported here are preliminary. A full report will be made available upon completion of the experiment. We evaluated all fruit in December and found Premier Honeycrisp to have varying degrees of splitting (0 or 22%), bitter pit (0 or 2%) and soft scald (0 or 7%) when stored in a common cold room (Table 3). Utilizing the Honeycrisp storage protocol reduced the incidence of soft scald from 7% to zero. Premier Honeycrisp displays harvest maturity, storage life, and eating quality after storage similar to other common Honeycrisp strains/selections (not shown).

Table 3: Strain, harvest date and types of cullage for nine batches of Honeycrisp representing commonly used strains. WTFRC 2017.

Honeycrisp	Harvest Date	% Bitterpit	% Softscald	%Splits	% Total Cullage
Premier Box B[Y]	8/28/17	-	-	-	0%
Premier Box A	8/28/17	-	7%	-	7%
Premier	8/30/17	2%	-	22%	24%
Firestorm	9/1/17	57%	-	-	57%
Royal Red	9/7/17	9%	-	-	9%
Standard	9/8/17	35%	-	-	35%
Firestorm A	9/15/17	2%	-	-	2%
Firestorm B	9/15/17	-	18%	1%	19%
Standard	10/13/17	8%	2%	-	10%

Footnote: Y = Samples stored following Honeycrisp protocol; all other samples stored at 33°F.

Collaborative research

We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre and postharvest fruit quality management (Table 4).

Table 4. 2017 Hanrahan/WTFRC collaborations on pre-and post- harvest fruit quality projects.

COLLABORATOR(S)	PROJECT	HANRAHAN ROLE
2017 (continuing and new)		
Evans/Auvil	WSU breeding: P3	Collaborator storage evaluation
Univ. of Talca*	Superficial scald control	Contract project
Blakey	NEW WSU Apple Disorder Guide	CO-PI
Willett	Apple rootstock and scion evaluation	collaborator

*project costs completely covered by companies/external projects

In addition, Hanrahan and her team participated in several events geared towards organic production to develop specific knowledge and expertise in this area. Further, since roughly 40% of all apple trees to be planted in 2018 will belong to one of ± 30 clubs, the team visited established plantings, obtained samples to observe and taste and gathered information on horticultural challenges, maturity, storability etc.

Lastly, Dr. Hanrahan went on two trips oversees to Europe (June/July), and Chile (Nov. 11-27). In Europe, Hanrahan attended an ISHS sponsored conference in Poland last summer and spent several additional weeks in Germany to learn about postharvest applications and to connect with research and extension personnel. In Chile, Hanrahan was hosted by Dr. Carolina Torres, Director for International Affairs and Professor in the Pome Fruit Research Center, University of Talca, Chile. We performed experiments to test a novel anti scalding agent developed and patented in the Torres lab. Hanrahan participated as invited speaker: Avances en Technologías en Postcosecha de Pomáceas y Cerezas (International Workshop: Advances in new Technologies for Pomefruit and Cherries) and visited local orchards and packing facilities for cherries, apples, and pears.

The information gathered on those trips is being shared with the WTFRC board and management, local collaborators, the extension team, and industry stakeholder groups (Pomclub, NCWFA) through a variety of means (report, ppts, personal visits). Further, we utilized the information to optimize our information transfer in the upcoming postharvest fruit school.

2017 Outreach Activities

Peer reviewed publications:
R. Karina Gallardo, Ines Hanrahan, Chengyan Yue, Vicki A. McCracken, James Luby, James R. McFerson, Carolyn Ross, and Lilian Carrillo-Rodriguez. 2017. Combining sensory evaluations and experimental auctions to assess consumers' preferences for fresh fruit quality characteristics. Agribusiness: an International Journal (accepted)

Hanrahan, I., Vorholz, M., DeEll, J. 2017. Identification and management of carbon dioxide injury in apples. Acta Hort. (submitted)

Leisso, R. S., Hanrahan, I., Mattheis, J., Rudell, D., 2017. Controlled atmosphere storage, temperature conditioning, and antioxidant treatment alter postharvest 'Honeycrisp' metabolism. HortScience. 52(3):423–431

Mattheis, J., Rudell, D., Hanrahan, I. 2017. Impacts of 1-Methylcyclopropene and controlled atmosphere established during conditioning on development of bitter pit in 'Honeycrisp' apples. Hort.Sci. 52(1): 132-137.

Abstracts/posters:
Hanrahan, I., Vorholz, M., DeEll, J.: Identification and management of carbon dioxide injury in apples (poster at CaMa)

D.A. Neuwald, N. Klein, C. Prunier, S. Gehweiler, L. Hart, C. F. Şerban, I. Hanrahan: Crop load affects the incidence of watercore in 'Fuji' apples (poster at CaMa)

Other publications:
Hanrahan, I. & Perrault M.: Honeycrisp Supermarket Survey Results. Published Aug. 12, 2017
http://treefruit.wsu.edu/news/honeycrisp-supermarket-survey-results/

Hanrahan, I. & Blakey, R.: Honeycrisp storage recommendations revisited. Published Sept. 8, 2017
http://treefruit.wsu.edu/news/honeycrisp-storage-recommendations-revisited/

Musacchi, S., Hanrahan, I., Lewis, K., Evans, K., DuPont, T. WA 38 Characteristics and Horticulture (WA 38 Factsheet); http://treefruit.wsu.edu/wa-38-characteristics-and-horticulture/

Klein, N. & Hanrahan, I. 2017. Ein aufgehender Stern am Apfelhimmel (a rising star in the apple sky)**.** Obstbau/Weinbau (accepted)

Hanrahan, I. & Perrault, M. The variabilities of Honeycrisp: Good to know
http://www.goodfruit.com/the-variabilities-of-honeycrisp-good-to-know/

Field days:
Sunburn prevention (presenter)
Cosmic Crisp field day at Sunrise: fruit quality and maturity demonstration (presenter)

Talks:
NY Fruit Growers Association: Current best Honeycrisp Management Practices in Washington State
NCWFA in Wenatchee: How to increase packouts

WSU Extension Yakima open house: apple varieties
Tree Fruit Production Trends in Washington State (Germany: Bavendorf and Esteburg, Chile: Talca)
Pomclub (2 talks): European Travel summary; Hanrahan program overview
Sunburn prevention (Spanish session, WSTFA Annual meeting, presented by Mendoza)

Media coverage:
Learning Cosmic lessons by S. Dininny, Dec. 2017, Good Fruit Grower:
http://www.goodfruit.com/learning-cosmic-lessons/

Other:
IFTA Annual Conference: harvest and postharvest (3 hour session, session manager)
MsSc committee member for Corina Serban (Bitter pit management in Honeycrisp)
Attended CaMa (ISHS) conference in Warsaw followed by Eurfin postharvest group meeting
Fielding fruit quality questions and troubleshooting storage problems (±200 contacts/yr.)
WA 38 to Dan Newhouse (delivered by Kate Woods, NHC) and Jay Inslee
WVC postharvest lab (WTFRC quality lab and storage disorders, 2 hour lesson plan)

CONTINUING PROJECT REPORT **YEAR**: 3 of 3
No Cost Extension Requested

PROJECT TITLE: Improving food safety of fresh apples by hot air impingement drying

PI:	Girish M. Ganjyal	**Co-PI**:	Meijun Zhu
Organization:	WSU, Food Science	**Organization**:	WSU, Food Science
Telephone:	509-335-5613	**Telephone**:	(509) 335-4016
Email:	girish.ganjyal@wsu.edu	**Email**:	meijun.zhu@wsu.edu
Address:	FSHN 110	**Address**:	FSHN 232
City/State/Zip:	Pullman, WA, 99164	**City/State/Zip**:	Pullman, WA, 99164

COOPERATORS: Van Doren Sales, Inc., Northwest Horticultural Council, Stemilt Growers LLC., Double Diamond Fruit Co., Pace International LLC., US Syntec, Hansen Fruit Company, Symms Fruit Ranch, Washington Fruit & Produce Company and others packing houses.

BUDGET: **Year 1**: $73,951 **Year 2**: $74,798 **Year 3**: $75,898

OTHER FUNDING SOURCES: Part of the new faculty start-up funds of Dr. Girish M. Ganjyal. Support from co-operators for some of the materials and time on their packing lines.

Organization Name: WSU **Contract Administrator:** Carrie Johnston/Ben Weller

Telephone: 335-4564 509-335-0052 **Email address:** carriej@wsu.edu / wellerb@wsu.edu

Item	2015-16	2016-17	2017-18
Salaries[1]	40,000	40,000	42,000
Benefits[1]	11,960	11,960	12,558
Wages[1]	3,750	6,000	4,500
Benefits	368	132	441
Equipment[3]	4,000	2,000	0
Supplies[2]	6,873	12,706	11,399
Travel[4]	7,000	2,000	5,000
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	$73,951	$74,798	$75,898

Footnotes:
[1] Salaries, Wages and Benefits for technical and student support
[2] Supplies and analysis fees, including for microbial testing
[3] Equipment related to biosafety level two microbial analysis
[4] Travel for industrial experiments

RECAP OF ORIGINAL OBJECTIVES

The objective of this proposal is to evaluate the potential of using hot air impingement drying to enhance the safety of the fresh packed apples. The specific objectives of the proposal are as detailed below:

1) Determine the impingement drying characteristics for broad range of wax coated apples and the impact on quality of the apples with in-plant testing.
2) Study the effectiveness of impingent drying in reducing the microbial levels in apples.
3) Develop scale-up strategies for commercial packing lines and complete the energy efficiency analysis.

SIGNIFICANT FINDINGS

As proposed in the original proposal, the first objective has been completed during the first year of the project and part of the 2nd objective has also been completed, during the year 2016. With the results from the year 2016, we did not see more than 1 log reduction of L. innocua (a surrogate strain of L. monocytogenes) with high temperature conditions in the dyer.

This made us to try to understand why we were not seeing the reduction even though we know from literature that the LM cannot survive at high temperatures. Please refer to Figure 1, on what we have done in the year 2017 to find a solution to this issue. The solution we have come up with may help make all other current industry practices more effective.

The following were the major findings from the year 2017:

Identifying the location of the Listeria spp. on apples

- We took a close look at the apple surface using microscopy. We identified many micro sized cracks with heavy amounts in the calyx and the stem bowl areas. These micro cracks are actually big for the Listeria spp. and other pathogen, but too small for the water or other chemicals to enter.
- By inoculating the apples with L. innocua we tried to understand where the bacteria hides. We location more L. innocua in the calyx and stem bowl micro cracks than on the other peel areas. The L. innocua was well hidden in the cracks, which are hard to reach by many of the interventions typically used, including the hot air.

Surface tension of the apple peel

- We had found that even with wetting the apples with water, before drying was not effective. We also know almost all the interventions (chemicals) are also not highly effective. This led us to look at the surface tension of the apple peel.
- We found that the water or other chemicals used with water, have very high contact angle, which can suggest they don't adhere to the apple skin closely and thus may not reach the hidden L. innocua in micro cracks.
- But with the addition of surfactant, the water drops adhere better to the skin and thus may get closer to the hidden LM.

Microbial Reduction Testing

- With the above new findings, we were able to do one set of microbial testing with the dryer, using a surfactant. This finally gave use some significant reduction in the L. innocua.
- We will conduct some final testing with different surfactants in the year 2018 and conclude the project with our final suggestions and recommendations, including the scale-up of the system to fit into the existing packing houses.

This project work, has led us to some great understandings that can help the reduction of the microbial loads on the fresh apples. We hope that these learnings will find practical applications in the industry in the years to come.

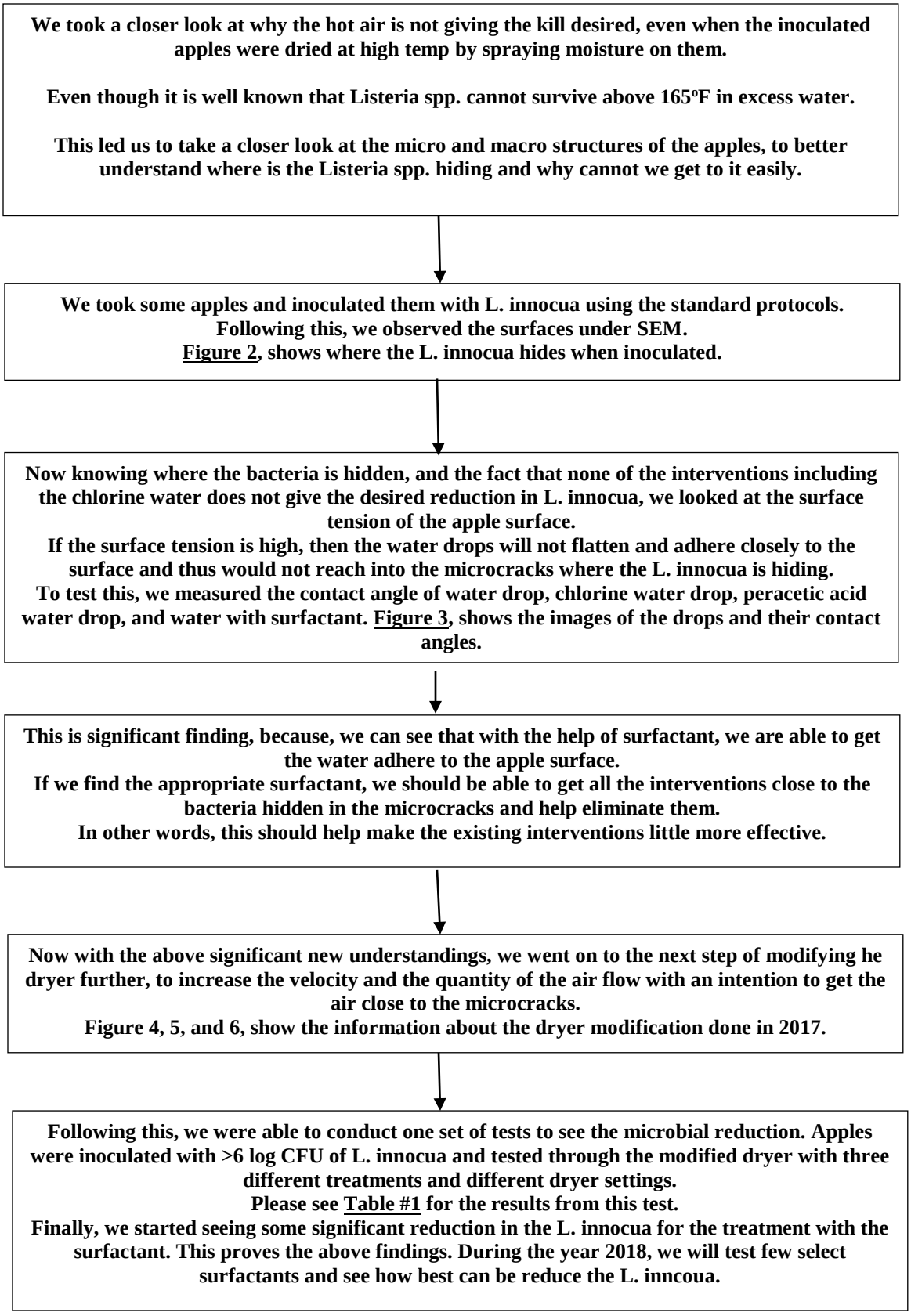


Figure 1. Summary of the project progress at end of 2017

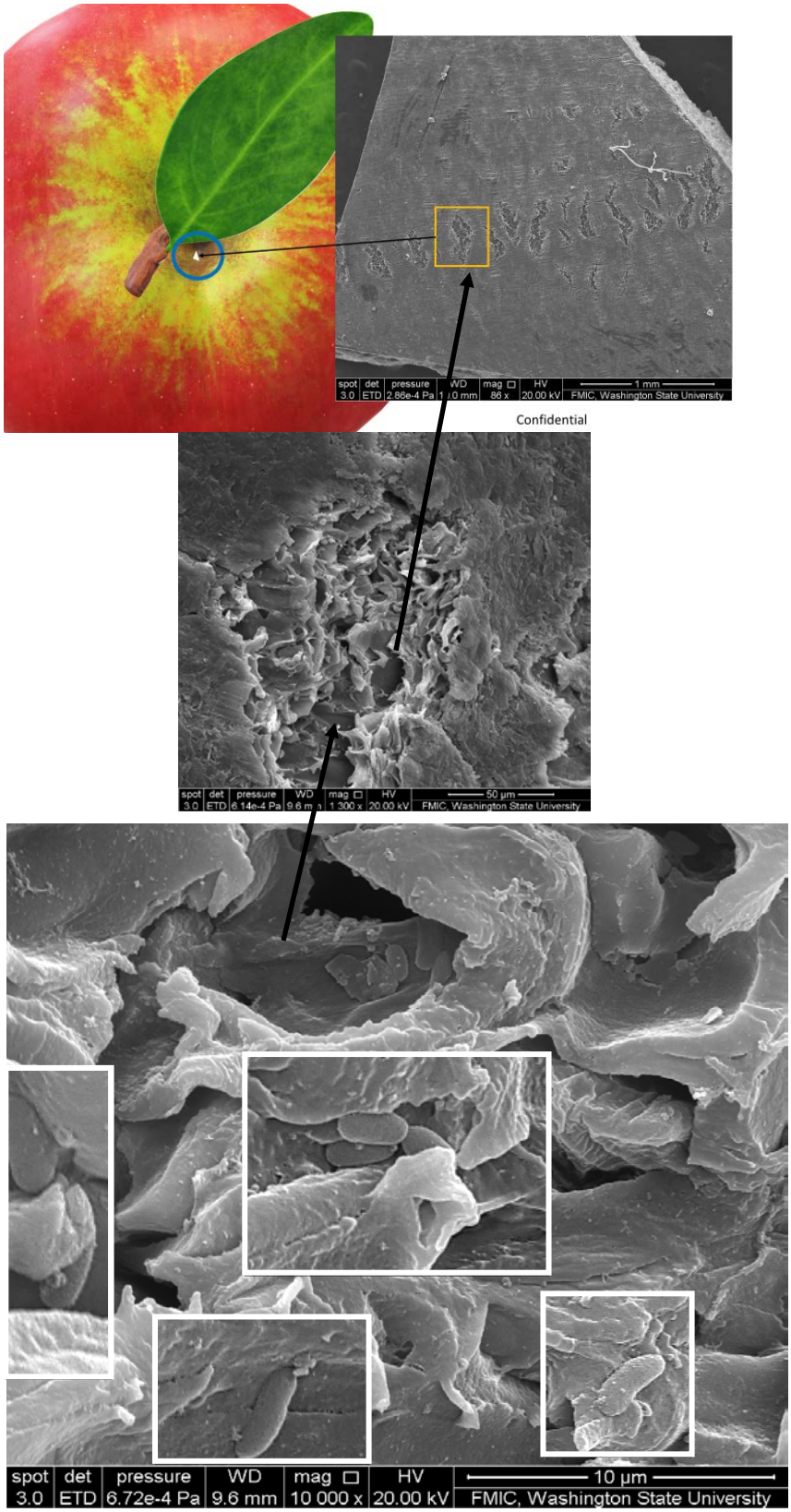
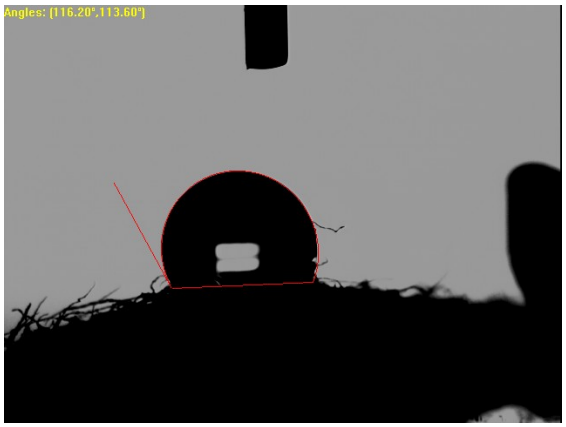
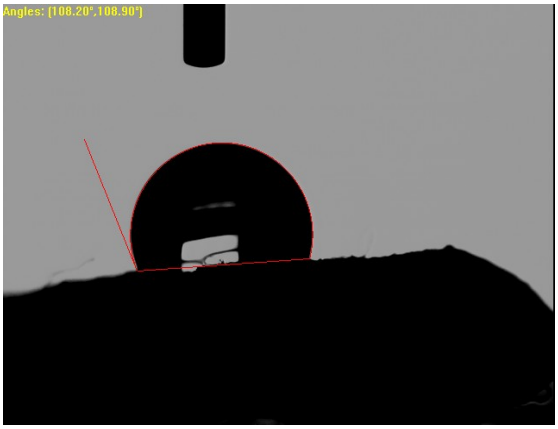


Figure 2. SEM images of the cracks in apple showing the LM hidden in very small microcracks

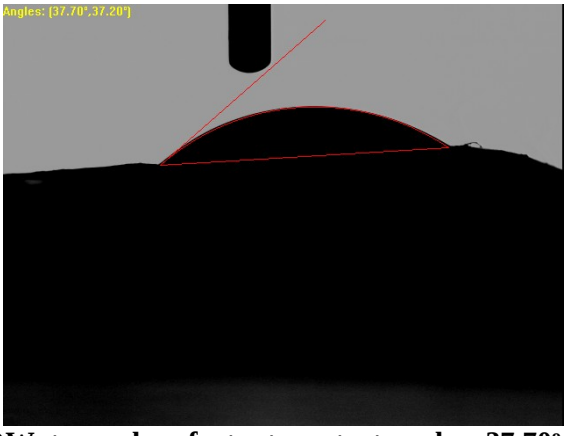


(a) Water, contact angle – 116.20º



(b) Chlorine water, contact angle – 108.20º

(c) PAA, contact angle –134.10º



(d)Water and surfactant, contact angle – 37.70º

Figure 3. Contact angle of drops of water, chlorine and water with surfactant

This shows the dryer modification in progress in the machine shop.

The goal was to –

1. Increase the air speed, impinging on the apples
2. Increase the quantity of the air flowing on to the apples

Figure 4. The modified oven/dryer in action

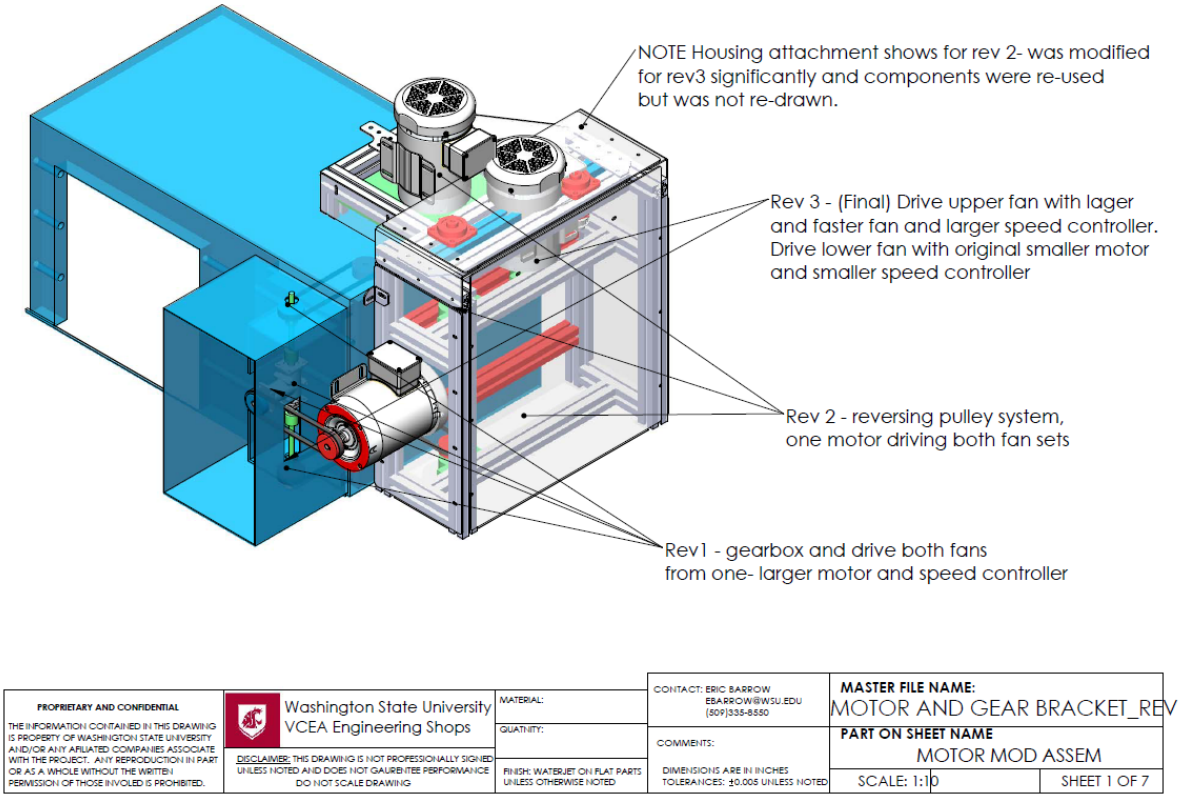


Figure 5. Modified design, showing the motor drive set-up was completely changed in 2017, to increase the air flow and speed.

Treatment(s)	Log CFU/apple	
	Average	Error Value
Control - 0h	6.14	0.03
Control - 24h	6.37	0.05
200°F+T1, 1 min	**4.73**	**0.20**
250°F+T1, 1min	**4.34**	**0.07**
200°F+T2, 1min	6.11	0.11
250°F+T2, 1min	6.07	0.12
200°F+T3, 1min	6.29	0.08
250°F+T3, 1min	6.02	0.08

Table 1. Survival of *L. innocua* on apples (for Trial 1 done in Nov 2017). Mean ± SEM, n=10.
T1 – Surfactant 1 (CytoGuard LA 2X – based on Lauric Arginate (LAE)) 2.5% ;
T2 – Surfactant 2 (Tween 20) 2.5%;
T3 – Water.

METHODS

We are providing here only the methods used for the data shown in this report. The drying protocols were provided in the previous reports and the original proposal.

Experimental procedure for microscopy and contact angle testing

- Apple peel pieces (approximately 4x4x2 mm) were gently cut from the different apple sections
- Freeze dried pieces (after fixation), were then mounted to aluminum stubs using Pelco tabs self-adhesive paper tracks.
- The stubs were gold-coated on a Hummer sputter coater and observed with a scanning electron microscope (SEM) at an accelerating voltage of 20 kV.
- Images were captured with the software **with magnifications ranging from 50x to 10,000x.**

Experimental procedure for microscopy and contact angle testing

- Contact angle was measure using VCA Optima video camera system (AST Products, Billerica, MA, USA)A drop of the liquid to be tested was dropped on the appropriate cut section of the apple peel and observed in the machine
- Images were captured along with the reading of the contact angle

Experimental procedure for microbial testing:

- Prepare 3-strain cocktail of *Listeria innocua* at ~ 10^6 CFU/ml
- Dip inoculate apples for 10 min
- Dry the inoculated apples at room temperature for 24 h
- Set the dryer to target temperature
- Spray inoculated apples with the right treatment and set on the dryer belt
- Start the conveyor with the belt speed set to give the residence time of the desired time
- Put each apple in one stomacher bag, rub, and plate immediately for enumeration

RESULTS & DISCUSSION

- Please refer to Figure 1, which gives the step by step discussion of the work accomplished in 2017.
- All the other figures and the table are discussed in the Figure 1.
- We thought this will be easier and quicker way to explain the work completed during this year.

CONCLUSIONS FROM THE STUDIES SO FAR

- The morphnology of the apple skin/peel, specifically including the microcracks plays a bigger role in hiding the microbes.
- Along with the skin morphology, another imporant aspect is the surface tension of the apple skin/peel. This plays a very critical role as well in determing the effectivesness of the different food safety interventions.
- If the contact angle of the water/chemical drops are decreased by using the appropriate surfactants, then they adhere to the skin/peel better and thus icnreasing the chances to reach the microbes hidden in the micro cracks.
- This drying method can be used effectively to reduce the current dryer footprint and thus providing the opportunity to use additional food safety interventions on the packing line.
- Drying times can be reduced significantly by using higher drying air temperatures and thus increasing the production capatcity.
- Overall, this drying technique can provide economic benefits to the packing houses

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-17-104

PROJECT TITLE: Complying with the FMSA Preventive Controls for Human Food Rule

PI: Girish M. Ganjyal
Organization: WSU, Food Science
Telephone: 509-335-5613
Email: girish.ganjyal@wsu.edu
Address: FSHN 110
City/State/Zip: Pullman, WA, 99164

Ph.D. Student: Ms. Ewa Pietrysiak

COOPERATORS: Claudia Coles (WSDA), Ines Hanrahan (WTFRC) and Various Packing Houses.

Budget: **Year 1:** $48,711 **Year 2**: **$50,260**

Other funding sources: The PI has some funds from the WSDA-SCBG program to support the one training on the PSFA-PCHF 2.5-day training. The event coordinator's (Cathy Blood) time will be covered through the WSDA grant.

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	2018
Salaries	28,418	29,555
Benefits	2,580	2,683
Wages	7,261	7,552
Benefits	452	470
Training Materials	5,000	5,000
Supplies	2,000	2,000
Travel	3,000	3,000
Miscellaneous	$0	$0
Plot Fees	$0	$0
Total	48,711	**50,260**

Footnotes:
The majority of the funding requested is to cover graduate student wages. Funds are also requested for wages to support an undergraduate student to help with the project. Funds are requested to cover travel costs related to the project work, such as trips to the packing facilities in Wenatchee and Yakima for the work related to first objective and for the trainings. Funds are also requested to cover supplies and training material costs that will be provided to the training attendees.

RECAP OF ORIGINAL OBJECTIVES

In this project, we proposed to conduct a thorough assessment of a range of apple packing lines and identify the common practices that can be improved. These assessments were further to be utilized to develop model food safety plan for the apple packing process.

The specific objectives of the proposal are as detailed below:

4) Develop a thorough assessment of the current apple and pear packing process and packing house environments.
5) Develop model food safety plans, for both apple and pear packing processes, to help comply with the FSMA-PCHF rule.
6) Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.
7) Offer one, 2.5-day training, on the FSMA-PCHF rule with an emphasis on apple and pear packing process, and two additional, 1-day trainings, focused on the implementation of the FSMA-PCHF rule.

SIGNIFICANT FINDINGS

The following were the major findings from the 1 year of work so far:

Assessment of current packing facility practices

- We visited various packing houses in the year 2017 both in Yakima and in Wenatchee areas.
- We selected packing houses of different sizes, so we get a better understanding of the current practices across the industry.
- The practices vary significantly across the industry.
- In general, there seems to be variations in the drenching of the incoming apples. The treatment of the drench water and the number of usage of this water vary a lot.
- Dump tank and flume water is generally dosed with chlorine or PAA. In some packing houses we observed the use of ozone as well.
- The constant monitoring of the level of the sanitizer in the dump tank is important. We saw variations in the readings.
- The sanitation practices across the packing houses vary significantly as well.

Food safety model plan development

- We have completed the first draft of the model food safety plan.
- The observations of the current practices from the first objective was incorporated into this plan.
- We presented this plan at the Hort Show in December in Pasco, WA.

Summary of literature on different interventions

- We reviewed a significant amount of the literature covering the various food safety interventions that are used in the fresh produce packing process including apples.
- Many interventions have shown significant log reduction of pathogens, although in ideal conditions in the laboratories.
- The surface morphology (including both the micro and macro structures) play a significant role in restricting the access to many of the interventions.

Overall, we are on track to complete all the proposed project objectives in the year 2018 well in time. As we are working on this project we are discovering new things that may help the industry in addressing the food safety concerns. We hope to report all our findings in early 2019 in the final report.

METHODS

Objective #1: Develop a thorough assessment of the current apple and pear packing process and packing house environments.

The apple packing houses vary a lot within the industry, in terms of the equipment and materials used, line set-up, food safety interventions, best practices and automation levels. The apple industry has evolved significantly in the last few years, with their approach to food safety. This has had positive effects on the level of food safety throughout the industry.

In this objective, we will expand on the information we already have, by thoroughly documenting the complexities of the apple packing process. This will be done by visiting many packing lines and having detailed discussions and completing the detailed survey on food safety practices by the experts in the different packing houses. Along with the packing houses, we will also work with the line manufacturers such as Van Doren Sales, Inc. and others. This effort will be very collaborative, and we expect to seek assistance from the WTFRC and the Northwest Horticultural Council for this effort.

Importance will be given to each and every step of the packing process during this thorough assessment process. For each of the steps, best practices will be documented. The outcome of this objective will include a document detailing, i) a detailed flow chart with description for each step in the process; ii) description of all the inputs at each step, including the ingredients, chemicals etc; iii) description of potential hazards at each step; and iv) best practices for each of the steps.

During the development of this document, efforts will be made to consider all aspects of the new FSMA-PCHF rule. This will assist in the compliance with the new rule.

Objective #2: Develop a model food safety plan for apple and pear packing processes to help comply with the FSMA Preventive Controls for Human Food Rule.

Thus in this objective, we will make an effort to develop a model food safety plan for the apple packing processes. Specific attention will be given to all the different preventive controls that can be used in the apple packing process.

The PI will work with all the cooperators to develop this plan and submit it to the FSPCA for approval. If this can be approved, it can be widely used within the industry for training purposes. This will also help all the packing houses to use the standardized model as a starting point, which can be updated based on their facility and specific situations.

Objective #3: Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.

The interventions in food safety have to be based on scientific data. There has been a tremendous amount of research conducted in the literature on various interventions in other industries. In the apple packing process, interventions such as use of ozone, chlorine, PAA, steam and others are

currently used. There has also been many other potential technologies and chemicals marketed to the industry.

It becomes the burden of the industry to find appropriate peer reviewed literature to support the use of these technologies. Thus in this objectives we will make an effort to conduct a thorough literature review on the effectiveness of these technologies. This will serve as useful information for the apple and pear packers as they make decisions on the use of the different interventions. This information will also be useful for developing the food safety plans.

Further, this will help identify appropriate research gaps that exist within the apple packing process. This information can potentially be used by the WTFRC for future direction of research.

Objective#4: Offer one, 2.5-day training, on the FSMA-PCHF rule with an emphasis on apple and pear packing process, and two additional, 1-day trainings, focused on the implementation of the FSMA-PCHF rule.

Training is essential component of the FSM-PCHF rule. Training with the developed food safety model specific to the apple packing process will help in adapting common practices across the industry.

RESULTS & DISCUSSION

i) Develop a thorough assessment of the current apple and pear packing process and packing house environments:

- The food safety practices vary significantly across the industry due to production size, facility design, equipment and chemical used.
- Variations are seen in application of fungicide, drench water treatment, and apple cleaning system.
- Dump tank and flume water is typically treated with chlorine or PAA at different concentration levels, with or without pH and temperature control. In some packing houses we have observed the use of ozone as well.
- In some facilities target concentration of sanitizer differed from actual value. The constant monitoring of the level of the sanitizer in the dump tank is important.
- In 2018 we would like to continue data collection by visiting different apple packing facilities and requesting completion of survey.
- Based on the gathered information we will create the report with a detailed description of apple packing process including inputs at each step, potential hazards and best practices.
- This will assist in sharing the examples of best practices which in turn will help to comply with FSMA-PCHF rule.

ii) Develop a model food safety plan for apple and pear packing processes to help comply with the FSMA Preventive Controls for Human Food Rule:

- The first draft of the model food safety plan for apple packing house has been completed.
- During January 2018, we will circulate this plan to the food safety teams across the apple industry for feedback.
- The plan will be revised based on industry feedback and submit to the FSPCA for approval.

- The food safety plan model can help all the packing houses to create or modify food safety plan and meet new regulation requirements.

(iii) Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.

- Review of numerous scientific publications on fresh produce decontamination during packing process have shown significant log reduction of pathogens, although in controled, laboratory conditions or with exceeding allowed concentration of chemical.
- Surface morphology analysis have indicate significant role of micro and macro structures in restricting the access to many of the interventions.
- The optimum intervention for apple decontamination is still to be developed. Findings about the surface morpholgy and bacteria attachment might help in future research.
- The review focuses on food safety interventions to control Listeria monocytogenes in fresh produce packing industry and will also include information about sources of contamination, bacteria attachement mechanism, and survival.
- That will provide complementary information about the L. monocytogenes, its nature and mechanisms of action.

CONCLUSIONS FROM THE STUDIES SO FAR

- The food safety practices, and level of advancement vary significantly across the industry. Visits and surrey have shown differences in chemicals, equipment, and procedures used.
- Based on (1) visits to various facilities, (2) survey outcomes, (3) scientific literature review, the first draft of food safety plan model was created and will be shared with the industry early January 2018.
- The apple peel surface morphology play a significant role in decreasing decontamination treatments efficiency.
- More research is needed to develop optimum method for apple decontamination.

CONTINUING PROJECT REPORT **Second Year Report**

WTFRC Project Number: AP-16-100A

Project Title: Ozone in apple storage: microbial safety and decay management

PI: Meijun Zhu
Organization: WSU
Telephone: 509-335-4016
Email: meijun.zhu@wsu.edu
Address: 100 Dairy Road, 106 FSHN
City/State/Zip: Pullman/WA/99164

Co-PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S. 18th St., Suite 100
City/State/Zip: Yakima, WA 98903

Co-PI: Achour Amiri
Organization: WSU-TFREC
Telephone: 509-663-8181 ext 268
Email: a.amiri@wsu.edu
Address: 1100 N Western Avenue
City/State/Zip: Wenatchee, WA, 98801

Cooperators: Allan Brothers. Inc., Stemilt Growers LLC.,

Total Project Request: **Year 1:** $104,707 **Year 2:** 108,515 **Year 3: no request**

Other funding sources

None

WTFRC Collaborative expenses: $7,000 per year

Item	2016	2017	2018
Salaries			**0**
Benefits			**0**
Wages	5,000	5,360.81	**0**
Benefits	2,000	1,769	**0**
Total	7,000	7,130	**0**

Footnotes: Hanrahan has spent 2% of her time on the project.

Budget 1: Meijun Zhu

Organization Name: WSU-Pullman **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** katy.roberts@wsu.edu

Item	2016	2017	2018
Salaries[1]	$22,000	$22,880	**0**
Benefits	$10,193	$10,601	**0**
Wages[2]	$27,565	$28,667	**0**
Benefits	$5,623	$5,848	**0**
Supplies[3]	$25,326	$26,219	**0**
Travel[4]	$2,000	$2,300	**0**
Miscellaneous[5]	$5,000	$5,000	**0**
Total	$97,707	$101,515	**0**

Footnotes:

[1/] Technical support: four-month salaries plus benefits are requested. For a research Intern (0.4 FTE) at 42.4% benefit rate.

[2/] PhD graduate student partial stipends and undergraduate assistant wages. Timeslip wages for 16 weeks.

[3/] Chrom *Listeria*, MOX plates, BLEB and selective reagents (Acriflavine hydrochloride, Nalidixic acid, Natamycin, Moxalactam), Chrom ECC, TSBYE and TSAYE, PCR reagents, other chemicals and medium for microbial culture and identification; Disposable consumable and supplies including dynabeads *Listeria*, filtration membrane, spreader, petri dishes, Whirl-PAK bags, PCR strips and 96 well plates, microtubes, pipette tips, serological pipettes, autoclave bags and others. Supplies include Petri dishes and artificial media for fungal growth.

[4/] Travel funds are requested to cover travel costs related to the project work such as trips to the packing facilities in central Washington for fruit collection and in plant testing. Travel to packing houses for trials and fruit sampling.

[5/] We have all instruments needed for proposed studies. Funds are requested to partially cover the Biosafety Level 2 facility and equipment maintaining fees and Biohazard disposal fees.

OBJECTIVES

1. Examine fate of *Listeria* and natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of continuous low doses of ozone.
2. Evaluate the efficacy of continuous low doses of ozone on postharvest decay
3. Examine the effect of ozone in the storage environment on final fruit quality.

SIGNIFICANT FINDINGS

1. *Listeria monocytogenes* is a tough foodborne pathogen. A limited reduction of *L. monocytogenes* on apple surfaces occurred during 12 weeks of refrigerated storage either at 1°C/33°F, 4°C/39.2°F or 10°C/50°F. There was about ~1 Log reduction when stored at room temperature (22 °C / 71-72 °F).
2. We determined a ~3-Log reduction of *Listeria* on Fuji apples after 30 weeks of cold storage under a commercial RA and CA storage environment. Continuously low dose ozone gas application (87.0 ± 38.8 ppb) in CA storage generated an additional 2-Log reduction.

   Additional 2-week storage under RA beyond their respective initial storage treatments had little influence on *L. innocua* survival.
3. Natural bacterial count of Fuji apples stored at CA/RA remained stable throughout storage. Continuous low dose ozone gradually resulted in about 1-Log reduction after 30-week storage.
4. Indigenous yeasts/molds (Y/M) count of un-inoculated Fuji apples stored in RA remained relatively stable during first 12 weeks of storage. By the end of the 30-week storage, the Y/M count of RA stored apples increased about one log. The Y/M count of Fuji apples in CA room remained relatively stable over 30 weeks of storage. There is about 0.6-Log reduction of Y/M count in Fuji apples of CA with ozone storage during first 12 weeks. Nevertheless, the inhibitory effect of ozone was compromised with prolonged storage time.
5. Continuous low doze ozone gas used at this study had no negative influence on apple visual quality, including both external and internal disorders.
6. Low dose ozone did not reduce the incidence of blue mold, gray mold and bull's eye rot and reduced severity (lesion diameters) very slightly (10 to 20%) on wounded and artificially inoculated Fuji and Granny Smith after 4 months of storage.
7. Low dose ozone reduced the density of spores of *Penicillium expansum*, *Botrytis cinerea*, and *Neofabraea perennans* on non-wounded fruit by 4 to 90%.
8. Low dose ozone did not reduce residue levels of TBZ, pyrimethanil or fludioxonil on the surface of the fruit after 5 months of storage.
9. Data show that cold storage with or without continuously low dose ozone can be an additional hurdle for controlling *Listeria* on apple fruits; however, it can not completely eliminate *Listeria*.
10. A systematic/hurdle approach is needed to ensure apple microbial safety.

