

2017 Technology Research Review
February 2, 2017
WA Cattlemen's Association, Ellensburg

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FINAL PROJECT REPORT

WTFRC Project Number: TR-15-102A

Project Title: Genetic analysis of Western Cherry Fruit Fly to facilitate species ID

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Percentage time per crop: Cherry: 70%

Apple: 30%

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1

Organization Name: UC Davis

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Item	2015	2016
Salaries	\$9,198	\$9,565.92
Benefits	\$3,716	\$3,864.63
Supplies	\$5,000	\$5,000.00
Miscellaneous	\$3,600	\$1,000.00
Total	\$21,514	\$19,430.55

Footnotes: Salary is to support a technician at 25% effort in both years 1 and 2 of the project. Benefits are calculated at UC Davis specified rate of 40.4%. Supplies include reagents for DNA/RNA extraction, Illumina sequencing library preparation, quality control of sequencing libraries, PCR enzymes, standard laboratory consumables and chemicals for molecular biology (PCR and agarose gel electrophoresis). Miscellaneous costs include transcriptome sequencing costs, which will be performed at BGI@UCD in Sacramento, CA, in Year 1 of the project. Miscellaneous costs for year 2 will be for publications and reporting costs to facilitate implementation of the diagnostic assay.

Budget 2

Organization Name: OSU-MCAREC

Contract Administrator: L. J. Koong

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Item	2015	2016
Miscellaneous	\$750	\$750
Total	\$750	\$750

Footnotes: Miscellaneous costs include costs for collecting and shipping insect samples from Oregon to California for transcriptome sequencing (Year 1) and testing and validating of the molecular diagnostic (Year 2).

JUSTIFICATION

The Western Cherry Fruit Fly (WCFF), *Rhagoletis indifferens*, is a serious pest of cultivated cherries in the Western U.S. and British Columbia, Canada (Yee et al. 2011; Yee et al. 2014a; Yee 2014b; Kumar et al. 2014). It damages the crop directly, and more importantly, becomes a quarantine and quality issue if found in fruit by domestic or overseas inspectors. Once fly larvae that remotely resemble WCFF are found by inspectors at packing houses or export facilities, fruit shipments are halted until a positive or negative species ID is determined. Distinguishing larvae from other insect species that infest cherries can be difficult, and rearing to adulthood for more reliable ID is not practical if marketability of the shipment were to be maintained. When even one suspect larva is found, an entire load of fruit can be rejected, and all subsequent fruit shipments will undergo intensive inspection to uphold the zero tolerance policy.

In order to (1) speed up species ID and (2) ensure reliability of ID to prevent false positives, which can lead to unnecessary quarantine measures and intensive inspection, both leading to increased economic burden to the Cherry industries, we propose to develop a molecular diagnostic test that can be used to rapidly (less than 2 hours) identify WCFF and differentiate it from other insect larvae, including the apple maggot (AMF), *Rhagoletis pomonella* (Green et al. 2013), which is an occasional pest in cherries, as well as the Spotted Wing Drosophila (SWD; *Drosophila suzukii*) (Beers et al. 2011; Walsh et al. 2011). There are a number of commonly used PCR-based molecular diagnostics that have been used for species ID, but these approaches often vary in cost and duration to obtain the results (Behura 2006, Garipey et al. 2007, Hebert et al. 2003, Williams et al. 1990, Wyman and White 1980). So far, only microsatellite markers have been designed for the molecular identification of WCFF (Maxwell et al. 2009; St. Jean et al. 2013), but results generated using microsatellites are generally difficult to interpret, even for trained scientists. The molecular diagnostic we propose to develop will be a simple, easy-to-interpret, one-step PCR amplification using WCFF-specific primers that is not dependent on sequencing or restriction enzyme digestion, procedures that add both cost and processing time. LAMP PCR approach, which will not require a thermocycler, will also be tested, and can potentially further reduce processing time. Our goal is to develop a molecular diagnostic that is easy to interpret, accurate, and require minimum processing time and equipment.

ORIGINAL OBJECTIVES OF PROJECT:

Objective 1: Sequence the transcriptomes (all expressed genes) of WCFF and perform bioinformatic and comparative sequence analysis with other insect pests of cherries (common and occasional) in the Pacific Northwest as well as closely related species to identify appropriate species-specific molecular diagnostic markers. Genetic analysis will also pave the way for future molecular analysis of WCFF to improve management strategies, e.g. evaluation of response to insecticide treatments and development of RNAi biopesticide (Year 1).

Objective 2: Develop an accurate PCR-based molecular diagnostic test to identify WCFF at all life stages from limited starting materials, e.g. a single larva. Both conventional and LAMP PCR will be tested to design a user-friendly and economical diagnostic assay. The assay will be validated using WCFF and closely related insect specimens (Year 2).

SIGNIFICANT FINDINGS:

- We obtained WCFF larvae, pupae, and adult samples and sequenced their transcriptomes.
- We completed the bioinformatic analysis to assemble the first transcriptome for WCFF.
- We collected closely related insect species as well as species that co-inhibit cherry hosts for validation of our molecular diagnostic (Table 1). Insect specimens include WCFF, apple maggot fly, olive fruit fly, walnut husk fly, blueberry maggot fly, and spotted wing Drosophila.

- We have successfully developed a molecular diagnostic to differentiate WCFF from apple maggot fly and spotted wing *Drosophila*. These three species co-inhabit the Pacific Northwest, and more importantly, at the larval stage, they share extensive similarity in morphology, which makes them difficult to differentiate and make a positive ID. We ensured that internal control primers (positive control for the PCR reaction) worked for all three species and that the divergent primers amplify only DNA from WCFF (diagnostic PCR). The protocol has been designed to be fast, simple, and reliable.

IN PROGRESS OR TO BE COMPLETED:

- A manuscript describing the transcriptome sequencing of WCFF and Apple Maggot fly, as well as development of the PCR diagnostic is in preparation.
- Currently, we are optimizing the protocol so that DNA extraction will no longer be necessary. We aim to finish the optimization and develop this direct tissue PCR diagnostic by April 2017, before the end of the project period.
- Three other closely related but non-target species, walnut husk fruit fly, olive fruit fly, and blueberry maggot fly, will be validated for the WCFF diagnostic. Based on sequence analysis of the diagnostic primers and internal control primers, we expect that the WCFF diagnostic primers will not amplify DNA from these three species.
- We will make a video protocol to illustrate the procedure of the diagnostic test, and it will be available to stakeholders and other interested parties.

RESULTS AND DISCUSSION:

Objective 1 (Year 1): Sequencing and assembly of WCFF transcriptome

Overview: In order to design an accurate and efficient one-step PCR diagnostic that can differentiate WCFF from other common and occasional cherry pest species, we first needed to obtain substantial sequence information of WCFF, which was not available. We therefore sequenced the transcriptomes of WCFF using different life stages as starting material. Sequencing the transcriptomes instead of full genomes will reduce cost of the project by at least 50%. Bioinformatic analysis was then performed to compare WCFF sequences with sequence data of other pest species that infest cherries, e.g. the apple maggot *Rhagoletis pomonella*, (Schwarz et al. 2009) and *Drosophila suzukii* (SWD) (Chiu et al. 2013) to design molecular diagnostic markers that can be used to differentiate these species (Figure 1).

RNA extraction, Transcriptome Sequencing, and assembly

Total RNA was extracted from individual specimens collected from the Pacific Northwest using Tri-reagent (Sigma). We collected WCFF larvae, pupae, and adults and generated three RNA sequencing libraries (Illumina) for each of the WCFF life stages. We then performed paired end sequencing on an Illumina HiSeq 3000 platform, and obtained a total of 778,742,672 100-bp reads.

Trimmomatic v0.35 was used to trim adaptor sequences and low quality ends for quality control. 99.07% of nucleotide bases were retained after trimming indicating high quality sequence data, and subsequently passed on to Trinity 2.1.1 (Grabherr et al. 2011) for transcriptome assembly. To reduce runtime and computing resource requirements, *in silico* read normalization was performed as part of the Trinity assembly process. A total of 230,770 transcript sequences and 204,659,650 bases were assembled. The transcript contig N50 is 1,943, demonstrating good quality sequence assembly. The GC content is 38.81%. Paired reads were mapped back to the assembly using STAR v2.5.0c and passed to Corset v1.04 for clustering into genes. Corset generated 96,628 clusters, representing possible number of expressed genes in WCFF. We anticipated that the number of expressed genes to be lower. Future genome sequencing can likely further improve WCFF

transcriptome assembly. Nevertheless, the transcriptome data we generated was sufficient for the development of molecular diagnostic for WCFF and apple maggot fly species ID.

Bioinformatic analysis to design species-specific diagnostic PCR primers

Bioinformatic analysis was performed to compare the WCFF transcriptome and sequence data from apple maggot fly, spotted wing *Drosophila*, and other closely related species which allowed us to design species-specific diagnostic PCR. Specimens of WCFF, apple maggot fly, olive fruit fly, walnut husk fly, blueberry maggot fly, spotted wing *Drosophila* have been collected in year 1 of the project to prepare for testing and validation of the molecular diagnostic. Although not within the scope of this project, comparative transcriptome analysis from different stages of WCFF will also advance our understanding of WCFF biology at different life stages.

Objective 2 (Year 2): Design a species-specific molecular diagnostic for WCFF

Overview: Our goal is to develop a PCR diagnostic that is easy to interpret, accurate, and requires minimum processing time and equipment. Upon completion of the project, we will work with stakeholders and scientists who are interested in implementing our species diagnostic into their monitoring or research programs.

Multiplex PCR primer design to identify WCFF

The multiplex PCR diagnostic test for WCFF relies on the use of two primer sets in a multiplex PCR reaction. The first set of primers was designed to amplify a product from WCFF, AMF, and SWD to confirm the presence of good quality DNA in the reaction and to verify the success of the PCR reaction. This represents our built-in quality control. Two separate internal control primer pairs were designed from the coding region of the “*Dark*” gene that is highly conserved among the three species based on our comparative genomic analysis to yield 427 and 462 base pair (bp) products (forward primer: 5'-TCAAATAAACACGAAGGCGC-3' and reverse primers: 5'-GTGGCACAAAATCGTATAATGC-3' and 5'-CATCAGATCGATCTGTGACGG-3' respectively) (Figure 2).

The second set of primers was designed from the Trehalose-6-phosphate synthase gene that is more divergent in sequence between the species of interest such that only the addition of WCFF DNA in the PCR reaction results in successful PCR amplification. We chose a primer pair that amplifies a 249 bp product (forward primer: 5'-GCGTTATGGATTATTCGCAG-3' and reverse primer: 5'-GGTATGTTGGAGGCTGAAATC-3').

Genomic DNA extraction and PCR reactions

To test the specificity of our multiplex PCR, we extracted genomic DNA from larvae, pupae or adult WCFF, AMF, and SWD, using a conventional CTAB protocol, which is routine in our lab, for use as template for PCR reactions. PCR was performed using Taq DNA polymerase (Life Technologies, Grand Island, NY) in a Mastercycler Pro PCR machine (Eppendorf, Hauppauge, NY). The amplified DNA products were resolved by agarose gel electrophoresis and visualized under UV light to assess (i) presence/absence of the two types of DNA bands, and (ii) size of DNA bands as compared to a size standard. To ensure utility of our WCFF diagnostic, we will validate the assay using specimens collected from a multiple geographical locations as genetic variations exist between populations from different collection sites.

Direct larval tissue PCR

Since PCR amplification using extracted genomic DNA has been successful, we are now proceeding to test our molecular diagnostic using crude larval extract as starting material. Our goal is to optimize our diagnostic to enable use of crude extract isolated from as little as one WCFF larva. The use of crude extract as starting material for the PCR will also greatly reduce necessary chemical

reagents as well as processing time to allow for SWD identification in less than 2 hours. Currently, DNA extraction adds about one hour to the entire procedure (total of 3 hours). Individual larvae will be cut in half with a sterile razor blade and incubated in PCR-grade water. The samples will be vortexed briefly and a small aliquot of the crude extract will be used directly as input to our PCR diagnostic. PCR amplification and DNA visualization will be the same as described above. We have previous success optimizing our species diagnostic for SWD (Murphy et al. 2015) to be performed using crude larval extract with DNA extraction.

EXECUTIVE SUMMARY:

We have designed an accurate PCR diagnostic that can unambiguously differentiate Western Cherry Fruit Fly from at least two other fly species that co-inhabit the Pacific Northwest in cherry crops, the Apple Maggot Fly and Spotted Wing Drosophila. These flies are difficult to ID through morphological examination, especially at the immature stages. Our diagnostic method relies on a multiplex PCR workflow that does not require sequencing or restriction digestion. We sequenced the transcriptome of the Western Cherry Fruit Fly and used a comparative genomic approach to facilitate the discovery of the diagnostic marker we presented here. In order to increase the utility of this PCR diagnostic, we are actively working to optimize this diagnostic method and further decrease the time of the workflow from 3 hours to 2 hours by eliminating the DNA extraction step. We anticipate the optimization to be completed by April 2017.

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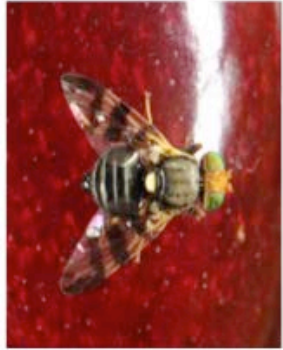
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Table 1: Specimens for validation of WCFF and Apple Maggot Fly molecular diagnostics

Common Name	Species Name	Source	Institution	Collector
Western Cherry Fruit Fly	<i>Rhagoletis indifferens</i>	Yakima, WA	USDA ARS Research Station	A. Abrams
Apple Maggot Fly	<i>Rhagoletis pomonella</i>	Hood River, OR	OSU	P. Shearer
Olive Fruit Fly	<i>Bactrocera oleae</i>	Davis, CA	Plant Pathology Field Station, UC Davis	N. Nicola
Walnut Husk Fly	<i>Rhagoletis completa</i>	CA	UC Berkeley	B. van Steenwyk
Blueberry Maggot Fly	<i>Rhagoletis mendax</i>	Benton Harbor, MI	Southwest Michigan Research and Extension Center	R. Isaacs
Spotted Wing Drosophila	<i>Drosophila suzukii</i>	Hood River, OR	OSU	P. Shearer



Rhagoletis indifferens
(Western Cherry Fruit Fly)



Rhagoletis pomonella
(Apple Maggot Fly)



Drosophila suzukii
(Spotted Wing
Drosophila)

Figure 1: Western Cherry Fruit Fly is difficult to differentiate phenotypically from closely related species, especially at immature stages. At the larval stage, it is difficult to identify WCFF from closely related species. More so, it does not vary much phenotypically from Apple Maggot Fly in the adult stage. All species co-inhabits the Pacific Northwestern of the U.S.

