Northwest Pear Research Review

Thursday, 2/15/2018

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8:10		Schmidt	Housekeeping	
			Final Project Reports	
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FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Evaluation of potential, new pear cultivars for the PNW

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Budget: **Year 1: $12,578** **Year 2: $17,334** **Year 3: $11,952**

Cooperators: Kate Evans

Other funding sources: None.

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: 541 737-4866 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017
Salaries[1]	2,291	4,720	2,421
Benefits	1,535	3,162	1,629
Wages[2]	0	0	500
Benefits	0	0	50
Equipment	0	0	0
Supplies[3]	500	500	500
Travel	0	0	0
Miscellaneous[4]	1,552	1,552	1,552
Total	5,878	9,934	6,652

Footnotes: **[1]Salaries are calculated as 5% of technician time (2.5 weeks) in year 1 and 10% of technician time in years 2 and 3 (5 weeks). The increase in salary in year 2 reflects a 3% rate increase. Benefits are calculated using OPE rate of 66%. [2]Wages are for part-time employee help harvesting fruit and general maintenance during the season; 80 hours at $13/hr. Part-time employee benefits are calculated at 10%. [3]Supplies are for tree training. [4]Miscellaneous costs account for MCAREC plot fees at a rate of $3,103/acre, prorated to 1/2 acre for field on-site field trials.**

Budget 2: Tom Auvil
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
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Item	2015	2016	2017
Salaries	3,000	3,500	2,000
Benefits	1,200	1,400	800
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1,000	1,000	1,000
Travel[1]	500	500	500
Miscellaneous[2]	1,000	1,000	1,000
Total	6,700	7,400	5,300

Footnotes: **[1]Ten trips to Wapato/Dryden from mid-August through mid-Oct. [2]RCA cold storage room charges.**

Objectives:

1. To test five new scion selections from the USDA-ARS pear breeding program in small-scale plantings in WA and OR.

2. To test two new pear cultivars from Prevar, Australia, in medium-scale plantings in WA and OR.

Significant Findings:

Objective 1

- Objective 1 was discontinued after year 2; hence, data were not collected in 2017. The budget was reduced accordingly. This decision followed Dr. Richard Bell's (USDA-ARS) communication that all selections had tested positive for viruses. Consequently, information gleaned from these trial evaluations may not appropriately represent tree growth, productivity or fruit quality attributes of these genotypes in a 'virus-free' condition.
- Notable results from the first two years (3^{rd} leaf and 4^{th} leaf production) of observations pertained only to one selection, 84907-166, which flowered profusely, had similar yields as Bartlett and produced attractive fruit with high percentages of red blush.
- We continued to observe 84907-166 in 2017. Trees to produced ~150 fruit per tree (2017 was the 5^{th} leaf). At this level of cropping, thinning would be required. Over-cropping resulted in small fruit (158 g); however, our previous data show fruit weight between 200-270 g at the appropriate crop load. Dr. Richard Bell submitted material to the Clean Plant Network to undergo therapy to produce virus free material.

Objective 2

- Tree growth in Hood River continued to be strong in 2017 (4^{th} leaf) despite small tree sizes at planting and poor growth in the establishment year.
- The selection 0118 is an early-maturing genotype, harvested ~2 weeks before 'Bartlett' (Aug 3, 2017). Fruit size, however, continued to be small (~142 g) and did not improve between the first and second pick (~1 week apart). These data were nearly equivalent to 2016 (fruit weight ~135 g; ~150 fruit per box). Fruit were attractive with fairly extensive red blush (nearly 50% of surface area), good sugar concentration (13.9%) but low acidity (0.24 % TA).
- Following 2 months of RA storage, 0118 ripened to dessert quality (FF, 2.9 lb)
- 0131 is a late-harvest selection, ~2 to 3 weeks after 'Bartlett' (approx. 'd'Anjou' timing). 0131 was not nearly as precocious as 0118, producing only a few fruit per tree in 2016 and 2017. Despite low crop load, 2017 fruit were small (150 g; harvested August 31, 2017). In 2016, fruit size was larger (180 g). Ripening was not evaluated given the limited number of fruit harvested.
- Given the lack of fire blight in Australia and the lineage of 0131 and 0118, we field-inoculated both genotypes and compared to 'd'Anjou' (control). Inoculation with 2×10^7 colony forming units/mL suspension of *Erwinia amylovora* at bloom (April 29) resulted in 90% of 0131 and 0118 tree mortality; in comparison, no 'd'Anjou' trees died from inoculations.
- The combination of small fruit size and apparent fire blight sensitivity does not support additional evaluation of these selections in the PNW.

Results:

1. **USDA-ARS cultivars**. Four fire-blight tolerant, summer pear selections were evaluated from Dr. Richard Bell's breeding program: 69426-038 (038), 84907-069 (069), 84907-078 (078) and

84907-166 (166). These were compared to commercial standards ('Bartlett', 'Bosc' and 'd'Anjou'). In addition, 71655-014 ('Gem') was planted in WA. As previously discussed, all accessions tested positive for viruses. 'Gem' is currently undergoing virus therapy at the Clean Plant Network. After notification of virus status, evaluations ceased, with the exception of 166 where we performed limited observations in 2017.

Data are provided from 2015-2017 in tables below. For most selections, tree size was about 2/3^{rds} the size of Anjou trees and similar or slightly smaller than 'Bartlett'. All trees were on OHxF 87. Selection 069 is a weak tree (~50% of 'Bartlett'); though, this should not be confused as beneficial dwarfing since trees appear to be in poor health. In Hood River, we observed a wide range of precocity among the four scions evaluated in the 3rd leaf (2015): 166 >> 038 = 069 > 078. In the fourth leaf, all scions bloomed at Anjou timing, except 166, which bloomed with Bartlett. Although 'Gem' was not included in this trial in Hood River, we have documented its bloom timing over 15 years to occur with 'Bartlett'. Fourth-leaf (2016) fruit set was highest for 078, followed by 069 and 166. Yields of these three selections were similar to 'Bartlett'. Fruit was not hand-thinned in the fourth leaf since crop loads were deemed adequate for tree sizes. Fruit maturity (to determine harvest timing) was monitored by FF weekly beginning mid-July based on preliminary data from 2015 and information from Dr. Richard Bell. Fruit size and quality was variable between sites and genotypes: Fruit size of 038 and 078 was small and unattractive at harvest and 069 had no appreciable distinguishing attributes compared to 'Bartlett'. Additionally, 078 was not precocious in 2015 compared to other selections. The only cultivar that appeared promising was 166 which had large fruit and produced yields similar to 'Bartlett' in OR. In WA, fruit size of 166 was small. In 2017, fruit was smaller than in 2016 but this was attributed to large cropload. Among years, fruit of 166 required different chilling in order to ripen to adequate firmness following a ripening treatment. Two months of RA storage were sufficient to satisfy chilling requirement in 2015 and 2017 (fruit softened to 3.3 lb after 7 d ripening) but not in 2016 (fruit did not soften below 6 lb), despite being harvested at lower pressure in 2016. Flavor profiles (informally evaluated) were quite similar to 'Bartlett' for all four selections.

Photos: An example of fruit from one replication of 166 following 2 months of RA storage (left) and after a 7 day ripening treatment at 68°F (right).

We previously documented 'Gem' storage and ripening behavior: Gem requires 30 days of chill to soften (Einhorn and Wang, 2016 *Journal of the American Pomological Society* 70 (1): 26-35).

2015, 3rd leaf bloom, fruit set, harvest data, and tree size of 4 USDA-ARS advanced selections in Hood River, OR compared to commercial standards.

Cultivar	Full Bloom (date)	Flower clusters (no. per tree)	Fruit set (fruit per cluster)	Fruit after thinning (no. tree)	Harvest (date)	Fruit weight (g)	Fruit shape (length:width)	Firmness (lbf)	Trunk cross-sectional area (cm^2)
69426-038	2-Apr	33	0.46	10.2	3-Aug	144.8	1.44	12.4	17.2
84907-069	1-Apr	35	0.11	4.6	3-Aug	226.7	1.29	14.4	11.2
84907-078	2-Apr	8	0.55	n.d.	n.d.	n.d.	n.d.	n.d.	17.1
84907-166	5-Apr	91	1.43	35	4-Aug	192.3	1.23	17.2	15.5
					19-Aug	249.2	1.18	16.4	
					29-Aug	249.7	1.21	15.3	
Anjou	1-Apr	5	0.1	0.8	n.d.	n.d.	n.d.	n.d.	23.1
Bartlett	5-Apr	122	0.83	40.2	3-Aug	222.4	1.29	18.9	20
Bosc*	n.d.	0	n.d.	0	n.d.	n.d.	n.d.	n.d.	2.8

n.d., no data

* Bosc trees were planted from small containers in 2015

2016, 4th leaf production for 4 USDA-ARS pear selections compared to standard cultivars at OSU- MCAREC, Hood River, OR.

Genotype	Tunk size (cm^2)	Flower clusters (no./tree)	Fruits/cluster (%)	Harvest (date)	Yield/tree (no. fruit)	Fruit wt. (g)	SSC (%)	TA (%)	FF (lbs)
69426-038	27.9	149	19.82	21-Jul	27.4	131.36	12.6	0.3326	13.49
69426-038				28-Jul		156.54	12.3	0.3039	12.18
84907-069	16.3	124.2	43.58	28-Jul	37.2	215.82	11.7	0.3166	13.36
84907-069				4-Aug		241.6	11.3	0.3125	12.94
84907-078	29.6	82.8	112.24	3-Aug	59.6	193.92	12.3	0.3489	11.81
84907-166	25.6	115	37.86	3-Aug	37.6	269.18	11	0.388	14.49
Anjou	40.8	34	10.95	n.a.	3.5				
Bartlett	31.2	180	29.12	3-Aug	44.8	275.78	12.2	0.3751	18.42
Bosc	6.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

2017, 5th leaf production of '166' at OSU MCAREC, Hood River, OR.

Cultivar	Harvest (date)	Yield/tree (no. fruit)	Fruit wt. (g)	FF (lb)	SSC (%)	TA (%)	Trunk size (cm2)
84907-166	9-Aug	148	157.8	16.5	11.9	0.44	32.2

means based on 5 single-tree replicates.

2. Australian (Prevar) cultivars.

All trees were exceptionally small when planted in 2014. Despite limited growth during the establishment year, trees recovered and, in fact, grew vigorously in 2016 and 2017 in Hood River, OR.

0118 is an early-maturing cultivar, harvesting ~ 2 weeks prior to Bartlett. Fruit size, however, has been quite small (130-140 g), in the range of 'Seckel' or 'Forelle' in both 2016 and 2017. The parentage of both selections is 'Corella', which is closely related to 'Forelle'. Providing an additional week on the tree did not improve fruit size of 0118 in either year. 0131 is a later-maturing cultivar, which harvested ~ between two and three weeks after 'Bartlett (closer to Anjou timing). Fruit size was equivalent to 110 box size in 2016 but markedly smaller in 2017 (131 g), despite low fruit set. Although harvest pressure was ~3 lb higher in 2017 than 2016, fruit growth had ceased between the two harvest dates of Aug 23 and Aug 31. Fire blight infection and sampling to determine harvest maturity severely reduced fruit quantities for 2017 evaluations. SSC and TA levels at harvest were moderate in 2016.

2016, 3rd leaf production for two Prevar, Australian pear selections at OSU- MCAREC, Hood River, OR.

Genotype	Tunk size (cm^2)	Flower clusters (no./tree)	Fruits/cluster (%)	Harvest (date)	Yield/tree (no. fruit)	Fruit wt. (g)	SSC (%)	TA (%)	FF (lbs)
118	16.3	4.9	154.9	21-Jul	6.3	132.0	12.4	0.31	12.9
118				28-Jul		134.3	12.4	0.31	10.4
131	14.3	7.6	98.5	18-Aug	7.4	175.1	12.8	0.46	14.9
131				24-Aug		180.6	12.0	0.40	13.4

Following 2 months of RA cold storage, fruit were assessed for quality and then exposed to a 7-day ripening treatment and evaluated for their ripened quality. 0118 fruits softened to acceptable dessert texture. 0131 fruits did not soften to a soft-buttery texture. 0131 has been characterized as a 'ready-to-eat' European pear. Results in 2017 were similar for 0118 but an insufficient quantity of 0131 precluded quality evaluations in 2017.

2016, 3rd leaf PH quality of Prevar, Australian pear selections at MCAREC, OR.

	2 months RA cold storage			+ 7 days at room temp.		
Genotype	SSC (%)	TA (%)	FF (lbs)	SS (%)	TA (%)	FF (lbs)
118 Harvest 1	13.2	0.34	11.0	13.6	0.29	3.3
118 Harvest 2	13.3	0.28	9.6	13	0.25	3.1
131 Harvest 1	13.5	0.50	15.1	14	0.50	6.1
131 Harvest 2	14	0.34	14.1	13.8	0.44	8.8

2017, 4th leaf production of Australian pear selections at OSU-MCAREC,Hood River, OR.

Scion	Bloom (no. clusters)	Fruit set (no. fruit)	Fruit set (%)	Yield (lb/tree)	Avg.fruit wt. (g)	Fruit diameter (mm)	Fruit height (mm)	SSC (%)	TA (%)	FF (lb)
118	59.76	45.65	74.6	9.4	142.4	75.68	61.68	13.7	0.28	12.62
131	60.3	9.1	14.7		130.9	73.7	61.9			17.01

data are means of four multi-tree replicates; fire blight spread from inoculated trees, infecting many 131 trees limiting yield and quality data
0118 trees were harvested on Aug 3 and Aug 9 (data shown for Aug 3), 131 were harvested Aug 23 and Aug 31 (data shown for Aug 31)

Given that 'Corella' is a parent of 0118 and 0131, we were concerned that these selections may be susceptible to fire blight. One L of suspension (2×10^7 colony forming units/mL suspension of *Erwinia amylovora*) was fogged onto trees (both selections plus 'd'Anjou' trees of the same age and location) at daybreak the morning of April 29 (courtesy of Drew Hubbard). All trees were considered to be within 1-2 d of full bloom. The infection risk was moderate according to the Cougarblight model. Inoculation resulted in high infection rates and 90% tree mortality for both 0131 and 0118; in comparison, no 'd'Anjou' trees died from inoculations. 'd'Anjou' had a significantly lower percentage of strikes than either 0131 or 0118 (roughly half). Application of Acitgard reduced the percentage of strikes but did not significantly affect lesion length or tree mortality.

2017 fire blight (*E. amylovora*) inoculation in the field (MCAREC). Trees were at full bloom

Selection	Treatment	Strikes (no./tree)		stdev	Strikes (%)		stdev	Mortality (no. of trees)	
131	utc	52.4	a	6.542171	0.352434	a	0.056922	4	a
131	actigard	34.8	ab	7.120393	0.205632	b	0.017778	3	a
118	utc	40.2	ab	20.25339	0.369294	a	0.102871	5	a
118	actigard	26.6	b	9.289779	0.26014	ab	0.085872	3	a
Anjou	utc	29.2	b	12.51799	0.173878	b	0.042833	0	b

Photos: An example of fruit from one replication of 0118 following 2 months of RA storage (left) and after a 7 day ripening treatment at 68°F (right).

Plant material, Sites and Planting Designs:

1. USDA-ARS cultivars. Five European pear scion selections from USDA-ARS were established in 2013 at two sites in Washington (Wapato, Chuck Peters; and, Wenatchee, Josh Koempel) and one site in Oregon (Hood River, MCAREC) via a 3-year project entitled, 'Pear scion trials in the Pacific Northwest' (see Evans et al. 2015 Final Report). At all sites, 5 single-tree replicates were randomized in high-density, modern training systems with 'd'Anjou', 'Bartlett', and 'Bosc' trees as controls. At Wenatchee, trees were planted 3 ft. in-row x 12 ft. between rows (1,210 trees per acre) without a trellis. Trees will be positioned ~70° from the vertical in year 4. At Wapato, trees were spaced 4 ft. in-row x 12 ft. between rows (908 trees per acre); each tree was tipped opposite its neighbor in a narrow V trellis. At MCAREC, spacing is 5 ft. in-row x 12 ft. between rows (726 trees per acre) and trained to a V, similar to Wapato.

2. Australian (Prevar) cultivars. Two bi-colored, Australian cultivars were to be established in medium-scale plantings in WA and OR in 2014. 'Lanya' (ANP-0118) was planted at two Washington sites (Dryden, Josh Koempel; and, Wapato, Chuck Peters) and at one site in Oregon (Hood River, MCAREC). Each site had a minimum of ~80 trees. At Dryden, trees were planted in a double-row design spaced 3 ft. x 12 ft. (1,210 trees per acre). At Wapato, trees are trained to a tall spindle and spaced 4 ft. x 12 ft. (908 trees per acre). In Hood River, trees were planted and trained identical to the USDA-ARS selections described above. The second cultivar, 'Deliza' (ANP-0131), however, was only established at MCAREC (40 trees) due to a shortage of nursery material. Additional trees were budded and cultured by a nursery collaborator for 2016 delivery (funding provided from the previous grant).

Executive Summary

New cultivars are needed to expand the pear market and excite new consumers. If promising, new cultivars are to be adopted, their performance needs to be evaluated in the PNW. This project evaluated five elite selections of fire blight tolerant European pear from the USDA-ARS pear breeding program and two Australian pear cultivars presently handled by Prevar. Preliminary data and proposed plantings were reported in the 2015 Final Report: Pear scion trials in the Pacific Northwest, led by Dr. Kate Evans.

The USDA-ARS pear selections were tested in small-scale plantings along with commercial standards ('Bartlett', 'GR Bosc' and 'd'Anjou') in WA and OR. The experimental design was a randomized complete block design with five single-tree replicates. The Australian selections were established as medium-scale plantings in WA and OR with roughly 10 trees per replicate in a randomized complete block design with four replicates.

Key findings are presented by objective.

1. USDA-ARS selections:

- We discontinued evaluation of these selections after year 2. This decision followed Dr. Richard Bell's (USDA-ARS) communication that all selections had tested positive for viruses. Consequently, results documented in reports from the first two years of the project may not appropriately represent tree growth, productivity or fruit quality attributes of these genotypes in a 'virus-free' condition.
- Notable results from the first two years (3rd leaf and 4th leaf production) of observations pertained only to one selection, 84907-166, which flowered profusely, had similar yields as Bartlett and produced attractive fruit with a high percentage of red blush.
- Fruit size varied for 84907-166 among years (ranging from 270 to 150 g). Small fruit size was an indirect effect of over-cropping. At the appropriate crop load, fruit weight of 200 to 270 g was achievable.
- An attempt to clean 84907-166 of virus is underway.

2. Australian selections:

- The selection 0118 is an early-maturing genotype, harvested ~2 weeks before 'Bartlett'. Fruit size, however, was small (~130-140 g) in both years and did not improve between the first and second pick (~1 week apart). Fruit were attractive with fairly extensive red blush (nearly 50% of surface area), good sugar concentration (13.9%) but low acidity (0.24 % TA).
- Following 2 months of RA storage, 0118 ripened to dessert quality (FF, 2.9 lb)
- 0131 is a late-harvest selection, ~2 to 3 weeks after 'Bartlett' (approx. 'd'Anjou' timing). 0131 was not nearly as precocious as 0118, producing only a few fruit per tree in 2016 and 2017. Despite low crop load, 2017 fruit were small (150 g; harvested August 31, 2017). In 2016, fruit size was larger (180 g). Ripening was not evaluated given the limited number of fruit harvested.
- Given the lack of fire blight in Australia and the lineage of 0131 and 0118, we field-inoculated both genotypes as well as 'd'Anjou' (control). Inoculation with 2 x 10^7 colony forming units/mL suspension of *Erwinia amylovora* at bloom resulted in a higher number of strikes (nearly double) for both Australian cultivars compared to 'd'Anjou' and 90% tree mortality; in comparison, no 'd'Anjou' trees died from inoculations.
- The combination of small fruit size and apparent fire blight sensitivity does not support additional evaluation of these selections in the PNW.

FINAL PROJECT REPORT

Project Title: Survey of Anjou pear conditioning in the Pacific Northwest

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Cooperators: D. Kihlstadius, PBN; K. Moffitt, Various ripeners

Total Project Request: **Year 1:** $30,480

Other funding sources
None

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

Item	**2017**
Wages	$6,210
Benefits	$1,350
Equipment	$17,920
Supplies	$1,000
Travel	$3,500
Miscellaneous	$500
Total	$30,480

Footnotes: Wages for 0.5FTE for 3 months, Equipment for ethylene and CO2 meter, firmness meters, and temperature loggers.

Objectives
This project was a gap analysis on the pear conditioning programs in the Pacific Northwest, surveying current commercial conditioning programs to identify the current and desired performance of these programs. It also provided a platform for direct interaction with participating warehouses, and develop material in preparation for a pear conditioning workshop after the project's conclusion.

Significant Findings
There is a gap between the "current state" and "desired state" of Anjou ethylene conditioning at most warehouses. The reasons for this are typically because of: (i) time pressure to condition fruit to meet an order, (ii) lack of understanding of pear ripening physiology, and possibly (iii) not appreciating the impact of eating fruit (good or bad) on consumer buying habits. Inferior eating quality, especially earlier in the season, is a consequence of an inadequate ethylene conditioning treatment.

Ripening trailers and warm rooms have no airflow, and consequently fruit warming is slow and variable. This can be managed, to some degree, by extending the treatment duration to allow the pulp temperature to reach a minimum of 60°F before ethylene treatment. Modern ripening rooms provide adequate infrastructure to ripen pears, but management needs to take into account the fruit pulp temperature and the required duration of ethylene treatment for conditioning to be effective. Operators should take into account – and ideally measure – the maturity, air flow, pulp temperature, time of treatment, ethylene concentration, and relative humidity during ethylene conditioning treatment to achieve an effective treatment.

A half day pear session and facility tour will be included in the WSU Postharvest Fruit School (March 20-22).

Materials & Methods
Four warehouses in Washington and Oregon with ethylene conditioning facilities were included in the study between June and October 2017.
The different types of ethylene conditioning treatments were:

- ripening trailer
- warm room
- ripening room (older and new)

Ethylene, CO_2, and O_2 concentrations were measured with a Felix F-950 Gas Analyzer. Air speed was measured with a Kestrel 5200 environmental meter. Air temperature, relative humidity (RH), and pulp temperature (two probes per logger) were measured with Onset Hobo U-12 data loggers. These loggers were installed at six locations around each room. Fruit firmness was measured with a Mohr MDT-2 fruit firmness meter. Non-conditioned and conditioned fruit were kept at room temperature. Firmness was measured every second day until firmness began to drop, and then every day until firmness dropped below 1lb.

Results

Detailed results were shared with the participating warehouses during the project. Summary results are provided here.

Ethylene & CO_2

Disappointingly, the gas analyzer did not always complete logging during the ethylene conditioning treatment, but when logging did complete, the ethylene concentration maximum was greater than 100ppm, and CO_2 was approximately 0.5%. One issue noted with the ethylene generator is that the ethylene concentration peaked and then declined during the treatment. A comparison between an ethylene generator and direct supply from a cylinder of 5 or 10% ethylene would be interesting. The CO_2 concentration was below the recommended 1.0% limit. The installation of meters for both ethylene and CO_2 would improve monitoring and could aid management decisions. Note that these meters should be calibrated regularly according to manufacturers' guidelines to be useful.

Temperature, Time, Air flow, and Relative Humidity

Summary results from the four facilities are provided in Table 1 below. Note, none of the facilities had active relative humidity management.

Ripening Trailer: Operating on a 24 h warming, 24 h ethylene conditioning treatment, and post-treatment cooling outside the room, this treatment was not adequate to consistently condition fruit. Because of the lack of air movement and time rather temperature based approach, fruit were slow to warm up, never reaching 65°F and only having 7:30 h above 60°F. The temperature variation was high and the relative humidity low (71%).

Warm Room: This facility used experience and pulp temperature to decide on an ethylene conditioning treatment and achieved an effective conditioning treatment. Time to warm the fruit was 39:00 – *i.e.* approximately 24 h longer than a modern ripening room, but fruit spent 32:30 h above 65°F. Temperature variation was high and relative humidity low (64%).

This approach has a low capital and operating cost, but treatment took almost 4 days (excluding post-treatment cooling) so capacity is limited, and it is relatively inflexible in terms of sales lead times – which could result in inadequate conditioning if the operator is not disciplined.

Ripening Room 1: This older ripening room has potential to adequately ripen pears, with adequate air flow to quickly warm and cool fruit, and minimize pulp temperature variation. The average time to warm fruit varied between 12:40 and 19:20 when fruit were loaded at approximately 35°F pulp temperature. The time above 60°F and 65°F pulp temperature improved after discussion with the operator (from 20:20 to 29:20 h above 60°F and 0:00 to 11:00 h above 65°F), but was still inadequate to ensure a consistent conditioning treatment. Pulp temperature was usually less variable than the trailer and warm room because of the forced air through each pallet. RH was higher (79-89%), but still below the recommended 90-95%. This facility could improve operations by moving from a time-based approach to a fruit-based approach, taking into account the pulp temperature of the fruit during the conditioning treatment, and have a longer lead time to adequately condition fruit – *i.e.* move away from spot conditioning to a conditioning program with customers.

Ripening Room 2: This new ripening room had near textbook results, with adequate air flow, 25:40 h above 60°F and 22:00 h above 65°F. Temperature variation was low, except for the pallets next to the door – which leads to a refrigerated corridor. Relative humidity was low (81%) – with could be problematic with regards to fruit shriveling. This could be exacerbated by rapid cooling of the fruit with a large difference between air and pulp temperature. Fruit began to show slight shrivel at the neck when mass loss was about 4% from the start of the ethylene conditioning treatment.

Table 1: Environmental conditions during ethylene conditioning treatments at four facilities, along with the Pear Handling Manual Recommendation.

	Manual Recommendation	Trailer	Warm Room	Ripening Room 1*	Ripening Room 2
Airflow (fpm)	-	0	0	453 532 551	122 ⁋
Time to Warm (h:m)	-	24:00 #	39:00 § #	12:40 19:20 15:50	11:30
Time above 60°F Pulp Temperature (h:m)	-	7:30	53:00	20:20 20:50 29:20	25:40
Time above 65°F Pulp Temperature (h:m)	24	0:00	32:30	0:00 4:40 11:00	22:00
Duration of ET (h)	24	24 #	43:00 #	24 24 24	24
Pulp Temperature during ET (°F)	65 ± 5	54 – 64	59 – 68	56 – 62 50 – 64 60 – 65	57 – 65
Relative Humidity (%)	90-95	71	64	85 79 89	81

* Surveys done 3 times in different rooms.
§ Pulp temperature started at about 45°F and continued to warm.
# No temperature control; figure is from loading to start of ethylene treatment.
⁋ Spot measurements before treatment were about 450 to 500 fpm.

Fruit Quality

When the conditioning treatment was adequate, the eating quality of conditioned fruit was far superior with fruit having a buttery juicy texture and good flavor at a higher firmness level than non-conditioned fruit. These results are entirely expected, but reinforce that ethylene conditioning results in pears with superior eating quality sooner. This provides convenience and superior eating experience for consumers, but there is a risk of fruit quality loss from scuffing, bruising, and general shrink so warehouses, distribution centers, and retailers may need to adapt handling protocols to minimize waste.

The Mohr MDT-2 is well-suited to measuring pear firmness because it measures flesh firmness from the skin to the core, and measures crispness (for pears the loss thereof). Although this unit is more expensive than a typical benchtop electronic fruit firmness meter, it provides more valuable information on fruit quality and it can also be used on apples to amortize the capital cost.

Discussion

Inadequate facilities (ripening trailer or warm room) can be managed to some degree by having a longer treatment duration because of slower fruit warming. Trailers are more difficult to manage because access

to all but the front two pallets is impossible with typical loading, and I recommend warehouses discontinue using ripening trailers. Access to fruit in warm rooms is possible, so pulp temperature around the room can be measured to inform management decisions.

Modern ripening rooms provide adequate infrastructure to ripen pears, but recommended minimum standards still need to be achieved for the ethylene conditioning treatment to be effective. This is easily achieved by monitoring pulp temperature and adjusting the treatment accordingly. “Spot conditioning” to an order is not conducive to effective ethylene conditioning and delivery of fruit with good eating quality because it typically results in an inadequate ethylene treatment from low pulp temperature and/or inadequate treatment duration. Considering the cost of ripening rooms, it is recommended that, if possible, warehouses work towards a conditioning program with their customers to supply fully conditioned fruit that will ripen with good eating quality. The pear industry needs to align with other ripened fruit (e.g. kiwi fruit, tomatoes and especially bananas and avocados) to supply a greater proportion of conditioned or ripened fruit to encourage repeat consumption and more super consumers. This may require a shift to more protective packaging to reduce scuffing, bruising, and shrink but such innovations are required to drive growth in pear consumption and rejuvenation in the pear industry in the US.

The adage, “you can’t manage what you can’t measure” is apt for pear conditioning. As such, the acronym “**MATTER**” (Maturity, Air flow, Temperature, Time, Ethylene and Relative humidity) is a good reminder of important factors to measure and manage during ethylene conditioning.

The recommendations for ethylene conditioning in the Pear Handling Manual are adequate to ripen Anjou pears. Two questions that still need to be answered are: (i) ‘What is the minimum ethylene conditioning treatment duration for early, mid- and late season fruit?’ and ‘Can 1-MCP-treated Anjou fruit ripen completely with an effective ethylene conditioning treatment?’ Potential avenues for future research and extension are:

- Refinement of the temperature, treatment duration, and ethylene concentration for different maturity levels, storage duration, and 1-MCP treatment,
- Minima and maxima for air flow and relative humidity to determine if these parameters have an appreciable effect on shrivel and eating quality.
- Standards for cooling of conditioned fruit to minimize firmness loss and shrivel.
- Refinement of maturity beyond firmness, possibly looking at dry matter.
- Use of ethylene gas vs an ethylene generator to maintain the ethylene concentration at 100ppm.

Executive Summary

This survey revealed that there is a gap between the 'current state' and 'desired state' at most warehouses that are conditioning fruit. The desired state being the recommendations provided in the USA Pears' Pear Handling Manual. The reasons for this are typically because of: (i) time pressure to condition fruit to meet an order, (ii) lack of understanding of pear ripening physiology, and possibly (iii) not appreciating the impact of eating fruit (good or bad) on consumer buying habits.

Facilities with poor air movement (trailers and warm rooms) have no air flow, resulting in slow and variable warming of the fruit. The facility using the **ripening trailer** achieved a weak ethylene conditioning treatment because pulp temperature was only above 60°F for 7:30 hours and 0:00 hours above 65°F in a 24 h period. The facility that used a **warm room** conditioned the fruit for almost 4 days, achieving an effective conditioning treatment (32:30 hours above 65°F).

Facilities with **forced air ripening rooms** had adequate air flow through the pallets (450 - 550 fpm) with faster warming and less variable pulp temperature. One facility did not allow the fruit to reach a minimum pulp temperature before treating with ethylene resulting in a weak ethylene conditioning treatment (20 – 29 hours above 60°F and 0 – 11 hours above 65°F), but did improve over the duration of the survey. One facility did follow the Manual's recommendation and achieved an effective ethylene conditioning treatment by treating with ethylene for 25:40 hours above 60°F and 22 hours above 65°F pulp temperature.

When the conditioning treatment was adequate, the eating quality of conditioned fruit was superior with fruit having a buttery juicy texture and good flavor at a higher firmness level than non-conditioned fruit.

The acronym "**MATTER**" (Maturity, Air flow, Temperature, Time, Ethylene, and Relative humidity) is a good reminder of important factors to measure and manage during pear ethylene conditioning.

Recommendations for further research are for (i) refinement of the temperature, time, and ethylene concentration, for different maturity levels and storage duration and 1-MCP treatment, (ii) minima and maxima for air flow and relative humidity, (iii) standards for cooling of conditioned fruit, (iv) comparison between ethylene gas and ethylene generator, and (v) refinement of maturity indices for pears.

FINAL PROJECT REPORT **YEAR: 4 of 3 (+1 yr NCE)**
Project number: PR14-108A

Project Title: Improving quality and maturity consistency of 'D'Anjou'

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Total Project Funding: **Year 1**: $ 65,992 **Year 2**: $ 67,272 **Year 3**: $ 68,602

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget History 1

Item	2014	2015	2016	2017 (NCE)
Salaries[1]	24,000	24,960	25,958	0
Benefit[1]	7,992	8,312	8,644	0
Travel[2]	500	500	500	0
Goods and Services[3]	3,000	3,000	3,000	0
Total	35,492	36,772	38,102	0

Footnotes:
[1]Salaries and benefits for 50% Ag. Research Assistant (Musacchi).
[2]Travel to different orchards and farm where the different trials will be conducted (Musacchi).
[3] Consumable lab ware and mineral analyses.

Budget History 2

Item	2014	2015[2]	2016[2]	2017 (NCE)
Wages[1]	15,000	15,000	15,000	0
Goods and Services[2]	15,500	15,500	15,500	0
Total	30,500	30,500	30,500	0

Footnotes:
[1] $12,500 for 25% annual instrument service contracts. $3,000 for consumables
[2]Add proposed same amount for year 1 if work is to be performed in years 2 or 3.

OBJECTIVES

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*
2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

SIGNIFICANT FINDINGS
Overall

- Considerable variability in fruit maturity exists within the large canopy of an open vase tree.
- The use of DA meter in pre-harvest on selected trees helps to be more aware of the maturity stage and variability within the canopy to address the harvest time.
- From year to year fruit maturity distribution (accordingly to the DA meter) at 2 weeks before harvest is variable. This indicated a potential use of this tool to determine the harvest time.
- The DA meter values (I_{AD}) for internal and external canopy fruit were different at harvest. External fruit on average tend to have lower I_{AD} values compared to Internal fruit.
- At harvest, external fruit had less green background, higher red blush coverage, higher dry matter %, and higher soluble solid content than internal fruit.
- Internal fruit tend to be greener than External up to 8 months of storage.
- Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
- Fruit ripening and potentially flavor is different depending upon canopy position.
- Canopy position impacts postharvest behavior including superficial scald risk. This can affect the need to repack fruit boxes.
- Levels of natural peel chemicals we have linked with light exposure may be exploited to develop in-field or warehouse sorting tools to reduce crop variability.

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.
Pre-harvest assessment and fruit maturity distribution

To assess the maturity on the 11th of August 2016 (18 days before harvest) a total of 677 fruit (included 640 good fruit and 37 of <60 mm size and/or with defects) were harvested. Total yield per tree was 121 kg and the average fruit weight was 179 g. Sunburned incidence was 1.8%, cork was 0.44% and no frost damaged fruit were observed.
By measuring I_{AD} before harvest, we determined the maturity stage of the fruit population, in fact, in 2016, more than 2 weeks before harvest, more than 95% of fruit were classified in the least mature I_{AD} classes (above 2.00 I_{AD}) and only a small percentage (0.2%) of fruit were classified in the more ripe classes (below 1.80 I_{AD}, Fig. 1).
From year to year the maturity distribution of fruit accordingly to the DA meter at 2 weeks before harvest is variable.

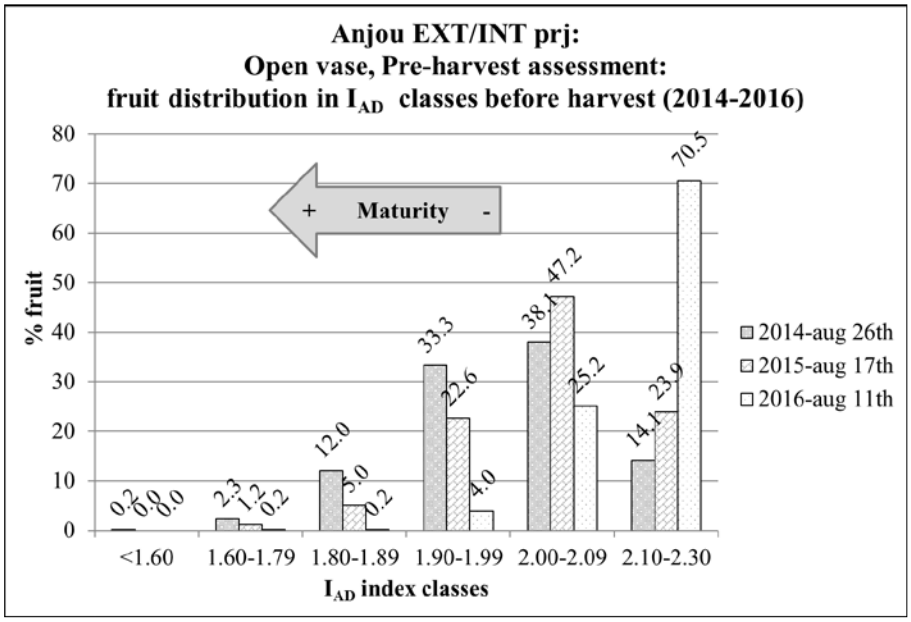

Figure 1: Pre-harvest assessment of fruit maturity distribution across the canopy of an open vase tree in 2014, 2015 and 2016 (≈2 weeks before harvest). Fruit % in each I_{AD} class of ripening is represented.

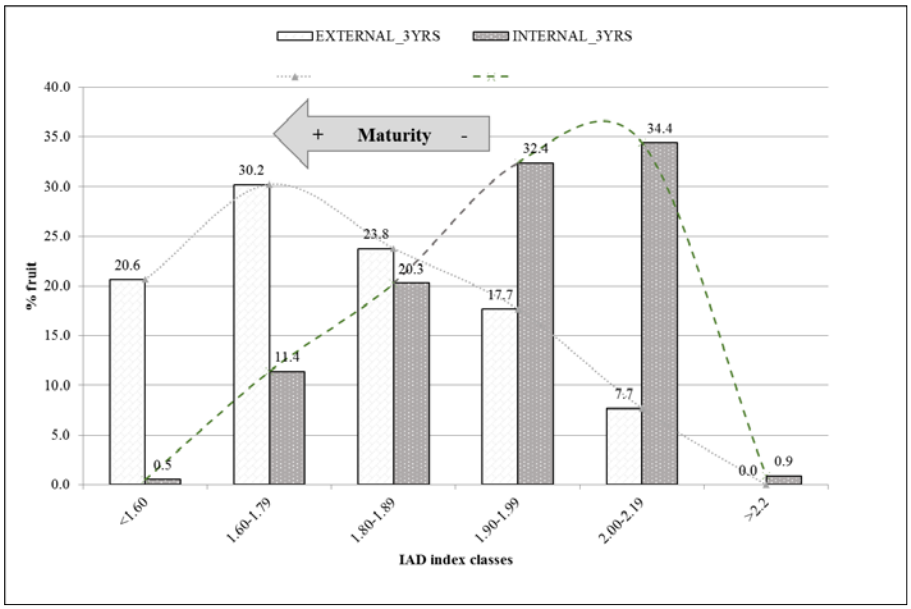

Figure 2: Distribution of fruit picked categorized by canopy position (external and internal) and I_{AD} class as well as in the 3 years, percentage are calculated on all fruit harvested in 3 yrs.

Fruit maturity distribution within I_{AD} classes at harvest divided by canopy position confirmed the observations done in the previous year where Internal fruit tend to be more unripe than the External one (Fig. 2). Looking at the distribution as all fruit harvested in 3 years, ≈34% of Internal fruit fell in the least ripe classes (I_{AD} <2.00), while only ≈8% of External fruit belonged to that class (Fig. 2). Almost 21% of the External fruit were classified in the most ripe categories (I_{AD} <1.60), while only 0.5% of the Internal ones resided in the same classes (Fig. 2).

This represents a strong example of how different are fruit belonging to those two extreme canopy positions. Harvesting as strip pick and collect all fruit in the same bin does not allow anymore to investigate canopy positions variations.

PAR measurement per single fruit and light in the canopy (2016)

PAR measurements of fruit marked for sampling allowed us to accurately choose fruit from the two canopy positions. The percentage of light intercepted by External fruit averaged 92.1% while only 1.4% by Internal fruit (Fig. 3A). Fruit belonging to light interception range from 30% to 70% were discarded. This type of precise harvest allowed us to track the behavior of the two type of pears in postharvest.
A qualitative measure of the light spectrum by a spectroradiometer (measure of photon flux in µmol s^{-1} m^{-2}) was done on 21st of July 2016 underneath one large canopy. A huge variability of light spectra hitting the trees in the four possible inner quadrants (South-West, North-West, North-East, South-East) was observed (Fig. 3B). Three quadrants on four showed lower radiation from 300 to 700 nm (PAR range) while the North-West quadrant was illuminated by direct sunlight and the trend looked similar to a full sun light spectrum (approx. External situation, Fig. 3B). Leaves in the inner part of the canopy have less energy available for photosynthesis so they may be subjected to a shortage of photo assimilates to translocate to the fruit.

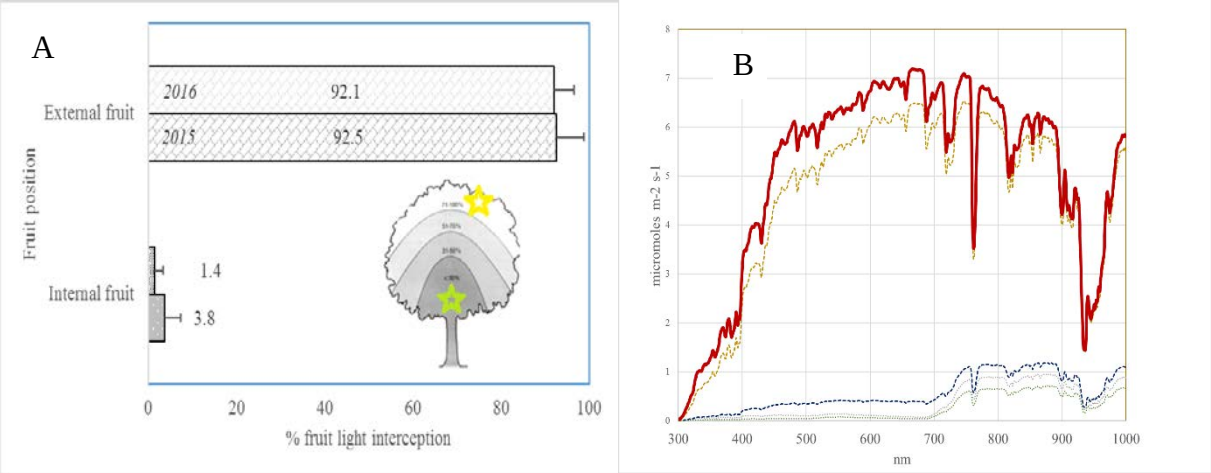

Figure 3: A) Percent of light interception of fruit harvested from the two canopy positions as determined by PAR measurement using the Q53292 quantum sensor in 2015 and 2016 (Li-Cor). Values are average ± stdDev. B) Photon flux measured in the large canopy on 21st of July 2016 between 10 am and 12 pm. Solid line is the light spectra of full sun measured above the canopy at 3.5 m from the ground, four different dashed lines are the four light spectra in the four quadrants (south-west, north-west, north-east, south-east) of a large tree at 40 cm from the trunk and 130 from the ground.