METHODS

We have established methods for proposed objective 1-3 studies as detailed in the following.

Objective 1: Examine fate of *Listeria* and natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of ozone.

a. Examine fate of *Listeria* on apple fruit surfaces under different cold storage conditions

1. 3-strain *Listeria* inoculum preparation and established on apple surface

A 3-strain *L. monocytogenes or L. innocua* cocktail was prepared via mixing equal numbers of each respective strain suspension. A 3-strain *L. monocytogenes* cocktail was used for examining the fate of *L. monocytogenes* on fresh apple under different temperatures in food microbiology lab

(Biosafety level 2), while 3-strain *L. innocua* cocktail was used for studying the fate of *Listeria* on fresh apple during different cold storage with or without ozone in a commercial packing facility.

Apples were individually and separately inoculated to establish 1×10^{6} or $1\times10^{3\text{-}4}$ CFU/apple of 3-strain cocktail of *Listeria* strains through dipping inoculation, and held at room temperature for 24 h prior to different storages.

2. Storage treatments in BSL2 food microbiology lab

Apples established with high and low levels of *L. monocytogenes* were randomly separated into four groups and subjected to different temperature storages (1 °C, 4 °C and 10 °C) for up to 12 weeks. Apples under different storage conditions were sampled at 0d, 1d, 4d, 7d, 14d, 28d, 56d and 84d of storage to analyze the survival of *L. monocytogenes* on fresh apples.

3. Cold storage treatments in a commercial packing facility

Fuji apples inoculated with ~1×10^{6} CFU/apple of *L. innocua* were randomly separated into three groups and subjected to three different storages: refrigerated air (RA, 33 °F), controlled atmosphere (CA, 33 °F, 2 % O_2, 1 % CO_2), and CA with a low dose (87.0 ± 38.8 ppb) ozone (CA+O_3) for up to 30 weeks. Apples under different storage conditions were sampled at 0, 1-, 3-, 6-, 12-, 18-, 24-, and 30-week of storage to analyze the survival of *L. innocua* on fresh apples.

4. Microbial analysis

At each sampling day, apples under the respective storage condition were sampled and transferred to sterile bags with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag to release attached microorganism, then serial diluted. Appropriate dilutions were plated on agar plates. Plates were incubated at 35˚C (95˚F) for 24 ± 2h and enumerated manually.

b. To evaluate natural microbial reduction on apple fruit surfaces under different cold storage conditions

1. Cold storage treatments in a commercial packing facility

Non-waxed, non-inoculated Fuji apples were subjected to different storage conditions (RA, CA and CA+O_3) as described previously. Apples were sampled at 0-, 6-, 12-, 24, and 30-week of storage for total plate count and yeast and mold enumeration.

2. Survival microorganism analysis

At each sampling day, apple was sampled and transferred to a sterile bag with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag, rub to release attached microorganism, then serial diluted. The appropriate dilution was plated onto TSAYE plates for total plate count and potato dextrose agar (PDA) plates for yeasts and molds, respectively. Colonies were counted manually after incubation at 35°C (95˚F) for 48h.

Objective 2. Evaluate the efficacy of continuous low doses of ozone on postharvest pathogens

a. Evaluate the efficacy of ozone on pre-wounded and inoculated apple fruits.

1. Apple surface disinfection and wounding

Freshly harvested organic apples of the selected varieties were surface-disinfected in 0.8% sodium hypochlorite, rinsed with tap water, and air-dried. Then, fruits were wounded using a sterile-3-mm diameter cork-borer at the stem-end zone of each fruit (2 wounds/fruit).

2. Fungal inoculation

Surface disinfected fruits were inoculated with 20 µl of a spore suspension of *Penicillium expansum* (blue mold), *Botrytis cinerea* (gray mold), *Neofabraea perennans* (bull's eye rot), *Sphaeropsis pyriputrescens* (Sphaeropsis rot), *Mucor piriformis* (Mucor rot*), Rhizopus stolonifer*

(Rhizopus rot) or *Phacidiopycnis washingtonensis* (Phacidiopycnis rot). Each pathogen was inoculated at three different concentrations i.e. 10^3, 10^4, and 10^5 spores/ml. Four replicates of 20 fruit each was used for each pathogen/spore concentration combination.

In October of 2017, four replicates of 20 fruit each on distinct Fuji trees at Sunrise Research orchard in Chelan county using a completely randomized design were sprayed with a spore suspension at 4×10^5 spores/ml of *P. expansum*, *B. cinerea*, *N. perennans*, and *Mucor piriformis* one week prior to harvest except for *P. expansum* and *M. piriformis,* which were inoculated postharvest.

3. Cold storages and apple analyses

Inoculated apples/fruits from each tree/pathogen combination were harvested and subjected to different cold storage (CA, and CA+O_3) as described above. The incidence and severity of blue and gray, mucor, and Rhizopus molds, bull's eye rot, and Sphaeropsis and Phacidiopycnis rots (slow pathogens) were determined after 2 weeks, 2 and 4 months, respectively.

4. Evaluate the efficacy of radical (wet) ozone versus gaseous ozone against aforementioned pathogens

Non-wounded fruits were sprayed with spore suspensions of *P. expansum* and *B. cinerea*, were treated with continuous low dose gaseous ozone (60-80ppm) or with 20 ppm radical ozone (fogged ozone) or 45 min. Disease incidence and severity were determined.

b. Assess ozone efficacy on natural infections caused by major pathogens in combination with or without fungicides.

1. Interaction of ozone and postharvest fungicides on natural infections occurring in the orchard or in storage rooms

Fuji apples at commercial maturity were drenched with either TBZ, fludioxonil (Scholar), pyrimethanil (Penbotec), or and difenoconazole suspension for 1 min at the label rates, then were immediately subjected to CA or CA plus low dose ozone cold storage, respectively. Apples were sampled before storage, 3-week and 4-month after storage to being analyzed for fungicide residue levels to determine whether ozone degraded fungicides when applied simultaneously.

2. Examine the potential negative effect of ozone on the efficacy of fungicides

Fuji apples were drenched with fungicides as described above, inoculated with spore suspensions of *P. expansum* at 5×10^4 spores/ml and immediately stored in CA, CA with low doses of ozone gas. Apples were sampled at 3 months of storage to assess fungicide efficacy in ozonated and non-ozonated rooms.

Objective 3: Examine the effect of ozone in the storage environment on final fruit quality.

1. Fruit quality analysis

Fruit maturity and quality measurements such as firmness, total soluble solids and titratable acidity were performed at harvest, after storage and following an additional week of storage at room temperature. Briefly, fruit firmness was assessed with a fruit texture analyzer using a 1 cm diameter probe on a peeled area of ~3 cm^2 on both sun and shade side of the apples. Total soluble solids (TSS) were evaluated using Atago PR-32 digital brix refractometer. Titratable acidity (TA) of fruit juice was measured with a potentiometric titrator. Measurements of each parameter were repeated four times independently with a sample size of 10 apples per replication per storage regimen.

2. Disorder analysis

The incidence of disorders was assessed after cold storage followed by one day at room temperature (RT) for external disorders and 7 days at RT for both internal and external disorders. The absence or presence of the following external disorders was visually inspected and recorded: ozone burn, superficial scald, lenticel decay, visible decay, sunburn, russet, and CO_2 damage. Apples were sliced 3 times to determine the presence of any internal disorders including watercore, internal

browning, or cavities. Sample size for both external and internal disorder analysis will be 50 apples per replication per storage regimen, with 4 replicates for each analysis.

RESULTS AND DISCUSSION

Objective 1

1. Fate of *L. monocytogenes* on fresh apples of selected varieties during storage at different temperatures

Currently, there is barely any information availbale on how easily *L. monocytogenes* survives on fresh apples under different storage conditions. Thus, we first did a short term storage study with *L. monocytogenes* established on fresh apples of selected varieties (Fuji, Granny Smith) under different storage temperatures. In this study, we chose the following storage temperatures per stated reasons.

- 1 °C (33 °F, a typical cold storage temperature).
- 4 °C (36 to 38 °F, a temperature commonly used for Honeycrisp long-term storage).
- 10 °C (50 °F, a temperature condition often used for Honeycrisp in preparation for storage).
- 22 °C (72°F, mimic situation of consumer purchased apples which are put on their kitchen count before consumption, though unlikely in commercial scenario).

During two weeks of short-term storage, *L. monocyotogenes* level on organic Granny Smith apples stored at 1, 4, and 10 °C stayed stable, though there was ~0.3 Log CFU/apple after 1-day storage (Figure 1A). More *L. monocyotogenes* reduction was observed when organic Granny Smith apples were stored at 22°C; there was ~1.0 Log CFU/apple reduction after 14-day storage (Figure 1A). Similar survival patterns of *L. monocytogenes* on conventional Granny Smith apples (Figure 1B) and Fuji apples (Figure 1C) were observed during the 14-day storage.

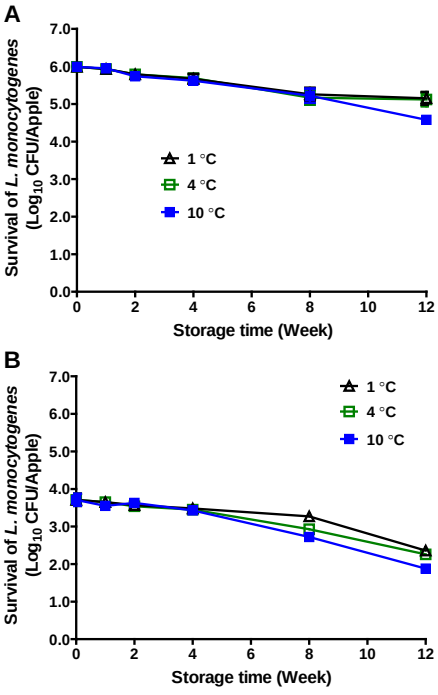


Fig. 2. Survival of *L. monocytogenes* on Fuji apples during 3-month cold storage. A. High inoculation level; B. Low inoculation level. Mean ± SEM, n=12.

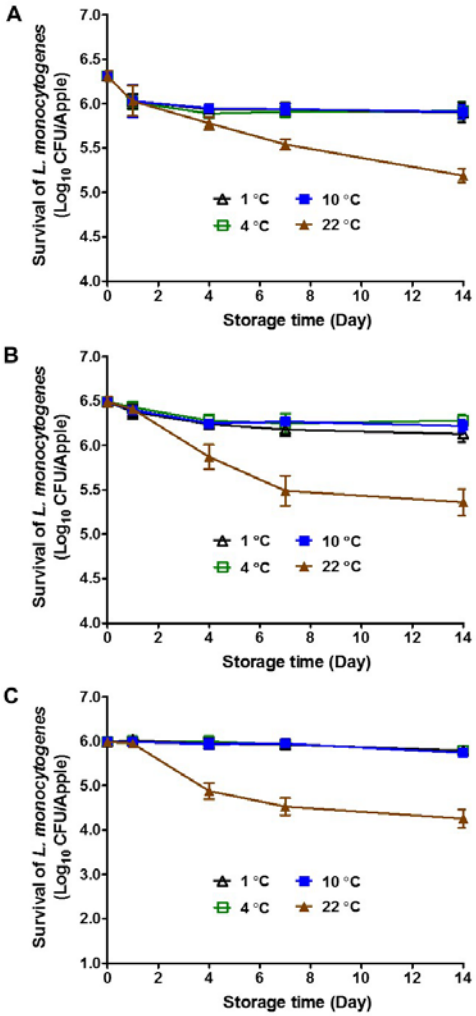


Fig. 1. Fate of *Listeria monocytogenes* on fresh apples during short-term storage under different temperatures when inoculated at 1×10^6 CFU/apple. A. Organic Granny Smith; B. Conventional Granny Smith; C. Conventional Fuji. Mean ± SEM, n=12.

We further examined fate of *L. monocytogenes* on fresh apples during 12-week cold storage. Very limited die-off of *L. monocytogenes* was observed on fresh Fuji (Fig. 2) and Granny Smith apples (data not shown) during the 12-week of cold storage, whether apples were inoculated with high level (Fig. 2A) or low level (Fig. 2B) of *L. monocytogenes*. There was no significant difference in survival of *L. monocytogenes* among the three storage temperatures (Fig. 2).

2. Fate of *L. innocua* established on fresh apples during under different cold storage at a commercial packing facility

We further conducted a cold storage experiment in a typical commercial apple facility using *L. innocua* inoculated apples in which Fuji apples were inoculated and established to 6.20 ± 0.06 Log_{10} CFU/apple before being subjected to RA, CA, and CA with 87 ppb ozone gas. A rapid reduction of 1.4-1.8 Log_{10} CFU/apple was observed within the first 3 weeks in all three storage conditions (Fig. 3). In the following 15-week storage, *L. innocua* on Fuji apples under RA and CA storage behaved similarly with about a 2.6 Log reduction. Interestingly, by the end of the 30-week storage, bacterial count on apples in RA decreased by ~0.9 Log_{10} CFU/apple compared with 18-week, while that in CA storage remained the same (Fig. 3). For CA storage with 87 ppb ozone gas, *L. innocua* population was reduced by ~5.0 Log after 24-week storage and remained at the similar level till 30-week. To mimic packing facility practice, apples in CA and CA with ozone storage were moved to RA by the end of the 30-week storage, at which time *L. innocua* was enumerated. Additional 2-week storage under RA beyond their respective initial storage treatments had little influence on *L. innocua* survival (Fig. 3).

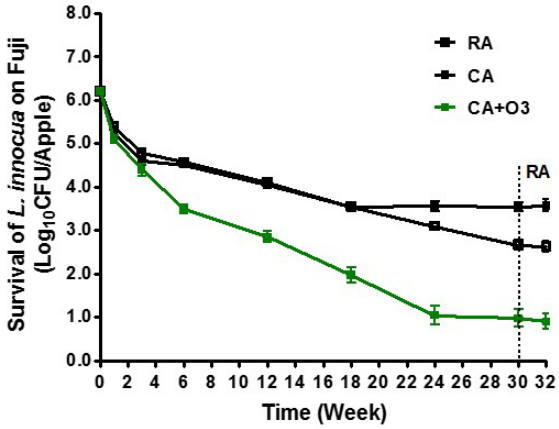


Fig. 3. Survival of *Listeria* on Fuji apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87±39 ppb ozone. Mean ± SEM, n=40.

3. Natural microbial reduction on apple fruit surfaces under different cold storage conditions

Another set of Fuji apple fruits (non-waxed and non-inoculation) was subjected to different storage conditions (RA, CA and CA with ozone), total plate count (TPC) and yeasts/molds (Y/M) count were evaluated as described above. The initial TPC and Y/M count were 3.44 ± 0.07 and 4.77 ± 0.07 Log_{10} CFU/apple, respectively (Fig. 4). TPC remained stable on Fuji apples in RA and CA storages throughout storage (Fig. 4A). Continuous application of ozone at low doses gradually achieved ~1.0 Log_{10} CFU/apple reduction of TPC after 30-week storage (Fig. 4A). At 12 weeks, Y/M count in RA and CA storage remained relatively stable, while the Y/M count in CA + O_3 was reduced by ~ 0.6 Log_{10} CFU/apple (Fig. 4B). Nevertheless, the inhibitory effect of CA ± O_3 was compromised with prolonged storage time. By the end of the 30-week storage, the Y/M count of apples in CA and CA ± O_3 reached 4.89 ± 0.05 and 4.63 ± 0.04 Log_{10} CFU/apple, respectively (Fig. 4B). Y/M count on apples under RA storage stayed at the same level within the first 12-week storage, increased by ~1.0 Log_{10} CFU/apple from the 12th to the 24th week of storage, then remained constant in the last 6 weeks of storage (Fig. 4B)

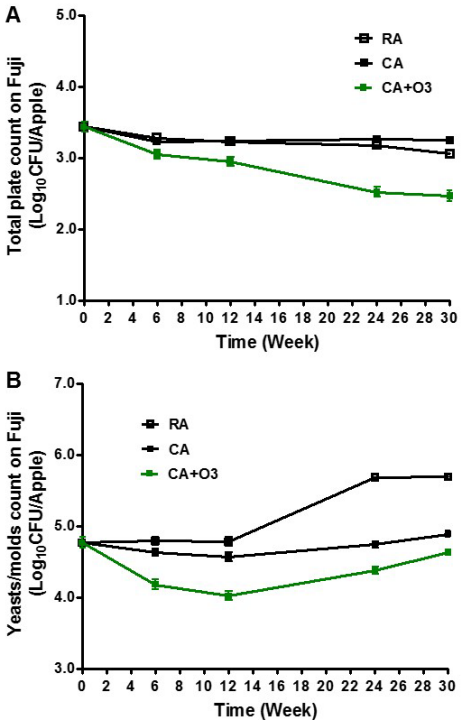


Fig. 4. Fuji apple decay during cold storages. A. TPC. B. Y/M count. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87±39 ppb ozone. Mean ± SEM, n=40.

Objective 2

1. Efficacy of ozone on artificially wounded and inoculated fruit and non-wounded fruit

After 60 days of storage, incidence of blue mold, gray mold and bull's eye rot was reduced by 52, 45 and 60%. After 90 days, respective reduction decreased to 18, 12 and 20%, respectively (Fig. 5A). On non-wounded fruit, ozone reduced spore loads of blue mold, gray mold and bull's eye by 78, 62 and 4%, respectively after 90 days of storage. After 180 days, reduction of blue mold spores decreased to 58% but remained steady for the two other pathogens (Fig. 5B).

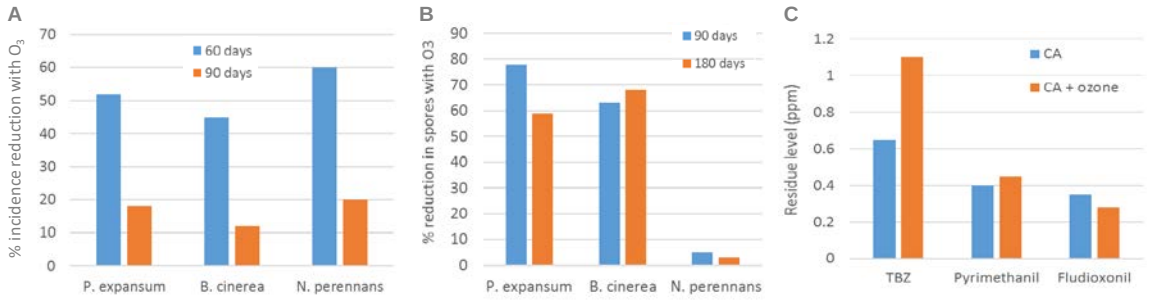


Fig. 5. Reduction of incidence of blue mold, gray mold and bulls eye rot on wounded fruit (A, wounded fruit) and of spore load on non-wounded fruit (B, surface inoculation) treated with ozone relative to non-ozonated fruit. Residue levels of TBZ, pyrimethanil, and fludioxonil on Fuji fruit stored in CA and CA with ozone for 6 months (C).

2. Interaction of postharvest fungicides with a low dose of ozone on non-wounded fruit

Gaseous ozone at 60 to 80 ppb did not decrease residue levels of TBZ, pyrimethanil or fludioxonil after 6 months of storage (Fig. 5C).

Objective 3

1. Examine the effect of ozone in the storage environment on final fruit quality

Quality parameters of apple fruits under different treatments were assessed both at harvest and after storage. Firmness decreased after storage for all apples, and no difference was found between CA and CA + O_3 storage. Apples subjected to RA storage had a significantly lower firmness than those in CA with or without gaseous ozone storage. Compared to harvest levels, TSS levels did not change in apples post-6-month storage in RA, CA, or CA + O_3. TA after storage decreased to between one third to two thirds of the TA at harvest. TA reduction in apples was significantly mitigated by CA storage, while addition of ozone had no impact on TA.

The incidence of external and internal disorders was visually evaluated at the end of each storage treatment. Overall, the parameters evaluated for either external disorders or internal disorders were not significantly different among apples stored under RA, CA, or CA + O_3. No ozone burn, lenticel breakdown, decay, or CO_2 damage was found in any apples subjected to 6-month of low-dose ozone gas storage. No watercore, internal browning, or cavity was observed in apples stored under CA or CA + O_3. A small number of apples under RA stored were found to have watercore and internal browning, but the incidence rate was not significantly different from those fruit kept under CA storage.

Conclusion

Continuous low dose ozone gas has the potential to be applied in the apple as well as other fresh produce industries with similar practices as a supplemental intervention method to ensure fresh produce safety.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-17-102

Project Title: Control of *Listeria* on processing surfaces in apple packing facilities

PI: Meijun Zhu
Organization: WSU
Telephone: 509-335-4016
Email: meijun.zhu@wsu.edu
Address: 100 Dairy Road, 106 FSHN
City/State/Zip: Pullman/WA/99164

Co-PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S. 18th St., Suite 100
City/State/Zip: Yakima, WA 98903

Cooperators: Stemilt Growers LLC.; McDougall & Sons; Hansen Fruit; Washington Fruit; Allan Bros Fruit; Josh Tucker, Pace International; Guardian Manufacturing, Inc.

Budget: **Year 1**: 98,447 **Year 2**: **101,752** **Year 3**: 105,882

Other funding sources: None

WTFRC collaborative expenses:

Item	2017	**2018**	2019
Salaries	1,573	2,172	2,172
Benefits	1,049	1,305	1,305
Wages	2,750	2,750	2,750
Benefits	825	825	825
Total	6,197	**7,052**	7,052

Footnotes: Dr. Hanrahan is committing to spend 2%/year of her time on this project, Mendoza will supervise Wenatchee based team members (0.5% of her time in year 1, 2% in years 2&3). Timeslip wages are calculated at $11/hr. plus benefits for 250 hours/year.

Budget 1: Meijun Zhu

Organization Name: WSU-Pullman **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	**2018**	**2019**
Salaries[1]	$13,562	$19,889	$20,685
Benefits[1]	$4,386	$6,094	$6,338
Wages[2]	$38,054	$30,773	$32,003
Benefits[2]	$3,248	$3,300	$3,432
Supplies[3]	$26,000	$26,644	$27,872
Travel[4]	$2,000	$3,000	$3,500
Miscellaneous[5]	$5,000	$5,000	$5,000
Total	$92,250	**$94,700**	$98,830

Footnotes:

[1/] Postdoc research associate and professor's salaries plus benefits.

[2/] PhD graduate student stipends and undergraduate assistant wages plus benefits.

[3/] Chrom *Listeria*, MOX plates, BLEB and selective reagents (Acriflavine hydrochloride, Nalidixic acid, Natamycin, Moxalactam), Chrom ECC, 3M Petrifilm™ for enterobacteriaceae and *E. coli*/Coliform, TSBYE and TSAYE, buffered peptone water, Dey/Engley neutralizing broth, PCR probe/primer and reagents, other chemicals and medium for microbial culture and identification; infrared pyrometer, Disposable consumable and supplies including filtration membrane, sponge swabs, large swabs, Q-tip swabs, Whirl-PAK bags, stainless steel, PVC/PS coupons, PCR strips and 96 well plates, microtubes, pipette tips, serological pipettes, spreader, petri dishes, autoclave bags and others.

[4/]Travel funds are requested to cover travel costs related to the proposed studies such as trips to the packing facilities in central Washington for sample collection and testing.

[5/] We have all instruments needed for proposed studies. Funds are requested to partially cover the Biosafety Level 2 facility and equipment maintaining fees and Biohazard disposal fees.

OBJECTIVES

4. Assess the antimicrobial efficacy of ozonated water against *L. monocytogenes* biofilm on different surfaces, which will be further comprehensively compared with commonly used sanitizers, quaternary ammonium compounds, chlorine dioxide and peroxyacetic acid.
5. Examine the antimicrobial efficacy of steam alone and in combination with selected sanitizers against *L. monocytogenes* biofilm on different surface materials.
6. Validate antimicrobial efficacy of superheated steam and/or selected sanitizers in apple packing lines using parameters selected based on laboratory testing

PRELIMINARY DATA AND SIGNIFICANT FINDINGS

11. Ozonated water 1-min treatment at 1.0, 2.0 and 4.0ppm resulted in ~0.9-, 3.4- and 4.1- log reducation of *L. monocytogenes* single strain biofilm grown on polystyrene (PS) surfaces, respectively.
12. QAC 1-min intervention at 100, 200 and 400ppm led to ~2.4-, 3.2- and 3.6- log reducation of *L. monocytogenes* single strain biofilm grown on PS surfaces, respectively.
13. Chlorine 1-min intervention at 100/200 ppm and chlorine dioxide at 2.5/5.0 ppm resulted in ~2.0-/3.1- and ~2.4/3.8 log reducation of *L. monocytogenes* single strain biofilm grown on polystyrene (PS) surfaces, respectively.
14. PAA 1-min treatment at 80 and 160 ppm resulted in ~2.9- and 3.3-log reducation of *L. monocytogenes* single strain biofilm grown on PS surfaces, respectively.
15. Antimicrobial efficacies of all sanitizers except PAA against *L. monocytogenes* mixed strain biofilm were reduced when compared to single strain *L. monocytogenes* biofilm.
16. Furthermore, antimicrobial efficacies of all sanitizers against 7-day-old biofilm were reduced when compared 2-day-old biofilm; antimicrobial efficacy of PAA is relative less influenced by age of biofilm.
17. Organic conditioning dramatically impacted antimicrobial efficacy of all sanitizers, with ozonated water was most affected. PAA 1-min treatment at 160ppm can still result in 3.4 log reduction against 7-day-old biofilm on PS conditioned with organic matter; further increase concentration did not improve its efficacy dramatically.

METHODS

We have established methods for proposed Objective 1 studies as detailed in the following.

Objective 1: Assess the antimicrobial efficacy of ozonated water against *L. monocytogenes* biofilm on different surfaces, which will be further comprehensively compared with commonly used sanitizers, quaternary ammonium compounds, chlorine dioxide or peracetic acid.

1. Strain selection

To elucidate the impact of strain variability on biofilm formation and sanitizer's antimicrobial efficacy, six strains of *L. monocytogenes* were evaluated. These *L. monocytogenes* strains were either outbreak strains or processing plant/food isolates. They have been kept at -80°C until used.

2. Selection and preparation of food contacting surfaces

Stainless steel, polyvinyl chloride (PVC), polyester (PET) and polyethylene (PE) along with PS were selected representing most commonly used surface materials. The selected surface sheet was cut into 0.75 cm × 1.5 cm coupons for *Listeria* biofilm growth. PS biofilm was conducted in sterile 96-well plates.

Preparation of test surfaces: The coupon of different surfaces was soaked in methanol overnight, ultra-sonicated once, rinsed thoroughly with distilled water, soaked in 70% ethanol overnight, finally air-dried under biosafety cabinet, which is then ready for use.

Organic matter conditioning: The above prepared surfaces were exposed with 1:10 diluted apple juice (Old Orchard) prior to be subjected to *Listeria* biofilm growth and sanitizer treatments.

3. *Listeria* biofilm formation on different surface materials

Inoculum preparation: Before inoculation, respective strains were twice activated in TSBYE broth, washed and re-suspended in Modified Welshimer's Broth (MWB) broth to achieve the target population density.

Biofilm formation on PS surface in 96 well plate: A100 µl of the above prepared cultures were transferred into each well of 96 well microtiter plate. The plates were covered and incubated at room temperature (22°C/72°F) for 2 or 7 days statically to form biofilm using our established method, 6 wells per sanitizer treatment of each experimental day.

Biofilm formation on different surfaces: All four surface coupons (conditioned with/without organic matter) will be transferred to 10 ml of *Listeria* suspension in MWB broth (10^7 CFU/ml) prepared as described above, and incubated at room temperature (22°C/72°F) for 2 or 7 days statically to form biofilm.

4. Sanitizer intervention against *Listeria* biofilm on different surfaces.

Sanitizer solution concentration: Ozonated water was use at 2.0 and 4.0 ppm, representing commonly used levels practiced on apple packing lines. Quaternary ammonium compound (QAC), chlorine dioxide and peroxyacetic acid (PAA) are commonly used for surface antimicrobial intervention. QAC (Stop It, Pace International) was prepared with water at a concentration of 200 and 400 ppm. PAA (Shield-Brite PPA 12.0, Pace International) were evaluated at 80, 160 and 200 ppm. Chlorine (Accu-Tab, Pace International) was used at 100 and 200 ppm, pH6.8. Chlorine dioxide was generated on site and used at 2.5 and 5.0 ppm.

Antimicrobial intervention: Wells of PS plates or coupons of the selected surface bearing *Listeria* biofilm cells were rinsed with sterile distilled water, then subjected to ozonated water and other sanitizer treatments by adding 100 µl of each sanitizer solution or immersing in 250 ml of sanitizer solution at appropriate concentrations for 1, 5 and 10 min. To ensure countable survivors, the contact times were adjusted for different surfaces based on initial testing and industry practice. Untreated control wells with biofilm were subjected to distill water instead of sanitizer solution treatments.

5. Microbiological analysis.

Detachment of biofilm from surface: At the end of antimicrobial intervention, biofilm in the well of 96 well plates was rinsed with Dey/Engley (D/E) neutralizer broth and resuspended in sterile PBS, sonicated for 1 min, then recovered by vigorous pipetting. Each post-treatment coupon was rinse and placed into a sterile 15-ml centrifuge tube containing 5.0 ml Dey/Engley (D/E) neutralizer broth and 5 glass beads. *Listeria* cells survived sanitizer treatment were detached from surface into the D/E neutralizer broth by vortexing with a benchtop vortex mixer at maximal speed for 1 min. Control coupons were placed directly into the D/E broth without sanitizer treatment.

Bacterial enumeration: The detached cell suspensions were serially diluted in 0.1% buffered peptone water and plated on duplicate agar plates. To enumerate the potential viable but injured cells, Tryptic Soy Agar (TSA) with yeast extract overlaid with Modified Oxford Agar (MOX) plates were used for enumeration. Colonies that had formed on the plates were counted after 24-48 h of incubation at 37°C (98°F).

If survival after specific sanitizer treatment is below the enumerative limit of detection, the suspension was enumerated for Presence/Absence after 24h enrichment in Buffered *Listeria* Enrichment Broth. The presumable colony was further confirmed by PCR analysis

Objective 2: **Examine the antimicrobial efficacy of steam alone and in combination with selected sanitizers against *L. monocytegenes* biofilm on different surface materials (Year 2)**

Methods developed in Objective 1 studies will be used for Objective 2 studies. The Objective 1 outcomes will guide standardization of sanitizer concentration in relation to the variable residence time. Additional methods will be updated in year 2.

Objective 3: Validate antimicrobial efficacy of steam treatment and/or selected sanitizers in apple packing lines using parameters selected based on laboratory testing (Year 3).

Methods developed in Objective 1 and 2 studies will be used for Objective 3 studies. The outcomes of Objective 1 & 2 studies will guide standardization of sanitizer concentration in relation to the variable residence time. Additional methods will be updated in year 3.

PROGRESS/RESULTS AND DISCUSSION

During the first year of the project in 2017, we have been conducting Objective 1 studies and made significant progresses on evaluating antimicrobial efficacies of different sanitizer solutions against *L. monocytogenes* biofilm at different stages grown on clean surfaces or surfaces conditioned with organic matter of apple origin. The following are the major findings from the first year of work.

1. Antimicrobial efficacy of selected sanitizers against single strain *L. monocytogenes* biofilm

First, a single *L. monocytogenes* strain biofilm was grown on PS surface (Figure 1). Wells bearing *Listeria* biofilm were rinsed with sterile distilled water three times, then subjected to different sanitizer treatments as described above. At the end of antimicrobial intervention, each post-treatment well was washed twice then subsequently detached and enumerated per description in the method section. Ozonated water 1-min treatment at 1.0, 2.0 and 4.0ppm resulted in ~0.9-, 3.4- and 4.1- log reduction of *L. monocytogenes* signal strain biofilm grown on PS surfaces, respectively (Figure 2A). QAC 1-min intervention at 100, 200 and 400ppm resulted in ~2.4-, 3.2- and 3.6- log reduction of *L. monocytogenes* signal strain biofilm grown on PS surfaces, respectively (Figure 2B). Chlorine 1-min intervention at 100/200 ppm and chlorine dioxide at 2.5/5.0 ppm resulted in ~2.0-/3.1- and ~2.4/3.8 log reducation of *L. monocytogenes* signal strain biofilm grown on PS surfaces, respectively (Figure 2 C & D). PAA 1-min treatment at 80 and 160 ppm resulted in ~2.9- and 3.3- log reducation of *L. monocytogenes* single strain biofilm, respectively (Figure 2E).

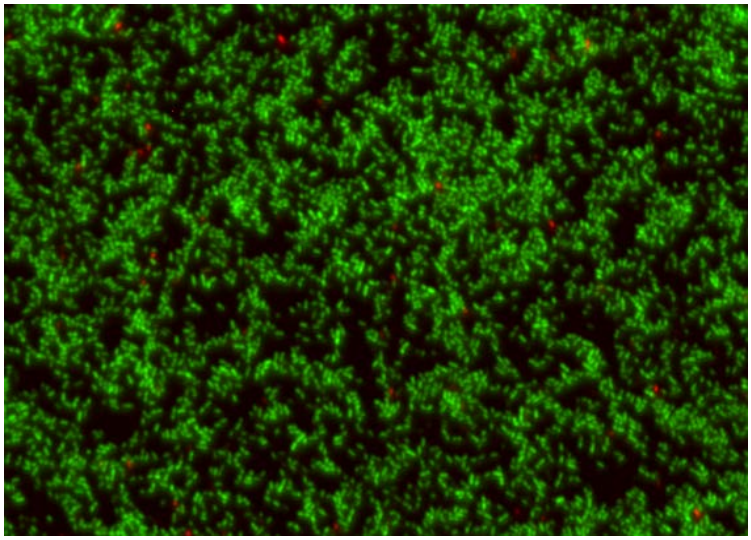


Figure 1. *L. monocytogenes* biofilm formed on polystyrene surface observed under fluorescence microscope at 100x magnification. Polystyrene surface was incubated in TSB statically at room temperature for 4 days. Biofilm was subjected to the BacLight Live/Dead bacterial staining.

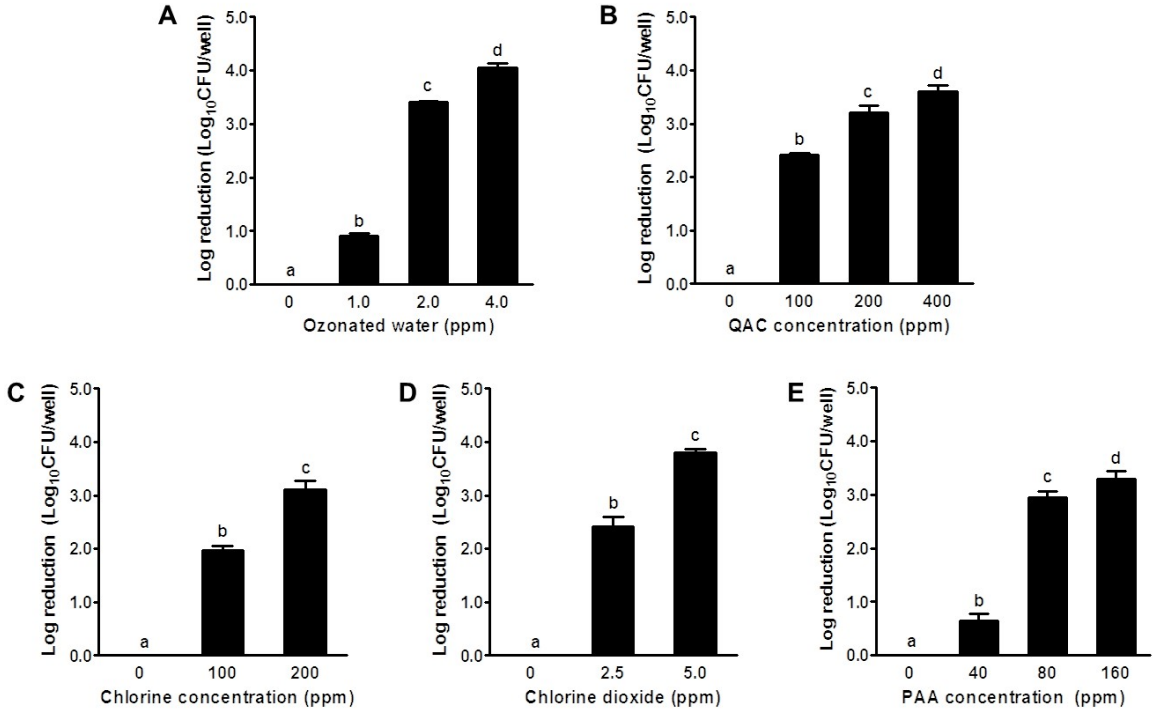


Figure 2. Antimicrobial efficacy of selected sanitizer intervention against biofilm of *Listeria monocytogenes* single strain at 2 days. A: Ozonated water; B: QAC; C: Chlorine; D: Chlorine dioxide; E: PAA. Mean ± SEM. Bars topped with same letter are not different at $P < 0.05$.

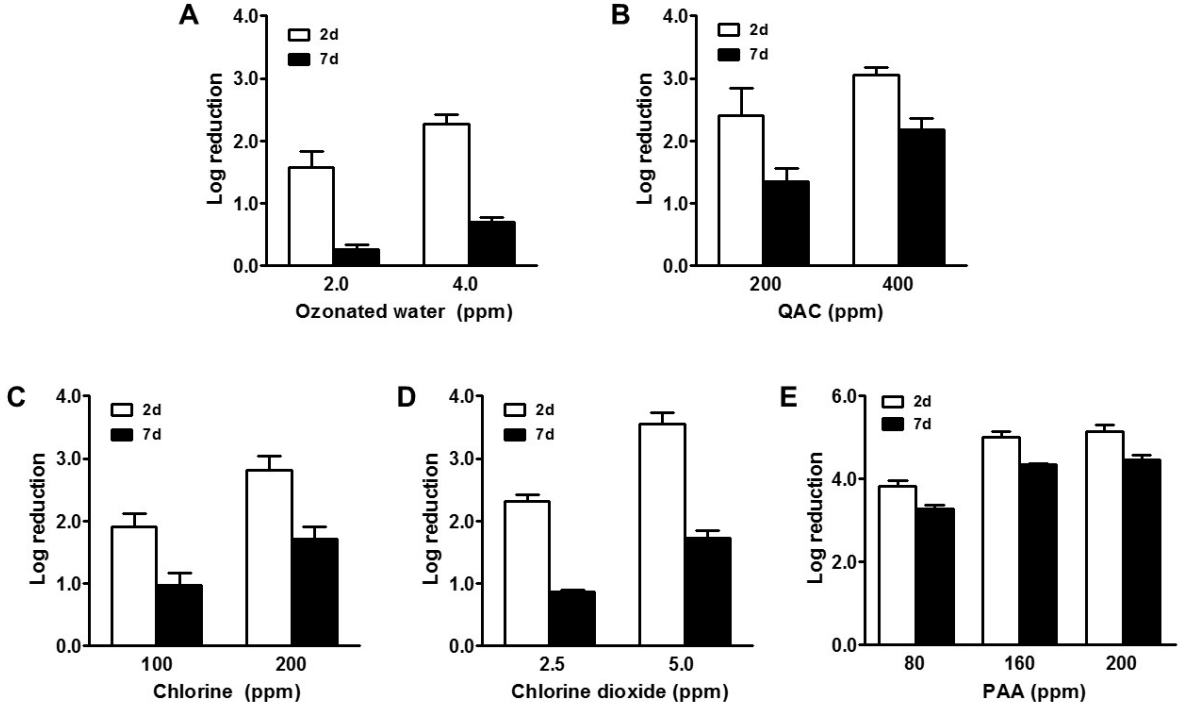


Figure 3. Antimicrobial efficacy of selected sanitizer intervention against biofilm of *Listeria monocytogenes* mixed strains. 2d, 2-day-old biofilm; 7d, 7-day-old biofilm. A: Ozonated water; B: QAC; C: Chlorine; D: Chlorine dioxide; E: PAA. Mean ± SEM.