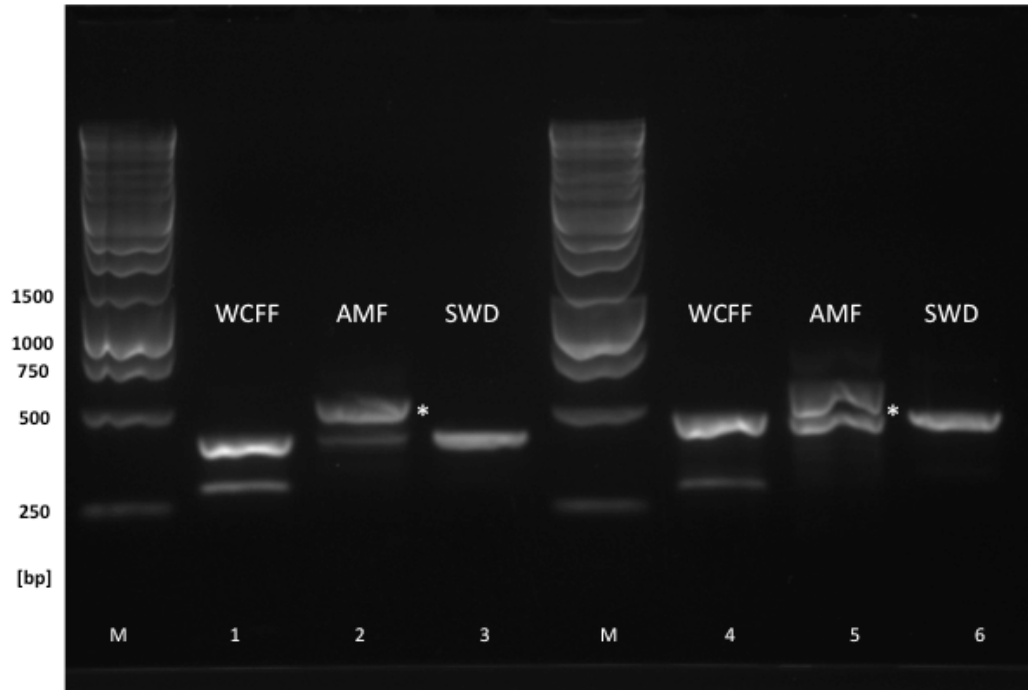


Figure 2: DNA agarose gel electrophoresis showing results of multiplex PCR. Multiplex PCR was performed using WCFF (Western Cherry Fruit Fly), AMF (Apple Maggot Fly), and SWD (Spotted Wing Drosophila). The top band (~427 bp for lanes 1-3 and ~462 bp for lanes 4-6) confirms the presence of high quality DNA in the PCR reaction. The lower band (~249 bp) confirms the identification of WCFF, e.g. in lanes 1 and 4. Therefore, two bands indicate that the organism is WCFF. The upper band present in lanes 2 and 5 (denoted by the asterisk) is a size variant of the gene amplified by the conserved internal control primers, and can be used to confirm the ID of AMF.

FINAL PROJECT REPORT

Project Title: Mechanical pruning in apple, pear and sweet cherry

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Cooperators: Olsen Brothers, Keith Oliver, McDougall & Sons, Columbia Fruit
Sara Serra (WSU-TFREC)

Percentage time per crop: Apple: 60% Pear:10% Cherry: 30% Stone Fruit:0

Other funding sources: None

Total Project Funding: 167,705

Budget History:

Item	Year 1: 2013	Year 2: 2014	Year 3: 2015
Salaries	26,295	26,307	27,359
Benefits	2,183	2,271	3,135
Wages	10,214	10,503	10,803
Benefits	844	878	913
Equipment	25,000		
Supplies	5,000	2,000	2,000
Travel	8,000	6,000	6,000
Plot Fees			
Miscellaneous			
Total	73,536	43,959	46,210

OBJECTIVES

The primary goal of this project is to establish best management practices for pruning PNW apple, pear and sweet cherry orchards mechanically.

- (1) Understand equipment and orchard requirements for successful operation of both a circular saw and sickle bar mechanical pruning system
- (2) Compare pruning technologies for their effects on fruit yield and quality
- (3) Conduct a preliminary economic assessment of mechanical pruning systems
- (4) Train an M.S. student in horticulture with extensive exposure to tree fruit horticulture, agricultural engineering and applied economics
- (5) Conduct demonstration trials and associated outreach activities

APPLE: *Fuji – September Wonder/Nic29, Spindle. Planted 2009*

SIGNIFICANT FINDINGS

- Machine dormant pruning is faster than hand pruning.
- The least amount of wood was removed in the dormant hand/summer mechanical (T3) plots, with an average of 0.42 lb wood/tree and 0.20 lb wood removed/tree, respectively. The greatest amount of wood was removed from dormant hand (T1) and dormant mechanical (T2).
- The number fruit/tree from dormant mechanical (T2) was 31% higher than number fruit/tree from dormant hand /summer mechanical (T3), with an average of 70.2 apples/tree. (Tab. 1)
- Apples from dormant hand treatment (T1) had 10% lower °Brix than those from dormant hand/summer mechanical (T3), with an average of 12.4 °Brix.
- Apples from dormant mechanical/summer mechanical (T4) had 46% more sunburn than the apples from dormant mechanical (T2), with an average of 7.6 apples with some degree of sunburn per tree.

METHODS

Trial block: Fuji/Nic 29 block trained to Slender Spindle at McDougalls & Sons (Mattawa) as a Complete Randomized Block Design. Pruning treatments are coded as follows:

Tmt code	2014		2015	
	Dormant pruning	Summer pruning	Dormant pruning	Summer pruning
T1	Hand	-	Hand (hedging and topping)	-
T2	Mechanical	-	Mechanical + hand cleanup (hedging and topping)	-
T3	Hand	Mech. 12-15 leaves	Hand cleanup (hedging and topping)	Mech. 12-15 leaves
T4	Mechanical	Mech. 12-15 leaves	Hand cleanup (hedging and topping)	Mech. 12-15 leaves

Dormant pruning performed on 3/10/2015 with the LaGasse hedger. All rows were topped manually with use of a platform. Data collection included time to prune each plot and weight of wood pruned per plot. Total time/tree includes hedging and topping (s/tree/person). Summer pruning at 12 leaves was done on 5/29/2015. Data collection included time to prune and weight of wood. Wood that was pruned was taken to the lab to record fresh and dry weights.

RESULTS AND DISCUSSION

Times recorded for mechanical pruning were 68%, 62% and 40% faster than times recorded for hand pruning. The average speed for hand pruning was 34.6 s/tree while the average time for mechanical was 12.3 s/tree and 20.6 s/tree. The average time to prune a tree by hand at dormant timing was 34.6 s/tree. This was 40% slower than dormant mechanical (T2) and 60%, and 54% slower than dormant hand/summer mechanical (T3), and dormant mechanical/summer mechanical (T4), respectively. T3 and T4 were the fastest pruning treatments, and did not differ among themselves

Yield

- Number fruit/tree from dormant mechanical (T2) was 31% higher than dormant hand/summer mechanical (T3).
- The lowest yield (lb/tree) was observed in the plots from dormant hand/summer mechanical (T3)
- Yield (lb/tree) from T3 was 29% and 25% lower than yield from T2 and T1, respectively.
- Yield and yield efficiency from T1 and T2 did not differ from each other.
- Yield efficiency from T1 did not differ from T3, as it did in lb/tree.
- Yield efficiency from T3 was 25% lower than yield efficiency in T2.
- The highest lb/fruit was observed from T1, which was 15% higher than T2, T3 and T4.

Tab. 1 Effect of four pruning treatments on averaged harvest metrics for 'Fuji'

TMT	TMT	Number fruit/tree	Kg/Tree	lb/tree	Yield Efficiency (kg/cm ²)	Yield Efficiency (lb/cm ²)	Total kg/tree	Total lb/tree
T1	Dormant Hand	54.47	14.09	31.06	1.14	2.51	0.26	.57
T2	Dormant Mechanical	70.27	15.01	33.09	1.27	2.80	0.22	.48
T3	Dormant Hand + Summer Mechanical	48.47	10.63	23.43	0.95	2.09	0.22	.48
T4	Dormant mechanical + Summer Mechanical	58.67	12.90	28.44	1.25	2.75	0.22	.48

APPLE: *Cripps Pink - Maslin /M9-337, Spindle. Planted 2012*

SIGNIFICANT FINDINGS

- Trees mechanically pruned in summer and winter + summer showed the same pruning weight.
- Trees that were mechanically pruned in summer only had higher yields than trees pruned in winter only by hand or machine and those that were mechanically pruned in winter and summer.
- At harvest, the number of fruit per tree, net weight of fruit, and yield efficiency was significantly lower in the control than the other treatments. However, the weight of the fruit in the control was significantly higher than other treatments

METHODS

Four treatments: dormant mechanical pruning, summer mechanical pruning, dormant and summer

mechanical pruning, and control (hand dormant pruning). All treatments trees were thinned to remove damaged and undersized fruit. Fruit was harvested on 9/28 for all the treatments; fruit from 9 representative trees per treatment were sized to assess the fruit size distribution at harvest. Some fruit in all treatments dropped a few days prior to harvest and fruit from 3 trees per winter and control treatments were sized. Weight, background and over-color, DA index, starch, firmness, and SSC (brix) were recorded.

RESULTS AND DISCUSSION

Hand pruning in the winter (control) removed more material than mechanical and hand pruning (Fig 1). Trees mechanically pruned in summer and winter + summer showed the same pruning weight, however significantly more fruits were removed when the trees were mechanically pruned in summer alone than in both seasons (Fig 2). Thinning in June, both total weight of fruit removed and weight of individual fruits per control tree were significantly heavier than other pruning treatments.

At harvest, the number of fruit per tree, net weight of fruit, and yield efficiency was significantly lower in the control than the other treatments. However, the weight of the fruit in the control was significantly higher than other treatments (Tab 1).

Tab. 1 - The effect of four pruning treatments on averaged harvest metrics for Cripps Pink

Treatment	Count of fruit/tree	Net weight fruit kg/tree	Net weight fruit lb/tree	Fruit weight g	TCS A cm ²	Yield eff kg/cm ²	Yield eff lb/c m ²	Metri c ton/A	US ton/A
winter pruning	111.67 a	22.04 ab	48.59 ab	197.6 2 b	15.0 9	1.48 a	3.26	32.0 0 ab	35.27 ab
summer pruning	115.89 a	23.64 a	52.11 a	205.6 4 b	15.6 1	1.40 a	3.09	34.3 2 a	37.83 a
mechanical winter+summer	97.44 a	19.28 b	42.51 b	199.3 9 b	14.4 5	1.36 a	2.99	28.0 0 b	30.87 b
Control hand only	62.33 b	14.10 c	31.09 c	226.3 1 a	14.3 2	1.01 b	2.22	20.4 7 c	22.57 c
Significance	***	***	***	***	NS	**		***	***

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

Arithmetic means are presented; post hoc tests were done with LSMEANS option and the Bonferonni adjustment provided letter.

PEAR: Bartlett/ OHF87, Spindle. Planted 2012

2015 SIGNIFICANT FINDINGS

- Hand pruning (control) Bartlett trees resulted in the removal of less total wood per tree than mechanical + hand pruning. (0.88 lb/tree and 1.34 lb/tree respectively)
- No statistical difference in vigor, as determined by trunk cross sectional area (TCSA), was found between the two pruning treatments.
- Hand pruning resulted in a greater yield than mechanical + hand (8.8 lb fruit/tree and 6.6 lb fruit/tree), but this difference was not found to be statistically significant.
- Mechanical + hand pruning produced a greater proportion of large fruit (>70mm diameter) than the control treatment (11.9% and 5.7%, respectively). (Fig. 1)
- Colorimetric readings indicated significant differences between fruit harvested from each pruning treatment; fruit from hand pruned trees were lighter in color, less green, and more yellow than fruit harvested from mechanical + hand pruned trees.

- No statistical differences between pruning treatments were found in fresh weight, dry weight, and percent over color, or SSC (Brix).

2016 SIGNIFICANT FINDINGS

- Mechanical winter pruning required 12 hours/acre, hand pruning took significantly longer at 19 hours/acre
- Hand pruning treatment resulted in twice the yield than mechanical + hand treatment (Table 1)
- Hand pruning treatment resulted in greater number of fruit per tree and higher average fruit weight than mechanical + hand treatment (Table 1)
- Mechanical + hand pruning produced less fruit in the larger category ($\geq 70\text{mm}$ diameter) than the hand pruned control (40% and 49%, respectively) and higher incidence of fruit in the 55mm diameter group (Fig. 2)
- Fruit harvested from hand pruned trees ($I_{AD} = 1.86$) were riper than fruit harvested from mechanical + hand pruned trees ($I_{AD} = 1.96$)
- Fruit harvested from hand pruned trees showed higher soluble solid content (SSC, Brix), titratable acidity and lower pH than fruit from mechanical + hand trees
- Fruit from hand pruned trees had a higher DM% than fruit from mechanical + hand trees (15.00% and 13.85% respectively)

METHODS

The mechanized pruning trial on Bartlett trees was conducted in Monitor, WA. The orchard was planted in 2012 at 5'x 12' and trained to a spindle (726 trees/Acre). The trees were separated into two treatments: 1) hand pruning (4 plots), and 2) mechanical pruning (4 plots) with additional hand pruning (mechanical + hand). Pruning was carried out in March (hand pruning) and in April (6.9 sec/tree). All pruning wood was collected and weighed.

All fruit was harvested from each tree in the trial and fruit was counted and weighed. Fruit were sized by diameter and separated into the following classes: $<55\text{mm}$, 55-60mm, 60-65mm, 65-70mm, and $>70\text{mm}$. Twenty-five fruit from each plot (4 plots per treatment = 100 fruit/treatment) and belonging to the 65-70mm size class were selected for fruit quality analysis and storage. After harvest all fruit were weighed and sorted into three I_{AD} classes (by DA meter readings): A, B and C (<1.9 , 1.9-1.99, and >2.0 , respectively), and split into two normal air (33°F) storage pull-out groups ($T_0=1$ month, $T_1=4$ months). The quality data reported here represent those only derived from the T_0 group. Quality parameters investigated include fresh weight and weight loss after storage, I_{AD} values before and after storage, flesh color ($L^*a^*b^*$, Minolta), percent red blushed overcolor, firmness (FTA, measured in kg), SSC (Brix), dry matter, pH and titratable acid (% malic acid).

RESULTS AND DISCUSSION - 2015

Pruning and vigor

In 2015, we found hand pruning Bartlett trees resulted in the removal of less total wood per tree than mechanical+hand pruning (0.88 lb/tree and 1.34 lb/tree respectively), but the difference was not statistically significant. No statistical difference in vigor, as determined by trunk cross sectional area (TCSA), was found between the two pruning treatments (18.9 cm^2 hand and 19.2 cm^2 mechanical+hand).

Yield and quality

Hand pruning resulted in a greater yield than mechanical+hand (8.8 lb fruit/tree and 6.6 lb fruit/tree), but this difference was not found to be statistically significant, while yield efficiency (yield per tree/average TCSA), differed significantly between the two pruning groups; hand pruning resulted in greater yield efficiency than mechanical+hand pruning (data not shown). In the comparison of

secondary fruit harvested from each pruning treatment, the mechanical+hand treatment averaged 33% fewer secondary fruit than the hand pruning treatment, but the difference was not statistically significant.

Mechanical+hand pruning produced a greater proportion of large fruit (>70mm diameter) than the control treatment (11.9% and 5.7%, respectively) and lower incidence of fruit categorized in the 55mm diameter group (20.2% compared to 25.5, Fig. 1).