2014 fruit storage and quality assessments

Fruit quality analysis at harvest (T0) showed that External fruit were significantly heavier, larger, and had higher titratable acidity and soluble solids compared to Internal fruit at harvest. Internal were greener. No difference in chroma and firmness.
Regarding I_{AD} index decrease in storage, Internal fruit reported always higher values (less ripe fruit) than External fruit from harvest to 8 months of storage and they showed a slower I_{AD} index decrease (without any ripening post-storage) than external one where each pullout registered a significant drop in this index, suggesting a faster kinetics of ripening of those fruit. The same behavior was noticed after 7 days of ripening at room temperature, where differences between Internal and External were maintained (Fig. 4).
Regarding firmness and storage duration, we did not find differences between External and Internal fruit from harvest up to 6 months, only after 8 months. Internal fruit were firmer than external immediately after removal from cold room. After 7 days ripening, Internal fruit were firmer than External except for no difference at 6 months of storage (Fig. 5).

Dry matter % was always higher in External fruit than Internal at both stages from 3 to 8 months of storage duration. In general, no big dry matter difference found among pullouts. Similar trend was reported for Soluble Solid content (SSC, Brix): External fruit showed higher SSC than Internal with or without ripening time. Correlation between dry matter % and SSC improved along storage moving from R^2=0.677 at 3 months (day 0) to R^2=0.782 at 8 months (day 0). Titratable acidity was significantly higher in the Internal fruit than External at day 0 only after 8 months, while exogenous ethylene was higher in the External than Internal at day 7 after 6 and 8 M.

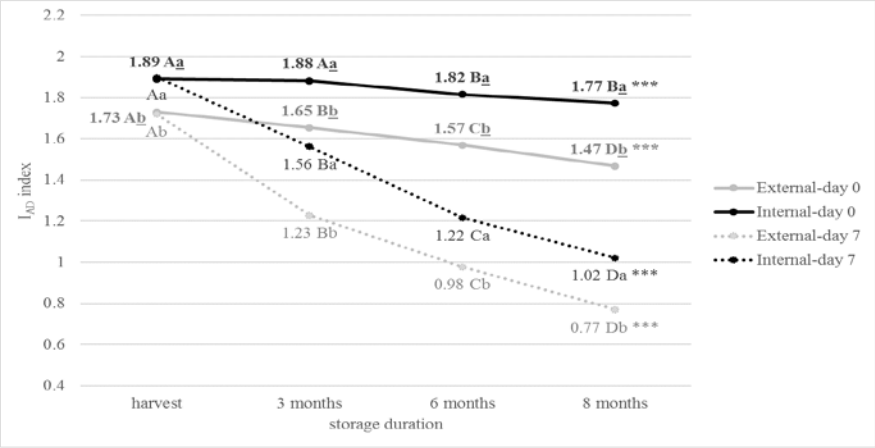

Figure 4: I_{AD} index decrease in storage (fruit harvest 2014). Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant Capital letters discriminate means among storage duration (horizontally), small letter between canopy position in pairs (vertically).**

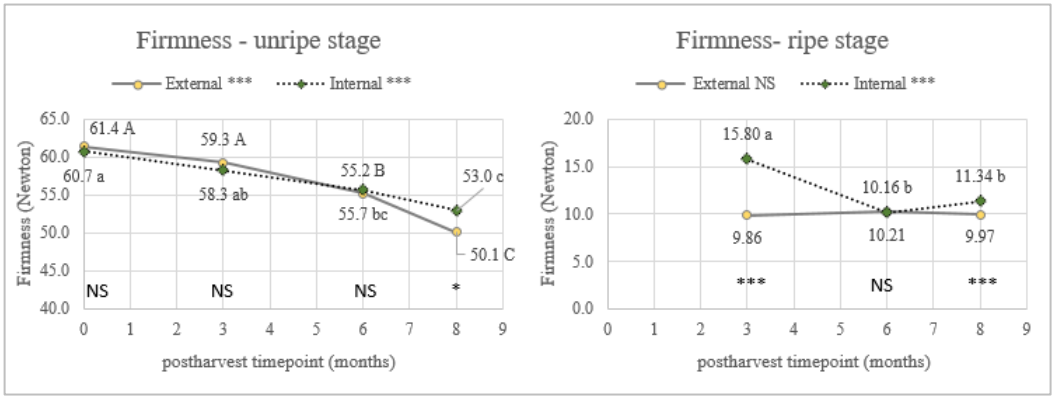

Figure 5: Firmness decrease at unripe and ripe stage in storage (fruit harvest 2014). Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant. Capital and small letters discriminate means among storage durations within the same canopy position (horizontally), while in a text box below significance between canopy positions in pairs (vertically) within each storage time.**

2015 fruit storage and quality assessments

Fruit from Internal and External canopy regions were picked separately on 31st August 2015. Fruit from each light condition were separated into two bins (containing 460 external and 486 internal pears) and immediately moved to 40°F for fruit maturity distribution analysis and sorting in DA classes.
Within each group, fruit were again classified using I_{AD} into 5 classes (I_{AD}<1.60, 1.60<I_{AD}<1.79, 1.80<I_{AD}<1.89, 1.90<I_{AD}<1.99, 2.00<I_{AD}<2.19). The first was only included in the External fruit (not present in Internal) and Internal fruit in 1.60<I_{AD}<1.79 class were not enough to cover all pullout so harvest and 8 months storage were chosen. Fruit belonging to each class were, then, equally divided into 3 groups for 0 (= harvest), 6, and 8 months CA storage. Fruit were stored in a research CA room (31°F, 2% O_2 and 0.8% CO_2). For each pullout, except for T0 at harvest, fruit were split in 2 subgroups: with or without 7 days of post-storage ripening time. Fruit quality analysis in 2015-2016 pullouts was performed in the same manner as 2014.
At harvest 2015, External fruit had less green background, higher red blush coverage (%), higher firmness, higher dry matter %, and higher soluble solid content (SSC, brix) than Internal fruit (data not shown). As reported in literature, sun-exposed 'Bartlett' pears had higher firmness than pears grown in the shade before and after ripening at room temperature probably due to the direct sun exposure (Raffo et al., 2011). This firmness difference between positions was a variation in comparison to 2014.
Within each canopy position fruit were divided accordingly to the I_{AD} index in classes and differences among them emerged. External fruit belonging to the least ripe class (2.00<I_{AD}<2.19) presented the highest background hue value (tended to more green) and the lowest SSC content (12.9 °Brix), while External fruit belonging to the most ripe class (I_{AD}<1.60) were bigger in diameter, less firm and higher SSC (14.0 °Brix). Similarly, the 2.00<I_{AD}<2.19 class for Internal fruit showed higher background hue and pH, lower SSC (11.0 °Brix), and lower acidity than the most ripe class for the same light condition (data not shown). No differences were detected in terms of dry matter %, total number of seed, viable vs dead seeds, ethylene production and weight. When all ripening classes and canopy positions were compared as combinations, significant differences of fruit weight, overcolor, dry matter %, firmness, diameter, pH and soluble solid contents, were found at harvest (Fig. 6).
After 6 months of storage in CA (T1), without any post-storage ripening time, External and Internal fruit differed for color/blush, firmness, SSC, dry matter % and pH with the most exposed fruit less green, firmer, higher in SSC and dry matter and lower pH. Same comparison done after 7 days of ripening (+6M storage + 7 days at room temperature) confirmed difference for color, SSC and dry matter. Among classes in External fruit without any post-storage ripening, 1.60<I_{AD}<1.79 class showed the highest drop in I_{AD} index, while 2.00<I_{AD}<2.19 class the lowest, confirming variation in ripening rate; similarly, between 1.80<I_{AD}<1.89 class and 2.00<I_{AD}<2.19 class for Internal (1.60<I_{AD}<1.79 was absent for internal at T1). This latter class showed also the lowest SSC among Internal fruit classes (data not shown).
Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 6 months of CA storage, 2.00<I_{AD}<2.19 class for Internal still showed the lowest drop in I_{AD} index in the 7 days of ripening at room temperature, the lowest SSC (13.1 °Brix) and dry matter %, the highest hue (more green), and the highest pH (Fig. 6).
After 8 months of CA storage (T2), without any post-storage ripening time, External and Internal fruit differed for weight, overcolor percentage and color, firmness, SSC, dry matter % and titratable acidity, with the most exposed fruit bigger, less green, with 15% overcolor, firmer, higher in SSC and dry matter and lower in acidity. In External fruit without any post-storage ripening, differences among classes were less than in shorter storage duration, in fact all destructive parameters like firmness, SSC, dry matter, pH and titratable acidity did not significantly differ. Ethylene production was higher for External fruit class I_{AD}<1.60 than the other classes (less ripe fruit). Internal fruit instead after 8 months and without any post-storage ripening presented differences in the comparison between DA classes with the most ripe class showing lowest firmness and highest SSC and dry matter % (data not shown).

After 7 days of ripening (+8M storage +7 days at room temperature) the comparison between External and Internal fruit reported difference for I_{AD} index drop in the 7 days, overcolor % and color, SSC. Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 8 months of CA storage, $2.00<I_{AD}<2.19$ class for Internal still showed the lowest drop in I_{AD} index in 7 days at room temperature, but the highest drop in weighs in 7 days (tendency to shriveling without proper ripening), the highest hue (still more green then the others), the lowest SSC and dry matter %, the highest hue (more green), and among the highest pH values (Fig. 6).

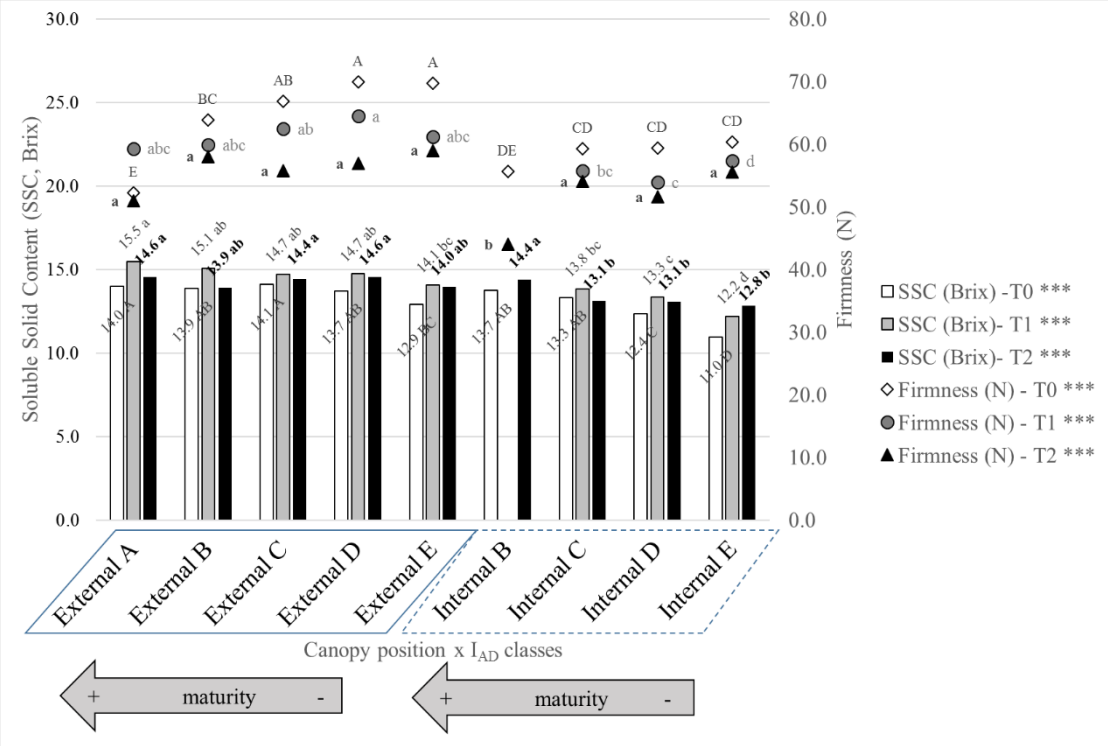

Figure 6: Comparison between combinations of DA classes and canopy position at harvest 2015 (T0), after 6 M of Ca storage (T1) and after 8 M of Ca storage (8M) for Soluble Solid Content and Firmness.

Regarding disorders observed during fruit assessment, cork incidence ranged from 10 to 14% in Internal fruit while for External fruit from 13 to 29%. Scuffing was absent at harvest (T0) in both fruit positions, while increased in the following pullouts, reaching a maximum of 96% of incidence in External fruit after 8 month of storage + 7 days of ripening (88% in the Internal fruit at the same time point). No superficial scald was noticed in the fruit from harvest up to after 8 months of CA without any post-storage ripening (day 0), while after 7 days of ripening at room temperature, superficial scald incidence was 37% in External fruit and 1.5% in Internal fruit (after 6 months) and 48% and 11% respectively (after 8 months). Superficial scald hue tended to get darker longer the storage duration but the affected area was similar approx. around 25% of fruit surface. So, in general, External fruit were more affected by superficial scald.

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.

Peel chemistry changed alongside fruit appearance and other quality traits. Differences of peel chemistry were most dramatic with tree position which changed as fruit ripened during storage (Fig. 7). Results indicate the greatest impact on fruit ripening and chemistry results from tree position more than any other factor in the experiment and, accordingly, it is the greatest source of quality and ripeness variability.

Differences were detected at harvest as well as throughout storage indicating the final product on the store shelf may also be different.

Differences of quality traits, including natural aroma and flavor, are clear within the chemical profile. These include sugars (sweetness), malic acid (tartness), phenolics (bitterness), and aroma volatiles. Pears may have more ripe or unripe aroma depending upon tree position, even at 8 months storage (Fig. 8). I_{AD} classification was reflected in the overall peel chemistry at harvest but this relationship declined with storage duration (data not shown). Peel chemical analysis results to date indicate that tree position will have a major impact on relative storability and eating quality.

Not only are flavor and maturity impacted by tree position but so are critical factors such as appearance. While we expect that external fruit may have more blush or, as fruit appear to ripen differentially, background color would be influenced by canopy position, there are also less obvious factors profoundly impacting finish. For instance, superficial scald incidence was higher in External fruit than Internal fruit, a factor linked with higher levels of key apple scald risk biomarkers detected in Internal peel (Fig. 9). As storage regimes and marketing strategies can be most effectively tailored to a consistent batch of fruit, it is clear that more consistent fruit at the beginning of storage would reduce losses and that these decisions are impacted by canopy position.

Shorter term strategies for reducing inconsistency of fruit going into storage may rely on the ability to "see" and sort fruit according to canopy position as that is the major contributor to inconsistency. Another outcome of our untargeted appraisal of peel chemistry are potential targets for just this task. External fruit have higher levels of compounds associated with light exposure and Internal fruit have higher levels of wax compounds involved in other pathways (Fig. 10). These metabolites associated with sun exposure are part of a fruit's natural defense to increased light exposure that are not apparent with the naked eye but can be detected using devices that focus on portions of the ultra-violet spectrum. This aspect could, potentially, be used to sort fruit in the orchard or warehouse according to tree position yielding a more consistent batch of fruit for tailored supply chain management, reducing downstream losses.

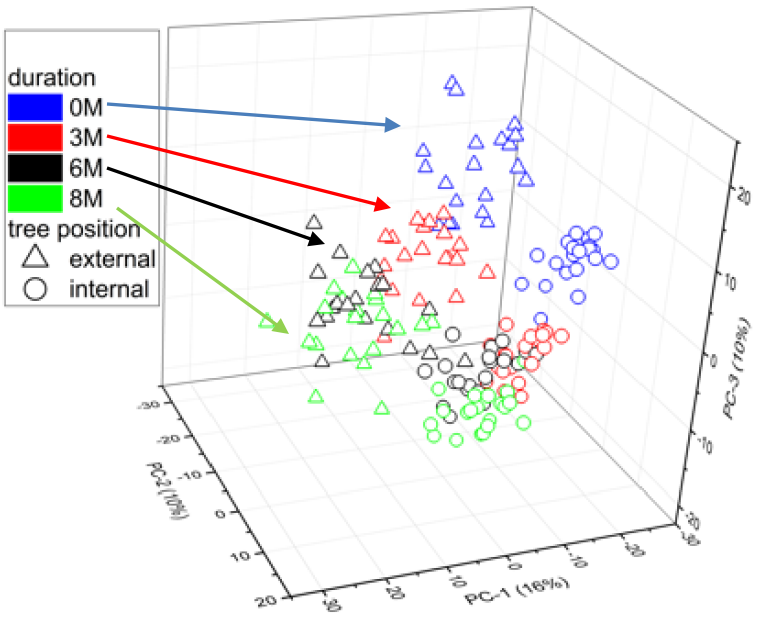

Figure 7: Principal components analysis (PCA) scores plot illustrating differences in overall natural chemical levels from Anjou pears harvested from the external or internal canopy and stored for up to 8 months in CA storage. Each point represents a summary of over 800 natural peel chemicals for a single peel sample. Triangles represent internal and circles represent external fruit peel. Storage duration is indicated by symbol color. Metabolism of internal and external peel changes during CA storage differentially.

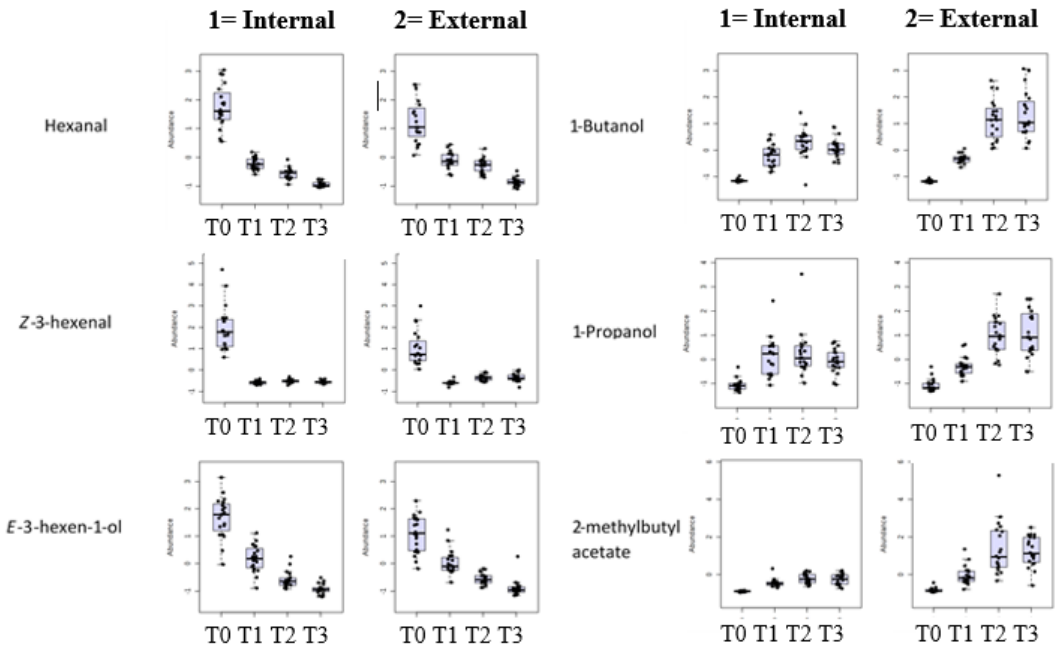

Figure 8: Changes of levels of peel chemicals different in d'Anjou pears from the Internal (1) or External (2) canopy over 8 month CA storage (from T0 to T3). Results suggest that "unripe" flavors (left) are higher in Internal fruit at harvest and are similar by 8 months while "ripe" flavors (right) are more prevalent in External fruit at the end of storage indicating fruit ripeness and quality are different depending upon tree position.

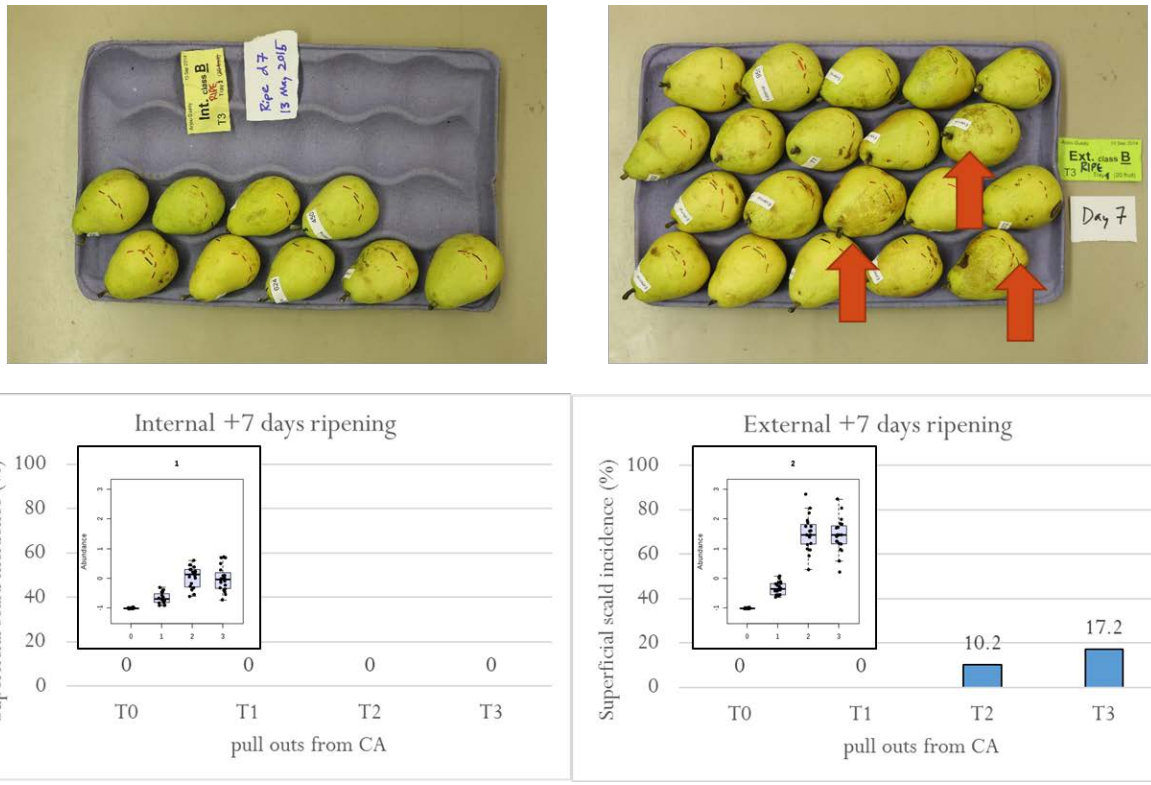

Figure 9: Superficial scald incidence (%) dependent upon canopy position for d'Anjou pears stored in CA for 8 months and left to ripen at 68 F for 1 week. In this case (orchard, year, storage conditions), External fruit (right) developed more scald than Internal fruit (left). Levels of an apple scald risk assessment biomarker (insets) were elevated in External fruit.

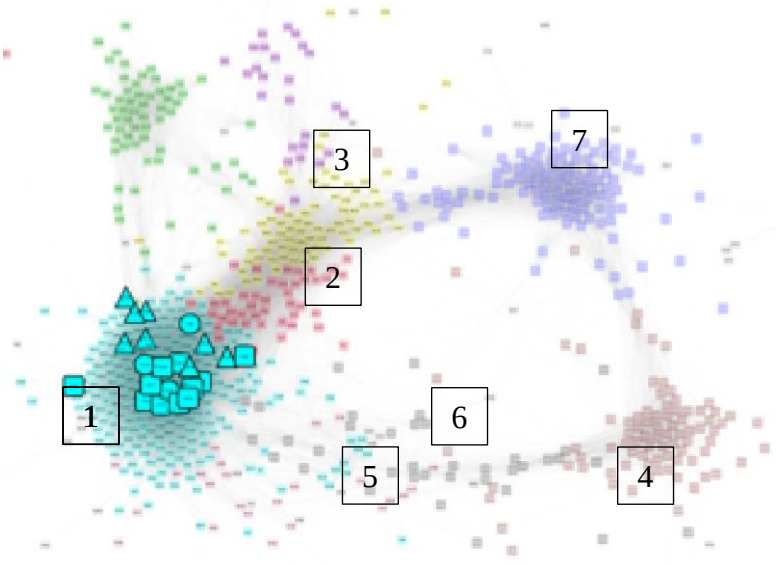

Figure 10: Associations among natural peel chemicals during 8 months CA. Chemicals (shapes) that are closer together indicate that their levels over the storage period change similarly with respect to other factors in the experiment such as tree position. Compounds associated with higher light environment are colored turquoise (1), red (2), and yellow (3), and those linked with lower light are brown (4), pink (5), and black (6). Compounds higher at harvest are blue (7). Turquoise compounds increase with storage more in external fruit. Chemicals we have identified that are associated with higher light conditions include flavonol glycosides with can be detected using UV reflectance imaging and possibly exploited for in-field or warehouse pre-storage sorting.

- **Publications:**
    - Zhang J., Serra S., Leisso R.S., Musacchi S. (2016) *"Effect of light microclimate on the quality of 'd'Anjou' pears in mature open-centre tree architecture"*. Biosystems Engineering, 141:1-11.
    - Rudell, D. R., Serra, S., Sullivan, N., Mattheis, J. P., & Musacchi, S. (2017). *"Survey of 'd'Anjou'Pear Metabolic Profile Following Harvest from Different Canopy Positions and Fruit Tissues"*. HortScience, 52(11), 1501-1510.

- **Presentations:**
    - Rudell D., Serra S., Sullivan N., Mattheis J., Musacchi S. *"Fruit position within pear trees impacts ripening and associated metabolism after harvest"* (oral presentation by Rudell D.). 12th Annual Conference of Metabolomics Society, Dublin, Ireland (June 2016).
    - Serra S., Rudell D., Mattheis J., Musacchi S. *"Evaluating Fruit Quality and Maturity in Large Open Vase-trained 'D'Anjou' Trees"* (Oral presentation by Serra S.) ASHS annual meeting, Atlanta, Georgia (August 2016).
    - Serra S. "DA Meter and Dry Matter" (oral presentation in IFTA session IV: New Instrument Panel discussion. 2017 IFTA Annual Conference, From bud to bin, Wenatchee WA (February 2017).

Executive Summary

Project Title: Improving quality and maturity consistency of 'D'Anjou'

Background

'D'Anjou has been trained for many years using an open vase. Single trees can reach 17 ft high with a very large canopy volume where fruits are distributed mostly in the upper-medium portion of the canopy. Fruit characteristics inside such a big and vigorous tree can be very different as less light can penetrate into the inside of the canopy and, consequently, light exposure can be quite different. Harvest in those orchards cannot be mechanized and is performed manually without any sorting. Consequently, many fruit quality characteristics, including maturity, can be highly variable within a single bin. This factor can dramatically impact fruit quality and storability often resulting in the need to repack to eliminate over-ripe, spoiled and scalded fruit from packed boxes.
Our preliminary work indicates a non-destructive approach using the DA-meter, which can be adopted to segregate pear fruit according to maturity by estimating associated chemical changes. We have found that fruit picked from the internal part of the canopy ripen more slowly, as estimated using the DA index, but lose weight more rapidly than fruit harvested from the outer part of the canopy. Our long-term goal is to develop tools and protocols that improve uniformity of fruit maturity and quality at harvest. Moreover, one possible long-term outcome is implementation of existing sorting technology to afford storage operators the ability to pre-sort pears by orchard or tree position/maturity. This sorting capacity would allow tailored storage regimes for improved ripening and quality consistency and reduced losses from postharvest disorders such as scald and possibly decay.

Project outcomes:

1. Method to prove that large 'D'Anjou open vase trees show inconsistency in ripening depending on light exposure.
2. Repacking problem and postharvest losses can be improved with fruit sorting at harvest and tailored storage conditions and durations.
3. New potential chemicals targets for sorting fruit accordingly to canopy position in the orchard or warehouse.

Significant Findings:

1. Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
2. Fruit ripening and potentially flavor is different depending upon canopy position.
3. Canopy position impacts postharvest behavior including superficial scald risk.

Future Directions:

1. Change 'D'Anjou trees architecture (and rootstocks) toward a narrower canopy and higher density planting and more planar canopy for more consistent crop.
2. Improve the picking process by canopy position and fruit sorting ability in the orchard.
3. Tailored storage duration depending on fruit sorted by maturity levels.
4. Tailored storage duration depending on fruit sorted by non-destructively predicted dry matter %.
5. Imaging to discriminate fruit by position.

FINAL PROJECT REPORT **YEAR: 4 of 3 (+1 of no cost extension)**
Project Number: PR14-104

Project Title: Fall and summer pruning to control vigor and psylla in Anjou pear

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Cooperators: Sara Serra (WSU/TFREC)

Total Project Request: **Year 1**: $72,707 **Year 2**: $71,589 **Year 3**: $71,170

Other funding sources:

Agency Name: USDA/ARS
Amt. awarded: Harvest and postharvest quality analyses conducted by Jim Mattheis to be supported with base USDA, ARS funds.

WTFRC Collaborative Expenses: None

Budget
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]	36,480	37,939	39,456	0
Wages[2]	11,440	11,898	12,374	0
Benefit[3]	14,130	14,695	15,283	0
Travel[4]	757	757	757	0
Goods and Services[5]	9,900	6,300	3,300	0
Total	72,707	71,589	71,170	0

Footnotes:
[1] Salary for a new hire Research Intern (Musacchi), a Research Intern (Beers).
[2] One non-Student temporary for 13 wks: 40/wk at $11/hr (Musacchi) and one non-Student temporary for 13 wks: 40/wk at $11/hr (Beers).
[3] Benefits at 9.7% (Musacchi and Beers).
[4] 676 miles/year for domestic travel to go to the orchard (Musacchi) and 676 miles/year for domestic travel to go to the orchard (Beers).
[5] Fruit mineral analyses, data loggers, light bar, laboratory supplies for fruit quality analyses (Musacchi).

OBJECTIVES

1. *Control vigor through pruning practices in a mature Anjou orchard while maintaining yield and quality, and reduce psylla densities throughout the tree.*

SIGNIFICANT FINDINGS

Vigor control and vegetative measurements

- Regardless of rootstock, more material was removed in 2017 winter pruning than 2016 fall.
- OHF97, OHF69, and OHF87 did not differ in weight pruned in winter 2017, while OHF87 reported the least amount of material removed in 2016 fall pruning respect to the other two rootstocks.
- Trunks of winter pruned trees were significantly larger than fall pruned trees for all rootstocks and, OHF97 trunks were the largest and OHF87 were the smallest in fall pruning only.
- There was no significant difference between annual trunk growth of trees pruned in different seasons.
- OHF87 had the most fruit set per branch and OHF69 had the least when considering both pruning treatments together (in 2016), while no differences in 2017 between rootstocks.

Yield (2016+2017) and fruit quality (2015)

- In the 2016 harvest, winter pruned trees had significantly more and heavier fruit, higher yield efficiencies and crop loads, but more fruit with sunburn and cork than trees pruned in the fall.
- There was no significant difference between the three rootstocks for productivity, average fruit weight, and incidence of sunburn and cork; however, OHF97 had significantly lower yield efficiencies and crop loads than the semi-vigorous rootstocks.
- In 2017, fall pruned trees produced significantly more fruit with a higher yield/ tree and higher yield efficiency and crop load than winter pruned trees.
- The average fruit weight for winter pruned trees was only 6 g higher than fall pruned trees.
- After 7 months, fruits from the winter pruning treatment were riper (by I_{AD} index) than fall+summer fruit: they lost significantly more weight in storage, ripened significantly faster and were less firm (only significant at 5 months) than fall+summer fruits.
- Winter pruned fruit from 2015 had more cork than fall+summer fruit after 5 and 7 months of storage. However, there were no differences in calcium content for pear tissue after 5 or 7 months of storage.

Psylla and Mite Densities

- Adult psylla densities were low in mid-April (2-3/tap) and remained low through early July; however, much higher numbers (8-10/tap) were found just before harvest in mid-September Nymph densities were also low (<0.05/leaf) except for a peak (0.2/leaf) in early July. Spider mites and predatory mites were low on all counts.
- No differences in seasonal average densities for mites or psylla were found among pruning treatments or rootstocks.
- For the first time in this experiment, fruit damage by psylla was significantly lower in the fall-pruned trees than in the standard (winter) timing. Psylla damage among rootstocks was OHF69>OHF97>OHF87. All fruit examined had russeting resembling rust mite damage, despite the absence of rust mites in leaf brush counts.

RESULTS AND DISCUSSION

Vigor and vegetative measurements

Regardless of rootstock, significantly (2.5 times) more material was removed in winter pruning than in fall in both 2015-2016 and 2016-2017 (Fig. 1). Among rootstocks, OHF97, OHF69, and OHF87 did not differ in weight pruned in the 2017 winter treatment (average. 13.7 kg/tree, Fig. 1 and 2) confirming the 2016 trend (Fig. 2), while OHF 87 reported a significant lower amount of material removed in the 2016 fall pruning in comparison to the other two rootstocks (Fig. 2). There was no significant difference between trunk growth of trees pruned at different times (Fig. 1), however OHF97 trunks grew the most and OHF87 trunks the least in 2016, while no difference reported for 2017 (data not shown).

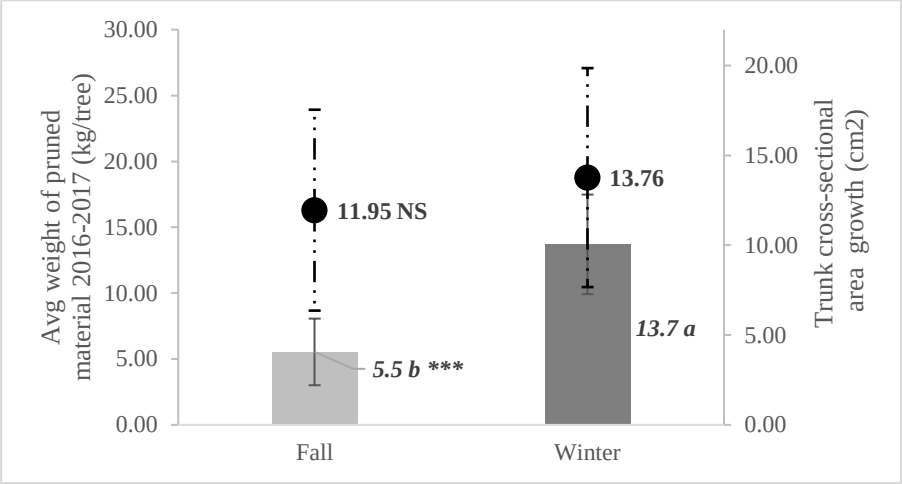

*Figure 1: Comparison between weight of wood (and leaves only in fall) removed per tree (kg) in 2016-2017and trunk growth (2016- 2017) for each pruning treatment (secondary axis, black dots). Significance: * p<0.05, ** p<0.01, ***p<0.001, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Error bars are ±SD.*

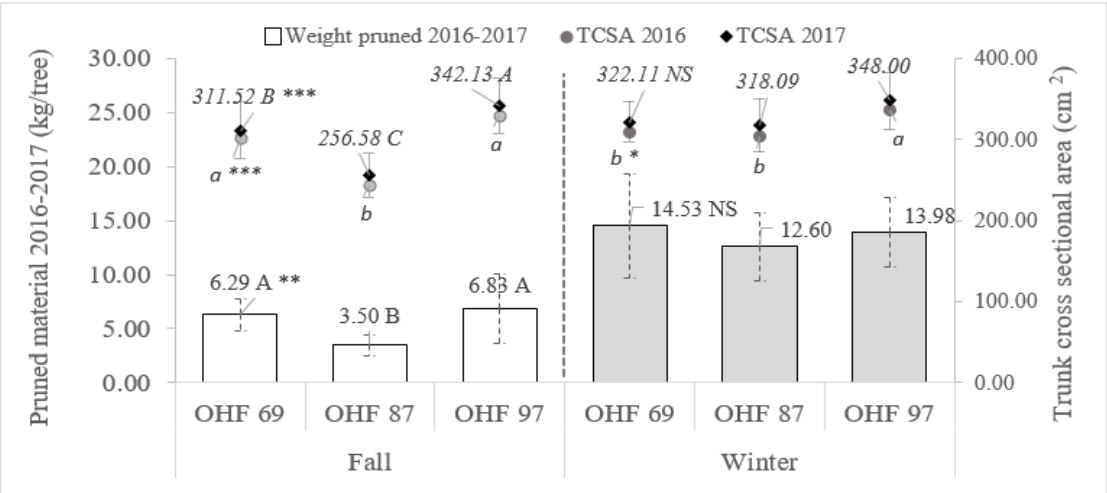

*Figure 2: Comparison between weight of wood (and leaves, fall only) removed per tree (kg) and trunk cross sectional area (TCSA cm^2) in 2016-2017 for each rootstock by pruning treatment. Significance: * p<0.05, ** p<0.01, ***p<0.001, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Error bars are ±SD.*

Trunks of winter pruned trees were significantly larger than fall pruned trees for all rootstocks and, regardless of pruning time, OHF97 trunks were the largest and OHF87 were the smallest. Fall pruning-OHF87 was significantly lower than all of the other combinations, while no difference between the three rootstock in the winter pruning for TCSA 2017 (Fig. 2).

Figure 3 describes all the pruning treatments performed in this trial from 2014 to 2017 reported as production years 1 to 4.

Pruning treatment and rootstock did not have a significant impact on average flower bud counts per m^3. In 2016, fall pruned trees reported 25 flower buds/m^3 while winter pruned had 21 flower buds/m^3, the resulting difference was not statistically significant. No difference between pruning treatments was reported for 2017 either. A generally lower amount of buds were counted in 2017 compared to 2016 (range 8.5-10.5 buds/m^3). Also in 2016, we noticed a general reduction in flower buds/m^3 in comparison to 2015, when they were 32 and 25 buds/m^3 for Fall+summer and winter pruned

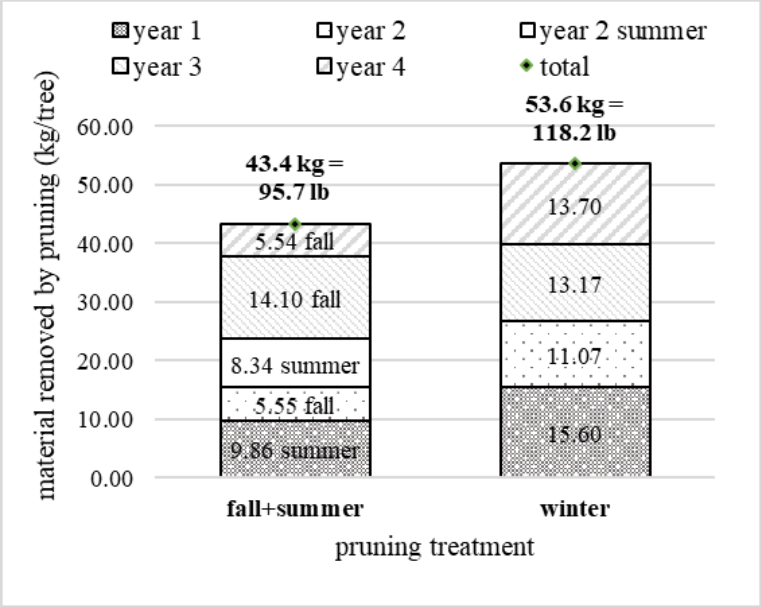

Figure 3: Pruning history of the experiment in 4 years by pruning treatments.

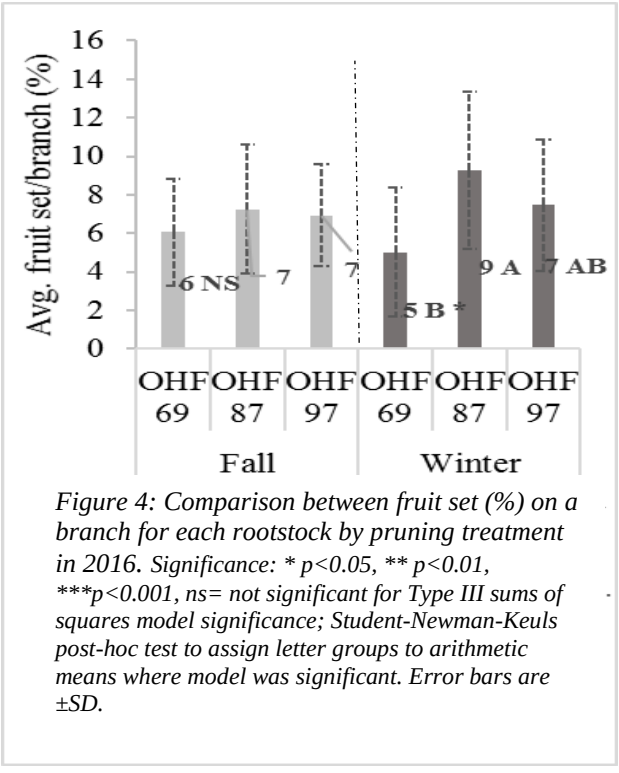

*Figure 4: Comparison between fruit set (%) on a branch for each rootstock by pruning treatment in 2016. Significance: * p<0.05, ** p<0.01, ***p<0.001, ns= not significant for Type III sums of squares model significance; Student-Newman-Keuls post-hoc test to assign letter groups to arithmetic means where model was significant. Error bars are ±SD.*

trees, respectively (difference not significant in 2015 as well). Also in the interaction means (pruning time x rootstock), there was no significant difference in number of flower buds/ m^3 in 2016 and 2017 (data not shown).

The fruit set (percentage of total flowers that set to fruit) per branch count showed no differences between pruning time, while significant differences were found between rootstocks in 2016. OHF87 had the highest percentage of fruit set per branch and OHF69 had the lowest when considering both pruning treatments ($p<0.05$). This difference is due to the behavior of the rootstocks in the winter treatment because there was no significant difference in fall (Fig. 4). OHF87 winter pruned trees had 1.8 times higher percentage of fruit set than OHF69 (Fig. 4).

Yield 2016 and 2017

The pre-harvest fruit ripening assessment by DA meter (I_{AD}=index of absorbance difference, indirect estimation of fruit ripening) in 2016 on OHF87 rootstock and both pruning treatments one week before harvest revealed that the majority of fruit (approx. 39%) was classified as $2.00<I_{AD}<2.09$ for both treatments, while fall pruned trees seemed to have riper fruit in $1.90< I_{AD}<1.99$ than fruit on winter-pruned trees. This behavior is opposite to that observed in the previous two years (Fig. 5A). In 2017, knowing it was a late season, the pre-harvest assessment was done on August 28th and it revealed a general delay in maturity approx. 46% of the fruit were classified as $2.00<I_{AD}<2.09$ for both treatments (Fig. 5B). The harvest in 2017 was done two weeks later than the assessment.