2. Antimicrobial efficacy of selected sanitizers against mixed strain *L. monocytogenes* biofilm

Antimicrobial efficacies of all sanitizers except PAA against mixed strain *L. monocytogenes* biofilm were reduced when compared to single strain *L. monocytogenes* biofilm (Figure 2 & 3). Furthermore, antimicrobial efficacies of all sanitizers against 7-day-old biofilm were reduced when compared to 2-day-old biofilm; antimicrobial efficacy of PAA was relative less influenced by age of bioflim (Figure 3). Organic matter of apple origin conditioning dramatically impacted antimicrobial efficacy of all sanitizers against biofilm on PS plates (Figure 2 and 4). Ozonated water was greatly influenced by organic matter, which almost completely abolished its antimicrobial efficacy (Figure 4A). QAC at 400ppm, chlorine at 200ppm and 5.0ppm chlorine dioxide were still able to reduce 1-2 log reduction of mixed strain *L. monocytogenes* biofilm on PS surface depending on the stage of biofilm (Figure 4B-D). PAA 1-min treatment at 160ppm resulted in 3.4 log reduction against 7-day-old biofilm conditioned with organic matter; further increase of PAA concentration seemed not quite effective.

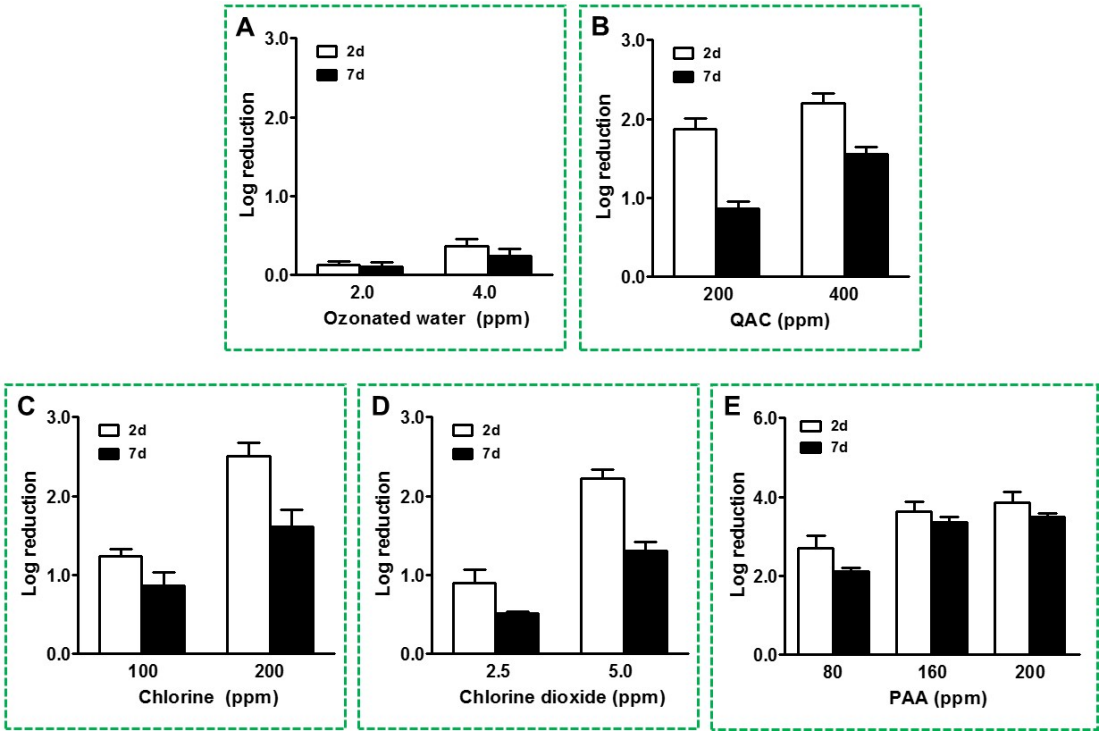


Figure 4. Antimicrobial efficacy of selected sanitizer intervention against biofilm of *Listeria monocytogenes* mixed strains in the presence of organic matter. 2d: 2-day-old biofilm; 7d: 7-day-old biofilm. A: Ozonated water; B: Chlorine; C: Chlorine dioxide; D: QAC; E: PAA. Mean ± SEM.

CONCLUSIONS

Eradication of *L. monocytogenes* biofilm in the processing surface is challenging. Antimicrobial efficacies of sanitizers against *L. monocytogenes* biofilm were dramatically impacted by biofilm stage, strains present and cleaniess of surfaces. Based on prelimnary data of the first year's study, PAA is a vaible sanitizer for surface decomtamination followed by QAC. More studies are ongoing in evaluating of efficacies of the selected sanitizers against biofilms formed on different surface coupons.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-16-106

Project Title: Managing rhizosphere/soil microbiology via apple rootstock biochemistry

PI:	Mark Mazzola	**Co-PI**:	Rachel Leisso
Organization:	USDA-ARS Tree Fruit Research Laboratory	**Organization**:	USDA-ARS Tree Fruit Research Laboratory
Telephone:	(509) 664-2280 ext. 209	**Telephone**:	(509) 664-2280 ext. 206
Email:	Mark.Mazzola@ars.usda.gov	**Email**:	Rachel.Leisso@ars.usda.gov

Cooperators: David Rudell, James Mattheis, USDA-ARS, Wenatchee, WA

Total Project Request: **Year 1**: $48,000 **Year 2**: $50,000 **Year 3**: **$52,000**

Other funding sources

NIFA-AFRI ELI postdoctoral fellowship, awarded in 2017 (award 2017-67012-26093) $152,000 over two years

WTFRC Collaborative expenses: None

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: 510-559-6019 **Email address:** Chuck.Myers@ars.usda.gov

Item	2016	2017	2018
Salaries[1]	$30,000	$31,000	$32,000
Benefits	$10,000	$10,200	$10,400
Wages			
Benefits			
Equipment			
Supplies	$7,500	$8,300	$9,100
Travel	$500	$500	$500
Miscellaneous			
Plot Fees			
Total	$48,000	$50,000	**$52,000**

Footnotes: [1]Salary support is requested from 0.5 FTE of a postdoctoral research associate.

OBJECTIVES

This report summarizes results in the second year of a three year project assessing the impact of rootstock genotype (cultivar) on the soil microbiome, specifically examining root exudates and rhizosphere soil pH changes. The project addresses three items identified as research priorities in the 2016 Apple Horticulture and Postharvest research needs assessment, including i.) Understanding and management of soil health and productivity in conventional and organic systems (critical priority) and ii.) Soil health and productivity – Interaction of rootstocks with rhizosphere microbiology (high priority) and contributes to additional priorities including iii.) apple replant (high priority) and iv.) improved scion and rootstock genetics (medium priority). The project objectives per the project proposal are below:

1. Characterize the effect of apple rootstock genotype on composition of the rhizosphere and orchard soil-inhabiting microbial community (microbiome).
2. Define differences in the natural chemical compound profile produced by rootstock cultivars that differ in inhibiting deleterious (pathogenic) or attracting beneficial rhizosphere micro-organisms.
3. Test the composite and independent (single compound instead of natural suite of compounds) impacts of natural chemical compounds on specific microbes or the soil microbiome to verify functional role in inhibition of deleterious microbes or attraction of beneficial microbes.
4. Determine effects of apple rootstock genotype on rhizosphere soil pH, contrasting rootstocks harboring different rhizosphere microbiomes of functional importance.

Year one, reported in 2016, focused on objectives 1, 2, and 4, with the outcomes of a) defining specific metabolites differing among rootstocks G41, G935, M9Nic29, and M26, b) establishing that root exudate quantity correlates to rootstock vigor / tree size, c) delineating preliminary data regarding rhizosphere pH, and d) new seedling growth and composition of the rhizosphere microbiome planted in replant soil is altered according to the genotype of the previous rootstock cultivated in the orchard soil. Activities in year two (2017) have continued objective 1, initiated work in objective 3, with additional experimentation in objective 3 extending beyond the stated goal; objectives 2 and 4 are considered complete with rhizosphere pH from additional experimentation reported here. Significant findings and discussion for year two (2017) are detailed below.

SIGNIFICANT FINDINGS

- In a greenhouse experiment assessing the impact of rootstock cultivar-specific (G41, G935, M9Nic29, and M26) exudates on a spatially separated orchard soil, the fungal community, as assessed by a molecular method (terminal restriction fragment length polymorphism analysis; T-RFLP), did not differ consistently among rootstock genotypes after 6 weeks of exudate percolation. Six additional weeks later (12 weeks post-planting) fungal community composition from soil treated with M9Nic29 exudates formed a unique cluster relative to other rootstock genotypes. In addition, fungal community composition from the control treatment was separated from other samples treated with rootstock exudates. Overall, the findings indicated that M9Nic29 rootstock exudates select for a specific fungal community and that for all rootstock genotypes examined, fungal communities were influenced by rootstock exudates relative to control communities (no rootstock) over time.
- Six phenolic metabolites -- 4-hydroxybenzoic acid, benzoic acid, chlorogenic acid, phloridzin, phloroglucinol, and rutin – which differed quantitatively among exudates from four apple rootstock genotypes -- G41, G935, M9Nic29, and M26 -- were tested for their ability to inhibit growth of the apple replant disease pathogens *Pythium ultimum* var. *ultimum*,

Phytophthora cactorum, and *Rhizoctonia solani* AG-5. Results indicate that phloridzin, benzoic acid, and 4-hydroxybenzoic acid can all inhibit these pathogens in a concentration-dependent manner. Inhibition of *Pythium ultimum* by rutin was minimal and chlorogenic acid and phloroglucinol moderate at the concentrations tested.

- Calibrations for chromatographic data relative to nanogram levels of several phenolic metabolites were prepared to enable quantitative (instead of relative) estimate of levels of phenolic metabolites in both exudates and in root samples. Preliminary findings suggest that specific metabolite concentrations required to inhibit pathogens when assayed independently are greater than the concentrations present in percolated exudates and root tissue.
- Soil treated with root exudates from G41, G935, M9Nic29, or M26 had lower pH than untreated soil; no statistically significant difference existed among rootstocks.

METHODS

Objective 1. For studies in year two, orchard soil treated with percolated root exudates from four apple rootstock genotypes (G41, G935, M9Nic29, and M26) was analyzed for differences in the fungal community composition utilizing molecular methods. Briefly, DNA was extracted from soil and a specific segment from the fungal ribosomal RNA gene was amplified via polymerase chain reaction using the fungal-specific primers ITSF and ITS4. Resulting PCR products (amplicons) were digested with restriction enzymes, and DNA fragments were analyzed to establish a community profile.

Objective 3. Root exudate metabolites which differed among rootstock genotypes G41, G935, M9Nic29 and M26 in year one – 4-hydroxybenzoic acid, benzoic acid, chlorogenic acid, phloridzin, phloroglucinol, and rutin – were tested for their ability to inhibit pathogen growth. Individual metabolites were added at varying concentrations to agar growth medium that was poured into Petri plates after autoclaving. Compounds were dissolved in ethanol or methanol and passed through a 0.22 µm sterile filter prior to addition to the agar; controls included agar amended with the same quantity of ethanol or methanol. A 0.5 cm agar plug containing the leading edge of an actively growing culture of *Pythium ultimum* var. *ultimum*, *Phytophthora cactorum*, or *Rhizoctonia solani* AG-5 (apple replant disease pathogens) were plated to the agar media and the diameter of the growing colony was measured daily between 24-72 hrs.

Additional methods for objective 3 were tested:

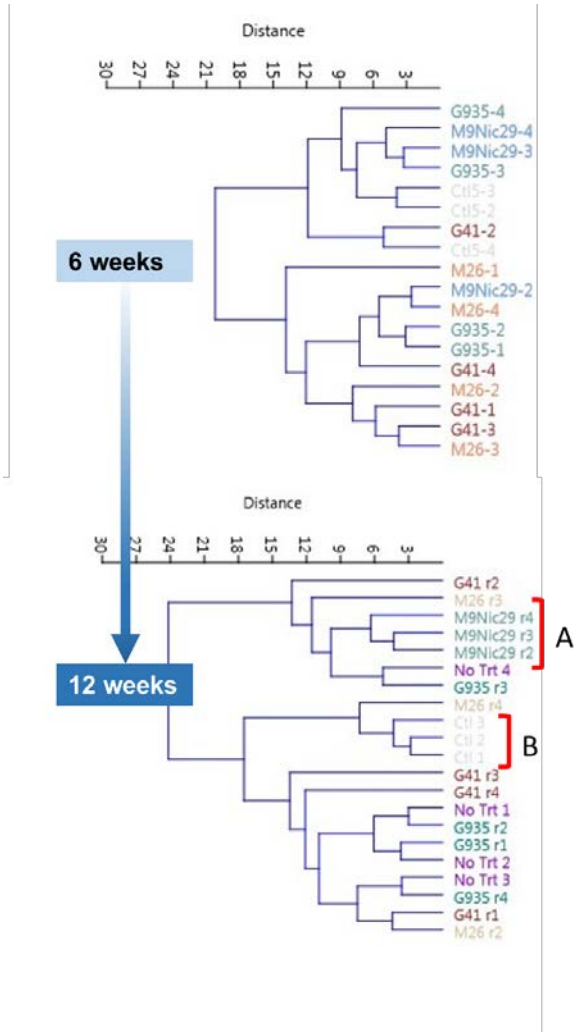
- In addition to sterile single compound addition to agar, a method for assessing the impacts of bulk tree exudates on specific pathogens was tested. Percolated exudates were collected from the root zone of G41 and M26 rootstock liners grown in controlled environment growth chambers. The 70 mL volume of exudates was filtered through a sterile filter (0.22 µm pore size) to remove microorganisms. A 0.5 cm agar plug from an actively growing culture of *Pythium ultimum* var *ultimum* was added to a sterile flask containing the filtered exudates, with the expectation that mycelia could be subsequently weighed.
- Root exudates in a subsequent third experiment were concentrated using Sep-Pak cartridges (a chemically binding column which selectively retains certain classes of metabolites) and eluted with a small volume of volatile solvents. Solvents were removed via a nitrogen gas drying procedure and resuspended in a small volume of methanol. Sterile filter discs were then soaked in this suspension and allowed to dry on a sterile Petri plate in a laminar flow hood. Filter discs were then placed on a Petri plate containing one-fifth potato dextrose agar and a 1-day old *Pythium ultimum* var *ultimum* culture.
- A fourth experiment is underway to test the effects of lyophilized exudates, which will retain the totality of the biochemical composition of the exudates.

Objective 4. Soil from pots containing orchard soil treated with percolated exudates from four apple rootstock genotypes (G41, G935, M9Nic29, and M26) were analyzed for differences in soil pH. Two experiments were performed.

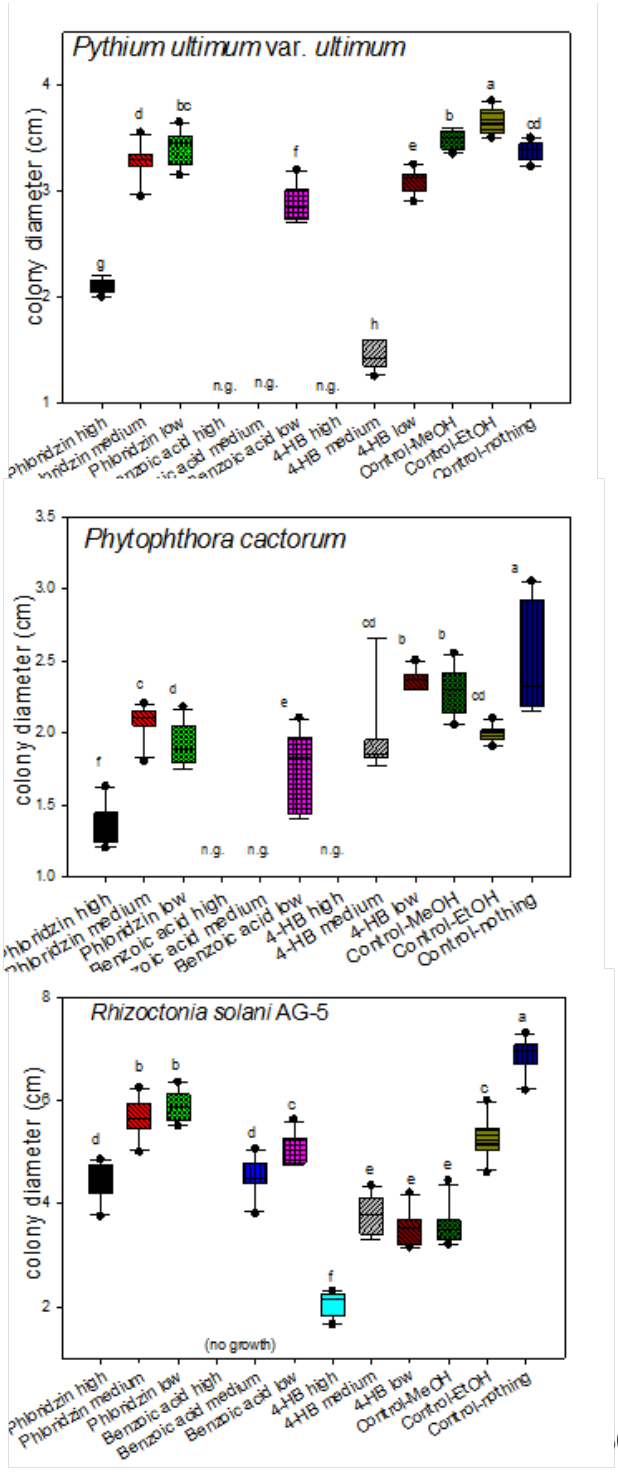
RESULTS & DISCUSSION

Significant finding 1 (results for objective 1, determining rootstock specific soil microbial communities): In a greenhouse experiment assessing the impact of rootstock genotype exudates on a spatially separated orchard soil, the soil microbiome did not differ consistently among rootstock genotypes after 6 weeks of exudate percolation. Rootstock cultivar-specific differences in the soil microbiome were apparent at 12 weeks demonstrating the temporal fashion at which changes in the microbial community are established in response to root exudates (Figure 1).

Figure 1. A molecular-based time-course assessment of the fungal microbiome (ITS amplicon) from soil treated with apple rootstock cultivar-specific root exudates: M9Nic29 profiles clustered at 12 weeks (A), as did control treatment profiles (B), indicating divergence of the rootstock fungal profile from original soil.



Significant finding 2 (results for objective 3, testing the impacts of root exudates on apple replant disease pathogens). Six phenolic metabolites -- 4-hydroxybenzoic acid, benzoic acid, chlorogenic acid, phloridzin, phloroglucinol, and rutin – which differed among exudates from four apple rootstock genotypes -- G41, G935, M9Nic29, and M26 -- were tested for their ability to inhibit growth of the apple replant disease pathogens *Pythium ultimum* var. *ultimum*, *Phytophthora cactorum*, and *Rhizoctonia solani* AG-5. Results indicate that phloridzin, benzoic acid, and 4-hydroxybenzoic acid can all inhibit these pathogens in a concentration-dependent manner (Figure 2). Inhibition of *Pythium ultimum* by rutin was minimal and chlorogenic acid and phloroglucinol moderate in the concentrations tested.



For the second method, which tested filter sterilized root exudates added to a liquid culture, visual examination indicated no differences (virtually no growth) from the agar plug for either of the treatments or control; the interpretation is presently that the food supply was too low / dilute for the oomycete to grow. For the third test method, the culture was not inhibited by the metabolites on the filter disc (the colony overgrew the plate after one day of incubation) and the filter discs subsequently exhibited signs of contamination. Further experimentation based on information from these tests will be performed.

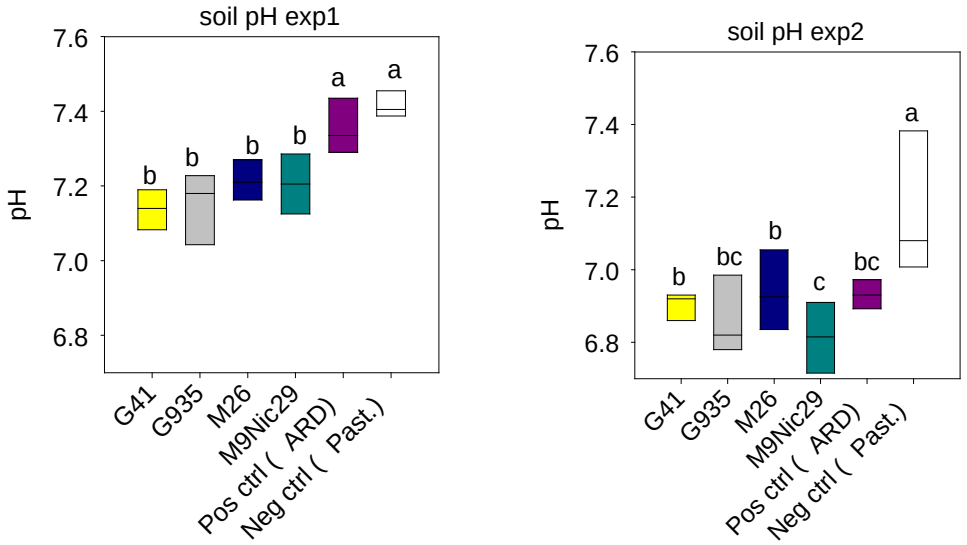
Figure 2 (left). Pathogen colony diameter on agar amended with varying concentrations of single apple root exudate metabolites which differed among rootstocks with varying tolerance to apple replant disease, indicating relative inhibition of pathogen growth by compound and/or concentration. *Abbreviations:* n.g., no growth of pathogen.

Significant finding 3 (expanded experimentation for objective 3, testing effects of exudates on pathogens). Calibrations of several phenolic metabolites were prepared to enable estimate of levels of these metabolites in both exudate and root samples. Preliminary findings indicate that concentrations required to inhibit pathogen growth *in vitro* are generally higher than that estimated to be present in either root exudates (concentration per mL exudate) or in root tissue (concentration per g of fresh root tissue). However, the synergistic effects of multiple compounds against pathogens as well as the point concentration of root exudate / tissue metabolites remains to be tested.

0]

Significant finding 4 (results for objective 4, effects of genotype specific exudates on soil pH). pH of soil treated by water percolated through the tree rhizosphere was altered by the presence of a rootstock but did not differ significantly among rootstocks (Figure 3). The presence of an apple rootstock lowered soil pH relative to the control soils where no trees were planted, but no statistically significant difference existed among rootstock cultivars.

Figure 3. pH of orchard soil treated with exudates separated by experiments. *Abbrevations:* Exp1, experiment 1; Exp2, experiment 2.



CONTINUING PROJECT REPORT **YEAR**: 4 months of 18
WTFRC Project Number: AP-17-108

Project Title: 'WA38' fruit size and dry matter for fruit quality/consumer preference

PI: Stefano Musacchi
Organization: WSU -TFREC
Telephone: 509 663 8181 (236)
Email: stefano.musacchi@wsu.edu
Address: 1100 Western Avenue
City/State/Zip: Wenatchee WA 98801

Co-PI: Kate Evans
Organization: WSU -TFREC
Telephone: 509 663 8181 (245)
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Co-PI: Carolyn Ross
Organization: WSU (Pullman)
Telephone: 509 335 2438
Email: cfross@wsu.edu
Address: Food/Nutrition 122
City/State/Zip: Pullman WA 99164-6376

Co-PI: Sara Serra
Organization: WSU-TFREC
Telephone: 509 663 8181 (251)
Email: sara.serra@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Cooperators: Alex Goke

Total Project Request: 18 months (2 harvest seasons): **$ 170,198**

Other funding sources
NONE

Budget 1
Organization Name: TFREC-WSU Contract Administrator: Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/5096638181-221 Email address: arcgrants@wsu.edu/ joni.cartwright@wsu.edu

Item	2017	**2018**
Salaries	24,000	24,960
Benefits		
Wages		
Benefits	8,597	8,941
Equipment¥	6,000	
Supplies		
Travel	6,500	6,500
Miscellaneous[1]	36,350	38,350
Plot Fees	5,000	5,000
Total	86,447	**83,751**

Footnotes:
[1] WSU sensory evaluation facility fees
¥ Originally we request the funds for Amilon (starch meter), instead in December 2017 our Minolta color meter broke and it is an old model so Konica Minolta is not servicing it anymore. We asked permission to Mike Willet to use the equipment money to buy a new Minolta Colormeter CR-400 (no request of increase in current year budget).

OBJECTIVES

1. Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (5th leaf and 4th crop in 2017 and 5th crop in 2018).
2. Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.

SIGNIFICANT FINDINGS

- A "pooled orchard" model for non-destructive dry matter prediction (pDM) by Felix F-750 Produce Quality meter was specifically developed for 'WA38' at harvest 2017 and performed reliably (accuracy 0.44% dry matter and R^2=0.87 at calibration).
- The pooled orchard model was also effective on the validation group of fruit, with an error of 0.40% dry matter and R^2 of 0.83 between predicted and destructive values.
- Apple sorting by size and pDM at harvest 2017 in 18 different combinations rootstock-fruit size-DM category showed significant differences in external and internal quality attributes one month after harvest.

METHODS

Harvest and fruit sampling 2017

To build accurate and flexible predictive models of dry matter, a broad range of variability and situations must be considered. For this reason, we utilized two locations, incorporating two rootstocks and three training systems. The two locations were 'WA38' orchards established in Rock Island (Sunrise Orchard) and Prosser, Washington (Roza Orchard) in 2013. The two rootstocks were G41 and NIC29, and the three-training systems Spindle, V and Bi-axis.

Harvest in Roza occurred on September 25th-26th, with fruit exhibiting an average starch index of 1.4 (scale WTFRC 1-6) and in Sunrise on October 9th-10th at an average starch index of approximately 2.

Apples were sorted in: harvest, T0 (+1M-25-26 October), T1 (+6M-26-27 March)

Fruit were stored at 32°F under regular atmosphere conditions until quality evaluation.

The major fruit sampling was done in Sunrise for quality assessment and consumer test at harvest and after storage. 36 trees trained at Spindle on Nic29 and G41 were harvested and graded for size and defects. Only Fancy and Extra fancy apples were collected from each of 36 trees and brought back to the cold room.

The 2017 'WA38' production from the mature orchard has been used to define the dry matter variability and distribution according to the following factors: rootstock (M9-Nic 29 and G41), fruit size (small, medium and large), dry matter content (low, medium and high).

In March 2016, we also established a new 'WA38' orchard from an existing 7-year-old 'Granny Smith' grafted on M9 T337 block with the top graft technique ('WA38' bud sticks) to simulate the common scenario for commercial orchards. In June 2016, healthy grafts were trained to become either single, double, or triple axis trees. These trees grew in 2017 as their hypothetical first year and they set very little fruit. So next year (2018), a first real crop will be harvested from 'WA38' young trees.

Non-Destructive Dry Matter Prediction

Predictive models of dry matter were developed on WA38 in order to classify fruit in to dry matter categories for the purpose of quality evaluation. At harvest, 768 fruit were selected from Roza and Sunrise orchards, incorporating both Nic29 and G41 rootstocks and spindle and V training systems in equal proportions. These fruit were randomly assigned in equal proportions to model and validation groups for each orchard, as well as to three storage groups (at harvest, 1 month of storage, T0, and 5

months of storage, T1, (Figure 1). The few fruits harvested from the top-grafted WA38 block were not used in model calibration or validation in 2017.

To construct a model, absorbance spectra of fruit (n=64) in the 729-975nm range were obtained using a Felix F-750 Produce Quality Meter (Felix Instruments, Camas, WA, USA). Two areas of each fruit, opposing blush and shade faces, were measured, increasing the effective sample size in each model to 128. During spectral acquisition, fruit were successively brought to thermal equilibrium at three separate temperatures (~1, 24, and 32°C) to simulate field, laboratory, and storage conditions and to reduce temperature-related noise in the models.

Dry matter values were obtained for each measured area using traditional destructive methods – a peeled tissue sample was dried to constant weight at 60°C for dry matter and expressed as a percentage of fresh weigh to dry weight.

A predictive dry matter model was developed by correlating smoothed second-derivative absorption spectra to the destructive dry matter and °Brix value for each tissue sample using partial least squares regression. Models from Roza and Sunrise orchards were pooled in order to incorporate a larger calibration sample set (n = 256). The model was validated by applying predictions to a separate validation fruit set (n=128 for pooled Roza and Sunrise orchards) in which predictions were made across blush and shade faces and averaged together. The prediction was compared to a traditional destructive dry matter value of each fruit (fresh weight/dry weight of a peeled and cored equatorial tissue slice).

The pooled orchard model developed at harvest performed well, with an average accuracy (RMSE) of 0.44% dry matter and an R^2 of 0.87 between predicted and destructive values at calibration. The pooled orchard model was also effective on the validation group of fruit, with an RMSE of 0.40% dry matter and an R^2 of 0.83 between predicted and destructive values.

At T0, the model calibration and validation procedure was repeated in a similar fashion in order to capture variance in fruit attributes introduced post- harvest and during storage. The procedures will again be replicated at T1 to further evaluate the impacts of long-term storage conditions on prediction accuracy.

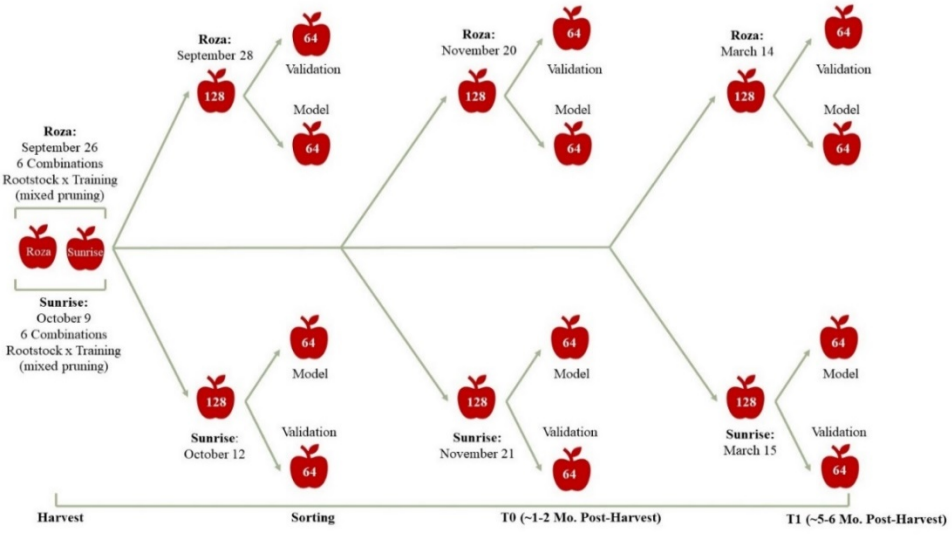


Figure 1: 'WA38' concept map for dry matter model building for Felix F750 from harvest to postharvest.

Apple size and dry matter sorting

All fruit divided by rootstock were assessed in cold storage for weight, size, I_{AD} (average of 2 cheeks with DA meter) and predicted dry matter (pDM) non-destructively estimated using the portable NIR

device Felix F750 Quality meter (average of 2 cheeks predicted by the pooled orchard model). Fruit size and pDM were the key parameters for our sorting purposes.
Size categories are defined as the following: small (70-75 mm), medium (80 mm) and large (85-90-95+ mm) - all fruit below 70 mm were considered cull.
pDM categories were classified as: low pDM (13.00-13.99 %), medium pDM (14.00-14.99 %) and high pDM (15.00-15.99 %). For more homogenous categories, we excluded apples with pDM <13.00 % and above 16.00% from the sorting into quality and consumer test samples.

Fruit were sorted within each size category in 3 pDM categories on the basis of the predicted DM values for T0 and T1 (respectively 1 and 5 months after harvest). T1 are stored at 32°F up to 5 months (March 2018).
Each treatment (combination of rootstock – size and pDM) was split for two purposes and two pullouts: fruit quality ($T0_q$ and $T1_q$) and sensory analysis by consumer test T0 $_{CT}$ and $T1_{CT}$) as reported in Figure 2.
Where possible, at least 16 apples for combination were analyzed/utilized, for T0q and T1q almost all the 18 possible theoretical combinations (17 for T1q) were satisfied, while for consumer test only 10 combinations were compared (see results and discussion).

WA 38 sorting scheme 2017

rootstock	size	mm	dry matter	DM class	T0q	$T0_{CT}$	T1q	$T1_{CT}$
G41	SMALL	70-75	DM LOW	13.00-13.99	13	16		16
G41	MEDIUM	80	DM LOW	13.00-13.99	16	27	16	27
G41	LARGE	85-90-95+	DM LOW	13.00-13.99	14		15	
G41	SMALL	70-75	DM MID	14.00-14.99	13		13	
G41	MEDIUM	80	DM MID	14.00-14.99	10	16	11	16
G41	LARGE	85-90-95+	DM MID	14.00-14.99	16	32	16	32
G41	SMALL	70-75	DM HIGH	15.00-15.99	6			
G41	MEDIUM	80	DM HIGH	15.00-15.99	12			
G41	LARGE	85-90-95+	DM HIGH	15.00-15.99	16	16	16	16
NIC29	SMALL	70-75	DM LOW	13.00-13.99	*16*	*32*	*16*	*32*
NIC29	MEDIUM	80	DM LOW	13.00-13.99	*16*	*32*	*16*	*32*
NIC29	LARGE	85-90-95+	DM LOW	13.00-13.99	*16*			
NIC29	SMALL	70-75	DM MID	14.00-14.99	*16*	*32*	*16*	*32*
NIC29	MEDIUM	80	DM MID	14.00-14.99	*16*	*32*	*16*	*32*
NIC29	LARGE	85-90-95+	DM MID	14.00-14.99	*16*	*16*	*16*	*16*
NIC29	SMALL	70-75	DM HIGH	15.00-15.99	*12*		*12*	
NIC29	MEDIUM	80	DM HIGH	15.00-15.99	*11*		*12*	
NIC29	LARGE	85-90-95+	DM HIGH	15.00-15.99	*12*		*12*	
					18	10 comb/18	*14*	10 comb/18

Figure 2: Schematic of all 'WA38' fruit sorting by rootstock, size (3 categories), dry matter (3 categories) and different purposes or investigation: fruit quality (T0q and T1q) and sensory analysis by consumer test T0 $_{CT}$ and $T1_{CT}$) from harvest 2017 in

'WA38' fruit quality assessment at T0

Fruit quality was assessed 1 month after harvest and the parameters measured were: percentage of red blushed overcolor, maximum red and background color (by Minolta colorimeter CR-300), external defects, exogenous ethylene concentrations (in a sealed static jar system by GC Agilent 7890B), firmness (sun/shade by Mohr® Digi-Test2), soluble solids concentration (°Brix by PAL-1 refractometer), starch index (1 to 6 WTFRC scale), actual dry matter %, titratable acidity and pH.
Actual dry matter % was destructively assessed on six slices for each combination of size/dry matter class/rootstock/duration of storage by taking the fresh weight of the peeled slices (after removing the core) and then weighing them again after drying at 60 ˚C until weight was stable.
Analysis will be repeated after storage at T1 (=5 months after harvest, March 2018) on fruit sorted in October 2017.
Few fruit produced from the young 'WA38' top grafted trees were assessed for fruit quality as described above but due to the limited number they were not art of the large sorting process in Figure 2).

'WA38'consumer test at T0 (*Ross C.*)

'WA38' apples were received on November 8th, 2017 and placed in 38°F storage at the WSU School of Food science in Pullman. Fruit from regular cold storage were brought up to room temperature 24 hours before analysis. Sensory analysis was accomplished 1 month after harvest, on November 15th-16th (one day per rootstock). On each of the two sensory panel days, the sensory panel for acceptance testing of five samples of 'WA38' apples was conducted. Each consumer was assigned a guest number to anonymize their identity. Ninety-four consumers were able to participate on 2 testing days.

Apples were washed in cool water and dried with paper towels. A representative sample was selected from each box per treatment for taste and appearance evaluation. Specifically, for flavor/taste evaluation, apples were cut into equal 1/8 parts with the seed core removed. Sampling from one apple served 8 panelists. For visual evaluation of whole fruit, one apple was presented in a bowl labeled with a 3-digit code. To provide a representative sample, five bowls of each treatment were prepared for random presentation to the consumers. Reagent grade water and unsalted-top saltine were provided to each consumer for palate cleansing between samples.
For each apple sample, consumers were presented with a 1/8 piece of apple (described above) on a white plate. From this samples, consumers were asked about their acceptance of the apple appearance, aroma, firmness, crunchiness, juiciness, sweetness, sourness, apple flavor and overall liking. All of these attributes were evaluated using a 9-pt hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely).
After evaluation of these attributes in all the 5 samples, consumers were presented with a whole apple of each of the samples. For these evaluations, consumers were instructed not to consume the sample but evaluate acceptance of the apple size, shape and color.
'WA38' fruit quality assessment at T1 as well as consumer test on T1 samples will be assessed on March 2018 and they will be reported together with consumer test at T0 in the 2019 project report.

RESULTS & DISCUSSION

1. Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (5th leaf and 4th crop in 2017 and 5th crop in 2018).

Apple size and dry matter sorting

Fancy and Extra Fancy fruit collected from 18 trees of 'WA38'/G41 and 18 trees 'WA38'/Nic29', totalling1360 fruit. A majority of fruits were produced by Nic29 (871 apples). G41 reported a higher percentage of cull fruit than Nic29 (42% and 32%, respectively), limiting the number of fruit available for sorting. From the production of those trees (reported in WTFRC Project Number: AP14-103A), Nic29-spindle trees produced significantly more fruit with no difference in yield (kg/tree), because 'WA38'/G41 fruit were on average significantly bigger than 'WA38'/Nic29 (respectively 263 g and 216 g). In Figure 3, fruit size distribution by rootstock is reported, showing similar percentage of fruit in the 80 mm size class (size medium) while 'WA38'/Nic29 was present in higher percentages in below 80 mm sizes (53 %, size small) and 'WA38'/G41 in the above 80 mm sizes (47 %, size large). In general, the comparison between fruit from the 2 rootstocks revealed significant differences also in the maturity stage: 'WA38'/Nic29 reported an average higher I_{AD} value (less ripe) than 'WA38'/G41. No significant difference in predicted dry matter ranging from 13.96 to 14.31 % as average values respectively for G41 and Nic29 (data not shown). The comparison between the 3 size categories regardless the rootstock showed highly significant difference in fruit of different size categories. In particular, small apples were less ripe (I_{AD} =1.00), with a smaller average fruit weight (190 g) and lower pDM (13.71 %) than large fruit (I_{AD} =0.61, weight=297 g and pDM=14.73 %) with medium fruit significantly

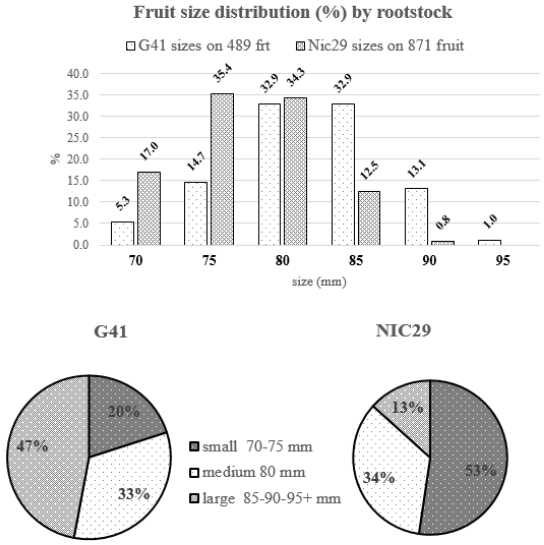


Figure 3: 'WA38' apple size distribution (%) by rootstock (G42 and Nic29) on sample fruit at harvest 2017 (top). Pie charts describing the subdivision of fruit in the 3 size categories determined for this trial by rootstock (bottom)

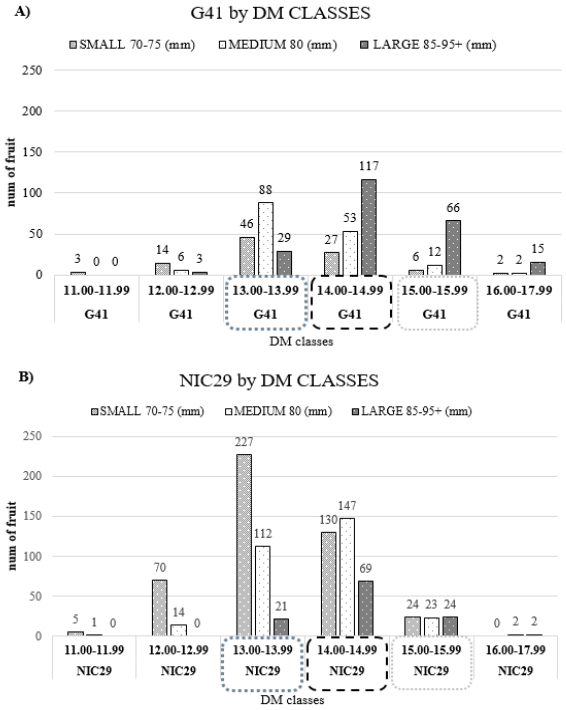


Figure 4: 'WA38' apple predicted dry matter (pDM) distribution (%) by rootstock: A) G41 and B) Nic29 in DM classes for each fruit size categories (small, medium and large) on 1360 sample fruit at harvest 2017. Dashed rectangular boxes in the X- axis show the 3 DM classes chosen for the trial and above each column the number of fruit belonging to that

distinguishable from the other two size categories (data not shown). Interaction rootstock*size was significant for weight and pDM (data not shown). Comparison between combination of rootstock- size revealed interesting different in maturity, weight and pDM at harvest. 'WA38'/G41-large apples were the most ripe, with highest weight and pDM% significantly distinguished from the others (data not shown).