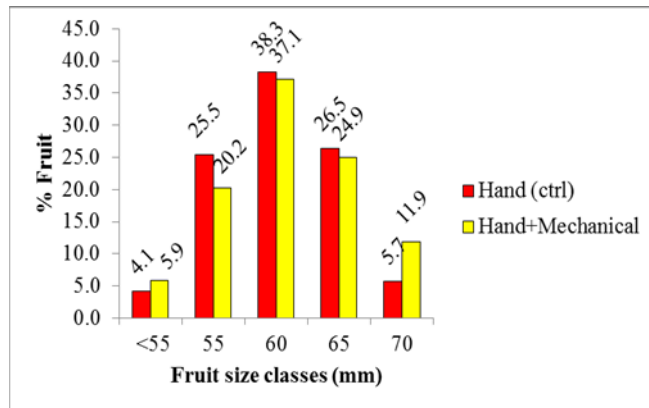


Figure 1: The effect of two pruning treatments on Bartlett fruit size distribution in 2015.

Fruit quality analysis revealed significant differences in measured I_{AD} values immediately after harvest, indicating fruit harvested from hand pruned trees ($I_{AD} = 1.96$) were more ripe than fruit harvested from mechanical+hand pruned trees ($I_{AD} = 1.99$). Differences in rate of I_{AD} change during ripening were statistically significant, suggesting fruit from hand pruned trees ripened quicker than fruit from mechanical+hand (data not shown). Comparison of firmness showed significant differences between fruit harvested from both pruning treatments (data not shown). Colorimetric readings indicated significant differences between fruit harvested from each pruning treatment; fruit from hand pruned trees were lighter in color, less green, and more yellow than fruit harvested from mechanical+hand pruned trees (Table 2). No statistical differences between pruning treatments were found in fresh weight, dry weight, percent over color, or SSC (Brix) (data not shown).

RESULTS AND DISCUSSION – 2016

Pruning and vigor

In 2016, hand pruning Bartlett trees resulted in less total wood removed per tree than mechanical+hand pruning (1.7 kg/tree and 2.8 kg/tree, respectively), but the difference was not statistically significant like the previous year (Fig. 1). No statistical differences in vigor, as determined by trunk cross sectional area (TCSA) and annual growth, were found between the two pruning treatments (27.45 cm² hand and 27.37 cm² mechanical+hand).

The mechanical pruning confirmed to save labor time: winter pruning was mechanically done in 12 hours/acre, while only hand pruning took significantly longer (19 hours/acre).

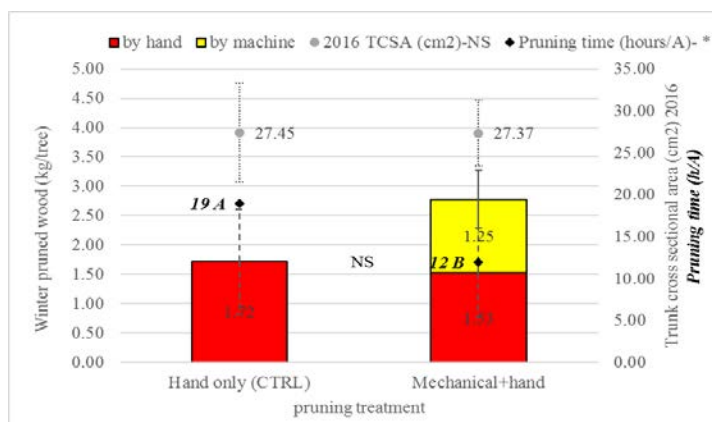


Figure 1: Weight of wood pruned by two pruning methods on Bartlett trees in March 2016, trunk cross sectional area (TCSA cm²) 2016 and pruning time.

Yield 2016

Hand pruning resulted in double yield than mechanical+hand (18 kg fruit/tree and 9 kg fruit/tree respectively), with higher number of fruit per tree and higher average fruit weight than mechanical+hand (188 g vs 162 g respectively). Hand pruning confirmed a greater yield efficiency than mechanical+hand pruning (Table 1). There was no significant difference in the number and weight of secondary fruit harvested from each pruning treatment. Mechanical+hand pruning produced less fruit in the larger category (≥ 70 mm diameter) than the hand pruned control (40% and 49%, respectively) and higher incidence of fruit in the 55mm diameter group (Fig. 2).

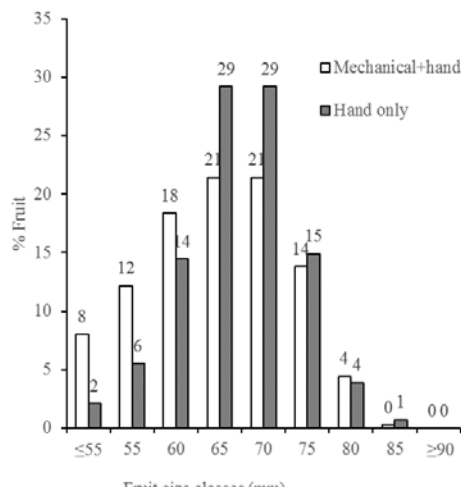


Figure 2: Comparison of Bartlett size at harvest 2016 between two pruning treatments.

Table 1: Bartlett yield in Monitor, WA August 2016

Treatment	Number fruit /tree		Net yield (lb/tree)		Avr. fruit weight (g)		Yield efficiency (lb /TCSA cm2)		Crop load (num. fruit /TCSA cm2)	
Pruning										
Mechanical+hand	53	B	18.9	B	162	B	0.69	B	1.93	B
Hand only	92	A	38.9	A	188	A	1.41	A	3.41	A
Significance	***		***		*		***		***	
Significance: * p<0.05, ** p<0.01, *** p<0.001. SNK as <i>post-hoc</i> test for means discrimination.										

Fruit quality 2016

Quality analysis revealed significant differences in measured I_{AD} values after harvest ($T_0=1$ month after harvest), indicating fruit harvested from hand pruned trees ($I_{AD} = 1.86$) were riper than fruit harvested from mechanical+hand pruned trees ($I_{AD} = 1.96$). Differences in rate of I_{AD} change during ripening were statistically significant, suggesting fruit from hand pruned trees ripened quicker than fruit from mechanical+hand (Table 2). Colorimetric parameters (hue and chroma) were not different between treatments, while % red overcolor seemed to be higher in mechanical+hand fruit (difference not statistically significant). Fruit harvested from both pruning treatments reported similar firmness values (Table 2). “Hand pruned” fruit showed higher soluble solid content (SSC, Brix), titratable

acidity and lower pH than “Mechanical+hand” fruit. The traditional destructive dry matter (DM) assessment showed significant differences between the two treatments revealing a higher DM% in “hand” fruit than “Mechanical+hand” fruit (15.00% and 13.85% respectively). I_{AD} classes (A, B, C) showed significant difference mainly in SSC: the ripest class had the highest SSC value (within “hand pruned” fruit $I_{AD} < 1.9$ had 14.13 Brix, while $I_{AD} > 2.0$ reported 12.99 Brix, similar difference for “Mechanical+hand” fruit) and lower titratable acidity only in the “Mechanical+hand” fruit, while not significant differences among the other parameters (data not shown). Fruit quality at T1 (4 months after harvest) is not presented in this report, all fruit after ripening at room temperature reported internal browning and several superficial scald.

Table 2: Bartlett fruit quality at T0 (harvest 2016).

BARTLETT 2016	weight (g)		I_{AD} at T0		Color parameter:		Color parameter:		I_{AD} drop in		weight (g)		% red		avr		SSC		destructive		pH		titratable	
	T0	day0	day0	day0	Chroma (T0 day7)	Chroma (T0 day7)	7days	@RT	drop in 7days	@RT	drop in 7days	@RT	overcolor	firmness (kg)	(Brix)	(Brix)	DM %	DM %					acidity (%)	malic acid (%)
hand only (ctrl)	211	A	1.86	B	95.04	52.37	1.56	A	8.53	9.98	0.85	13.74	A	15.00	A	3.69	B	0.38	A					
mech+hand	196	B	1.96	A	96.11	52.56	1.48	B	8.76	12.10	0.79	12.67	B	13.85	B	3.79	A	0.33	B					
Significance trt	**	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	***	*	**	**	**								
Significance class	NS	***	NS	NS	NS	NS	*	NS	NS	NS	NS	***	NS	NS	NS	*								
Significance trt*class	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS								

CHERRY: Tieton / Gisela 5, UFO. Planted 2008

SIGNIFICANT FINDINGS

Sweet Cherry (Mechanical pruning vs/+ hand pruning)

- Mechanical pruning was 29 times faster than hand pruning alone at a tractor speed of 1.3 m/h, and 17 times faster than the combination of both approaches.
- Hand pruning removed 2.6 times more wood/tree than mechanical pruning, and 1.2 times more wood/tree than the combination of mechanical and hand pruning.
- Mechanical pruning was 11 times more efficient than hand pruning, the combination of mechanical and hand pruning was 1.4 times more efficient than hand pruning alone.
- Mechanical pruning had no effect on yield or yield efficiency. There was no difference between treatments regarding yield efficiency and kg fruit/tree.

Sweet Cherry (Preharvest and postharvest topping)

- Total current season shoot length per upright was significantly greater from uprights pruned during the dormant season and full bloom compared to those topped 2 and 3 months after full bloom. Pruning performed 2 and 3 months after full bloom removed 73% more wood than full bloom pruning and 48% more than DP.
- Timing of pruning affected yield per upright but not yield efficiency in 2015.
- Yield was 35% higher on unpruned uprights compared to uprights pruned in the dormant season, and no different from the other treatments.
- Fruit quality traits were not affected by timing of topping, except for soluble solids content.

METHODS

Mechanical pruning vs/+ hand pruning: The experiment was designed to assess the effect of pruning over two years. The three treatments are as follows (2014/2015): 1) hand pruning/hand pruning, 2) mechanical pruning/mechanical pruning, and 3) mechanical pruning/mechanical pruning + hand pruning follow-up. Each treatment has 5 replications of 20-tree blocks (i.e., 100 trees/treatment).

Mechanical pruning was performed with the sickle bar (Gillison's Center Mount Topper and Hedger) with 3 passes of the machine (hedging on each side of the row and topping), topping was performed at 11 feet height. The motor of the sickle bar was positioned at the top to avoid hitting the lower branches of the trees, and the speed of the tractor was fixed at 1.2 m/h in 2014 and 1.3 m/h in 2015. Hand pruning was performed by the commercial crew (4 people) using ladders, and with the practices that they regularly perform at the orchard. The time to prune each plot and the weight of the wood pruned were recorded for each treatment. Additionally types of cuts and wood damage were observed, as well as the general performance of the machine. The block was picked at commercial harvest (6/4/2015), 3 trees/rep/treatment were randomly chosen for yield and fruit quality evaluation and samples of 25 cherries/tree. Fruit quality was evaluated in the laboratory with weight, firmness, soluble solids content, stem pull force and diameter measurements.

Preharvest and postharvest topping: this trial was also established at Olsen Brothers in Benton City with 'Tieton'/'Gisela®5' trained to the UFO system in a complete randomized design, consisting of 5 different timings of hand pruning (treatments) with 5 replications, and 3 trees/rep. Trials were initiated in 2014, the trees were topped by hand at 11-12' high at different timings: 1) dormant, 2) full bloom, 3) full bloom + 1 month, 4) full bloom + 2 months and 5) full bloom + 3 months. Fruit was picked at commercial harvest (6/11/2014) with samples of 25 cherries per upright. Data of the diameter at the cut site and the length of the removed branches were recorded. Fruit quality was evaluated in the laboratory with firmness, total soluble solids, titratable acidity, weight, and diameter measurements. Regrowth was measured during the winter 2015. The experiment was replicated in 2015 on 4 different rows, fruit was picked at commercial harvest (6/4/2015) and fruit quality was evaluated in the laboratory. Regrowth is being measured during the winter 2016.

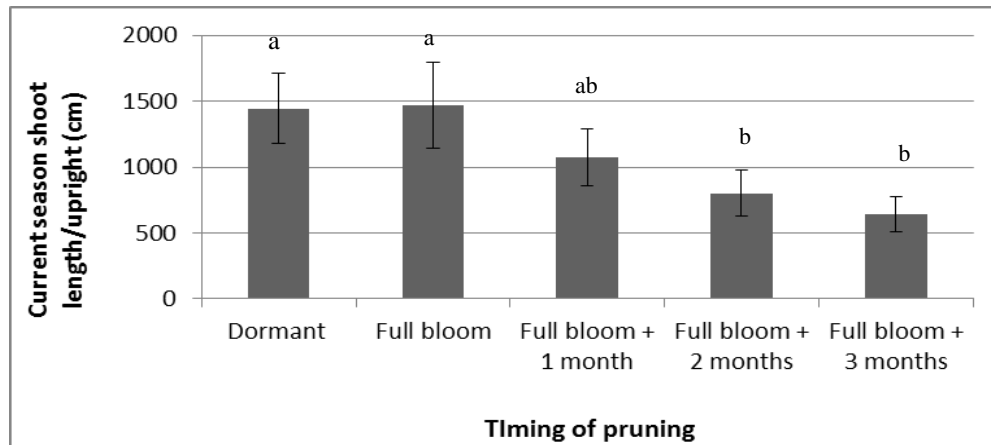
RESULTS AND DISCUSSION

Mechanical vs/+ hand pruning: Our 2015 results showed that mechanical pruning was 29 times faster than hand pruning alone and 17 times faster than the combination of both approaches, mechanical pruning followed by a hand cleanup was 1.6 times faster than hand pruning alone (Figure 1). Each tree was pruned approximately in 14 s with the sickle bar, 408 s per person by hand (6.8 min) and 245 s (4 min) with the sickle bar followed by a hand cleanup.

Hand pruning removed 2.6 times more wood/tree than mechanical pruning, and 1.2 times more wood/tree than the combination of mechanical and hand pruning, with 6.5 kg wood removed/tree approximately. With these results, we evaluated pruning efficiency as kg of wood removed/min/tree and mechanical pruning was 11 times more efficient than hand pruning, and 8.2 times more efficient than mechanical pruning followed by a hand cleanup. The combination of mechanical and hand pruning was 1.4 times more efficient than hand pruning alone. It is believed that hand pruning removed more wood than mechanical pruning because it is more selective. Mechanical pruning had no effect on yield or yield efficiency. Yield efficiency ranged from 0.05 to 0.06 kg/cm² TCSA, which represents 7.6 tons/acre for hand pruning, 9.1 tons/acre for mechanical pruning and 7.5 tons/acre for mechanical pruning 2 (Figure 2). Mechanical pruning had an effect on fruit diameter and weight but in a very small percentage. Fruit diameter from mechanical pruning₁ and mechanical pruning₂ was 3% (0.9 mm) and 2% (0.7 mm) lower than fruit from trees that were hand pruned, but row size was the same (9) for all treatments. Fruit weight from mechanical pruning was 7% lower (0.8 g) than fruit from trees than were hand pruned. We believe that these results might be due to the fruit yield of our trial, even though the difference was not significant, yield from mechanical pruning was 10% higher than yield from hand pruning.