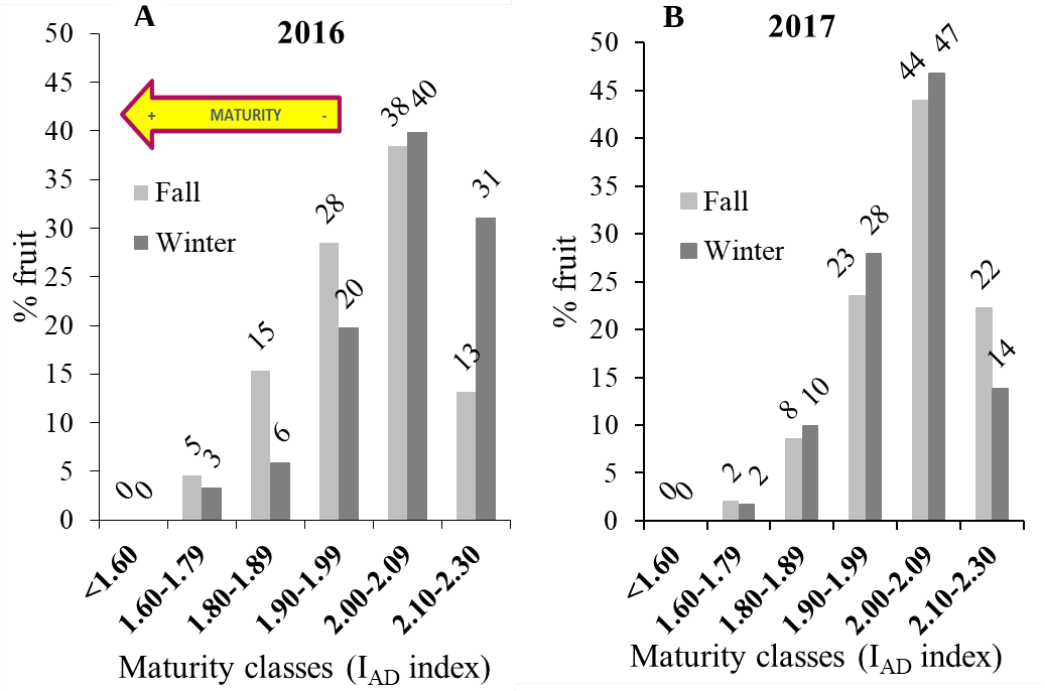

Figure 5: Fruit distribution in I_{AD} classes (indirect maturity assessment) one-two week before harvest in fall and winter pruned trees in 2016 (A) and 2017 (B).

Yield in 2016 had significantly more and heavier fruit from trees pruned in the winter than those in the fall (Table 1). The difference between treatments was around 35 lb/tree or 71 fruit/tree (Table 1). The average fruit weight for winter pruned trees was 7 g higher than fall pruned trees and they were commercially sized between 90-100 fruit/box and 100-110 fruit/box, respectively (Fig. 5A). Winter pruned trees had significantly higher yield efficiencies, crop loads, but more fruit with sunburn and cork than trees pruned in the fall, as in 2015. No frost damage was detected in 2016. There was no significant difference between the three rootstocks for productivity, average fruit weight, and incidence of sunburn and cork. However, OHF97 had significantly lower yield efficiencies and crop loads than the less vigorous rootstocks (Table 1).

In contrast to 2016, the 2017 Fall treatment produced significantly more fruit with a higher yield/tree and higher yield efficiency and crop load than winter pruned trees (Table 2). The difference between treatments averaged 16 lb/tree or 42 fruit/tree (Table 2). The average fruit weight for winter pruned trees was only 6 g higher than fall-pruned trees. Pears harvested in 2017 were commercially sized between 80-90 fruit/box (Fig. 6B). No frost damage was detected in 2017. Cork and sunburn were negligible in 2017, and no significant treatment differences occurred (Table 2). Among the three rootstocks, OHF87 produced more

fruit with a higher yield/tree, yield efficiency and crop load than the others, although average fruit weight did not differ, ranging between 209 and 217 g (Table 2). Sunburn and cork incidences did not show any significant differences between rootstocks. From significance in the interaction between pruning treatment and rootstock we noticed that all the significance between rootstock was only confirmed within the fall pruning, while the three rootstocks performed the same if pruned with winter pruning. This lack of significance between means in pruning could also suggest a higher variability of those trees that hid differences between rootstocks.

Table 1: Anjou yield and disorders in Cashmere, WA in August 2016.

Treatment	Count fruit /tree		Net yield (lb /tree)		Fruit weight (g)		Yield efficiency (lb /TCSA)		Crop load (num. fruit /TCSA)		Sunburned fruit (%)		Fruit with cork (%)	
Pruning season														
Fall	251	**B**	108.8	**B**	198	**B**	0.38	**B**	0.88	**B**	0.74	**B**	0.08	**B**
Winter	322	**A**	143.6	**A**	205	**A**	0.46	**A**	1.04	**A**	1.77	**A**	0.20	**A**
Significance	***		***		*		**		*		***		*	
Rootstock														
OHF69	295		131.1		205		0.43	**A**	0.98	**AB**	0.87		0.16	
OHF87	294		129.5		201		0.47	**A**	1.08	**A**	1.62		0.03	
OHF97	269		118.1		199		0.36	**B**	0.82	**B**	1.22		0.24	
Significance	NS		**NS**		**NS**		**		*		NS		NS	
Signif. Prun.XRoot.	**NS**		**NS**		**NS**		**NS**		**NS**		**NS**		**NS**	

*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.

Table 2: Anjou yield and disorders in Cashmere, WA in September 2017.

treatment - 2017	Count fruit/tree		Net yield (lb)		Fruit weight (g)		Yield efficiency (lb/cm^2 TCSA)		Crop load (num. fruit/TCSA)		Sunburned fruit (%)	Fruit with cork (%)
Pruning season												
Fall	322	**A**	149	**A**	211	**B**	0.51	**A**	1.12	**A**	0.01	0.90
Winter	280	**B**	133	**B**	217	**A**	0.41	**B**	0.87	**B**	0.01	1.27
Significance	**		*		*		**		**		**NS**	**NS**
Rootstock												
OHF 69	282	**B**	133	**B**	216.04		0.43	**B**	0.90	**B**	0.00	1.39
OHF 87	344	**A**	157	**A**	209.14		0.57	**A**	1.25	**A**	0.03	0.75
OHF 97	278	**B**	133	**B**	216.82		0.39	**B**	0.82	**B**	0.00	1.11
Significance	**		**		**NS**		***		***		**NS**	**NS**
Sign. pruning x root.	*		**		**NS**		***		***		**NS**	**NS**

*p<0.05= *, p<0.01=**, p<0.001= ***, NS = not significant for Type III sums of squares model significance. Student-Newman-Keils post-hoc test to assign letter groups to arithmetc means where model was significant.*

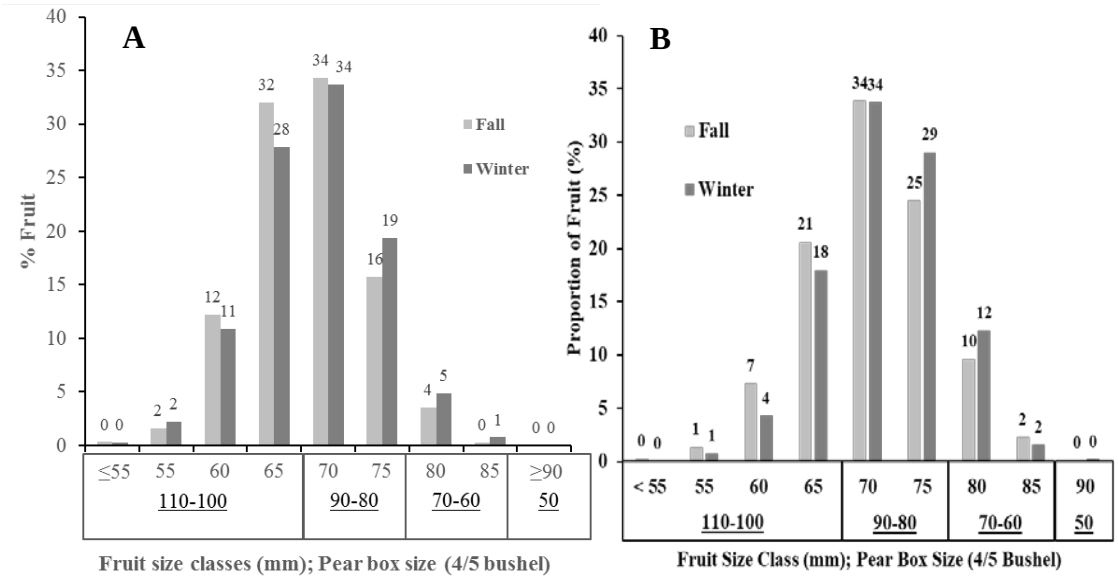

Figure 6: Fruit size distribution (in mm diameter) for fall and winter pruning at harvest 2016 (A) and 2017 (B). Correspondence in 4/5 bushel pear box underlined below diameters in mm.

Fruit quality (harvest 2015)

Fruit from 2015 harvest on OHF87 rootstock had differences in post-storage quality between pruning treatments. After 5 months, fruits from the winter pruning treatment ripened significantly faster (according to the I_{AD} drop) and had a lower firmness than fall+summer pruned trees (Table 3). Winter fruits also lost significantly more weight and ripened faster after 7 days of ripening than fall+summer fruits after 7 months of storage. At harvest, fruits from both treatments were similar in hue (color) and chroma (shade), but fall+summer pruned fruit were significantly greener color after 7 months of storage than winter fruit (Table 3). At harvest fruits from both treatments were similar in firmness, but fall+summer fruits were significantly firmer after 5 months of storage than the winter fruit and the trend continued (although not significant) in the 7th month pullout. At harvest, fall+summer pruned trees had significantly more soluble solid content (SSC) than winter, but after storage there was no significant difference among the treatments (data not shown). At harvest and after 5 months, fall+summer fruits showed lower titratable acid (TA, $p<0.05$) than winter fruits and after 5 months, higher pH than winter fruit. Incidence of cork was similar at harvest among the pruning treatments, but winter fruit had more cork after 5 and 7 months of storage than fall+summer fruit. The I_{AD} ripening classes were distinguished at harvest and the ripest class in both treatments ripened the most and was the most yellow after 5 and 7 months in storage. The opposite was observed for the most unripe class. At 5 months for both treatments, the ripest class (Z) was the least firm, had the highest SSC, and winter only had the highest percentage of dry matter. At 7 months considering both treatments, the second and third ripest classes (B, C) was least firm and classes A and B had the highest dry matter %.

Samples of pear flesh tissue from T1 and T2 (harvest 2015) were analyzed for calcium, nitrogen, and other macro and micronutrients and there were no significant differences between winter and fall+summer pruned fruit except for a higher percentage of potassium (K%) in winter fruit than fall (data not shown).

Table 3: Fruit quality parameters (Anjou/OHF87 fruit harvested in 2015 and stored up to 7 months) T1 =5 months of storage, and T2= 7 months of storage on quality.

Storage 2015	Treatment	Weight drop (g) after storage	Weight drop (g) after 7 days of ripening + storage	IAD index drop after storage	IAD index drop after 7 days of ripening + storage	Color parameter: hue	Color parameter: chroma	Firmness (lb) avr of 2 faces	SSC (Brix)	pH	Titr. Acidity (% malic ac.)
5 months (T1)	Fall +sum pr.	5.7	7.2	0.28 B	0.19	108.5	41.9 B	7.82 A	14.2	3.89 A	0.26
	Winter pr.	5.9	7.5	0.32 A	0.21	107.6	42.8 A	6.49 B	14.3	3.73 B	0.27
	Significance	NS	NS (5.3)	**	NS	NS	***	***	NS	***	NS
7 month (T2)	Fall +sum pr.	7.0 B	8.4 B	0.47 B	0.41 B	105.8 A	42.6	4.27	14.4	3.66	0.20
	Winter pr.	8.0 A	9.0 A	0.52 A	0.46 A	104.2 B	42.2	3.79	14.1	3.68	0.22
	Significance	***	**	*	**	***	NS (5.2)	NS (5.2)	NS	NS	NS

*Pr = pruning 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.

Psylla and Mite Densities

Overwintering psylla adult densities were high in 2016 before insecticide applications were made; however, they were low throughout the rest of the season. No pre-treatment counts were made in 2017, but post-treatment adult counts indicated low densities throughout the season until September, when populations began to rise again.

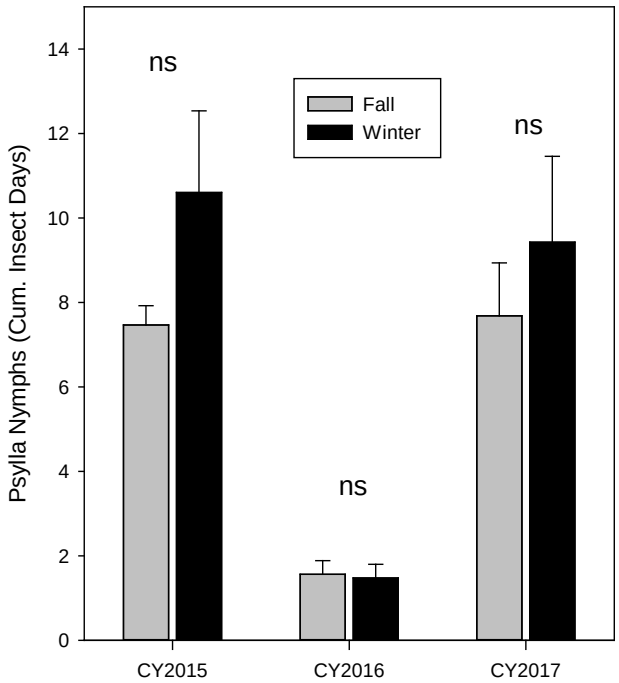

Fig. 7. Seasonal cumulative insect days for psylla nymphs, 2015-2017 (mixed model ANOVA with Tukey mean separation, P<0.05).

Leaf counts of insect densities (psylla and mites) indicated low populations in 2016 and 2017, with no significant treatment, rootstock, or interaction differences among means. The only exception was the psylla nymphs in 2017, which peaked in mid-July. Overall, densities of psylla nymphs was not different between pruning treatments (Fig. 7).

Fruit damage from psylla was moderate in 2015 and 2016; however, in a very high pressure year, 2017, it increased ca. 40% in the highest treatment (winter-pruned), which was significantly higher than the fall+summer pruned treatments (Fig. 8). This is the first indication in this experiment that the fall+summer pruning regime, presumably with lower vigor, may have promoted lower psylla populations. While some (non-significant) variations occurred in lepidopteran damage (codling moth and surface feeding), these are difficult to attribute to the pruning regime, except perhaps through improved coverage in the fall+summer where greater light penetration may correlate with greater spray penetration.

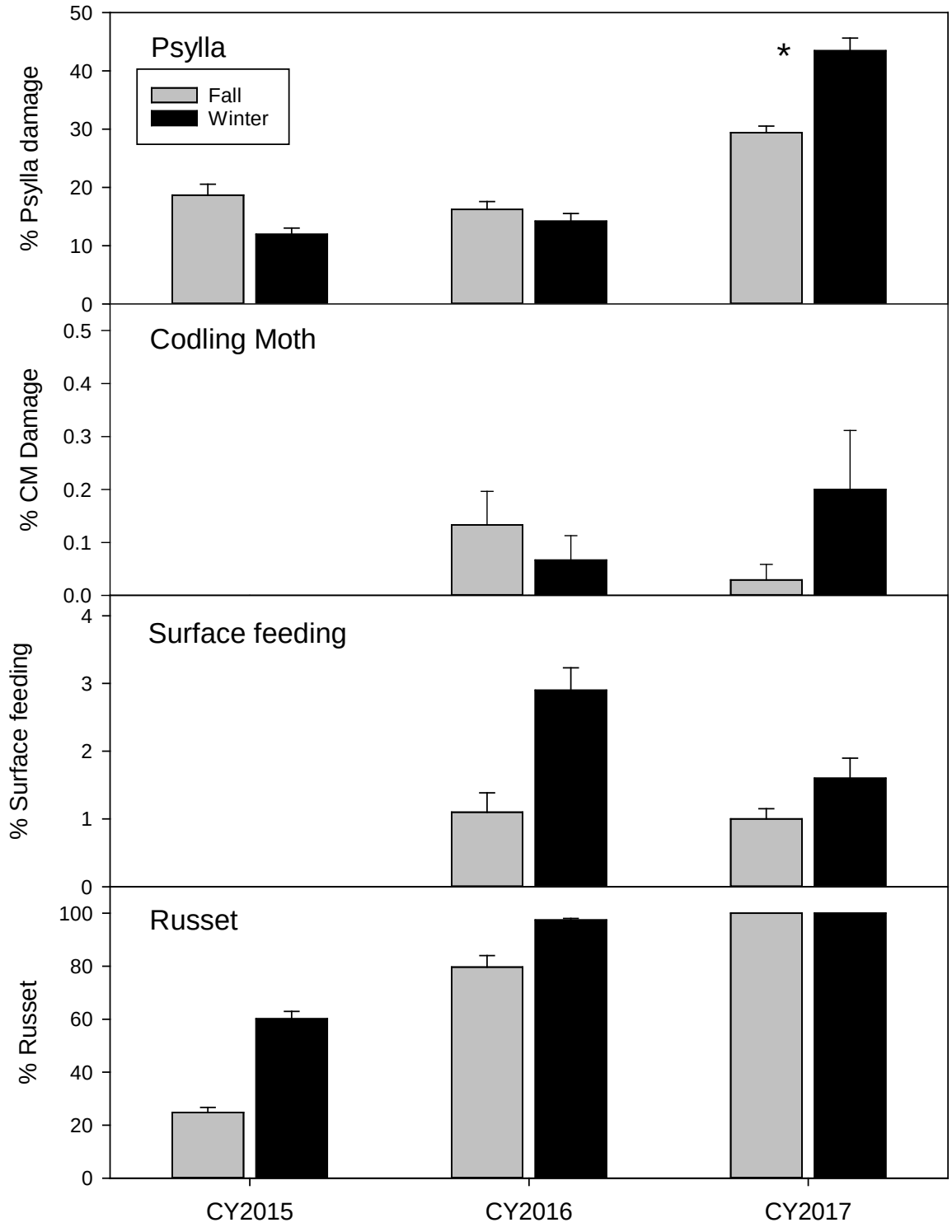

Fig. 8. Fruit damage from pear pests in two pruning timing treatments, 2015-2017. Differences between means indicated by an asterisk (*)(mixed model ANOVA with Tukey mean separation, P<0.05).

EXECUTIVE SUMMARY

Controlling vigor in Anjou pear trees is still an ongoing challenge in Washington pear orchards. The tree vigor depends on many factors like cultivar, rootstock, nitrogen fertilization, and pruning/training systems. The cultivar 'Anjou' is inherently more vigorous than other cultivars, notably 'Bartlett', which produces a smaller, more manageable tree. Dwarfing rootstocks, largely adopted in the apple industry are not utilized in pear orchards. The only available dwarfing rootstock for pear are quince genotypes, but they are seldom planted due to the poor winter hardiness and compatibility issues.
Vigor is also the main driver of higher pear psylla populations, one of the key pests of pear. This phloem-feeding pest thrives on high nitrogen levels, driving up its reproductive capacity. In some regions of the state, this pest threatens crop yield and quality annually, despite intensive pesticide control programs. The presence of honeydew at harvest also discourages pickers from working in pear orchards.
In this project we aimed to achieve the best possible horticultural and entomological outcomes to control vigor, limit psylla and maintain fruit quality.
The trial was carried out in an 'Anjou' orchard planted in 1998 (Cashmere, WA) on three different rootstocks: Old Home x Farmingdale OHxF97, OHxF69 and OHxF87. OHxF97 is considered a vigorous rootstock in comparison with the other two (semi-vigorous). Specifically, we proposed to alter pruning management (fall and summer pruning versus the current standard winter pruning) to reduce tree vigor while maintaining yield and quality (including cork spot). After 4 years, trees pruned with fall (+summer) technique showed a better light penetration and a more homogeneous fruit bud distribution in the canopy that reflected in a higher yield per tree and yield efficiency with fruit just slightly smaller than winter pruning, but no significant difference in the main quality traits. In the fourth year only, fruit damage by pear psylla was lower in the fall (+summer) pruned trees, an indication of vigor reduction by this pruning regime.

Project outcomes:

- **Field days**
  Anjou and Bartlett pruning, January 10, 2017 Tonasket (S. Musacchi)
  The young growers pruning tour, March 3, 2016 Cashmere/Monitor (S. Musacchi)
- **Video**
  2017 https://www.youtube.com/watch?v=Iykwa4VxFrA&t=14s. How to use the Click Pruning Method with Stefano Musacchi - Hort Show, 2016. Published on Jan. 23, 2017 (2,180 views).
  2015 https://www.youtube.com/watch?v=5h5aQ5DwYOo. Pruning Bartlett Pear to Optimize Fruit Quality. Published on Feb. 17, 2015. (44,408 views).
- **Web articles**
  http://www.goodfruit.com/understanding-the-click-pruning-technique-video/
  http://www.goodfruit.com/dynamic-pruning-keeps-trees-productive/
  https://www.youtube.com/watch?v=5h5aQ5DwYOo
- **Professional presentations/conferences**
  Musacchi S., Serra S. and Mattheis J. "Fall and summer pruning to control vigor in d'Anjou pear" (oral presentation by Musacchi S.). XI International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems, Bologna, Italy (August 2016).

Future direction:

- how pruning impacts dry matter accumulation in pear fruit
- moving toward a high density-fruit wall-machine friendly pear orchard on dwarfing rootstocks.

FINAL PROJECT REPORT

Project Title: Molecular gut content analysis to pinpoint where psylla overwinter

PI:	W. Rodney Cooper	**Co-PI**:	David Horton
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Other funding sources: None

Total Project Funding: $29,000

Budget History:

Item	Year 1: 2016	Year 2: 2017
WTFRC expenses		
Salaries	$7500	
Benefits	$2500	
Wages		
Benefits		
Equipment		
Supplies	$17,500	
Travel		
Plot Fees	$1500	
Miscellaneous		
Total	$29,000	No-cost extension

OBJECTIVES

1. Design PCR primers to detect shelter plant DNA.
2. Determine the number of sequences required to identify previous shelter hosts.
3. Determine how long the plant DNA signal persists in winterform psylla.
4. Develop and test flight interception traps that (a) effectively trap returning psylla before they have entered the orchard and fed extensively on pear, but (b) do not compromise the plant (DNA) signal within the guts of those returning insects.

SIGNIFICANT FINDINGS

1. Verification that pear psylla do indeed feed on multiple species of shelter plants
2. Successful development of methods for direct sequencing
3. Evidence that specimens from sticky traps may degrade too extensively to provide consistent sequencing results; tests with a preservative-filled trap (developed for citrus psyllid) show promise in collecting dispersing winterforms.
4. Several hundred specimens of winterform psylla from multiple habitats, with and without known dietary history, collected and stored for assay.

RESULTS AND DISCUSSION

Many winterform pear psylla disperse from pear orchards beginning in early- to mid-September following leaf fall in pear, and colonize a wide-variety of shelter plants including conifer and deciduous windbreaks and other fruit tree orchards such as peach, apple, or cherry (Kaloostian 1970, Fye 1982, Horton et al. 1994a). Psylla adults begin returning to pear orchards in late February and March. Although dispersal of winterform psylla from pear orchards is well documented, it is not known what shelter habitats are preferred by dispersing psylla, or what proportion of the winterform population remains in pear. Since habitats surrounding orchards can vary by location, better knowledge of winterform dispersal and use of shelter plants could improve predictions of which orchards or regions within orchards are most at risk of colonization by overwintered pear psylla.

Technology to investigate landscape-level movements of pear psylla are not currently available. We previously developed a PCR-based method to identify dietary history of the potato psyllid, *Bactericera cockerelli* (Cooper and Horton 2016). This method mimics aspects of molecular gut content analyses of insect predators (Harwood and Obrycki 2005). Although psyllids primarily feed on phloem contents that presumably lack plant DNA, nearly 40% of the time spent stylet-probing involves contact with non-vascular tissues including DNA-containing parenchyma cells (Civolani et al. 2011, Sandanayaka et al. 2014). Potato psyllid apparently acquires plant DNA during these stylet penetrations within parenchyma tissues.

Feeding behavior of winterform pear psylla is poorly understood, but published and preliminary results indicate that winterform pear psylla likely obtain water from shelter hosts. Horton et al. (1994b) reported that winterform psylla caged on shelter plants during the winter survived, but psylla confined to dead pear limbs died confirming the need for a moisture source. Also, dispersing winterform pear psylla are known vectors of the pathogen that causes peach yellow leaf roll disease in peach (Purcell and Suslow 1985, Blomquist and Kirkpatrick 2002). This disease is caused by a phloem-limited bacterium that is transmitted to peach when the insect feeds and salivates. It seems possible that winterform psylla may acquire shelter plant DNA during the stylet-probing activities. Acquisition of shelter plant DNA would allow us to identify which shelter plants pear psylla had previously visited and fed upon.

Our goal was to adapt methods that we developed for analyzing gut contents of potato psyllid (Cooper et al. 2016) to identify plant species that are fed upon by wintering pear psylla. The technology would allow us basically to look back in time at the winter diet of psylla that are captured and assayed weeks later as they return to the orchard. While the basic premise of this technique is simple – amplify plant DNA from psylla using PCR, clone PCR products into bacteria vectors, sequence PCR products to

identify plants that had been visited by the insect (Figure 1) – many challenges remained in the development of this technology: (1) designing PCR primers that efficiently amplify short but variable regions of chloroplast DNA from a wide-variety of possible shelter plants, (2) establishing the minimum number of sequenced clones required to identify the most recent shelter plants visited by any given insect, (3) determining how long chloroplast DNA persists in living pear psylla, and (4) developing flight interception traps that capture returning psylla but that will not complicate DNA extraction or the detection of the plant signal in captured insects

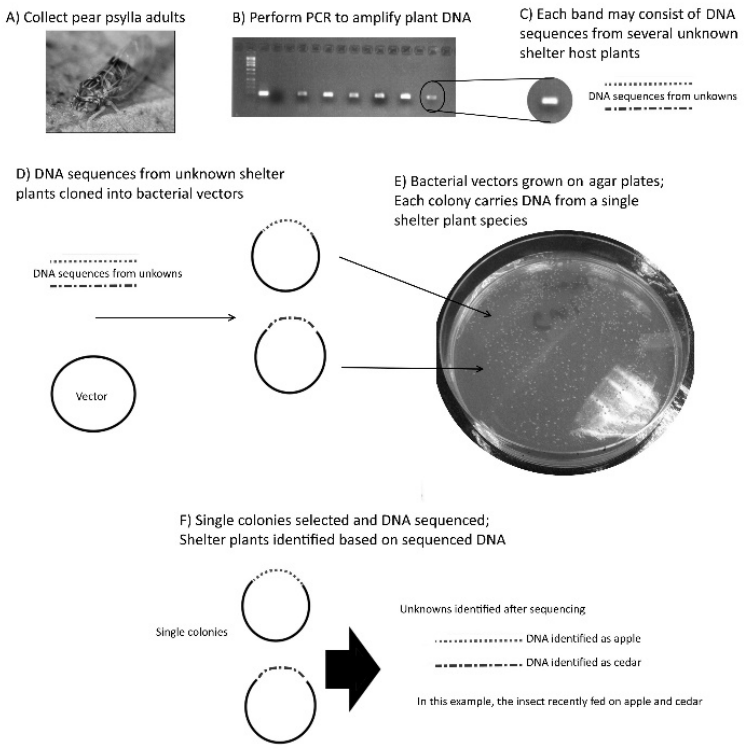


Figure 1. Basic process for identifying dietary history of psyllids using Sanger-based sequencing.

Objective 1: Design PCR primers to detect shelter plant DNA

Our previously published primers for chloroplast DNA (Cooper et al. 2016) amplify sequences from plants within the Solanaceae with high efficiency, but do not adequately amplify sequences from other plant Families. Several other universal primer sets were tested, but most did not consistently amplify plant DNA from psylla. We identified primers which consistently amplify 400-500 bp regions of the host chloroplast genes, *trnL* and *trnF*, from pear psylla. Both regions of chloroplast are highly variable and are suitable for identifying host plants to Family, and in many cases to genus or species. These primers were used for PacBio sequencing (Obj. 2).

Objective 2: Determine the number of sequences required to identify previous shelter plants

Studies in Year 1 indicated that a psylla visited a large number of shelter hosts or feeding hosts. The large number of sequences required to fully assess the dietary history of winterform pear psylla would be cost prohibitive using our previously described methods involving cloning and sequencing PCR products (Figure 1). We requested a no-cost extension for 2017 to explore methods that would reduce costs and improve our ability to detect plant DNA signals.

Following discussions with other researchers and managers of University CORE facilities, we concluded that direct sequencing of PCR products using a PacBio system at the WSU CORE facility in Pullman, WA would be cost-effect and provide a large sequence database to identify dietary history of psylla. This process includes DNA extraction from psylla and PCR using barcoded primers to amplify plant DNA in the guts of psylla. The primer barcodes allow us to identify which samples the sequences belong to after sequencing. Products from all samples are pooled and shipped to the CORE facility at WSU where they are processed for direct sequencing. The resulting dataset is far more extensive that could be achieved by cloning and sequences (Figure 1).

During winter and spring of 2016/2017, we collected a large number of winterform psylla having known and unknown dietary histories (Table 1). An initial experiment was designed using PacBio direct sequencing to 1) determine whether direct sequencing was suitable for gut content analysis of pear psylla, 2) examine to what extent psylla feed on non-pear plants, and 3) determine whether capture of psylla on sticky traps leads to unacceptable levels of DNA degradation (Objective 4).

Pear psylla were collected in November of 2016 from a pear orchard located at the USDA research farm near Moxee, WA (Figure 2A). Sequencing results indicated that psylla within these collections included specimens that had fed upon one or more of the following: pear, apple, Juniper, *Salix*, *Solanum*, pine, or plants within the Asteraceae. The detection of pear sequences was expected because psylla were collected directly from the canopies of pear trees. Windbreaks composed of Juniper and *Salix* are located to the west of the orchard, and an apple orchard is located to the east of the orchard (Figure 2A). Potato was planted to the north of the orchard between the pear orchard and the Juniper windbreak (Figure 2A). Pine trees are also located on the farm. Asteraceae is a large plant family that includes many weed species located on the orchard floor. The results indicate that some winterform psylla present in pear orchards in November had at one time left the orchard and fed upon trees and plants outside of the orchard, and then subsequently returned to the orchard where they were collected (in November). This behavior by winterform psylla has not previously been documented. The presence of Asteraceae sequences in winterform psylla suggests that the insects also fed upon annual weeds located on the orchard floor. These feeding events may have occurred as psylla were displaced from trees by autumn leaf-fall, suggesting that psylla which have dropped from the canopy (either associated with leaf fall or voluntarily), will 'drink' from herbaceous weeds before returning to the pear canopy.

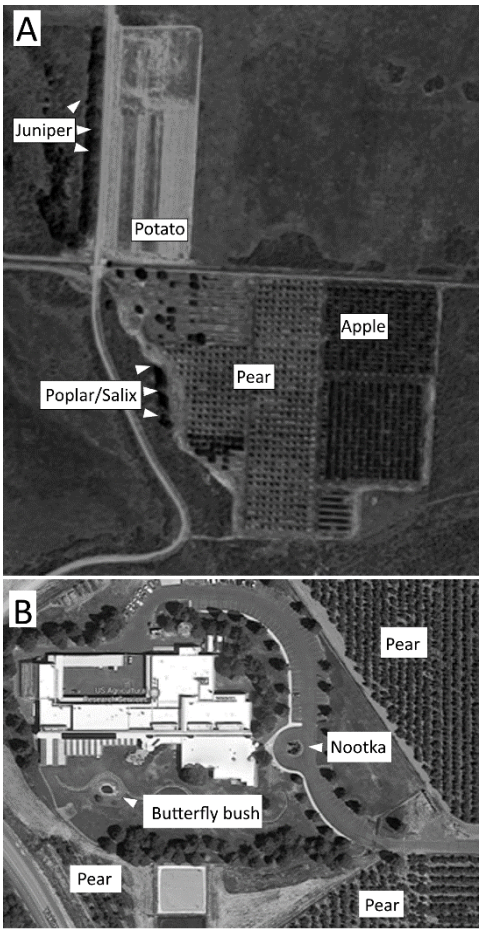


Figure 2. Winterform pear psylla were collected in November 2016 from a pear orchard located at the USDA experimental farm near Moxee, WA (A) and from weeping Nootka located near the ARS laboratory in Wapato, WA (B).

Pear psylla were also collected from Weeping Nootka (an ornamental conifer) located near the ARS laboratory in Wapato, WA in November of 2016 (Figure 2B). Sequences identified from these psylla included Juniper and butterfly bush, which are both planted on the grounds of the Wapato lab. Although the presence of butterfly bush was not known to Cooper before identification of sequences, Horton has seen exceptionally large populations of winterform pear psylla accumulating on this large bush during leaf-fall in pear, followed by disappearance from the bush as the ornamental in turn drops its leaves in late autumn. Our sequence results indicate that many psylla migrated from pear to butterfly bush, then migrated to weeping Nootka from where they were collected for gut content analysis.

Our results confirm that winterform psylla feed from non-host shelter plants, and that direct sequencing using the PacBio platform provides a cost- and labor-effective method for gut content analysis of pear psylla. Results of our pilot study also reveal compelling patterns in autumn migrations of diapausing winterform pear psylla. We have collected and stored a large number of pear psylla with known and unknown dietary histories, and will continue to add to this collection (Table 1). These psylla will be used during the spring of 2018 for a more comprehensive study of shelter plant use by winterform pear psylla using methods developed from this industry-funded pilot study.

Objective 3: Determine how long the plant DNA signal persists in winterform psylla

Pear psylla were collected from Juniper and confined to pear shoots using sleeve cages every two weeks from 20-January 2017 to 3-March 2017. Psylla were also collected directly from Juniper when psylla were retrieved from the sleeve cages on 17-March. Dietary history of psylla was assessed using methods described in Figure 1. The Juniper DNA signal was detected in psylla that were moved from Juniper to pear on 3-March indicating that the Juniper signal persisted in psylla confined to pear for at least 2 weeks. DNA other than pear, including maple and several perennial weed species, were detected in psylla moved from Juniper to pear in February and January. These same weed species were also detected in psylla collected directly from Juniper on 17-March. These results confirm our previous finding with potato psyllid that the plant DNA signal persists in psyllids for an extended period of time. We anticipate the length of time in which the plant DNA signal persists in psyllids to be dependent upon temperature, and to therefore be substantially shorter in psylla collected later in the year when temperatures rise.

Objective 4: Develop and test flight interception traps that (a) effectively trap returning psylla before they have entered the orchard and fed extensively on pear, but (b) do not compromise the plant (DNA) signal within the guts of those returning insects.

Psylla were collected by cooperator Louis Nottingham from yellow sticky traps placed on the perimeter of pear orchards near Wenatchee WA during the spring re-entry period. Traps were hung for a week, and most of the collected insects were highly desiccated and coated in TangleTrap. We were unable to detect plant DNA from these insects suggesting that the DNA was too highly degraded for gut content analysis.

Efficiency of several alternative interception traps for capture of winterform adults were compared in spring of 2016. Interception traps with low-tack tape were not effective at capturing psylla, and will not be suitable for capturing psylla for gut content analysis. Mesh traps treated with horticultural oil were very effective at capturing psylla, but were messy to work with. Brown and olive green traps developed for citrus psyllid (Figure 3) successfully captured winterform psylla. Because these traps capture psylla directly into preservative, there is no need to remove horticultural oil or sticky trap residue from psylla before DNA extraction. We will continue work this winter and spring with mesh traps and 3D traps, and determine whether trapping methods compromise the plant DNA signal.

Figure 3. Prototype 3D-printed traps that capture winterform pear psylla directly into a preservative.

Conclusions. Our results provide the strongest evidence to date that winterform pear psylla indeed do feed extensively upon multiple species of non-developmental shelter plants, and that PacBio sequencing of plant barcoding genes can be used to identify the sometimes highly complex dietary history of dispersing pear psylla. Our long-term objective is to use molecular gut content analysis along with other landscape-ecology approaches to study the landscape-level movements of winterform psylla. To this end we have collected a large number of winterform pear psylla with known and unknown dietary histories from various locations. These specimens along with psylla collected by collaborators in other pear growing regions of the Pacific Northwest will allow a more comprehensive investigation of shelter plant use by winterform pear psylla. A better understanding of winterform dispersal and overwintering habitats could then enable growers to predict which orchards or

areas within orchards are most at-risk of being colonized by overwintered psylla. This information will also enable researchers to develop and test landscape-level approaches of managing the overwintering population of pear psylla.

Table 1. Winterform psylla were collected mid-November to early-December 2015, 2016 and 2017 from miscellaneous orchard and shelter plants at four locations. Collections (1)-(4): specimens were collected directly from shelter plants and are being used to confirm the utility of our molecular methods for psyllids having a partially known dietary history. Collection (5): dispersing winterforms were collected in mid-November from the side of a house in West Yakima, located some 2 miles from the nearest pear orchard; these specimens will allow us to examine our methods for psylla having an unknown dietary history.

	Numbers of winterforms collected and now in storage (-80 °C)
(1) Known plant sources (Moxee farm; winter 2015-2017)	
Pear orchard, apple orchard, rabbitbrush, sagebrush coniferous windbreak	200+
(2) Known plant sources (West Yakima; Nov-Dec 2016)	
Juniperus windbreak	22
Mixed creekside vegetation (*Rosa, Populus, Salix, Cornus*)	6
Ponderosa pine (*Pinus ponderosa*)	5
Weeping Nootka false cypress (*Chamaecyparis nootkatensis*)	7
Unidentified coniferous	3
Golden currant (*Ribes* sp.)	2
Unknown ornamental fir (*Abies* sp.)	9
Lilac bush (*Syringa vulgaris*)	3
Gold Cone Cedar (*Cedrus deodara*)	28
(3) Known plant sources (YARL-Wapato; Nov-Dec 2017)	
Butterfly bush (*Buddleja* sp.)	41
Unknown ornamental fir (*Abies* sp.)	35
Western Cedar (*Thuja plicata*)	42
Weeping Nootka false cypress (*Chamaecyparis nootkatensis*)	64
Ponderosa pine (*Pinus ponderosa*)	30
Oregon grape (*Mahonia aquifolium*)	39
Rosa	3
(4) Known plant sources (Naches region; Nov-Dec 2016)	
Ponderosa pine (*Pinus ponderosa*)	1
Douglas fir (*Pseudotsuga menziesii*)	4
Western cedar (*Thuja plicata*)	14
Mugo pine (*Pinus mugo*)	9
(5) Unknown dietary history (West Yakima Nov-Dec 2017)	300+
Unknown dietary history (preservative-filled traps to be placed on perimeter of orchards)	

EXECUTIVE SUMMARY

Preventing unacceptably high densities of pear psylla during the growing season requires effective management of the post-winter generation. A factor complicating these efforts is the tendency of winterform psylla to disperse from orchards in autumn and overwinter on non-pear shelter plants. In late winter prior to pear budbreak, psylla leave these shelter plants, return to pear orchards, and begin laying eggs destined to become the first summerform generation. We have a very poor understanding of what habitats are preferred by wintering psylla, other than that plants suitable for maintenance feeding by psylla apparently are necessary, and that many different types of plants can provide the needed resources. Better understanding of this part of psylla's life cycle would help us predict whether a given orchard is likely to receive a large post-winter influx of psylla (i.e., orchards near favorable overwintering habitat) versus a small influx (i.e., orchards surrounded by less-favorable habitat). The objective of our study was to develop methods for gut content analysis to identify the dietary history of winterform pear psylla as they return to the pear orchard in spring.

Summary of Findings. We previously demonstrated that plant DNA can be PCR-amplified from potato psyllid, and that the dietary history of the potato psyllid could be identified by cloning and sequencing the PCR products. Although this method was appropriate as a proof-of-concept, it is not cost- or labor-effective for wild psyllids that potentially feed upon numerous plant species. Our initial studies in 2016 demonstrated that overwintering pear psylla may feed upon a large number of shelter plant species. We therefore requested a no-cost extension in 2017 to examine whether direct sequencing using a PacBio platform would provide suitable and cost-effective data. Psylla were collected in November from a pear orchard near Moxee, WA, and from a coniferous ornamental (weeping Nootka) located on the grounds of the ARS laboratory in Wapato, WA. Sequences from pear, apple, *Salix*, and juniper were identified in psylla collected from Moxee. All plant species that were detected in psylla specimens occur somewhere on the farm-grounds and within dispersal distance from the source pear orchard. In addition, sequences from potato and from weeds within the Asteraceae were identified from these psylla. A potato field was located immediately below the pear orchard, suggesting that specimens of winterform psylla collected in our source pear orchard in November had at some time preceding the November collection date visited this stand of potatoes. This result was completely unexpected. Asteraceae is a large plant family that includes weeds that are common on the orchard floor, and we suggest that the Asteraceae signal in winterforms is evidence that psylla had visited the orchard floor and fed on weedy Asteraceae before being collected from the tree canopy in November. Sequences from butterfly bush and juniper were identified in psylla collected on the grounds of the Wapato laboratory. Large numbers of winterform psylla often can be found on this stand of butterfly bush during leaf drop in pear, but those psylla then disappear from this plant as it loses its leaves in late autumn. The butterfly bush signal was found in late-autumn in psyllids collected from a coniferous ornamental, indicating that the signal was detectable even following movement onto the coniferous shelter host. Collectively, our results demonstrate that winterform pear psylla feed upon and acquire DNA from non-pear shelter plants and that direct sequencing provides quality data useful for identifying dietary history of winterform psylla. Results also reveal insight into patterns of autumn dispersal by winterform pear psylla that would be impossible to demonstrate using other approaches. These results will be useful as we design more broadly ranging studies in the future.

Another goal of our study was to develop a trap that captures pear psylla but does not interfere with our ability to detect plant DNA. DNA isolated from psylla captured on yellow sticky cards was too degraded to amply plant sequences. We evaluated capture of psylla using 3D-printed traps originally designed for monitoring citrus psyllid and adapted for monitoring potato psyllid. Although the traps are not as efficient as yellow sticky cards, they are less messy and capture psylla directly into a preservative that prevents degradation of plant DNA.