Predicted dry matter percentage distribution by rootstock and sizes is reported in Figure 4, the most representative pDM categories for 'WA38'/G41-medium fruit was 13.00-13.99 % (54.7 % of all G41 medium fruit), while for 'WA38'/Nic29-large was 14.00-14.99 % (59.5 % of all Nic29 large fruit).

'WA38' fruit quality assessment at T0

Fruit quality analysis one month after harvest revealed several significant differences in the different possible comparisons. Between rootstocks (regardless of other factors), 'WA38'/Nic29 apples were on average smaller, with higher overcolor %, red intensity, less starch degradation, pH and less yellow background, lower firmness and TA than 'WA38'/G41. There was no notable difference for SSC and ethylene. The comparison between the 3 size categories revealed the least number of significant differences among all parameters. Small 'WA38' fruit were firmer (than medium and large), with highest starch degradation, lower ethylene (than large) and large fruit were less firm, more ripe (for I_{AD}). No differences in terms of color or SSC nor dry matter appeared in the comparison between sizes. 'WA38' apples compared by dry matter category (low, medium, high) presented more differences than by size. High dry matter 'WA38' (regardless size and rootstock) reported on average the lowest I_{AD}, the highest fruit weight, % blush overcolor, red intensity (lowest overcolor hue and highest background chroma), higher firmness, SSC and TA than medium and low pDM fruit. Actual dry matter percentage confirmed the discrimination in the 3 categories done at sorting step by Felix F-750, with the high class a little overestimated than the lowest one (data not shown).

The comparison between 18 combinations of rootstock-fruit size-dry matter for fruit quality attributes at T0 (1 month after harvest 2017) resulted in significant differences, among over 20 parameters analyzed in Figure 6 are reported just average red intensity, SSC and actual dry matter % by destructive method. Average red intensity was similar across combinations, but Nic29-medium-high pDM and Nic29large-high pDM stood out with highest values (above 4 in a 1-5 scale), while G41-small-low pDM emerged as the lowest value (3.04). Actual dry matter (%) revealed always a significant difference between high pDM and low pDM within each rootstock and fruit size, while the medium pDM was always statistically similar to the low pDM or/and to the high pDM category suggesting a narrow discrimination between pDM categories at sorting. SSC were more discriminated between combinations than actual dry matter %, in fact in general almost all pDM categories resulted statistically separate with higher SSC in high pDM combination despite the size, except for Nic29-medium-medium pDM and high pDM and Nic29-large-medium pDM and high pDM (Figure 5). Highest TA was found in some of the combinations in the high pDM (Nic29-medium, G41-smallG41-large) while lowest values in combination of Nic29-low pDM (small, medium and large). Firmer fruit were those in the small-medium size high and medium pDM, while the least firm low pDM ones (Nic29, despite the size).

The few fruit produced from the young 'WA38' top grafted trees were assessed for fruit quality and they did not show many significant differences between the 3 types of axis, just apples picked from 3-axis trees were less ripe with higher average red intensity (1-5) then other systems. In general, those young fruit were pretty large (283 g avr.), firm (9.3 kg), with high DM (16-17%), 15% SSC, high TA (0.89%).

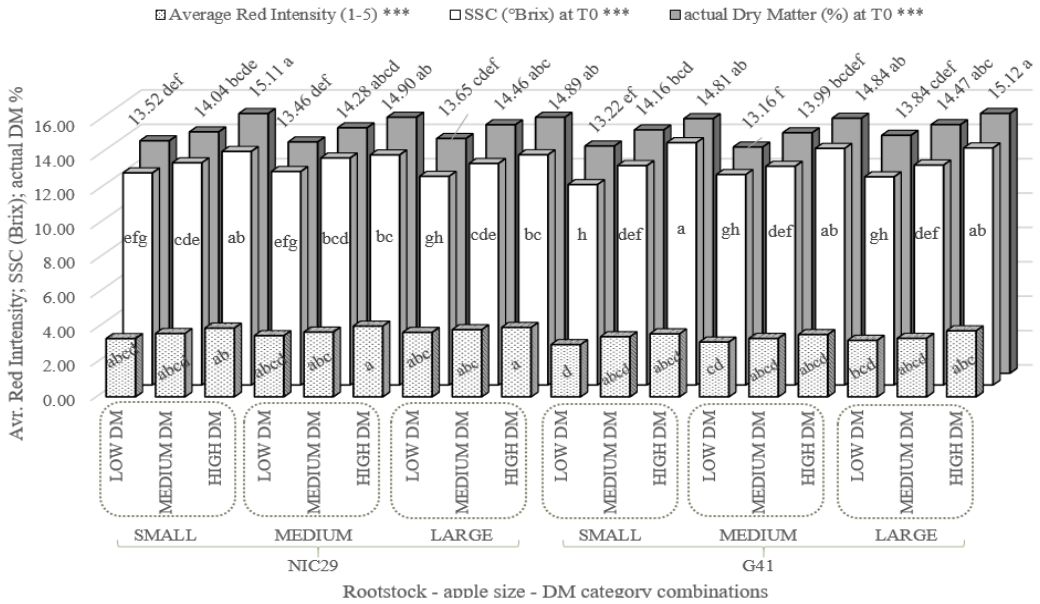


*Figure 5: 'WA38' fruit quality analysis at T0 (1 month after harvest 2017): comparison between 18 combinations of rootstock-fruit size-dry matter for average red intensity, SSC and actual dry matter % by destructive method. Significance is reported in the legend as *=p<0.05, **=p<0.01, ***=p<0.001 and same letters within each parameter do not distinguish means*

2. Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.

This objective is in progress, consumer test at T0 (1 month after harvest) was done on 10 combination of apples, but results need still to be elaborated. The project report in 2016 will include all the consumer preference results on 'WA38' fruit harvested in 2017 and stored up to 5 months.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-17-101

Project Title: Control of fruit size and bitter pit in Honeycrisp using irrigation

PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-663-8181
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Jeff Cleveringa, Oneonta Starr Ranch; Dave Allan and Suzanne Niemann, Allan Brothers

Total Project Request: **Year 1:** $53,442 **Year 2:** **$74,199** **Year 3:** $84,972

Other funding sources

Agency Name: WTFRC Technology Review
Amt. awarded: $84,000
Notes: This was funded in March 2017 and will be entering its second year of the project in 2018. Dr. Dave Brown is the project lead and this work complements efforts made here.

Agency Name: Lawrence Berkeley National Lab, Berkeley, CA
Amt. awarded: ~$32,000
Notes: This is work in collaboration with scientists at the University of California, University of Kentucky, and Lawrence Berkeley National Lab to look at how irrigation regimes change fruit structure, porosity and how it relates to quality. The funding supports beamline access to make measurements in the spring and fall.

Agency Name: Canadian Light Source Synchrotron, Saskatoon, SK
Amt. awarded: ~$30,000
Notes: This is work in collaboration with scientists at the Canadian Light Source to look at how irrigation regimes change fruit vasculature in developing fruit. The funding supports beamline access to make measurements in the summer.

Agency Name: Pacific Northwest National Lab, Richland, WA
Amt. awarded: ~$20,000
Notes: This is work in collaboration with scientists at the PNNL to look at how irrigation regimes change fruit vasculature in developing fruit. The funding supported instrumentation access to make measurements in the fall of 2017 from these experiments.

Agency Name: USDA Specialty Crop Block Grant - Planned
Amt. requested: $250,000
Notes: This will be submitted in January 2018 to work more closely with the industry and test new plant water status tools to implement effective irrigation management in orchard to improve pack outs for Honeycrisp.

Budget 1

Organization Name: Washington State University Contract Administrator: Joni Cartwright
Telephone: 509-663-8181 Email address: joni.cartwright@wsu.edu

Item	2017	**2018**	2019
Salaries[1]	18,000	46,026	47,867
Benefits	7,942	10,809	11,306
Wages[2]	0	5,223	12,192
Benefits	0	141	1,607
Equipment[3]	8,000	0	0
Supplies[4]	17,000	9,500	9,500
Travel	2,000	2,000	2,000
Miscellaneous	0	0	0
Plot Fees	500	500	500
Total	53,442	**74,199**	84,972

Footnotes:
[1] Salaries are budgeted to support a research technician at 50% for three years and the salary for a M.S. student for two years.
[2] Wages provide summer salary for a M.S. student and a summer student for year 3
[3] Equipment in year 1 will be for the purchase of a pressure bomb to measure stem water potential
[4] Supplies are for irrigation set-up supplies in year 1 combined with lab consumables, leaf and fruit nutrient testing and fruit quality analysis for years 1-3.

OBJECTIVES

1. Test how early, middle and late-season deficit irrigation affects fruit size, quality and return bloom in Honeycrisp.

2. Identify whether bitter pit occurrence can be reduced by reducing fruit size in a bitter pit susceptible orchard.

3. Develop horticultural indicators (e.g. visual indicators, leaf water potential and/or soil moisture) for monitoring plant water status to guide the deployment of deficit irrigation for the control of fruit size.

SIGNIFICANT FINDINGS

- In a sandy loam soil, soil water was not difficult to manipulate.
- Visual stress indicators on Honeycrisp are difficult to spot and the cultivar does not visually show early signs of drought. Visual symptoms only showed during water limitations late in the season and even at that point, did not negatively impact fruit quality or tree health.
- Stomatal conductance was strongly affected by irrigation regime.
- Photosynthesis decreased during periods of water limitation indicating a change in stomatal conductance and plant water-status.
- Midday leaf water potential increased during drought treatments and then recovered once irrigation was brought back to normal
- When water limitations were applied, fruit size was reduced in all treatments
- Bitter pit was lower when water was limited during the middle and later part of the season (45-105 DAFB)
- Red color increased when water was limited later in the season (75-105 DAFB)

METHODS

Experimental site and tree management

An experiment was set up at the WSU Sunrise Research Orchard using 240 Honeycrisp trees on M9-T337 that were planted in 2015 at a spacing of 3’ x 12’ (1210 trees/acre). The soil is an alluvial shallow sandy loam soil. The trees filled their canopy space in 2015 and 2016. The first year crop was in 2017. Using a randomized complete block design (See Figure 3), irrigation regimes were used that will withhold irrigation either early, middle or late in the season and compare it to a fully watered control. Trees were not sprayed with calcium to be sure that bitter pit incidence will be high enough to see differences among treatments.

Experimental design and irrigation treatments

The irrigation system at Sunrise was controlled with a variable speed pump drive and electrovalve controlled. Using exclusion valves and by-pass lines, the entire block was appropriately randomized. Irrigation was applied using emitters at 1 foot spacing at 0.42 gal/hour and supplemented with microsprinkler irrigation to maintain the grass between rows. The well irrigated control will be irrigated to meet water demand, usually for 30-60 minutes per day early in the season and between 90-120 minutes per day, depending on the conditions, during the summer.

The early irrigation deficit where irrigation was reduced by approximately 80-90% from 15-45 days after full bloom (DAFB), middle irrigation deficit with irrigation was reduced by approximately 80-90% from 45-75 DAFB and late irrigation deficit where irrigation was reduced by approximately 80-90% from 75-105 DAFB. Full bloom occurred on May 3rd, 2017. All treatments were returned to the well-watered irrigation schedule after the predetermined deficit irrigation period.

Tree Selection

Sample trees were selected for uniformity. Caliper measurements were taken 15 cm above grafting union on all trees in the plot. Three trees were selected per replication with caliper measurements between 22-23 mm (~1") diameter and uniform growth throughout the tree. Bloom clusters were counted to continue checking uniformity.

Physiological Measurements

At the beginning, middle, and end of each deficit irrigation period, physiological measurements were made including mid-day leaf water potential and photosynthesis. Plant water status, measured as Ψ_{md} was assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} were bagged for at least one hour in silver reflective bags to equalize the leaf and xylem water potential before readings were taken. Ψ_{md} was measured around solar noon. Leaf gas exchange was measured using a LI-6400XT infrared gas analyzer (Li-COR, Lincoln, NE, USA). Reference carbon dioxide concentration was set at 400 ppm, leaf temperature at 25°C, and photosynthetic photon flux density (PPFD) to 1500 μmol CO_2 m^{-2} s^{-1}.

Harvest and Fruit Quality

All of the fruit was harvested from sample trees on September 6th. The total amount of fruit from each tree was weighed in the field and counted. Then, 20 fruits were randomly subsampled from each tree, 10 for at-harvest fruit quality and then 10 fruit for quality evaluation after storage were placed in RA storage for 3 months. Quality analyses was performed two days after harvest. Both diameter and weight were measured to capture variation in fruit size and density which can both affect quality. Color classification and background color were evaluated according to the Washington State Tree Fruit Reseach Commision's color classification scale and background color scale. Fruit was then rated for bitter pit incidence and fruit firmness was measured using a Güss fruit texter analyser (Güss Manufacturing Ltd, Strand, South Africa.

After firmness was measured, the apples were cut in half on the horizontal axis. The bottom half was used for starch index and the top half of the fruit was used the measurement of soluble solids content (°Brix). Starch was measured by spraying with potassium iodide solution (0.04M. The starch was measured using a scale developed for 'Honeycrisp' apple by the Washington Tree Fruit Research Commission that contained values from 1-6, where 1 indicated maximal starch concentration and a value of 6 indicated no apparent strarch. From the top portion of the apple a small section of fruit was pressed in a garlic press and two drops of juice were pipetted onto the measurement surface of an Atago (3810) handheld refractometer (Bellevue, WA).

RESULTS

In this type of soil, a shallow sandy loam soil, the maximum volumetric soil water content was between 35-40% vol/vol. Water limitations were imposed at three different times; from May 18th-June 18th, June 18th-July 19th, and July 19th-August 18th. Water was turned off at the beginning of

these three periods in the associated treatments. Volumetric soil water content was allowed to decrease until it reached approximately 12% vol/vol. At this point, water was turned on for 1-2 hours to deliver small amounts of water every 3-4 days but still keeping soil volumetric water content well below the well-watered control (Figure 1). We used soil moisture to determine when to water, not when visual symptoms were present. These periodic water limitations translated into real responses in the tree. At the end of the early, middle, and late water-limitation periods, leaf photosynthetic rates were lower than the well-watered controls. After the water limitation periods, the trees quickly recovered and photosynthetic rates increased back to levels that were not signficantly different than the controls (Figure 2). Midday leaf water potential followed a similar pattern where the highest leaf water potential was observed during periods when there were water limitations and water potential quickly increased again once irrigation patterns returned to normal (Figure 3).

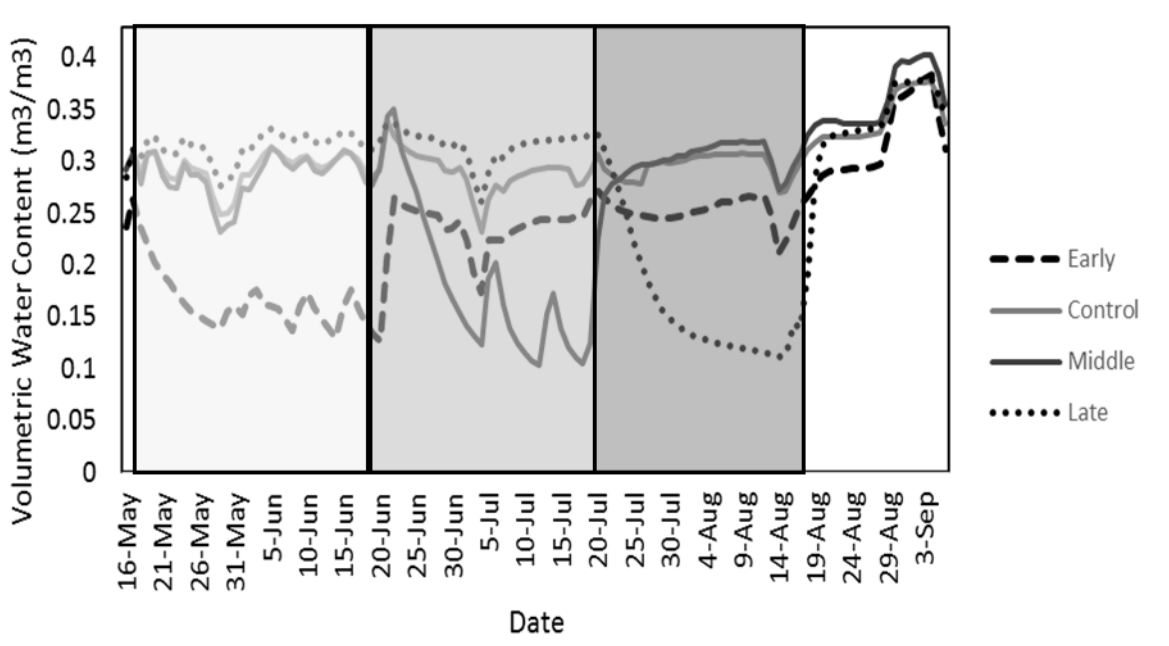


Figure 1: Volumetric water content (m^3/m^3) during the 2017 growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line; 75-105 DAFB). Light grey, medium grey, and dark grey squares represents the early, middle and late periods of water limitation, respectively.

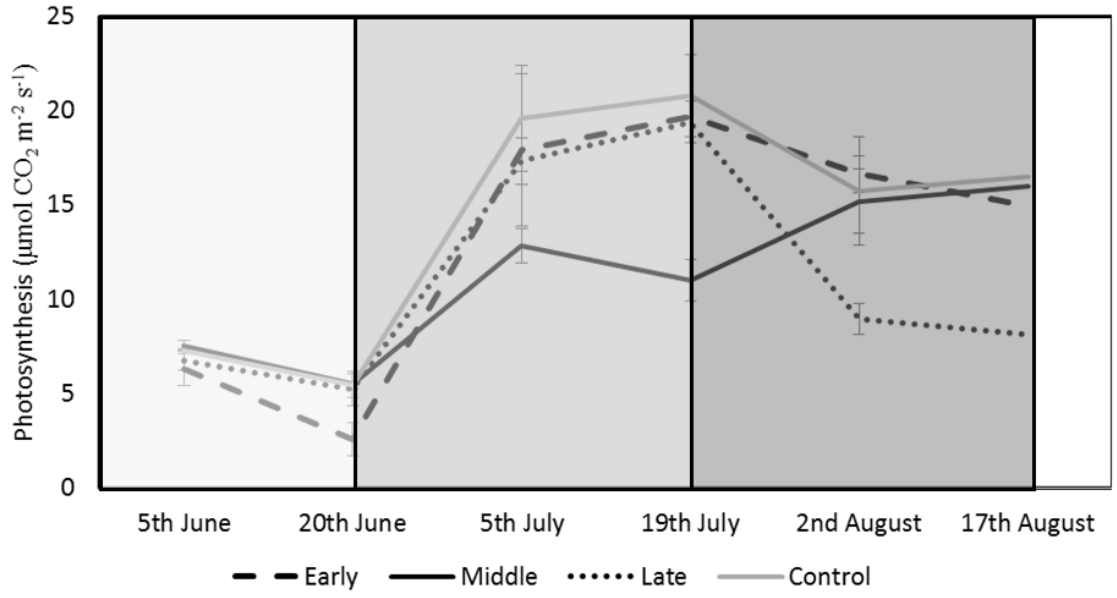


Figure 2: Mean leaf photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) during the 2017 growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line; 75-105 DAFB). Light grey, medium grey, and dark grey squares represents the early, middle and late periods of water limitation, respectively.

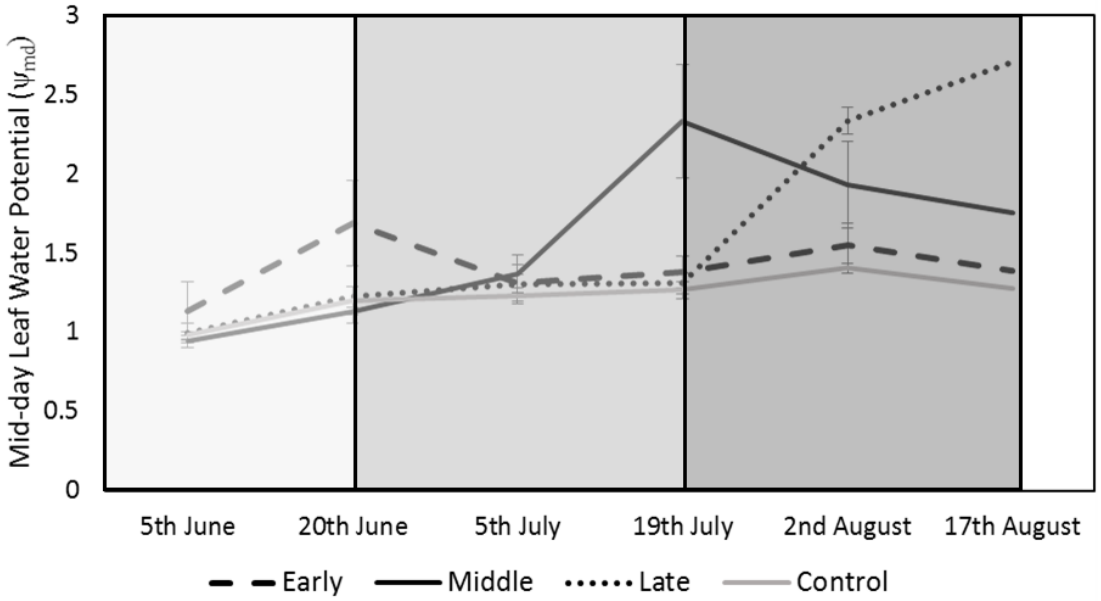


Figure 3: Mean midday leaf water potential (ψ_{md}) during the 2017 growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line; 75-105 DAFB). Higher values indicate greater water stress. Light grey, medium grey, and dark grey squares represents the early, middle and late periods of water limitation, respectively.

Fruit from trees that were either well-watered or were exposed to water-deficits early in the season had an average fruit diameter of ~84.5 mm. Fruit size decreased in water-limited treatments with the greatest impact being late in the season compared to the well-watered control (Table 1). Fruit harvested from trees that were exposed to water-deficits either during the middle or late part of the season averaged 81.8 and 79.2 mm, respectively. Similarly, fruit weight was lower from trees that were water limited later in the growing season (Figure 4).

Red color was greater in fruit from trees that were water limited compared to the well-watered control. Fruit from trees that were exposed to water deficits late in the season had an average overall red color classification of 2.94. A color classification of 3 is where 50-75% of the fruit is red. Firmness (8.93 kg), starch degradation classification (5.52) and soluble solids content (15.61 °Brix) were also highest in fruit harvested from trees that were exposed to water deficits late in the season (Table 1). In contrast, fruit from trees within the well-watered control had the lowest firmness (7.79 kg), starch degradation value (4.85) and ° Brix (13.76). Fruit from trees exposed to water deficits either early or mid-season were in-between fruit from the well-watered control and fruit from trees that were water-limited later in the season.

Bitter pit incidence was high immediately after harvest in fruit from trees that were water-limited early in the season where 24% of the fruit had bitter pit. Bitter pit incidence at harvest was low in the well-watered control and for fruit from trees that were exposed to water deficits in either the middle or later part of the growing season with 6%, 1%, and 7%, respectively (Fig 5). After 3 months of regular atmosphere storage at 2 °C, bitter pit incidence increased in all treatments. Periodic water limitations had a significant impact on bitter pit incidence. 77% of the fruit from trees that were water-limited early in the season had bitter pit compared to 59% for fruit from the well- watered control. Fruit from trees that were water- limited during the middle or late times in the growing season had 34% and 42% bitter pit incidence, respectively.

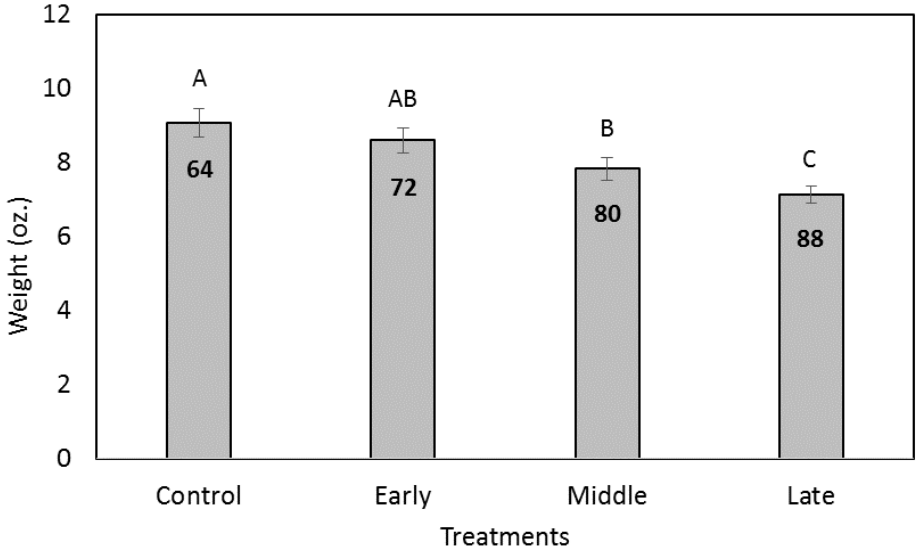


Figure 4: Fruit weight (ounces) at harvest for early (dotted; 15-45 DAFB), middle (striped; 45-75 DAFB), and late (diagonal lines; 75-105 DAFB) water-limitations compared to a well-watered control. Letter denote significant differences among treatments determined using a Tukey's test (α=0.05). Numbers within each box represent the average box size.

Table 1: Fruit quality after storage averages for each treatment from 90 fruits per treatment.

Treatment	Size (mm)	Weight (g)	Color Class (1-4)	Background Color (1-4)	Firmness (lb)	Starch (1-6)	Brix (%)
Control	84.36 a	254.99 a	2.57 b	1.68 a	17.1 a	4.85 a	13.76 a
Early	84.55 a	247.34 ab	2.67 b	1.85 a	17.4 a	5.24 ab	15.29 c
Middle	81.81 b	225.97 b	2.32 b	1.83 a	18.1 a	4.95 a	14.54 b
Late	79.24 b	209.36 c	2.94 a	1.67 a	19.6 b	5.52 b	15.61 c

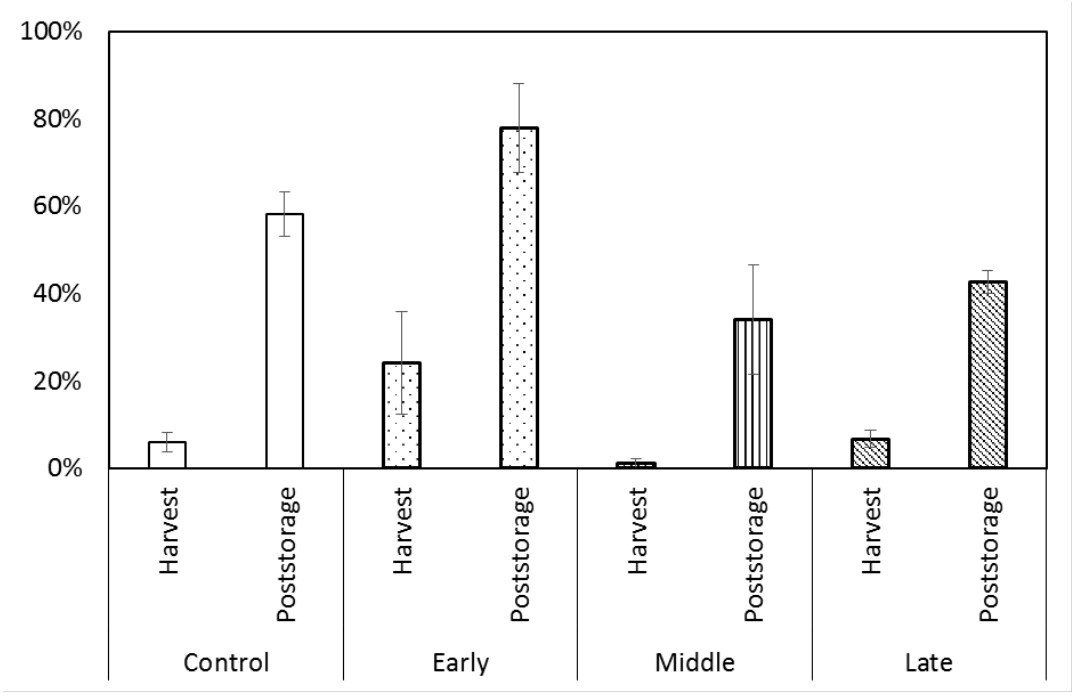


Figure 5: Bitter pit incidence during water limitation periods during early (dotted; 15-45 DAFB), middle (striped; 45-75 DAFB), and late (diagonal lines; 75-105 DAFB) compared to a well-watered control at harvest and after 3 months of regular atmosphere storage at 2°C. Vertical bars represent the SE of the total (N=3).

2018 Plans

- Repeat the experiment at Sunrise Research Orchard but incorporate regular calcium sprays into the management to reduce overall bitter pit incidence to commercial levels.
- Measure return bloom in the different treatments.
- Fruitlets and fruit from the 2017 and 2018 experiments will be analyzed for structure including cell density, size, and number to account for why early water limitations increased bitter pit in 2017.
- Work with interested grower orchards with soil moisture monitoring already in place to regulate irrigation during the middle and late period in a small plot in one of their orchard blocks. They can choose their management strategy as long as their soil moisture values can be reported to my group. My lab group will then come and sample fruit and make one set of physiological measurements on the trees to check on their status during the water limitation period.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-16-107

Project Title: At-harvest protocols for apple fruit disorder and quality management

PI: Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: USDA, ARS
Address 2: 1104 N. Western Avenue
City/State/Zip: Wenatchee, WA 98801

Co-PI: Dave Rudell
Organization: USDA, ARS
Telephone: 509-664-2280 x 245
Email: david.rudell@ars.usda.gov
Address: USDA, ARS
Address 2: 1104 N. Western Avenue
City/State/Zip: Wenatchee, WA 98801

Collaborators: Lee Kalcsits, Corina Serban, WSU TFREC, Wenatchee

Total Project Request: **Year 1:** $53,441 **Year 2:** $53,956 **Year 3: $54,476**

Other funding sources: USDA, ARS ($25,462 salary and benefits, GS-9 technician)

Budget
Organization Name: USDA, ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** Chuck.Myers@ARS.USDA.GOV

Item	2016	2017	2018
Salaries	$35,135	$35,239	$35,828
Benefits	$17,306	$17,716	$17,647
Wages			
Benefits			
Equipment			
Supplies	$1000	$1000	$1000
Travel			
Miscellaneous			
Plot Fees			
Total	$53,441	$53,956	**$54,476**

Footnotes: 0.75 Salary, benefits for GS-7 technician

OBJECTIVES

1) Identify optimum controlled atmosphere conditions during 'Honeycrisp' conditioning.
2) Determine impacts of CA established during temperature conditioning on fruit quality and disorder development of 'Gala', 'Fuji', and 'Granny Smith' apples.
3) Compare how MCP and rapid CA establishment during temperature conditioning impact disorders and fruit quality.

SIGNIFICANT FINDINGS

- 'Honeycrisp' bitter pit incidence was reduced by one to four weeks in CA followed by storage in air, or by MCP treatment the day after harvest followed by CA or air storage.
- No CO_2 disorders developed through 8 months storage on 'Honeycrisp' apples stored in CA with O_2 initially at 3% then reduced to 2% with up to 2% CO_2.
- CA during conditioning of 'Fuji' and 'Gala' has potential for disorder and quality management.
- CA storage of 'Gala' at 31°F increased internal browning and shrivel compared to fruit stored at 33°F.
- Cavity development in 'Honeycrisp' treated with MCP and stored in CA was reduced if fruit were also treated with DPA.

METHODS

Fruit will be obtained from commercial orchards at commercial maturity. At harvest, maturity analyses (starch index, firmness, soluble solids content, titratable acidity, weight, color, internal ethylene content, dry matter) will be performed. Fruit from each orchard will be conditioned 7 days at 50°F or cooled immediately to 33 or 34°F (all except 'Honeycrisp'). 1-MCP treatment will be applied the day fruit is received. CA will be initiated 1 or 7 days after receipt. Fruit will also be stored in air. All fruit will be stored in the CA cold storage facility at the USDA, ARS Wenatchee laboratory.

RESULTS & DISCUSSION

Delayed cooling: 'Gala' Two Gala lots, one with a history of internal browning development (Vantage), were obtained at commercial maturity. Fruit were held at 50°F for 7 days then at 33°F. During the week at 50°F, some fruit were treated with MCP and/or CA (1% O_2, 1% CO_2) was established. CA was also established 2 days after the storage temperature was reduced to 33°F. Senescent browning was reduced by CA and by MCP for fruit stored in air (Table 2). CA during conditioning reduced browning compared to CA after conditioning (lot 1 only). CA during conditioning also reduced firmness loss compared to CA after conditioning, however, firmness of fruit held at 33°F continuously with CA started after 7 days was similar or higher compared to CA during conditioning. Trends were similar for titratable acidity.

Orchard	weight g	ground color 1-5	starch 1-6	SSC %	TA %	lbs	IEC ppm
Vantage	202±14	3.9±0.2	1.7±0.5	10.5±0.3	0.355±0.015	19.0±1.8	0.88±0.62
Saddle Mtn	197±26	3.8±0.5	2.6±1.6	13.5±0.7	0.323±0.013	18.0±2.3	3.4±1.4

Table 1. 'Gala' maturity at harvest. Values are mean ± standard deviation for 18 fruit. Ground color: 1=green, 5=yellow; starch: 1=full, 6=none; SSC: soluble solids content; TA: titratable acidity; IEC: internal ethylene content.

Orchard	Temp °F	Atm CA: 1% O_2, 1% CO_2	MCP	Senescent browning %	Lbs	SSC %	TA %	Ground color 1-5
Vantage	50 7d/33	CAd9	No	14cd	12.7c	12.9a	0.293c	4.0
		CAd1	No	0e	15.3b	12.8a	0.321ab	3.9
		Air	No	56a	8.8d	12.1b	0.200e	4.0
		CAd9	Yes	0e	17.6a	12.2b	0.311b	4.0
		CAd1	Yes	0e	17.4a	12.7a	0.332a	4.0
		Air	Yes	18bc	16.4a	12.8a	0.313b	4.0
	33	Air	No	24b	11.8c	12.2b	0.220d	4.0
		CA d7	No	6de	17.4a	12.6a	0.315b	3.9
Saddle Mtn	50 7d/33	CAd9	No	3c	12.7d	15.0ab	0.278c	4.8a
		CAd1	No	0c	15.9bc	14.6bcd	0.282bc	3.9bc
		Air	No	13b	10.9e	14.3cd	0.186e	4.7a
		CAd9	Yes	0c	16.4ab	15.0ab	0.306a	4.2b
		CAd1	Yes	0c	16.9a	15.2a	0.307a	4.1bc
		Air	Yes	0c	15.1c	14.3cd	0.276cd	4.9a
	33	Air	No	18a	11.0e	14.2d	0.185e	4.9a
		CA	No	1c	15.1c	14.6bc	0.270cd	3.9bc
		Air	Yes	1c	16.0bc	14.8ab	0.262d	4.9a
		CA	Yes	0c	17.1a	14.8ab	0.295ab	3.8c

Table 2. 'Gala' fruit quality after 6 months. Fruit held 7 days at 68°F after removal from cold storage. Values are means of 18 fruit (54 fruit for senescent browning). Ground color: 1=green, 5=yellow; SSC: soluble solids content; TA: titratable acidity. Values followed by different letters are significantly different, $p \leq 0.05$.

Delayed cooling: 'Fuji'. 'Fuji' apples (starch = 5.9) were held at 50 °F for 7 days then at 34°F or continuously at 34°F. One or nine days after receipt CA (3% O_2, 0.5% CO_2) was initiated, two days later O_2 was reduced to 1.5%. Senescent browning was reduced by CA regardless of cooling regime (Table 3). At 8 months, browning incidence was similar in fruit immediately cooled to 34°F with CA on days 1 or 9 and conditioned fruit with CA started on day 1. CA regardless of cooling regime resulted in higher SSC, TA and firmness compared with fruit stored in air. At 8 months peel ground color was greener in fruit immediately cooled and stored in CA compared with conditioned fruit where CA was initiated on day 1 or for fruit stored in air.

Month	Temp °F	Atm.	SB %	SSC %	TA %	Lbs	Ground color 1-5
0	Harvest	---	0	12.5	0.274	13.9	2.8
4	50/34	CA d9	6c	12.9bc	0.199a	13.2b	2.9
		CA d1	3c	12.7c	0.186ab	13.5b	2.9
		Air	30b	13.2ab	0.132c	10.9c	3.1
	34	CA d9	4c	12.5c	0.183b	13.4b	2.8
		CA d1	8c	13.5a	0.187ab	14.3a	2.6
		Air	87a	11.7d	0.109d	10.4c	3.0
8	50/34	CA d9	28b	13.2a	0.127b	13.2a	2.8bc
		CA d1	1d	12.8ab	0.135ab	12.8ab	3.1ab
		Air	82a	12.2c	0.062d	12.2c	3.1ab
	34	CA d9	3cd	12.5bc	0.133ab	12.5bc	2.6c
		CA d1	12c	12.2c	0.144a	12.5bc	2.4c
		Air	84a	10.9d	0.080c	10.9d	3.4a

Table 3. 'Fuji' fruit quality after 4 and 8 months. Fruit held 7 days at 68°F after removal from cold storage. Values are means of 18 fruit (54 fruit for senescent browning). Ground color: 1=green, 5=yellow; SSC: soluble solids content; TA: titratable acidity. Values followed by different letters are significantly different, $p \leq 0.05$.

Ultra-low O_2 storage. The same **t**wo 'Gala' lots as above were cooled after harvest to 31 or 33°F. CA was established one day after receipt with O_2 at either 0.7 or 1.0% O_2 with 0.5% CO_2. One day 3 0.7% O_2 was reduced to 0.5%. CA at 31°F increased internal browning and shrivel compared to fruit held at 33°F (Table 4). While one of two lots had higher firmness in the exterior 0.25" of cortex and both lots had lower firmness in the fruit interior (0.25" to coreline) when stored at 31°F, %O_2 had no effect on firmness. Outer firmness for 33°F fruit where CA was started 1 day after harvest compared to 7 days (Table 4) appeared similar. TA was higher and peel color greener for Saddle Mtn. fruit stored at 31°F, however no effect of O_2 concentration was observed.

Orchard	Temp °F	O_2 %	Internal Browning %	Shrivel %	Lbs outer	Lbs inner	SSC %	TA %	Ground Color 1-5
Vantage	31	0.7	11a	1	17.3	21.5b	12.8b	0.328	4.0
		1.0	18a	1	17.5	21.9b	13.0ab	0.328	4.0
	33	0.7	3b	0	17.4	23.7a	13.0ab	0.321	4.0
		1.0	0b	0	17.6	24.0a	13.2a	0.320	4.0
Saddle Mtn	31	0.7	0	11a	17.7a	20.1ab	15.7a	0.303a	3.3c
		1.0	1	11a	17.2a	19.9b	15.3ab	0.303a	3.6bc
	33	0.7	0	0b	15.8b	21.4a	15.2ab	0.281b	3.9ab
		1.0	1	0b	15.8b	21.4a	14.9a	0.294ab	4.2a

Table 4. 'Gala' fruit quality after 7 months in CA storage. "Lbs outer" = maximum firmness of outer 0.25" cortex; "Lbs inner" = maximum firmness of cortex 0.25" to coreline. Values are averages (n=54 internal browning, shrivel; n=18 firmness, SSC, TA, peel ground color). Values followed by different letters are significantly different, $p \leq 0.05$.

'Honeycrisp' experiments. **Short-term CA.** (2016-17) Fruit from two commercial orchards near Quincy, WA were held at 50ºF for 7 days. MCP was applied to some fruit the day of receipt. Fruit was stored in air or CA (2.5% O_2, 0.5% CO_2) established 1 day after receipt. After 1, 2, or 4 weeks, CA fruit was moved to air. Through 8 months, storage in CA for 1-4 weeks reduced bitter pit development compared to fruit stored continuously in air (Figure 1). Treatment with MCP reduced bitter pit development in fruit stored in air or CA. No internal disorders were observed in any fruit. Titratable acidity and peel green color were generally highest in fruit stored the longest in CA. Results from 2017 harvest are consistent with previous years (Figure 2). Average bitter pit reduction from CA during conditioning is 60% or higher for all lots (5) over 3 years (Figure 3). No increase in other disorders, external or internal, has been observed due to rapid CA.