Preharvest and postharvest topping: Our 2015 results showed that pruning performed full bloom + 2 months (FB2) and full bloom + 3 months (FB3) removed 73% more wood than full bloom (FB) pruning and 48% more than dormant pruning (DP). DP and FB were the treatments with the lowest amount of wood removed, but they were not different from each other. There was a positive correlation between length and caliper of wood removed at different timings of pruning, R²

coefficient was lower for 2015 than for 2014. Differently from our 2014 trial, timing of pruning affected yield per upright but not yield efficiency. Yield was 35% higher on unpruned uprights compared to uprights from DP, and no different from the other treatments. Yield efficiency ranged from 0.12 kg/cm² TCSA to 0.18 Kg fruit/cm² TCSA. Timing of pruning showed an effect on fruit SSC and stem pull force. Fruit SSC ranged from 14.1 °Brix to 15.5 °Brix. The lowest value was observed from full bloom + 1 month (FB1), SSC from FB1 was 4%, 5% and 7% lower than fruit SSC from DP, FB and unpruned uprights, respectively. There was no difference between unpruned trees and DP and FB1. Fruit from all treatments were row size 9.



Executive Summary

The primary goal of this project is to establish best management practices for pruning PNW apple, pear and sweet cherry orchards mechanically. In all cases, trials included mechanical hedging and hand pruned treatments with hand pruned only being the control. In some cases, treatments included hand plus mechanical.

This project included replicated trials (two consecutive seasons) and demonstration trials in Apple, Pear and Sweet Cherry. Apple trials were conducted in 'Fuji' (planted 2009), 'Cripps Pink' (planted 2012) and 'Kanzi' (planted 2013). The one pear trial was conducted in 2015 and 2016 in a 'Bartlett' spindle block planted in 2012. Sweet Cherry replicated trials were conducted in a 2008 Tieton block trained to UFO architecture. Significant findings for each trial can be found in the full report.

The project included the purchase and operation of 2 commercial mechanical hedgers. The Gillison's hedger (Benzonia, MI) has the capacity to hedge on the horizontal and vertical. This hedger was used in year 1 in all species and was a good fit in cherries for topping and hedging. This implement is heavy, difficult to mount and has more bounce than we want. The second hedger is the Lagasse (Lyons, NY) and it can only vertically hedge (at various angles). This is a simple, relatively light weight, front loaded implement that is a good fit for narrow row spacing and tree heights and commonly used orchard tractors. Ground speed ranged from 1.2 to 1.5mph.

To use or not to use a hedger should be decided when development plans for a block are in the initial stage. If this is a tool you want to use, than you should establish blocks and pruning strategies accordingly. As an example, mechanical hedgers do not prune wood that is parallel to the row, so tree architecture and development would need to take this into consideration. All the blocks we used were established without the plan to use mechanical hedging resulting in more hand and structural pruning.

Mechanical hedging should not be considered for the sole purpose of saving money. Mechanical hedging should be considered for the following reasons: 1) filling in blind wood near the trunk, 2) restricting canopy depth and height for various reasons including to allow for additional mechanization and automation, 3) light management in late winter/early spring, summer and close to harvest. You need to know what response you after to determine when and how to use mechanical hedging. Our trials were designed to evaluate the response to time of hedging on tree vigor, yield and fruit quality and return bloom.

Project investigators agree that there is a place in the toolbox for mechanical hedgers in canopy management of modern apple, pear and cherry orchards. Results in the field are dependent on many factors including tree architecture (type of wood and placement), timing, cultivar, equipment type and operation, and follow up / clean up pruning.

It is important to assess blocks for fireblight before using a mechanical hedger. This tool like any other tool going from tree to tree can spread bacteria.

The hedger has been demonstrated to roughly 400 people. Trade articles:

<http://www.goodfruit.com/be-careful-in-adopting-summer-hedging-to-build-those-fruiting-walls/>

<http://www.goodfruit.com/help-with-hedging/>

<https://youtu.be/Pm0ppPdt1M4?list=PLvq9oom2vWpc7icQdCKb0VDJuNb-pELod>

www.goodfruit.com/keeping-limbs-in-line-with-mechanical-pruning-video/

www.goodfruit.com/hedging-to-improve-quality/

A Best Management Practice document will be prepared in the next few months and will include information learned in Europe and the Eastern US.

FINAL PROJECT REPORT

Project Title: Development and validation of pest and natural enemy models

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Cooperators: Betsy Beers, WSU-TFREC

Percentage time per crop: Apple: 50 Pear: 20 Cherry: 20 Stone Fruit: 10

Other funding sources

Agency Name: WSU-Extension

Amt. awarded: \$266,344

Notes: The amount funded is the contribution that WSU-Extension provides for DAS support and maintenance + an additional 1 FTE for a second programmer for one year.

Total Project Funding: **Year 1:** 75,154 **Year 2:** 78,160 **Year 3:** 81,306

Budget History

Item	2014	2015	2016
Salaries ¹	42,129	43,814	46,354
Benefits ²	14,983	15,582	16,668
Wages	12,480	12,979	12,480
Benefits ³	262	273	300
Equipment	0	0	0
Supplies ⁴	2,500	2,600	2,704
Travel ⁵	2,800	2,912	2,800
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	75,154	78,160	81,306

Footnotes:

¹ U. Chambers Y1-3 (0.5 FTE); T. Melton Y1-3 (0.25 FTE)

² 33.5%

³ 2.1%

⁴ includes lab and field supplies

⁵ w/in state travel

Objectives:

1. Develop models for mites and aphids using literature data and validate the information as needed.
2. Validate natural enemy models already developed in the SCRI biological control grant.
3. Re-evaluate the San Jose scale model and its biofix and accuracy.

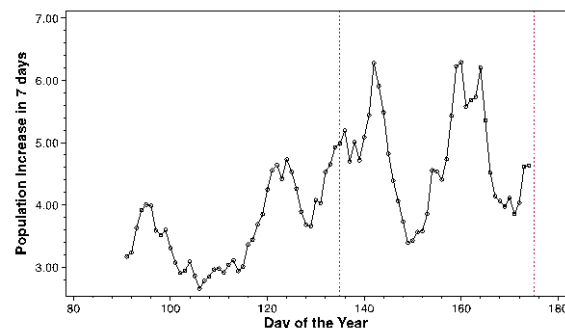
Significant Findings:

- We developed models for two-spotted spider mite, European red mite, woolly apple aphid, green apple aphid, rosy apple aphid, and the western predatory mite that predict the population growth rate throughout the season
- Our field surveys showed both RAA and Apple grain aphid populations tend to be restricted to narrow windows in time in the field.
- Breaking diapause by two-spotted spider mite in the spring was shown to be predictable by photoperiod alone; this gives us information on the development of their populations that are critical for management
- We completed phenology models for the following natural enemies over the course of the grant: *Deraeocoris brevis* (a predator of spider mites, aphids, and pear psylla), the WAA parasitoid *Aphelinus mali*, the syrphid fly, *Eupeodes fumipennis* (aphid predator), and the green lacewing, *Chrysoperla plorabunda* (generalist predator on aphids, mites, soft-bodied insects).
- The current San Jose scale model was significantly improved and does a good job of predicting the second crawler generation and no longer requires a biofix

Objective 1. Develop models for mites and aphids using literature data and validate the information as needed

Over the course of the grant, we have developed models from literature data to predict population growth for two spotted spider mite (TSSM), European red mite (ERM), woolly apple aphid (WAA), green apple aphid (GAA), rosy apple aphid (RAA), and the western predatory mite (WPM), *Galendromus* (= *Typhlodromus*) *occidentalis* (see Fig. 1 as an example). These models do not predict phenology, but as we collect more data, we should be able to develop the models further. The RAA, GAA, ERM, TSSM, and WPM models have been developed into complete life tables that can easily be converted to demographic degree-day models that can predict pesticide effects as soon as we get the phenology quantified.

Fig. 1. Population growth rate for RAA using data from WSU TFREC in 2014. Dotted lines indicate time for greatest numbers found in the orchard.



Rosy apple aphid

We have been collecting data in the field since 2015 to provide the actual phenology of the different stages so that we can make this into a pesticide effects model, but need more data for complete validation. However, there is considerable information that our field studies have generated that provide us with information that insights into the population dynamics.

Our data this past two years where we randomly chose trees and estimated the population levels showed that 92.4% of the total number of RAA found were found between 14 May and 24 June at all locations (Fig. 2). Winged adults were commonly found during this period as well as throughout the summer in very limited numbers. In 2016, we also performed a targeted sample after 24 June where we specifically looked for RAA by examining each tree for presence of WAA before sampling. When we compare the two sampling types, we found that the targeted samples detected 7-fold more RAA throughout the summer compared to the random samples.

The samples also showed that the winged forms appeared at the same time as the wingless forms throughout the summer, generally in multiples of 270 DD apart (\approx immature development period). Samples in the future need to be taken more frequently to adequately characterize the phenology. In addition, we need to collect overwintering eggs in the spring to evaluate hatching which our data summary suggests should be predictable.

The numbers throughout the season suggest that controls should not be orchard-wide except during the period of early population growth (14 May to 24 June). Populations after this point are sporadic and highly clumped, which suggests that only spot treatments or use of natural enemy attractants would be sufficient for population control. Movement back into the orchard from the summer hosts starts in the late season (after first week of September), but populations are low enough at that time that they would not be easy to treat. In addition, they come in over a fairly long period which would make it harder to target the populations.

Apple Grain Aphid

The literature data on the AGA has not been synthesized at this time. However, our sampling for RAA also provided information on AGA phenology. Similar to the RAA, the populations of AGA are most abundant early in the season and by 24 June they also moved out of the orchard and moved back into the orchard in much higher numbers starting at about the same time (first-second week of September) as RAA. In between those dates, AGA was not found in any significant numbers in any of the six orchard/years of data (Fig. 3).

Green Apple Aphid

Our data for GAA are much more complete than for either the RAA or AGA. Unlike the other two species, the GAA is found in the orchard throughout the season in very high levels and does not move out of the orchard during the summer (Fig. 4). We still have a significant amount of analysis to do before we can estimate the phenology, but will be working on this over the next few months.

Fig. 2. No. RAA per 75 terminals found over the last two years at 3 different orchards. Dotted lines show the populations between 14 May and 24 June.

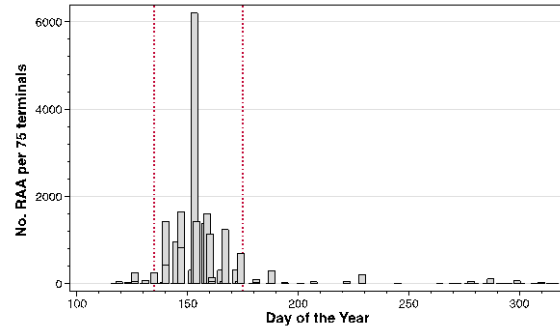
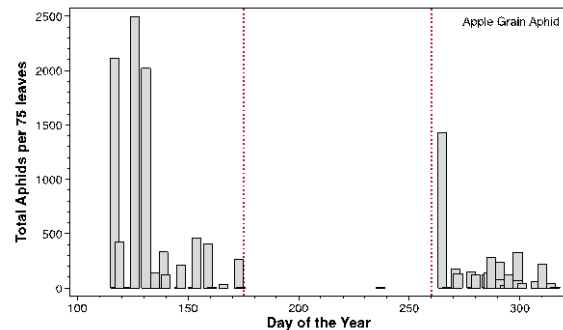


Fig. 3. Phenology of AGA over the years 2014-2015 at 3 different orchards in NCW. Dotted lines at 24 June and 15 Sept.



Effect of leaf age on aphid populations

Literature data suggests that aphid population growth is heavily influenced by whether the foliage is young and quickly growing or starting to age and hardened off. We classified all the terminals as either young and expanding, and then categorized the aphid populations as 0, or >5 per leaf. For the GAA and RAA, there were significantly higher aphid populations on younger leaves. However, for the AGA, higher populations were found significantly more often on the older leaves.

Mite models

The data from 2014-2016 show that diapause for the two spotted spider mite (TSSM) is broken by photoperiods >13.3 hours long (Fig. 5) which happens roughly around the third week of March (in NCW) depending on the latitude. The studies for the egg hatch of European red mite egg hatch did not work well and need to be re-done. We ran studies in 2014 that showed the emergence was about 30% longer than literature data indicated and when they were repeated in 2016, there was a problem with the photoperiod where it was not changed over the course of the experiment to correspond to the outside conditions. That resulted in the egg hatch period being greatly expanded, well beyond compared to what would be reasonable. Examining egg numbers in the field taken at weekly intervals did not clarify the issue. We will collect egg masses at different times very early in February and continuing weekly until late April, bring them to the lab, and put them in a growth chamber with a constant temperature, but photoperiod adjusted weekly to the average of the outside photoperiod. This will allow us to determine how the egg hatch changes with photoperiod and whether we can predict egg hatch to help clarify the best ways to control the eggs and resulting adults.

We did develop population growth models to determine how fast the population develops over the season for both TSSM and ERM, and our data show that the TSSM growth is about 25% higher and developmental rate is about 25% quicker at any time during the season than ERM, suggesting it would be more of a problem when it is present. However, because the populations start at different stages in the spring (ERM as eggs, TSSM as adult females in diapause), there are differences which affect the generalization particularly at the start of the season.

Fig. 4. GAA phenology over the years 2015 and 2016 at 3 orchards in NCW.

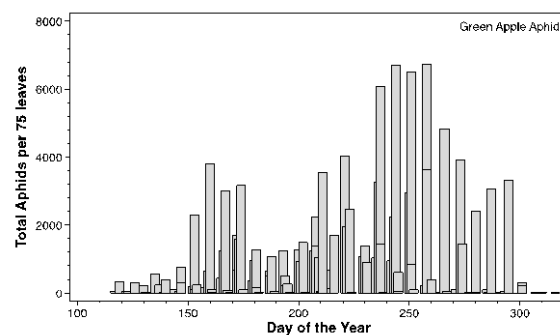
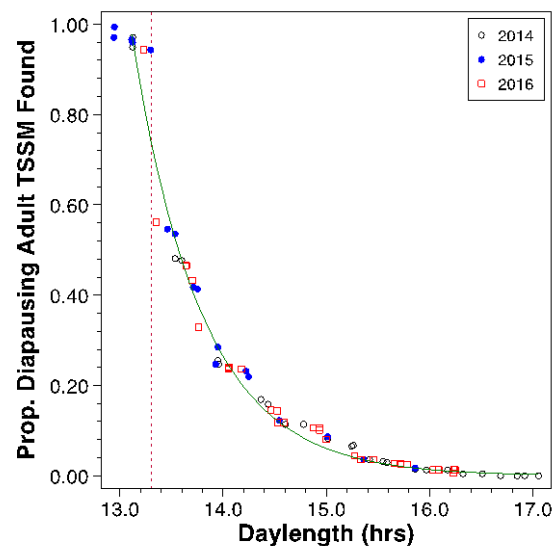


Fig. 5. Proportion of diapausing adult TSSM over a three-year period at x different orchards as day length changes in the spring. Dotted line shows the rapid drop at 13.3 hrs daylight, which corresponds to late March in NCW.



Obj. 2. Validate natural enemy models already developed in the SCRI biological control grant.

Over the course of this grant, we have completed models for the syrphid fly (*Eupeodes fumipennis*), the green lacewing (*Chrysoperla plorabunda* (= *C. carnea*)), the predatory bug, *Deraeocoris brevis*, and the parasitoid of the woolly apple aphid, *Aphelinus mali*. The models predict phenology of the adult stage for the syrphid, *Deraeocoris*, and *A. mali*, and all stages for *C. plorabunda*. We are still working on the *Deraeocoris* model and hope to have it developed into a pesticide effect model, similar to those for codling moth, OBLR, PLR, and the two lacewings (*Chrysopa nigricornis* and *C. plorabunda*). We hope to complete this in the spring and it will be part of the training system that will allow the user to evaluate spray programs on pests and the natural enemies that are being developed over the next three years on a WSDA/USDA block grant already funded.