Our longer term objectives are to use these methods to examine landscape-level movements and shelter plant use by winterform psylla from pear growing regions occupying any of a range of native habitats (coniferous forest [Wenatchee, Hood River] to native rangeland [Medford, Wapato]).

FINAL REPORT
WTFRC Project 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)

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-17-100

Project Title: Fire blight management: new products and effective rates

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

Cooperators: None

Total Project Request: Year 1: 14,134 **Year 2**: **13,812** **Year 3**: 14,256

Other funding sources

Agency Name: Industry Gift Grants
Amt. : $1,500 per product/rate screened.
Notes: For screening of individual new products. Does not include multiple rates or individual products proposed here.

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

Item	2017	**2018**	2019
Salaries[1]	7,800	8,112	8,436
Benefits[2]	2,884	3,000	3,120
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	950	200	200
Travel[4]	500	500	500
Miscellaneous	0	0	0
Plot Fees[5]	$2,000	$2,000	$2,000
Total	14,134	**13,812**	14,256

Footnotes:
[1]Salary for one technician at $3,900 per month for two months.
[2] Benefits at 37% for one technician.
[3]Supplies include a new power misting backpack sprayer in year one ($750), and safety and application materials in all years.
[4]925 miles per year for travel to research plots, to organize project and present results.
[5]Plot fees included here are for a pear block at Sunrise Research Orchard for russet trials.

OBJECTIVES

1. Test the efficacy of three commercially available copper and biological products (Cueva, Previsto, Blossom Protect) and one experimental product (Alum) at five rates in order to determine at which rates products are effective. Treatments will be assigned randomly to plots within a randomized complete block and compared to untreated inoculated and untreated non-inoculated controls.
2. Investigate russet potential in order to determine when products are effective with little or no russet risk. Four products will be applied at four rates in a randomized complete block and assessed for russet.
3. Provide research based recommendations to pear producers on appropriate rates for new products.

SIGNIFICANT FINDINGS

- **Blossom Protect with 1.5x the Buffer labeled rate (13 lbs vs 8.75 lbs/100 gal) had a relative control of 77% statistically the same as the oxytet standard, while 4.4 and 8.75 lbs of buffer provided only 44 and 56% control.**
- Cueva at the 5 quart per 100 gallons per acre rate provided a rate of control (72%) not significantly different than the oxytet standard (84%). 1-4 quart rates provided less control (44 to 51%).
- Previsto at 2-4 quarts had levels of control of 74-84%, statistically the same as the oxytet check. The 1 and 5 quart rate performed lower at 50 and 54%.
- Alum provided 67 to 81% relative control. All rates were statistically equivalent to the oxytet and strep standards and statistically better than the untreated control.
- **Regression analysis were not significant and further years of analysis will be needed to support initial conclusions.**

METHODS

Site: A 0.42 acre mature Bartlett & Anjou pear block at WSU Columbia View Orchard Orondo, WA was used for russet evaluations. A two-acre research block of mature Red Delicious & Golden Delicious apples at WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA 98802-8283 was used for the inoculated trial. Soils are a Cashmont Gravely Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.

Plots: Four blocks of 40 trees (apples) and three blocks of 21 trees (pears) were designated (1-2 tree rows each). Individual trees were marked as plots in a randomized complete block where suitable trees were selected based on sufficient bloom (100+ flowers on lower branches).

Inoculum: Ultrafreeze-preserved cultures (−80°C) of the *Erwinia amylovora* 153 (streptomycin sensitive fireblight strain) were grown for 72 hours 28°C in NYDA agar to propagate dormant colonies. Subsequent inoculations were made transferring cultures to fresh NYDA plates every 24 hours to ensure fresh (<48 hrs old) plates.

Cluster Inoculation: Fresh cultures were diluted to $1x10^7$ CFU ml^{-1} and verified using an optical density spectrometer. A 1:9 dilution of the $1x10^7$ CFU ml^{-1} solution was used to obtain $1x10^6$ CFU ml^{-1} solution used in field inoculation. A one-liter sprayer was used to lightly wet each cluster. 100 clusters per plot were inoculated when the blooms were at an average of 100% bloom on the branch. An untreated and un-inoculated check treatment was included. Inoculation was on May 2, 2017.

Treatments: Products were applied by tree to the area of the tree to be inoculated according to manufacturer recommendations (see Table 1) using a Stihl SR420 blow mister backpack sprayer with a wetting agent (Biolink, organic; Regulaid, conventional). Products were applied to wet, near dripping previously calibrated to equal 100 gal/A. 2017 application dates were; April 28 (20% bloom); April 29 (50% bloom); April 30 (80%); May 2 (full bloom); May 9 (Petal fall).

Included in this trial as a comparison and as "treated checks" were FireLine (oxytetracycline 17%) at 1.5 lbs. / 100 gal. / A and FireWall (streptomycin sulfate 17%), at 1.5 lbs. / 100 gal. / A, both antibiotics from AgroSource, Inc., and critical for comparisons as long-term standards). An untreated and inoculated check treatment and an untreated non-inoculated check treatment were included.

RESULTS & DISCUSSION

Blossom Protect: Blossom protect was tested with three levels of buffer and compared to treated and untreated controls (Table 1). This preliminary data shows that a rate 1.5x the labeled rate of buffer (13 lbs vs 8.75 lbs/100 gal) provided increased efficacy. Blossom protect with 13 lbs of buffer had a relative control of 77% statistically the same as the oxytet standard, while 4.4 and 8.75 lbs of buffer provided only 44 and 56% control. However, the regression analysis was not significant and further years of analysis will be needed to support initial conclusions.

Table 1. Blossom Protect with three rates of buffer.

Treatment	Rate per 100 gal H2O		Timing*	Strikes				Infection (%)**				Relative Infection (%)***				Relative Control (%)****			
Firewall 17 standard strep w Tech Mg	28.8 oz	c	50% bloom, 100% bloom, PF	0	±	0	c	0%	±	0%	c	1%	±	1%	c	99%	±	1%	a
Untreated, NOT Inoculated Check	water	na	100% bloom	0	±	0	c	1%	±	1%	c	3%	±	3%	c	98%	±	3%	a
Fireline 17 (standard oxytet) w Tech Mg	24 oz	c	50% bloom, 100% bloom, PF	5	±	2	bc	4%	±	2%	cb	17%	±	7%	bc	84%	±	7%	ab
Blossom Protect + Buffer Pro. (1.5x)	1.25 lb 13 lb	o	20% bloom, 80% bloom	6	±	3	bc	6%	±	2%	cb	24%	±	11%	bc	77%	±	11%	ab
Blossom Protect + Buffer Pro.	1.25 lb 8.75 lb	o	20% bloom, 80% bloom	11	±	4	abc	10%	±	4%	b	44%	±	17%	b	56%	±	17%	b
Blossom Protect + Buffer Pro. (0.5x)	1.25 lb 4.4 lb	o	20% bloom, 80% bloom	12	±	5	ab	13%	±	5%	b	56%	±	19%	b	44%	±	19%	b
Untreated, Inoculated Check	water	na	100% bloom	19	±	8	a	23%	±	5%	a	98%	±	22%	a	2%	±	22%	c

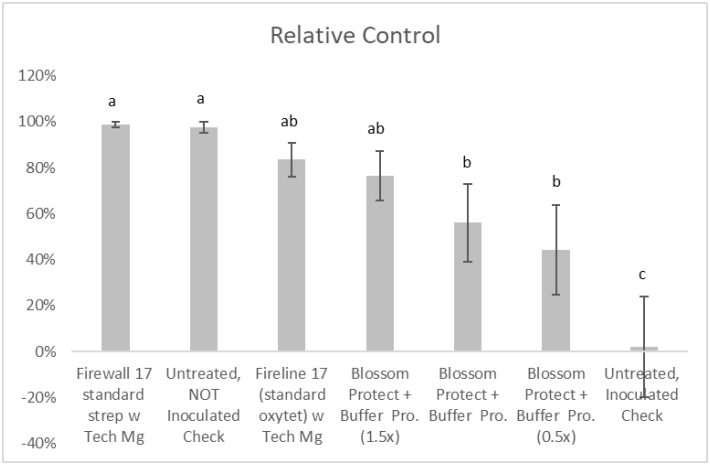


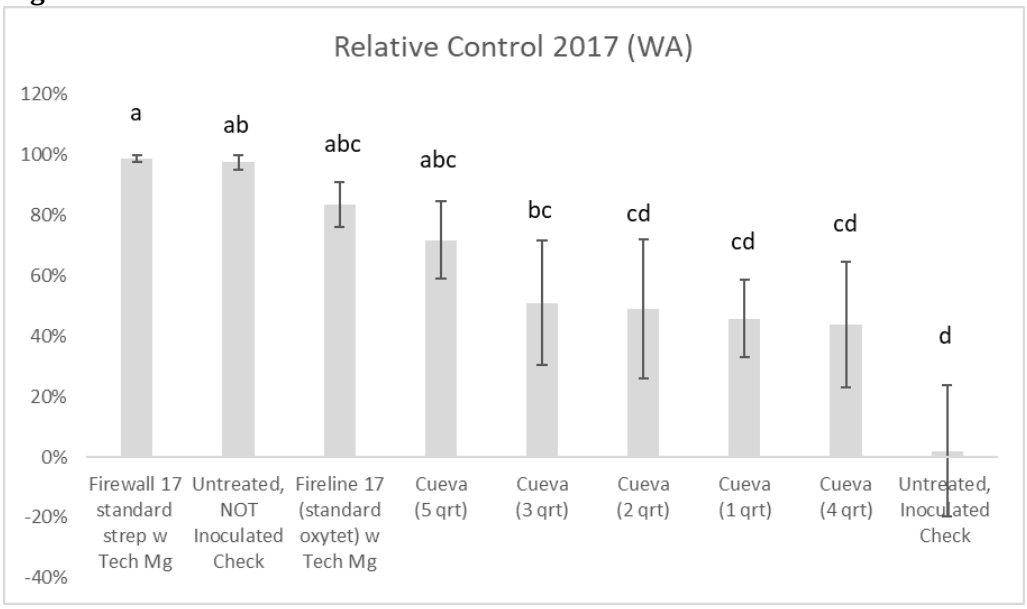
Figure 1. Relative control of Blossom Protect with three rates of buffer

Cueva: Cueva at the 5 quart per 100 gallons per acre rate provided a rate of control (72%) not significantly different than the oxytet standard (84%). 1-4 quart rates provided less control (44 to 51%). **However, the regression analysis was not significant and further years of analysis will be needed to support initial conclusions.**

Table 2: Cueva at five rates.

Treatment	Rate per 100 gal H2O		Timing*	Strikes				Infection (%)**				Relative Infection (%)***				Relative Control (%)****			
standard strep w Tech Mg	28.8 oz	c	50% bloom, 100% bloom, PF	0	±	0	c	0%	±	0%	d	1%	±	1%	d	99%	±	1%	a
Untreated, NOT Inoculated Check	water	na	100% bloom	0	±	0	a	1%	±	1%	a	3%	±	3%	a	98%	±	3%	ab
Fireline 17 (standard oxytet) w Tech Mg	24 oz	c	50% bloom, 100% bloom, PF	5	±	2	bc	4%	±	2%	bcd	17%	±	7%	bcd	84%	±	7%	abc
Cueva	5 qrt	o	day before and day after 100% bloom	5	±	2	bc	7%	±	3%	bcd	28%	±	13%	bcd	72%	±	13%	abc
Cueva	3 qrt	o	day before and day after 100% bloom	10	±	3	bac	11%	±	5%	bc	49%	±	21%	bc	51%	±	21%	bc
Cueva	2 qrt	o	day before and day after 100% bloom	11	±	5	bac	12%	±	5%	b	51%	±	23%	ab	49%	±	23%	dc
Cueva	1 qrt	o	day before and day after 100% bloom	11	±	4	bac	13%	±	3%	ab	54%	±	13%	ab	46%	±	13%	dc
Cueva	4 qrt	o	day before and day after 100% bloom	14	±	4	ba	13%	±	5%	ab	56%	±	21%	ab	44%	±	21%	dc
Untreated, Inoculated Check	water	na	100% bloom	19	±	8	c	23%	±	5%	cd	98%	±	22%	cd	2%	±	22%	d

Figure 2: Cueva at five rates.

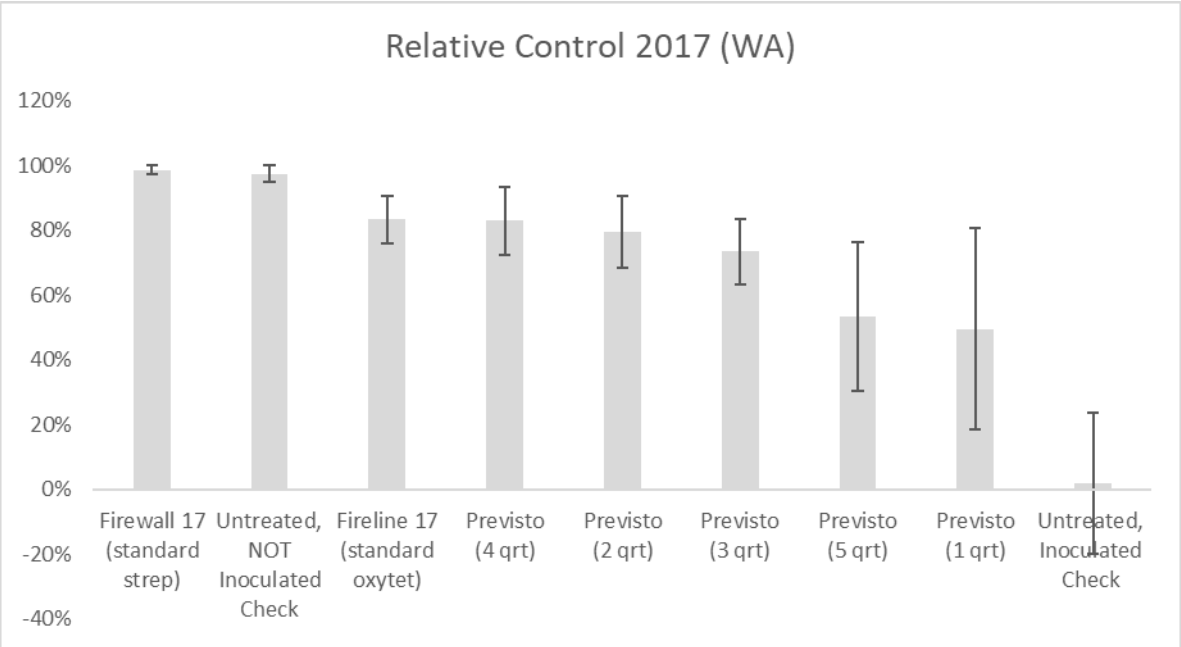


Previsto: Previsto at 2-4 quarts had levels of control of 74-84%, statistically the same as the oxytet check. The 1 and 5 quart rate performed lower at 50 and 54%. **However, the regression analysis was not significant and further years of analysis will be needed to support initial conclusions.**

Table 3: Previsto at 5 rates.

Treatment	Rate per 100 gal H2O		Timing*	Strikes				Infection (%)**				Relative Infection (%)***				Relative Control (%)****			
Firewall 17 standard strep w Tech Mg	28.8 oz	c	50% bloom, 100% bloom, PF	0	±	0	b	0%	±	0%	d	1%	±	1%	d	99%	±	1%	a
Untreated, NOT Inoculated Check	water	na	100% bloom	0	±	0	b	1%	±	1%	cd	3%	±	3%	cd	98%	±	3%	ab
Fireline 17 (standard oxytet) w Tech Mg	24 oz	c	50% bloom, 100% bloom, PF	5	±	2	b	4%	±	2%	bcd	17%	±	7%	bcd	84%	±	7%	abc
Previsto	4 qrt	o	day before and day after 100% bloom	4	±	2	b	4%	±	2%	bcd	17%	±	10%	bcd	83%	±	10%	abc
Previsto	2 qrt	o	day before and day after 100% bloom	5	±	3	b	5%	±	3%	bcd	21%	±	11%	bcd	80%	±	11%	abc
Previsto	3 qrt	o	day before and day after 100% bloom	5	±	1	b	6%	±	2%	bcd	27%	±	10%	bcd	74%	±	10%	abc
Previsto	5 qrt	o	day before and day after 100% bloom	10	±	5	ab	11%	±	5%	bc	47%	±	23%	bc	54%	±	23%	bc
Previsto	1 qrt	o	day before and day after 100% bloom	10	±	6	ab	12%	±	7%	b	50%	±	31%	b	50%	±	31%	c
Untreated, Inoculated Check	water	na	100% bloom	19	±	8	a	23%	±	5%	a	98%	±	22%	a	2%	±	22%	d

Figure 3: Previsto at 5 rates.



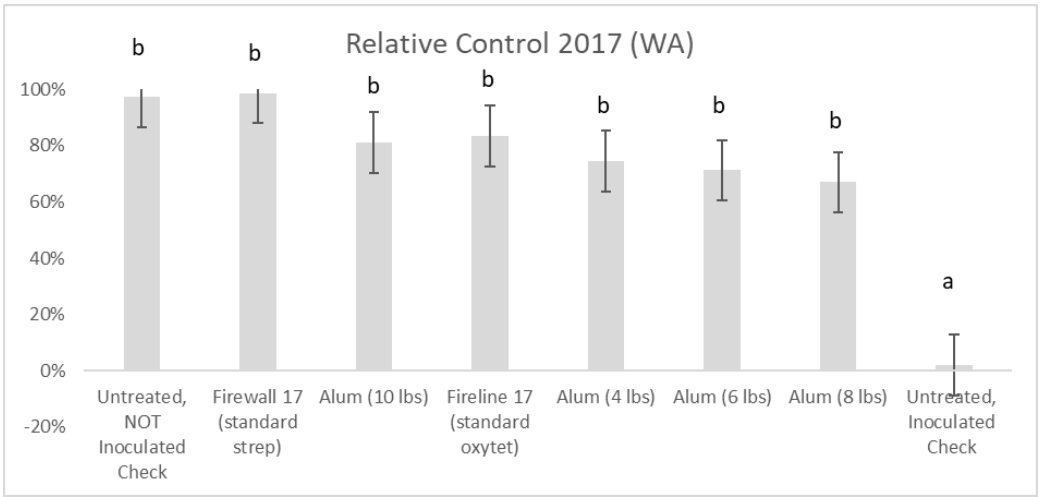
Alum: Alum provided 67 to 81% relative control. All rates were statistically equivalent to the oxytet and strep standards and statistically better than the untreated control. Rates of control were similar to Previsto, higher rates of Cueva and Oxytet. This product shows considerable promise and should be explored further for commercialization.

Table 4. Alum at 4 rates.

Treatment	Rate per 100 gal H2O		Timing*	Strikes				Infection (%)**				Relative Infection (%)***				Relative Control (%)****			
Untreated, NOT Inoculated Check	water	na	100% bloom	0	±	0	b	1%	±	1%	b	3%	±	3%	b	98%	±	3%	b
Firewall 17 standard strep w Tech Mg	28.8 oz	c	50% bloom, 100% bloom, PF	0	±	0	b	0%	±	0%	b	1%	±	1%	b	99%	±	1%	b
Alum (1.25%)	10 lb	o	100% bloom, petal fall	4	±	1	b	5%	±	1%	b	19%	±	3%	b	81%	±	3%	b
Fireline 17 (standard oxytet) w Tech Mg	24 oz	c	50% bloom, 100% bloom, PF	5	±	2	b	4%	±	2%	b	17%	±	7%	b	84%	±	7%	b
Alum (0.5%)	4 lb	o	100% bloom, petal fall	5	±	3	b	6%	±	4%	b	26%	±	15%	b	75%	±	15%	b
Alum (0.75%)	6 lb	o	100% bloom, petal fall	5	±	1	b	7%	±	1%	b	29%	±	6%	b	71%	±	6%	b
Alum 1%	8 lb	o	100% bloom, petal fall	8	±	3	b	8%	±	3%	b	33%	±	14%	b	67%	±	14%	b
Untreated, Inoculated Check	water	na	100% bloom	19	±	8	a	23%	±	5%	a	98%	±	22%	a	2%	±	22%	a

*= % bloom open, FB = Full Bloom, PF = Petal Fall
**Number of blighted clusters per 100 blossom clusters. Trees inoculated on April 11 with 5x10 6 CFU/ml Erwinia amylovora strain Ea153 (streptomycin sensitive fireblight strain)
***Percent infection relative to the inoculated untreated control (23% in 2017). {(%infection*100)/%infection inoculated untreated control*100)}
****Percent control relative to the inoculated untreated control. { 1-(%infection*100)/%infection inoculated untreated control*100)}
Treatments with different letters are significantly different (T LSD). o=Biolink, c=Regulaid

Figure 4. Alum at 4 rates



CONTINUING PROJECT REPORT **YEAR: Year 1**

Project Title: Acoustically based mating disruption of winterform psylla

PI: David Horton
Organization: USDA-ARS
Telephone: (509) 454-5639
Email: david.horton@ars.usda.gov
Address: USDA-ARS
Address 2: 5230 Konnowac Pass Road
City/State/Zip: Wapato, WA 98951

Co-PI (2): Elizabeth Beers
Organization: Washington State University
Telephone: (509) 663-8181
Email: ebeers@wsu.edu
Address:
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI (3): David Crowder
Organization: Washington State University
Telephone: (509) 335-7965
Email: dcrowder@wsu.edu
Address: 166 FSHN Building
Address 2: PO Box 646382
City/State/Zip: Pullman, WA 99164

Total Project Request: Year 1: $52,761 Year 2: $49,733 Year 3: $53,166

Other funding sources: None

Budget 1
Organization Name: WSU Pullman **Contract Administrator: Ben Weller**
Telephone: 509-335-0052 **Email address: grants.fsclark@wsu.edu**

Item	6/1/2017 to 5/31/2018	6/1/2018 to 5/31/2019	6/1/2019 to 5/31/2020
Salaries[1]	$28,417	$29,554	$30,736
Benefits[2]	$2,580	$2,683	$2,791
Wages[3]	$11,040	$11,251	$11,471
Benefits[4]	$1,124	$1,145	$1,168
Equipment			
Supplies[5]	$6,000	$3,000	$3,000
Travel[6]	$3,600	$2,100	$4,000
Miscellaneous			
Plot Fees			
Total	$52,761	**$49,733**	$53,166

Footnotes:
[1] Salary for the PhD student for the academic year
[2] Benefits for the PhD student for the academic year include health insurance and fringe
[3] Wages for the PhD student for the summer; also includes a time-slip employee who will work 40 hours a week for 12 weeks each summer during the project
[4] Fringe benefits for the PhD student and time-slip employee during the non-academic year
[5] Yr 1 – supplies for the laser vibrometer, minishakers, and for conducting the vibrational studies (objective 2). Yrs 2 and 3 - Experimental supplies for Objectives 3 and 4
[6] Yr 1 – Funds will support travel to the USDA-ARS facility in Gainesville, FL. Yrs 2/3 - Vehicle lease through the state motor pool; this vehicle will be used to complete field research objectives

OBJECTIVES

1. Recruit Ph.D. student (co-supervisors E. Beers and D. Crowder). COMPLETED
   a.Student: Visit the ARS laboratory in Gainesville FL for training in methods that are being used to examine citrus psyllid acoustics (likely to be dropped from Objectives; see Methods).
2. Describe vibrational signals used by psylla in mate location activities (to begin summer 2018).
3. Show (in large cage studies with potted trees) that it is possible to slow or disrupt mating by mechanically transmitting these signals to the tree substrate.
4. Show that it is possible to slow or disrupt mating in a field setting by mechanically transmitting signals through the support wires of a trellised pear orchard.

Delay in achieving 2017 objectives:
Recruiting a Ph.D. student required longer time than anticipated, which has led to delays in beginning the assay work scheduled for Objective 2.

SIGNIFICANT FINDINGS

A Ph.D. candidate has been recruited and will arrive in Pullman this summer to begin research. Course-work will begin in autumn 2018.

METHODS

Source of insects and plants. We will use field-collected and lab-reared winterforms and summerforms in developing methods for recording acoustic cues and testing synthesized mimics of those cues. Horton will provide the needed psyllids and host material to the student to begin assays (Objective 2) in late summer at the Pullman location (Crowder lab).

Objective 1. Recruitment of Ph.D. student. Completed (see Results and Discussion). ***Train in methods to examine citrus psyllid acoustics***. Our initial plans were to have the student visit the USDA-ARS citrus psyllid acoustics lab in Gainesville FL to learn techniques in acoustic mating disruption. We are likely to drop this sub-objective, as the recruited student has extensive hands-on experience in use of these methods developed during her studies of treehoppers (M.S. thesis).

Objective 2. Describe vibrational signals.

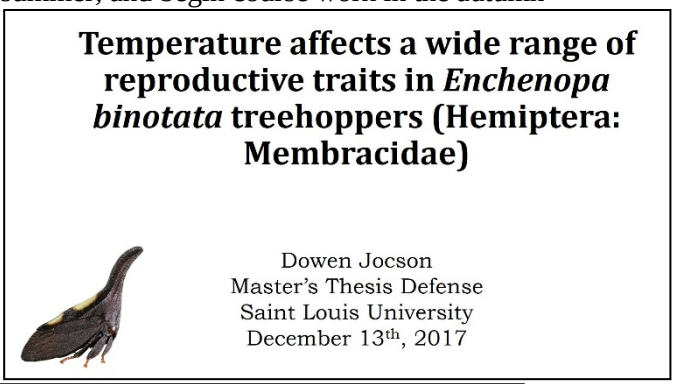
***Detecting and recording vibrational signals*.** We will record vibrational signals of pear psylla using a laser vibrometer, as the student has familiarity with this technology. The vibrometer will be used to measure the amplitude and frequency of vibrations on the plant surface based on the reflectance of the laser beam off of the vibrating surface. Vibrational signals are forwarded from the recording device to a PC for digitization and analysis using freely available software. *Playback tests of signal*. Synthetic mimics of vibrational signals will be examined with playback tests to confirm that the signal does indeed prompt vibrational response by psyllids. Synthetic signals will be examined for biological activity by disseminating signals through the plant by use of minishaker. The shaker will be in physical contact with the plant surface through a small push rod attached to the minishaker. Vibrations from the minishaker are passed to the push rod and from there to the plant surface. The minishaker will be under control of a laptop computer.

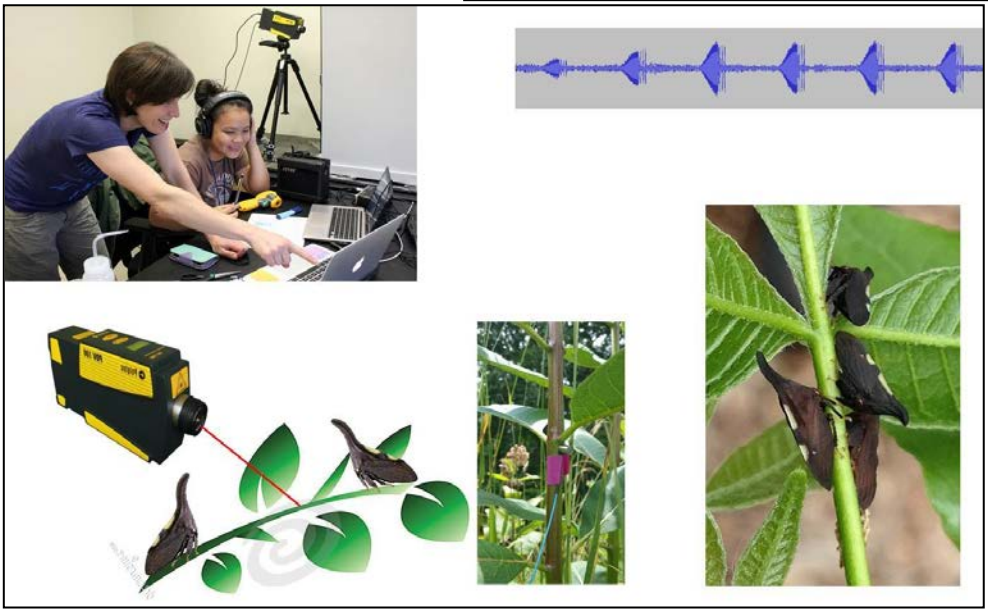
Objective 3. Large cage studies to prove disruption. We will use a cage study to examine the effects of synthetic mimics of vibrational signals on mating success of winterform psylla. The tests will be done out-of-doors in large "Bugdorm" cages (6 x 4 x 4 foot) each containing a potted pear tree 4-5 foot in height. A minishaker will be used to transmit the female-signal to trees. Fifty virgin female winterforms will be introduced into each cage and allowed to settle on trees. After 48 hrs, 50 male winterforms will be added to each cage, and the buzzer apparatus activated. Females will be collected from each cage after 2 days and dissected to determine mating status. Control cages will be treated identically to treatment cages, with the exception that no buzzer system will be present.

Objective 4. Field tests in trellised pear orchard. We will conduct a field test of the concept under an orchard situation. Tests will be done in March at a high density pear orchard under a wire trellis system. Electromagnetic minishakers attached to trellis wires will be used to disseminate the acoustic signals to trees. A laptop computer will control the minishakers and signal production. We will collect winterforms from target trees (those receiving the signal mimics) and control trees located a few rows away. Females will be dissected to determine mating status.

RESULTS AND DISCUSSION (YEAR 1)

Objective 1: Recruitment of Ph.D. student. An opening for a student to pursue a Ph.D. degree in Entomology (dissertation topic acoustic communication by pear psylla) was advertised in late spring 2017, with course-work to begin in the autumn semester. The student is to be co-supervised by Crowder, Beers, and Horton. We received a number of applications. Two applicants were interviewed by phone. Neither applicant seemed entirely suitable, so the position was re-advertised in summer 2017. Ms. Dowen Jocson, a graduate student at St. Louis University, applied for the position. Ms. Jocson completed a Master's Degree in Entomology in December 2017, studying acoustic communication by treehoppers. We interviewed Ms. Jocson by phone and then in person during an invited visit to Pullman and Wenatchee. We found her to be a very strong candidate. She has accepted our offer to enter the Ph.D. program in Entomology at Washington State University to conduct the psylla acoustics work for her dissertation. Ms. Jocson will arrive in Pullman and work in David Crowder's laboratory beginning in late summer, and begin course work in the autumn semester. David Horton will be supplying all of the pear psylla and pear plants needed for her to work in Pullman. Once course work and lab assays have been mostly completed, Ms. Jocson will move either to Wenatchee or Wapato to begin field trials.





CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-16-104

Project Title: Integrated fruit production for pears

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 x 234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: None

Total Project Request: **Year 1:** $105,424 **Year 2:** $121,474 **Year 3**: **$125,811**

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** K. Roberts/J. Cartwright
Phone: 509-335-2885/509-663-8181 **Email address:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	2017	**2018**
Salaries[1]	63,597	75,054	78,056
Benefits[2]	21,932	26,250	27,300
Wages[3]	6,240	6,490	6,749
Benefits[4]	626	651	677
Equipment	0	0	0
Supplies[5]	4,000	4,000	4,000
Travel[6]	3,529	3,529	3,529
Miscellaneous	0	0	0
Plot Fees[7]	5,500	5,500	5,500
Total	105,424	121,474	**125,811**

Footnotes: [1]Research Intern, 7 months (year 1), 12 months (years 2 and 3) 0.40 FTE. Post-Doc, 3 years [2]Benefits for Research Intern 38.6%, Post-Doc 33.5%. [3]Wages for time-slip help, 1.0 FTE, summer. [4]Benefits for time-slip 10%. [5]Supplies – office and lab supplies, electronics, statistical consulting. [6]Travel to plots – motor pool rental. [7]5.5 acres total: 2.7 acres (TF8,9), 2.8 acres (WSU Sunrise)/yr x $1,000/acre, 3 years.

Objectives

1. *Evaluate selective pesticides and non-insecticidal tactics for supplementing broad-spectrum insecticides for pear pests*. The large field plot trial comparing soft vs. conventional programs will continue along with tests on reflective mulch, particle repellents and plant elicitors.
2. *Determine the potential for the use of insect growth regulators (IGRs) as pre-bloom and post-harvest sprays for reducing overwintering psylla populations.* Pre-bloom evaluation will continue in field trials and lab bioassays. Post-harvest evaluations are scheduled for the fall of 2018 using the methods of Krysan (1990).
3. *Evaluate tree washing techniques for control of pear psylla and mites.* Overhead tree washing sprinklers were added to soft plots described in Objective 1. Airblast sprayer field trials will be supplemented with greenhouse trials on potted trees in the summer of 2018.
4. *Evaluate non-target effects on the predatory mite Galendromus occidentalis for commonly used pear miticides.* This work was completed in 2016.
5. *Evaluate pesticide efficacy for specific pesticide and pest issues.* Work on this objective will continue with input from the pear industry.
6. *Communicate project results as they become available using electronic outlets (websites, email lists).* The results from this project will continue being posted on the WSU Tree Fruit website under the link '*Pear IPM'* and on the newsletter *Fruit Matters*. We will continue to use an email list to provide updates to interested growers and fieldmen.

2017 Significant Findings

- Our soft program orchard started the year with fewer overwintering adult psylla. Psylla nymph levels were higher in soft plots than in the conventional plot in mid-summer, but lower in fall. The soft plot had higher levels of natural enemies (lacewings, earwigs, spiders, and *Trechnites*) throughout the season.
- Surround (kaolin) was the most effective repellent for psylla adults, with the lowest oviposition.
- Metallized plastic film (reflective mulch) significantly reduced densities of psylla adults, eggs, and nymphs compared with bare soil from delayed dormant through petal fall.
- Malathion, Lorsban, Bexar and Delegate were the most acutely toxic products to adult psylla in lab bioassays; Warrior and Exirel were not statistically different than water.
- Bexar and Assail were the only insecticides that were acutely toxic to psylla eggs.
- Assail, Actara, Delegate and Bexar were the most effective insecticides against young and old nymphs (>90% mortality).
- The organic products Cinnerate and TetraCURB caused 80-90% mortality of pear rust mites.

Obj. 1. **Soft vs. Conventional Plots**. *Materials and Methods.* Two 2.3-acre research blocks at WSU's Sunrise orchard were used to compare a soft vs. conventional insecticide program. Effects on pear psylla, mites, and natural enemies were of primary interest. Blocks are identical mixed plantings of 'Anjou' and 'Bartlett' pears planted in 2007. Both blocks received the same nutrient, weed and disease management programs, but different insecticides. All insecticides were applied with oil.

The conventional pesticide program was developed in collaboration with local fieldmen to closely resemble standard programs for orchards with high psylla pressure. Prior to bloom, conventional sprays used Surround, Cobalt, Malathion, wettable sulfur, Centaur, Rimon, Agri-Mek and Assail. Post-bloom sprays used Ultor, Rimon, Exirel, Delegate, Centaur, FujiMite, Actara, Assail, and Nealta. The soft program used fewer insecticidal products (mainly IGRs) overall, and incorporated overhead tree-washing. Pre-bloom sprays used lime-sulfur, Surround, wettable sulfur, Esteem, Centaur and Vendex. Post-bloom sprays used Centaur, Intrepid, Cyd-X, Dimilin, and Envidor.

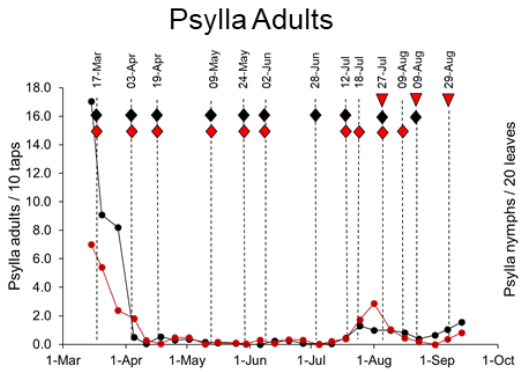


Fig. 1. Psylla adult counts, 'soft' and 'conventional' programs, 2017.

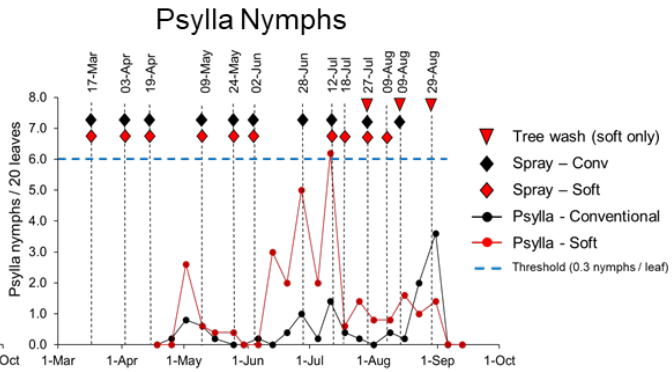


Fig. 2. Psylla nymphs counts, 'soft' and 'conventional' programs, 2017.

The soft and conventional blocks were sampled for pests and natural enemies each week from early March (dormant) to September (Anjou harvest + 1 week) using the same methods as 2016: beating trays for adult psylla and natural enemies; cut spurs for early psylla eggs and nymphs; leaf brushing for summer psylla eggs, nymphs and mites; sticky pheromone traps for syrphids and lacewings; and rolled corrugated cardboard traps for earwigs and spiders.

Results and Discussion. Psylla adult densities were lower in soft plots at the beginning and end of the growing season (Fig. 1). Nymph numbers were higher in the soft plot mid-summer, but sharply decreased in mid-July and became lower in the soft plot by the end of the season (Fig. 2). Higher densities of lacewings, earwigs, spiders, and *Trechnites* were found in the soft plot (data not shown). Despite having higher psylla nymph densities in the soft plot, the percentage of fruit downgraded from honeydew russet (3.7%) was slightly lower than in the conventional plot (4.3%), in contrast to the 2016 results. The addition of overhead tree washing is probably the responsible factor for reduced injury in the soft plot compared with 2016.

Repellent Sprays. *Materials and Methods.* Psylla adult repellency was evaluated for various spray materials using potted 'Anjou' trees in a greenhouse experiment. Materials were applied to individual trees, ca. 2.5 ft tall, about 2 weeks prior to bud break. Trees were sprayed with hand spray bottles until completely wet, ca. 50 ml (1.7 fl oz) per tree. After treatments dried, the trees were placed in a 4 × 4 × 16 ft mesh cage in a greenhouse. Adult psylla were collected from pear trees at the TFREC orchard in Wenatchee, and 1,200 were released into the cage. Six days after release, the trees were visually inspected for adults and eggs.

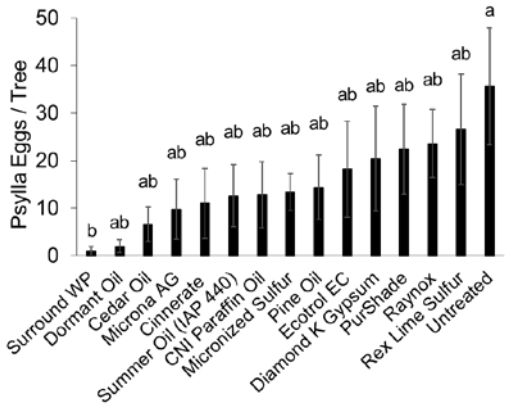


Fig. 3. Psylla eggs on potted trees after treatment with repellent materials

Results and Discussion. Few statistical differences were observed among treatments. Only one treatment, Surround, had significantly fewer eggs than the water check (Fig. 3), and no treatments had fewer adults (not shown). No phytotoxicity from treatments was observed.

Reflective Mulch. *Materials and methods*. Reflective mulch has been used for control of various insects including Asian citrus psyllid, and was therefore of interest for pear psylla control. A field experiment was conducted in the 2017 growing season to evaluate its effects on psylla, mites and natural enemies. Single tree plots with the herbicide strip covered by reflective mulch, black mulch, or left as bare ground were established at the TFREC pear orchard (TF8&9) in mid-March. Each

treatment was replicated 6 times. Samples were performed weekly beginning 17 March (prior to mulch installation) and continued into September. Beat trays were used throughout the season to sample adults and natural enemies. Prior to canopy development, psylla eggs and nymphs were sampled from 6 excised spurs per tree. Once leaves had expanded, psylla eggs and nymphs were sampled by brushing 50 excised leaves per tree. Leaf counts were also used to count parasitized psylla nymphs (mummies).

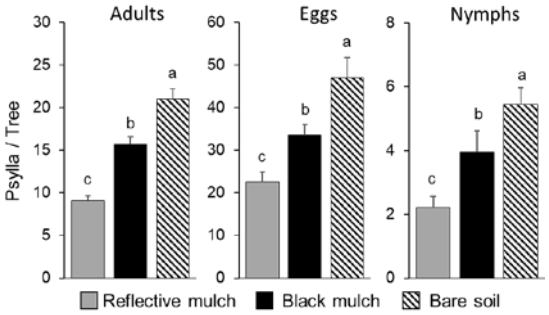


Fig. 4. Average densities of psylla from dormant to petal fall on plastic mulches

Results and Discussion. Mite densities remained too low for evaluation. Fewer psylla adults, eggs, and nymphs were found in reflective plots compared to black mulch and bare soil plots from the first post-treatment count on 23 March through petal fall (Fig. 4). From July through harvest adults and egg numbers were not different among treatments, and nymphs were often higher in reflective plots than black plastic and bare ground plots. Early season pest control followed by late season pest increases has been observed for reflective mulch in other crops as well, and has a few possible causes. This adverse effect can likely be mitigated by removing reflective mulch around petal fall, or using canopy thinning methods like summer shoot pruning. Overall, these data suggest that reflective mulch is likely to repel psylla adult colonization and oviposition prior to petal fall, thus reducing the first generation of nymphs. After this point, it is probably necessary to remove the mulch and implement other management techniques.

Obj. 2. Insect Growth Regulators. IGRs were tested in various experiments described in Objective 5. The post-harvest aspect of this objective will be conducted in the fall of 2018.

Obj. 3. Tree Washing, Overhead Sprinklers. *Materials and Methods*. An overhead sprinkler system was installed in the soft pear block at Sunrise research orchard in the spring of 2017. This system is separate (both mechanically and functionally) from the under-tree sprinkler system used for irrigation. Overhead sprinklers wash honeydew from trees to prevent fruit injury, and potentially remove psylla nymphs and mites. In the summer of 2017, the overhead washing system was run on three dates: 27 July, 16 August, and 29 August. Each wash ran for 3 hours. A non-ionic surfactant, Regulaid at 1 pint/acre, was injected into the system within the last hour of washing to aid the removal of honeydew, psylla and mites.

Results and Discussion. Although we could not directly examine cause and effect relationships of overhead washing on honeydew and pest populations, it seems likely that the overhead tree washing system helped reduce honeydew injury in soft plots, as was discussed in the results section of Objective 1. Additionally, psylla nymph densities dropped after all three washes; however, nymph densities also dropped in conventional plots on 2 of these 3 dates, making it difficult draw conclusions about this effect. Mite densities were too low in both plots for elucidate trends.