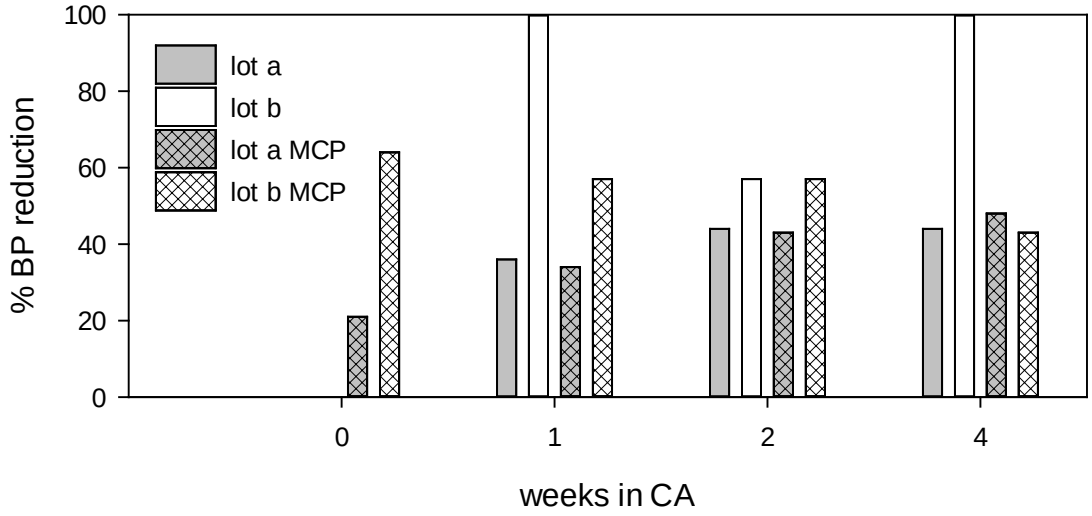


Figure 1. 2016 bitter pit incidence in 'Honeycrisp' apples through 4 months in storage (orchard B). All fruit were held 7 days at 50ºF then at 37ºF. Fruit were held in air or a CA (2.5% O_2, 0.5% CO_2) established 1 day after harvest for 1,2, or 4 weeks then in air at 37ºF.

Lot	Weeks CA	MCP	Cortex Browning %	Cavities %	SSC %	TA %	Lbs	Ground color 1-5
A	0	No	0	0	12.3	0.389bc	13.0	4.2a
		Yes	0	0	12.9	0.412ab	12.5	3.9ab
	1	No	0	0	12.6	0.345c	12.9	3.6bc
		Yes	0	0	12.1	0.400ab	12.2	3.8ab
	2	No	0	0	11.9	0.373bc	13.1	3.3c
		Yes	0	0	12.3	0.415ab	12.6	3.4bc
	4	No	0	0	11.9	0.392bc	12.7	3.2c
		Yes	0	0	12.7	0.434a	12.6	3.3c

Table 5. 'Honeycrisp' quality and disorders after 4 months. Values are averages (n=54 cortex browning, cavities; n=18 firmness, SSC, TA, peel ground color). Values followed by different letters are significantly different, $p \leq 0.05$.

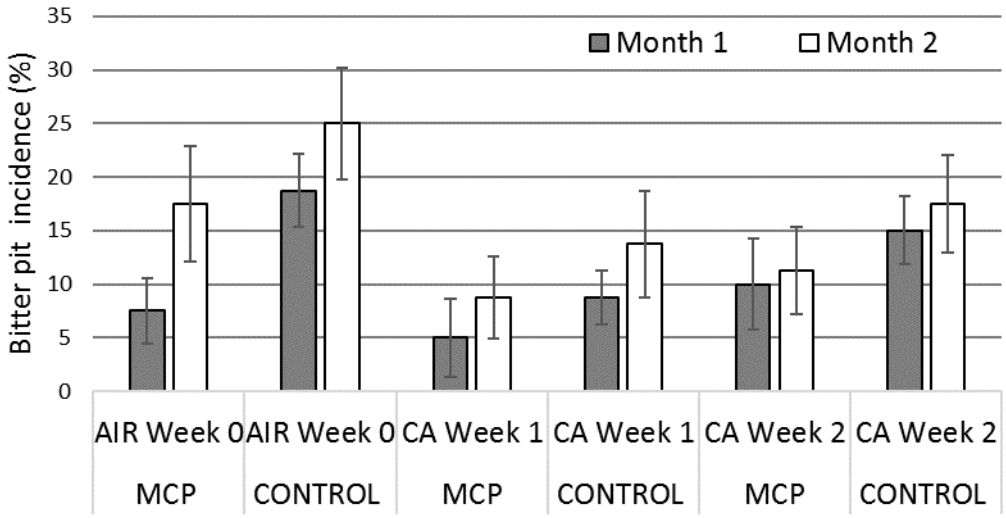


Figure 2. 2017 bitter pit incidence in 'Honeycrisp' apples at 1 and 2 months in storage (lot A). All fruit were held 7 days at 50°F then cooled to 37°F. Fruit were held in air or CA (2.5% O_2, 0.5% CO_2) established 1 day after harvest for 1 or 2 weeks then in air at 37°F. Each value represents the mean of bitter pit incidence (%) per treatment. Each treatment had 5 replications with 16 fruit per replication. C.Serban figure.

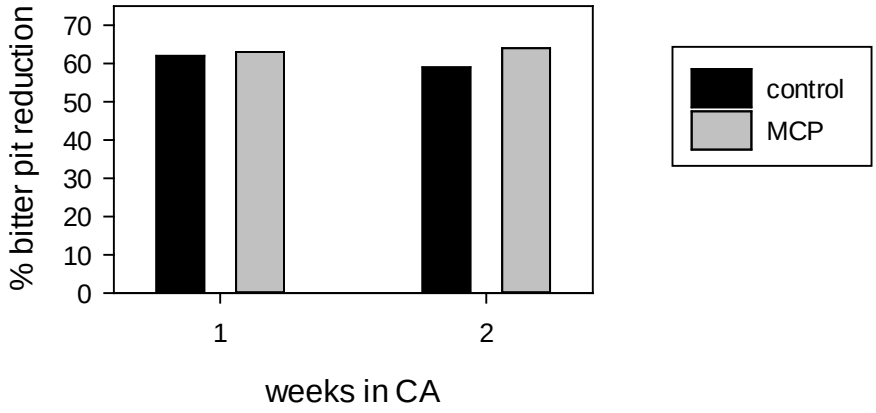


Figure 3. Average reduction in bitter pit incidence for 5 orchard lots over 3 harvest years where fruit were held one or two weeks in CA (initiated during conditioning) with or without prior MCP treatment, then through 4 months in air.

DPA and Rapid CA. 'Honeycrisp' apples can develop internal browning during low O_2/high CO_2 storage. DPA can prevent this type of injury in 'Honeycrisp' and other varieties but has not been evaluated when CA is established during conditioning. Fruit were treated with 1000 ppm DPA and/or 1-MCP after harvest then stored in CA established during conditioning. MCP fruit from lot B developed cavities (9%), controls and MCP/DPA fruit did not.

Lot	Treatment	Cavities %	Stem-end browning %	Soft-scald %	Soggy breakdown %
A	Control	1	1	0	0
	MCP	3	3	0	1
	MCP/DPA	0	0	0	0
B	Control	0b	0	0	2
	MCP	9a	0	0	3
	MCP/DPA	0b	0	0	0

Table 6. 'Honeycrisp' disorders after 8 months CA storage. Values are averages (n=54). Values followed by different letters are significantly different, $p \leq 0.05$.

CA Settings during Conditioning: CA during conditioning to date has reduced bitter pit development and not caused development of other disorders. In addition to the CA protocol used previously (3% O_2 for 2 days then 2%, 0.5% CO_2), fruit were stored in 2% O_2, 0.5% CO_2 continuously, or the 3% O_2 for 2 days then 2% protocol with 1 or 2% CO_2.
Overall MCP treated fruit had less bitter pit, more peel blotch, but the same total number of fruit with an external disorder (Table 7). No absolute relationships between O2 and CO2 protocols and internal disorders or lenticel breakdown were observed.

CA	MCP	BP %	peel blotch %	Lenticel breakdown %	Core Browning %	Internal browning %	Cavities %
2% O_2, 0.5% CO_2	no	33b	0b	0b	3	12ab	1b
3/2% O_2, 0.5% CO_2		37ab	23a	7ab	0	12ab	12a
3/2% O_2, 1% CO_2		47ab	0b	0b	1	6b	9ab
3/2% O_2, 2% CO_2		45ab	20a	6ab	4	10ab	15a
2% O_2, 0.5% CO_2	yes	55a	0b	10a	1	10ab	4b
3/2% O_2, 0.5% CO_2		27b	24a	6ab	3	10ab	14a
3/2% O_2, 1% CO_2		52a	0	4ab	3	24a	8ab
3/2% O_2, 2% CO_2		32b	14a	10a	3	11ab	10ab

Table 7. 'Honeycrisp' disorders after 8 months storage. BP: bitter pit; BP severity: 1=none; 2=1-25% peel with bitter pit; 3=26-50% peel with bitter pit; 4=51-100% peel with bitter pit. Peel blotch=peel lesions larger than bitter pit.

CONTINUING PROJECT REPORT **YEAR:** 2 of 3
WTFRC Project Number: AP-16-101

Project Title: Reducing scald after long-term CA storage

PI:	David Rudell	**Co-PI**:	James Mattheis
Organization:	USDA-ARS, TFRL	**Organization**:	USDA-ARS, TFRL
Telephone:	509 664 2280 (ext. 245)	**Telephone**:	509 664 2280 (ext. 249)
Email:	David.Rudell@ars.usda.gov	**Email**:	James.Mattheis@ars.usda.gov

Budget: **Year 1**: $30,690 **Year 2**: $63,095 **Year 3**: **$72,508**

Collaborators: Brenton Poirier, Ed Valdez, Loren Honaas, Girish Ganjyal, Ines Hanrahan, Heidi Hargarten

WTFRC Collaborative expenses:

Item	2016	2017	2018
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental	$6,300	$6,300	$6,300
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$6,300	$6,300	**$6,300**

Footnotes: Costs for 1 RCA room

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** chuck.myers@ars.usda.gov

Item	2016	2017	2018
Salaries	$18,338	$39,004	$41,344
Benefits	$6,052	$12,871	$13,644
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous *		$11,220	$11,220
Plot Fees			
Total	$24,390	$75,695	**$66,208**

Footnotes: One-third of instrument service contract

OBJECTIVES:
1. Identify rapid, stress provoking, at-harvest treatments that reduce scald levels during a prolonged supply chain.
2. Validate changes in peel chemistry as indicators of efficacy for stress-based scald treatments.
3. Determine how at-harvest treatments that provoke stress impact other fruit quality factors.
4. Determine if post-storage reduction of ethylene action is a feasible post-storage scald control technique.

Goals and activities for the next year:
Determine how harvest maturity influences acclimation treatments. Determine factors influencing post-storage heat treatment/acclimation and ethylene mitigation for scald control. Determine supply chain temperature recommendations for the best scald outcomes. Defining if and how the scald induction window is influenced by storage atmosphere and CA imposition delay.

SIGNIFICANT FINDINGS:
1. Physical stress most effectively controlling superficial scald- must occur prior to 1 week of cold storage.
2. Stress responses that prevent scald from developing may require warmer temperatures prior to cold storage.
3. The effectiveness of acclimation treatments is dependent on whether fruit are stored in CA or air.
4. Post-storage (3 or 6 months 0.6% O_2 CA storage) 1-MCP treatment reduced scald to varying degrees up to 4 months in 37 °F air when storage atmosphere was imposed within a day following harvest.
5. Results suggest proper CA conditions imposed in a timely manner extend the scald induction period.
6. Reducing temperature from 37 °F to 33 °F following CA storage substantially improves scald outcome in the supply chain.
7. New scald risk assessment biomarkers based on gene expression indicate if storage environment was effective after up to 6 months (Honaas Lab).

METHODS:
Equipment and Cooperative Summary: Stress treatments (excluding impingement drier) as well as fruit quality, tissue sampling, processing and analysis of SRABs using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. Treatment using the impingement drier was performed at BSYSE, WSU-Pullman in collaboration with Drs. Ganjyal and Hanrahan. Pressure treatment was performed by Dr. Honaas and staff at ARS-Wenatchee. Storage experiments will be performed both at ARS-Wenatchee and in Stemilt RCA storages. New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

<u>Year 1 (includes activities outlined for Objectives 1, 2, and 3)</u>

Temperature conditioning (3 experiments)
Delayed cooling and intermittent warming: Granny Smith apples were conditioned at 68 °F for 0, 3, 5, and 7 days prior to storage in 0.7 or 1 % O_2 (0.5% CO_2) for 6 months. (All of the stress treatments below included fruit that went immediately into storage and a group that were left at 68 °F for 2 days). Another treatment included fruit that were immediately placed in 33 °F for 1 week, then 2 days at 68 °F, then back to 33 °F air or 1 % O_2 (0.5% CO_2) for up to 6 months. Fruit stored in CA

underwent scald and quality evaluation during and following a 3 month supply chain simulation at 37 °F.

Impingement oven: Granny Smith apples were treated using the impingement drier at the WSU Biosystems Engineering (Girish Ganjyal) at ~130 °F (upper surface temperature for ~ 20 s). Fruit were placed immediately into 33 °F air and scald evaluated at 3 and 6 months.

Stress amendment treatment

Impact injury: To indicate the time required for scald-free zones to form around a bruise, the impact of physical stress on scald was assessed using a standardized impact force to equally bruise Granny Smith apples at different time points beginning and then 2, 4, and 8 weeks after putting fruit in cold air storage at 33 °F. To bruise fruit, a 5.6 g ball-bearing was dropped directly onto two positions on the unexposed side of the fruit in a glass tube to assure an equidistant (38 cm) drop and therefore equal force. The initial indentation was 0.58 cm. Scald incidence and severity as well as diameter of scald free area around each bruise was evaluated monthly until 6 months.

Chemical stressors: A variety of other stress *treatments were applied at harvest with the idea of improving the effectiveness and/or reducing the duration of effective temperature acclimation of Granny Smith apples. Following treatment, apples will be placed immediately in 33 °F air or CA (0.6% O_2: 0.5% CO_2) storage or left at 68 °F for 2 d before being placed into CA. Subsequent years will focus on optimizing those treatments with the best supply chain quality outcomes. Fruit from the dry heat or pressure treatment were not stored in CA. Year 1 treatments included nitric oxide/nitrogen dioxide fumigation, H_2O_2 solution drenching, and superoxide solution drenching, and O_3 treatment for different durations (up to 3 h) and concentration (up to 50 ppm for 1 h).

Post-(CA)storage supply chain optimization

Ethylene mitigation. Granny Smith apples were placed in CA chambers at Tree Fruit Research Laboratory and stored in 0.6% O_2: 0.5% CO_2 at 33 °F within 24 h after harvest. Fruit were removed after 3 and 6 months storage. At each pullout, a subset was treated with 1 ppm 1-MCP for 12 h. A second trial using 4 lots of Granny Smith stored commercially (multiple rooms) at 0.6-0.8% O_2:1% CO_2 for 6 months was treated with 1 ppm 1-MCP for 12 h or submerged in 3.33 g gallon-1 Retain solution. Controls and treated fruit were moved to 37 °F air storage and scald evaluated monthly.

Supply chain temperature study. Granny Smith were sampled from 4 lots stored commercially (multiple rooms) at 0.6-0.8% O_2:1% CO_2 for 6 months. Within 2 days following removal from CA, apples were placed in 33 °F, 37 °F, or 40 °F air for 5 months and scald evaluated monthly.

Tissue sampling for metabolic profiling and SRAB monitoring

Peel has been and will be sampled following each stress treatment and/or temperature conditioning combination and then following 14, 30, 60, 90, 120, and 180 days after cold storage inception. Metabolic profiling will be performed on these samples with the idea of continuing to associate risk, as impacted by stress, with changes in peel chemistry. We will use untargeted metabolic profiling to better understand how peel chemistry changes with respect to different stresses imposed to find chemistries that indicate a treatment is effective at controlling scald as well as common changes associated with other postharvest disorders. Existing scald risk assessment biomarkers (SRABs) are continually monitored by extracting wax and estimating levels using a spectrophotometer as outlined as an outcome of our previous project. These will be monitored for every treatment at least monthly for up to 4 months. This process will further test the existing risk assessment under these conditions.

Testing gene expression candidates. Peel from 12 lots of commercially stored Granny Smith apples (0.6-0.8% O_2:1% CO_2; multiple rooms) was taken 2 days following removal from storage. Ninety fruit from each lot was stored up to 5 months evaluating scald monthly. Peel was analyzed using qPCR of candidate genes selected for scald risk assessment during previous projects (Honaas group).

Quality and scald incidence assessment
Quality assessment (Firmness, TA, soluble solids) will be performed at harvest, following treatment, and upon removal from storage throughout the simulated supply chain period for all experiments except supply chain experiments. Incidence of superficial scald and other defects will be identified and quantified with all quality assessments as well as repeatedly on all samples that are not destroyed.

Year 2 (includes objectives 1, 2, and 3)

Temperature conditioning
Granny Smith apples were harvested at commercial maturity and two weeks prior. Apples were subjected to three different heat treatments prior to storage: (1) hot water submergence (118°F for 3 min); (2) warm air (100°F for 72 h); and (3) hot air (108°F for 24 h). For each treatment, apples were either placed immediately into cold storage (33 °F air) or held at 68°F for 2 days prior to cold storage; untreated controls were included for both storage conditions

Delayed DPA in different CA environments
Granny Smith apples will be stored at 33°F in air and three different CA storage conditions: (1) 2% O_2 (0.5% CO_2); (2) 1% O_2 (0.5% CO_2); and (3) 0.5% O_2 (0.5% CO_2). Apples were treated with DPA immediately following harvest and additional treatments will be performed on separate apples at 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, and 6 months postharvest. Untreated controls have been included for each storage condition. All apples will be moved to 37°F air storage after 6 months for continued evaluation.

Post-storage 1-MCP treatment with CA storage delays
Granny Smith apples were placed into CA storage (33°F, 0.6% O_2:0.5% CO_2) immediately after harvest or following a 2 week or 4 week delay. After 6 months, all apples will be removed from CA and placed in 37°F air storage. Apples from each of the three storage conditions will be treated with 1-MCP (1ppm for 12h) immediately upon removal from CA. Separate sets of apples from immediate CA storage will be treated with 1-MCP following a 1 day or 2 day delay after removal from CA. Untreated controls have also been included for each CA storage condition.

Post-storage heat treatment and wounding experiments
Granny Smith apples were placed into CA storage (33°F, 0.6% O2:0.5% CO_2) immediately after harvest. After removal from CA at 6 months, apples will be subjected to either hot water (118°F for 3 min) or warm air (68°F for 2 days) temperature treatments. Additional apples will undergo an injury treatment of bruising with a 5.6 g ball-bearing drop, as described above.

RESULTS AND DISCUSSION:
Physical damage assessment
Physical injuries to the fruit surface including bruises, limb rubs, sunburn, as well as disorders that appear prior to or soon after harvest, such as bitter pit can develop regions of the peel within and/or immediately around the pre-existing injury that do not develop scald symptoms. These regions are typically attributed to some, yet undefined, form of innate immunity triggered by the prior injury that renders the peel insensitive to chilling stress during the first month when superficial scald is initiated. While it can be assumed that stress-induced immunity occurs prior to the induction of superficial scald (the first 1-2 months in cold air storage), the optimal time when a stress most effectively leads to immunity is unknown.

This set of experiments assessed whether the injury must occur prior to storage and how long into harvest injury would form a scald-clear zone. Injury was assessed at 4 and then 6 months. These results (Fig. 1) and other similar tests (not shown) indicated the immunity is initiated at harvest and less so 1 week following cold storage initiation. Evidence indicates that physical stress (limb rubs, finger bruises, and the other aforementioned injuries) must occur prior to or very near the beginning of cold storage and that the resulting immunity is most likely an active metabolic process that may best arise during warmer temperatures.

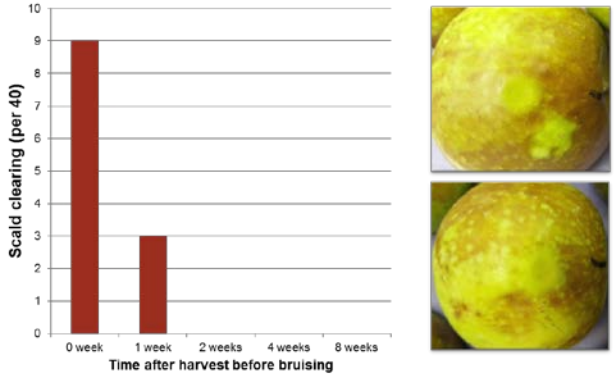


Fig. 1. Incidence of "scald-free" region (right) after 6 months 33 ºF air storage in and around peel intentionally bruised at 0, 1, 2, 4, and 8 weeks following cold storage inception.

Other stress response treatments

Our findings from the preliminary experiment indicate that some form of stress similar to bruising, wounding, sunburn, or bitter pit prior to cold storage could provide some degree of scald protection. Of course, any stress employed as a control measure could not visibly injure the fruit and would optimally have no impact on internal quality. Consequently, in the first season, we focused on screening a number of known chemical and physical stressors that might meet these criteria. These focused on gaseous treatments expected to provoke oxidative stress (ozone, nitrogen dioxide, hydrogen peroxide, superoxide) as well as non-bruising physical stressors at this key time point prior to storage. In each case, fruit were either placed immediately into 33 °F or left for 2 days at 68 °F to indicate if any process leading to scald immunity musters more effectively at warmer temperatures.

Results indicate that stress response processes were mustered during this 2 day adaptation period prior to cold storage. O_3 was applied for 1 h to Granny Smith at 3 different rates prior to cold air and CA storage. As of 3 months storage, the 50 ppm treatment developed typical O_3 damage (a lenticel blotch) but only on fruit that were placed in air storage immediately. This may be indicative of a stress response process that transpired within the first few days following O_3 treatment but was impeded by chilling temperatures, resulting in cell death around the lenticels. However, superficial scald incidence was not reduced by any O_3 treatment either in air or CA storage. Scald was reduced by the 2 day delay before cold storage for the air but not the CA stored treatments. Other chemical stress treatments had similar results. No treatment had an impact similar to physical injury or sun exposure.

Intermittent warming

As with results from earlier projects, a period of intermittent warming as well as a 2 day delay before placement in cold storage at harvest reduced scald incidence during air storage but not during a prolonged supply chain following effective CA storage (Fig 2). Our further work will focus on confirming if fruit maturity impacts the effectiveness of warming treatments at harvest and investigating whether temperature acclimation may actually be effective for scald control following removal from long-term CA storage.

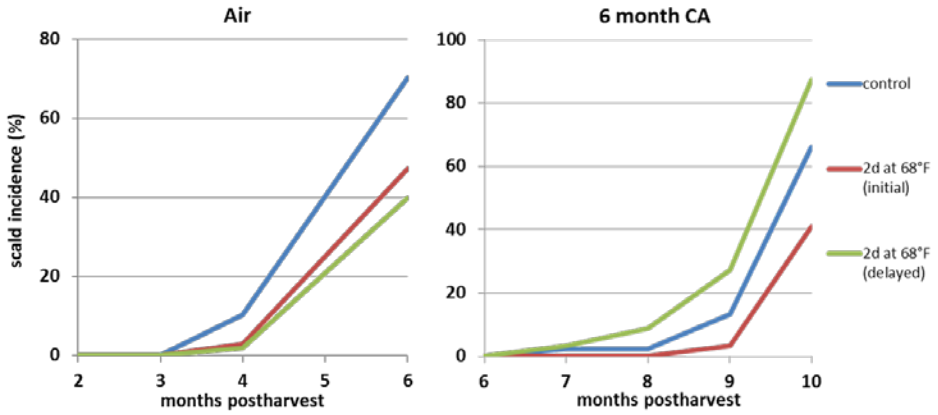


Fig. 2. Temperature acclimation treatments. An initial warming treatment at 68 °F for 2 days reduced scald for both CA and air-stored apples. A delayed warming treatment (after 1 week of cold storage) resulted in scald reduction for air-stored apples, but not for CA-stored apples.

Post-storage 1-MCP treatment

One consistent theme from the results throughout this report is that temperature acclimation, even the 2 day delay imposed in many of the experiments, was only effective for controlling scald during air storage but not always following effective CA storage. A completely different test may have revealed why storage conditions influence the efficacy of acclimation regimes. 1-MCP treatment following 3 or 6 months of immediately imposed, effective CA conditions delayed scald development for 5 months in 37 ºF air storage (Fig. 3). However, 1-MCP had no impact after 6 months storage in commercial rooms. One principal difference between the in-house test and the experiment carried out on the commercial fruit was the time it took to load and impose the CA environment. It is well known that scald induction occurs during the first 1-2 months in air storage. This evidence indicates that the scald induction period may be delayed or the rate of injury reduced when effective CA is employed. It also serves as another illustration of how critical rapid room loading and CA imposition is for scald prevention. As post-storage acclimation and ethylene mitigation for scald control may be useful tools at the end of or following removal from storage and prior to packing, we are focusing on determining if the injury induction period is indeed extended by CA. It also opens up the possibility of effective heat treatment following cold storage and prior to packing, which is another focus of the current year's experiments.

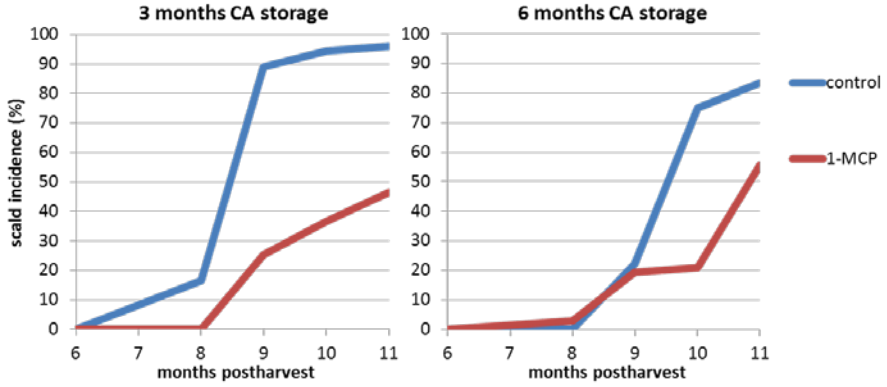


Fig. 3. Delayed 1-MCP treatment following controlled atmosphere storage. Treatment with 1-MCP immediately after removal from CA at 3 and 6 months decreased scald incidence during a prolonged supply chain.

Supply chain temperature

Supply chain temperature had a significant impact on apples remaining scald-free. Organic Granny Smith stored in commercial CA (0.6-0.8% O_2) did not develop scald until between 3-4 months in 33 ºF air, 2-3 months in 36 ºF air, and 1-2 months in 40 ºF air following removal from CA (Fig 4). Results indicate that, especially for fruit not treated with 1-MCP or DPA, the window for scald free fruit can be significantly extended "simply" by reducing the post-CA storage temperature. Our current experiments are focusing on determining if temperature between 33 and 36 ºF can be as effective as 33 ºF and the best conditions for organic apples on the retail shelf.

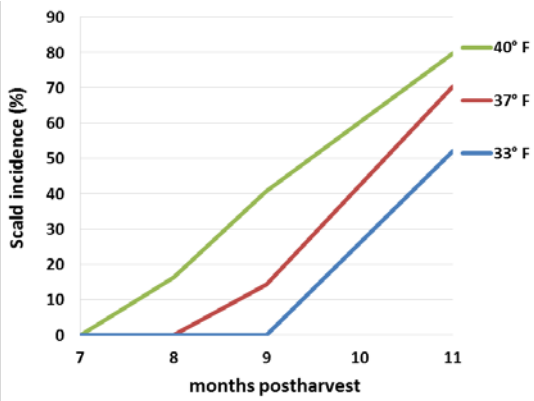


Fig. 4. Supply chain temperature optimization. Results indicate that 33 °F is the optimal temperature apples remaining scald-free for a longer period in the supply chain.

Scald risk assessment

CTOL evidence from commercial trials predicted problems based on less than adequate room O_2 settings. As the CTOL-based scald risk assessment test (Blakey and Rudell, 2017) is not accurate following 2-3 months in storage, we have sought other tests for latter storage periods. Other scald risk assessment biomarkers, based on gene expression (tested by the Honaas lab), also indicated scald risk at the 6 month pull out resulting from less adequate CA settings but did not accurately predict supply chain performance among lots when lots were stored in adequate CA environment. Instead, this may indicate that injury began following CA rather than during CA in those lots, and we should continue risk assessment using the gene expression biomarkers during the supply chain to indicate lot to lot performance. Current work will look into monitoring expression of these genes during the supply chain as a basis for assessing scald risk and managing stock accordingly.

Publications

Blakey, R. and D.R. Rudell. 2017. Superficial scald risk assessment assay for apples. WSU Extension Bulletin FS287E. (www.extension.wsu.edu/publications/)

CONTINUING PROJECT REPORT **YEAR:** 2 of 3
WTFRC Project Number: AP-16-102

Project Title: Risk assessment for delayed sunburn and sunscald

PI: David Rudell
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 245)
Email: David.Rudell@ars.usda.gov

Co-PI: James Mattheis
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 249)
Email: James.Mattheis@ars.usda.gov

Co-PI: Carolina Torres
Organization: Centro de Pomáceas, Univ. of Talca, Chile
Telephone: +56 9 6847 0541
Email: cartorres@utalca.cl

Budget: **Year 1**: $67,427 **Year 2**: $70,865 **Year 3**: **$72,595**

Collaborators: Christine McTavish, Omar Hernández, Brenton Poirier

Other funding sources

Agency Name: CONICYT, Chile (proposed)
Amt. awarded: $88,700 (total over 3 years)
Notes: Funds for supplies and materials, travel, and analytical services.

Budget
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510) 559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2016	2017	2018
Salaries	$40,757	$43,342	$44,620
Benefits	$13,450	$14,303	$14,755
Wages			
Benefits			
Equipment			
Supplies			
Travel	$2,000	$2,000	$2,000
Miscellaneous*	$11,220	$11,220	$11,220
Plot Fees			
Total	$67,427	$70,865	**$72,595**

Footnotes: One-third instrument service contract

Objectives:

1. Identify changes in apple peel chemistry associated with response to light prior to and during cold storage.

2. Determine if changes in peel chemistry are specifically indicative of delayed sunscald and other sun-related postharvest peel disorder risk prior to symptom development.

3. Develop protocols to establish tissue viability before and during cold storage.

Goals and activities for the next year:
Separate the impacts of heat and light on metabolism to determine what targets associated with risk are light and/or heat related and if heat can cause sunscald alone. Test if UV reflectance imaging that targets a peel chemical associated with risk is a basis for non-destructively determining risk at harvest and if patterns of absorbance indicate the exact area where sunscald will occur. Focus on analysis of novel natural chemicals in the wax and cuticle coating that may be enhanced or depleted by the sun leading to sunscald. Continue work assessing the ability of the peel to produce energy during the period leading up to sunscald development.

SIGNIFICANT FINDINGS:

8. Peel appearance (symptoms) changes in different ways during storage depending upon cultivar.
9. Peel chemistry changes differentially depending upon pre-harvest sun exposure, indicating continued stress on the exposed side of the fruit during cold storage.
10. Heating following harvest lead to symptoms similar to sunscald.
11. Visible/near infrared reflectance models (Torres lab) effectively indicated sunscald risk for Washington state Granny Smith apples.
12. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.

Methods:

Equipment and Cooperative Summary: Fruit quality, tissue sampling, processing and peel chemistry analysis using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. UV-vis reflectance spectral deconvolution and modelling is being performed by Dr. Torres. Both storage experiments will be performed at ARS-Wenatchee. New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

2016-2017 (primarily Objective 1)
The influence of pre-harvest light environment alone and during the transition to cold storage on peel metabolism is different among apple cultivars. We analyzed all apple peel chemicals included in our full metabolic analysis with special focus on lipids, oils, and waxes and other oil-soluble metabolites that may be most impacted by light and temperature together. To begin to address this, we analyzed peel chemistry of 4 apple cultivars that are differentially impacted by the combination of high light and chilling. We harvested approximately 360 apples each of ‘Granny Smith’ (9/20/16), Gala (8/5/2016), ‘September Fuji’ (8/23/2016), and ‘Honeycrisp’ (8/22/16) at around commercial harvest. Orchards were selected for smaller trees with high sun exposure. Fruit that were obviously exposed in clear contrast with back side were picked. The exposed sides were marked on the stem end. Once back in the lab, apples were further sorted to obtain the best front to back contrasts and cull any fruit

that were not exposed enough on the exposed side or too exposed on the backside. Fruit with sunburn above the median level representing that orchard were retained in their own category. Starch index and internal ethylene were assessed at harvest. Fruit were stored in air at 33 ºF.

At 0, 2, 4, and 8 weeks (and then monthly until 6 months) sun damage incidence was monitored on Gala, Fuji, and Honeycrisp and categorized on Granny Smith by rating the exposed side 0-4 (Table 1). At 0, 2, 4, and 8 weeks (and, finally, at 6 months), color was also monitored on both sides using a Minolta colorimeter as well as peel sampled. One goal was to carefully characterize visual differences in any delayed symptoms among cultivars, and changes were photographically recorded on the front and back of the fruit. Peel samples taken from those time points were analyzed for over 800 metabolites. Metabolite data analysis focused on determining differential changes between the exposed and unexposed sides of the fruit upon chilling stress, possibly linking specific changes in peel chemistry with specific delayed conditions. Additional samples consisting exclusively of sunscald tissue were analyzed to determine where peel chemistry was most linked with the disorder.

Table 1. Sun damage severity rating table for Granny Smith. A rating of 4 constitutes delayed sunscald.

Rating	Description
0	Green (no damage)
1	Sun (yellow)
2	Blush
3	Darkening brown
4	Brown/black

Sunscald prediction model and UV-vis reflectance characterization

A model to predict delayed sunscald of Granny Smith based on the degree of sun exposure at harvest has been developed for Chilean apple producers by the Torres laboratory. To test this model and work towards adaptation to our climate, 90 fruit from 5 different Granny Smith lots were sampled and the degree of sun exposure of each fruit was cataloged. These values were entered into the mathematical model which, in turn, generated a % sunscald prediction. Afterwards, fruit were placed into 33 ºF air storage. Sunscald final ratings at 6 months were compared to the model predictions.

Ten other Granny Smith lots were picked from bins for analysis using a UV-Vis reflectance spectrometer at harvest. This work was performed by a visiting collaborator from the Torres Laboratory. Again, fruit were immediately placed in 33 ºF cold storage and final sunscald ratings were taken at 4 months of storage. The UV-Vis spectra from 108 fruit from each lot were incorporated into existing models from Chile and were tested against actual sunscald incidence.

2017-2018 (Objectives 1 and 2)

Hand thinned Granny Smith apples (645) in the Columbia View experimental orchard were heated on both the sun facing and shaded sides to 130 ºF for 3 minutes at 4 and 2, and 1 week(s) before commercial harvest. Fifteen fruit were bagged with green sleeved paper apple bags at each time point and remained so until harvest. Another 15 fruit were heated to 130 ºF and bagged. Four days before harvest, 45 additional fruit were treated at 130, 115, and 100 ºF. Unheated controls were included at every treatment date.

Injury on each fruit will be tracked using image analysis. Peel from the front and back will be sampled at 0, 3, and 6 months to determine if compounds associated with sunscald in year 1 were more associated with sun stress, heat stress, or a combination and levels of which compounds are most inextricably associated with injury. Peel from both heat damage and sunscald from unheated fruit will be compared.

An additional tool was developed using a camera adapted to detect specific wavelengths of light. Target metabolites associated with sunscald risk absorb light in specific wavelengths. Peel will be taken from regions of different absorbance in this bandpass (region of the spectrum) to verify if target peel chemicals are represented by absorption (darkness). Darkness will be quantified and compared with peel injury to determine if a larger experiment is warranted in the next year.

RESULTS AND DISCUSSION:

Evaluation of the sun damage incidence indicated differential changes of appearance depending on sun exposure of Fuji, Honeycrisp, or Granny Smith (Figs. 1, left and 2). The sun facing side of Gala tended to darken some but no disorders were noted. In Fuji and Honeycrisp there were changes of symptom appearance, although these changes were more towards a "muddy" background to even greening resulting from anthocyanin (red color) loss much like "stain". Honeycrisp developed severe lenticel blotch on the sun side of many fruit. Sunscald severity (0-4) of Granny Smith demonstrates the transition of blushed or sunburned sides of the fruit to more severe sun damage, including delayed sunscald, over the first 4 months of storage (Fig. 1, right). These changes and the timing of the changes in every one of these cultivars reference those from multiple previous studies. It is important to note that solar radiation also includes heat which is thought to be a principal cause of sunburn rather than only ultraviolet/visible light. Delayed sunscald is typically the progressive darkening or browning of the exposed side and is not thought to be related to cold stress but, rather, the continuation of the effects of pre-harvest irradiation well into storage. However, stain is a combination of irradiation and chilling stress, as it can be reduced using cold acclimation techniques at the start of cold storage. Honeycrisp and Fuji can develop stain in the Washington climate and Gala can in the Maule, Chile climate. Granny Smith develops delayed sunscald in both climates.

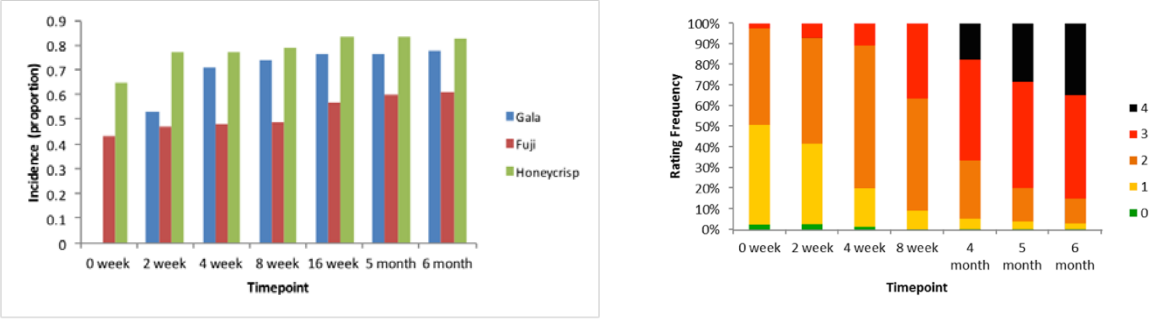


Fig. 1. (left) Incidence of sun damage for 6 months of storage. Sun damage incidence remained largely unchanged for Gala, Fuji, and Honeycrisp, instead changing in appearance where existing symptoms and/or surrounding areas acquired a muddy appearance. (right) Sunscald severity (0-4) (Table 1) on Granny Smith as rated for 6 months of 33 ºF air storage. Sunscald severity had already increased between 2 and 4 weeks as can be noted by the decrease in fruit in category 1 and increases in categories 2 and 3. At 4 months, a "sooty" browning indicative of delayed sunscald (see Fig. 2) appeared and increased in frequency throughout storage.

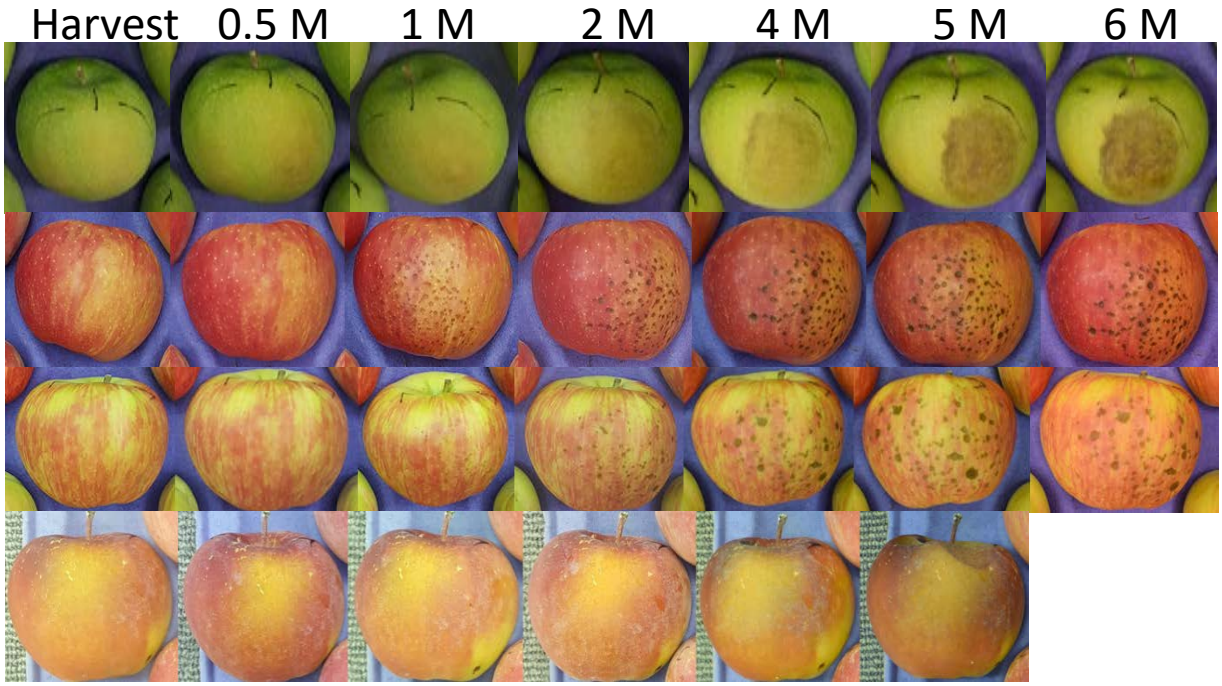


Fig. 2. Examples of different peel disorders occurring on the sun side of (top to bottom) on different cultivars. Highlighted disorders include (top to bottom) sunscald on Granny Smith, 2 presentations of lenticel blotch on Honeycrisp, and sunscald on Fuji. In each case, the injury was not present at harvest and began to develop after 1 month in 33 ºF air storage.