All of the models have been previously reported on except the *Deraeocoris* and *A. mali* models. We found that the lower threshold was 50 °F and the upper threshold was 88°F and uses a horizontal cutoff – the same DD calculations as codling moth. *Deraeocoris* overwinters as the adult stage underneath the bark scales, and the overwintering emergence starts almost immediately and is mostly complete by 400 DDF, with immatures starting to occur fairly quickly and give rise to the first summer generation adults around 700 DDF. We had enough data to model the first four generations (overwintering and 3 summer generations) (Fig. 6); there are probably more generations in warmer years, but we don't have enough data to model those other generations at this point.

The *Aphelinus mali* (parasitoid of woolly apple aphid) model provides phenology for each adult generation and we found at least 8 generations per year. The first generation is very short, primarily because they overwinter in the pupal stage, but after that, the generations are separated by about 450 DD (length of the egg-adult stage) (Fig. 7). Phenology was very similar between the developmental and validation data sets. We are not sure if we can develop this into a pesticide effects model yet, we will work on it in the spring.

Objective 3. Re-evaluate the San Jose scale model and its biofix and accuracy.

The San Jose scale model suggested that the codling moth biofix be used to predict the first flight of males. Unfortunately, males are hard to capture reliably because they are small, fragile, and don't fly well if any wind is present. However, the target for spray programs is actually the crawler stage, so predicting the emergence of crawlers is of greater importance for IPM. The model used on DAS was the old PETE model from Michigan State University and studies done in California and Washington.

Fig. 6. *Deraeocoris brevis* adult phenology over a six year period from 24 orchard/years data. Open circles are from the model development data set and solid circles from the validation data set.

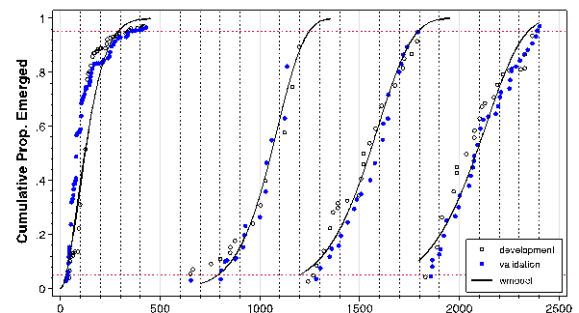
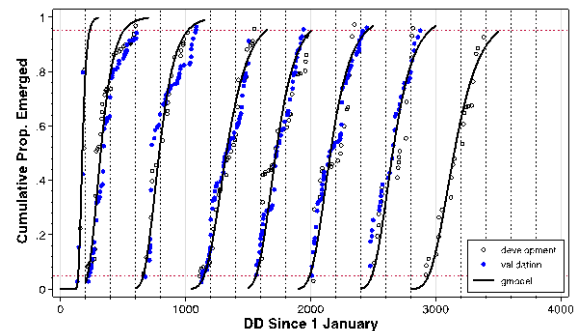


Fig. 7. Phenology of adult *A. mali* over a six-year period from 24 orchard/years data. Open circles are from the model development data set and solid circles from the validation data set.



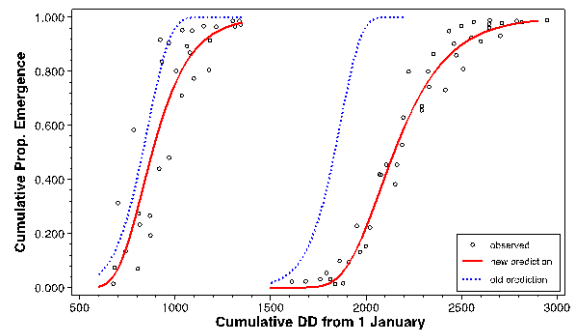
To test the model and update it as needed, we collected SJS crawlers (and adults) using double sided sticky tape placed around infested branches during the years 2014-2016 at three different orchards. Tapes were replaced weekly and the number of crawlers were recorded for each orchard/year combination.

Results & Discussion:

Our data showed the current model, even when updated with the codling moth biofix did not predict the crawler emergence well (Fig. 8, dotted line). In general, the old model predicted first crawler generation earlier than observed in our studies, although the shape of the distribution was similar. The second generation was considerably earlier and had a much shorter window of emergence than what we observed. Some of the difference in the window of emergence may have to do with our warmer spring and summer periods that would tend to make a greater percentage of the population miss the diapause triggers that may be important in cooler years. However, the big discrepancy in our situation and the old model means that if the second generation had ever been targeted (**note: DAS never reported or suggested controls for the second generation**) using the model timing, it would have been way too early to have any impact on the population.

Our new model predicts the emergence of both crawler generations with greater accuracy than that of the old model (Fig. 8). Control recommendations in DAS will be updated with the new model and remain concentrated on the first crawler generation. However, we can also make some recommendations for timing of the second generation if the first generation was missed and populations are high. This would not normally be a good target, because by this time crawlers may have already moved to the fruit, but as a rescue treatment, it might be a useful strategy.

Fig. 8. Comparison of the old and new San Jose scale models for predicting crawler emergence.



Executive Summary:

The work done on this grant has provided a number of new tools that greatly improve our ability to predict the best times for management and times to withhold treatments because of their impacts on natural enemies. In addition to the development of various models, we have also clarified areas where we need more information to complete models for different pests.

Overall, the grant provided six new population growth models for Green apple aphid (*Aphis pomi*), the rosy apple aphid (*Dysaphis plantaginea*), the woolly apple aphid (*Eriosoma lanigerum*), two-spotted spider mite (*Tetranychus urticae*), European red mite (*Panonychus ulmi*), and the western predatory mite (*Galendromus* (= *Typhlodromus*) *occidentalis*). Combined with what we already have in terms of phenology and what we hope to gain over the next few years, we should be able to clarify the best management timings and needs.

In terms of validating a series of natural enemy phenology models developed previously from our SCRI biological control grant, we were able to validate four of those models. The model for the green lacewing *Chrysoperla plorabunda* has already been developed into a pesticide effects model (similar to those already developed for codling moth, *Pandemis* leafroller, oblique-banded leafroller, and the lacewing, *Chrysopa nigricornis*), and we will be working on the development of those models for *Deraeocoris brevis* and *Aphelinus mali*. If those models can be developed, we will have a robust picture of how different pesticide programs affect both the pests, non-target pests, and natural enemies. We currently have a grant that will develop these models into a training module for consultants, but we hope to provide feedback on WSU DAS to show the effects of different options on the overall complex of pests and natural enemies.

The San Jose scale model will be a fairly minor change in our current management programs (mainly moving treatments slightly later in the season), but it should give better timing for the first crawler generation and open a window for rescue treatments in the second generation. We will incorporate these timings in DAS during the next season.

FINAL PROJECT REPORT

Project Title: Engineering analysis for high density trellis structures

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Cooperators: Travis Allan, Brue Allen, Jeff Cleveringa, Dave Gleason, Dale Goldy, Scott Jacky, Mark Stennes

Percentage time per crop: Apple: 100%

Total Project Request: Year 1: \$92,500

Other funding sources: None
WTFRC Collaborative expenses: None

Budget 1

Organization Name: De Kleine Machine Company
Telephone: 509.832.1108

Contract Administrator: Mark De Kleine
Email address: mdk@mdekleine.com

Item	2016
Salaries	12,000 ¹
Benefits	0
Wages	71,500 ²
Benefits	0
Equipment	3000 ³
Supplies	2500 ³
Travel	3500 ⁴
Miscellaneous	0
Plot Fees	0
Total	92,500

Footnotes:

¹ Cost for WSU Mechanical Engineering student capstone project

² Wages for engineering

³ Materials for testing and related project equipment

⁴ Travel cost for meetings, orchard visits, and engineering reviews

1. Objectives

The goal for this project was to develop a standardized design practice for selecting a trellis structure based on biological and environmental factors. Specific objectives of this proposal were:

1. Perform an engineering analysis of fruit loads exerted on a trellis structure based on expected yield for high density orchard plantings.
2. Perform an engineering analysis of fixed and variable loads exerted on a superstructure.
3. Develop a trellis design tool as a planning guide for enterprise use.

2. Significant Findings:

Industry Survey Results (11 responses)

- 90% of failures were less than 10 years old and of that, 78% were less than 7 years old
- Failures in both angled and vertical systems at approximately 50-50 ratio
- In-row post spacing ranged from 40-55 feet; more than half were greater than 40 feet between posts
- Half the failures occurred in September and October with high wind events (40mph +)
- Roughly 40% were reported as anchor/anchor wire related (loose anchor, rusted anchor wires)
- 60% were reported as in-row post related (poles snapped or up ended)

Engineering Analysis

- Embedment depth can be predicted for adequate foundation
- Above ground forces need to be balanced through a trellis member to below ground forces
- A fixed base, or fully embedded trellis member, reduces the soil variability to a predictable value
- Wind loads are proportional to canopy area and porosity factor
- Wind load is the critical design variable for trellis members that are embedded correctly
- Post spacing can be determined using the Trellis Engineering Model

3. Results and Discussions

The purpose of trellis is to provide support for horticulture by balancing above ground forces (loads) to below ground forces (foundation), through a desired material (member). General trellises consist of: members, wires, and anchors. Environmental factors include wind, tree structure, fruit, and soil foundation. Trellis components act together as a system and should be considered as such.

Each trellis component has a maximum limit (load) for which permanent yielding will occur when the applied loads exceed the ultimate load. Permanent yielding is considered failure, and significant decreases in material properties occur once permanent deformation starts. The focus of this analysis is to limit members to a value below the maximum, by including factors of safety.

Above ground forces include wind, canopy, fruit, fabric, wire, and not all forces act in the same plane. For example, wind acts perpendicular to a member whereas fruit loads act axially. Wires, that are tensioned, will act perpendicular to a trellis member but not necessarily in the same direction as the wind. Therefore, a trellis member must provide adequate support in all directions.

3.1 Soils and Foundation

EMBEDDING A TRELLIS MEMBER PROPERLY IS AN IMPORTANT ASPECT OF TRELLIS INSTALLATION AND STRENGTH. IF THE MEMBER IS NOT EMBEDDED TO THE PROPER DEPTH, THE SOIL WILL BE THE LIMITING FACTOR FOR THE TRELLIS'S STRENGTH. A SOIL LIMITING STRUCTURE IS UNSTABLE IN PRACTICE AND NOT RECOMMENDED WITHOUT ADDITIONAL DESIGN CONSIDERATIONS MADE BY SOMEONE SKILLED IN THE ART.

There are two general types of trellis post configurations: constrained and non-constrained. Most WA orchards have non-constrained posts which are pounded or set into augured holes. Soil and/or restraints provide the lateral support (strength and stiffness) for a trellis member foundation. Adequately embedding a member ensures that the soil will not be the limiting factor when selecting materials. A properly embedded member is considered a 'fixed base' for engineering analysis.

Embedment depth is determined from either ASABE EP486.2 [3] or ICC/IBC Soils and Foundation [4]. In general, these two references have similar outputs however when soil layers are present, ASABE EP486.2 employs more exact solutions. From ICC equations 18-1:

$$d = 0.5A \{1 + [1 + (4.36 H_p / A)]^{1/2}\}$$

Where: $A = 2.34P/S_1b$; b = diameter of round post or footing or diagonal of square post or footing (ft), d = embedment depth (ft), H_p = distance to above ground applied load 'P' (ft), P = applied lateral force (lbf), S_1 = allowable lateral soil bearing pressure based on a depth of one-third the depth of embedment (psf). ***Note: embedment equations are for round or square vertical members. For angled trellises, increase depth by minimum of 3b.**

If shallow soils exist and become inadequate for proper embedding, restraints should be used. Restraints reduce the required depth and are used above ground, at the ground, or below ground. Embedment depth can vary by 20% for a 12ft trellis member (H_T) depending on soil type. Soil tests are recommended using standard lab tests or preferably in-situ tests. Rock layers require less depth than clays or organic silts.

Use the range values in Table 1 in-lieu of proper soil testing. Lateral pressure values may be doubled for (isolated) trellis posts, per 1806.3.4[4]. Moisture content will decrease the lateral strength for soils which hold moisture; Quincy soils typically found in Eastern Washington, have virtually no (< 25psf) lateral strength when fully saturated. ***A failure occurred in central WA when the irrigation system was on for 20 hrs consecutively (with no strong wind event).** Consider proper drainage techniques when installing trellises in soft soils. Backfilling with concrete-attached to the member-increases the effective width of the trellis member. Material properties of the backfill should be included in a geotechnical analysis.

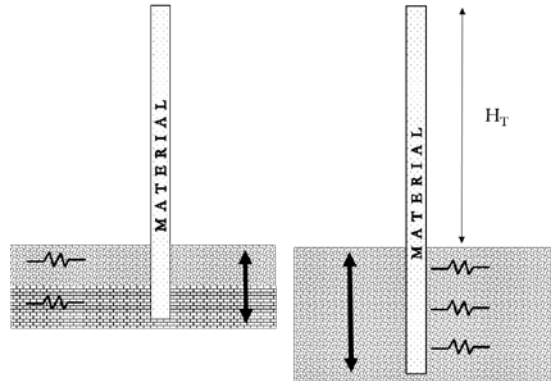


Figure 1. Layered soil (left) and uniform soil (right) represented as springs.

Table 1. Soil properties as given in ASABE EP 486.1 for 5 general soil classes and estimated post embedment depth for a 4" round wood post as percent of above ground height.

Soil Class	Lateral Pressure per unit depth (lb/ft ² *ft)	Friction Angle (deg)	Density (lb/ft ³)	Approximate Embedment depth for 4in wood post (% of H _T)
Bedrock	1200	-	-	20%
Sedimentary rock	400	-	-	30%
Sandy Gravel	200-300	32-38	90-110	37%
Sand, Clayey Sand	150-200	26-30	85-105	41%
Clay, Clayey Silt	100-130	10-15	90-120	47%

3.2 Wind Loads

Each trellis member must support a representative load within the length of a row as shown in figure 2. One extreme case resembles a solid wall, the other extreme- only wires. A practical consideration of porosity is somewhere in the middle depending on canopy and horticultural training. Consider material on the orchard floor or superstructure that could potentially get blown onto the canopy. A trellis can only hold this material if it is designed for the additional loading. ***A failure occurred when reflective fabric on the orchard floor uplifted and caught on the trellis row.**

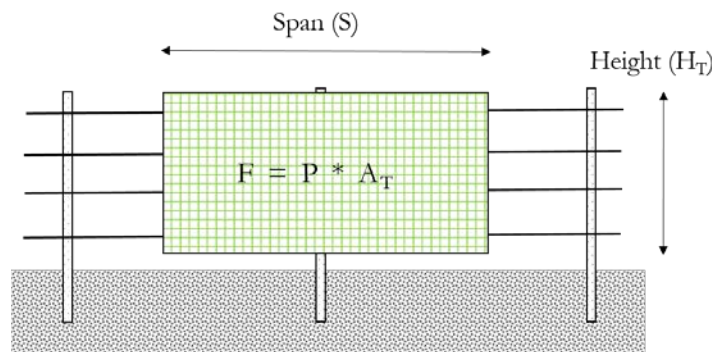


Figure 2. Example of required member support in a general vertical trellis system.