Tree Washing, Airblast Sprayer. *Materials and Methods*. A field experiment was conducted to test the hypothesis that honeydew removal via tree washing prior to an insecticide spray will increase psylla mortality. Six treatments were examined: 1) water+Regulaid followed by Delegate; 2) water followed by Delegate; 3) Delegate only; 4) Regulaid+water only; 5) water only; 6) untreated control. This experiment was conducted in mid-July at the TFREC research block (TF8&9) on 'Anjou' and 'Bartlett' trees planted in 1972. At the time of the experiment, trees were heavily infested with psylla and honeydew. Treatments had 6 replicates organized in a randomized complete block; each replicate consisted of three consecutive trees, with three replicates in each cultivar. Water and Regulaid sprays were applied using a PTO airblast sprayer at 400 gpa, and Delegate was applied at 200 gpa. Nymphs

were sampled by excising and brushing 24 leaves per replicate the day before treatment, and again 4 and 13 days after treatment.

Results and Discussion. Psylla nymph densities did not differ significantly among treatments 4 or 13 days after treatment (data not shown). Nymph densities 4 days after treatment seemed to suggest that the prewash with Regulaid (Treatment 1) reduced psylla numbers compared with other treatments; but overall the findings of this experiment were inconclusive. Some changes may be necessary to achieve efficacy from airblast prewashing (timing, pressure, droplet size, tractor speed and/or using more water for the prewash). In 2018, experiments will be conducted on a smaller scale using potted trees in the greenhouse to test the hypothesis more directly.

Obj. 4. Evaluate Non-Target Effects on the Predatory Mite *Galendromus occidentalis* for Commonly used Pear Miticides. Experiments for this objective were completed in 2016. Corresponding lethal/sublethal bioassays were conducted with twospotted spider mite in 2017 to develop selectivity ratios.

Obj. 5. Evaluate Pesticide Efficacy for Specific Pesticide and Pest Issues. Lab and greenhouse bioassays were conducted throughout the season to determine the efficacy of various materials on different life-stages of pear psylla and mites. Different methods were used depending on the pest species and life-stage.

Psylla Adults. *Materials and Methods*. Winterform psylla adults were collected from a commercial orchard in Cashmere, WA and transported to the lab. Psylla were anesthetized using CO_2 for sorting; only females were used in this experiment. Twenty females were placed in 4.5 × 7.5 cm (1.8 × 3 in.) plastic cups for treatment. Nine insecticides (Warrior II+PBO, Danitol+PBO, Cobalt Advanced+PBO, Lorsban, Malathion, Bexar, Exirel, Delegate, and Dimilin) and a water check were compared. Five replicates (individual cups) of each treatment were tested. Treatments were applied at high field rates to each replicate using a laboratory sprayer. Live and dead psylla were counted after 48 hours.

Results and Discussion. Lorsban, Malathion, Bexar and Delegate all produced corrected mortalities above 80% (Fig. 5.). Danitol, Cobalt, and Dimilin resulted in 45-70% mortality; Warrior, Exirel and the check resulted in 0-20% mortality. Organophosphates have not been widely used for adult psylla control in over a decade, which may have contributed to the efficacy of this insecticide group seen in this experiment. Bexar has demonstrated efficacy against adults and other psylla life-stages in this and other experiments (see eggs and nymphs below); but registration of this product is still pending. The lack of efficacy demonstrated by the pyrethroids in this trial is congruent with reports from growers and fieldmen indicative of high levels of resistance to these compounds.

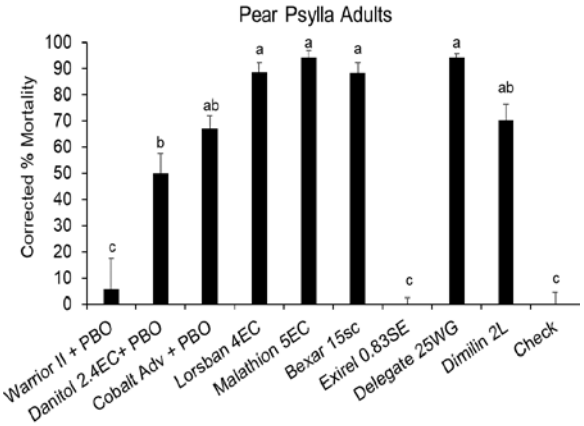


Fig. 5. Corrected percentage mortality of pear psylla adults following treatment with

Psylla Eggs. *Materials and Methods*. Two egg bioassays were conducted with different methods. In the first bioassay, eggs were obtained from a greenhouse colony maintained on potted Anjou trees. This colony was started in the spring of 2017, from adults collected in a commercial orchard in Cashmere, WA. Individual leaves with 15-20 eggs were excised and transported to the lab. Individual leaves were placed on moistened cotton in 4.5 × 7.5 cm (1.8 × 3 in.) plastic cups, with the underside facing up to expose eggs to sprays. Each cup was a replicate. Treatments were applied using a laboratory sprayer Microna AG (lime nutrient), Manzate (fungicide), diatomaceous earth, Agri-Mek, Cinnerate, Exirel, Centaur, FujiMite, Envidor, Dimilin,

Esteem, Bexar, Ultor, Rimon, and Assail were compared. Each treatment had five replicates. Leaves were inspected 5 days after treatment for live and dead eggs and nymphs.

In the second egg bioassay, instead of excising leaves, treatments were applied directly to potted Anjou trees. Two experiments using identical methods were conducted. The first experiment compared Envidor, Ultor, Exirel, Neemix, Centaur, Esteem, Rimon, Dimilin, Intrepid and a water check; the second compared Bexar, Assail and a water check. Psylla adults were collected from a commercial orchard in Cashmere, WA, brought back to the greenhouse, and 20 adults were contained on the top portion of uninfested potted Anjou trees using mesh bags. Adults were allowed 24 hours to deposit eggs on the leaves before bags and adults were removed. Eggs were counted before treatment to ensure that each tree had at least 30 eggs. Because number of eggs per tree varied significantly, blocking was used to evenly distribute egg numbers among treatments. Trees were then treated with products at high field rates using 1-liter spray bottles, and sprayed until fully covered. The trees were kept in a psylla-free greenhouse for 9 days before being inspected for live and dead eggs and nymphs.

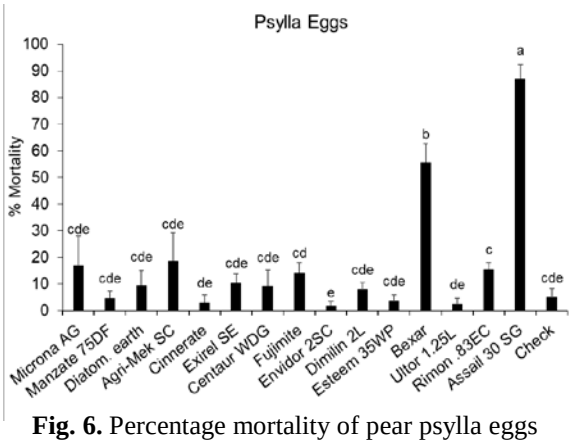


Fig. 6. Percentage mortality of pear psylla eggs following treatment with insecticides

Results and Discussion. For the first bioassay, the only treatments that exhibited significantly greater mortality than the check were Bexar (56% mortality) and Assail (87% mortality) (Fig. 6). In the second bioassay (data not shown), none of the IGRs tested reduced egg hatch. However, Bexar and Assail significantly reduced egg hatch compared with the control, with fewer than 5% of eggs hatching into nymphs for both product. The results of these assays strongly suggest that Bexar and Assail provide acute mortality of psylla eggs.

Psylla Nymphs. *Materials and Methods*. Two nymph assays were performed, one on young nymphs (1^{st}, 2^{nd}, and 3^{rd} instars) and another on old nymphs (4^{th} and 5^{th} instars). Both assays used the same insecticides and methods. Nymphs were collected from a greenhouse colony described in the first egg bioassay. Ten insecticides (Delegate, Exirel, Nexter, FujiMite, Altacor, Agri-Mek, Bexar, Assail, Actara, and Admire Pro) and a water check were evaluated. Leaves with about 10 nymphs were used in experiments. After leaves were excised from plants, each was placed on moistened cotton in 4.5 × 7.5 cm (1.8 × 3 in.) plastic cups, with the underside facing up to expose nymphs. Each treatment had 5 replicates. Treatments were applied at high field rates using a laboratory sprayer, then checked after 48 hours for living and dead nymphs.

Results and Discussion. For young nymphs, all products tested resulted in significantly greater mortalities than the check (Fig. 7). Products that resulted in >90% mortality were Delegate, Nexter, Assail, and Admire Pro. Those between 70 and 90% were Exirel, FujiMite, Bexar and Actara. Those below 70% were Altacor (40%) and AgriMek (60%).

For older nymphs, all products except FujiMite and Altacor resulted in significantly greater mortalities than the check (Fig. 8). Products that resulted around 80-90% or greater mortalities were Delegate, Agri-Mek, Bexar, Assail, and Actara. Exirel and Nexter both produced just under 70% mortality, and Admire Pro resulted in 55% mortality. Predictably, mortality was similar or lower for older vs younger nymphs, other than Agri-Mek, which curiously exhibited increased mortality in older nymphs. It is important to note that these nymph bioassays only measured acute toxicity. A new method will be used in 2018 using cut shoots in water (See pear rust mite *methods*, Fig. 9) which will allow for longer assays testing slower acting insecticides, such as IGRs.

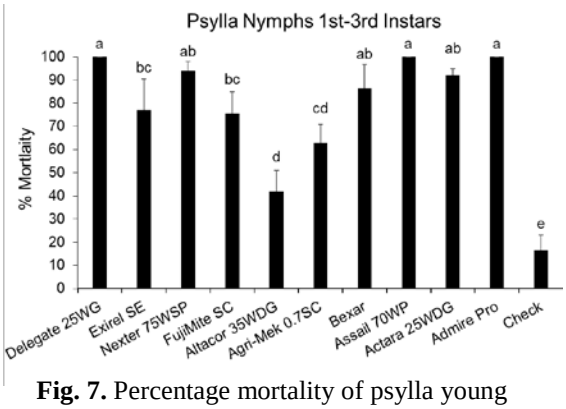


Fig. 7. Percentage mortality of psylla young nymphs following treatment with insecticides

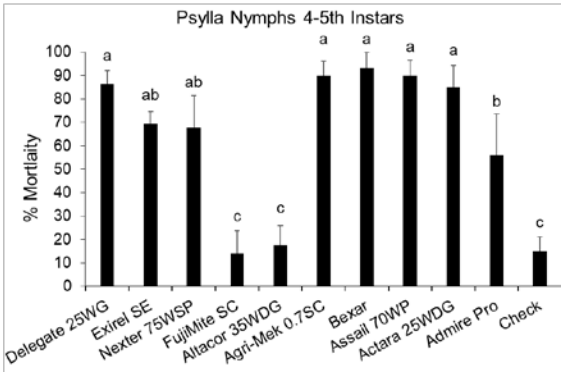


Fig. 8. Percent mortality of psylla old nymphs following treatment with insecticides

Pear Rust Mite. *Materials and Methods*. A bioassay was conducted to test the efficacy of various organic products against pear rust mite (PRM), a perennial problem in organic pear production. Shoots were collected from heavily infested Anjou trees at the TFREC orchard (TF8&9), and trimmed to four leaves. Seven organic products we compared with the convention miticide, Nexter, 1% summer oil, and a water check; products were not mixed with oil. PRM were counted on each shoot, then treated using spray bottles until leaves were thoroughly covered. Shoots were placed in cut shoot containers for storage (Fig. 9), and inspected for living and dead mites 48 hours after treatment.

Fig. 9. Cut shoot bioassay arena

Results and Discussion. The standard, Nexter, resulted in nearly 100% mortality of PRM (Fig. 10). Cinnerate (cinnamon oil) at both rates, TetraCURB (rosemary oil) and 1% summer oil caused similar levels of mortality (80-95%). Neemix, Pyganic, Azera, and SucraShield caused 50-70% mortality, while Entrust resulted in the lowest mortality (ca. 30%).

Obj. 6. Dissemination of Project Results. An email list consisting of growers, fieldmen, researchers, and extension agents was established in February 2017 for dissemination of trial results. Eight emails with results from the most recent trials were sent to this group from February to October 2017. The group expanded from 15 to 24 members and it will likely keep growing.

In addition to the email list, summaries of trials are posted on the WSU Tree Fruit website under the *Pear IPM* link (http://treefruit.wsu.edu/crop-protection/insect-mite-pests/pear-ipm/) and in the *Fruit Matters* newsletter. There is also discussion of starting a Facebook or other social media outlet for results dissemination and general discussion among members.

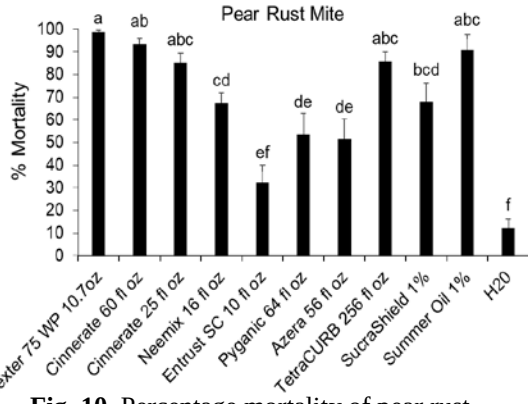


Fig. 10. Percentage mortality of pear rust mite following treatment with insecticides.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-16-103

Project Title: Enhancement of postharvest decay management in pear

PI: Achour Amiri
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Co-PI (2): Richard Kim
Organization: Pace Int. LLC
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City/State/Zip: Wapato, WA 98951

Cooperators: Kelly Wallis (Oregon), multiple packers in WA and OR, Craig Christensen (Cashmere, WA).

Total Project Request: **Year 1:** $32,284 **Year 2:** $33,284 **Year 3: $34,323**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
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]	17,550	18,252	18,982
Benefits[1]	7,434	7,732	8,041
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[2]	4,100	4,100	4,100
Travel[3]	2,000	2,000	2,000
Miscellaneous	0	0	0
Plot Fees[4]	1,200	1,200	1,200
Total	32,284	33,284	**34,323**

Footnotes:
[1] Salaries for a research intern (Laxmi Pandit, 0.65 FTE) at 42.4% benefit rate.
[2] Supplies include Petri dishes, multi-well plates, microbiological media for fungi growth and fungicide sensitivity tests.
[3] Travel to multiple packinghouses in WA and OR for fruit collection.
[4] Plot fees for an experimental orchard to be used for field studies.

OBJECTIVES

1- Conduct a general disease survey to identify and quantify major postharvest rots.

2- Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR to TBZ, pyraclostrobin, boscalid, fludioxonil and pyrimethanil.

3- Evaluate the efficacy of fungicides applied by thermofogging and investigate the possibility of reducing fungicide input.

4- Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a general disease survey to identify and quantify major postharvest rots*

- 124 grower lots from 9 packinghouses, including 4 packinghouses in WA and 5 packinghouses in OR, were surveyed from February to April of 2017 on 2016 crop. 88 lots where from OR and 36 where from WA.
- Gay mold followed by Nectria rot and Cladosporium rot were most predominant in Washington, whereas blue mold followed by gray mold and Mucor rot were most predominant in Oregon.
- The "export" quarantine pathogen *Phacidiopycnis pyri* was found at about 8 and 4% of total decay in OR and WA, respectively.

Objective 2: *Conduct a general resistance monitoring program across multiple pear orchards and packinghouses in WA and OR*

- A total of 700 isolates of *Penicillium expansum* (blue mold) and 974 isolates of *Botrytis cinerea* (gray mold) were collected from the different packinghouses surveyed in objective 1. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), 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, resistance frequencies of *P. expansum* (blue mold) and B. cinerea (gray mold) were higher in OR than in WA.
- Resistance as high as 80% to TBZ in *P. expansum* was seen versus 20% in *B. cinerea*.
- Resistance in gray mold-*B. cinerea* to orchard fungicides Pristine and Merivon was lower than 10% in both state.
- 124 decay and resistance profiles were created and sent 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.

Objectives 3: Because it was not possible to identify a packer who drenches, fogs or aerosols at the same time, this objective was not conducted. An attempt will be made in 2018-19 season to identify a packer. Moreover, based on the low residue levels we have seen in trials done on apple using fog or aerosol, the option of reducing the fungicide rate is not worth consider at this time.

Objective 4: *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.*

- Harvesting a week to 10 days earlier resulted in significantly less decay after 8 months of storage.
- Adding Ziram to Pristine or Merivon preharvest, reduced postharvest disease losses by 15 to 50% compared to Pristine or Merivon solo.

METHODS

Objective 1. *Conduct a regional decay survey program.*

In 2018, we plan to start in December 2017 to April 2018 and include a larger number of grower lots from Washington and Oregon. For this, 50 decayed fruit will be sampled on the packing line. Ten grower lots (orchards) will be surveyed from each single packinghouse. Fruit will be sampled between February and May and 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 molecularly.

Objective 2. *Conduct a multiyear regional resistance monitoring program.*

Fruit collected for decay survey (Objective 1) will be used to conduct a fungicide resistance monitoring. 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 2018 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 using an Air-Test sampler. This will help in understanding the buildup and spread of resistance inside storage rooms.

Objective 3. *Evaluate the efficacy of fungicides applied by thermofogging and investigate the possibility of reducing fungicide input*

If a packer is identified in 2018 where a comparison side by side of wet and dry application, trial will be conducted to evaluate impact on decay and fungicide residues. Currently, 5 formulations, i.e. Shield-Brite TBZ 99WP or Deccozole A for TBZ, ecoFOG-160 for pyrimethanil, and eFOG-80 or Scholar EZ for fludioxonil, are available for postharvest applications. We will evaluate the efficacy of the pyrimethanil and fludioxonil based formulations in select commercial packinghouses in the Cashmere area, WA. Fifty bins of fruit stored in rooms fogged with the aforementioned fungicides will be evaluated at the end of cold storage. Bins will be run through packing lines to determine decay incidence on multiple grower lots. Because of potential logistical difficulties, if a commercial packinghouse is not identified, smaller-size trials will be conducted at Pace International facilities in Wapato.

To determine potential impact of the different treatments on fungicide resistance development, symptomatic fruit from each treatment/rep will be used to collect fungal isolates that will be evaluated for fungicide sensitivity as described in objective 2.

Objective 4. *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development.*

Fruit from trials conducted in 2017 are in storage and disease incidence will be determined in April of 2018 and results will be compared to those from 2016. If significant differences are seen between the two years, a 3rd-year trial will be conducted in the in the summer of 2018 at a commercial d 'Anjou pear orchard in Cashmere, WA. The objective is to evaluate six different fungicide rotation programs on disease development in postharvest and potential for resistance development. Fruit were harvested in September and will be evaluated after 6 months of storage at 33°F.

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue mold with almost 35% of total decay was predominant (Figure 1) in OR but found at low frequency (7%) in WA. On the other hand, gray mold accounted for 26% of decay in OR versus 18% in WA. Mucor was higher (16%) in Oregon, whereas Nectria rot was higher (13%) in WA (Figure 1).

Besides these three major decays, the "export" quarantine pathogens *Phacidiopycnis pyri* were twice higher in OR (8%) than in WA (4%), whereas bull's eye rot frequency was around 1% in both states. Interesting to note that the postharvest rot Cladosporium was found at 6 and 9% in OR and WA, respectively (Figure 1).

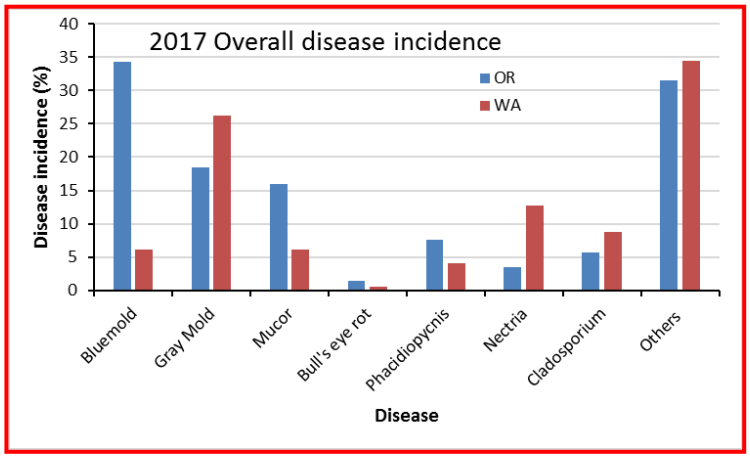


Figure 1. Overall incidence of major postharvest diseases found in in 2017 in Washington State (red bars) and Oregon (blue bars). Results are from 124 growers lots.

Objective 2. *Fungicide resistance occurrence and frequencies*

A total of 700 isolates of *Penicillium expansum* (blue mold) and 974 isolates of *Botrytis cinerea* (gray mold) were collected from the different packinghouses surveyed in objective 1. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), 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, resistance frequencies of *P. expansum* (blue mold) and B. cinerea (gray mold) were higher in OR than in WA. Resistance as high as 80% to TBZ

in *P. expansum* was seen versus 20% in *B. cinerea*. Resistance in gray mold-*B. cinerea* to orchard fungicides Pristine and Merivon was lower than 10% in both state.

More details and specific numbers will be shared at the per review meeting in February 2018.

Objectives 4. *Evaluate the impact of applying fungicide mixtures in orchards on postharvest decay and resistance development*

In 2016, two new pre-harvest fungicides Pristine (new to pear but commonly used on apple) and Merivon were tested as solo or tank-mixed with the multi-site Ziram. Two harvest dates were tested, one at the end of August and the second one at early September.

Except for the untreated control, all treatments resulted in disease incidence lower than 10% on fruit harvested late August, whereas disease incidences ranged from 15 to 33% when fruit were harvested 10 days later in September (Figure 2).\

Interestingly, Ziram' s efficacy was equal to that of Pristine or Merivon tank-mixed with Ziram. The inconveniency of irritation caused by Ziram to pickers should be avoided by wearing proper clothing during harvest. Moreover, economically it should be more beneficial to growers to include Ziram in their management programs. We have not tested for fungicide resistance in plots where Ziram was used, but previous studies on mixing single-sites with multi-sites fungicides such as Ziram, thiram or captan has delayed selection for resistance to single-sites such as TBZ and boscalid (Pristine).

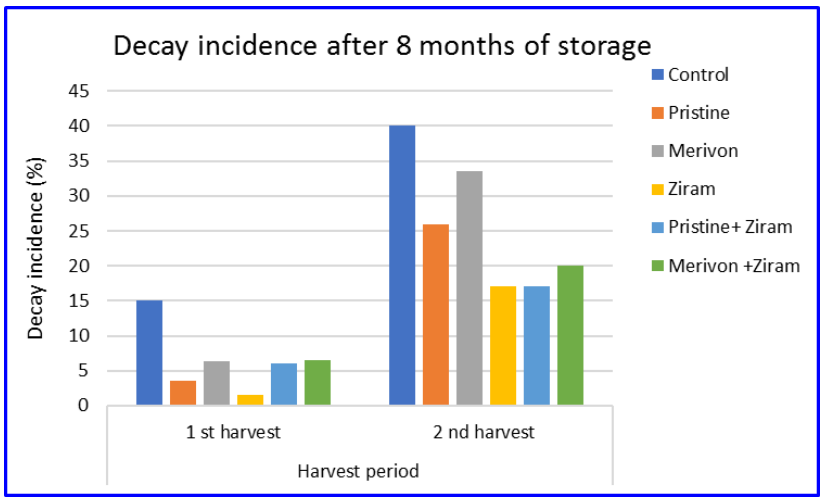


Figure 2. Overall decay incidence on d 'Anjou pear treated with Pristine, Merivon, or Ziram preharvest after 8 months of storage at 33F in a regular atmosphere. 1st harvest was done in 1st week of August 2016 followed by a 2nd harvest 10 days slater.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: Epidemiology and management of postharvest decay on pears

PI: Achala N KC
Organization: Oregon State University
Telephone: 541-772-5165 Ext 222
Email: achala.kc@oregonstate.edu
Address: 569 Hanley Rd.
City/State/Zip: Central Point, OR-97502

Cooperators: Mike Naumes (Naumes Inc, Medford, OR), Matt Borman (Harry&David, Medford, OR),

Total Project Request: Year 1: 44,698 **Year 2: 46,039**

Other funding sources
USDA-Specialty Crop Multi-State Program: Amount requested $450K (Amiri $261K, KC $189K)

Budget
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	**2017-18**	**2018-19**
Salaries Faculty Research Assistant	22,500	23,175
Benefits OPE 63%	14,198	14,624
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies	6,000	6,180
Travel	2,000	2,060
Miscellaneous	0	0
Plot Fees	0	0
Total	$44,698	**$46,039**

Footnotes: Annually: FRA 6 mo + fringe, 6K supplies and consumables, 2K local and in-state travel, 3% inflation

OBJECTIVES

1. Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology
2. In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management
3. Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

SIGNIFICANT FINDINGS

The postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum, Alternaria sp., Neofabrea sp., Phacidiopycnis washingtonensis, Sphaeriopsis pyriputrescens,* and *Potebniamyces pyri,* were isolated in equal frequencies from early stages of fruit development (full white stage). Pathogens such as *Botrytis cinerea, Alternaria sp.,* and *Cladosporium herbarum* were consistently isolated in higher frequencies throughout the blossom period. Several unknown fungi ranging from 9-18% of total isolation per sampling period were also isolated; however, the pathogenicity of these fungi needs to be confirmed.

METHODS

Objective 1.

Experimental Design: Two commercial Bosc orchards in Southern Oregon were included for periodic monitoring of postharvest rot pathogens. Stratified random sampling method was used for sampling the tissues. There were five strata with four trees in each strata. Twenty trees in each location with three branches per tree were marked before sample collection. Samples were collected from the same branch throughout the season. Altogether 60 samples were collected from each stage of fruit development, white bud, full bloom, petal fall, and fruitlets. Total of 40 fruits were also collected randomly from field bins immediately after harvest that were stored in cold storage at 30^{o}F.

Pathogen isolation and identification: Samples were processed with an initial rinse in sterile water, surface sterilization for one minute in 1% sodium hypochlorite solution, and a final rinse in sterile water. Samples were then blotted on a lab wipe to dry and cultured on half-strength potato dextrose agar (PDA) amended with streptomycin and ampicillin. For the white bud, full bloom, and petal fall stages, the entire sample was cultured. For the fruitlet stage, where disease symptoms were evident, the edge of the affected area was cultured, otherwise the blossom end of the fruitlet was cultured. The culture plates were incubated at room temperature (70^{o} F) under 12 hrs light and dark cycles. Based on the culture morphology, each unique culture was sub-cultured on full-strength potato dextrose agar. Pure culture of each fungus was obtained by single spore culture or hyphal tip method in water agar. The obtained pure culture was transferred to full strength PDA and the fungi was identified based on culture and spore morphology. After identification, the culture was air dried under laminar flow hood and stored in -112^{o} F for long-term storage. The fungi that could not be identified based on culture and spore morphology were marked as unknowns and proceeded for DNA extraction and sequencing. DNA extraction followed a CTAB protocol. The ITS region was amplified using the ITS1 and ITS 4 primers that will be sequenced for identification.

The field bin samples in cold storage were monitored every week for prevalence of any disease symptoms. The symptomatic tissues were cultured on half-strength potato dextrose agar (PDA) amended with streptomycin and ampicillin. The pure culture, identification, and storage followed the similar methods as described before.

Objective 2

Fungicide sensitivity tests: Frequently prevalent pathogens *Botrytis cinerea* and *Alternaria* sp. will be tested for sensitivity against fungicides from different FRAC groups. Pure cultures of 28 *Botrytis cinerea* isolates and 31 *Alternaria* isolates are collected from this study which will be proceeded to sensitivity tests against fungicides from FRAC groups 2, 3, 7, 9, 11, and U12.

Objective 3

Preharvest fungicide application: The trial was conducted in Southern Oregon Research and Extension Center, pear research orchard. This trial included foliar calcium application followed by fungicides application. The treatments were applied using 4 x 25 gallon air blast sprayer on Anjou, Bartlett, Bosc, and Comice pears as described in table 1, 2, 3, and 4 respectively. The treatments were arranged in randomized complete block design with four replications.

Eighty fruits from each treatment were harvested within a week of fungicide application, of which 40 fruits were stored in cold storage at 30º F. The fruits were monitored every week for prevalence of any disease symptoms. The symptomatic tissues were cultured on half-strength potato dextrose agar (PDA) amended with streptomycin and ampicillin. The pure culture, identification, and storage will follow the similar methods as described before.

Another 40 fruits were artificially inoculated with *Botrytis cinerea* after surface sterilization of fruits by 1% sodium hypochlorite solution. The conidial suspension of *B. cinerea* was adjusted to 1 x 10^5 spores/ml. A 5 mm diameter nail head was used to make one 5 mm deep wound on fruits surface. The wound was inoculated with 50 µl spore suspension. The inoculated fruits were then stored in cold storage at 30º F. The inoculated fruits were monitored every week and two directional lesion diameter was recorded every two weeks once the lesion started to expand. The treatments effect were compared using analysis of variance test for area under disease progress curve (AUDPC).

Preharvest 1-MCP application: The trial was conducted in Southern Oregon Research and Extension Center, pear research orchard. This trial included Harvista application a week and two weeks prior to commercial harvest at minimum and maximum rates. The treatments were applied in Bosc and Comice pears as described in table 5, and 6 respectively. The treatments were arranged in randomized complete block design with four replications. Rears Harvista kit and the product were supplied by AgroFresh. The kit was attached to Rears air blast sprayer and applied per AgroFresh recommendations.

Ninety fruits were harvested from each treatment, of which 80 fruits were divided into four boxes of 20 fruits each. The fruits were stored in cold storage at 30º F and each box was labeled as 2, 4, 6, and 8 months. The fruits will be examined for disease incidence and fruit texture at 2, 4, 6, and 8 months after storage. The rest ten fruits were surface sterilized and artificially inoculated with conidial suspension of *B. cinerea.* The inoculated fruits were then stored in cold storage at 30º F and the lesion diameter was recorded following the same method as described under preharvest fungicide application trial.

RESULTS & DISCUSSION

Objective 1

Altogether 90 fungal cultures were isolated from white bud stage of fruit development. The postharvest rot pathogens, *Botrytis cinerea*, *Cladosporium herbarum, Penicillium expansum, Alternaria* sp., *Neofabrea* sp., *Phacidiopycnis washingtonensis, Sphaeriopsis pyriputrescens, Potebniamyces pyri* were isolated in more or less equal frequencies at this stage of fruit development. The unknown groups of fungi were also isolated at significant frequency (Figure 1A). The isolation frequencies of these pathogens altered as the developmental stages progressed.

At full bloom stage, 103 fungal cultures were isolated. *Botrytis cinerea*, and *Alternaria* sp. were dominant pathogens isolated at this stage with significant frequencies of unknown fungi. Other pathogens such as *Cladosporium herbarum, Penicillium expansum, Neofabrea* sp., *Phacidiopycnis washingtonensis, Sphaeriopsis pyriputrescens,* and *Potebniamyces pyri* were also isolated however with lower frequencies. The unknown fungi comprised of 16% of total isolation (Figure 1B). Similar results were obtained at petal fall stage. Altogether 113 fungal cultures were isolated with dominant *Botrytis cinerea*, and *Alternaria* sp. frequencies. Other pathogens were also isolated with lower frequencies. The unknown fungi comprised of 18% of total isolation (Figure 1C).

At fruitlet stage, 34 fungal cultures were isolated of which *Alternaria* sp. was dominant followed by *Cladosporium herbarum,* and *Botrytis cinerea.* The unknown fungi comprised of 9% of total isolation (Figure 1D). The disease monitoring of fruit at field bin stage is still under progress as not all fruits have expressed the rot symptoms.

Results from this objective is significant to the industry as these pathogens reside in the lower tissues and remain latent until the fruit health is compromised in storage with ripening. Isolation of gray mold pathogen, *Botrytis cinerea* and alternaria rot pathogen, *Alternaria* sp. consistently over the fruit development stage even from symptomless tissues is indicative of latent infection. Isolation of these and other economically important pathogens even at lower frequencies necessitates disease management program targeted at bloom stages of fruit development. Our current practice rely on disease management program few weeks before harvest followed by postharvest spray programs. This program is still viable, however addition of management program at bloom would reduce the sources of inoculum going to storage resulting lowered disease pressure and increased efficiency of current program. An integrated program with bloom fungicide application followed by preharvest and postharvest application will be tested in 2018.

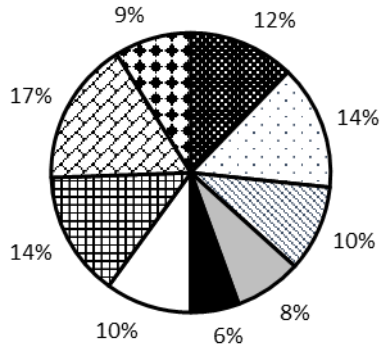


Figure 1A

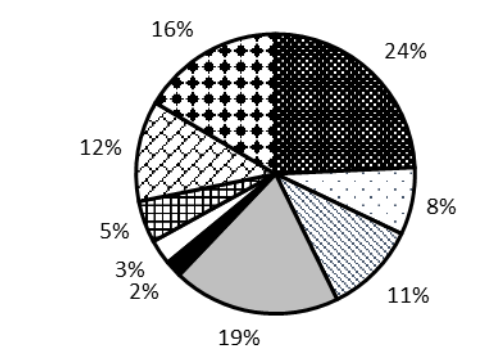


Figure 1B

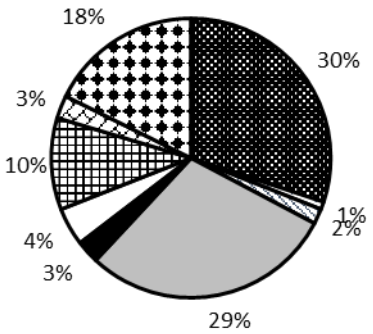
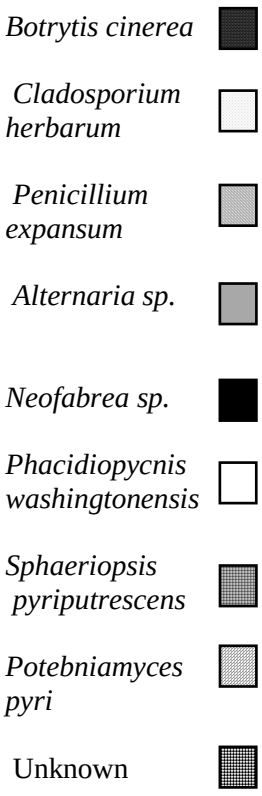


Figure 1C



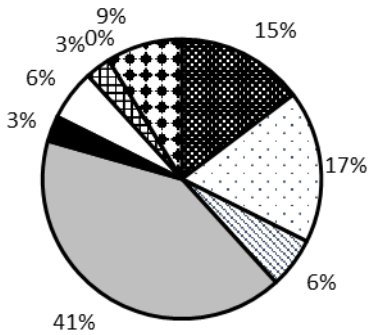


Figure 1D

Figure 1. Relative prevalence of postharvest rot pathogens at white bud (1A), full bloom (1B), petal fall (1C), and fruitlet (1D) stages of Bosc pear fruit development in Medford, OR.

Objective 2

Pure cultures of 28 *Botrytis cinerea* isolates and 31 *Alternaria* isolates are collected from this study which will be proceeded to sensitivity tests against fungicides from FRAC groups 2, 3, 7, 9, 11, and U12. Results from this objective will help decide the most effective fungicides for the control of most prevalent pathogens in Southern Oregon pear orchards.

Objective 3

Preharvest fungicide application: Preharvest application of foliar calcium and single application of preharvest fungicides alone did not significantly reduce the wound initiated *Botrytis cinerea* infection in cold storage. However, foliar calcium spray combined with foliar Syllit FL spray significantly reduced the disease progress over time on Bartlett and Bosc pears compared to water treated controls (Table 2 and 3). Similalry, foliar calcium spray combined with foliar Procure 480 SC significantly reduced disease progress by *Botrytis cinerea* on Anjou pear (Table 1). None of the applied treatments significantly reduced the disease progress on Comice pear.

The effect of preharvest application of foliar calcium and single application of preharvest fungicides alone on the storability of non-wounded fruits are still in progress as not all fruits have expressed the rot symptoms. The fruits are monitored every week for prevalence of any disease symptoms.

The results from this study will allow us to identify the best preharvest fungicide that can be combined with season long disease management program.

Preharvest 1-MCP application: Preharvest application of foliar 1-MCP alone did not significantly reduce the wound initiated *Botrytis cinerea* infection in cold storage for both Bosc and Comice pears. The disease progress over time was lower on Bosc fruits treated with 1-MCP a week prior to harvest at minimum rate; however, it was not statistically significant. Disease progress on other treatments were significantly higher than water control treatments. Similar result was observed on Comice fruits treated with 1-MCP.

The effect of preharvest application of foliar 1-MCP alone on the storability of non-wounded fruits are still in progress. The fruits are stored in cold storage at 30^{o} F and will be examined for disease incidence and fruit texture at 2, 4, 6, and 8 months after storage.

Application of 1-MCP at preharvest is not fungicidal enough to control the disease caused by wound initiated pathogens. The information generated from disease incidence and fruit texture analysis of 1-MCP treated non-wounded fruits will be helpful in determining the storability of fruits by 1-MCP treatment. Moreover, this information can be combined with integrated disease management program including fungicide applications.

Table 1. Effect of preharvest fungicides application on *Botrytis cinerea* inoculated Anjou pear

Treatment	Rate per 100 gallons water	Date treatment applied [x]			Harvest	Average
		Aug 11	Aug 25	Aug 31	Sept 6	AUDPC [y]
Foli Cal	2 qt	x	x	-	x	1826.16 a
plus Merivon	5.5 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1841.96 a
plus Vangard WG	5 oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1798.44 a
plus Syllit FL	58 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1724.38 b
plus Procure 480 SC	16 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1832.21 a
plus Nevado 4F	38 fl oz	-	-	x		
Water		x	x	x	x	1821.42 a
p >F						*0.006*

Table 2. Effect of preharvest fungicides application on *Botrytis cinerea* inoculated Bartlett pear

Treatment	Rate per 100 gallons water	Date treatment applied [x]		Harvest	Average
		Aug 11	Aug 18	Aug 24	AUDPC [y]
Foli Cal	2 qt	x	-	x	1726.24 a
plus Merivon	5.5 fl oz	-	x		
Foli Cal	2 qt	x	-	x	1675.23 ab
plus Vangard WG	5 oz	-	-		
Foli Cal	2 qt	x	-	x	1583.75 c
plus Syllit FL	58 fl oz	-	x		
Foli Cal	2 qt	x	-	x	1623.78 bc
plus Procure 480 SC	16 fl oz	-	x		
Foli Cal	2 qt	x	-	x	1742.65 a
plus Nevado 4F	38 fl oz	-	x		
Water		x	x	x	1627.72 bc
p >F					*0.001*

Table 3. Effect of preharvest fungicides application on *Botrytis cinerea* inoculated Bosc pear

Treatment	Rate per 100 gallons water	Date treatment applied [x]			Harvest	Average
		Aug 11	Aug 25	Sept 7	Sept 13	AUDPC [y]
Foli Cal	2 qt	x	x	-	x	1687.66 bc
plus Merivon	5.5 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1696.43 bc
plus Vangard WG	5 oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1658.21 c
plus Syllit FL	58 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1723.18 abc
plus Procure 480 SC	16 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1751.36 ab
plus Nevado 4F	38 fl oz	-	-	x		
Water		x	x	x	x	1796.86 a
p >F						*0.021*

Table 4. Effect of preharvest fungicides application on *Botrytis cinerea* inoculated Comice pear

Treatment	Rate per 100 gallons water	Date treatment applied [x]			Harvest	Average
		Aug 11	Aug 25	Aug 31	Sept 6	AUDPC [y]
Foli Cal	2 qt	x	x	-	x	1766.01 b
plus Merivon	5.5 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1725.65 b
plus Vangard WG	5 oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1969.49 a
plus Syllit FL	58 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1802.3 b
plus Procure 480 SC	16 fl oz	-	-	x		
Foli Cal	2 qt	x	x	-	x	1795.86 b
plus Nevado 4F	38 fl oz	-	-	x		
Water		x	x	x	x	1828.58 ab
p >F						*0.017*

Table 5. Effect of preharvest 1-MCP application on *Botrytis cinerea* inoculated Bosc pear

Treatment	Rate (fl oz/acre)	Date treatment applied [x]		Harvest	Average
		Aug 30	Sept 6	Sept 13	AUDPC [y]
Water	0	x	x	x	2207.33 cd
One week prior to harvest Low rate	48	-	x	x	2085.82 d
One week prior to harvest High rate	96	-	x	x	2286.19 bc
Two weeks prior to harvest Low rate	48	x	-	x	2473.71 a
Two weeks prior to harvest High rate	96	x	-	x	2373.63 ab
p >F					*0.0004*

Table 6. Effect of preharvest 1-MCP application on *Botrytis cinerea* inoculated Comice pear

Treatment	Rate (fl oz/acre)	Date treatment applied [x]		Harvest	Average
		Aug 23	Aug 30	Sept 6	AUDPC [y]
Water	0	x	x	x	1577.57 a
One week prior to harvest Low rate	48	-	x	x	1631.4 a
One week prior to harvest High rate	96	-	x	x	1674.33 a
Two weeks prior to harvest Low rate	48	x	-	x	1678.5 a
Two weeks prior to harvest High rate	96	x	-	x	1577.65 a
p >F					0.085

[x] – Product was not applied;

[y] Means within a column followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference

CONTINUING PROJECT REPORT **YEAR**: 1/3 years

Project Title: Mechanisms and practical solutions to control scald of pears

PI: Yu Dong
Organization: MCAREC
Telephone: 541-386-2030 (EXT. 38229)
Email: dongyu@oregonstate.edu

Cooperators: Yingli Li, Shaoying Zhang, Paul Chen, Steve Castagnoli, Ines Hanrahan

Total Project Request: **Year 1:** 36,916 **Year 2: 39,011** **Year 3:** 40.061

Other funding sources: none

Budget:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2017	2018	2019
Salaries	20,222[1]	20,829	21,454
Benefits	1,950[2]	2,009	2,069
Wages	10,744[3]	11,066	11,398
Benefits	1,074[4]	1,107	1,140
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	37,990	**39,011**	40,061

Footnotes:
[1]Postdoctoral Research Associate: 1/2 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 800hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold storage and CA storage rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES

1. Understand completely the physiological mechanisms of scald development; understand growing season conditions and harvest maturity effects on the natural antioxidant capacity associated with the oxidation of α-farnesene into conjugated trienols (CTols) and therefore scald susceptibility of Anjou pear.
2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear; the potential of the combination treatments of Harvista/ReTain + ethoxyquin + low-O_2.
3. Study the potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.
4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

SIGNIFICANT FINDINGS

1. Physiological mechanism of scald development:
   - The reduction of α-farnesene and increase in CTols during storage are associated with superficial scald development.
   - Sunlight exposure prior to harvest delayed the accumulation of α-farnesene and CTols, and inhibited the development of superficial scald development.
   - Harvest maturity affected scald development. More mature fruit developed more scald.
2. Commercially feasible methods of controlling scald:
   - Harvista at 120 g/acre applied 10 d before harvest to 'Anjou' pears inhibited ethylene production (EPR) and respiration rates (RR) compared to the control, and reduced scald development.