To investigate any difference in chemical composition during the first 6 months of storage caused by the combination of cold stress and sun exposure, peel from either side of each cultivar was sampled during storage as outlined above. Sun exposure impacted levels of a number of chemicals produced by multiple metabolic pathways and residing in different layers of apple peel. Aside from many of the differences in levels of both water soluble and oily pigments, levels of compounds residing in the wax layer, cellular membranes, and aroma differed depending upon sun exposure. Using a statistical analysis that finds the main influence of experimental factors (treatments or differences in appearance we expect or employ in our tests), we determined that peel differed depending upon sun exposure. We distinguished peel according to sun exposure for all cultivars on this basis (Fig. 3). Peel chemistry continued to diverge between sides of the fruit during storage. Honeycrisp and Fuji were more mature when picked and the contrast between sides was not as dramatic. Some of the major differences were the elevated levels of pigments and related compounds including quercetin glycosides known to be associated with light exposure and sun damage. Other compounds included volatile metabolites that are associated with high light stress in leaves, suggesting oxidative events responsible for the genesis of these compounds continue after the fruit have been removed from the orchard. Possibly some of the most striking differences were levels of compounds that potentially modify the structure of the wax and cutin layer on the outside of the fruit. Changes in relative concentration of these could alter the “breathability” or consistency of these layers at different temperatures.

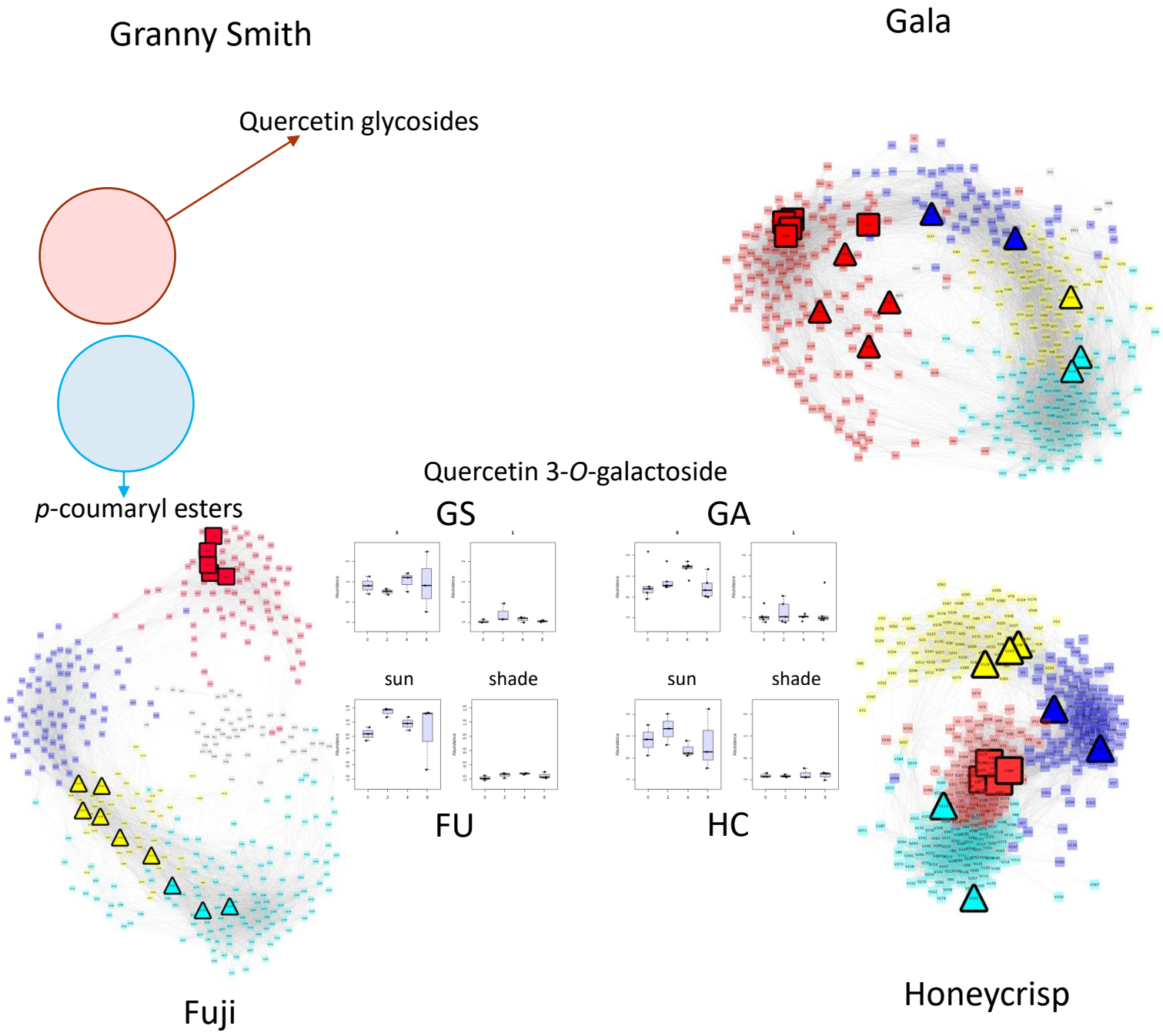


Fig. 3. Network of apple peel chemicals demonstrating differences between sun exposed and shaded sides during the first 8 weeks of air storage (33 ºF). Each data point represents a peel chemical and lines between metabolites indicate that they change similarly according to tree position and over the storage period. Peel chemicals designated with the same color and residing in the same region of the network represent exposed/increasing over storage (blue), exposed /unchanged over storage (red), shade/increasing over storage (yellow), and shade/unchanged over storage (turquoise). Highlighted examples include the quercetin glycosides (with an example of changes over time in the inset between the networks) and novel peel chemicals, the *p*-coumaryl fatty acid esters which reside in the cuticle/wax. We are pursuing non-destructive techniques to detect the former peel chemicals.

While there is potential to use spectrometric properties to visualize many peel chemicals linked with sunscald risk, the light absorbance properties of certain chemicals with better established links with high light conditions provide a good first basis for proof-of-concept. Quercetin glycosides have a unique absorption spectrum (less interference), therefore, we chose to target this area of the near ultraviolet spectrum to image peel according to light exposure. We modified a digital camera to acquire images within this absorption range. Our preliminary results indicate that peel on the exposed side is darker, or absorbs more light within this spectral band ostensibly due to the elevated presence of quercetin glycosides, and fruit from internal portions of the tree, while still maintaining the contrast between sides, are, overall, lighter than external fruit (Fig. 4).

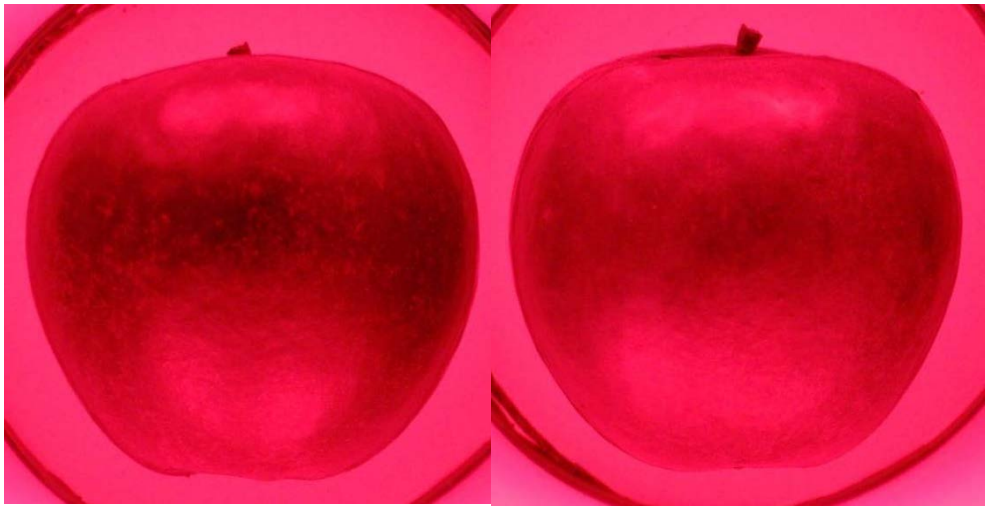


Fig. 4. Granny Smith apple sun side (left) and shade side (right) after one month of storage using a modified camera that we expect displays primarily quercetin glycosides, one group of target compounds highlighted in our metabolic analysis. Notice the sun side is darker (between the glare caused by the light source on the tops and bottoms of the fruit) indicating the target compound related to higher light exposure may be elevated which could not be visually determined without the aid of the filter. We are investigating this as a basis for sorting apples non-destructively according to their relative sun exposure.

Visible light/near infrared risk detection

Sunscald prediction models using Vis/NIR reflection of both non-sunburned and sunburned apples at harvest (Grandón, 2017) were developed by Dr. Torres's group. These models were tested on lots from two local packing sheds. Both models tested revealed significant differences in accumulated reflectance between fruit that did and did not develop sunscald after storage (not shown) indicating they may be useful as a non-destructive prediction test for Granny Smith in Washington state.

Other results

Another experiment from our other superficial scald project revealed the possibility that sunscald-like symptoms could be induced with direct heat. An impingement drier provoked symptoms where the hot rollers on the line came into contact with peel. Symptoms developed during cold storage and warranted further investigation of this factor in the context of separating heat from the light with regard to sunscald incidence. Another observation supporting that light and heat may have separate roles in sunscald development is the differential expression of the disorder on equally blushed regions of the peel. Taken together, a large portion of our effort in this season are directed at contrasting heat and light. This experiment also feeds into our superficial scald project, as scald typically develops on the opposite side of the fruit.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Crop load and canopy management of apple

PI: Tory Schmidt
Organization: WTFRC
Telephone/email: (509) 665-8271 tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Jim McFerson, Ines Hanrahan, Manoella Mendoza, Tom Auvil - WTFRC

Budget 1:
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: (509) 665-8271 **Email address:** kathy@treefruitresearch.com

Year	2015	2016	2017
Salaries	3000	2000	2000
Benefits	900	600	600
Wages	35,000	26,000	26,000
Benefits	12,000	8,600	8,600
Equipment			
Supplies	500	500	500
Travel	2,500	2,000	2,000
Stemilt lab fees	1,500	500	500
WSU plot fees		6,400	0
Total gross costs	55,400	46,600	40,200
Reimbursements	(87,000)	(70,000)	(43,200)
Total net costs	(31,600)	(23,400)	(3000)

Footnotes:

- Salary and benefits reflect contributions from exempt WTFRC staff other than project managers
- Supply costs primarily covered by private industry cooperators
- Travel includes fuel costs for driving to trial sites
- Stemilt lab fees for use of single lane Aweta color grader
- 2017 WSU plot fees waived due to donation of ag chemicals to WSU by WTFRC cooperators

NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

1) Continue screening PGRs and chemical thinners for apple
2) Refine practical PGR programs to manipulate floral initiation and promote annual bearing
3) Expand collaborative efforts with other research programs working on crop load and canopy management

2015-2017 CONCLUSIONS:

K-Pax, an alternative lime sulfur formulation, performed similarly to Rex Lime Sulfur in two years of thinning studies (Table 2)

The most efficacious options for chemical bloom thinning of apple continue to be spray oil + lime sulfur programs (Table 3)

Metamitron can effectively reduce fruit set and boost fruit size in WA conditions (Tables 4, 5)

Metamitron efficacy can be promoted by tank mixing with non-ionic surfactants or lightweight summer petroleum oils (Table 4)

Aggressive metamitron programs can induce phytotoxicity in apple trees when applied in temperatures above 85F; leaf burn can sometimes occur during cooler temperatures, but effects are largely temporary

Warm temperatures combined with low light conditions following applications of postbloom thinners can amplify treatment effects, potentially resulting in over-thinning (Table 4)

Thinning efficacy of BA can be improved with use of surfactants (Table 4)

BA + NAA programs are as effective as any postbloom thinning program featuring carbaryl (Table 5)

Multiple applications of 100 ppm GA_3 have effectively reduced return bloom in apple over several years of study, including a 2016 trial (Table 6)

New formulation of GA shows promise for reducing flowering in apple (Table 6)

Multiple formulations of prohexadione-calcium were effective at reducing Fuji shoot extension in a 2015 trial; efficacy was increased by acidifying spray tanks with ammonium sulfate (data not shown)

Collaborative research efforts continue to help develop new models, information, and technologies to improve crop load management of WA apples

BACKGROUND:

After years of robust efforts to evaluate various aspects of bloom and postbloom chemical thinning programs, our current focus is to screen new chemistries and provide collaborative support for external research programs working on crop load and canopy management. Most of our current trials

are funded in part or wholly by third party companies that contract our services to independently evaluate their products alongside industry standard programs. We continue to evaluate the relative success of thinning programs through three measurable targets which are directly tied to a grower's economic bottom line:

1. Reduction of green fruitlet hand-thinning
2. Improved fruit size and quality
3. Increased return bloom/annual bearing

The degrees to which our chemical thinning programs achieve each of these goals are reflected in our data labeled fruitlets/100 floral clusters, harvest fruit size, and percent return bloom, respectively.

Chemical thinning programs evaluated over the last 3 years are listed in Table 1. Due to the potentially risky nature of many of our treatments, we conducted most of our trials at WSU's Sunrise Research Orchard, which also allowed us to ensure no other thinning applications were superimposed on our plots. Historically, however, additional bloom or postbloom chemical thinning applications have been left to the discretion of individual commercial grower-cooperators, provided that each experimental plot received the same programs.

Table 1. Chemical thinning programs evaluated (applied in 100 gal water/acre). WTFRC 2015-2017.

BLOOM THINNERS
4-10% Rex Lime Sulfur (LS)
6% K-Pax
4-8% K-Pax II
2% Crocker's Fish Oil (CFO) + 1.5-3% K-Pax II
2% Crocker's Fish Oil (CFO) + 3% Rex Lime Sulfur (LS)
4% ATS
2% WES + 10% Rex Lime Sulfur (LS)
48 oz Carbaryl
48 oz Carbaryl + 5 oz Fruitone L
400-800 ppm Brevis
POSTBLOOM THINNERS
300-800 ppm Brevis (metamitron)
400-800 ppm Brevis + 1% Wilbur Ellis 440 summer oil (WES)
300-800 ppm Brevis + 16-32 oz Regulaid
400-600 ppm Brevis + 4-6 oz Fruitone L
400-800 ppm Brevis + 6 oz Sylgard
400-800 ppm Brevis + 64 oz IAP dormant oil
400 ppm Brevis + 122 oz Exilis Plus
400 ppm Brevis + 48 oz carbaryl 4L/4F
600-800 ppm ADA 46342
400-600 ppm ADA 46342 + 16 oz Regulaid
24 oz Exilis 9.5SC + 4 oz Fruitone L
122 oz Exilis Plus + 4-6 oz Fruitone L
48 oz Carbaryl 4L + 4-6 oz Fruitone L
128 oz MaxCel + 4-5 oz Fruitone L
24 oz FAL-551 + 4 oz Fruitone L
25.6 oz FAL-551
25.6 oz FAL-551 + 64 oz Surfactant A
25.6 oz FAL-551 + 64 oz Surfactant B
25.6 oz FAL-551 + 64 oz Surfactant C

2-3.3 lb ADA 46343
2-3.3 lb ADA 46343 + 32 oz Regulaid
2-3.3 lb ADA 46343 + 1% WES
36-48 oz Sevin 4F + 3-5 oz Fruitone L

BLOOM THINNING:

The focus of our chemical bloom thinning work in recent years has been to screen new products that could potentially become commercially viable materials used by industry. The main new candidates in that arena have been alternative formulations of lime sulfur developed by Orcal Inc. known as K-Pax and K-Pax II. These products handle and perform much the same as Orcal's standard Rex Lime Sulfur product. The new formulations include potassium and potentially produce a higher yield of hydrogen sulfide, one of the active byproducts of lime sulfur.

In three years of testing as chemical thinners, we saw no obvious differences between Rex and K-Pax products in terms of handling, thinning efficacy, or side effects on tree health or fruit finish; in short, the products seemed relatively indistinguishable from a chemical thinning perspective. Results from the fullest test of K-Pax II in 2016 are detailed in Table 3 below; even though no thinning treatments produced significant results in that trial, earlier studies with the products did demonstrate reductions in fruit set (data not shown). According to the company, Orcal will not market K-Pax nor K-Pax II as distinct products, but plan to modify the formulation of Rex Lime Sulfur to include more potassium under its current label.

Table 2. Crop load and fruit quality effects of bloom chemical thinning programs. WTFRC 2016.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit	Return bloom
		%	*%*	*g*		*%*	*%*
Gala / M.9 Nic.29 – Rock Island							
2 gal CFO + 1.5 gal K-Pax II	93 a	44 ns	32 ns	152 ns	119	93 ns	13 ns
2 gal CFO + 3 gal K-Pax II	88 ab	49	29	152	119	97	22
2 gal CFO + 3 gal Rex LS	61 b	57	29	164	111	93	7
4 gal K-Pax II	65 ab	56	28	160	114	87	14
8 gal K-Pax II	72 ab	49	34	151	120	95	12
4 gal Rex LS	73 ab	52	31	154	118	100	11
8 gal Rex LS	62 b	57	29	158	115	85	10
Control	67 ab	55	28	153	119	98	10

Table 3 reflects the cumulative success rates of our most frequently tested chemical bloom thinners over time at achieving our three main criteria for effective thinning and demonstrates the overall superiority of programs featuring lime sulfur.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials. WTFRC 1999-2017.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 60 (25%)	10 / 63 (16%)	4 / 55 (7%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	26 / 58 (45%)	12 / 52 (23%)	9 / 52 (17%)
CFO + LS	62 / 115 (54%)	27 / 106 (25%)	22 / 105 (21%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	15 / 30 (50%)	5 / 29 (17%)	4 / 29 (14%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12 (0%)

[1]Does not include data from 2017 trials.

[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

POSTBLOOM THINNING:

The cornerstone of postbloom chemical thinning in Washington for decades has been carbaryl, which has delivered generally efficacious results at a relatively low price point for apple growers. The US EPA is currently reviewing the registration of carbaryl products and could eventually recommend restrictions in its usage or even complete deregistration. TKI NovaSource, the current registrant of carbaryl, is confident they will be able to successfully preserve its labeled use as a postbloom thinner of apple, but even if carbaryl survives the review process relatively unscathed, some major retailers have already announced they will no longer purchase produce which has been treated with carbaryl, a trend that is likely to expand in the future. As such, our program has focused for several years on identifying and developing thinning programs which do not rely on the use of carbaryl.

Historically, we have not found 6-BA products to be adequate as stand-alone chemical thinners, but that they partner well with other thinning chemistries such as carbaryl or NAA (Table 5). This year, however, we had better results in trials on Honeycrisp and Fuji with FAL-551, a formulation of BA analogous to Exilis 9.5SC. When applied by itself, FAL-551 provided modest thinning but no clear increase in fruit size; its performance was improved in both categories by the addition of a range of proprietary surfactants (Table 4). These preliminary results are encouraging and merit further investigation.

One promising new chemistry is metamitron, a sugar beet herbicide that has been recently registered by Adama under the trade name “Brevis” as a postbloom thinning agent in several countries including Italy, France, Spain, and South Africa. We have worked with small quantities of metamitron since 2011, finding it to be a promising chemistry when used aggressively in our relatively low plant stress environment. While trials in Europe and the Eastern US have found single applications of 200-400 ppm metamitron to be efficacious, our results indicate that two applications of 600-800 ppm are often necessary to produce similar effects in Washington conditions. With these aggressive use patterns, we continue to produce trial results which indicate metamitron can be a viable thinning chemistry for our industry, particularly if carbaryl use becomes more restricted.

For the second consecutive year, our trials at WSU’s Sunrise Research Orchard near Rock Island were confounded by unusual weather patterns which contributed to significant overthinning in nearly all treatments (Table 4). Both Granny Smith and Jonagold trial blocks experienced several days of cloudy, dark weather after application, followed several days of high temperatures in the 90s and nighttime lows in the 60s. These types of weather conditions add stress to fruit trees, limit their

capacity to generate carbohydrates via photosynthesis, and generally amplify the effects of most postbloom chemical thinners. Even though we have now observed multiple instances of overthinning from metamitron, it is encouraging that industry standard thinning programs such as carbaryl + NAA have overthinned at least as much if not often more significantly, suggesting that thinning results from metamitron may be somewhat more predictable and perhaps less subject to weather-related volatility. More typical Central Washington weather conditions bracketed the spray applications in commercial trials on Fuji near Wapato and Honeycrisp near Bridgeport, and the thinning responses at those sites were more modest (Table 4).

This year, we evaluated Brevis, the commercial formulation of metamitron used in Europe, alongside a numbered formulation (ADA 46343) from Adama which contains a different package of inert ingredients, but comparable loading of metamitron, the active ingredient. As in we have seen in the past, our 2017 metamitron treatments were generally equal to or better than industry standards like carbaryl and BA in terms of reducing fruit set and/or promoting fruit size across sites and cultivars (Table 4). Generally speaking, we have found that metamitron can pair well in tank mixes with a non-ionic surfactant (Regulaid), a summer oil (Wilbur Ellis Superior Oil), or NAA (Fruitone L); in most instances, a reduced concentration of metamitron in a tank mix with one of those partner chemistries has produced similar results to those of higher rates of metamitron alone. Previous WTFRC studies found that adding silicone-based surfactants or heavier-weight dormant oil to metamitron produced significant levels of phytotoxicity without clear improvements in thinning.

Table 4. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2017.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	*%*	*g*		*%*
Honeycrisp/sdlg with Cameo interstem - Bridgeport						
ADA 46343	75 a	45 de	38 a	252 b	72	0 ns
ADA 46343 + Reg	52 bc	57 bcd	35 ab	233 b	78	3
FAL-551	69 ab	48 cde	39 a	227 b	80	4
FAL-551 + Surfactant A	40 cd	67 ab	28 bc	235 b	77	0
FAL-551 + Surfactant B	52 bc	58 bc	34 ab	230 b	79	0
FAL-551 + Surfactant C	44 cd	62 b	33 abc	242 b	75	0
Sevin 4F + Fruitone L	31 d	73 a	23 c	298 a	61	0
Control	86 a	43 e	35 ab	234 b	78	0
Fuji / M.9 – Wapato						
ADA 46343	23 cde	81 b	16 cd	245 ab	74	45 ns
ADA 46343 + Reg	12 e	91 a	7 e	256 a	71	78
FAL-551	40 b	68 de	26 ab	223 bc	81	61
FAL-551 + Surfactant A	18 de	84 b	14 de	248 ab	73	61
FAL-551 + Surfactant B	36 bc	71 cd	23 abc	237 abc	77	60
FAL-551 + Surfactant C	30 bcd	77 bc	17 bcd	246 ab	74	68
Sevin 4F + Fruitone L	23 cde	80 bc	18 bcd	253 a	72	68
Control	56 a	58 e	31 a	213 c	85	66
Granny Smith / M.9 – Rock Island						
ADA 46343 2lb	25 b	78 cd	18 b	232 a	78	90 ns
ADA 46343 2lb + Reg	13 bcd	89 bc	9 c	237 a	77	85

ADA 46343 2lb + WES	13 bcd	88 bc	10 c	214 a	85	75
ADA 46343 3.3lb	11 d	91 bc	8 c	240 a	79	81
ADA 46343 3.3lb + Reg	6 d	95 ab	4 c	239 a	76	88
ADA 46343 3.3lb + WES	4 d	97 a	3 c	224 a	81	90
Brevis	11 d	91 bc	8 c	229 a	79	78
Brevis + Reg	9 d	92 ab	7 c	228 a	80	79
Brevis + WES	7 d	94 ab	4 c	213 a	85	74
Exilis Plus + Fruitone L	23 bc	78 d	21 b	232 a	78	84
Sevin 4F + Fruitone L	12 cd	89 b	10 c	246 a	74	78
Control	87 a	32 e	52 a	155 b	117	90
Jonagold / M.26 – Rock Island						
ADA 46343 8-10 & 12-14mm	45 b	62 d	31 b	274 bc	66	80 ns
ADA 46343 12-14 & 16-18mm	9 cd	92 c	8 cd	318 ab	57	68
ADA 46343 16-18 & 20-22mm	13 c	87 c	12 c	308 ab	59	83
ADA 46343 + Reg 8-10 & 12-14mm	7 cd	93 bc	6 cd	307 ab	59	81
ADA 46343 + Reg 12-14 & 16-18mm	2 d	98 ab	2 d	305 ab	60	80
ADA 46343 + Reg 16-18 & 20-22mm	1 d	99 a	1 d	281 ab	65	91
Sevin 4F + Fruitone L	0 d	100 a	0 d	338 a	54	90
Control	64 a	46 e	45 a	218 c	83	76

One positive outcome of the unusual weather at our Rock Island trials was the opportunity to observe the consequences of applying metamitron during hot weather. While overthinning was observed across most treatments and application timings, the incidence of leaf phytotoxicity was most pronounced in treatments which were sprayed at 16mm fruitlet size on May 22; the high temperature that day was 88F, followed by 93F the next day. Leaf damage similar to that depicted in Figure 1 was far more common in our Rock Island trials than those in Wapato or Bridgeport, which were sprayed in cooler conditions (data not shown). In 20 total trials, we have yet to observe any deleterious effect of metamitron on fruit finish, regardless of cultivar, spray conditions, or incidence of leaf phytotoxicity.

Figure 1. Phytotoxicity in untreated control (L) and metamitron + Regulaid treated (R) leaves. Jonagold/M.26, Rock Island, WA. WTFRC 2017.

Our confidence in the potential of metamitron as a thinner in WA conditions continues to grow as we gain more experience with this chemistry. Table 5 demonstrates that after several years of testing, our success rates for producing satisfactory results from metamitron thinning treatments are comparable or superior to any standard industry programs; when metamitron is partnered with materials like a non-ionic surfactant, a summer oil, or another thinner such as NAA, our results have consistently improved. Even though metamitron is unlikely to complete registration with the EPA within the next few years, WA growers should be able to achieve satisfactory results with currently available products. We continue to find good results in postbloom thinning programs that feature tank mixes of carbaryl, BA, and/or NAA (Table 5).

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2017.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	4 / 25 (16%)	0 / 26 (0%)	0 / 22 (0%)
Carb + BA	33 / 91 (36%)	10 / 89 (11%)	13 / 86 (15%)
Carb + NAA	22 / 69 (32%)	16 / 69 (23%)	7 / 63 (11%)
BA + NAA	18 / 40 (45%)	9 / 39 (23%)	7 / 35 (20%)
Metamitron	10 / 20 (50%)	6 / 19 (32%)	3 / 16 (19%)

[1]Does not include data from 2017 trials.

[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

GIBBERELLIC ACID FOR BLOOM INHIBITION:

Despite the annual cropping tendencies of modern dwarfing rootstocks and improved chemical thinning programs, biennial bearing continues to present a major challenge to many apple growers, especially in organic production systems which have limited options for postbloom thinning and plant growth regulators (PGRs). Over the years, we have investigated a number of PGR programs to promote bloom, but had very poor results with industry standards such as summer applications of ethephon and/or NAA. Consequently, we shifted our focus to investigate cost-effective PGRs, namely gibberellic acids (GAs), which could help excessive cropping in an "on" year of an alternate bearing cycle by inhibiting flower formation after a season of light bloom (i.e. the "off" year). Our work showed that several isomers of GA can reduce return bloom in WA conditions, but our primary focus was on GA_3 due to its potential for use in organic orchards and effective rates of that isomer would potentially be less expensive to growers than effective rates of more potent isomers.

After many years of studying product rates and timings, we determined that 2-4 applications of 100-200 ppm of GA_3 in the month after petal fall yielded the most consistent reductions in return bloom across numerous sites and cultivars. Single applications of higher concentrations of product were also sometimes effective, but not as reliably as multiple applications at 7-14 day intervals. Table 6 reports results from GA trials launched in 2016 which featured Falgro 2XLV, a commercial formulation of GA_3 registered for use on cherry to promote size and delay maturity.

As in the past, our recent trials demonstrate the inherent challenge of generating statistically significant results due to pronounced variability within return bloom data; even though a grower would consider trees with either 2 or 20 flower clusters to have insufficient bloom, results like those still reflect a 10X degree of variability, which can thoroughly confound an analysis of variance. Despite these mathematical challenges, roughly half of our GA_3 trials through the years have produced statistically significant reductions in return bloom. Further, another 20-30% of our studies

have yielded results where apparent numeric reductions in return bloom were observed without statistical significance.

The fundamental question remaining for these programs is not their efficacy, but whether registrants of GA_3 products will decide to amend their labels to accommodate this use pattern on apple. Several companies manufacture GA_3 for use in tree fruit, and we have lobbied the key PGR suppliers for the Pacific Northwest tree fruit market for years to consider relevant label expansions. Unfortunately, these companies find little financial incentive to assume the costs and potential liabilities for doing so given the availability of several other analogous competitor products in the market.

Given the well-established track record of our GA_3 programs, it seems of little marginal value to continue demonstrating their efficacy in ongoing trials, so our current focus in this arena is to evaluate new GA formulations to inhibit flowering. We initiated two trials in 2016 that featured just such a product with a unique blend of GA isomers, comparing it to our "standard" GA_3 program of 4 applications of 100ppm Falgro 2XLV at weekly intervals. The single application of the new product was not quite as potent as the Falgro program on Honeycrisp in Othello, but showed some ability to reduce flowering (Table 6); no treatments were effective on Honeycrisp in Naches, which were nearly devoid of any flowering spurs at the time of application and may have been alternating too severely to be impacted by our treatments. Nonetheless, this new formulation of GA has produced good results in other trials and could potentially be brought to market with a label for inhibition of apple bloom within a few years. We currently have another trial in the field featuring this product that will be evaluated this spring.

Table 6. Effects on tree vigor, fruit size, and return bloom of GA applications. WTFRC 2016.

Treatment	2016 shoot length	2016 harvest fruit weight	2016 relative box size	2017 return bloom	2017 return bloom per CSA
	cm	*g*		*%*	*clusters/cm²*
Honeycrisp / M.9 337 - Othello					
New GA product 25ppm	28.7 ns	249 ns	73	1239 ns	2.1 ab
New GA product 100ppm	30.4	243	75	804	1.9 ab
Falgro 2XLV (4 x 100 ppm)	30.4	232	78	639	1.4 b
Control	30.2	223	81	1270	2.4 a
Honeycrisp / M.106 on Red Delicious interstem - Naches					
New GA product 25ppm	11.4 b	262 ns	69	2376 ns	12.9 ns
New GA product 100ppm	13.8 ab	260	70	2582	11.7
Falgro 2XLV (4 x 100 ppm)	14.7 a	263	69	2019	12.3
Control	12.5 ab	244	74	2394	13.2

COLLABORATIVE CROP LOAD MANAGEMENT RESEARCH:

"Effects of physiology of apple under photoselective anti-hail nets" (AP-15-104; PI: Kalcsits) – support for labor intensive data collection, harvest sampling, and postharvest fruit quality analysis; also support for project leadership team including sharing of relevant WTFRC projects and protocols, as well as editing of project manuscripts

“Pollen tube growth model validation & utilization for flower thinning” (AP-15-105; PI: Yoder) – local support for coordination with WSU-AgWeatherNet, beta testers, and flower sample collection for shipment to VTU for microscopic analysis; leadership of extension/education efforts regarding industry adoption of models

“Validation of Honeycrisp and Granny Smith pollen tube growth models” (AP-15-103; PI: Yoder) – local support for coordination of beta testers and flower sample collection for shipment to VTU for microscopic analysis

“Validation of the Red Delicious pollen tube growth model” (AP-16-108; PI: Yoder) – local support for coordination of beta testers and flower sample collection for shipment to VTU for microscopic analysis

“Development and validation of a precision pollination model” (TR-16-102; PI: DeGrandi-Hoffman) – coordination of local data collection for bee foraging, bloom phenology, and fruit sampling activity at sites near Yakima and Chelan; active member of project leadership team (project funded through WTFRC technology committee)

“Developing and validating models for tree fruit” (TR-17-102; PI: Jones) – coordination of data collection for fruit growth at 39 blocks throughout Central Washington (primarily Golden Delicious, Fuji, and Honeycrisp); help with outreach activities for new horticultural models (project funded through WTFRC technology committee)

EXECUTIVE SUMMARY

- Efficacious programs for chemical bloom and postbloom thinning of Washington apples are well established in industry. Ongoing WTFRC thinning trials focus on identifying new chemistries to expand and enhance current options.
- New formulations of Rex **Lime Sulfur** handled and thinned much the same as the original product in three trials, but may have more potency as a fungicide.
- Efficacy of **BA** products may be improved with use of some surfactants; further study is warranted to corroborate preliminary results.
- **Metamitron** products have established a strong record of successfully thinning multiple apple varieties in WTFRC trials. Effective application rates and timings have been established, but effects of tank mixes with other products on results would benefit from more investigation to further develop best practices for use of this product prior to its registration and use by commercial growers.
- **Metamitron** has the potential to overthin during high stress periods for apple trees (prolonged periods of low light with warm temperatures), but seemingly no more so than current standard postbloom thinning programs. Application of metamitron in high temperatures (85F +) can cause significant leaf phytotoxicity, especially when tank mixed with an oil or surfactant. These observations would be strengthened by further study of metamitron programs in variable weather conditions.
- Many formulations of **gibberellic acid (GA)** can help inhibit floral initiation in apple; when applied in the "off" year of a biennial bearing cycle, this strategy can help pull trees out of alternation and promote annual cropping. After several years of study, we found that 2-4 weekly applications of 100ppm GA_3 starting at petal fall were most effective at reducing return bloom. While GA_3 products are unlikely to be labeled for this use pattern in the near future, at least one other GA material is in commercial development which may provide similar results. We will need to work further with this formulation to help determine practical recommendations for its usage.
- Acidification of spray tanks with ammonium sulfate improved the performance of **prohexadione-calcium** products at inhibiting shoot growth in Fuji apple trees.
- WTFRC collaboration with other scientists has significantly aided the development of several **models** with implications for crop load management including the pollen tube growth model, bee foraging model, bloom phenology model, and fruit growth model. We have also helped assist research partners to study horticultural impacts of **protective overhead netting** and hope to help further refine its practical use by evaluating the impacts of various net shade factors and the deployment of reflective ground cloth underneath netting to help offset the reduction of ambient light.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-17-103

Project Title: How do we measure and manage soil health for productive orchards?

PI: S. Tianna DuPont
Organization: Washington State University
Telephone: (509) 663-8181
Email: tianna.dupont@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI: Lee Kalcsits
Organization: Washington State University
Telephone: (509) 663-8181
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: None

Budget: **Year 1**: $48,884 **Year 2**: **$51,258** **Year 3**: $51,686

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Joni Cartwright/Kate Roberts
Telephone: 509.663.8181/509.335.2885 **Email:** joni.cartwright@wsu.edu/arcgrants@wsu.edu

Item	2017	**2018**	2019
Salaries[1]	24,600	25,584	26,607
Benefits[2]	9,740	10,130	10,535
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	10,272	11,272	10,272
Travel[4]	4,272	4,272	4,272
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	48,884	**51,258**	51,686

Footnotes:
[1]Salaries for a 25% scientific assistant (Kalcsits) and a 33% scientific assistant (DuPont).
[2]Benefits at 44.1% for scientific assistant (Kalcsits) and 37% for scientific assistant (DuPont).
[3]Goods and services include soil nutrient analysis, soil quality analysis, plant tissues tests, fruit quality analysis, sampling and lab materials.
[4]Travel to collect soil, yield, and fruit quality samples from farm sites.

OBJECTIVES

1. Test the relationship between soil quality and fruit productivity.
2. Determine which of a suite of 21 soil quality indicators are appropriate for tree fruit production systems in the irrigated west.
3. Increase grower understanding of soil quality indicators, what they mean, and how to use the information they provide to improve management.

SIGNIFICANT FINDINGS

- **Preliminary data shows that when soil quality indices were combined into a single "score," the score was significantly higher in sites growers rated as highly productive (High) compared to sites growers rated as low productivity (Low).**
- Estimated 40 lb packs/acre was greater in grower identified sites with high productivity than paired sites with self-identified low productivity.
- Individual fruit quality metrics can vary with other factors such as crop load, orchard age, planting density, etc and were less closely aligned with the grower identified high and low productivity sites. However, even with these not accounted for, higher productivity orchards are linked to specific soil quality metrics (refer to the modelling work you have done).

METHODS

Site description: 49 plots were soil sampled to date (20 pairs), 10 were well matched pairs with available/measured yield data, and analysis performed on collected soils. A subset of plots (5 pairs, 10 plots) was sampled for fruit yield and fruit quality.

Paired plots were on the same general soil type with matching variety, tree age and training system. One plot in each pair was high performing based on grower description and the other site in the pair was underperforming.

Soil sampling: Fifty to one hundred soil probe subsamples to an 8-inch depth just inside the drip line of the canopy were taken for nutrient, soil health and nematode analysis. Four four-inch deep intact soil cores were taken for bulk density analysis. Five intact cores two inches deep by two-inch diameter were taken for micro-arthropod analysis.

Soil health analysis: Compaction, and water infiltration were measured in the field (for methods see DuPontNew). Micro-arthropods were measured using a modified Berlese-Tullegren funnel extraction. Nematodes were extracted using a combination of decanting, sieving and Baermann funnel methods, counted and identified. An apple seedling bioassay (adapted from Laurent 2008) and a bean bioassay (Cornell 2010) were performed. Nutrient analysis, aggregate stability, soil protein, respiration, active carbon, and potentially available N were analyzed by Oregon State University Soil Health lab (per Clune et al 2016). Water holding capacity analysis was performed by Cornell University.

RESULTS & DISCUSSION

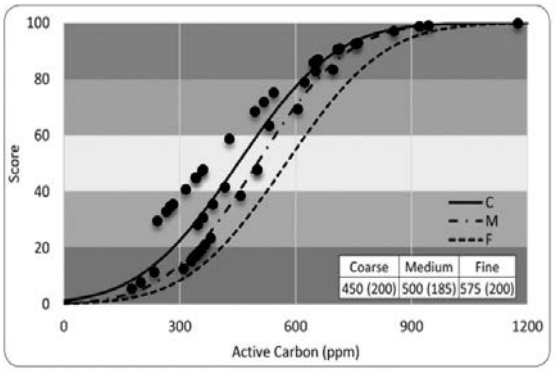
Of 49 plots soil sampled to date (20 pairs), 10 were well matched pairs with available/measured yield data. Figures 1 to 7 show the results of soil quality measurement results for some of the more interesting measurements compared to the scoring function curves where available (Cornell 2011). Individual dots indicate the value for that indice at a single site. For most indices we have measured a range of values from high to low. For some indices such as water stable aggregates (WSA) and potentially mineralizable nitrogen (PMN) (figure 2&3),

Washington Orchard soils have tended to score in the lower end of the scale compared to the scoring curve functions which were developed in the East. Conversely, subsurface hardness (figure 4) tends to score high (low compaction levels). Root health indicators based on both apple seedling growth in pasteurized and unpasteurized soil as well as bean root bioassays are starting to display a normal distribution now that a larger number of soils have been sampled (compared to the pilot study) (figure 5).

When soil quality indices were combined into a single "score" the score was significantly higher in sites growers rated as highly productive (High) compared to sites growers rated as low productivity (Low) (figure 8). When a multiple regression was performed between soil quality indices and yield/fruit quality (measured as packs per acre) there were four significant models. (I.E

Fruit quality was variable across sites and no individual fruit quality indicator could be linked to grower identified productivity. However, when we accounted for downgraded and culled fruit to calculate an estimated number of 40 lb packs per acre, we observed strong trends in overall productivity that was linked to the grower identified sites (figure 9-10; table 1). This was especially true for Gala and Granny Smith. For Honeycrisp, there was no difference between high and low sites for disorder incidence or packs per acre.