Factors included in the wind force calculation coefficient are drag, height, exposure category, importance factor, gust factor, and topography. Special cases that will affect the value of the coefficient are gorges, mountainous areas, or hurricane prone regions. MWPS-1 [1] and ASCE 7 [2] were used to develop the wind equation used for the trellis engineering model (TEM). Simply stated, and assuming a drag coefficient (C_d) of 0.5 for porous canopies, the force of the wind can be approximated by:

50% Porous Canopy $F_w = 0.001 * V^2 * A_T$	70% Porous Canopy $F_w = 0.0015 * V^2 * A_T$	Solid Wall (& $C_d = 1$) $F_w = 0.0043 * V^2 * A_T$
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Where: F_w = Force of the wind (lbf); V = Velocity (mph); A_T = Trellis Area (sqft). The trellis area is considered the span between the trellis members and the height of the trellis (top wire). For angled

canopies, A_T is the projected area perpendicular to the windward direction. *A trellis should be designed to support the actual/expected tree height and fruit region.

When each member is embedded properly, the material can be sized based on geometric and material properties. Stated obviously, a solid wall needs more support, or decreased span, than a wall with holes. The reaction force, against the wind, of each tree was neglected as a conservative approach.

Wind force is considered uniformly distributed along the height of the trellis member (this assumption disregards any support from trellis wires and trees). Use good judgement for estimating the resolved wind force based on uniform distributed load. A triangular load distribution may be appropriate where applicable, thus changing the height of the resolved load. For angled trellis, use projected area to calculate wind loads.

A commentary on portable windbreak fences from the Saskatchewan Agriculture and Natural Resources department [7] depicts wind effects on solid and porous fences as shown in figure 4. It is important to note that both windward and leeward velocities need consideration when designing the trellis.

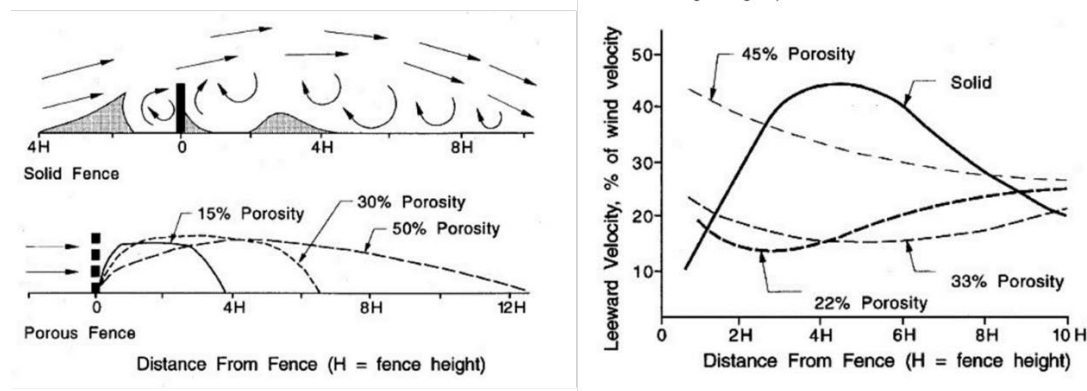


Figure 3. Wind effects shown on a solid and porous windbreak and leeward velocity representation as percent of windward velocity.

3.3 Anchors

Anchors provide additional support to trellis members, usually used at end posts (although not restricted to end posts). Typical anchors used in WA are screw anchors which rely on depth of soil and diameter of screw to determine holding capacity. Consider weld strength when appropriate (at greater depths).

A 'frustum' volume equation can approximate the weight of the anchor system for a given soil and friction angle assuming the anchor rod is small compared to the overall volume. Use soil friction angle to determine top radius (R). Consider collars and post volumes during calculations. Refer to ASABE EP 486.1 for posts with concrete and wood collars.

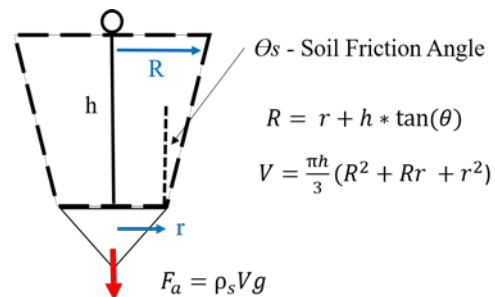


Figure 4. Force related with screw type anchors.

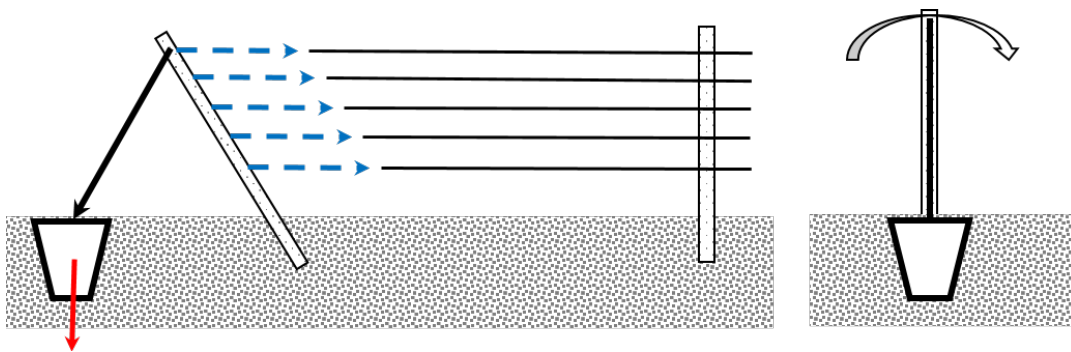


Figure 5. Typical anchor and end post configuration.

Anchors placed in-line with the trellis provide little side-to-side support. Consider alternative anchor setup if side-to-side stability is needed.

Figure 6 shows an aerial view of a failed trellis that did not cause the end posts to fail. The end posts in figure 6 where similar style to figure 5.



Figure 6. Aerial view of failed trellis (right) with end posts still standing.

An anchor and wire connected to an end post can reduce the internal shear and moment of a member depending on the location of the wire. In general, a wire will cause a point load. Design for member shear less than allowable shear. Point loads can crush wood members and cause local failures.

Other anchor considerations are concrete ecology blocks ($\sim 145 \text{ lb ft}^{-3}$). The ecology blocks will provide 3900 and 7800 lbf (F_a) for a half block (3x3x3 ft) and full block (3x3x6 ft) respectively.

3.4 Beam Theory

Beam theory is used to analyze a member that is loaded axially or laterally and distinct equations exist for each situation. In a formally trained system, buckling will occur if the wire loads all acting on the member exceed Euler's formula for critical buckling load (figure 7(b)). A high slenderness ratio (for long thin posts) will buckle easier than a post with low slenderness ratio. When superstructure is present, dead loads such as ice, hail, rain, and snow, will add loads and buckling and foundation design need consideration.

Beam theory assumes perfect bending with no torsion component and such is the case with wood posts and closed section metal posts of 'moderate length'. For non-symmetrical open section metal shapes such as hat channel, torsion and subsequent buckling need to be addressed. Design should be taken to limit the torsion in

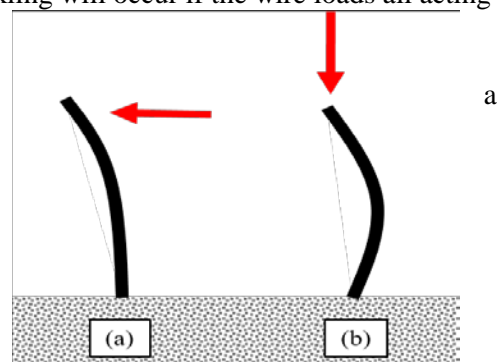


Figure 7. Bending (a) and buckling (b) of a "fixed-end free-end" trellis members.

system using open section shapes. Significant reduction in material strength will occur when a member is twisted beyond its ultimate yield.

3.5 Wire and Wire Sag

High tensile wire is typically used to support horticulture and in some cases fabric. For a 12.5ga wire tensioned to 250lb, an additional load of 1200 lbs is permitted [5]. There is no apparent situation when wire becomes the limiting factor of strength for securing horticulture and fruit loads in between trellis members. **If trellis members are not fixed and allowed to pivot (lean), the load on the wire becomes cumulative to adjacent members and can potentially cause overloading.**

Consider this: a 12.5ga wire is not sufficient to ‘lift up’ a half ecology block or a 6” dia screw anchor, 5 ft deep in 105 lb ft⁻³ soil. Therefore, adequately size anchor-to-end-post wire(s) based on resolved forces in the row, trellis member, and anchor weight (force). A six-wire system tensioned at 250lbs each, will pre-stress a member by 1500 lbs.

Wire will sag according to a catenary curve. The equation used to describe wire sag is:

$$\Delta = (W * S^2) / 8T$$

Where: Δ = Wire sag (ft), W = weight of distributed load (lb ft⁻¹), S = wire span (ft), T = Tension (lb). Wire tensioned at 250lbs with a 100ft and 500ft span will sag approximately 1.6in and 3.3ft, respectively. A short wire span can provide additional support for trellis members if designed to do so.

3.6 Fruit Load

Fruit loading is not a significant factor for vertical wood posts (3-4-5”) with a ‘normal’ span using 12.5ga wire. A normal wire span has enough capacity for heavy fruit loads between trellis members. Branches will also support some fruit load even in formally trained systems.

Fruit loads should be included for angled canopies or when a vertical system starts to lean (see section 3.1). Two common trellis structures for angled canopies are shown in figure 9. Fruit loads on an intra-row brace (a) system will be transferred to each member and their respective soil structure. **It is not correct to assume an intra-row brace will provide additional support in-lieu of embedment depth. Leaning angled posts are an indication of improper embedment.**

A similar concept that was used to predict wind loading can be applied to predict fruit loads on angled members. Fruit can either be broken down into formally trained loads (fruit per linear foot) or informal loads (density based).

Consider this: a 12 ft tall angled trellis (figure 8a) with 14 ft spacing, growing 125 (900lb) bins/ac will add 1044lbs to a trellis member at a 30ft span. This load would be equivalent to 15% of an ultimate yield stress for a wood post. The same trellis growing 85 (900lb) bin/ac would add an equivalent load equal to 10% of the ultimate yield stress. Decrease span accordingly.

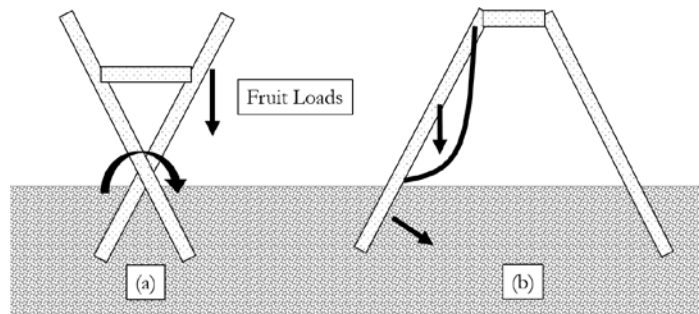


Figure 8. Two common angled canopy configurations: (a) intra-row bracing and (b) inter-row bracing.

Angled trellises are statically indeterminate and need proper design considerations for shear, moment, and deflection in the member. Connection members such as bolts, nails, and wires should be sized correctly. Likewise, welding steel members requires a professional skilled in the art. In general, braces will reduce the shear and moment within a member. For “fixed-end free-end” members, the maximum shear and moment are at ground level.

Two members placed (almost) in plane with each other, as in figure 8(a), will have less lateral soil pressure available if one member begins to disrupt the foundation. Disturbing the soil effectively decreases the lateral pressure capacity of the remaining soil. **Increasing embedment depth will reduce the risk of the aforementioned.** Angled trellis members should not be considered isolated posts with respect to lateral soil pressure (section 3.1).

4.0 Trellis Engineering Model (TEM)

The trellis engineering model was developed using the criteria from sections 3.1-3.3 described above. The purpose of the TEM is to provide a simple baseline for planning or evaluating a trellis system. The TEM is conservative from an engineering analysis point of view. TEM output(s) are shown in Figure 10 depicting span versus wood post diameter for a given height, wind speed, and post member design value.

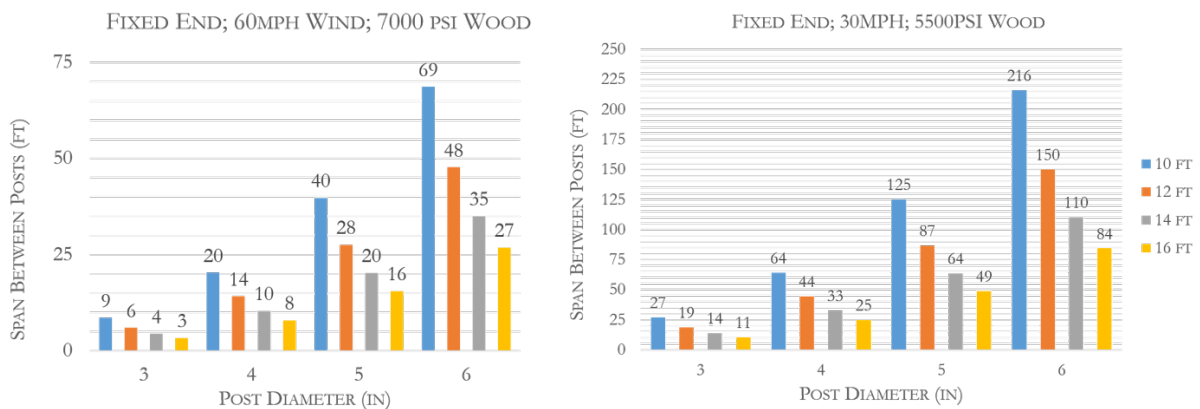


Figure 9. Trellis Engineering Model used to predict trellis member span versus diameter for 4 trellis heights.

The TEM is interactive which allows users to change input variables. Figure 9 shows a TEM output with different input variables.

5.0 Testing

Tests were performed on 30 treated posts bought from local suppliers in central WA. It was not the scope of this project to evaluate suppliers' material quality. Consider material quality when predicting the trellis span using the TEM. ***Wood is a biological material and strength decreases for knots, splits, young wood, and high moisture content. A higher factor of safety will reduce the risk of this variance.**

For example: the Timber Pile Design and Construction Manual from the Timber Piling Counsel of the American Wood Preserves Institute uses an allowable bending stress for treated round southern pine piles as 2400 psi [6] but reduces by 10% for a > 10yr application. Extreme outdoor conditions will also decrease the allowable stress value of wood members.

In theory, a treated pine wood member will fail around 7000psi. The actual value used in practice is equated to application use, i.e. a shelter for people uses a lower design value than a pump-house. Risk and cost can be adjusted in these values but sound judgement should be used by someone skilled in the art of mechanics and materials.