METHODS

Objective 1a.

Determine the role of antioxidants in inhibiting the oxidation of α-farnesene into CTols in fruit peel and scald incidence and severity after storage using fruit having varied ACU, different sunlight exposure, and varied fruit tissue Ca concentration.

Fruit with varied ACU will be collected from 5 orchards located at elevations of 500 to 2,000 ft. in the Mid-Columbia area. Temperature loggers will be used to log the temperature profile from full bloom to commercial harvest date in different orchards. ACU (hours < 50°F during 42 d prior to harvest) will be calculated based on the temperature profile in each orchard.

Fruit with varied Ca concentrations will be generated by pre-harvest Ca sprays. The non-sprayed Anjou in MCAREC have ~600ppm fruit tissue Ca content. Fruit with Ca content from ~600 to ~900 ppm are expected.

Fruit with different sunlight exposure will be collected from the outer and inner canopy. Fruit bagged about 2 months before harvest will be included.

All the fruit will be harvested at FF=14-15 lbs. and stored at 30°F in RA for 6 months. Physiological, biochemical, and scald evaluations will be performed monthly.

The antioxidants being determined in fruit peel include ***antioxidant metabolites***: total polyphenol (TP), total flavonoids (TFO), total flavonols (TFA), total anthocyanins (TA); and ***antioxidant enzymes***: superoxide dismutase (SOD), catalase (CAT), monodehydroascorbate reductase (MDAR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR). ***Total antioxidant capacity*** is determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and by ferric reducing antioxidant power (FRAP). The oxidation of ***α-farnesene*** into ***CTols*** and ethylene synthesis will be monitored.

Objective 1b.

Determine the influence of harvest maturity on fruit scald susceptibility:

Fruit from two orchards will be harvested at maturity ranging from FF=15-16 lbs., FF=14-15 lbs., FF=13-14 lbs., FF=12-13 lbs. and stored at 30°F for 6 months. Physiological, biochemical, and scald evaluations will be performed monthly.

Objective 1c.

Determine the influence of NAA Stop Drop on fruit scald susceptibility:

NAA, the active ingredient in stop drop treatments, is commonly applied for reducing preharvest fruit drop. Our preliminary research has shown that preharvest NAA application may influence Anjou pear maturity and susceptibility to disorders during storage through increasing ethylene production. The effect of NAA on scald susceptibility will be further tested. Fruit sprayed with NAA at different rate and preharvest timing will be harvested and stored at 30°F for 5-6 months. Physiological, biochemical, and scald evaluations will be performed monthly.

Objective 2a.

Determine the effects of low-O_2 CA on fruit scald susceptibility: Fruit will be collected from two low-elevation orchards at commercial maturity of FF = 14-15 lbs. and stored under CA at 30°F for 10months. O_2 treatment levels will include: O_2 = 21%, O_2 = 2%, O_2 = 1.2%, O_2 = 1%, and O_2 = 0.5%; 15 gas-tight CA cabinets will be used in this study (5 O_2 trt x 3 replications). O_2 concentration in each cabinet will first be reduced to the desired level within 5d by flushing with purified N_2 generated from a membrane gas generator (Model CPA-5, Permea, St. Louis, MO). The desired O_2 levels will then be maintained for the test period by mixing purified N_2 with compressed air. Two-stage regulators will be used to regulate each type of gas that is mixed in a mixing tube and the mixed gas delivered into each CA cabinet with a flow rate of approximately 50 ml/min, replacing the atmosphere in each cabinet about every 4 h. CO_2 in each cabinet will be controlled at <0.03% by adding hydrated lime (1:20, w:w). Concentrations of O_2 and CO_2 in each cabinet are monitored daily using an O_2 and CO_2 analyzer (Storex, Gravendeel, The Netherlands). Physiological (ethylene synthesis and respiration rate), biochemical (antioxidants, α-farnesene, CTols) and scald evaluations will be performed every two-three months.

Objective 2b.

Determine the effects of ethylene inhibitors 1-MCP, and AVG on fruit scald susceptibility

Test effect of preharvest 1-MCP or AVG treatments on fruit susceptibility to scald of Anjou pear.

Objective 2c.

Test the efficacy of the combination of preharvest Harvista/ReTain + postharvest ethoxyquin + normal/low O_2 CA on controlling scald of susceptible Anjou pears collected from orchards in low elevations.

Objective 3.
Mixing Lovastatin and AsA at different rates and maybe other naturally-occurring food-grade antioxidants with commercially available edible coatings (i.e., SemperFresh, Carnauba) will be tested by comparing with ethoxyquin on controlling scald of Anjou pear.

Objective 4.
Summarize pre/postharvest practices to reduce scald susceptibility by increasing the natural antioxidant capacity in fruit peel and commercially feasible methods to reduce storage losses due to scald.

RESULTS

1. Physiological mechanisms of scald development:

α-farnesene metabolism

For Anjou pear harvested at 16-15 lbs. and stored in regular-air (RA) at 30 °F, the incidence of superficial scald after 7 d at 68 °F remained relatively low through three months of storage but increased thereafter (Fig 1). α-Farnesene showed marked increase from one month of storage, peaking at three months and then declining. CTols concentration showed a similar pattern but peaked one month later than α-farnesene and preceded the large increase in scald.

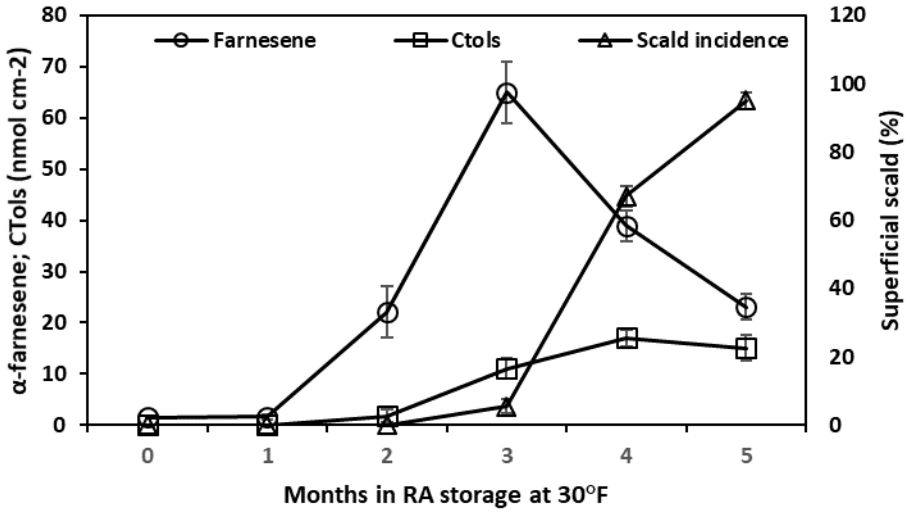


Fig. 1 α-Farnesene, conjugated trienols (CTols), and superficial scald incidence of Anjou pear in 7 d at 68 °F during 5 months storage in regular-air (RA) at 30 °F.

Fruit with varied accumulated cold units (ACU)

Fruit was collected at commercial harvest maturity from 5 orchards located at elevations ranging from 500 to 2,000 ft. and placed in RA storage. Fruit will be evaluated after 3, 5, and 7 months of storage and results will be reported next year.

Fruit with varied Ca concentration

Ca application (0.15%) was made prior to harvest. Fruit was collected at commercial harvest maturity and placed in RA storage. Fruit will be evaluated after 5 months of storage, and results will be reported next year.

Different sunlight exposure (completed in 2016-2017)
Bagged and unbagged fruit were collected at commercial harvest maturity and placed in RA storage. Fruit were evaluated at harvest, and 2, 3, 4, and 5 months after storage. There were no significant differences in α-farnesene concentration of blushed and shaded peels of unbagged fruit from harvest through five months of storage (Fig. 2). Both had the highest level of α-farnesene at three to four months of storage. In bagged fruit, the concentration of α-farnesene was similar to that of unbagged fruit at harvest but was higher by two months of storage, and peaked earlier and at a much higher level. The blushed peel of unbagged fruit had the lowest concentration of CTols throughout the experimental period. The blushed and shaded peels of unbagged fruit accumulated the highest CTols concentrations at 4 months, while the bagged fruit reached the highest level of CTols one month earlier. The shaded peels of unbagged fruit and peels of bagged fruit had similar CTol concentrations at four and five months of storage. The blushed peel of unbagged fruit did not develop scald throughout the five-month storage period, while the shaded peel developed 0, 8, 89 and 100% scald incidence at 2, 3, 4 and 5 months, respectively. Bagged fruit developed 0, 39, 95, and 100% scald incidences at 2, 3, 4 and 5 months storage, respectively.

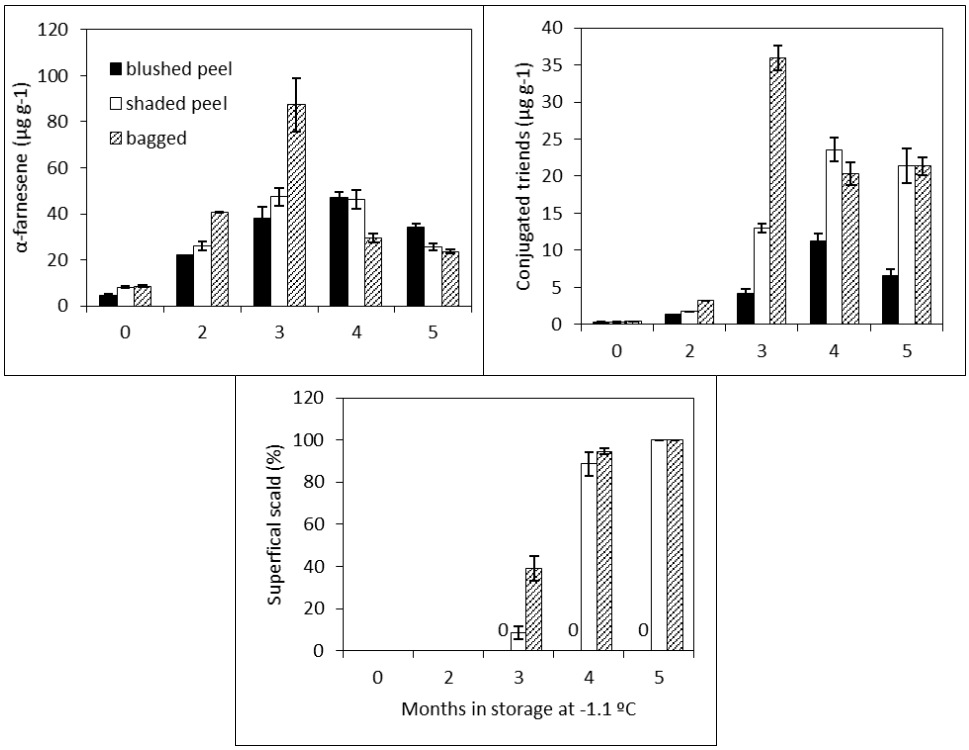


Fig. 2 α-Farnesene, conjugated trienols (CTols), and superficial scald incidence affected by sunlight of Anjou pear in 7 d at 68 °F following 5 months storage in regular-air (RA) at 30 °F.

Influence of harvest maturity. Anjou pear harvested at 13.9-11.8 lbs. had relatively high incidence of superficial scald development after 3-4 months in regular-air storage and 5-6 months in CA storage following 7 d at 68 °F (Fig. 3). Anjou pear harvested at 15.9-13.9 lbs. developed less superficial scald than that harvested at 13.9-11.8 lbs. after 3-4 months of storage and 3-7 months in regular CA at 30 °F following 7 days at 68 °F. For fruit stored in RA, all harvest maturities developed nearly 100% incidence of scald by 5 months of storage. Scald incidence of all harvest maturities of CA stored fruit increased up to 9 months of storage.

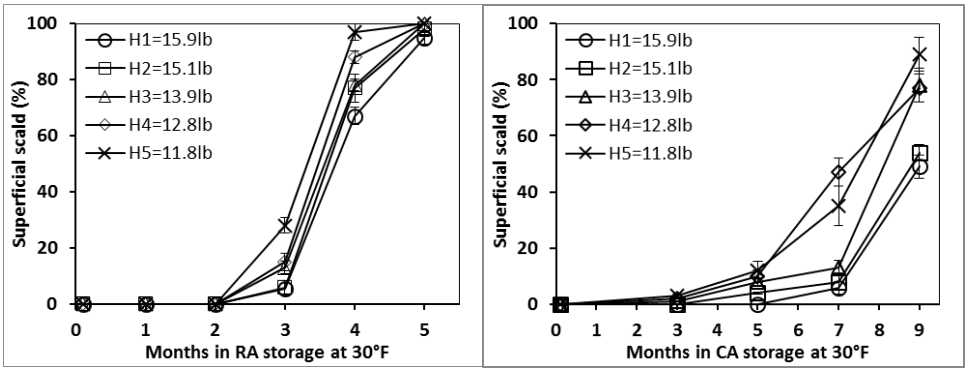


Fig. 3 Superficial scald incidence affected by harvest maturity of Anjou pear in 7 d at 68 °F following 5 months storage in regular-air (RA) at 30 °F or 9 months storage at controlled atmosphere (CA) at 30 °F.

Influence of NAA Stop Drop

NAA (30 ppm) was applied prior to harvest. Fruit was collected at commercial harvest maturity and 10 days later and placed in RA storage. Fruit will be evaluated after 3, 5, and 7 months of storage, and results will be reported next year.

2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear

Effects of preharvest 1-MCP on superficial scald (completed in 2016-2017)

Harvista (1-MCP) was applied at 120g/acre applied 10 d before harvest. Fruit was collected at commercial harvest maturity and 4 days later and placed in RA storage. Fruit was evaluated after 2, 4, 6, and 8 months of storage. Based on reductions of FF and green color (data not shown), Harvista extended the harvest window by 3-4 days. Harvista also inhibited the ethylene production rate (EPR) and respiration rate (RR) during 2-8 months of storage at 30 °F following 7 d at 68 °F (Fig. 4). After 8 months of storage, both Harvista and delay-harvest pears significantly reduced the superficial scald incidence (Fig. 5).

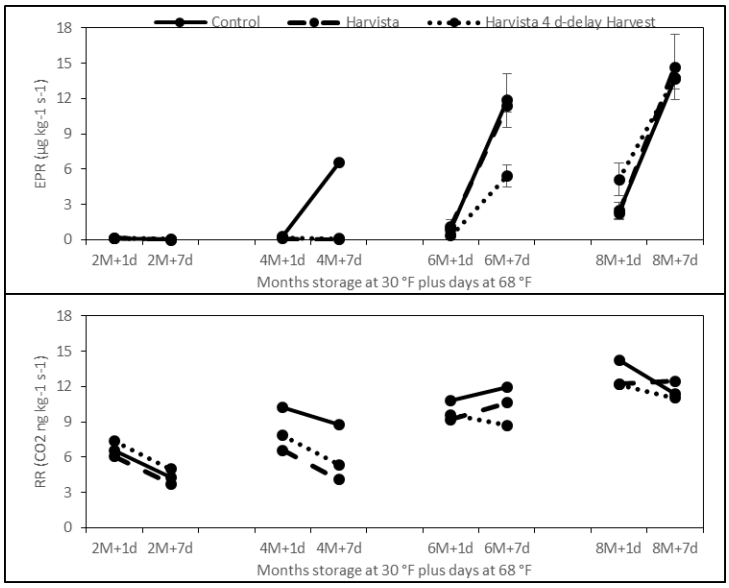


Fig. 4. Effect of Harvista on EPR and RR of 'Anjou' pears after 7 d at 68 °F following 2-8 months of storage at 30 °F plus. Values are means ± standard deviation.

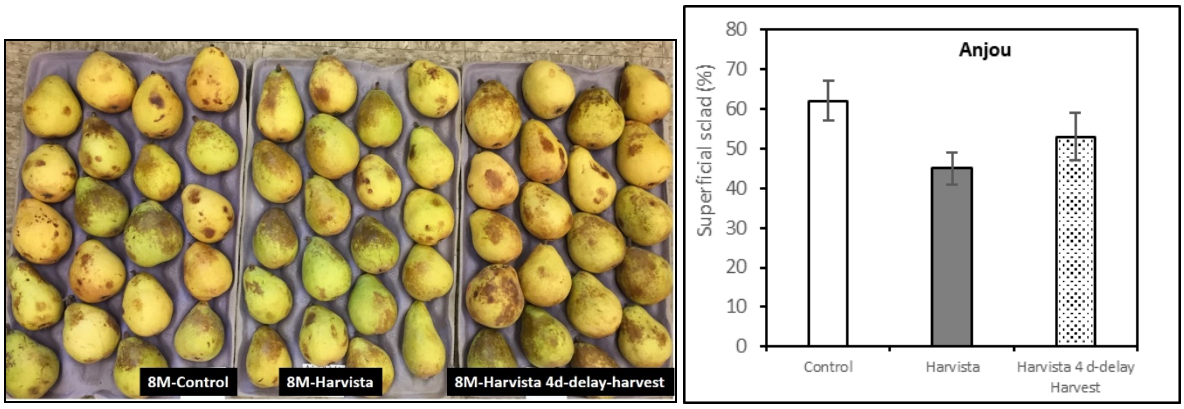


Fig. 5. Superficial scald of Anjou pear after 8 months of storage at 30 °F and 7 d at 68 °F.

3. ***The potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.***
Work on this objective will be initiated in 2018.

4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.
This summary will be developed for the final report.

CONTINUNING PROJECT REPORT **Third year report** **YEAR:** 3 of 3
WTFRC Project number:

Project Title: Delivering quality pear fruit to consumers

PI: Yu Dong
Organization: MCAREC
Telephone: 541-386-2030 (38229)
Email: dongyu@oregonstate.edu
Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR97031

Cooperators: Steve Castagnoli, Paul Chen
Drs. Shunchang Cheng, Yingli Li, Shaoying Zhang

Total Project Budget: **Year 1**: $25,725 **Year 2**: $26,390 **Year 3**: $27,073

Other funding sources: none

Budget 1
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017	2018
Salaries	13,088[1]	13,481	13,885	
Benefits	1,250[2]	1,300	1,352	
Wages	6,715[3]	6,917	7,124	
Benefits	672[4]	692	712	
Equipment				
Supplies	3,500[5]	3,500	3,500	
Travel	500[6]	500	500	
Miscellaneous				
Total	25,725	26,390	27,073	0

Footnotes:
[1]Postdoctoral Research Associate: 1/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 500hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), and gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES:

1. Elucidate the cell metabolic mechanisms and pre/postharvest factors affecting the development of buttery-juicy melting texture (BJMT) during ripening of pears.
2. Study pre/postharvest factors influencing the chilling requirement for ripening capacity (CRRC) of pears.
3. Develop conditioning protocols for 1-MCP treated 'Anjou' pear.

SIGNIFICANT FINDINGS

Elucidating the cell metabolic mechanisms and pre/postharvest factors affecting the development of buttery-juicy melting texture (BJMT) during ripening of pears.

Cell wall pectin metabolism

- Water soluble pectin (WSP), CDTA-soluble pectin (CSP), and pectin methylesterase (PME) are positively correlated with BJMT. WSP are hygroscopic and give consumers the BJMT feeling.

Factors affecting the development of BJMT

- **Harvest maturity** -Anjou pear harvested between 14-15 lbs. had excellent BJMT and flavor after 4-7 months of RA storage at 30°F plus 7 d at 68°F, while pears harvested between 12-13 lbs. had inferior BJMT and flavor.
- **1-MCP+ethylene** - 150 ppb 1-MCP treated Anjou pear (15-14 lb.) failed to develop BJMT following 8 months of RA storage plus 7d at 68°F. However, 1-MCP+ethylene treated pears developed BJMT after 8 months.
- **Storage temperature**. Similar to fruit stored at 30°F, Anjou pear stored at 32°F could develop BJMT after 5-7 months, but also resulted in high incidence of storage disorders during ripening. Higher storage temperature, such as 34°F, accelerated fruit ripening in the early ripening test.

Developing conditioning protocols for 1-MCP treated 'Anjou' pear.

- Late-harvest of Anjou pears (FF=12-13 lbs.) helped increase ripening of 1-MCP treated pears while controlling scald.
- The combination treatment of 300 ppb 1-MCP and 300 ppb ethylene improved ripening capacity of Anjou pear after long-term CA storage (i.e. > 7-8 months).

METHODS

Objective 1. Lab procedures were developed to quantify cell wall total pectin substances (TPS), WSP, CDTA-soluble pectin (CSP), and sodium carbonate-soluble pectin (SSP). The key enzymes (polygalacturonase (PG) and pectin methylesterase (PME)) regulating the pectin degradation process were also be monitored. Anjou pear fruit harvested at commercial maturity was ripened for 7 d at 68 °F after storing at 30 °F for 0, 1, 2,3,4,5,6,7,8 months in RA and 5, 6, 7, 8, 9 months in RA at 30°F. Tissue samples were frozen in liquid N_2 and stored at -80 °C until analysis. The effects of accumulated cold unit, fruit tissue Ca content, harvest maturity (FF = 15-12 lb.), 1-MCP+ethylene, storage temperatures (30, 32, and 34°F), and CA storage on cell wall pectin metabolism and buttery-juicy texture development were studied. An industry standard methodology is being developed to objectively quantify the buttery-juicy texture.

Objective 2. To facilitate early marketing of Anjou, commercially feasible conditioning protocols will be developed based on orchard elevation (500, 2,000 ft), fruit nutrition (Ca concentration at ~600

ppm and ~900ppm), cultivars (green and Columbia Red Anjou) to ensure conditioned fruit with ripening capacity but having optimal shipping firmness and post-conditioning storage life. Conditioning parameters will include ethylene conditioning, intermediate temperature conditioning, and ethylene + intermediate temperature conditioning.

Objective 3. Develop conditioning protocols for 1-MCP treated 'Anjou' pear.

RESULTS

Elucidating the cell metabolic mechanisms and pre/postharvest factors affecting the development of buttery-juicy melting texture (BJMT) during ripening of pears.

a. Cell wall pectin metabolism

Anjou pears harvested at 14-15 lbs. from MCAREC showed no ripening capacity (RC) expressed by fruit firmness following 1-3 months of regular-air (RA) storage at 30°F plus 7 d at 68°F (Fig. 1A). After 4 months of storage, RC and BJMT were well developed (Fig. 1A&B). Following 8 months in RA storage at 30°F BJMT declined resulting in coarse and dry texture. The development of BJMT was negatively correlated with the RC, and was positively correlated with WSP, CSP, and PME (Table 1). TPS, SSP, and PG were not correlated with BJMT over the entire storage period.

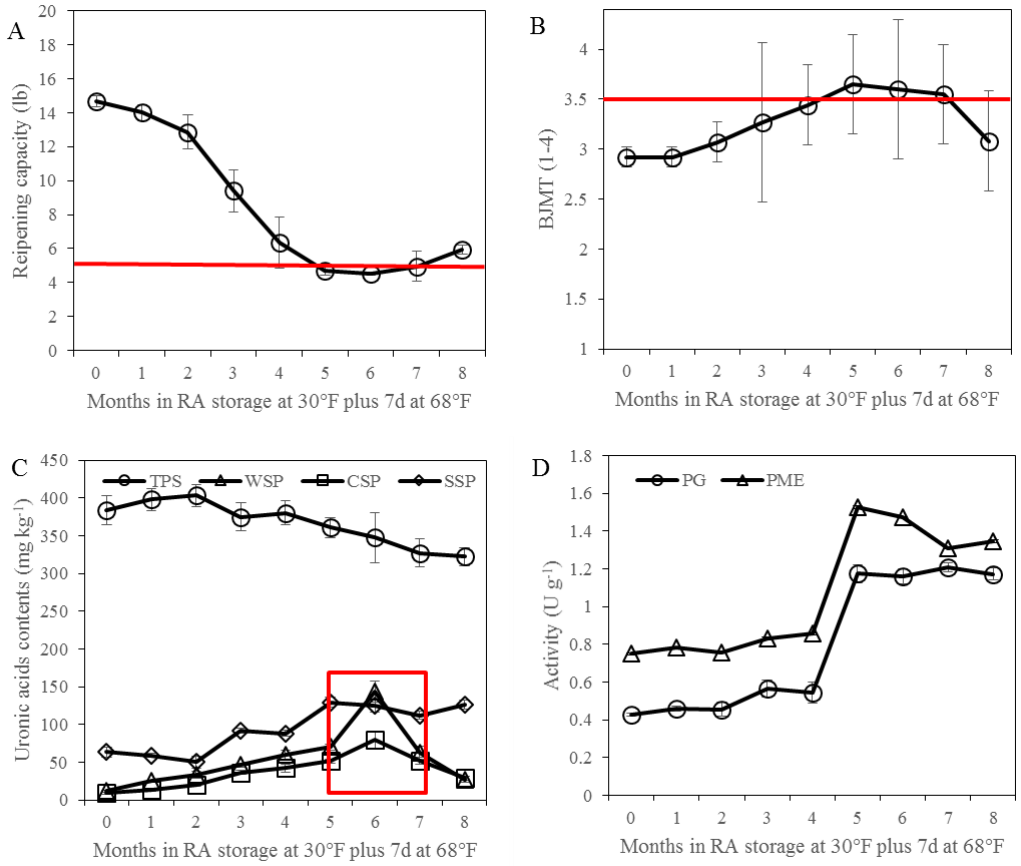


Fig. 1. Ripening capacity (RC) expressed by fruit firmness (A); BJMT (B); total pectin substances (TPS), water-soluble pectin (WSP), CDTA-soluble pectin (CSP), sodium carbonate-soluble pectin (SSP) (C); polygalacturonase (PG), and pectin methylesterase (PME) (D) of Anjou pears following 8 months of RA storage at 30°F plus 7 d at 68°F.

Table 1. Correlation analysis among RC, BJMT, TPS, WSP, CSP, SSP, PG, and PME.

	RC	BJMT	TPS	WSP	CSP	SSP	PG	PME
RC								
BJMT	**-0.909****							
TPS	0.781*	-0.488						
WSP	-0.691*	**0.919****	-0.366					
CSP	-0.863**	**0.818****	-0.562	0.953**				
SSP	-0.396*	0.299	-0.866**	0.599	0.368			
PG	-0.001	0.001	0.727*	0.074	-0.130	-0.348		
PME	-0.859**	**0.668***	-0.501	0.870**	0.724*	0.427	-0.486	

*, ** indicated the difference at P = 0.05 and 0.01 levels, respectively.

b. Ethylene - (Data were shown in 2016-2017.)

c. BJMT index - (Data were shown in 2016-2017.)

d. Factors affecting the development of BJMT

Accumulated cold unit (ACU) - (Data were shown in 2016-2017.)

Harvest maturity - In 2016, Anjou pears were harvested between 11-15 lbs. from MCAREC. For H1, FF was 14.7 lbs; For H2, FF was 12.8 lbs; For H3, FF was 11.2 lbs. H2 and H3 pears developed BJMT following 2-4 months of RA storage at 30°F plus 7 d at 68°F, while H1 showed BJMT after 4-6 months. Although FF in H2 and H3 was below 5 lbs. following 4-6 months of RA at 30°F plus 7 d at 68°F, coarse and dry texture was observed in both H2 and H3 pears and a dramatic reduction of WSP was observed (Fig. 2).

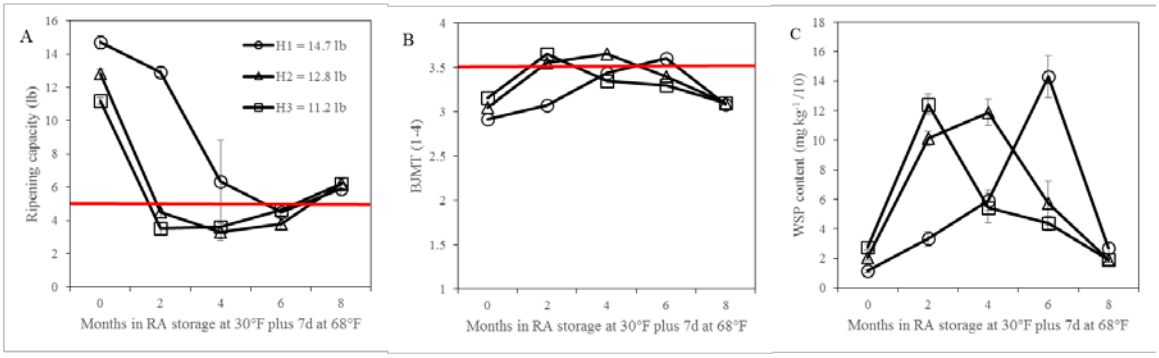


Fig. 2. RC (A), BJMT (B), and WSP (C) affected by harvest maturity of Anjou pears in MCAREC following 8 months of RA storage at 30°F plus 7d at 68°F.

1-MCP+ethylene - Anjou pears harvested at 15-14 lbs. from MCAREC developed BJMT after 5-7 months of RA storage at 30°F plus 7 d at 68°F. 1-MCP treated pears failed to develop BJMT over the whole storage period, while fruit treated with 1-MCP combined with ethylene could recover RC and develop BJMT with higher WSP content after 8 months of RA storage at 30°F plus 7 d at 68°F (Fig. 3).

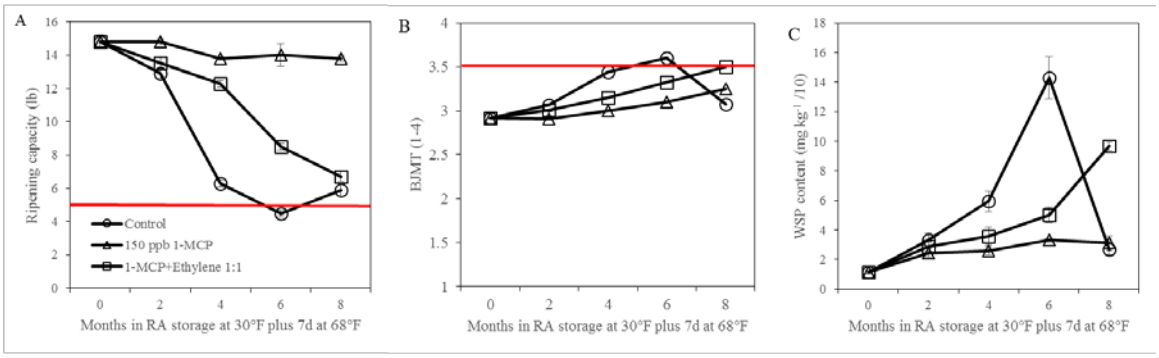


Fig. 3. RC (A), BJMT (B), and WSP (C) affected by 150 ppb 1-MCP alone or combination with 150 ppb ethylene of Anjou pears in MCAREC following 8 months of RA storage at 30°F plus 7d at 68°F.

Storage conditions - Although Anjou pears harvested at 15-14 lbs. from MCAREC developed BJMT after 5-7 months of stored at 32°F, fruit had poor appearance and higher incidence of storage disorders during ripening. After 4 months of 34°F, fruit had developed BJMT, indicating that the higher storage temperature can promote the ability to ripen. 30°F appeared to be the most effective storage temperature for Anjou pears (Fig.4).

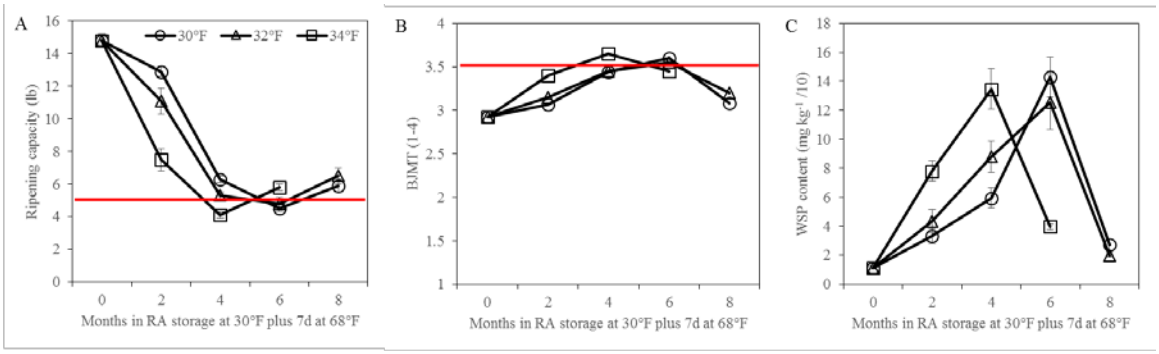


Fig. 4. RC (A), BJMT (B), and WSP (C) affected by storage temperature of Anjou pears in MACREC following 8 months of RA storage at 30°F plus 7d at 68°F.

CA storage - (Data were shown 2016-2017.)

<u>Study pre/postharvest factors influencing the chilling requirement for ripening capacity (CRRC) of pears.</u>

ACU and harvest maturity - (Data were shown 2016-2017.)

Ca content – (Data are under analysis and will be included in the final report.)

Temperature and ethylene conditioning – (Data are under analysis and will be included in the final report.)

<u>Develop conditioning protocols for 1-MCP treated 'Anjou' pears.</u>

Late-harvest pears treated with 1-MCP - Late-harvest Anjou pears are prone to losses in firmness and green color, are more susceptible to superficial scald after removal from RA storage,

and develop coarse and dry texture at ripening. In recent hot seasons and years with labor shortages a significant amount of fruit may be harvested at over-mature stage with reduced storability. In this study, partial late-harvest pears treated with 150 ppb 1-MCP at LM1 and LM2 developed BJMT and controlled superficial scald after 6 months storage in RA storage at 30°F plus 7 d at 68°F (Table 2).

Table 2. Changes in RC, soluble solids content (SSC), titratable acidity (TA), BJMT, and superficial scald (SS) of commercial maturity (CM) and late-harvest maturity (LM1 and LM2) of 'Anjou' pears from MACREC on day 7 at 20 °C affected by 150 ppb 1-MCP following storage at –1.1 °C for 4 and 6 months.

Harvest periods	Treatment	Storage periods (months)	RC (lb.)	SSC (%)	TA (meq. L^{-1})	BJMT (1-4)	SS (%)
CM – (14.8 lb.)	Control	4	4.9 ± 0.6 b	13.0± 0.2 a	33.88 ± 1.77 b	3.6 ± 0.2 b	5.7 ± 1.2 b
	Control	6	3.9 ± 0.4 c	13.0± 0.3 a	22.33 ± 1.17 d	3.8 ± 0.1 a	25.34 ± 3.7 a
	1-MCP	4	11.9 ± 0.6 a	13.1± 0.3 a	37.77 ± 1.39 a	3.2 ± 0.2 c	0 c
	1-MCP	6	11.6 ± 0.3 a	13.2± 0.5 a	25.03 ± 0.64 c	3.1 ± 0.2 c	0 c
LM1 – (12.8 lb.)	Control	4	3.3 ± 0.5 c	13.0 ± 0.2 a	28.97 ± 0.31 b	3.6 ± 0.2 a	11.9 ± 2.1 b
	Control	6	3.6 ± 0.4 c	13.2 ± 0.3 a	24.07 ± 0.31 c	3.5 ± 0.2 ab	40.3 ± 5.9 a
	1-MCP	4	8.3 ± 0.6 a	12.9 ± 0.3 a	31.34 ± 0.12 a	3.1 ± 0.2 c	0 d
	1-MCP	6	6.6 ± 0.2 b	13.3 ± 0.4 a	28.04 ± 0.25 b	3.4 ± 0.2 b	3.7 ± 1.0 c
LM2 – (11.2 lb.)	Control	4	3.6 ± 0.4 c	13.2 ± 0.3 a	25.69 ± 0.03 c	3.6 ± 0.2 a	32.5 ± 5.6 b
	Control	6	4.6 ± 0.3 b	13.3 ± 0.5 a	22.87 ± 0.11 d	3.5 ± 0.2 a	58.7 ± 7.9 a
	1-MCP	4	6.6 ± 0.3 a	13.1 ± 0.4 a	28.97 ± 0.51 a	3.4 ± 0.1 a	3.6 ± 0.6 d
	1-MCP	6	6.1 ± 0.5 a	13.4 ± 0.5 a	26.49 ± 0.34 b	3.5 ± 0.1 a	5.9 ± 1.3 c

Different letters indicate significant differences between treatments at each harvest period according to Fisher's protected LSD test at $P < 0.05$.

Production elevation influenced the effect of 1-MCP on later-harvest pears (Table 3). Fruit treated with 1-MCP at FF ~12.5 lbs. from elevation at 688 ft. developed BJMT after 7 months storage in RA storage at 30°F plus 7 d at 68°F. Pears treated with 1-MCP from elevation at 1752 ft failed to develop BJMT after 7 months and did not develop RC after long-term storage.

Table 3. Changes in RC, SSC, TA, BJMT, and SS of late-harvest 'Anjou' pears on day 7 at 20 °C affected by 150 ppb 1-MCP and production elevation (Orchard 1 = 688 ft and Orchard 2 = 1752 ft) following storage at –1.1 °C for 5 and 7 months.

Production elevation	Treatment	Storage periods (months)	RC (lb.)	SSC (%)	TA (meq. L^{-1})	BJMT (1-4)	SS (%)
Orchard1 – 688 ft (12.5 lb.)	Control	5	3.3 ± 0.2 c	13.1 ± 0.3 b	24.29 ± 0.83 b	3.7 ± 0.1 a	51.3 ± 4.5 b
	Control	7	3.7 ± 0.2 b	12.5 ± 0.2 c	22.15 ± 0.47 c	3.5 ± 0.2 ab	63.3 ± 7.2 a
	1-MCP	5	6.7 ± 0.4 a	13.5 ± 0.1 a	26.15 ± 0.99 a	3.3 ± 0.1 b	2.6 ± 0.6 c
	1-MCP	7	3.7 ± 0.3 b	13.1 ± 0.4 b	24.34 ± 0.02 b	3.6 ± 0.2 a	5.1 ± 0.5 c
Orchard2 – 1752 ft (12.6 lb.)	Control	5	3.0 ± 0.1 d	13.2 ± 0.4 a	23.67 ± 0.02 c	3.8 ± 0.2 a	55.0 ± 4.7 b
	Control	7	4.2 ± 0.1 c	12.3 ± 0.2 c	21.74 ± 0.51 d	3.6 ± 0.2 a	66.0 ± 5.5 a
	1-MCP	5	10.3 ± 0.2 a	13.1 ± 0.4 a	29.67 ± 0.78 a	2.8 ± 0.1 c	1.1 ± 0.2 d
	1-MCP	7	8.3 ± 0.3 b	12.7 ± 0.2 b	28.11 ± 0.34 b	3.2 ± 0.2 b	5.3 ± 0.3 c

Different letters indicate significant differences between treatments at each harvest period according to Fisher's protected LSD test at $P < 0.05$.

***b.* Combination treatment of 1-MCP and ethylene in CA storage**

The 1-MCP+ethylene (1-MCP (300 ppb) + ethylene (300 ppb)) treatment recovered the RC of Anjou pears and controlled superficial scald development. Control and ethylene-treated fruit developed RC following 4-8 months storage in CA storage at 30°F plus 7 d at 68°F (Fig. 5A). 1-MCP inhibited RC for 8 months. The 1-MCP+ethylene treated fruit softened to 7.9 and 5.6 lb. after 6 and 8 months in CA storage at 30°F plus 7 d at 68°F, respectively. SSC of 1-MCP+ethylene treated fruit increased slightly during 8 months storage (Fig. 5B). TA in control fruit declined from 0.40% at harvest to 0.16% after 8 months of CA storage (Fig. 5C). TA in 1-MCP+ethylene treated fruit was higher than in the control or ethylene treated fruit, but lower than in 1-MCP treated fruit. BJMT of the control fruit was 3.8, 3.8, and 3.6 in 7 d at 68°F after 4, 6, and 8 months, respectively. 1-MCP treated fruit had BJMT lower than 2.7 during the same period of storage. The 1-MCP+ethylene treated fruit had BJMT at 2.8, 3.2, and 3.4 in 7 d at 68°F after 4, 6, and 8 months storage, respectively.