Figure 1. Soil Organic Matter Indicators



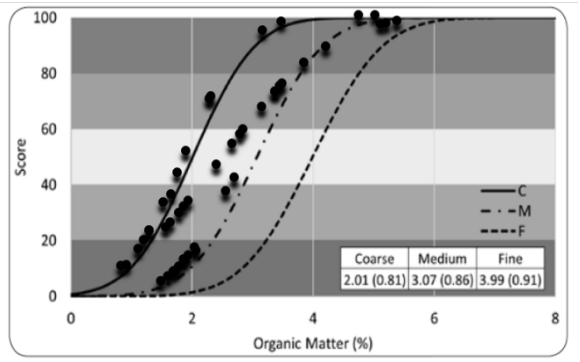


Figure 2. Soil Structure Indicator

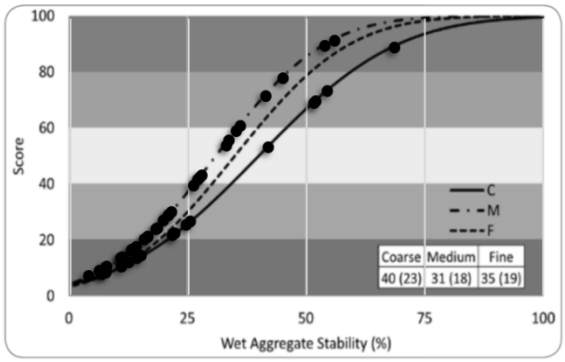
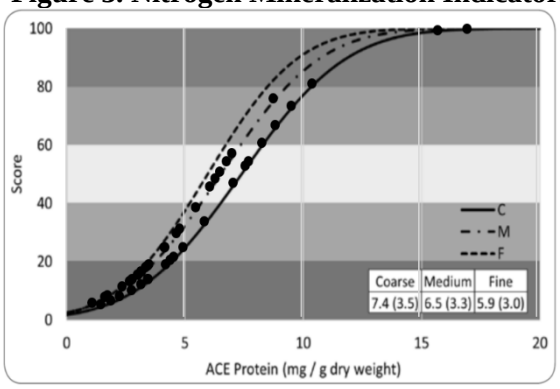


Figure 3. Nitrogen Mineralization Indicators



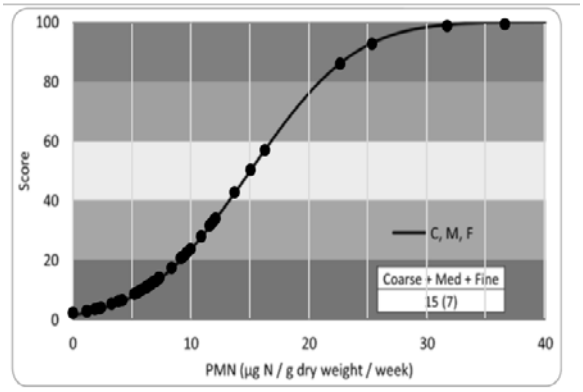
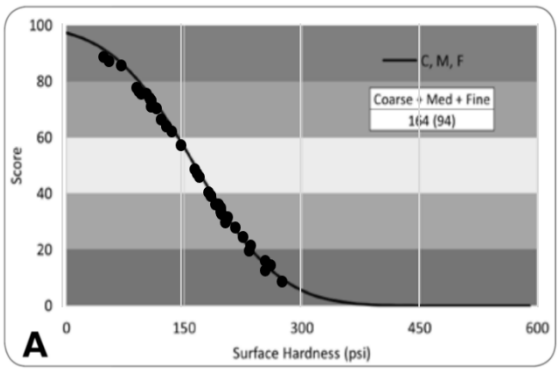


Figure 4. Soil Compaction Indicators



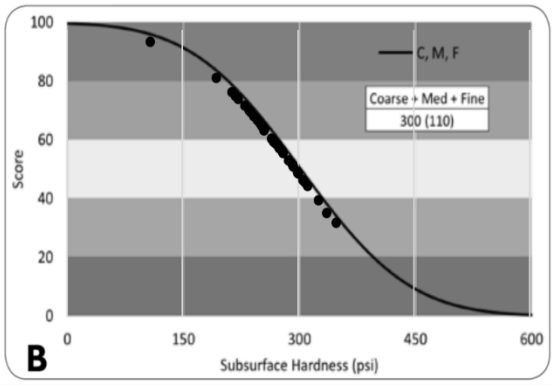
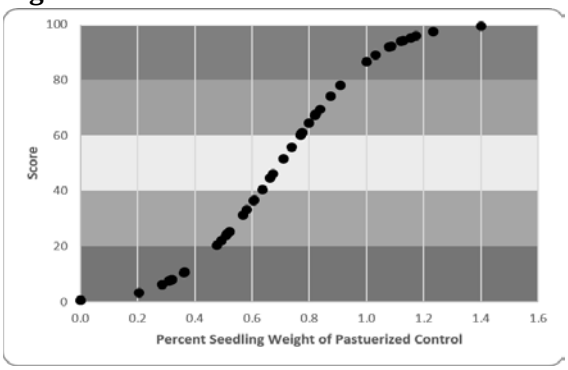


Figure 5. Root Health Indicators



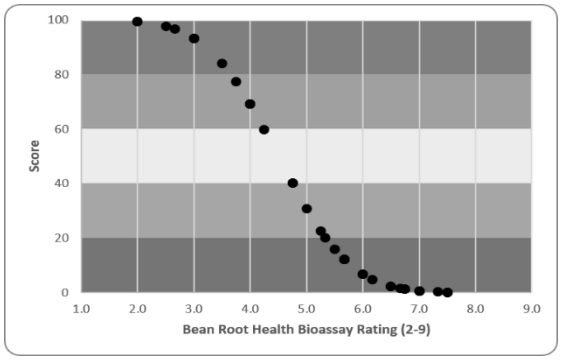


Figure 6. Nematode Soil Food Web Indicators

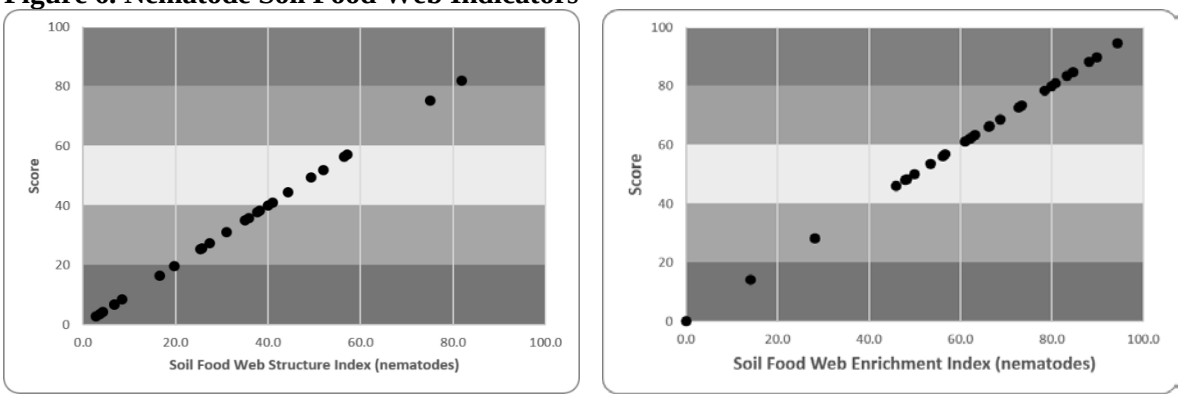


Figure 7. Water Availability Indicators

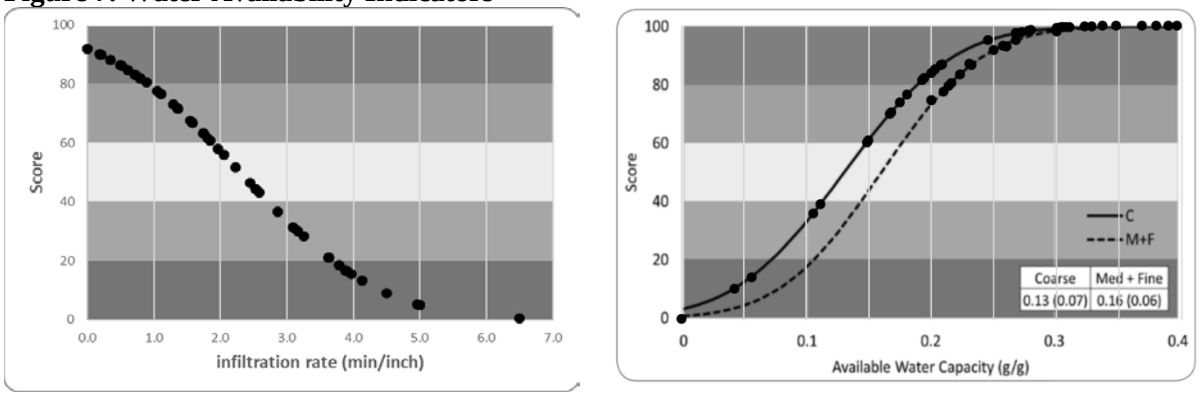


Figure 8. Differences Soil Quality Score based on a weighted average of twenty one soil quality indices between grower rated high productivity (High) and low productivity (Low) sites.

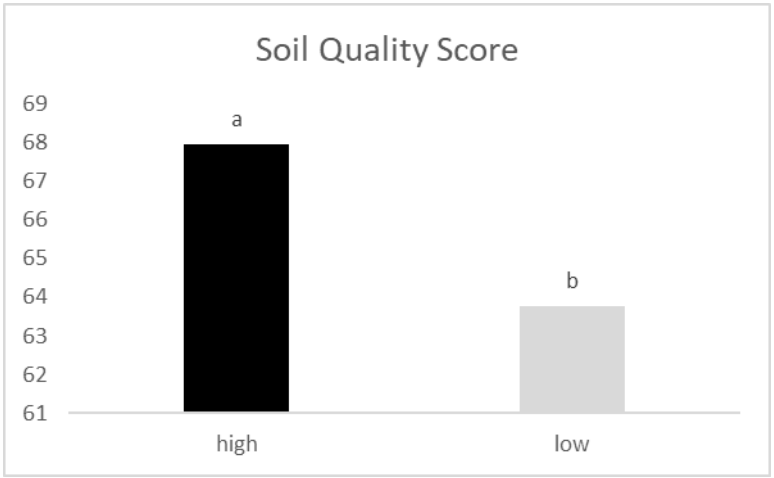


Figure 9. Estimated 40 lb. packs/acre for Gala (white), Honeycrisp (light grey), and Granny Smith (dark grey) apple harvested from a grower-selected high (black stripes) or low producing site (no stripes) paired orchard site.

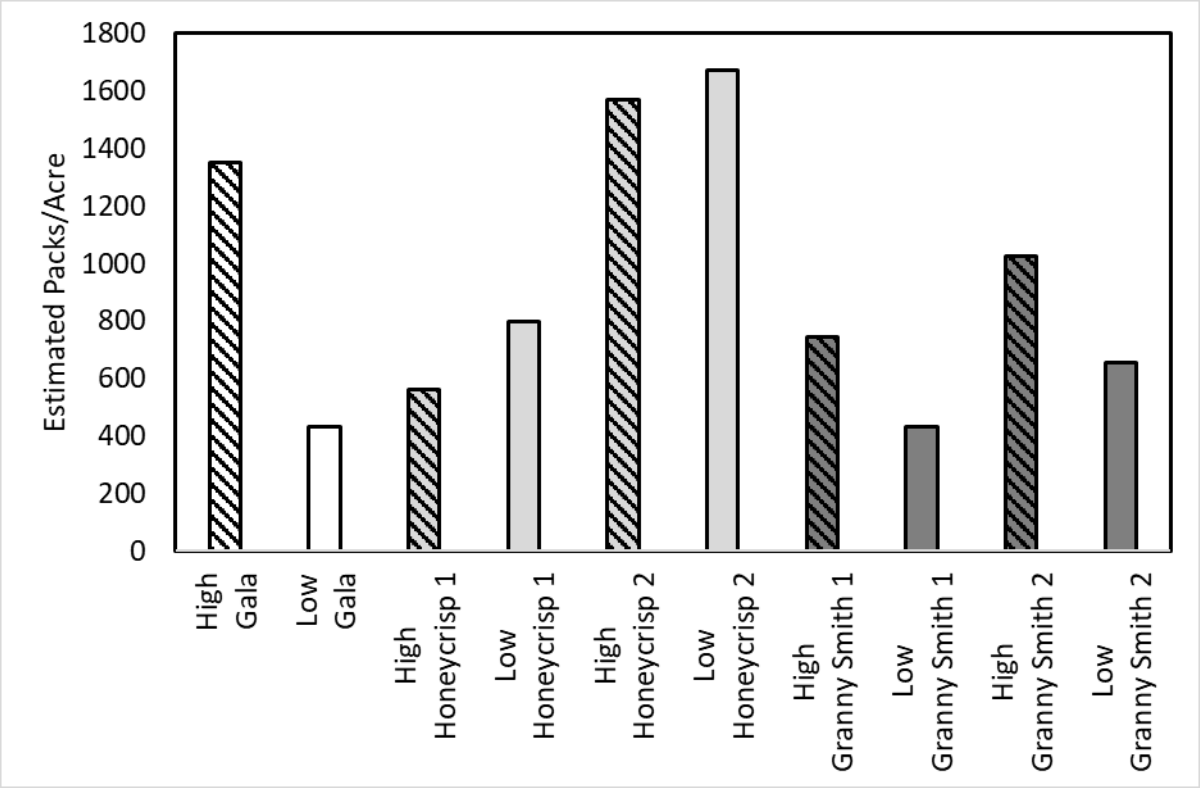


Figure 10. Calculated cull percentage Gala, Honeycrisp and Granny Smith fruit sampled from grower-identified paired sites with either high or low productivity. Culls were calculated based on sunburn above Y2 of the WTFRC sunburn scale, bitter pit and other external disorders visually assessed at harvest.

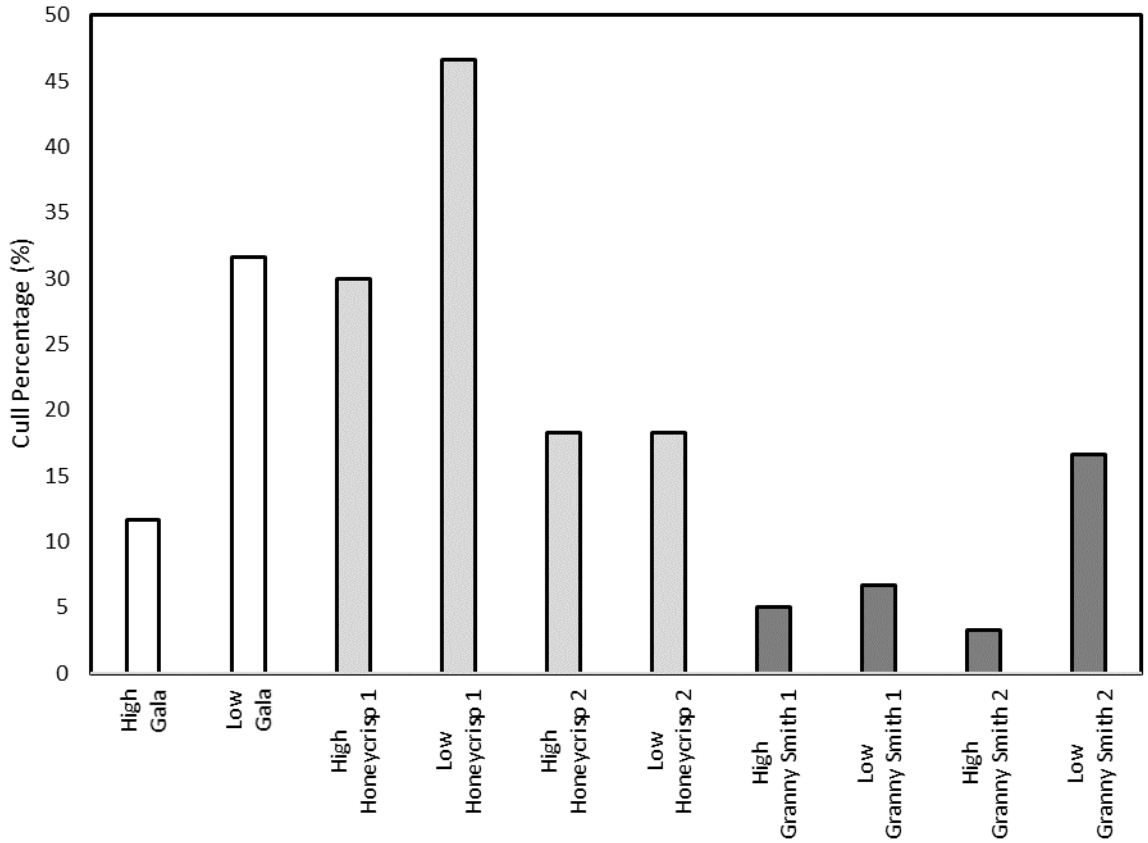


Table 1. Mean 40 lb box size class for Gala, Honeycrisp and Granny Smith fruit sampled from grower-identified paired sites with either high or low productivity.

	Gala		Honeycrisp 1		Honeycrisp 2		Granny Smith 1		Granny Smith 2	
	High	Low	High	Low	High	Low	High	Low	High	Low
Mean Size Class	125	113	72	80	113	100	150	100	113	100

CONTINUING PROJECT REPORT **Second year report**
WTFRC Project Number: AP-16-104A

Project Title: Evaluation of fungicide application methods for improved fruit quality

PI: Achour Amiri
Organization: WSU TFREC
Telephone: 509-633-8181 ext268
Email: a.amiri@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI: R. Karina Gallardo
Organization: WSU SES PREC
Telephone: 253-445-4584
Email: karina_gallardo@wsu.edu
Address: 2606 West Pioneer
City/State/Zip: Puyallup/WA/98371

Cooperators: Gebbers Fruit, Northern Fruit, McDougall Fruit, Stemilt. Richard Kim (Pace Int. LLC), Tim Mowry (Decco).

Total Project Request: **Year 1**: 70,950 **Year 2:** 73,767

Other funding sources
None

WTFRC Collaborative expenses:

Item	2016	2017
RCA Room Rental	6,300	6,300
Shipping	0	0
Plot Fees	0	0
Miscellaneous	0	0
Total	6,300	6,300

Budget 1. (Achour Amiri)
Organization name: WSU-TFREC **Contact Administrator:** Katy Roberts; Joni Cartwright
Telephone: 509-335-2885; 509-663-8181 x221 **Email:**arcgrants@wsu.edu;joni.cartwright@wsu.edu

Item	2016	2017	2018
Salaries[1]	39,000	40,560	
Benefits	15,639	16,265	
Wages[2]	2,592	2,696	
Benefits	260	270	
Equipment	0	0	
Supplies[3]	4,000	4,000	
Travel	1,500	1,500	
Miscellaneous[4]	7,967	8,476	
Plot Fees	0	0	
Total	70,958	73,767	**0**

[1] Salaries are for a PostDoc (Vikas Koundal, 1.0 FTE) at 40.1% benefit rate.
[2] Wages are for an hourly person for 12 weeks.
[3] Supplies include Petri dishes and microbiological media for lab use
[4] Miscellaneous include budget for Dr. Gallardo cost/benefit estimates and residue level analysis.

OBJECTIVES

1. Evaluate the efficacy of the different methods used currently to apply fungicides to control major postharvest pathogens including artificial and natural infections.

2. Investigate (a) residue levels related to each method and (b) impact on fungicide resistance development.

3. Evaluate the effect of different rates of fludioxonil and pyrimethanil on efficacy, residue levels, and resistance risks.

4. Evaluate the economic impact of each application method by estimating costs and benefits.

SIGNIFICANT FINDINGS:

- ❖ On wounded and artificially inoculated Fuji and Red Delicious fruit, drenching with TBZ, pyrimethanil (PYR) or fludioxonil (FDL) delayed decay development compared to dry application (fog or aerosol) of the same fungicides.
- ❖ On naturally infected fruit at commercial packing houses, fresh drenching of Fuji resulted in less decay compared to dry applications.
- ❖ Decay incidence on Red Delicious drenched with FDL +TBZ was not statistically different from dry (fog/aerosol) application.
- ❖ Fungicide residue levels for pyrimethanil above the minimum required level (MiRL) and below MRLs when fog or drench was used.
- ❖ Fungicide residue levels seem to be more consistent and similar inside the room when FDL and TBZ are aerosolled but TBZ residue levels were below MiRL.
- ❖ When fog was used, significant disparities in residue levels of TBZ and FDL were observed in different at different spots of the room or bin position in the piles.

METHODS

OBJECTIVE 1: *Evaluate the efficacy of the different methods used currently to apply fungicides to control major postharvest pathogens on artificially inoculated fruit*

In September and October of 2017, experiments were conducted on Fuji and Red Delicious apples. To study the efficacy of different fungicide application methods on artificially wounded fruit, Fuji, fruit were wounded near the stem-end and inoculated with 20 µl of a spore suspension of *Penicillium expansum* (blue mold) and *Botrytis cinerea* (gray mold). Because *N. perennans* are not typical wound pathogens, we used non-wounded fruit inoculated 6 days prior to fungicide treatments. All fruit were inoculated with spore suspensions of each pathogen at 5×10^4 spore/ml. Four replicates of 10 fruit each were used for each pathogen/spore concentration combination.

Fruit (each rep in separate mesh bags) were drenched with labeled rates of two fludioxonil formulations i.e. Scholar SC (Syngenta) and Shield-Brite FDL 230SC (Pace Int) and with pyrimethanil (Shield-Brite Penbotec), fogged with pyrimethanil (ecoFog^{TM}-160, Pace Int) or fludioxonil (ecoFOG^{80}, Pace Int.), or aerosoled with fludioxonil (Scholar EZ, Syngenta) and Penbotec Aerosol (Decco). Fruit are stored in a controlled atmosphere and verified for disease incidence and severity after 1, 3, and 5 months for blue and gray molds, and after 2, 4, and 6 months for bull's eye rot (slow growing pathogen).
To investigate naturally infected fruit, trials were conducted using organic Fuji fruit which were either drenched, fogged or aerosoled with the fungicides described above. Fruit are stored in a controlled

atmosphere and will be verified for disease incidence and severity after 6 months of storage. 4 reps of 20 fruit were used for each treatment.

OBJECTIVE 2: *Impact of application methods on residue levels and resistance development*

Plugs including the wounded area (non-inoculated) as explained in objective 1 were taken from fruit treated with different fungicides and are being analyzed for residue levels on wounds. Samples were taken immediately after the fungicide was applied (for drenching) and after one week for fogged or aerosoled fruits and will be taken again after 4 and 6 months of storage. To evaluate residue levels on commercial fruit (natural infection), sample fruit consisting of 10 fruit from three bins (one top, one in the middle and one from the ground) will be used to estimate in-bins and between bins variability. Wound tissues or whole fruit (commercial trial) are immediately stored in a cooler and transferred to analytical labs for residue analysis.

Decayed lesions form wounded fruit (objective 1) will be transferred to agar plates and will be tested for sensitivity to pyrimethanil and fludioxonil using protocol used in the lab. The sensitivity of isolates used for wound inoculation is known and potential shifts in sensitivity will be detected after 4 or 6 months of storage.
In fall of 2017, we used agar plates and fruit from Objective 1. The sensitivity of each fungal isolate to each fungicide will be determined prior to the beginning of the experiment and will be expressed as the effective concentration necessary to inhibit 50% mycelial growth (EC_{50}). If decay is not observed on fruit after the aforementioned incubation periods, fruit will be stored for an additional period at same temperature and CA conditions followed by a seven-day period at room temperature. New fungal isolates will be made from fruit showing decay and their EC_{50} values will be determined for each fungicide and compared to the original values to detect potential change in sensitivity.

OBJECTIVE 3. *Evaluate the effect of different rates of dry applications of fludioxonil and pyrimethanil on disease control, residue levels, and resistance development*

Because of the low residue levels observed for fog or aerosol in some cases, reducing the rate of fungicide would not be applicable. Therefore, this objective will not be carried out as initially described. However, because MiRL applied for dry application have been developed based on efficacy of the fungicides through drench application and do not seem to be adequate for dry application. These MiRL values need to be updated in the future.

OBJECTIVE 4. *Evaluate the costs and benefits related to different application methods*.

Methods: Drenching is the status quo method to prevent and control postharvest diseases for apples in controlled atmosphere storage. To compare the costs of the different methods, we collected data on fungicide costs (material and application costs) for each method using Penbotec or Fludioxonil+TBZ as fungicide and decay incidence at the end of the season.

We have a first estimation based on data from the first year (2017). To be fair and accurate, the cost and benefit estimates associated with drench, fog, or aerosolization will be fully conducted at the end of the second year based on 2 years of data.

RESULTS AND DISCUSSION

Decay development on artificially wounded and inoculated fruit. In fall 2016, Gala, Fuji and Red Delicious apples were inoculated and then either drenched, fogged or aerosolled. Fruit used for fog or

aerosol were placed at the front of the rooms where fungicide residue levels are expected to be adequately high. On all cultivars, decay developed after 70 days for fog or aerosol, whereas drenched fruit showed significant decays after 140 days. For blue and gray molds, decay reduction with drenched ranged between 35 to 90% compared to dry application.
Non-inoculated wounds treated with different fungicides and methods were cut and subjected for fungicide residue analyses. Residue levels found on wound were similar to levels on fruit surface (cuticle). This indicates that something other than the fungicide concentration affect decay control by fungicides applied through fog or aerosol.

Decay development on naturally infected fruit at commercial packinghouses in 2016. Fuji fruit and Red Delicious apples grown conventionally were harvested, stored and treated with fungicides following standard procedures used in commercial packing houses. Rooms were opened after 5 to 7 months and 15 bins located at the front (6 bins), center (3 bins) and back (6 bins) of each room were used. Decayed fruit were separated healthy fruit to determine decay incidence.
For Red Delicious, Fludioxonil + TBZ was applied through drench, fog or aerosol. Decay incidence was 0.52, 0.70 and 0.84%, respectively.

For Fuji apples, 3 different lots were used. Total decay incidence ranged between 0.32% and 2.2% in drenched fruit and between 0.58% and 9.3% for fog and aerosol with FDL + TBZ. For Penbotec (PYR), decay rate ranged between 0.7 to 1.8 for drench and from 0.27 and 5.2% for fog.

While the overall trend seems to indicate increased decay rate following dry application, there are nuances that should be taken into consideration:

- In Fuji trial, there were significant differences between lots. When orchard disease pressure is high, drench seem to provide a better efficacy than dry applications.
- Therefore, management should start from orchard especially for diseases such as gray mold and particularly bull's eye rot, especially in lots with a known history of disease problems.
- Drench used in our trials on Red Delicious was similar to standard packinghouse practices (700 to 1000 bins). But for Fuji, our drench can be considered as a "fresh" drench because the fungicide solution was only recycled for about 100 bins. When a fungicide solution gets "dirtier", the risk for cross-contaminations with Mucor or blue mold to occur may be higher but efficacy should not decrease as long as there is enough active ingredient (fungicide) in the solution.

Fungicide residue levels on fruit drenched, fogged or aerosolled

Drench:
All fruit sampled from drenched lots showed residue levels at or slightly above the minimum level required for the fungicide to be effective (MiRL). We sampled from three bins on the truck (top, middle and bottom) and there were no significant differences in term of residue levels.

Fog:
Residue levels for Pyrimethanil (a.i.) were above the MiRL and below the MRL at the front and center of the room but lower than MiRL in floor bin regardless of the position of the room. Significant variability in term of residue of TBZ and FDL was observed between bins next to the ceiling compared to the floor bins and between the front and the back of the room. Residue higher than MRL were seen at ceiling and lower then MiRL at back and center of the rooms.

Aerosol:
We tested only TBZ and FDL for aerosol in 2016. Overall, fungicide distribution throughout the room was more consistent compared to fog. For FDL residue levels were all above MiRL and below MRL

(max levels allowed) but TBZ residues were lower than minimum required (MiRL) level in all rooms tested.

PS: Preliminary data of the results obtained from 2016 were presented at the HortShow (Kennewick) in December 2017. Specific results and figures will be presented at the review meeting in 2018.

Costs and benefits related to different application methods: The estimated fungicide costs of different treatment methods are shown in Table 1. If drenching only, using Penbotec is cheaper compared to using Fludioxonil+TBZ. Note that drenching includes the cost of the fungicides and water and power but not labor cost. For aerosolization or thermofogging, the cost ranges from $8,000 to $10,000 per 2,000 bins if using Penbotec, and $8,000 to $12,000 per 2,000 bins if using Fludioxonil+TBZ. Thermofogging and aerosolization are both custom work which means their respective costs include material and application costs.

Table 1. Comparison of fungicide costs ($ per 2,000 bins): drench vs dry application.

Chemicals	Drench*	Aerosol/Thermo-fogging	
		Lower rate	Higher rate
Penbotec	$4,300	$8,000	$10,000
Fluodioxonil+TBZ	$4,500	$8,000	$12,000

*Includes costs of chemical, water and power only. Estimates do not include labor cost.

Table 2 shows the estimated revenues of 'Fuji' and 'Red Delicious' that are lost due to decay in fruits while in storage. This considers an average price of $530/bin for 'Fuji' and $420/bin for 'Red Delicious'. Compared to the other treatment methods, *drench* using Fludioxonil+TBZ has the smallest amount of revenues lost for 'Fuji' (i.e., $11,554 – $11,660 per 2,000 bins) and 'Red Delicious' (i.e., $4,368 per 2,000 bins).

Table 2. Comparison of lost revenues due to decay ($ per 2,000 bins) given different treatment methods.

Method	Chemical	Decay rate (per 2,000 bins)		Lost revenues ($ per 2,000 bin	
		Fuji	Red Delicious	Fuji	Red Deliciou
Drench	Penbotec	1.49%	0.52%	$15,794	$4,368
Drench	Fludioxonil+TBZ	1.09%–1.10%	0.52%	$11,554–$11,660	$4,368
Thermofogging	Penbotec	3.80%	0.70%	$40,280	$5,880
Thermofogging	Fludioxonil+TBZ	2.95%–3.92%	0.84%	$31,270–$41,552	$7,056
Aerosolization	Fludioxonil+TBZ	3.92%	0.84%	$41,552	$7,056

On-going: The labor costs for *drenching* and capital investment on a drencher will be estimated. Results are expected to show whether it is more feasible to invest on a drencher (considering both variable costs and fixed costs) or pay a company to do thermofogging or aerosolization. The next project report will utilize 2 years of data on fungicide application costs and postharvest decay rates.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-16-105

Project Title: Improved risk assessment and management of apple postharvest diseases

PI: Achour Amiri
Organization: WSU-Wenatchee
Telephone: 509-663-8181
Email: a.amiri@wsu.edu
Address: 1100 N. Western
City/State/Zip: Wenatchee, WA, 98801

Cooperators: Multiple packers in Washington. Decco, Pace, Syngenta.

Other funding sources: None

WTFRC Collaborative Expenses: None

Total Project Request: **Year 1**: 67,121 **Year 2**: 67,534 **Year 3**: **67,635**

Budget 1
Organization name: WSU-TFREC **Contact Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885;509-663-8181x 221 **Email:**arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017	**2018**
Salaries[1]	39,600	41,184	42,831
Benefits	15,721	16,350	17,004
Wages	0	0	0
Benefits	0	0	0
Equipment[2]	2,000	0	0
Supplies[3]	8,000	8,000	6,000
Travel[4]	1,800	2,000	1,800
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	67,121	67,534	**67,635**

Footnotes:
[1] Salaries are for PostDoc (Ali Emran, 1.0 FTE) at 39.7% benefit rate.
[2] Equipment will include costs for an Air-Sampler to monitor the airborne fungal population.
[3] Include costs for lab supplies i.e. sampling tubes, microbiological media and plates for fungal growth and fungicide sensitivity tests.
[4] Travel to packinghouses and orchards.

OBJECTIVES

5. Conduct a multiyear statewide decay survey program to detect and quantify decay risks.
6. Evaluate risks related to fungicide resistance
    a. Develop rapid and accurate methods for fungicide sensitivity evaluation.
    b. Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres.
7. Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type.
8. Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens.
9. Evaluate pathogenicity and fungicide sensitivity of *Lambertella* and *Phacidium* rots, newly reported in pome fruit in WA.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a multiyear statewide decay survey program to detect and quantify decay risks*

- 165 grower lots were surveyed across 10 counties in central Washington between February and June, 2017. Blue and gray molds were predominant and accounted for 44 and 25% of total decay, respectively. These frequencies are slightly lower than 2016.
- Bull's eye rot incidence was 3 times higher in 2017 compared to 2016.
- The "export" quarantine pathogens Speck rot (Phacidiopycnis) was at about 2% whereas Sphaeropsis and Phacidium rots were found very sporadically.
- The newly reported yellow (Lambertella) rot, now known as Yellow rot was found in 36% of lots surveyed with an incidence ranging from 2 and 40% per grower lots.

Objective 2: *Evaluate risks related to fungicide resistance*

2-a- Develop rapid and accurate methods for fungicide sensitivity evaluation

- A lab assay consisting of 15-cm plates has been developed and validated to test for fungicide sensitivity. The assay can be used to screen for fungicide sensitivity of 60 isolates/plate simultaneously and results are obtained within 24 hours.

2-b- Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres

- In 2017, 3200 isolates of *Penicillium expansum* (blue mold) and 1,900 isolates of *Botrytis cinerea* (gray mold) were collected from different packinghouses. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (TBZ), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *B. cinerea* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *B. cinerea* only.
- Overall statewide resistance frequencies of *P. expansum* to thiabendazole (TBZ) and pyrimethanil (Penbotec) was slightly (2 to 3%) higher in 2017 compared to 2016.
- Resistance of *B. cinerea* to pyrimethanil, TBZ, Pristine, and fluxapyroxad (Merivon) was found in 52%, 40%, 38% and 12% of the 165 lots surveyed, respectively.
- Populations of *B. cinerea* and *P. expansum* with reduced sensitivity (tolerance) to fludioxonil were found in 50% and 48% grower lots, respectively. These populations are controlled by the higher label rate of the fungicide. However, continuous use of Scholar/FDL and related products can cause these populations to become actually resistant.

- 165 decay and resistance profiles were provided back to the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their locations.

Objective 3:

- Early results from 2016 trial indicate that the expression of some genes playing a role in fungicide resistance development is increased at cold temperatures (33°F) in Botrytis (gray mold) when Penbotec or Scholar/FDL are used but not TBZ.
- No major impact of controlled atmospheres (CA) was seen compared to regular atmospheres (RA).
- Work is ongoing to verify if cold temperatures have similar effect in *Penicillium* (blue mold).
- This finding may indicate increased resistance risks for Penbotec and Scholar/FDL in cold storage.

Objective 4:

- The 1,900 *Botrytis* isolates collected in 2017 were tested for sensitivity to fluopyram (Luna sensation = same FRAC group as Pristine) and to difenoconazole (in Inspire Super and Academy)
- Very low resistance frequency (2%) was found to Luna and no resistance to difenoconazole was found

Objective 5: *Evaluate pathogenicity and fungicide sensitivity of Lambertella and Phacidium rots, newly reported in pome fruit in WA.*

- Susceptibility of 10 major apple cultivars to Lambertella corni-maris and fungicide sensitivity of this pathogen is determined and results have been published.
- Ongoing work is conducted for Phacidium rot.

METHODS

Objective 1. *Conduct a second-year statewide decay survey program.*

To assess season variability due to changing weather conditions or different spray regimes, we will conduct a second-year survey. Fifty 50 decayed fruit will be sampled on the packing line from same 15 packinghouses surveyed in 2017 across the state. Ten grower lots (orchards) will be surveyed from each single packinghouse. Fruit will be sampled between February and June. Fruit will be placed in clamshells to avoid crashing and cross contamination and transported to the Pathology lab at WSU-TFREC for decay identification and culturing on agar media. Decay identification will be done based on symptoms, spore shape and colony morphology on agar plates. If needed, some pathogens will be identified using molecularly.

Objective 2b. *Conduct a multiyear statewide resistance monitoring program.*

Fruit collected for decay survey (objective 1) will be used to conduct the fungicide resistance monitoring. Fifty decayed fruit will be collected from the same lots surveyed in 2016 and 2017. Additional lots may be included. We will test *Penicillium*, *Botrytis*, and *Neofabraea* (Bull's) isolates from each orchard lot. All *Botrytis* and *Neofabraea* isolates will be tested for sensitivity to boscalid, and fluxapyroxad (Merivon), from the same chemical group (FRAC7), and to difenoconazole, TBZ, pyrimethanil, and fludioxonil whereas *Penicillium* will be tested for the last four fungicides only. Results from the second year will be compared to those from 2016 to produce a map with location-specific resistance profiles to help understanding resistance development and spread. Because storage room can harbor tremendous amount of airborne fungal population, we will survey resistant

population of *Penicillium* in storage room atmospheres across the State using an Air-Test sampler. This will help in understanding the buildup and spread of resistance inside storage rooms.

Objective 3. *Evaluate the impact of storage conditions on resistance development in the blue and gray molds.*

In November 2017, in vitro and in vivo experiments aimed to understand the impact of low temperatures and CA conditions on some of the biochemical and molecular mechanisms related to fungicide resistance development were started. Six *Penicillium* isolates and 6 *Botrytis* isolates having different sensitivity phenotypes were used. In vitro, isolates were inoculated to plates with a medium amended with a sub-lethal dose of fludioxonil, pyrimethanil, or thiabendazole. Plates are incubated in the lab at 0°C (33°F) for 6 months, or for 2 months at 22°C (68°F) and 28°C (84°F) or for 6 months at 0°C (33°F) in CA at Stemilt facility. A similar experiment was conducted on organic Fuji pre-wounded, sprayed with half and full label rate of each fungicide and inoculated with the same isolates used in vitro. This trial is currently ongoing. For each incubation period, the sensitivity of isolates will be assessed to all postharvest fungicides to check for shift in sensitivity. RNA will be extracted from some cultures from each treatment and used to evaluate the expression of ABC transporter gene and mrr1 genes, known to be overexpressed in resistant isolates. Based on the first-year results, we plan to reassess the impact of multiple temperatures and CA conditions on resistance development.

Objective 5. *Fungicide sensitivity of Phacidium.*

Phacidium is a newly reported pome fruit in WA. We will continue monitoring it as explained in objective 1. Herein, we will assess the sensitivity of 100 isolates previously collected (part of the collection at TFREC-Pathology lab) to boscalid, pyraclostrobin, TBZ, pyrimethanil, difenoconazole, and fludioxonil using mycelial growth inhibition assay on 10-cm Petri plates containing appropriate media amended with 0, 0.001, 0.01, 1, and 10 µg/ml for each fungicide. Plates will be incubated 4-5 days at 20°C and growth inhibition will be expressed compared to the control (0 µg/ml) and effective concentration necessary to inhibit 50% mycelial growth (EC_{50}) values will be determined. These values will serve as a baseline to monitor future sensitivity shifts. Efficacy of the aforementioned fungicides against *Phacidium* on fruit will be evaluated.

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue and gray molds accounted for almost 69% of total decay observed with blue mold being predominant with 44% of total decay (Figure 1). Blue mold was detected in all lots surveyed, whereas gray mold was found in 94 % of lots surveyed. A majority of lots had less than 20% incidence of gray mold, whereas a higher number of lots had between 40 and 80% blue mold. Besides these two mains decays, bull's eye rot accounted for 7% of total decay, up 3 times compared to 2016. The frequency of the "crabapple diseases" Speck rot and Sphaeropsis rot was 6 and 0.7%, respectively.

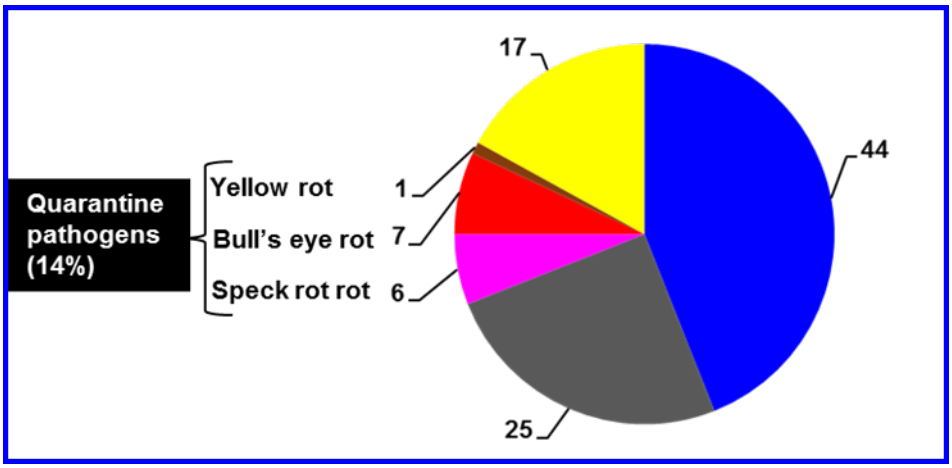


Figure 1. Overall incidence of major postharvest diseases found in Washington in 2017. Blue and gray mold are shown in blue and gray color, respectively.

Objective 2. *Fungicide resistance occurrence and frequencies*

In *Penicillium expansum* (blue mold) isolates tested, 22.5% and 17.4% of isolates were resistant to TBZ and pyrimethanil (Penbotec), respectively. About 11% had reduced sensitivity (tolerance) to fludioxonil. In Botrytis, resistance frequencies were higher for Penbotec than for TBZ and reduced sensitivity to fludioxonil was found as well.

For both blue anf gray molds, a majority (60%) of lost surveyed had a resistance frequency lower than 20% and less then 10% showed frequencies higher than 50% resistance.

Objective 3: *Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type (**ongoing**)*

- Fuji apples treated with TBZ, Penbotec or Scholar and inoculated with *B. cinerea* or *P. expansum* were stored for 6 months at 33F in a regular or a controlled atmosphere.
- ABC and mrr1 genes were checked for expression in comparison to inoculated but untreated fruit.
- Early results indicate an over-expression of the above genes in B. cinerea isolates treated with the fungicides compared to non-treated.
- Other genes are being checked for *P. expansum*.
- Impact of other temperatures is being evaluated.

Objective 4: *Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens*

- Results from 2017 assays indicate that resistance of B. cinerea to fluopyram (Luna), which is not registered for gray mold, is present in apple orchards but at a very low frequency of 2%. The danger with this fungicide is that all isolates resistant to it are automatically resistant to other SDHIs (Pristine, Merivon) which are registered for gray mold. This requires caution when using Luna to control powdery mildew early in the season when Botrytis inoculum can be present in orchards.

Objective 5: *Prevalence of yellow rot and its sensitivity to pre- and postharvest fungicides*

The incidence of yellow rot (Lambertella) was slightly lower in 2017 (1.2%) compared to 2016 (2.4) and found in 24% of lots in 2017 compared to 34% of lots surveyed in 2016.