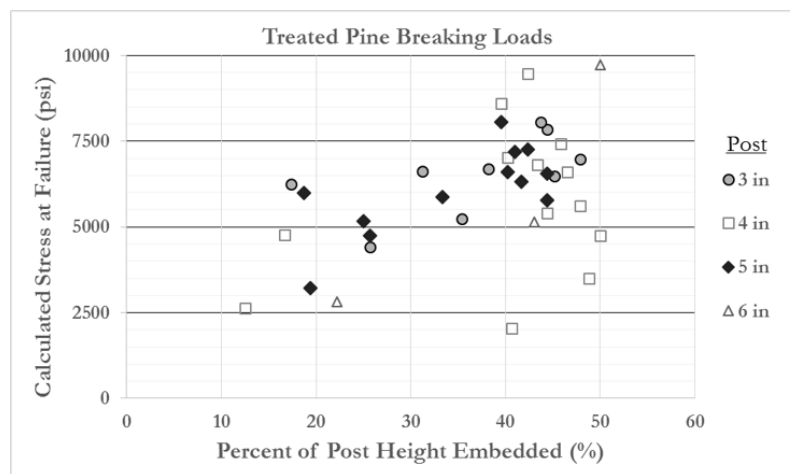


Figure 10. Results for pulling tests on 30 treated pine posts in Quincy type soil.

6.0 Superstructures

The TEM model can be used to determine wind loads for a solid wall, resembling a fabric installed vertically on an outside row. Other considerations when fabric is used to enclose an orchard are dead loads including snow, rain, and hail. Refer to ASCE 7 section 7 for snow loads and section 8 for values of rain loads. A permanent superstructure should be considered a building and designed accordingly. Additional loads will increase the required axial strength of each superstructure member. It is more probable that the fabric will be the limiting factor, depending on installation style, and fail before a wire or trellis member. This is largely dependent upon the setup of the wires in relationship to trellis members and spacing.

The pressure coefficient associated with a seasonal superstructure can be assumed as an 'agricultural building'. Pressure coefficients given in figure 101-8 [1] should be used to estimate uplift force. Pressure coefficients of 0.2 for windward side and 0.7 for leeward side roofs seem reasonable for an enclosed superstructure. In general, a trellis system is not likely to lift from the soil by means of uplift force on the fabric. However, good judgement is needed when considering the connection between

the fabric and posts and additional foundational collars or anchors will help secure trellises that are not connected to individual trees. Trees themselves can act as anchors in a formally trained system.

Disclaimers and General Assumptions

This information is intended to be used to provide fundamental (preliminary) understanding of principle variables acting on a trellis system. For the design of an actual trellis, a competent professional should be consulted.

Wood post refers generally to treated pin post typically found at an orchard supply company in Eastern Washington. Trees do not contribute to resistance loading. Thermal changes were neglected. Trellis heights are less than 15ft for wind calculations. A prerequisite understanding of listed reference material.

References

- [1] Midwest Plan Service- Structures and Environment Handbook. 1983. Iowa State University, Ames, IA 50011.
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- [4] International Code Council. International Building Code, Soils and Foundation (Chp 18). 2009.
- [6] Collin, J.G. Timber Pile Design and Construction Manual. Timber Piling Council, American Wood Preservers Institute. 2002.
- [7] Government of Saskatchewan. 2017. Using mobile windbreak fences for winter feeding on pastures and cropland. Retrieved from: <https://www.saskatchewan.ca/business/agriculture-natural-resources-and-industry/agribusiness-farmers-and-ranchers/livestock/cattle-poultry-and-other-livestock/cattle/portable-windbreak-fences>
- [8] Knapp, John W. 1982. How to Build Orchard and Vineyard Trellises with USS Max-Ten 200 High-Tensile Fence Wire. USS Catalog No. T-111578, U.S.A.

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- [9] Van Dalfsen, K. Bert. 1989. Support Systems for High-Density Orchards. "First presented at the British Columbia Horticultural Forum, November 5, 1986. Providence of British Columbia, Ministry of Agriculture and Fisheries, Victoria B.C., Canada. ISBN-0-7726-1009-6.
- [10] Mollah, M. R. 1989. Review of Trellis Structures for Horticultural Crops. Department of Agriculture and Rural Affairs. Mildura, Victoria. ISBN 0-7306-0546-9.

4. Executive Summary

Trellis systems are a collection of individual components that must collectively work together to resist external loading. The weakest link often gets exploited and creates a potential for costly capital failure. A model to predict loads on trellises was developed as a tool to aid in the analysis and planning of current and future trellis systems.

The trellis equation is a product of multiple variables, for which numerous solutions exist. However, each component must be sized according to the collective system for proper trellis design. The most important factors are: trellis style, soil structure, canopy wind loading, and span between members.

The trellis engineering model can be used to determine canopy wind loading, embedment depth for a given soil type and span between posts. Wind loads can be sized according to canopy style where denser canopies will 'catch' more wind. Trellis members require an embedment depth that results in a proper fixed base; deeper for weaker soils. After wind, soil, and some other variables are quantified, the span between members gives the orchardist the plan to implement a proper trellis for their crop.

Each trellis is a unique system when all variables are assembled together. It is important to understand each trellis system's structural integrity and the potential areas of weakness in that (style) system.

The following are good practices and considerations for trellis design and planning:

- Design the trellis to protect capital investment
- Design the trellis to a horticulture preference
- Use the TrellX model to estimate wind loads
- Conduct soil tests at locations in orchard where soil depth may vary: hills and valleys
- Use restraints in shallow soils
- Increase embedment depth for angled trellis with intra-row braces
- Fruit loads are additive on angled canopies
- Minimize twisting (torsion) when using non-symmetric metal shapes
- Use the TrellX model to estimate trellis span between posts
- Consider unexpected loads and use a factor of safety to reduce risk of failure
- Size secondary trellises properly when 'adding on' to existing trellis
- Limit pre-stressing members caused from overtightening wires and cables
- Consult an expert

CONTINUING PROJECT REPORT
WTFRC Project Number: TR-16-102

YEAR: Year 1 of 3

Project Title: Development and validation of a precision pollination model

PI: Gloria DeGrandi-Hoffman*

Organization: USDA-ARS Tucson

Telephone: 520-647-9187

Email: Gloria.Hoffman@ars.usda.gov

Address: Carl Hayden Bee Research Center

Address 2: 2000 East Allen Rd

City/State/Zip: Tucson, AZ 85719

Co-PI (2): Vincent P. Jones

Organization: WSU-TFREC

Telephone: 509-663-8181 x291

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Address: Dept. Entomology/TFREC

Address 2: 1100 N. Western Ave

City/State/Zip: Wenatchee, WA 98801

Co-PI (3): Tory Schmidt

Organization: WTFRC

Telephone: 509-665-8271 x4

Email: tory@treefruitresearch.com

Address: 1719 Springwater Ave

City/State/Zip: Wenatchee, WA 98801

Cooperators: Dr. Stefano Musacchi (WSU-TFREC), Karen Lewis (WSU-Extension), Dr. Melba Salazar-Gutierrez (WSU-Prosser), Dr. Lee Kalcsits (WSU-TFREC), Dr. Steve Sheppard, WSU-Entomology

*Nicole Rafferty was previously the PI, but has left WSU to take a job at UC Riverside. Gloria DeGrandi-Hoffman will be the PI, and Vince Jones will be the WSU PI. Steve Sheppard has indicated his role is best as a co-operator.

Total Project Request: Year 1: \$95,834 Year 2: **\$102,842** Year 3: \$104,632

Percentage time per crop: Apple: 80% Pear: 0% Cherry: 20% Stone Fruit: 0%

Other funding sources: None

WTFRC Collaborative Expenses:

Item	2016	2017	2018
Salaries	5,000	5,000	5,000
Benefits	2,000	2,000	2,000
Wages	8,000	12,000	12,000
Benefits	2,400	3,600	3,600
RCA Room Rental			
Supplies			
Travel	1,800	2,000	2,000
Miscellaneous			
Total	19,200	24,600	24,600

Budget 1**Organization:** WSU-TFREC**Contract Administrator:** Carrie Johnston/Joni Cartwright**Telephone:** 509-335-4564/509-663-8181 x221 **Email:** carriej@wsu.edu / joni_cartwright@wsu.edu

Item	2016	2017	2018
Salaries ¹	35,000	45,000	46,800
Benefits ¹	15,120	11,493	11,953
Wages ²	18,800	11,440	11,898
Benefits ²	1,214	309	321
Equipment			
Supplies	3,500	3,500	2,500
Travel ³	3,000	1,500	1,560
Miscellaneous			
Plot Fees			
Total	76,634 73,634	73,242	75,032

Footnotes:¹ Salaries and benefits are for a half-time grant manager² Wages and benefits are for student temporary employees.³ Travel (\$3,000) moved to USDA-ARS to cover PI DeGrandi-Hoffman travel**Budget 2****Organization Name:** USDA-ARS**Contract Administrator:** Kathleen Vandebur**Telephone:** 520-647-9160**Email address:** Kathleen.Vanderbur@ars.usda.gov

Item	2016	2017	2018
Salaries		1,000	1,000
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel		4,000	4,000
Plot Fees			
Miscellaneous			
Total	3,000	5,000	5,000

Footnotes:¹ Travel expenses will enable Co-PI DeGrandi-Hoffman to travel between Tucson to Wenatchee at least once per year and will cover other related travel costs for the technician.

Objectives:

1. Update DeGrandi-Hoffman's original apple bloom phenology model to incorporate newer cultivars and horticultural advances.
2. Examine the effects of netting on honey bee foraging and modify foraging model accordingly.
3. Incorporate information on honey bee foraging and cross-pollination rates into the pollen tube growth model to improve decision making and thinning practices. Also evaluate foraging model on cherry.
4. Evaluate the effects of variability in spring weather conditions, as well as directional shifts toward earlier bloom, on fruit set and best pollination management strategies.

Significant findings:

- Models for bloom phenology were developed for the cultivars Red Delicious, Cripps Pink, and Gala using WTFRC data from 2010-2014.
- Work from this proposal will allow us to develop bloom phenology models for Honeycrisp.
- The model of honeybee foraging was updated to allow us to predict foraging quality which depends on temperature, wind speed, rainfall, and solar radiation.
- Honeycrisp was tested for self-compatibility using paternity testing of fruit collected from a solid block planting. The testing revealed that all fruit was the product of cross-pollination.

Results and Discussion

Objective 1. Update DeGrandi-Hoffman's original apple bloom phenology model to incorporate newer cultivars and horticultural advances.

The bloom phenology data from a study done by WTFRC and WSU personnel was evaluated for bloom phenology. The data were taken at 2-3 day intervals during the period from dormancy through petal fall and came from nine sites over the years 2010-2014. Most of the years had 30 flowers per cultivar sampled, except for 2014, when 50 flowers/cultivar were done from the majority of the sites. The different stages available in the data set were green tip, 1/2" green, tight cluster, first pink, full pink, first bloom, full bloom, and petal fall.

The sampling procedures and the short duration of the different flowering stages made the data very "granular" because often only a few observations on a particular stage occurred at a particular location. This really required the multiple site and multiple year data set to give a reasonable sample size that allowed us to define the length of time that a particular stage lasted. Using the complete dataset, the number of observations was sufficient to get the timings for each stage, although the sample size decreased with the latter stages as some of the flowers aborted early.

The data were paired with the weather data from AWN and the DD since 1 January were calculated using a 40.1°F LT and a 75.7 UT with a vertical cutoff. We examined the Weibull, Gumbel, and normal distributions for fit, with the normal distribution providing the best fit. The use of the normal distribution allows us to predict the progress through the stages using only the mean and standard deviation. Each cultivar has a slightly different offset on a DD scale, but all stages are well represented by the cumulative normal distribution (Fig. 1).

In addition to the WTFRC/WSU data analysis mentioned above, data were also taken this year for the cultivars Honeycrisp at Chelan and Yakima and Gala at Yakima. That data has not yet been analyzed.

Work this coming year:

Bloom data will continue to be taken in 2017 and used to validate equations that will be developed to predicting bloom phenology from 2016 data. The analysis will use the same methods as those described above for the WTFRC/WSU data set.

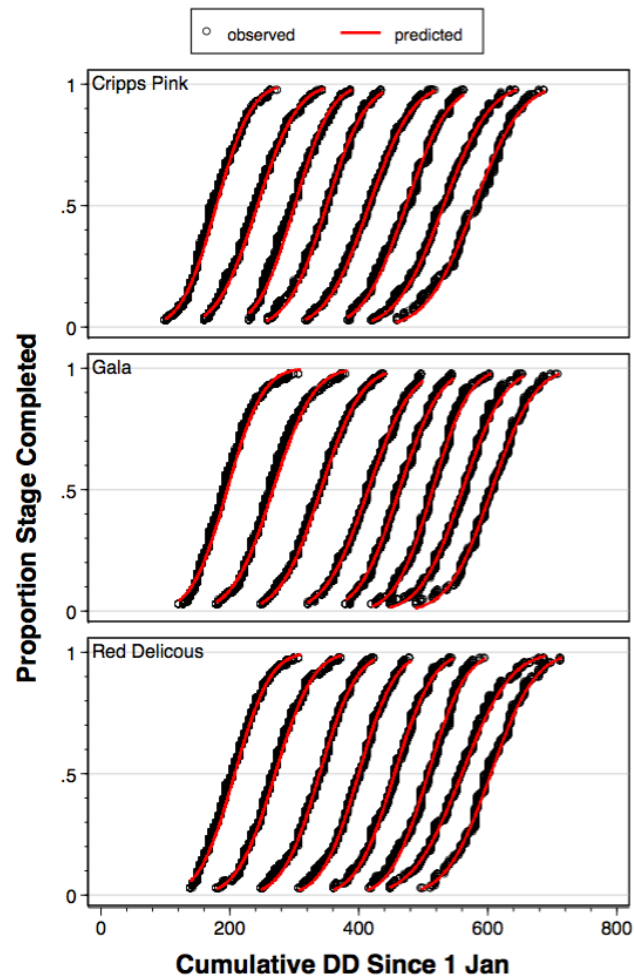
Objective 2. Examine the effects of netting on honey bee foraging and modify foraging model accordingly.

This objective will be started this coming year. We will count open blossoms on tree of the same cultivar that are enclosed in shade houses and those that are not (without shading). Ten trees per row in blocks with and without shading will be used for blossom counts. We will count bees in the rows on the marked trees. Foraging data will be expressed as bees per open blossom. Repeated measures ANOVA of the foraging data taken throughout bloom will be used to determine if foraging activity differs on trees with and without shading. In addition, the bloom data and temperatures will be compared between blocks with and without shading to determine if significant differences in the number of heat units are accumulating under the shading to accelerate bloom.

Objective 3. Incorporate information on honey bee foraging and cross-pollination rates into the pollen tube growth model to improve decision making and thinning practices. Also evaluate foraging model on cherry.

We have modified Dr. DeGrandi-Hoffman's honeybee model to provide a stand-alone model that can predict the foraging quality of any given day. This model is based on rainfall, solar radiation, wind speed, and temperature. The model uses the 15-minute AWN data over the period from 7am to 6pm each day to give a relative score (from 0 = poor to 10 best possible) for foraging that is averaged over the entire day. We have validated the solar radiation sub-model at locations at the extreme north, east, south and west parts of the state, so that we can accurately predict the solar radiation over the day in a cloud-free day and

Fig. 1. Proportion of different bloom stages completed versus DD. From left to right: green tip, 1/2" green, tight cluster, pink, full pink, first bloom, full bloom, and petal fall.



additionally found that data can be modified using cloud cover for future predictions. For each date, we can also provide how each of the factors affected the overall foraging score (*e.g.*, foraging might be limited more by wind speed than temperature on a given date versus cloud cover and rainfall on another day). As this model is based solely on environmental variables, it should be applicable to all honeybee pollinated crops. However, for the final fruit set model, the problems of bloom overlap, cross pollination, are being addressed in the other experiments that are being performed.