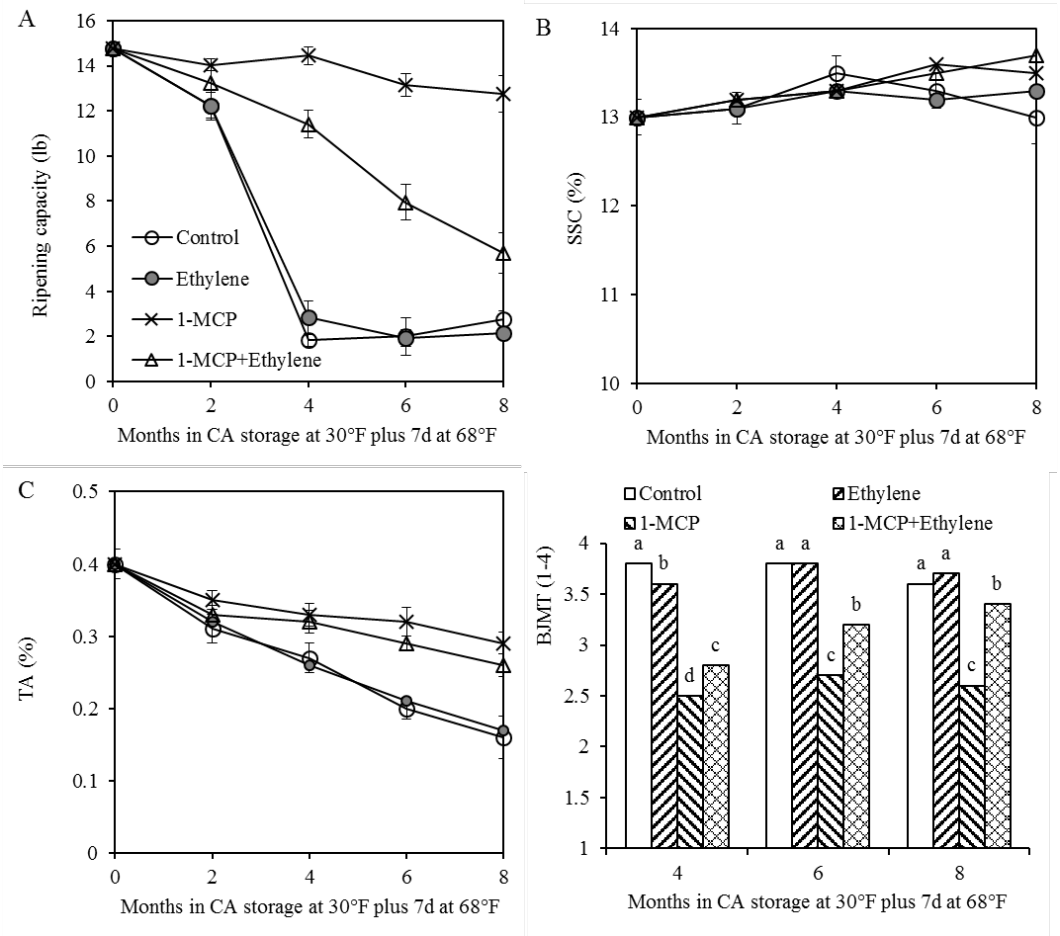


*Fig. 5. RC (A), SSC (B), TA (C), and BJMT (D) affected by 300 ppb 1-MCP and 300 ppb ethylene, alone or in combination with, in Anjou pears following 8 months of CA storage (*1.5% O_2 + < 0.05% CO_2*) at 30°F plus 7d at 68°F.*

CONTINUING PROJECT REPORT **YEAR:** 2 of 2 (NCE)
WTFRC PROJECT NUMBER: PR16-105

PROJECT TITLE: Dry matter assessment in pear and consumer perception

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Total Project Request: Year 1: $ 51,655 **Year 2**: $ 56,172

Other funding sources: None

WTFRC Collaborative Expenses: None

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	2016	2017	**2018 (NCE)**
Salaries[1]	24,000	24,960	**0**
Benefits[2]	8,414	8,750	**0**
Wages[3]	2,880	2,995	**0**
Benefits[4]	289	300	**0**
Equipment	0	0	**0**
Goods/Services[5]	14,572	17,667	**0**
Travel[6]	1,500	1,500	**0**
Plot Fees	0	0	**0**
Miscellaneous	0	0	**0**
Total	51,655	56,172	**0**

Footnotes:
[1] Salary for a new hire 50% Research Intern (Serra-Musacchi) paid the other 50% on other grant.
[2] Benefit on salary at 31.5%
[3] One non-Student temporary for 12 wks: 20hrs/wk at $12/hr (Serra-Musacchi).
[4] Benefits on temporary at 10% (Serra-Musacchi).
[5] Labware/consumable, fruit sample reimbursement (Serra-Musacchi), sensory panel costs (consumable and incentive advertising), electronic tongue: sensors, chemicals and glassware (Ross), publication (all).
[6] 2778 miles/year for domestic travel ($0.54/mile) to go to the orchard and to Pullman to meet co-pi and deliver fruit.

OBJECTIVES

1) Determine the reliability of the Felix F-750 Produce Quality Meter and therefore if this non-destructive dry matter assessment tool can be used as at harvest sorting step for more consistent fruit quality categories.

- **Test Reliability of d'Anjou Model Created in Prior Year (2016) On 2017 Harvest**
- **Develop a Bartlett-Specific Model and Evaluate its Accuracy**
- **On-Tree Monitoring of Dry Matter and Soluble Solids Content Prior to Harvest**
- **Instrumental Eating Quality Among Dry Matter Classes Determined at Harvest**

2) Assess if higher dry matter in pear translates into greater consumer liking and acceptability through consumer preference and sensory analysis studies.

- **Consumer Preference and Sensory Analysis (conducted in 2017 from 2016 Harvest)**

SIGNIFICANT FINDINGS

1) Objective 1- 2017 Priorities

Test Reliability of d'Anjou Model Created in Prior Year (2016) on 2017 Harvest

- "2016 d'Anjou models" performed similarly on both 2016 and 2017 harvests with no apparent reduction in accuracy between years.
- Predicted vs. actual dry matter and soluble solids content were highly correlated (>0.80 R^{2*} in most scenarios) with small magnitudes of error (< 0.60 RMSE[¥] in most scenarios) in applications both 1 and 6 months after 2016 harvest, and 1 month after 2017 harvest.

Develop a Bartlett-Specific Model and Evaluate Its Accuracy

- Models for dry matter and soluble solids content was developed for Bartlett variety with 0.86 and 0.84 R^2 and 0.39 and 0.43 RMSE for dry matter and soluble solids content, respectively.
- Model performance for both parameters decreased when model trained on d'Anjou was used to predict parameters on Bartlett, and vice-versa.

On-Tree Monitoring of Dry Matter and Soluble Solids Content Prior to Harvest

- Models for dry matter and soluble solids content can be developed up to two months prior to harvest with fair accuracy (0.20 to 0.95 % dry matter RMSE[¥]; 0.36 to 0.48 °Brix RMSE).
- Model performance degrades substantially when applied to time points other than what the model was calibrated for, particularly for dry matter models.
- Using a combined model developed over time is a fair compromise.
- Models can be used to detect quality differences in the field between fall and winter pruning treatments, though the differences were similar in magnitude to the average error of the models.

Instrumental Eating Quality Among Dry Matter Classes Determined at Harvest

- Sorting fruit in to predicted dry matter classes at harvest in 2017 produced similar results to 2016 – higher predicted dry matter groups had significantly lower I_{AD} index and higher soluble solids content on average.
- Weight, firmness, pH, and titratable acidity did not consistently vary among predicted dry matter classifications.

2) Objective 2- 2017 Priorities

Consumer Preference and Sensory Analysis (conducted in 2017 from 2016 Harvest)

- Fall and summer pruned trees produced the most preferred pears among all pruning treatments, with superior sweetness, pear flavor, and overall liking scores.
- Perceived juiciness, sweetness, and pear flavor increased with increasing dry matter classes.
- Higher dry matter fruit were significantly more favored overall, supporting fruit sorting by DM.
- Overall liking was best associated with perceived "pear flavor" ($\rho = 0.88$), followed by perceived sweetness ($\rho = 0.82$) and juiciness ($\rho = 0.70$).

* R^2 (coefficient of determination) =1 equals a perfectly linear correlation. ¥ RMSE = root mean square error of a model provides indication of the level of uncertainty to associate to future predictions.

MATERIALS AND METHODS

Test Reliability of d'Anjou Model Created in Prior Year (2016) on 2017 Harvest

A predictive dry matter and soluble solids content model (herein referred to as "2016 d'Anjou Model") was developed in 2016 on d'Anjou fruit harvested that year, the results of which were reported in the previous annual report. To evaluate the reliability of the model over time, it was applied to a second, 2017 harvest of the same orchard ("Orchard 1" as referred to in previous report). In a block of Anjou/OHF87 trained to central leader (planted in 1998 at 4.30 m x 2.45 m) four trees were selected for each of four pruning practices:

- Winter pruning 2017 (27 March 2017) + NO summer pruning 2017 =WP
- Winter pruning 2017 (27 March 2017) + Summer pruning 2017 (22 June 2017) =W+SP
- Fall pruning 2016 (1 November 2016) + NO summer pruning 2017 =FP
- Fall pruning 2016 (1 November 2016) + Summer pruning 2017 (22 June 2017) =F+SP.

Harvest 2017 occurred on September 11-12th (16 trees total). Fruit were immediately washed and placed in regular atmosphere cold storage (1 °C = 33.8 °F) for sorting purposes. Fruit were sized and measured (two readings per fruit) by a Felix F-750 Produce Quality Meter to acquire predicted dry matter (%) and soluble solids content (°Brix) prediction with the "2016 d'Anjou model" developed in the prior year. Fruit were sorted by dry matter from the lowest to highest % in to four dry matter classes (11.00-12.99, 13.00-13.99, 14.00-15.99, 16.00-16.99 % predicted dry matter) and among three experimental pullouts (T0 quality, 1 month after storage in October 2017; T1 quality, 5 months after storage to be conducted in February 2018; and T1 consumer testing, also to be conducted in February 2018). The accuracy of the "2016 d'Anjou model" was evaluated by comparing destructive values obtained at 2017 T0 quality to the predictions made by the model of dry matter and soluble solids content at harvest. Instrumental fruit quality (fruit firmness, dry matter, soluble solids content, pH, and % malic acid) were evaluated between predicted dry matter classes of both 2016 T0 and T1 and 2017 T0.

Develop a Bartlett-Specific Model and Evaluate Its Accuracy

Predictive dry matter and soluble solids content models may or may not be cultivar specific for pear. To test this, dry matter and soluble solids content models were developed on Bartlett variety harvested in 2016 ("Orchard 3" in previous report) using similar methods as those used in the calibration of "2016 d'Anjou model" as previously described. 100 Bartlett fruit were used in model calibration with the incorporation three internal fruit temperatures (approximately 34, 68, and 90 °F) to reduce temperature-associated deviations in spectral signal. Model performance at calibration was evaluated on the basis of its coefficient of determination (R^2) and root mean squared error (RMSE). Both Bartlett and d'Anjou models were validated on separate fruit material of both varieties to evaluate performance outside of the calibration environment.

On-Tree Monitoring of Dry Matter and Soluble Solids Content Prior to Harvest

In order to monitor fruit quality on the tree during development, dry matter and soluble solids content prediction models were developed at 84, 112, and 140 days after full bloom (DAFB; 24 April 2017 as date of full bloom) using methods previously described (140 DAFB as harvest date; alternatively 2, 1, and 0 months prior to harvest). Models were developed on 24, 24, and 64 fruit samples for 84, 112, and 140 DAFB, respectively. Fruit used in model calibration were randomly sampled from WP and FP trees not used in the current study. Simultaneous to model calibration, one WP and one FP tree was selected for on-tree monitoring through the growing season. 15 fruit from each tree were randomly selected and measured with the Felix F-750 at 84, 112, and 140 DAFB. Models developed at each time point were applied to the respective data captured on-tree in order to estimate dry matter and soluble solids content through time as the fruit matured. Individual model performance, as well as a combined model integrating all time points, was evaluated on the basis of root mean squared error (RMSE). Pruning treatments were compared by their predicted dry matter and soluble solids content values in order to detect differences in fruit quality arising from a result of those pruning practices.

Instrumental Eating Quality Among Dry Matter Classes Determined at Harvest

At T0 quality assessment, weight, I_{AD} index, firmness, soluble solids content (°Brix), dry matter (%), titratable acidity (% malic acid), and pH were evaluated after seven days of room-temperature ripening. Dry matter classes were evaluated for differences in these parameters.

Consumer Preference and Sensory Analysis (conducted in 2017 from 2016 Harvest)

A sensory acceptance test was conducted to determine the degree of liking reported by untrained consumers of 2016 harvested pears representing four pruning treatments and six dry matter classes. Consumers were asked to rate their acceptance of the sensory attributes of a one-eighth slice of fresh pear using a 9-point 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). Applying this scale, consumers were asked about their acceptance of each pear's appearance, aroma, firmness, crunchiness, juiciness, sweetness, bitterness, pear flavor, and overall acceptability. Between samples, panelists were allowed a 30-second break and 45 seconds after the fourth sample for rinsing the palate with filtered water and unsalted cracker. Following an overall comment, each consumer was presented with a bowl of whole fruit of each treatment for visual evaluation of overall appearance. Responses were evaluated with ANOVA and post hoc SNK with pruning treatment and dry matter class as factors, and the relationships between sensory patterns investigated with a Pearson product-moment correlation coefficient (ρ).

RESULTS AND DISCUSSION

Test Reliability of d'Anjou Model Created in Prior Year (2016) on 2017 Harvest

d'Anjou model developed in 2016 performed comparably on fruit harvested in 2017 relative to fruit harvested in 2016 (Figure 1). Pooling all fruit among these 2016 (T0 and T1) and 2017 (T0 only) resulted in RMSE of 0.62 and R^2 of 0.85 between actual and predicted dry matter (436 fruit evaluated), and RMSE of 0.56 and R^2 of 0.83 between actual and predicted soluble solids content (1291 fruit evaluated). This indicates that one well-developed model can perform reliably year-over-year.

Develop a Bartlett-Specific Model and Evaluate Its Accuracy

At calibration, "2016 Bartlett model" performed well with an R^2 0.86 and 0.84 and RMSE of 0.39 and 0.43 for dry matter and soluble solids content models, respectively. These values are comparable to the "2016 d'Anjou model", which had R^2 0.94 and 0.91 and RMSE of 0.36 and 0.42 for dry matter and soluble solids content, respectively. Dry matter models performed better than soluble solids content models overall. Applying models to fruit not used in calibration, and of other varieties, reduced model performance (Table 1). Performance most notably decreased when applying either model to a pooled group of fruit consisting of both varieties originating from three different orchards, where both models displayed a high RMSE for dry matter of 1.08% and 0.82% for Bartlett and d'Anjou model,

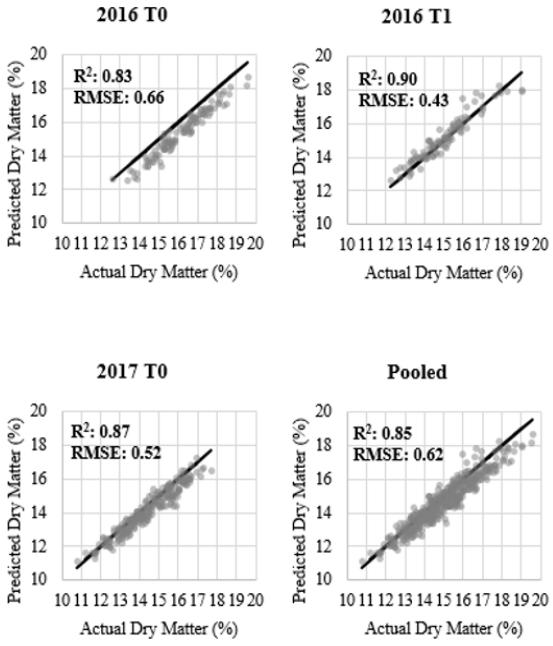

Figure 1: Actual vs. predicted dry matter for d'Anjou 2016 T0 (upper left) and T1 (upper right), 2017 T0 (lower left), and pooled quality pullouts (lower right). Solid line indicates a perfect (1:1) prediction.

respectively (Table 1). This high of an error is likely due to differences in fruit characteristics between those used in model calibration (at a uniform ripening and storage stage) and those used in this validation exercise (e.g. fruit of both varieties held in storage for either 1 or 6 months). Model performance for use on both varieties could likely be improved by incorporating both varieties in the calibration, as well as different ripening, and storage stages. A second Bartlett model will be developed in 2018 taking in account such differences in ripening stage and storage durations.

Table 1: Performance statistics for dry matter and soluble solids content models developed for d'Anjou and Bartlett pear applied to fruit material from 2016 harvest and evaluated for quality.

			Dry Matter (%)			Soluble Solids Content (°Brix)		
Variety	**Origin of fruit (2016 Harvest)**	**Model Used**	**n. fruit**	**RMSE**	**R^2**	**n. fruit**	**RMSE**	**R^2**
Bartlett	"Orchard 3"	Bartlett	120	0.78	0.78	382	0.69	0.77
		d'Anjou	120	0.78	0.78	382	0.71	0.76
d'Anjou	"Orchard 1"	Bartlett	203	0.84	0.72	944	0.50	0.81
		d'Anjou	203	0.71	0.80	944	0.52	0.79
	"Orchard 2"	Bartlett	75	0.74	0.87	217	0.76	0.83
		d'Anjou	75	0.66	0.90	217	0.72	0.84
d'Anjou & Bartlett	Pooled	Bartlett	398	1.08	0.67	1543	0.84	0.65
		d'Anjou	398	0.82	0.81	1543	0.68	0.77

On-Tree Monitoring of Dry Matter and Soluble Solids Content Prior to Harvest

Individual predictive models of dry matter and soluble solids content were developed two months prior to harvest with reasonably low error of prediction (RMSE values in Figure 2). However, when these models were applied to fruit at other stages of maturity, model performance was dramatically reduced (e.g. a dry matter model developed at 84 DAFB and applied at 140 DAFB suffers an 0.53 increase in RMSE, it means a less accurate prediction; Figure 2).

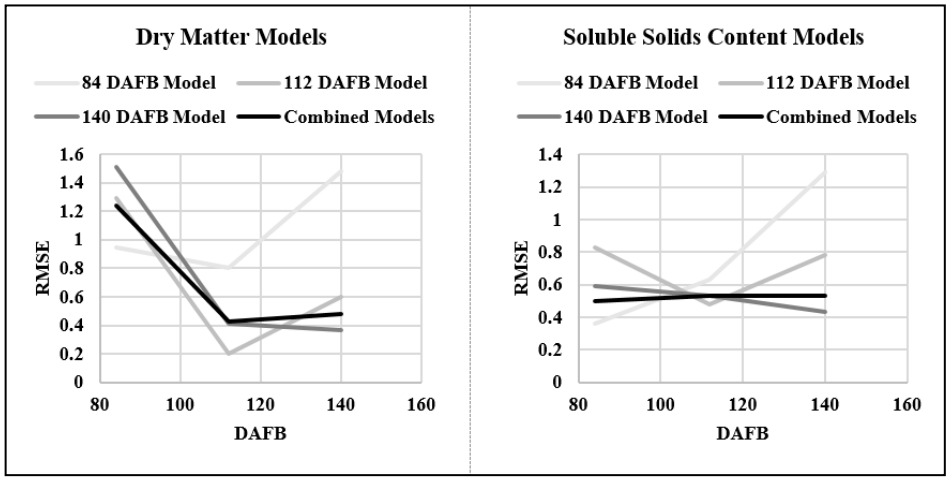

Figure 2: Dry matter (left) and soluble solids content (right) predictive model performance measured as root mean squared error (RMSE) as a function of time of calibration (DAFB) and application.

Combing models across all time points resulted in stable performance through time for soluble solids content, but not for dry matter predictions (Figure 2). This would indicate that changes in fruit tissue ultrastructure and water content throughout development strongly impact model performance, much more so for dry matter than soluble solids content.

A strong model for field use would therefore be made specifically for the anticipated time of use, or be the merging of a broad collection of time points to accommodate the rapid fruit development. Using the model developed at each time point, non-destructive predictions were able to be made for fruit left to mature on the tree. Averages of the predictions for 15 fruit each of winter and fall pruning (no summer) treatments revealed that fall-pruned trees had higher dry matter and soluble solids content in their fruit as early as two months prior to harvest (Figure 3). Through time, soluble solids content generally increased on average, while dry matter decreased, most notably between 1 and 2 months prior to harvest. This is likely due to rapid cellular expansion between 1 and 2 months prior to harvest, and the slowing down of growth approaching harvest. However, the prediction error for the models used for the predictions approached (or in some cases, exceeded) the difference in average dry matter or soluble solids content between pruning treatments. More accurate models are needed in order to reliably discern quantitative differences in fruit quality among pruning treatments.

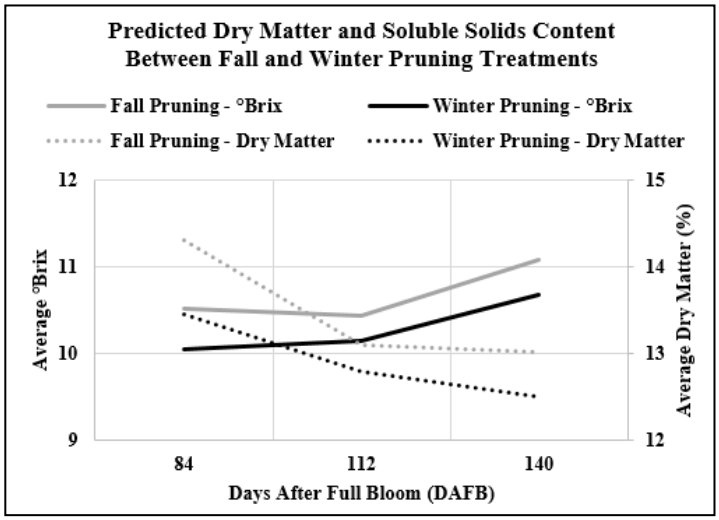

Figure 3: Average predicted dry matter and soluble solids content between fall and winter pruning treatments.

Instrumental Eating Quality Among Dry Matter Classes Determined at Harvest

At T0 in 2017, sorting fruit in to predicted dry matter categories also revealed similar trends in quality parameters between categories compared to 2016 (Table 2).

Table 2: Fruit quality parameters among predicted dry matter classes at T0 and T1 from 2016 harvest and T0 from 2017 harvest.

Harvest	Pullout	Predicted Dry Matter Class	Weight (g)		I_{AD} Index		Firmness (kg)		Dry Matter (%)		Soluble Solids Content (°Brix)		pH		Titratable Acidity (% Malic Acid)	
2016	T1	11-12.99	169	CD	1.36	A	0.9	CD	13.5	F	12.2	F	4.14		0.25	
		13-13.99	187	BC	1.03	B	0.8	D	14.1	E	13.3	E	4.07		0.25	
		14-14.99	205	AB	0.89	C	0.8	CD	15	D	14	D	4.07		0.24	
		15-15.99	217	A	0.82	CD	0.9	BC	15.7	C	14.6	C	4.07		0.24	
		16-16.99	205	AB	0.74	DE	1.1	B	16.7	B	15.4	B	4.07		0.24	
		17-18.14	165	D	0.63	E	1.3	A	18.3	A	16.6	A	4.03		0.23	
		Significance	***		***		***		***		***		NS		NS	
2017	T0	10-11.99	163	D	1.89	A	5.8	AB	12.5	E	10.5	E	4.20	A	0.28	B
		12-12.99	188	C	1.84	AB	6.2	A	13.7	D	11.6	D	4.06	B	0.31	AB
		13-13.99	208	B	1.79	B	6.0	A	14.3	C	12.5	C	3.98	C	0.33	A
		14-14.99	231	A	1.69	C	5.4	B	15.5	B	13.4	B	3.92	C	0.33	A
		15-15.99	242	A	1.68	C	5.3	B	16.2	A	14.5	A	3.95	C	0.33	A
		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.*

In all pullouts evaluated to-date (T0 and T1 2016, T0 2017), fruit in predicted higher dry matter classes regardless of pruning treatment had lower I_{AD} index and higher soluble solids content.
Differences in weight, firmness, pH, and titratable acidity were subtle or inconsistent. Dry matter predicted at harvest, seen here as related to both I_{AD} as a ripeness indicator and soluble solids content as a quality index, can therefore be used to create more consistent groups of fruit in terms of these parameters both at harvest and after cold storage. Quality evaluation of 2017 harvest in February 2018 will confirm these findings.

Consumer Preference and Sensory Analysis (conducted in 2017 from 2016 Harvest)
1896 pear samples were tested by lay consumers representing four pruning treatments and four dry matter classes. Among pruning treatments, fall and summer pruning (F+SP) ranked superior in perceived aroma, sweetness, pear flavor, and overall liking (data not shown). Generally, pruning in fall instead of winter produced more favorable fruit in terms of sweetness, pear flavor, and overall liking. Among predicted dry matter classes, overall liking notably increases along with increasing predicted dry matter class (Table 3). Perceived aroma, juiciness, sweetness, and pear flavor also exhibit this relationship with predicted dry matter with varying magnitudes and significance. The ranges defining each predicted dry matter class were unequal, and therefore it is unclear whether consumer liking scales linearly with increasing predicted dry matter. A second consumer panel planned for February 2018 will address this concern with more consistent class sizes.

Table 3: Perceived sensory attributes among predicted dry matter classes of 2016 harvested fruit.

DM Class	Appearance	Aroma		Firmness	Crunchiness	Juiciness		Sweetness		Bitterness	Pear Flavor		Overall Liking	
11.00-12.99	6.88	6.25	C	6.81	6.26	6.32	B	5.98	C	5.47	5.94	B	5.87	B
13.00-13.99	6.77	6.47	BC	6.68	6.18	6.85	A	6.34	B	5.66	6.47	A	6.38	A
14.00-15.99	6.55	6.64	B	6.75	6.32	7.06	A	6.75	A	5.67	6.77	A	6.68	A
16.00-16.99	6.78	7.06	A	6.99	6.46	6.92	A	6.85	A	5.72	6.83	A	6.68	A
	NS	***		NS	NS	***		***		NS	***		***	

*$p<0.05$= *, $p<0.01$=**, $p<0.001$= ***, 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.*

Sensory attributes were each related to each other to varying degrees (Table 4). Each pairwise correlation to overall liking was highly significant at $p < 0.001$, indicating the sensory attributes measured exhibit some degree of multicollinearity with overall liking. Overall liking itself was best associated with perceived pear flavor ($\rho = 0.88$), followed by perceived sweetness ($\rho = 0.82$) and juiciness ($\rho = 0.70$). Perceived appearance, aroma, firmness, and crunchiness were poorly associated with overall liking relative to sweetness and juiciness ($\rho \leq 0.52$). These divergent, poorly-associated responses in consumer preference to perceived firmness and crunchiness indicate that instrumental measures of sugar content (and by extension, dry matter), as a proxy for sensory perception of sweetness, may be better equipped to predict customer satisfaction. This can support and justify the idea to sort in the future pear fruit by dry matter and soluble solid content via in-line grader machines in order to better satisfy consumer expectations.

Table 4: Correlation matrix of sensory attributes of 2016 harvested fruit.
*Values indicate Pearson product-moment correlation coefficients (ρ); asterisks indicate level of significance. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.*

Sensory Attribute	Appearance	Aroma	Firmness	Crunchiness	Juiciness	Sweetness	Bitterness	Pear Flavor	Overall Liking
Appearance	1	0.35	0.3	0.28	0.26	0.23	0.22	0.27	0.32
Aroma	***	1	0.32	0.29	0.33	0.38	0.28	0.41	0.41
Firmness	***	***	1	0.7	0.42	0.4	0.33	0.42	0.52
Crunchiness	***	***	***	1	0.4	0.38	0.37	0.38	0.45
Juiciness	***	***	***	***	1	0.7	0.44	0.64	0.7
Sweetness	***	***	***	***	***	1	0.56	0.81	0.82
Bitterness	***	***	***	***	***	***	1	0.59	0.63
Pear Flavor	***	***	***	***	***	***	***	1	0.88
Overall Liking	***	***	***	***	***	***	***	***	1

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (NCE)
WTFRC Project Number: PR-15-105

Project Title: Pear rootstock breeding

PI: Kate Evans
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Co-PI (2): Amit Dhingra
Organization: Washington State University
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Address: P O Box 616414
City/State/Zip: Pullman WA 99164

Cooperators: David Neale (UC-Davis); Stefano Musacchi (WSU-TFREC); Richard Bell (USDA-ARS WV); Joseph Postman (USDA-ARS Corvallis).

Total Project Request: **Year 1:** $63,499 **Year 2:** $112,138 **Year 3:** $97,616

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $34,133 (2017-2019)
Notes: "Greenhouse screening of 49 dwarf rootstock candidates" PI Dhingra, Co-PI Evans. Synergistic project to develop and establish pear rootstock seedlings.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

WTFRC Collaborative Expenses: None

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

Item	2015	2016	2017	2018
Salaries[1]	29,064	67,666	58,406	
Benefits[1]	10,501	22,116	17,463	
Wages[2]	5,760	5,990	6,230	
Benefits[2]	1,094	3,786	3,937	
Equipment & Supplies Pullman	6,500	6,500	6,500	
Equipment & Supplies TFREC	6,000	2,500	1,500	
Travel[3]	4,580	3,080	3,080	
Plot Fees	0	500	500	
Total	63,499	112,138	97,616	**0**

Footnotes:
[1]Salaries for Nathan Tarlyn (Research intern, Dhingra lab) and researcher to be appointed (Evans lab);
[2]Wages for time-slip labor for orchard management and trait phenotyping;
[3]In-state travel between collaborators and year 1 trip to Corvallis, OR for collection of propagating wood.

OBJECTIVES

1. Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.
2. Phenotyping established seedling populations for dwarfing.
3. Establish the Pear Rootstock Breeding Program.

This project aims to build on recent (and concurrent) research to develop a long-term, dedicated pear rootstock breeding program at the Tree Fruit Research and Extension Center, Wenatchee. Diverse germplasm collected from USDA-ARS Corvallis and seedlings derived from previously performed crosses, currently growing in Pullman, will be transferred to Wenatchee for establishment in the orchard and development of high quality phenotypic data essential to exploit the genomic data being generated in the Neale project (*PR-14-111*) and others. New germplasm will be produced using the traditional breeding method of crossing and selection. Parents for crossing within this 3-year proposal will focus on *Pyrus;* however, it is expected that should the breeding program continue, parents will also be sourced from other species, for example *Amelanchier* and Quince (*Cydonia oblongata*).

SIGNIFICANT FINDINGS

- Of the 64 accessions collected from the USDA-ARS Corvallis collection, 11 accessions have been successfully established in the micropropagation process ready for mass multiplication, 13 accessions are currently in the rooting tissue culture media, and 9 accessions have multiple clones that have rooted and are being maintained in the greenhouse. Remaining selections, that were initially established in TC were lost to infection or did not survive in TC. A new set of collection of the germplasm will be performed in spring 2018.
- A selected subset of the seedling populations were planted in replicated trials in Wenatchee spring 2017.
- First seedlings raised specifically for establishing pear rootstocks in 2017.

METHODS

Objective 1: Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.

a. Greenhouse phenotyping of rooting potential.

A diverse subset of accessions from the US pear germplasm repository (Corvallis, OR) has already been selected for genotypic analysis in the Neale project (*PR-14-111*). Hardwood cuttings of this set (plus commercial controls and as many other accessions as possible) will be collected straight after leaf fall of the germplasm to be tested. The absolute number of accessions tested will depend on the availability of sufficient propagating wood and on the size and number of wooden bins that we are able to obtain. Following removal of spines, the cuttings will be bundled into 50's and the ends cut flat and dipped into rooting hormone. Tops of the cuttings will also be sealed to stop dehydration. The bundles will be placed upside down in wooden bins lined with black plastic liners and filled with peat moss and maintained at temperatures around 15^{o}C (59F) until root callus starts to form (usually by the following January). Appearance of callus will be scored as an indication of rooting potential. Callused cuttings can be potted into soil-less media or stored at 4^{o}C (39F) until ready to plant. After 3 months of growth, plants will be uprooted, medium removed and extent of rooting and architecture documented.

Accessions that fail to produce roots as hardwood cuttings will be micropropagated to provide rooted shoots for (Objective 1b, below). Although typically in the breeding program these would be selected against, this germplasm may provide valuable parental alleles for size control of the scion.

Although new micropropagation facilities are available at the TFREC (Musacchi lab), making use of the considerable expertise and resources available in the Dhingra lab for micropropagation of *Pyrus* should expedite this process.

b. Phenotyping of dwarfing potential.
Ten rooted cuttings from each of the accessions rooted in Objective 1a (above) will be budded with a standard scion variety (to be determined, but most likely d'Anjou) and grown in pots in the greenhouse prior to planting in the field in a randomized block design. It is expected that this will be in two waves of planting, the accessions that root from hardwood cuttings would be the first wave followed by those that require micropropagation.

Trees will be grown in the field for the remainder of the project and shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. One problem that may be encountered is incompatibility of the scion to the rootstock. If this is the case, an alternative scion variety will be considered.

Depending on how fast we can determine a good dwarfing phenotype (which may be beyond the time frame of this project), we will also test the genomic loci previously reported to be involved in dwarfing (pear - *PcDw* locus [Wang et al., 2011]; apple - *Dw1* and *Dw2* loci [Celton et al., 2009, Rusholme Pilcher et al., 2008, Fazio et al., 2014]) to determine whether or not there is a good correlation in this germplasm. If well-correlated, these DNA-based tools will be a useful indication of dwarfing in new populations of seedlings. Should new DNA-based tools be developed from other projects within the timeframe of this project, we will also attempt to incorporate them where relevant.

Objective 2: Phenotyping established seedling populations for dwarfing.
Seedlings will be selected using the growth habit, precocity and floriferousness data generated in the Dhingra/Evans project and will be propagated *in vitro* and budded with a standard scion cultivar (most likely 'd'Anjou'). These seedlings are predominantly derived from the crosses 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' (reminder: the true parentage of OH×F 87 was recently identified as 'Old Home' × 'Bartlett'). The most dwarf individuals (short inter-noded) will form the bulk of those selected but some individuals from medium and high vigor groups will also be selected (up to a maximum of 50 individuals). Budded trees will be planted in the field; shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. Seedlings derived from the irradiated pollen that can be rescued in the Dhingra/Evans project will also feed into this phenotyping when available.

Objective 3: Establish the Rootstock Breeding Program.
A crossing program will be initiated to generate seedlings focused on the principal targets determined in the earlier PNW-funded project of size-controlling, precocity, good fruit size and finish, resistance to fire blight and pear decline, ease of propagation and winter hardiness.

Crosses will be made in year 1, fruit harvested and seeds collected in the fall. Those seeds will be vernalized and then germinated in the TFREC greenhouse in spring of year 2. Seedlings will be planted at close spacing in the orchard in Wenatchee (year 2) and budded with a popular scion cultivar (most likely 'd'Anjou') in year 3. Crosses will also be made in year 2 and year 3.

These seedlings would form the basis for an on-going, long-term breeding program. They will be grown using standard orchard practices and assessed annually (beyond the scope of this project) for vigor by measuring shoot length and trunk diameter. Bloom date and amount will be recorded

annually to determine the precocity of the seedling rootstock. Fruit data recorded will include harvest date, yield, size, skin finish, firmness, titratable acidity and ºBrix. Seedlings that are selected as dwarfing and precocious will be cut back to remove the scion and earthed up to promote the production of rooted suckers. This method has been successfully used by PI Evans in her previous rootstock breeding program at East Malling Research, UK.

RESULTS AND DISCUSSION

Objective 1a: Greenhouse phenotyping of rooting potential.

In 2016, shoots from all 64 accessions were collected. At the time, most of the trees were actively growing with some entering paradormancy. This variability is expected since the germplasm represented a diverse set of genotypes. Shoots from each accession were sorted into three categories for further processing. Majority of the shoots were surface cleaned and sterilized and divided into nodes and initiated in micropropagation process. A total of 50 – 75 buds each were initiated for each accession. Remaining shoots were divided into half with each half going into direct rooting in the greenhouse and the other half being placed in the cold to go dormant.

None of the shoots that were directly processed for rooting in the greenhouse rooted. The dormant material was surface cleaned and sterilized and divided into nodes and introduced into the micropropagation process over a couple of months into fall of 2016. The nodes in the micropropagation system required constant attention and triaging as and when fungal and bacterial infection appeared. At the time of this report, of the 64 accessions that were collected from USDA-Corvallis Pear Germplasm Repository in 2016, multiple clones from 11 accessions have been successfully established in the micropropagation process ready for mass multiplication in the future, 13 accessions are currently in the rooting tissue culture media, and 9 accessions have multiple clones that have rooted and are being maintained in the greenhouse (Figure 1 A – C).

The trees from which plant material was collected are old and have a substantial amount of pathogen load. This became evident from the initial amount of fungal and bacterial growth observed when the plants were introduced into the micropropagation system. At the start of the project, 25 accessions had been successfully established in the micropropagation system of which nearly 50% succumbed to infection. The ones that are now firmly established in the micropropagation process needed to go through several rounds of cleaning and sub-culturing consuming a large amount of time. Therefore, no repeat collection was done in 2017. We have since learned that it might be best to obtain both dormant material in Feb-March and actively growing material in April-May to increase our chances of having all of the material successfully established in micropropagation process. The remaining germplasm will be collected twice in 2018, and introduced into the micropropagation system.

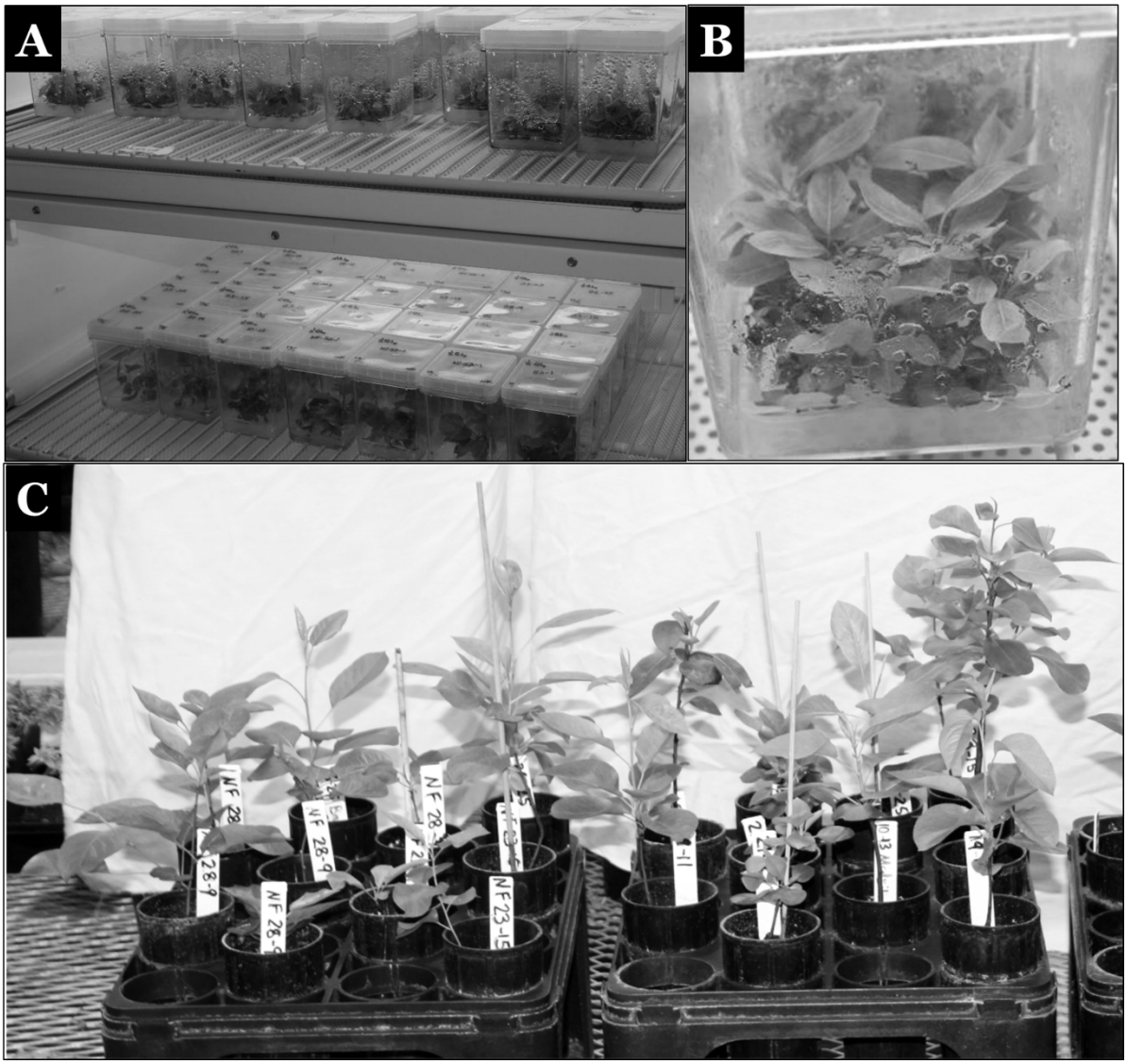

Figure 1: Establishment of USDA- Corvallis Germplasm in various stages of the micropropagation process. A. Various accessions with multiple shoots in tissue culture containers. B. A close up of one of the accessions. C. Various clones of some of the USDA accessions in the greenhouse.

Objective 2: Phenotyping established seedling populations for dwarfing

A subset of 13 individuals was selected for propagation from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' and three trees of each were planted in a randomized complete block design at the Columbia View orchard, Wenatchee, in spring 2017. The trees were budded with a standard scion (d'Anjou) in August 2017. Vigor data will be taken in 2018 and 2019.

Objective 3: Establish the Rootstock Breeding Program.

Five crosses were made in spring 2016 using parents such as 'Bartlett', OHF333, 'Old Home', two dwarf *P. communis* varieties and three interspecific hybrids. Just over 3000 seeds were extracted of which approximately 1000 were germinated. Extensive phenotypic data was collected through the 2017 season while the seedlings remained in the greenhouse. Seedlings were not planted immediately in the orchard due to concerns of predation. Instead, the potted seedlings were

overwintered in cold storage and will be planted at WSU Columbia View orchard in spring 2018. These seedlings will be budded with a standard scion in August 2018. Due to this delay, the no-cost extension was requested.
Nine crosses were made in spring 2017 using parents such as 'Nain vert', 'Bartlett', and 'Mustabey. Approximately 4,500 seeds were extracted. Seed will be sown in spring 2019.

Outreach

Dr Gokhan Ozturk, a pear breeder from Isparta, Turkey joined the Evans program in May 2017 for 9 months. He presented 3 Research News Flash presentations, Washington Horticultural Association Show, December 2017 and a HORT 509/510 seminar (January 2018). Dr Ozturk's visit was reported in the Good Fruit Grower (November 2017).

Amit Dhingra presented 'The foundation for the future of pear production in the PNW' Research News Flash talk at the Washington Horticultural Association Show, December 2017.

Chevreau E, Evans K, Chagné D, Montanari S. (in press) *Pyrus* spp. Pear and *Cydonia* spp. Quince. In: *Biotechnology of Fruit and Nut Crops*. Ed Litz, Alfaro and Hormaza. CABI.

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

Project Title: Greenhouse screening of 49 dwarf rootstock candidates

PI:	Amit Dhingra	**Co-PI**:	Kate Evans
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Email:	adhingra@wsu.edu	**Email**:	kate_evans@wsu.edu
Address:	155 Johnson Hall	**Address**:	1100 N. Western Ave
City/State/Zip:	Pullman, WA 99164	**City/State/Zip**:	Wenatchee, WA 98801

Cooperators: UC Davis project funded by Pear Bureau NW and Cal Pears.

Total Project Request: **Year 1**: 34,133 **Year 2**: **19,289** **Year 3**: 20,037

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $273,253 (2015-2018)
Notes: "Pear Rootstock Breeding" PI Evans, Co- PI Dhingra. Synergistic project to advance the selected pear rootstock seedlings via phenotyping and propagation.

Agency Name: WSU CAHNRS Ignite Program
Amt. awarded: $2500
Notes: Support in 2017 for an undergraduate student to perform phenotyping and tissue culture of selected seedlings.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2017)
Notes: Support for Danielle Guzman, Graduate student in the Dhingra lab.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

WTFRC Collaborative Expenses: None

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

Item	2017	**2018**	2019
Salaries[1]	21,000	10,920	11,357
Benefits	10,133	5,269	5480
Supplies[2]	1000	1000	1000
Travel	500	500	500
Plot Fees[3]	1500	1600	1700
Total	34,133	**19,289**	20,037

Footnotes:
1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants
2 – Greenhouse soil and supplies
3 – Greenhouse space usage fee per year

OBJECTIVES

1. Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform rooted material for subsequent grafting experiments.
2. Graft 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

This project represents one of the three distinct but complementary approaches to identify a source of dwarfing within *Pyrus communis*. The aim of the project is to evaluate if the dwarf habit of the seedlings will transmit to the scion. Promising selections out of the 49 dwarf seedlings are expected to be used as a rootstock selection, or a parent for the pear rootstock breeding program.