The susceptibility of 9 apple cultivars to yellow rot (*L. corni-maris*) shown in Figure 4 indicates that Red Delicious is the least susceptible cultivar together with Cameo and Granny Smith being significantly less susceptible than the remaining cultivars. On the other hand, Honeycrisp and Gala are among the most susceptible ones (Figure 2-left).

Yellow rot is totally controlled by fludioxonil at label rate while pyrimethanil provided a high efficacy (>94% control) on fruit wounded and inoculated with the fungus (Figure 5). To the contrary, TBZ failed to provide any efficacy against yellow rot. This is not due to fungicide resistance but rather to inherent inefficacy of this group of fungicide against yellow rot. It is not clear yet if *L. corni-maris* infect fruit pre- or postharvest, however the preharvest fungicides Topsin-M (same group as TBZ) and Pristine (pyraclostrobin + boscalid) may not provide adequate control if fruit are infected in the orchard.

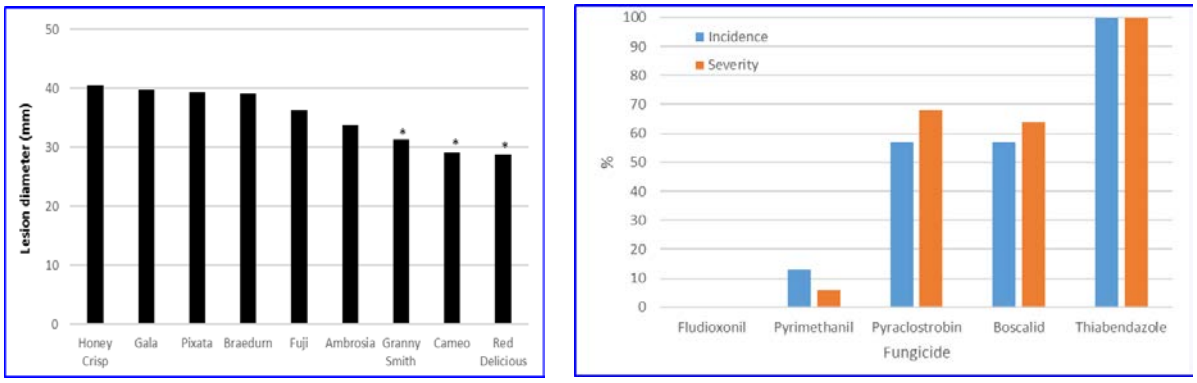


Figure 2. Susceptibility (expressed as lesion diameter) of most common apple cultivars to yellow rot (left) and efficacy of pre- and postharvest fungicides against yellow rot on apple fruit (Amiri et al. Plant Disease, 2017). * indicate cultivars significantly less susceptible.

Phacidium rot was very sporadic in 2017 and was only found in 12 lots. Work is being planned to determine the susceptibility of trees and fruit from different cultivars to Phacidium and to assess the efficacy of labeled fungicides to control this disease.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Apple rootstock evaluation

PI: WTFRC Staff
Organization: WTFRC
Telephone: 509-665-8271
Email: Kathy@treefruitfresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

WTFRC Staff cooperators: Mike Willett, Ines Hanrahan, Mano Mendoza, Tory Schmidt

Cooperators: Jim Divis, Scott McDougall, Dave Taber. Tianna DuPont (WSU Extension)

Total Project Request: **Year 1:** 57,900 **Year 2: 110,165** **Year 3:** 50,200

WTFRC Expenses:

Item	2016	2017[3,4]	**2018[4]**
Salaries[2,3]	30,500	40,500	14,000
Benefits[2,3]	10,000	13,365	4,800
Crew Wages[3,4]	5,000	33,000	12,000
Crew Benefits[3,4]	1,000	4,900	5,000
Stemilt RCA room	8,400	8,400	8,400
Shipping			
Supplies			
Travel[1,4]	3,000	10,000	6,000
Miscellaneous			
Total	57,900	110,165	**50,200**

[1]Fuel and maintenance plus hours for time slip to travel to plots
[2]Salaries and benefits for Hanrahan, Schmidt, and Mendoza apportioned to this project
[3]2017 will see large increase in field activity, fruit harvest, storage and lab activity.
[4]Minimum wage increase

Note: WTFRC work on Phase 3 trials of the apple breeding program is available in the apple breeding program report along with the WTFRC collaborative budget for the scion project.

OBJECTIVES:

1. Evaluate performance of replant tolerant Geneva apple rootstocks in new ground and replant sites compared to commercial standards, in commercial settings in Washington State.
2. Conduct outreach activities to provide opportunity for nurseries and industry to see new commercially available rootstocks in the Geneva family as the trees grow canopy and become productive.

2017 Honeycrisp Average Yield per Tree (Figure 1) and Yield Efficiency (kg/cm^2) (Figure 2) for Eleven Geneva and Malling Series Rootstocks Planted in 2015 at Two Sites in Northcentral Washington.

Figure 1

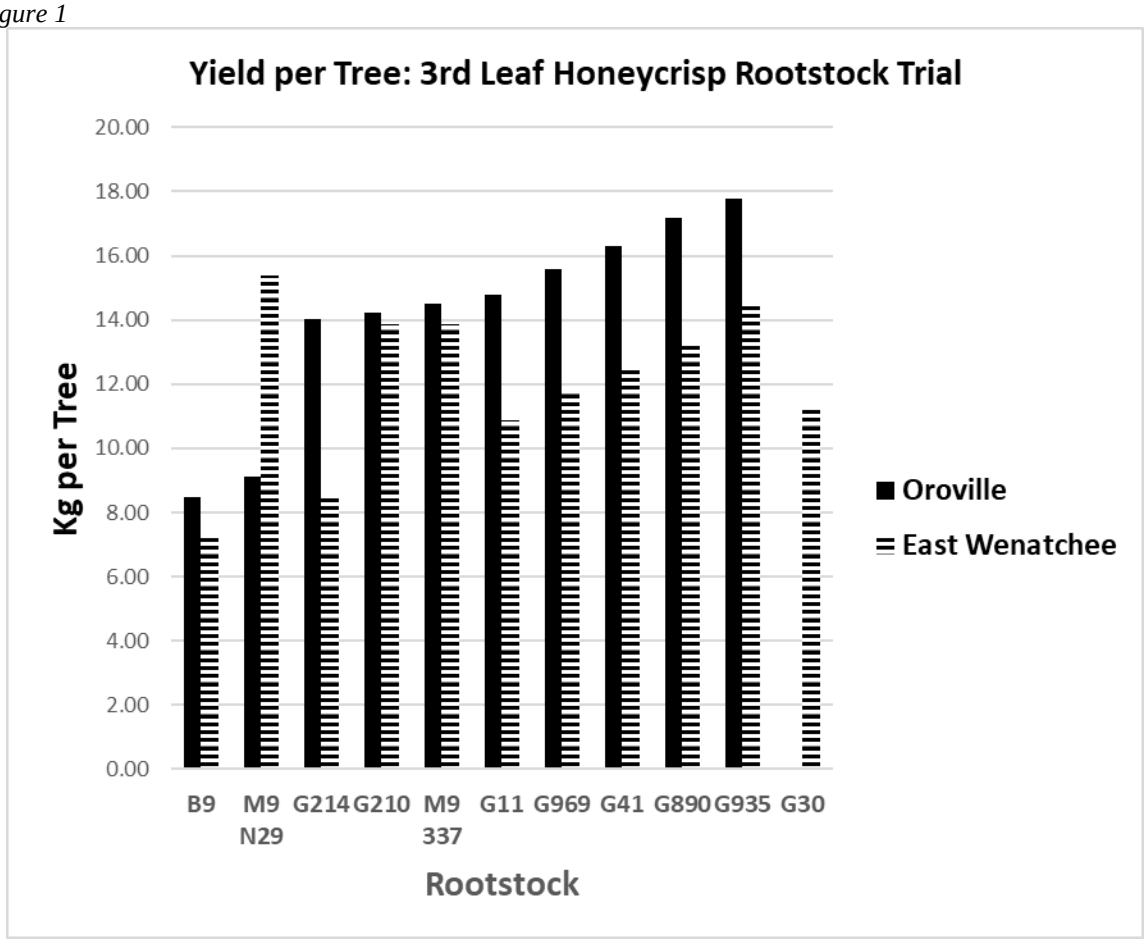


Figure 2

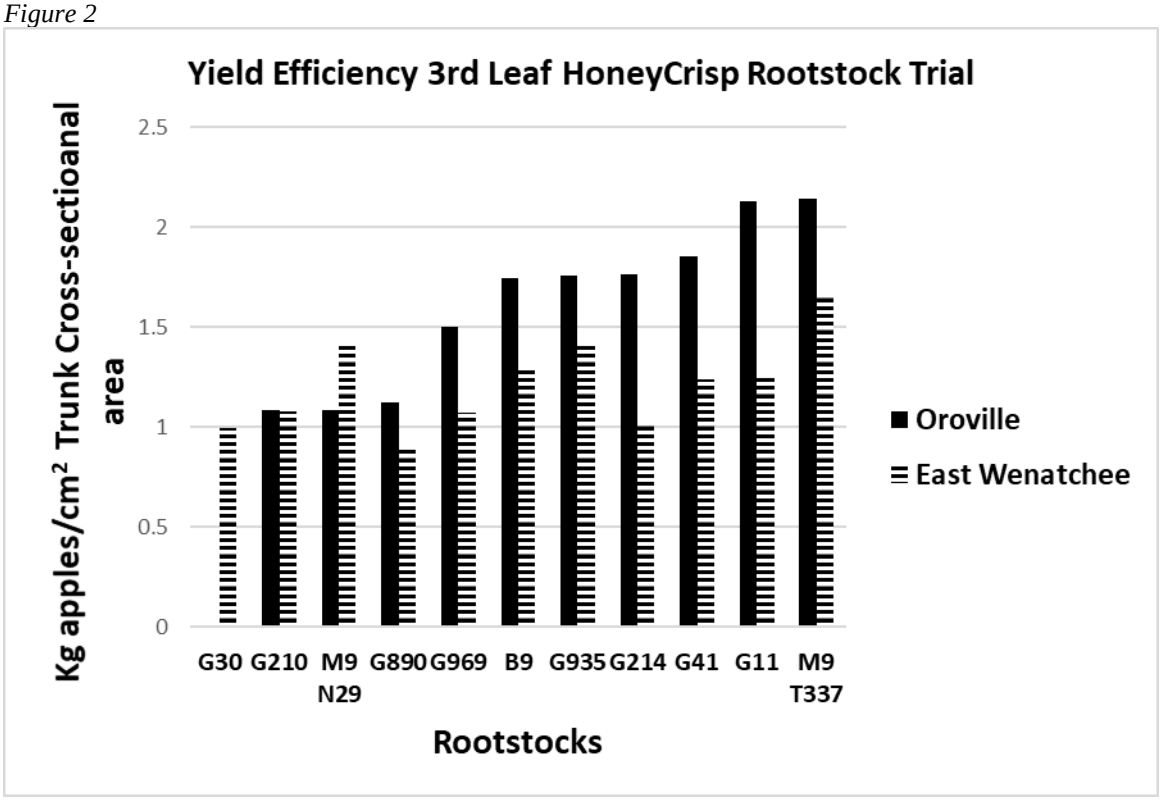


Figure 3: 2016 Oroville Honeycrisp trunk cross sectional area (TCSA)

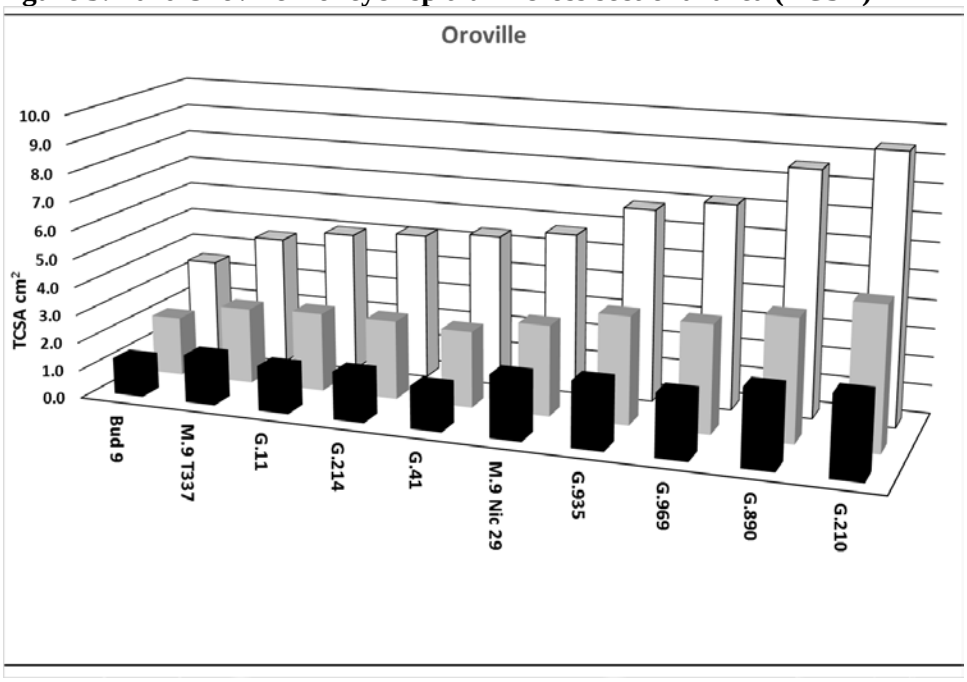
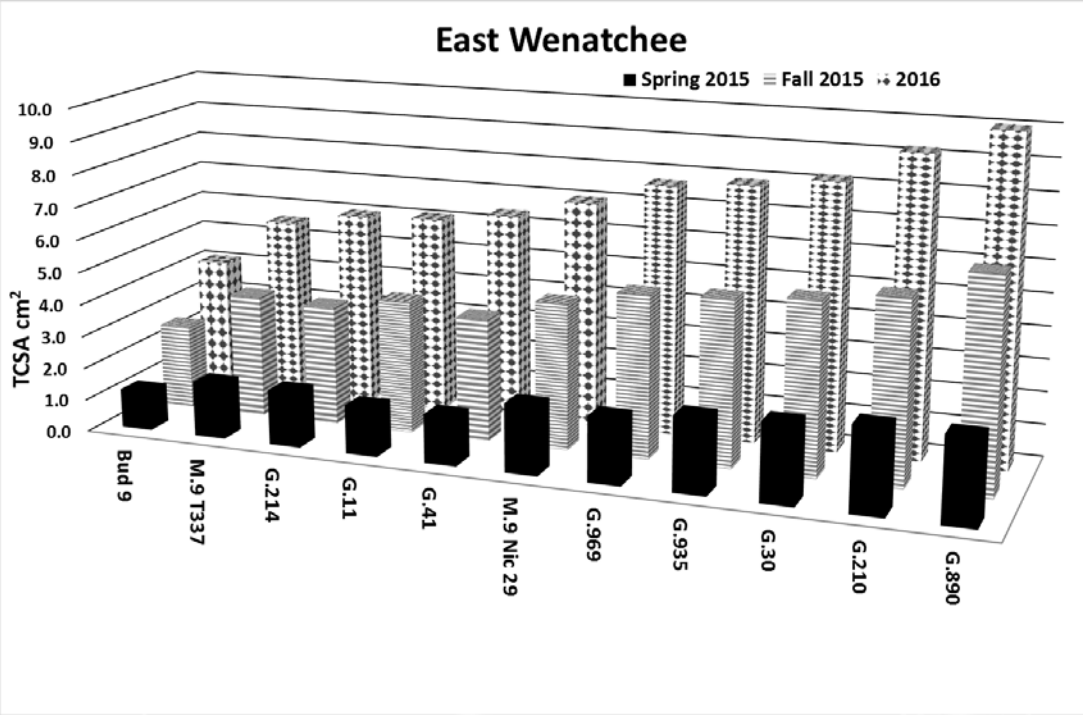


Figure 4: 2016 East Wenatchee Honeycrisp trunk cross sectional area (TCSA)



Rootstock findings and activities:

- A grower field day was held on August 25 in cooperation with WSU Extension-Tianna DuPont
- Rootstock information is updated and is on the treefruit.wsu.edu website.
- G.969 continues to look very promising in many aspects including nursery propagation, yield, woolly aphid resistance and replant tolerance.
- G.935, G.30, G.11 and CG. 4011 are NOT woolly aphid resistant. We encourage growers and nurseries to pursue the woolly aphid resistant genotypes.
- Availability of G.30 is declining due to its unreliable propagation performance.
- G.41 has encountered broken unions especially with large caliper trees from some, but not all nurseries. ½" and smaller caliper trees have very minimal union breakage. ¾" caliper and larger trees on G.41 can have serious losses.
- G.41 has issues in transplanting which may be related to the number of roots on the plant being transplanted (fewer roots = less transplant success). Tissue culture sourced liners seem to have more consistent and higher root count.
- G.969 has good to excellent finished tree propagation traits. Yield data indicates G.969 will be similar to other members of the replant tolerant Geneva's in productivity.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project Number: AP-15-102A

Project Title: Apple scion breeding

PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-663-8181 x245
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Co-PI (2): Cameron Peace
Organization: WSU-Horticulture
Telephone: 509-335-6899
Email: cpeace@wsu.edu
Address: PO Box 616414
City/State/Zip: Pullman WA 99164

Cooperators: Bruce Barritt, Professor Emeritus, WSU; Amit Dhingra, Dorrie Main, Carolyn Ross, WSU Pullman; Tom Auvil, Ines Hanrahan, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata; Craig Hardner, Australian Crop Genetic Services, Brisbane, Australia

Total Project Request: **Year 1:** $249,881 **Year 2:** $266,445 **Year 3**: $260,362

Other funding sources

Agency Name: WTFRC Apple Review
Amount awarded: $107,000 (2015-2018)
Notes: "Combining fire blight resistance and horticultural quality in Washington apples" PI: Norelli. Co-PI: Evans. Synergistic project to identify sources of fire blight resistance.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $5.72M (2015-2017 with 2 more years likely)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" PI: Iezzoni. Co-PIs: Peace, Evans et al. To further develop MAB for U.S. Rosaceae crops.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2.7M (2014-2019)
Notes: "Genome Database for Rosaceae: Empowering Specialty Crop Research through Big-Data Driven Discovery and Application in Breeding" PI: Main. Co-PIs: Evans, Peace et al. Synergistic project for application of bioinformatics to tree fruit crops.

WTFRC Collaborative expenses:

Item	2015	2016	2017
Wages	21,500	11,700	14,700
Benefits	8,600	7,800	9,800
RCA Room Rental (x2)	8,100	8,100	8,100
Shipping	0	0	0
Supplies	1,000	1,000	1,000
Travel	3,500	3,500	3,500
Plot Fees	0	0	0
Total	42,700	32,100	37,100

Budget 1

Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnson & Joni Cartwright

Telephone: 509 335 7667,509 663 8181 **Email address:** carriej@wsu.edu; joni.cartwright@wsu.edu

Item	2015	2016	2017
Salaries[1]	59,205	61,573	64,036
Benefits	20,697	21,525	22,386
Wages[2]	22,680	23,587	24,530
Benefits	4,309	4,482	4,661
Orchard establishment supplies	20,000	20,800	18,060
Genotyping supplies	17,000	18,500	20,000
Travel[3]	14,690	15,278	15,889
Miscellaneous (virus testing)	1,500	4,500	1,500
Plot Fees	8,800	8,800	8,000
Total	168,881	179,045	179,062

Footnotes:

[1]Salaries for Agricultural Research Technologist (Bonnie Schonberg @ 1.0 FTE) and for 3 months for genetic screening technician (Terence Rowland @ 0.25FTE)

[2]Wages for time-slip labor for orchard management and trait phenotyping

[3]In-state travel to research plots which are spread across the state.

Budget 2

Organization Name: Willow Drive **Contract Administrator:** Brett Adams

Telephone: 509 787 1555 **Email address:** brett@willowdrive.com

Item	2015	2016	2017
Seedling propagation	35,400	53,300	35,700
Phase 2 & 3 trees	2,900	2,000	8,500
Total	38,300	55,300	44,200

OBJECTIVES

1. Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.
2. Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

This project continues the existing WSU apple breeding program that has the specific focus of producing new improved apple varieties for the Washington industry. This project addresses the highest priority of the WTFRC apple horticulture and post-harvest committee of 'Fruit quality pre and post-harvest'.

SIGNIFICANT FINDINGS

1. Twenty-two new families were made in 2017 with approximately 22,000 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 12,500 seeds from 2016 crosses were grown in the greenhouse.
3. Approximately 10,000 seedlings were screened with DNA markers for fruit quality; just over 7000 were culled leaving the remaining seedlings to be transplanted to Willow Drive nursery.
4. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. Approximately 3,000 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2018.
5. The final count of new Phase 1 trees planted in 2017 was approximately 2,600.
6. Promising selections already in Phase 2 trials (planted in 2007-2016) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Seven new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2017.
8. Ten promising selections made in 2016 were propagated in 2017 for planting in 2019 Phase 2 trials at three diverse sites in Central Washington.
9. One new promising Phase 3 selection was planted (on Geneva 41 rootstock) at the Quincy and Prosser sites.
10. Fruit was harvested on four elite selections in Phase 3.
11. A WA 38 field day was held in September.
12. Genetic identity was confirmed for all mother trees of WA 38 planted in the nursery mother tree blocks. All trees tested as true to type using several DNA markers.
13. *S*-incompatibility alleles of most parents and selections were deduced by the Peace lab.

METHODS

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

RESULTS & DISCUSSION

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

Crosses for this season were designed taking into account all the available DNA test information as well as phenotypic trait knowledge. DNA testing focused on the Ma-indel test and worked very efficiently to reduce population sizes. In addition, three progenies were screened with the LG1-Fru test for fructose content.

S-incompatibility alleles for the majority of WABP parents and advanced/elite selections were determined by the Peace lab using a new DNA test. This information is particularly useful when designing crosses to eliminate the risk of a fully incompatible cross combination.

More than 1,600 seedlings were screened in the greenhouse for resistance to fire blight. These seedlings were created from WA 38 crossed with three *Malus sieversii* accessions selected from the Norelli project ('Fire blight resistance and fruit quality in new Washington cultivars', CP-15-100). Resistant individuals were planted in the Columbia View orchard and re-inoculated later in the season.

Stored fruit from 2017 is still being evaluated for fruit quality and storage potential.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

Evaluation of fruit from the 2016 season harvest was completed early in the year with a total of 179 seedling trees evaluated after storage. In addition, fruit from 73 Phase 1 'keeper' selections (selected in previous years for re-evaluation) was evaluated (229 samples in total), plus 34 Phase 2 selections and controls (590 samples) and four Phase 3 selections (21 samples).

Performance data were analyzed at the end of the season with 'Elite Advance' software, trait by trait, and top-ranking individuals were selected using a combination of this data and breeding team discussion. New selections were made for Phase 2 and Phase 3. Six promising seedling selections were propagated in spring 2017 for inclusion in Phase 2 plantings in 2018. Four more promising seedling selections were propagated in fall 2017 for inclusion in Phase 2 plantings in 2019.

Jamie Coggins (a Masters student in the Evans lab) has demonstrated that the Felix F750 Quality meter can be used to accurately predict the dry matter content and soluble sugars content of Phase 2 advanced selections. The prediction models were considerably less robust for estimating acidity content or firmness. Consequently, we will continue to use destructive measurements to evaluate these acidity and firmness. Correlation analysis of the dry matter data with other routinely collected sensory and instrumental measures is on-going.

As a result of collaboration with Dorrie Main's NRSP10 Big Data project, we have successfully transitioned all our sensory data collection into the Tablet-based Field Book App, streamlining the last remaining part of the program that required streamlined data entry.

One hundred trees of a new Phase 3 selection (on G.41) were planted in both Quincy and Prosser in spring 2017.

Fruit was harvested on four elite selections in Phase 3 from the Quincy site (three from the Prosser site). Results from three elite selections were discussed at the November BPAC meeting and fruit samples were provided for tasting. The observation of a relatively large proportion of culled fruit at harvest (23% total yield) of a 'Honeycrisp' × 'Cripps Pink' selection validated performance problems that were experienced in the 2016 season. Consequently, the decision was taken to discontinue this selection. Sufficient volumes of fruit from two other 'Golden Delicious'-season elite selections (another 'Honeycrisp' × 'Cripps Pink' selection and a 'Cripps Pink' × 'Honeycrisp' selection) will enable samples to be provided at multiple stakeholder meetings in the 2017/18 season.

The breeding program benefits from input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the horticultural aspects of the elite selections in Phase 3. An annual meeting in November provided opportunity to get BPAC feedback on the quality of the Phase 3 selections and provide input on the future relationship between the WABP and its Washington stakeholders.

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscol and Dale Goldy for the Quincy trial and Ray Fuller for the Phase 2 planting in Chelan. Also thanks to AgroFresh for providing 1-MCP, Stemilt for accommodating our complex needs through the storage season and to Legacy fruit packers.

WABP Publicity

Numerous fruit samples of WA 38 and Phase 3 selections were distributed to the industry, allied industry and target audiences. Two field days were organized to showcase WA 38 at various times through the growing season.

CBS coverage. https://www.youtube.com/watch?v=PxkpWUktiJw

NPR coverage. http://www.npr.org/sections/thesalt/2017/05/03/525421226/washington-apple-growers-sink-their-teeth-into-the-new-cosmic-crisp

Guardian newspaper. https://www.theguardian.com/science/2017/jun/18/cosmic-crisp-apple-hybrid-washington-state

Talks, publications and posters

Aug 2017 – National Association of Plant Breeders poster, Raleigh, NC. (*Coggins, Evans Grad student*): 'Utilizing dry matter and Near-Infrared spectroscopy for selection in the WSU apple breeding program.'

Aug 2017 – WSU CSS 512 Field crop breeding students tour of the apple breeding program (*Evans*).

Sep 2017 – Pacific Science Center WellBeing Curiosity Day, Seattle, WA (*Schonberg, Kostick, Evans team*) Apple breeding program fruit evaluation.

Sep 2017 – American Society for Horticultural Science Annual Conference 2017, Waikoloa, HI. (*Peace*): 'What you see is what you can improve: Breeding utility of genome-wide haplotype mosaics'.

Sep 2017 – NRSP10: Bioinformatic and Database Resources for Specialty Crops workshop, American Society for Horticultural Science Annual Conference 2017, Waikoloa, HI. (*Peace*): 'NRSP10 resources for translational tree fruit research'.

Sep 2017 – American Society for Horticultural Science Annual Conference 2017, Waikoloa, HI. (*Coggins, Evans graduate student*): 'Utilizing visible/Near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program'.

Oct 2017 – Our Valley Our Future seminar series, Wenatchee Valley College, WA. (*Evans*): 'Apple breeding 101'.

Nov 2017 - The breeding program hosted the Fruit evaluation class from Wenatchee Valley College. (*Kostick & Coggins, Evans graduate students*)

Nov 2017 – NC-140 multi-state project annual meeting, Wenatchee, WA. (*Kostick*): 'Identifying sources of fire blight resistance and associated heritable loci in apple'.

Dec 2017 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association meeting in Wenatchee, WA.

Dec 2017 - Washington State Horticultural Association meeting, News flash presentations, Kennewick, WA. (*Barritt, Coggins, Kostick*): 'Cosmic Crisp® cv WA 38 and Sunrise Magic® cv WA 2: different opportunities to satisfy consumers', 'Utilizing visible/near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program', 'Identifying sources of fire blight resistance and associated heritable loci in apple'

Evans K, Peace C. (2017). Advances in marker-assisted breeding for apple. In: *Achieving Sustainable Cultivation of Apples*. Ed. K. Evans. Burleigh Dodds Science Publishing, Cambridge, UK. (ISBN: 978 1 78676 032 6) pp 165-194

Harshman J, Evans K, Allen H, Potts R, Flamenco J, Aldwinckle HS, Wisniewski M, Norelli JL. (2017) Fire blight resistance in wild accessions of *Malus sieversii*. *Plant Disease* 101:1738-1745.

Howard NP, van de Weg E, Bedford DS, Peace CP, Vanderzande S, Clark MD, The SL, Cai L, Luby JJ (2017). Elucidation of the 'Honeycrisp' pedigree through haplotype analysis with a multi-family integrated SNP linkage map and a large apple (*Malus* × *domestica*) pedigree-connected SNP data set. *Horticulture Research* 4:17003.

Peace C (2017). DNA-informed breeding of rosaceous crops: Promises, progress, and prospects. *Horticulture Research* 4:17006.

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 3 Years

Project Title: Apple scion breeding program

PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-663-8181 x245
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Co-PI (2): Cameron Peace
Organization: WSU
Telephone: 509-335-6899
Email: cpeace@wsu.edu
Address: PO Box 616414
City/State/Zip: Pullman WA 99164

Cooperators: Bruce Barritt, Professor Emeritus, WSU; Amit Dhingra, Dorrie Main, Carolyn Ross, WSU Pullman; Ines Hanrahan, WTFRC; Brett Adams, Willow Drive Nursery, Ephrata; Craig Hardner, Australian Crop Genetic Services, Brisbane, Australia

Total Project Request: **Year 1: $268,142** Year 2: $279,948 Year 3: $287,540

Other funding sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2.7M (2014-2019)
Notes: "Genome Database for Rosaceae: Empowering Specialty Crop Research through Big-Data Driven Discovery and Application in Breeding" PI: Main. Co-PIs: Evans, Peace et al. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: WTFRC Apple Review
Amount requested: $107,000 (2015-2018)
Notes: "Combining fire blight resistance and horticultural quality in Washington apples" PI: Norelli. Co-PI: Evans. Synergistic project to identify sources of fire blight resistance.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.82M (2014-2018 with 1 more year likely)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" PI: Iezzoni. Co-PIs: Peace, Evans et al. To further develop MAB for U.S. Rosaceae crops.

WTFRC Budget:

Item	2018	2019	2020
Salaries[1]	10,935	11,044	11,153
Benefits	3,609	3,681	3,753
Wages[2]	15,000	15,675	17,807
Benefits	5,000	5,680	5,936
RCA Room Rental[3]	12,600	12,600	12,600
Shipping	---	---	---
Supplies[4]	500	500	500
Travel[5]	500	500	500
Total	**48,144**	49,680	52,249

Footnotes:
[1]Estimate of percent of time spent for Mendoza (10%) and Hanrahan (6%), a 33% benefit rate and 2% annual increases.
[2]Based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00, 2010=$13.50)
[3]2 rooms @ $6,300 p.a.
[4]Consumables for fruit quality lab (KOH, distilled water, iodine solution etc.)
[5]In-state travel for Hanrahan (mainly lodging in Wenatchee)

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 x221 **Email address:** arcgrants@wsu.edu/ joni.cartwright@wsu.edu

Item	2018	2019	2020
Salaries[1]	64,469	67,047	69,729
Benefits	25,629	26,654	27,721
Wages[2]	24,381	25,356	26,370
Benefits	2,309	2,401	2,497
Orchard establishment supplies	20,000	20,800	21,632
Genotyping supplies	20,000	20,800	21,632
Travel[3]	13,910	13,910	13,910
Miscellaneous (virus testing)	3,000	3,000	3,000
Plot Fees	8,800	8,800	8,800
Total	**182,498**	188,768	195,291

Footnotes:
[1]Salaries for Agricultural Research Technologist (Bonnie Schonberg@ 1.0 FTE) and for 3 months for genetic screening technician (to be appointed @ 0.25FTE)
[2]Wages for time-slip labor for orchard management and trait phenotyping
[3]In-state travel to research plots which are spread across the state.

Budget 2
Organization Name: Willow Drive Nursery **Contract Administrator:** Brett Adams
Telephone: 509-787-1555 **Email address:** brett@willowdrive.com

Item	2018	2019	2020
Seedling propagation	32,500	40,000	35,000
Phase 2 & 3 trees	5,000	1,500	5,000
Total	**37,500**	41,500	40,000

JUSTIFICATION

New improved apple varieties are essential to continue to enhance a successful Washington industry. The WSU apple breeding program (WABP) has exciting new material in the selection pipeline including several populations derived from the recently released WA 38 which are especially suited to Washington growing conditions.

Application of DNA-informed breeding is now routine in the WABP and the program continues to implement the many new selection tools coming from WTFRC-funded projects and the USDA SCRI-funded "RosBREED 2" project.

This proposed project spans the three years in which there is expected to be a significant increase in royalty income to WSU from the release of WA 38. The WSU faculty manual states that 50% of the adjusted WSU royalty will be allocated to the Agricultural Research Center for enhancement of vegetatively-propagated variety programs in consultation with the breeders who generated the income for this category. Administrative guidelines for allocation of royalties are currently being developed and will involve the establishment of a governing body with industry representation. As the guidelines are still not finalized, this proposal requests full support of the WABP for the next three years. This request can of course be reviewed and adjusted annually.

The continued involvement of the WTFRC staff provides invaluable independent evaluation of Phase 3 elite selections which reinforces the credibility of the results presented.

OBJECTIVES

1. Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.
2. Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

METHODS

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

a. Marker-assisted parent selection will be used to determine the most suitable combinations of parents for crossing to achieve our aim of a portfolio of new improved apple varieties. Using data from the SCRI-funded RosBREED project and the Peace lab, facilitated by the new Breeders Toolbox for breeding database interfacing developed by the Main lab, we will choose the optimum cross combinations from among available germplasm. As new parental germplasm is identified, samples will be genotyped with the full range of DNA tools available. Introgression of resistance to mildew or resistance to fire blight remains a target in the crossing program.
b. Crosses will be made each spring, most likely aiming at annual production of around 20,000 seeds. Following vernalization, seedlings will be germinated and grown on in the greenhouse at the TFREC. To optimize efficiency and accuracy of sample collection, leaf samples will be collected in the greenhouse from some of these seedling progenies and sent to the Peace lab or an external service provider for DNA testing. Genetic tests used will depend on the

particular cross combination. Some progenies will be inoculated with fire blight to enable phenotypic selection for resistance. Only the un-culled seedlings will be planted in the nursery and then budded onto M.9 rootstock for further evaluation. If deemed appropriate, seedlings will be maintained in pots until sufficient propagating wood is available for propagation for Phase 2 (or Phase 1.5).

c. Budded trees will be planted at the TFREC Columbia View orchard for Phase 1 trials where their resulting fruit will be evaluated. Selection in the orchard will be initially based on fruit appearance (primarily color, uniformity, freedom from defects) followed by eating quality (primarily firmness, crispness, sugar/acid balance).

d. Promising selections will be propagated on G41 rootstock and planted in replicated Phase 2 trials (five trees/selection) at up to three diverse sites in central Washington. Data will be collected on fruit quality, productivity and tree health. DNA samples will be collected from all Phase 2 selections for screening with predictive markers to provide DNA-based information on genetic potential to enhance subsequent selection decisions.

e. Outstanding selections will be propagated as 'elites' for Phase 3 trialing with an aim of approximately 75 trees in up to three diverse grower sites in central Washington. Phase 3 is conducted in cooperation with the WTFRC, with trial sites managed by Ines Hanrahan. When appropriate, propagated selections may be planted in just one site (Phase 2.5 or 'staggered start') before propagation for full Phase 3. Harvested fruit will be subjected to a range of storage treatments. Budwood from all Phase 3 selections will be sent to the Clean Plant Center to establish certified, virus tested material ready for distribution to nurseries.

f. Outstanding selections will be proposed for commercialization, patent data will be collected and submitted and the nursery mother trees will be confirmed as true-to-type by genetic fingerprinting.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

a. Using the labeling system initiated in the Phase 1 orchard, fruit samples collected will be bar-code labeled to correspond to the source tree, the pick number and the harvest date. These labels will then remain with the fruit as it is evaluated in the fruit laboratory thus minimizing mixing of samples and data-entry errors. Harvest date is determined using the starch-iodine test and the Cornell starch chart however identification of the optimum harvest maturity continues to be challenging with the seedling fruit.

b. Ten fruit samples will be divided into five fruit for instrumental evaluation and five for sensory evaluation. The fruit for instrumental evaluation will be tested for maturity using the Cornell starch chart. Texture, size and weight will be recorded with the Mohr® DigiTest and the remaining fruit will be juiced for soluble solids concentration and titratable acidity measurements.

c. Sensory analysis will usually be performed by a team of four, producing a detailed breakdown of appearance and eating quality attributes. All data will be recorded making use of the Field Book App and bar-code labels. The breeding team was trained in sensory profiling by the Ross lab in Pullman in 2010.

d. First-season seedling fruit will be stored in regular atmosphere storage at the TFREC at 34ºF for two months followed by one week at room temperature prior to evaluation. If a sample achieves the appropriate overall rating, the same seedling tree will be harvested at more than one pick date the following year (subject to fruit availability). Second- and third- season samples will be evaluated at harvest as well as after two months (plus one week) storage. If

sufficient fruit is available, a four-month stored sample with one week at room temperature will also be evaluated.

e. Fruit evaluation will continue as selections move forward through Phases 2 and 3, with samples taken at up to four pick dates and evaluated at harvest and, after two and four months of regular storage with one week at room temperature. Data from Phase 2 fruit evaluation will be analyzed using the R-based statistical software 'Elite Advance' developed by Craig Hardner which will rank the selections trait by trait based on genetic potential. This data, together with accumulated knowledge of the specific character of each advanced selection, guides the decision of which to move into Phase 3. Larger volumes of fruit from Phase 3 will be drenched with a post-harvest fungicide, tested with and without a 1-MCP treatment and stored in regular and controlled-atmosphere storage using the Stemilt RCA facility. Fruit out of storage will be tested in the WTFRC lab as well as the TFREC lab.

f. Fruit from promising selections from Phase 3 will be sent to the Ross lab in Pullman for consumer evaluation as required.

The breeding program benefits from regular input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the elite selections in Phase 3. Orchard visits and an annual meeting provide several opportunities to get BPAC feedback on the quality of the selections and also the priorities and targets for the program itself.

Expected results are primarily new elite selections progressing into the Phase 3 trial and beyond. Decisions to release new varieties are dependent on the amount and quality of data available. Once a selection is identified for release, the program focuses on communicating results regularly through Field days, providing multiple opportunities to sample fruit and publishing reports usually in the Good Fruit Grower.

Weather-related events can cause potential problems both in terms of the failure of the crossing program (frost damage to bloom) or the lack of quality fruit data if the crop is affected by e.g. frost or hail damage. The risks from these potential problems are mitigated by spreading the crossing program and the test plantings across the state. Rodent pressure in the greenhouse and orchard has increased. Appropriate rodent control measures will be applied. TFREC refrigerated storage for the WABP is now fully alarmed in case of potential high or low temperature threats.

LITERATURE REVIEW

Retailers have welcomed a steady stream of attractive, new apple varieties as they can help differentiate their produce departments from other retailers (1) and because they stimulate interest from the consumer. Washington apple growers need to position themselves to provide these new exciting eating experiences to consumers worldwide by having a range of improved apple varieties especially selected for the growing conditions of central Washington.

The WSU apple breeding program is well-positioned, with its established, rigorous testing protocols (2), to provide new improved apple varieties for the Washington industry as we have seen with the recent unprecedented uptake of WA 38 (3).

The WSU apple program is also at the forefront of application of DNA-informed breeding (4). It is perfectly positioned to take advantage of DNA tests coming out of the USDA-SCRI funded RosBREED projects (5) and is linked into WSU's Genome Database for the Rosaeace for enabling data management (6).

The program also takes advantage of new technologies wherever possible to improve its efficiency (7). Recent additions include the use of the Field Book App (8) for recording sensory evaluation data in the lab and full evaluation of the Felix F750 Quality meter (9).

1. O'Rourke, D. (2017) Consumer trends in apple sales. In: *Achieving Sustainable Cultivation of Apples*. Ed. K. Evans. Burleigh Dodds Science Publishing, Cambridge, UK. (ISBN: 978 1 78676 032 6) pp 511-522.
2. Courtney R (2016) Cosmic Crisp insights: Hort show preview. http://www.goodfruit.com/cosmic-crisp-insights-hort-show-preview/
3. Charles D. (2017) Washington apple growers sink their teeth into the new Cosmic Crisp. https://www.npr.org/sections/thesalt/2017/05/03/525421226/washington-apple-growers-sink-their-teeth-into-the-new-cosmic-crisp.
4. Evans K, Peace C. (2017). Advances in marker-assisted breeding for apple. In: *Achieving Sustainable Cultivation of Apples*. Ed. K. Evans. Burleigh Dodds Science Publishing, Cambridge, UK. (ISBN: 978 1 78676 032 6) pp 165-194.
5. Evans K, Iezzoni A, Peace C, Luby J, Brown S, van de Weg E, Main D, Bassil N, Fazio G, Yue C, Weebadde C, McFerson J. (2010) RosBREED – Enabling marker-assisted breeding in WSU's apple breeding program. *Journal of Fruit Science*. 27 Suppl:43-47.
6. Evans K, Jung S, Lee T, Brutcher L, Cho I, Peace C, Main D (2013) Addition of a breeding database in the Genome Database for Rosaceae. *Database.* DOI 10.1093/database/bat078.
7. Evans KM**,** Brutcher LJ, Konishi BS. (2012) Implementation of bar-code technology in a tree fruit breeding program. *HortScience*. 47(1):149.
8. Rife TW, Poland J (2014) Field Book: an open-source application for field data collection on Android. Crop Science. 54. 10.2135/cropsci2013.08.0579.
9. Coggins JL, Evans KM. (in press) Utilizing visible/Near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program. HortScience (ASHS 2017 abstracts)