The project plan is to introduce all selections into tissue culture and establish enough clones for each selection in the greenhouse. These will then be grafted over with budwood from Bartlett and Anjou. OHxF 87 rootstock will be used as a control. The grafted plants will be grown and maintained in the WSU greenhouse to assess if the dwarfing trait is transmitted to the scion. Data on internode length, height and ratio between the two; crotch angle will be recorded. Seedlings that impart dwarfing to the scions will be evaluated as rootstock candidates in field trials to be performed after the completion of this project.

SIGNIFICANT FINDINGS

The seedlings being cycled through rapid growth process in the greenhouse have recently emerged from dormancy. Actively growing shoots are being processed and initiated in the micropropagation system during the months of Jan – Feb 2018.

METHODS

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

For the greenhouse screening, each of the 49 dwarf seedlings will be established in vitro to establish a source of developmentally and physiologically uniform, clean and genetically true to type plant material. These dwarf rootstocks have already been genetically screened and DNA fingerprint for each of the selections is already available which will be utilized to ensure genetic uniformity.

Briefly, axially buds from dormant or actively growing plant material will be surface sterilized with bleach and washed with autoclaved water prior to being initiated on to the basic pear initiation media standardized in the Dhingra lab. Once the buds have swollen and elongate into an initial shoot, the nodes would be excised and placed onto the pear bud multiplication media. Usually, a 3-4x rate of multiplication, obtained via suckering and elongation, per 4-5 weeks is achieved routinely in the lab. Give the genetic variability of the material being used, it is expected that the media may need to be standardized for some of the genotypes to achieve optimal growth and multiplication.

The goal would be to have a minimum of 50 plantlets per seedling established in tissue culture. This will provide a good and constant source of plant material for subsequent steps. For this experiment, 25 plantlets will be moved from bud multiplication media to rooting media. The rooted plantlets will be moved to the greenhouse, acclimatized and grown to a height of 18-24 inches in the greenhouse to achieve a minimum caliper of $1/4^{th}$ inches. Thereafter the rootstock plants will be forced into dormancy and maintained at 42 degree Fahrenheit till they are ready to be budded. Along with the 49 dwarf aneuploid selections, the current industry standard rootstock OH × F 87 will also be processed

in a similar way and will be used as a reference material in the experiment. Therefore, there will be a total of 50 selections each with 50 plants each in tissue culture which totals to 2500 plants. In the greenhouse, 1250 rootstocks (50 selections x 25 plants each) will be prepared for objective 2.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

Virus and disease free, genetically true to type Bartlett and Anjou budwood will be used to perform chip budding of 10 clones for each of the 50 rootstocks. Once the buds have callused and swollen, only 5 plants of each selection per scion will be maintained in the greenhouse for phenotyping of the habit imparted to the scions. The budded plants will be screened for number of nodes produced and height of the plant achieved over a set period of time till the plants go into paradormancy. Thereafter the plants will be provided 1000 hours of chilling and placed back in the greenhouse to initiate another spurt of growth. This aspect will be repeated for 2-3 cycles to identify the potential rootstock selections that are not dwarf on their own but also transmit the trait to the scion. The desirable aneuploid rootstocks will then be selected for field based evaluations as this project nears its conclusion.

RESULTS AND DISCUSSION

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

The 49 dwarf seedlings were obtained from crosses made in 2013. The growth of these seedlings has been fast tracked using horticultural rapid cycling process which includes providing ecodormancy (cold requirement) treatments in a cold room. The plant material was incubated in the cold for 4 months and has been recently moved to the green house, where the plant material is undergoing active growth (Figure 1). We are in the process of initiating nearly 50 buds per selection. It seems that we should be able to get all the selections introduced into the micropropagation system by end of March. The selections that fail to establish will be reinitiated after the next ecodormancy cycle for best response when introduced into the micropropagation process.

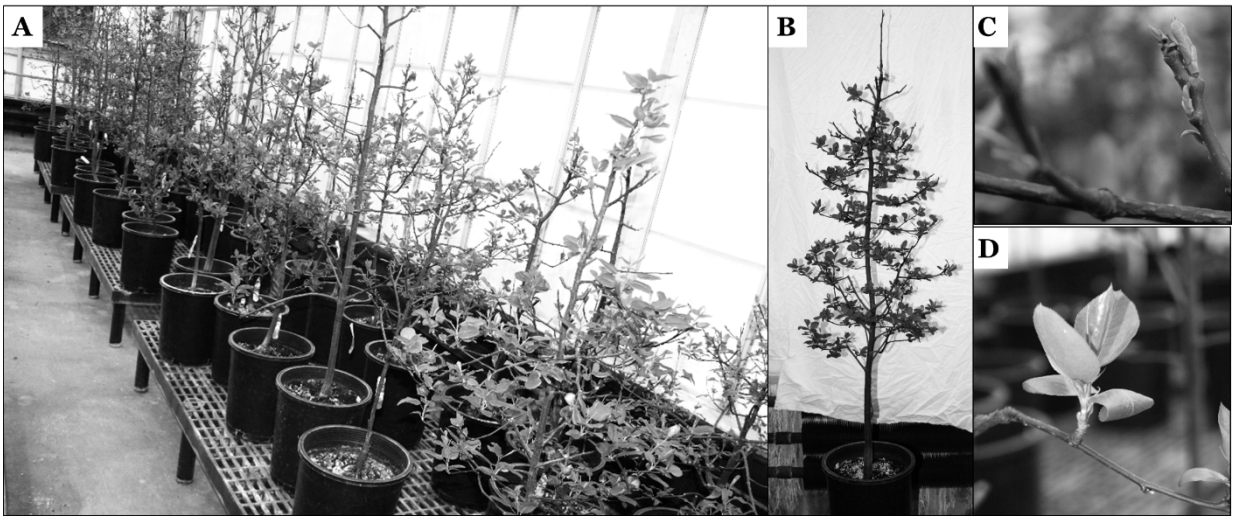

Figure 1: 49 dwarf seedlings in the greenhouse. A. An overview of all the seedlings. B. One of the selections exhibiting a compact growth habit. C and D – actively growing shoots that are being processed to be initiated in the micropropagation system.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

No results to report at this time.

Outreach

Presentation by Amit Dhingra - 'The foundation for the future of pear production in the PNW' Research News Flash talk at the Washington State Tree Fruit Association Meeting, December 2017.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: Interstem grafts to evaluate pear germplasm for dwarfing potential

PI: Joseph Postman
Organization: USDA Agricultural Research Service
Telephone: 541-738-4220
Email: Joseph.Postman@ars.usda.gov

Cooperators: Kate Evans, Washington State University

Total Project Request: **Year 1**: $18,000 **Year 2**: **$9,000**

Other funding sources

Agency Name: **USDA Agricultural Research Service, National Plant Germplasm System**
Amount awarded: **$22,000**
Notes: A collaboration with Nahla Bassil (USDA-ARS, Corvallis) and Sara Montanari (UC Davis) to work on Pyrus tree architecture and dwarfing rootstock potential. Half of the funds supported tree architecture evaluations and half supported assessing the SSR marker Hi01c04 to collect genotypic data on the presence of a locus in pear homologous to the apple *Dw1* locus.

Budget:

Organization Name: USDA-ARS **Contract Administrator: Richard Kimball**
Telephone: 510-559-6019 **Email address: Richard.Kimball@ars.usda.gov**

Item	2017	2018	
Salaries			
Benefits			
Wages	$12,000	$9,000	
Benefits			
Equipment			
Supplies	$6,000		
Travel			
Miscellaneous			
Plot Fees			
Total	$18,000	**$9,000**	

OBJECTIVES

It is difficult to clonally propagate prospective pear clones as self-rooted trees for rootstock trials, and a procedure to pre-screen selections for dwarfing ability will help focus resources. The USDA living pear germplasm collection includes numerous pear selections identified as potential rootstocks, and a very large and diverse assortment of pear selections and species that have never been evaluated as rootstocks or for dwarfing potential. The objective of this project is to evaluate whether interstem grafts can be used to identify pear selections that have dwarfing potential, and provide a relatively rapid assay for screening pear germplasm for prospective rootstock candidates. The goal was to top-work 10 grafts of each interstem with Bartlett and 10 with Bosc in order to obtain 8 successful combinations for field planting in year 2.

SIGNIFICANT FINDINGS

- The concept of generating interstem trees in the greenhouse in one season was accomplished, but a single season was not adequate for evaluating dwarfing potential.
- Interstem graft success with 20 grafts per candidate clone ranged from 0% to 100%, with a mean of 74% interstem survival after 4 months.
- 21 of 31 Bartlett/interstem combinations resulted in 7 or more successful combination trees.
- 16 of 31 Bosc/interstem combinations resulted in 7 or more successful combination trees.
- 610 interstem grafts were made. Nearly 400 cultivar/interstem trees are suitable for growing on in a field planting for size control evaluation.

METHODS

Interstem Grafts. Scions were collected in January 2017 from interstem candidates and from virus free mother trees of Bartlett and Bosc (for top-working) and stored at 4 °C (40 °F). After Tree Fruit Research Commission funding was awarded in April 2017 pear seedling rootstocks were planted in 2” x 10” deepots. An undergraduate student intern was hired for the summer to graft and manage the trees. Beginning in late May, 15 cm (6 in) long interstems were grafted onto seedling rootstocks in a cool greenhouse. Twenty grafts were made with each of 31 interstem candidates (Table 1). Approximately 2-3 weeks after interstem grafts were made, 10 were chip-budded with Bartlett and 10 with Bosc at the top of the interstem (Figure 1). Grafted trees were maintained in pots and flood irrigated in a shade-house during the growing season. Rootstock and interstem shoots were regularly removed during the growing season to force the cultivar buds. Interstem graft survival and cultivar top-graft survival was assessed in mid-July and again in mid-October. Length of cultivar bud growth was measured in mid-October.

Tree Architecture. Funding from USDA-ARS allowed collection of tree architecture traits for the suite of interstem candidates, continuing the work of Richard Bell to evaluate for traits potentially correlated with dwarfing potential. Amount and angle of side branching, tree form, stem spininess, fruit crop, and mean interstem length was measured on 10 current season shoots per rootstock candidate.

Goals for 2018. Interstem trees (29 interstem candidates, 397 total top-worked trees) will be lined out in a field nursery on 24 in. centers and grown for at least 1 additional year to assess dwarfing potential (Table 2). An inadequate number of combination trees was obtained for two candidates (*P. calleryana* ‘Crown Point’ and *P. elaeagrifolia* ‘Turkish Mist’) and these will not be included in further evaluations. Cultivar shoot height, stem diameter (to calculate stem cross sectional area), and branching will be evaluated at the end of year 2 and measurements will be compared to Bartlett and Bosc interstem controls (Bartlett on Bartlett interstem and Bosc on Bosc interstem). Tree architectural traits will again be evaluated for mother trees of interstem candidates. Funding is requested for seasonal student labor to establish trees and collect data.

RESULTS & DISCUSSION

Interstem Graft Success. Interstem graft success ranged from 0% to 100%, with a mean of 74% interstem survival after 4 months (Table 1). Graft success was ≥ 75% for 21 of 31 interstem candidates.

Bartlett & Bosc Top-graft Survival and Growth. Survival of Bartlett and Bosc top-grafts was 97% and 84% respectively on surviving interstems (Table 2). Low actual numbers of surviving cultivar grafts on some interstems was largely due to failure of the interstem graft. Had the interstems been grafted earlier in the season, instead of late May a greater success rate might be expected. Extension growth of Bartlett and Bosc top-grafts had tremendous variation due to some of the buds pushing very late in the season (Tables 3 and 4). Stem diameter measurements were meaningless as a result of this variation and should be much more meaningful at the end of the 2018 growing season.

Tree Architecture. Due to the large difference in ages of Genebank trees used in this study, tree form, precocity and fruit crop data is difficult to evaluate and likely cannot be correlated with interstem graft performance. Side branching angles, stem spininess and mean interstem length in current season growth may be more meaningful than other traits under germplasm collection conditions.

Discussion. The most useful result of this study is a proof of concept that combination interstem plus top-cultivar grafted trees can be easily produced in a single growing season. Of course, adequate size and quality of interstem scion pieces is necessary for success. Interstem grafts should have been made earlier in the year to get better survival and growth of cultivar grafts. It would be helpful to hard-prune prospective interstem candidates a year in advance to force the growth of good quality scions.

Conclusion. While interstem grafts may not be practical for assessing dwarfing potential in a single growing season, it is a much faster method for establishing cultivar grafts on large numbers of potential rootstock candidates for further evaluation.

Figure 1 – Successful interstem grafts recently chip-budded to Bosc and Bartlett.

Table 1 - Interstem Graft Survival

Interstems Grafted Late May - Early June 2017			July	October
interstem	anticipated dwarfing	grafts	% interstem survival	% interstem survival
×Crataegosorbus miczurinii	unknown	20	65.0%	45%
×Pyronia veitchii	unknown	20	100.0%	95%
Bartlett	control	20	100.0%	95%
Bosc	control	20	100.0%	95%
BP-1	semidwarf	20	85.0%	75%
BP-2	vigorous	20	100.0%	100%
BU 2/33 - Pyro II	dwarf	20	60.0%	45%
Le Nain Vert	(compact CV)	10	80.0%	80%
Mustafabey	(compact CV)	20	100.0%	100%
OHxF 69	semidwarf	20	100.0%	100%
OHxF 87	semidwarf	20	95.0%	95%
OHxF 97	vigorous	20	100.0%	80%
OHxF 333	semidwarf	20	100.0%	90%
P. betulifolia – Shaanxi	vigorous	20	90.0%	75%
P. calleryana CP-5-67	vigorous	20	15.0%	5%
P. calleryana D6	semidwarf	20	100.0%	75%
P. elaeagrifolia Gasparian 38	dwarf?	20	80.0%	70%
P. elaeagrifolia 'Sbkta'	unknown	20	100.0%	95%
P. elaeagrifolia 'Turkish Mist'	vigorous	20	0.0%	0%
P. fauriei 12-14	dwarf	20	95.0%	95%
P. korshinskyi	unknown	20	100.0%	90%
P. nivalis compact hybrid	(compact CV)	20	50.0%	40%
P. regelii	unknown	20	80.0%	55%
P. sachokiana	unknown	20	100.0%	80%
P. salicifolia (hybrid 2)	dwarf?	20	100.0%	95%
P. salicifolia (hybrid R)	unknown	20	100.0%	95%
P. spinosa ALB-038	vigorous	20	60.0%	60%
P. syriaca – Armenia	dwarf	20	85.0%	65%
P. syriaca – Israel	dwarf	20	15.0%	15%
Passe Crassane	(compact CV)	20	95.0%	90%
QR 708-12	dwarf?	20	95.0%	95%
		610	**82%**	**74%**

Table 2 - Bartlett and Bosc Top-graft Survival
Late May to Early June Interstem grafts - Late June to Early July Top Grafts

% of top grafts **on surviving interstems** only

	Survival October 2017		Survival October 2017	
Interstem	**Bartlett**	**Bartlett %**	**Bosc**	**Bosc %**
×Crataegosorbus miczurinii	3	100%	5	83%
×Pyronia veitchii	10	100%	9	100%
Bartlett	8	89%	8	80%
Bosc	8	80%	8	89%
BP-1 (Bien Donne 1)	7	88%	7	100%
BP-2 (Bien Donne 2)	10	100%	9	90%
BU 2/33 - Pyro II	4	100%	5	100%
Le Nain Vert	4	100%	3	75%
Mustafabey	10	100%	9	90%
OHxF 69	10	100%	8	80%
OHxF 87	9	100%	9	90%
OHxF 97	9	100%	4	57%
OHxF 333	7	88%	10	100%
P. betulifolia - Shaanxi	7	100%	6	75%
P. calleryana - Crown Point 5-67	n/a	-	1	100%
P. calleryana D6	8	100%	7	100%
P. elaeagrifolia Gasparian 38	4	67%	5	63%
P. elaeagrifolia 'Sbkta'	10	100%	7	78%
P. elaeagrifolia 'Turkish Mist'	n/a	-	n/a	-
P. fauriei Selection 12-14	8	89%	4	40%
P. korshinskyi 94011 - Kyrgyzstan	8	100%	10	100%
P. nivalis compact hybrid	3	100%	5	100%
P. regelii	3	100%	4	50%
P. sachokiana GE-2006-115	7	100%	5	56%
P. salicifolia (hybrid) sdlg. 2	10	100%	8	89%
P. salicifolia hybrid - Russia	9	100%	9	90%
P. spinosa ALB-2011-038	3	100%	6	67%
P. syriaca - Armenia No. 1087/62	7	100%	6	100%
P. syriaca No. 1 - Israel	1	100%	2	100%
Passe Crassane	8	100%	10	100%
QR 708-12	10	100%	8	89%
interstem trees for year 2 plot	**204**		**194**	

Table 3 - Mean length of Bartlett shoot growth after top-grafting on interstems

interstem	n	mean scion growth (cm)	min*	max
×Crataegosorbus miczurinii	3	14.7	6.5	21.5
×Pyronia veitchii	10	19.1	3	37
Bartlett	8	23.4	1	41
Bosc	8	19.4	14.5	29.0
BP-1 B92	7	25.3	2	44.5
BP-2	10	25.0	2.5	58
BU 2/33 - Pyro II	4	12.4	3.0	30.0
Le Nain Vert	4	11.5	7.0	16.0
Mustafabey	10	14.8	2.5	35
OHxF 69	10	14.3	6.5	36.0
OHxF 87	9	23.4	9.5	41.0
OHxF 97	9	8.9	0.5	19.0
OHxF 333	7	16.9	10.0	25.0
P. betulifolia - Shaanxi	7	16.4	8.0	36.0
P. calleryana CP-5-67	n/a	n/a	-	-
P. calleryana D6	8	8.7	2.0	18.5
P. elaeagrifolia Gasparian 38	4	11.4	9.5	15.0
P. elaeagrifolia 'Turkish Mist'	n/a	n/a	-	-
P. elaeagrifolia 'Sbkta'	10	20.5	5.5	40.0
P. fauriei 12-14	8	27.8	10.5	60.0
P. korshinskyi	8	9.3	4.0	14.0
P. nivalis compact hybrid	3	11.7	6.0	16.0
P. regelii	3	17.8	15.0	22.0
P. sachokiana	7	12.9	2.5	22.0
P. salicifolia (hybrid 2)	10	13.0	9.5	20.0
P. salicifolia (hybrid R)	9	16.2	6.5	28.0
P. spinosa ALB-038	3	22.8	10.0	39.5
P. syriaca - Armenia	7	20.5	12.0	35.0
P. syriaca - Israel	1	22.0	22.0	22.0
Passe Crassane	8	36.1	3.5	58
QR 708-12	10	9.5	0.5	18.0

* some bud grafts were late to form graft unions and produced very little new growth.

Table 4 - Mean length of Bosc shoot growth after top-grafting on interstems

interstem	n	mean scion growth (cm)	min*	max
×Crataegosorbus miczurinii	5	38.5	24.5	48
×Pyronia veitchii	9	25.9	1.5	55.5
Bartlett	8	21.4	1.5	59
Bosc	8	21.4	8.0	38.0
BP-1 B92	7	30.6	3	66
BP-2	9	23.5	1	71
BU 2/33 - Pyro II	5	13.2	2.0	26.0
Le Nain Vert	3	5.0	0.5	14.0
Mustafabey	9	23.9	1.5	58.5
OHxF 69	8	7.3	1.0	41.5
OHxF 87	9	15.0	1.0	37.0
OHxF 97	4	11.6	1.0	29.5
OHxF 333	10	25.9	1.0	43.0
P. betulifolia - Shaanxi	6	22.6	8.0	38.0
P. calleryana CP-5-67	1	31.5	31.5	31.5
P. calleryana D6	7	9.6	0.5	37.0
P. elaeagifolia Gasparian 38	5	3.4	1.0	12.0
P. elaeagrifolia 'Sbkta'	7	19.2	1.0	45.0
P. elaeagrifolia 'Turkish Mist'	n/a	n/a	-	-
P. fauriei 12-14	4	10.5	5.0	16.0
P. korshinskyi	10	18.8	1.0	49.0
P. nivalis compact hybrid	5	15.8	7.5	26.0
P. regelii	4	17.4	1.5	35.0
P. sachokiana	5	25.6	1.5	60.5
P. salicifolia (hybrid 2)	8	11.7	1.5	43.5
P. salicifolia (hybrid R)	9	14.4	1.0	30.5
P. spinosa ALB-038	6	20.5	1.0	37.0
P. syriaca - Armenia	6	16.5	3.0	34.0
P. syriaca - Israel	2	18.5	0.5	36.5
Passe Crassane	10	28.4	0.5	68
QR 708-12	8	17.4	0.5	54.0

* some bud grafts were late to form graft unions and produced very little new growth.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: Functional genomics of 'D'Anjou' pear fruit quality and maturity

PI: Loren Honaas
Organization: USDA-ARS
Telephone: 509.664.2280 x211
Email: loren.honaas@ars.usda.gov
Address: 1104 North Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Stefano Musacchi & Sara Serra (WSU-TFREC), David Rudell & Jim Mattheis (USDA-ARS), Claude dePamphilis (PennState)

Total Project Request: Year 1: $52,707 **Year 2: $33,488**

Other funding sources: USDA-ARS technician salary and benefits - $31,734

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

Item	2017	2018
Wages[1]	$12,500	$12,500
Equipment[2]	$1,980	NA
Supplies	$8,407	$5,483
Miscellaneous[3]	$29,820	$15,505
Total	$52,707	**$33,488**

Footnotes:
[1] Cooperative Agreement to Penn State for data processing and data analysis
[2] Service contract for CLC genomics workbench support
[3] Illumina sequencing & library prep at Penn State Genomics Core via Cooperative Agreement

Objectives:
1) **identify gene activity** correlated with fruit quality and maturity as it relates to on-tree fruit position
2) **discover genes** in 'D'Anjou' pear for comparative genomics with 'Bartlett' pear
3) **generate a list of potential biomarkers** for use in research and fruit production

Year 2 goals:
In year 2 initially we will focus on the final steps of objective 2 that includes validation of our "from scratch" gene predictions. Once validation is complete, we will use statistical analyses (including time course tests and correlated gene activity networks) to identify gene activity associated with fruit quality and maturity. Genes of interest that emerge from the statistical analysis represent an initial list of potential biomarkers. To begin the long process of validating biomarkers, we will examine other biological samples from "Improving Quality and Maturity Consistency of 'D'Anjou'" led by Stefano Musacchi (WSU Wenatchee). This examination will include targeted analyses of select genes and sequencing of additional samples.

Significant Findings:
- RNA from stored pear was high quality and resulted in successful library preparation
- The Bartlett genome is not a preferred reference for D'Anjou RNA-Seq
- RNA-Seq data from pear fruit was used successfully to build gene models *de novo*

Methods:
Tissues that are in storage from project "Improving Quality and Maturity Consistency of 'D'Anjou'" led by Stefano Musacchi (WSU Wenatchee) were extracted to obtain RNA using the Honaas lab's published protocol made specifically for European pear. The roughly 200 RNA samples were rigorously evaluated for quality and after pooling, cleanup, and concentration were provided to the Genomics core at Penn State for transcriptome sequencing. Library construction was optimized by pilot library preparation using test samples of pear fruit RNA. Each library was sequenced to a depth of ~20 million reads per sample (80 samples) for a total read volume of ~1.6billion. Reads were mapped to the Bartlett Genome (obtained from phytozome.jgi.doe.gov). Because the read mapping rate was very low to the genome (~50%), we constructed gene models from scratch (called *de novo* assembly) using a subset of the raw D'Anjou transcriptome data with CLC Genomics Workbench 9.5.3. To determine if the subset of data was enough to build the best possible gene predictions, we doubled the data amount and verified no improvement (expected based on previous published work from Honaas). We evaluated the gene predictions using metrics from the CLC assembly, plus additional analysis and improvement steps using state of the art tools (TransRate, PlantTribes, and Benchmarking Universal Single-Copy Orthologs - BUSCO).

Results & Discussion:
Stored D'Anjou fruit yield sufficient high-quality RNA for transcriptome analysis We processed tissue for 80 biological samples of D'Anjou pear with our in-house protocol. The RNA was of excellent quality (Figure 1) and the yield was sufficient for transcriptome library construction. Fully intact RNA has a RNA Integrity Number (RIN) of 10 and >8.0 is required for RNA-Seq. RNA of very high purity ($A_{260}/A_{280} \approx 2.0$) is also required. Our preps averaged RIN of 8.29 and A_{260}/A_{280} of 2.03 (Table 1). A_{260}/A_{230} (also in Table 1) is a useful, but not critical, assessment of protein contamination that is highly susceptible to RNA extraction protocol residues; our pilot library preparations combined with other metrics indicated the RNA was of sufficient quality. Because plant total RNA contains widely variable amounts of mRNA (the target of RNA-Seq) we pilot tested library preparation with preliminary samples. After optimization, library prep was 100% successful.

The 80 libraries were sequenced to a depth sufficient for robust statistical analysis (~20 million reads each). The overall data quality was very high, afforded in part by excellent quality starting material. The accepted minimum data quality is Q20 indicating 95% accuracy. Our data had an average score of >Q35, that is >99.5% accuracy, making it ideal for RNA-Seq and especially for gene discovery.

The Barlett Genome is not suitable for RNA-Seq data analysis in its current form We used the *Pyrus communis* Bartlett genome as a mapping reference for our D'Anjou RNA-Seq data. The cross-cultivar genetic differences presumably were very high resulting in reduced mapping rates of ~50%, meaning half of our D'Anjou data was not assigned to a Bartlett gene. A typical mapping rate for this type of experiment is 65-75% in a variety with a sequenced genome (for example Golden Delicious apple). The low rate is not surprising because this method is sensitive to genetic differences and other recent reports in the literature indicate similar mapping rates. Until my lab develops and tests a reference polishing algorithm, the Bartlett genome will have minimal direct utility. It will be useful to classify our custom gene predictions and develop a polished D'Anjou pear reference.

Gene predictions from raw D'Anjou data bring in 20-30% more data We hypothesized that the reason roughly half of our D'Anjou data cannot be matched to Bartlett gene models is due to genetic differences between the cultivars. To test this, we predicted gene models directly from the raw D'Anjou RNA-Seq data using an approach called *de novo* transcriptome assembly. This approach leverages a powerful computer (roughly 25 times more powerful than a modern laptop computer) and uses a sophisticated computer algorithm to transform hundreds of millions of reads (in our case each read is 150 letters or basepairs (bp) of DNA code) into tens of thousands of gene models that are typically 1000bp each of DNA code. Previous work from Honaas has shown that assembly errors include extensive fragmentation and high levels of redundancy in the predictions.

To address this problem Honaas has been part of a years-long project to develop error correction strategies. These proven and published strategies have been implemented by cooperator Claude dePamphilis (Penn State, and Eric Wafula in dePamphilis' lab) as a full standalone software in the latest release of a phylogenomics platform called PlantTribes. Within this pear project we are beta-testing the software that executes these proven strategies. Using PlantTribes, we have error corrected D'Anjou *de novo* assemblies to reduce redundancy, resolve fragmentation, and screen out bad predictions. Table 2 shows how assembly statistics change during each of these steps. It is expected that these "clean up" steps will remove a small amount of useful gene predictions, but the majority of the removed data represent low quality and redundant data. Using our raw predictions as read mapping references we mapped fully 80% of the RNA-Seq data. After error correction, in which half the predictions were removed due to low quality, we could still map ~70% of the RNA-Seq data, a 20% improvement over using the Bartlett Genome. This result supports our hypothesis that genetic differences are causing reduced mapping rates of D'Anjou RNA-Seq data to the Bartlett genome.

Perspectives:

The use of a high-quality RNA-Seq reference (genome or *de novo* transcriptome) is a necessary prerequisite for reliable data analysis. Because the Bartlett Genome is not suitable as a reference in its current form, we have focused on the gene discovery objective so as to provide the best possible RNA-Seq reference. Our other WTFRC project "Enhancing Reference Genomes for Cross-cultivar Functional Genomics" is utilizing *de novo* transcriptomes together with existing genomes to create a new RNA-Seq reference that overcomes the limitations of using either reference type alone. The genome sequence for *Pyrus communis* 'Bartlett' is a resource that should be useful to correct errors in the *de novo* assembly, and vise versa.

For the current project however, we will continue to refine and test the *de novo* (i.e. from scratch) assembly to meet the community standards that were, in part, articulated by my previous work. This refined and tested gene set will be sufficient for statistical analysis of gene activity for the current project.

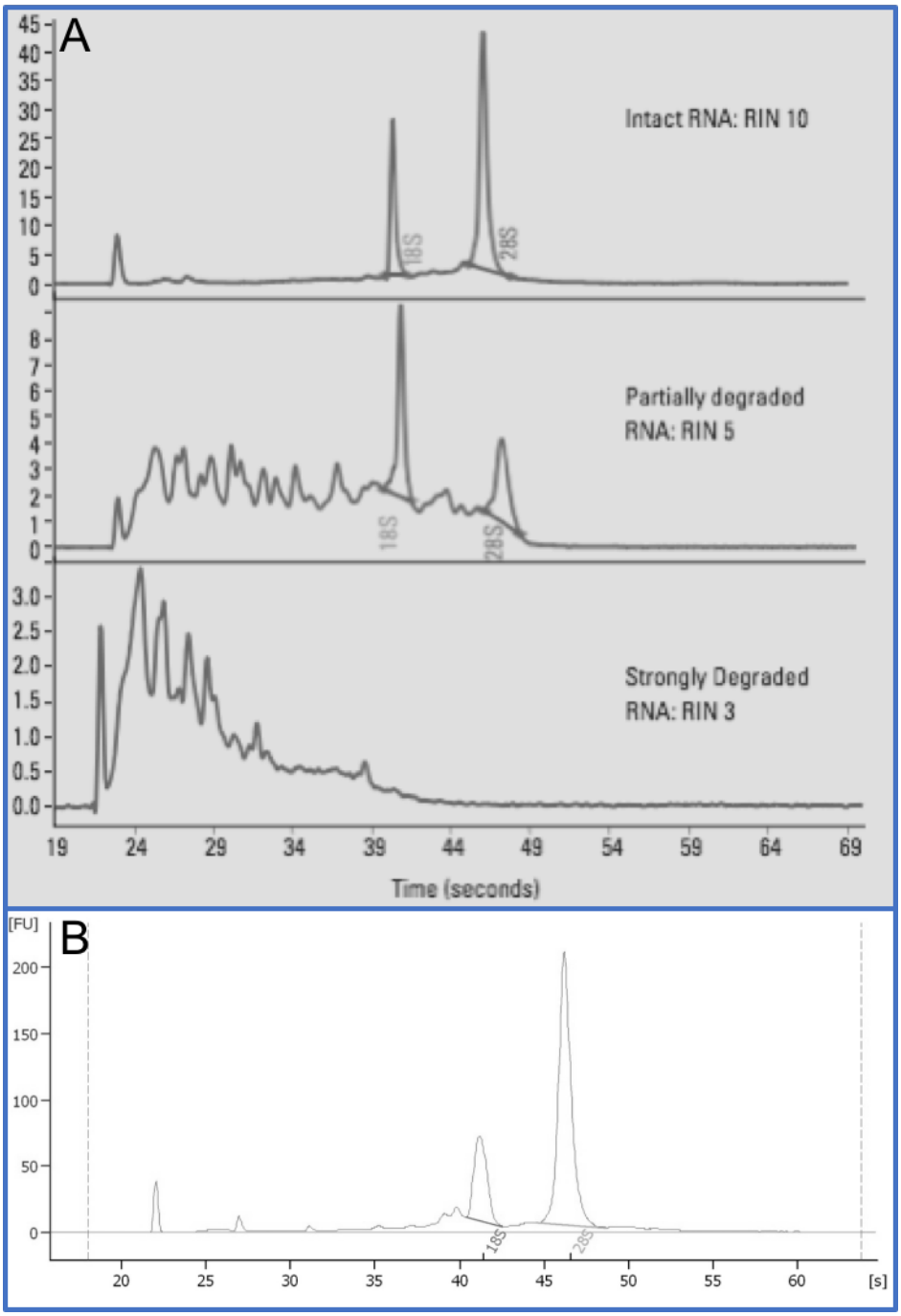

Figure 1. **A. Examples of RNA quality assessment using state of the art gel electrophoresis analysis with the Agilent Bioanalyzer (credit www.agilent.com).** The X axis is signal intensity, the Y axis is resolution time. The top panel shows fully intact RNA, the middle panel shows partial degradation of RNA, and the bottom panel shows severely degraded RNA. **B. RNA from stored pears is intact.** Clear 18s and 28s peaks indicate minimal degradation of RNA from stored fruit. This is excellent for a plant fruit RNA sample and better than results using a commercially available RNA kit.

Averages / Sample ID	RNA Conc. ng/µL	Nanodrop A260/280	Nanodrop A260/230	Bioanalyzer RIN
all	48.53	2.03	1.26	8.29
peel	52.77	2.11	1.32	8.29
cortex	43.89	1.95	1.20	8.30
external all	51.37	2.07	1.33	8.26
internal all	44.98	1.98	1.17	8.33
external peel	49.22	2.10	1.41	8.25
internal peel	47.99	2.11	1.19	8.34
external cortex	51.39	2.06	1.13	8.28
internal cortex	38.27	1.83	1.22	8.32
Time 0	71.53	1.97	1.42	8.29
Time 1	48.82	1.91	1.23	8.32
Time 2	45.77	2.08	0.90	8.37
Time 3	29.19	2.15	1.41	8.21
Time 0 peel	90.07	2.09	1.50	8.27
Time 3 peel	28.01	2.18	1.36	8.15
Time 0 cortex	53.00	1.85	1.34	8.32
Time 3 cortex	30.78	2.10	1.48	8.30

Table 1. **RNA quality and quantity summary.** Summary includes a selection of samples from multiple tissues and sample collection time points showing consistent high quality across the experiment.

Assembly quality categories	Primary assembly	Corrected Assembly
Number of gene predictions	83,395	41,435
Average gene prediction length	677	836
Encodes putative Protein (including redundant)	26,344	25,565
Matches to Pear Proteins (including redundant)	39,414	28,188
Pear proteins >90% coverage (including redundant)	5,287	4,290
Read data matched	~80%	~70%
TransRate Score (bigger is better)	0.119	0.150
Match to known plant gene	NA	41,435
Recovered BUSCOs	68.3%	63.8%

Table 2. **The *de novo* D'Anjou transcriptome is a suitable reference for RNA-Seq.** Redundancy is a frequent error in *de novo* assemblies, and correction of this error while maintaining gene content is challenging. Our PlantTribes error correction shows that while more than 40,000 gene predictions were removed, the ones that encode proteins are largely retained. **Transrate Score:** this score uses raw data information to validate gene predictions – it is highly conservative. **Match to known plant gene:** the PlantTribes analysis finds matches to known plant genes from other plant genomes, so this can be seen as a kind of filter for bad gene predictions. **Recovered BUSCOs:** BUSCO = Benchmarking Universal Single-Copy Orthologs. This is a similar process to PlantTribes, except that it looks for small set of widely shared genes (all land plants) - it can be used to estimate completeness of the predicted gene set. In this case, because we are only surveying genes activated in fruit we do not expect to capture more than roughly 2/3 of BUSCOs. Here we are using it to see *changes* in completeness for our predicted fruit genes.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: PR-17-105

Project Title: Assessment of organoleptic traits in sliced pears

PI: Amit Dhingra
Organization: Washington State University
Telephone: 509 335 3625
Email: adhingra@wsu.edu
Address: 155 Johnson Hall
City/State/Zip: Pullman, WA 99164

Cooperators: Blue Bird Growers – Ron Gonzales; Blue Star Growers – Smart fresh treated fruit; Crunch Pak: Ozgur Koc WSU: Seanna Hewitt and Scott Mattinson

Total Project Request: **Year 1:** $33,406 **Year 2: $34,402**

Other funding sources

Agency Name: WSDA
Amt. requested/awarded: $204,000 **(awarded)**
Notes: Willingness to pay and consumer preference studies

Agency Name: NIH Protein Biotech Training Program
Amt. requested/awarded: $52,234 **(awarded)**
Notes: Support for Seanna Hewitt, Ph.D. student includes stipend, travel, medical, tuition and fees

Agency Name: Crunch Pak
Amt. requested/awarded: $20,000 **(Requested)**
Notes: Support for pear slicing, packaging, purchase of fruit, labor and fruit quality analysis

Agency Name: USA Pears
Amt. requested/awarded: $7,000 **(requested)**
Notes: Support for economic analysis and consumer surveys

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

Item	2017	**2018**
Salaries[1]	16,800	17,472
Benefits	8,106	8,430
Supplies[2]	7500	7500
Travel[3]	1000	1000
Total	33,406	**34,402**

Footnotes:
1. Technical support for evaluation and analysis of fruit
2. Support for purchasing fruit, chemical compounds, and modified atmosphere bags
3. Travel to warehouses for fruit procurement

OBJECTIVES

1. Assess commercially valuable traits of sliced pears
2. Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment

The objectives of this project aim to quantify the profile and content of volatile compounds critical for a positive consumer experience. This information will complement the exciting results obtained from the consumer trials and willingness to pay study that has been recently concluded.

SIGNIFICANT FINDINGS

- Trial timelines were modified for them to be conducted in January and May of 2018 and then during the same months in 2019. Pears used for the first slicing trial in November had an average firmness of 14.5 lbf and were most likely treated with 150 ppb of 1-MCP. They were treated with 1% of ripening compound (RC) to induce ripening. However, the rate of ripening was too slow to quantify the volatiles. The fruit obtained in January has been pre-tested for ability to ripen with 1% RC and the fruit ripens as expected.
- Established a new collaboration with a colleague in Biosystems Engineering who plans to contribute to the evaluation of custom modified atmosphere bags with optimal gas transmission rates.

METHODS

Objective 1: Assess commercially valuable traits of sliced pears
Smart fresh (1-MCP)-treated and untreated Bartlett and Anjou pears will be sliced. The ripening compound treatment will be applied in conjunction with anti-browning solution provided by Crunch Pak. Typically, 1 gallon of this solution will be prepared with varying concentrations of the ripening compound. The sliced fruit will then be dipped in the solution for 1 minute prior to being packaged in three different types of modified atmosphere bags. The table below outlines the experimental plan to be used for this project.

MA Bags	**Control and 2 types of RC**			
Type 1	Packing dates	November	March	**Sampling strategy**
	Time line	Day 3	Day 3	5 sliced fruit bags/day/treatment
		Day 6	Day 6	
		Day 9	Day 9	
		Day 12	Day 12	**Objective 1 assessments**
		Day 15	Day 15	firmness
		Day 18	Day 18	brix
		Day 21	Day 21	shelf life
		Day 24	Day 24	

Type 2	Packing dates	November	March	**Objective 2 assessments**
	Time line	Day 3	Day 3	HPLC - sugars/
		Day 6	Day 6	GCMS - volatiles
		Day 9	Day 9	
		Day 12	Day 12	
		Day 15	Day 15	
		Day 18	Day 18	
		Day 21	Day 21	
		Day 24	Day 24	
Type 3	Packing dates	November	March	
	Time line	Day 3	Day 3	
		Day 6	Day 6	
		Day 9	Day 9	
		Day 12	Day 12	
		Day 15	Day 15	
		Day 18	Day 18	
		Day 21	Day 21	
		Day 24	Day 24	

Shelf life, firmness and brix of the sliced product with smart fresh treated fruit packed in November and March and packed in 3 different types of modified atmosphere bags will be assessed. Five, 2 oz. bags of sliced fruit will be sampled every 3 days till day 24 for these analysis.

Objective 2: Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment

Volatile compounds from dynamic headspace will be collected and identified by GC/MS. The volatile profiles are expected to characterize compounds in groups of esters, alcohols, hydrocarbons, aldehydes, and ketones. Five, 2 oz. bags of sliced fruit will be used for HPLC analysis which will quantify the relative amounts of sugars. Fruit will be sampled every 3 days till day 24 for these analysis.

RESULTS AND DISCUSSION

Objective 1: Assess commercially valuable traits of sliced pears

Since the sliced fruit derived from high firmness and 150 ppb of 1-MCP trialed in November demonstrated a very slow rate of ripening. The experimental timeline has been modified to conduct the trials in January and May of 2018 and 2019.

Objective 2: Evaluate flavor profile of the sliced fruit using HPLC and GCMS in a time course experiment

No results to report at present.

DISCUSSION

As the experiments are proceeding, consideration has been given to the fact that the modified atmosphere bags have a range of oxygen transmission rates (OTRs) and carbon transmission rates (CO2TR). It is known that the transmission of gases across packaging structures is governed by factors described by the Fick's law:

Jgas = *A x ACgas/R*

Jgas is the total flux of gas (cm3/s)
A is the surface area of the film (cm2)
Cgas is the concentration gradient across the film
R is the resistance of the film to gas diffusion (s/cm)

For any given fresh – cut produce, the choice of optimal OTR and CO2TR is dependent upon its respiration rate, weight, the internal package dimensions, the targeted atmosphere composition, and product handling temperature. Therefore it is necessary to select a modified atmosphere bag where oxygen and carbon dioxide transmission rates match the needs of the product. Carbon dioxide diffusion rates are two to five times faster than oxygen and the ratio of carbon dioxide transmission rate to oxygen transmission rate of a polymer in the bag is called as the beta value of a particular polymer. We are using bags of beta value 2, 3.5 and 5. It follows from this that the bags with beta value of 2, will release carbon dioxide slower, which may be useful for pears that have a lower respiration rates. In the past most of our experiments have been done with bags from Crunch Pak which are more likely suited for higher respiration rates of apples.

Outreach

Peer-reviewed publication - Ikiz D, Gallardo RK, Dhingra A, Hewitt S (2017) Assessing consumers' preferences and willingness to pay for novel sliced packed fresh pears: A latent class approach. Agribusiness

Good Fruit Grower article - Dhingra A, Gallardo K (2017) Customers are willing to pay a premium only on high quality, fresh sliced pears. In: Good Fruit Grower. Washington State Fruit Commission, Wenatchee, WA, pp 36-39

Presentation - Sliced Pears—How to add $1 million to the pear market's bottom line in the PNW. Seanna Hewitt and Amit Dhingra. Annual Washington State Tree Fruit Association Meeting, December 2017.