A big part of the original model for the DeGrandi-Hoffman apple bloom phenology/fruit set model is the importance of cross-pollination. To clarify this, samples were sent to Phytelligence to evaluate paternity of Honeycrisp seeds from a block planted solely to Honeycrisp (*i.e.*, no pollinizers inter-planted in the block). The testing revealed that all fruit was the product of cross-pollination. Our previous studies revealed that bees can acquire cross-pollen from nest mates in the hive that are foraging cultivars within the flight range of the hive. The source of pollen on foragers that cross-pollinated the Honeycrisp blossoms in the block probably was from nest mates foraging compatible cultivars in neighboring blocks.

Based on findings from paternity testing of Honeycrisp seeds, we will program the cross-pollination and fruit set model so that Honeycrisp blossoms will require cross-pollination for fruit set. Estimates of cross-pollination will be based on varieties in bloom within the foraging distance of colonies in Honeycrisp orchards.

Work this coming year: We will use this model in conjunction with WSU DAS to provide growers with a running average of the quality of foraging for the last 3 days and use NOAA's forecast data to examine the potential quality in the next 3 days. This data can be used by managers to help guide pollination decisions. We expect to have this model up for our beta users this year and release it for general use the following year. The sub-model will also be used in the final model that predicts fruit set. ***NOTE: We are not using any of these funds to implement the model on DAS, that process is being handled from other funding sources.***

We will also contact Dr. Dave Gibeau (Oregon State Univ.-MCAREC) about his cherry bloom model to evaluate whether we can use that data to develop the bloom phenology for cherry. In year 3, we will evaluate the honeybee foraging model on cherry during the bloom time to make sure that there are no changes for foraging needed on that crop.

Objective 4. Evaluate the effects of variability in spring weather conditions, as well as directional shifts toward earlier bloom on fruit set and best pollination management strategies.

This objective was not scheduled to start until year 2 and has been complicated by the departure of PI Nicole Rafferty from this project and WSU. Without the expertise of Rafferty in this area, we have scaled-down our plan. We will be obtaining down-scaled climate change projections for the next 50 years and run the bloom foraging models to evaluate how climate change will affect bloom timing and honeybee foraging to see if there will be any serious issues related to asynchrony related to mis-match of the temperature profiles needed for honeybee pollination and bloom timing (*i.e.*, will full bloom happen too early in the season for reasonable honeybee foraging?).

CONTINUING PROJECT REPORT
WTFRC Project Number: TR-16-101

YEAR: 1 of 2

Project Title: Calibration development for nutrient analysis using a handheld XRF

PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-663-8181 ext. 229
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Jeff Cleveringa (Oneonta Starr Ranch), Glade Brosi (Stemilt), Rob Lynch (Redox), Lee Drake (Bruker Instruments)

Total Project Request: Year 1: \$32,757 **Year 2: \$33,818**

Percentage time per crop: Apple: 80% Pear: 15% Cherry: 5% Stone Fruit: 0%

Other funding sources:

Agency Name: Washington State University
Amt. requested: \$66,575

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
Salaries¹	16,000	16,640
Benefits²	5,610	5,834
Wages¹	4,800	4,992
Benefits²	115	120
Supplies³	5,840	5,840
Travel⁴	392	392
Plot Fees	0	0
Total	32,757	33,818

Footnotes:

¹ Salaries for a 33% FTE research intern (Kalcsits) and summer wages for a M.S. student (Corina Serban).

² Benefits at 35.1% for research intern and 2.4% for M.S. student.

³ Goods and services include lab consumables cost for nutrient analysis and service fees in Pullman and California for elemental analysis.

⁴ Travel to collect fruit and to Kennewick, WA to meet with Bruker for calibration analysis.

OBJECTIVES

1. Identify how correlations between x-ray and lab analysis differ among apple and pear varieties with known differences in skin thickness.
2. Develop cultivar-specific and skin-thickness specific calibrations for non-destructive analysis of calcium and potassium in apple and pear.
3. Incorporate quantitative calibrations into the Bruker software for industry-friendly instrument use.

This project has the goal of looking at how surface measurements using a portable x-ray fluorometer relates with traditional lab analysis. In the previously funded project, the focus was to validate that the instrument measurements agree with traditional lab analysis. In the current project, we are seeking to develop calibrations that can be inserted into the commercially available unit for measurements of fruitlet, fruit at harvest or fruit in storage. However, there is evidence that each cultivar might behave differently with the instrument. This may be due to differences in skin thickness or possibly flesh density. Once these factors have been identified, corrections can be made to develop calibrations across tree fruit crops to increase the range of applications in which the PXRF can be used.

The previous year (2016) included sampling of cherries (Chelan, Sweetheart and Selah), Pears (Anjou and Bartlett), and apples (Honeycrisp, Pink Lady, Fuji). Sampling occurred in June, at harvest and is continuing post-storage. The approach was to compare PXRF measurements with traditional techniques of lab analysis to look at the comparisons. Current lab analysis sampling protocols can differ depending on individual lab protocols but usually a core is sampled from the outer flesh that usually includes the skin in the analysis. We have looked at the relationship between just skin analysis and the PXRF and when the flesh to the core is included in the analysis.

SIGNIFICANT FINDINGS

- There needs to be a re-evaluation of sampling protocols of fruit for traditional lab analysis. My findings are supported by Lailiang Cheng's group in Cornell that have reported that skin analysis is a better indicator of fruit nutrient status than the flesh, particularly for Honeycrisp apple since skin can account for a large portion of the cations in a sample even though the volume occupied by the skin is a small fraction of the overall sample volume.
- We are developing a modified standardized approach for nutrient sampling that only samples the outer 1/4" of peel and flesh of the fruit. When tested, this approach will identify critical differences between fruit samples in nutrient status and will better relate to non-destructive measurements using the PXRF to develop more accurate calibrations.
- Across several fields at equal points of maturity, the slope of the lines remain similar indicating that one calibration could be used for a single cultivar if the sampling protocol is clear and uniform.
- Fruit development impacts the slope for calcium but not potassium (Figures 2 and 3). A calibration for fruitlets and mature fruits will need to be developed because of differences in flesh density and nutrient concentrations.

- The PXRF appears to work for cherries as well and more work will be done to develop a calibration for cherries in 2017.

METHODS

For 2017, the direction outlined in the original proposal will largely remain the same. The goal is to look at how skin thickness and peel to flesh ratios affect the non-destructive measurements using the PXRF. This will help develop a stronger calibration than what can be derived right now. Currently, a calibration curve has been developed and can be used for quantitative measurement of Honeycrisp measured at harvest or in storage. While this has been shown to be reasonably accurate, we don't yet know whether skin thickness can vary and what impact that has on measurements from orchard to orchard. Using multiple locations for each cultivar, we can get an understanding of the range in skin thickness for a cultivar and whether that impacts measurements.

Most of the activities that were planned for 2016 are still in progress since samples are sent to external labs and post storage samples are pulled out of storage for analysis. The skin thickness measurements and calibration sampling for apples will occur in January – March 2017 (Table 1). This will provide the datasets to develop the calibrations for Honeycrisp and Pink Lady apple and Anjou pear. The drastically different slopes shown in Figure 2 in June and measurements at harvest for calcium and not for potassium suggest that more work is needed on standardizing the sampling depth for lab analysis to be sure that everything is uniform. In 2017, we will take corresponding samples using just the peel, ¼ deep core, ½” deep core and a sample to the core of the fruit. These samples will be related to the non-destructive PXRF analysis. Lastly, once all of the calibration curves have been collected, I will make the calibration curves available for non-destructive analysis to use in PXRF devices.

Table 1. Completed and planned activities for the completion of the proposed project

Objective	Activity	Completed or Anticipated Completion Date
1	Looked at how peel and flesh differ in nutrient concentrations in Honeycrisp	Completed 2016
1	Looked at how the relationship between lab analysis and PXRF differs between fruitlets and fruit at harvest	Completed 2016
1	Analyzed groups of apples, pears and cherries using PXRF and then lab analysis	Completed 2016
2	Calibration sampling for Anjou pear	December 2016 - January 2017
2	Look at how lab sampling depth affects the relationship between PXRF and lab analysis	February 2017
2	Calibration sampling of Honeycrisp and Pink Lady	January 2017
2	Skin thickness measurements of Honeycrisp and Pink Lady	January 2017
2	Calibration development for Honeycrisp and Pink Lady	June 2017
1	Fruitlet and cherry sampling	June and July 2017
2	Cherry calibration sampling	July 2017
2	Cherry skin thickness measurements	July 2017

2	Calibration sampling for Gala apple and Bartlett pear	August 2017
2	Skin thickness measurements for Gala apple and Bartlett pear	August 2017
2	Calibration sampling for Fuji	October 2017
2	Skin thickness measurements for Fuji	October 2017
3	Calibration input into PXRF device and open source for industry use	December 2017

RESULTS & DISCUSSION

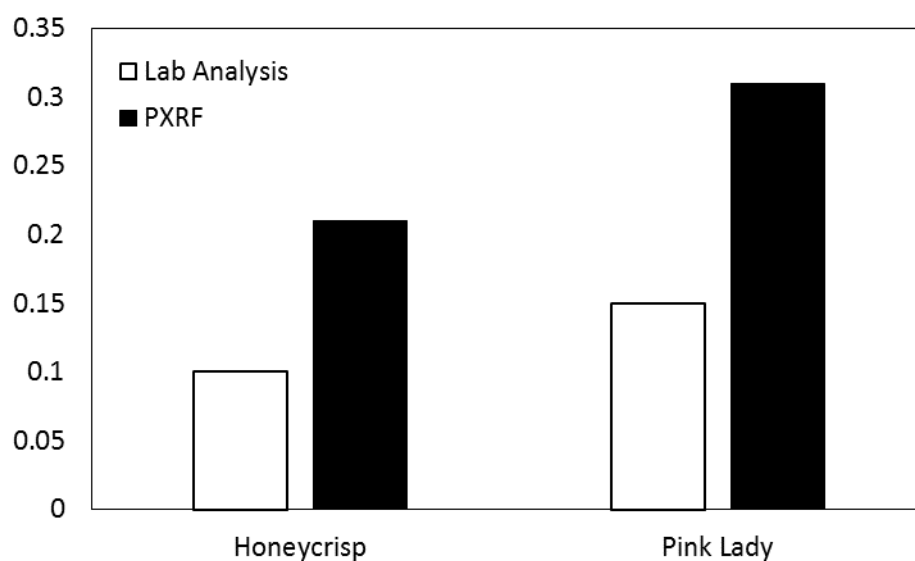


Figure 11. Lab analysis (% D.W.) compared to PXRF (standardized PXRF Ca counts/Rh counts) analysis for calcium concentrations in Honeycrisp and Pink Lady apple

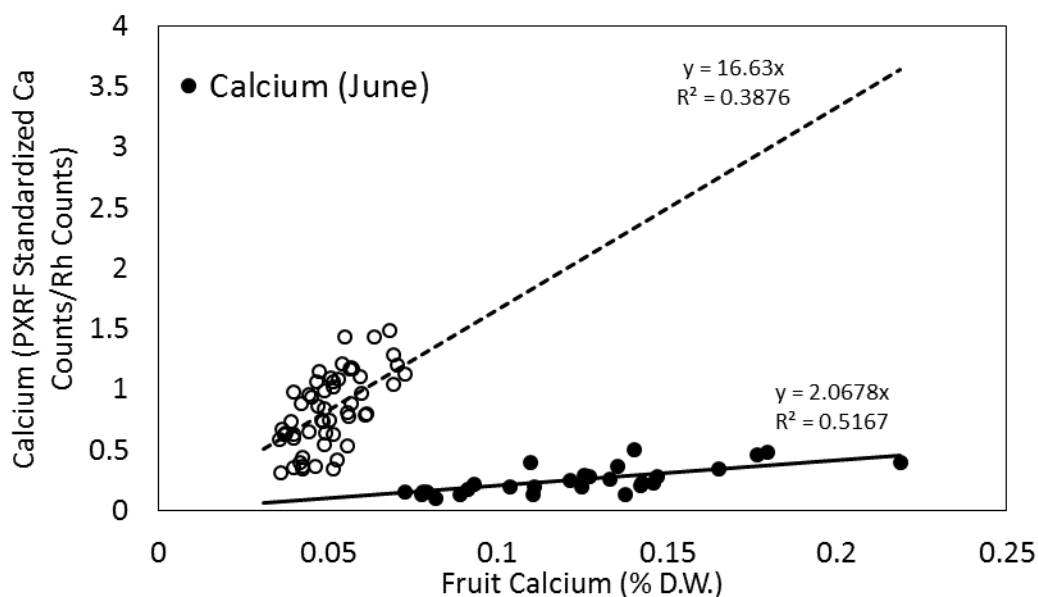


Figure 2. Non-destructive PXRF calcium measurements compared with lab analysis for Honeycrisp apple sampled either in June (closed circles) or at Harvest (open circles)

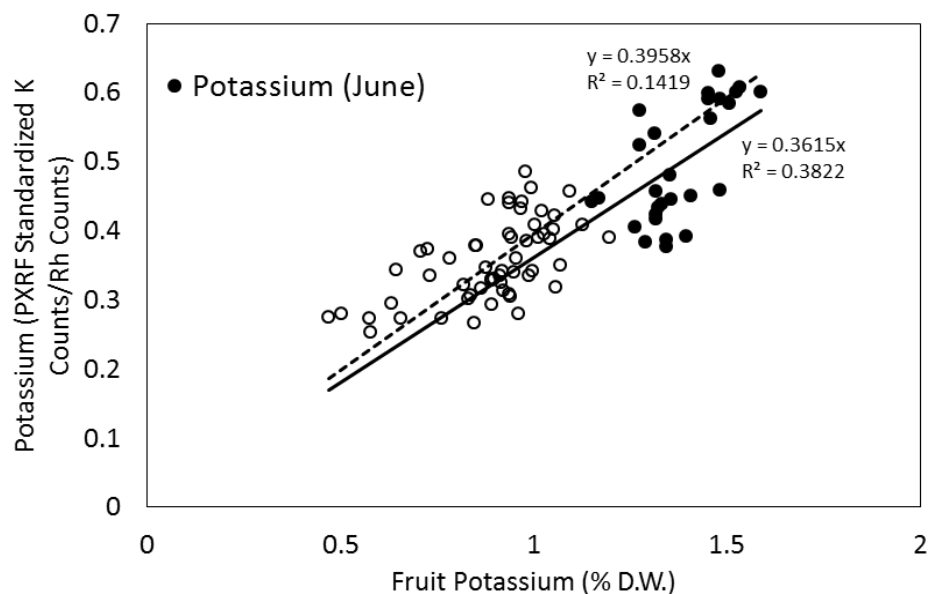


Figure 3. Non-destructive PXRF potassium measurements compared with lab analysis for Honeycrisp apple sampled either in June (closed circles) or at Harvest (open circles)

Table 2. Lab analysis of calcium and potassium concentrations in ‘Honeycrisp’ apple (N=50)

	Peel (% D.W)	Flesh (% D.W.)
Calcium	0.039±0.002	0.015±0.002
Potassium	0.818±0.006	0.761±0.005

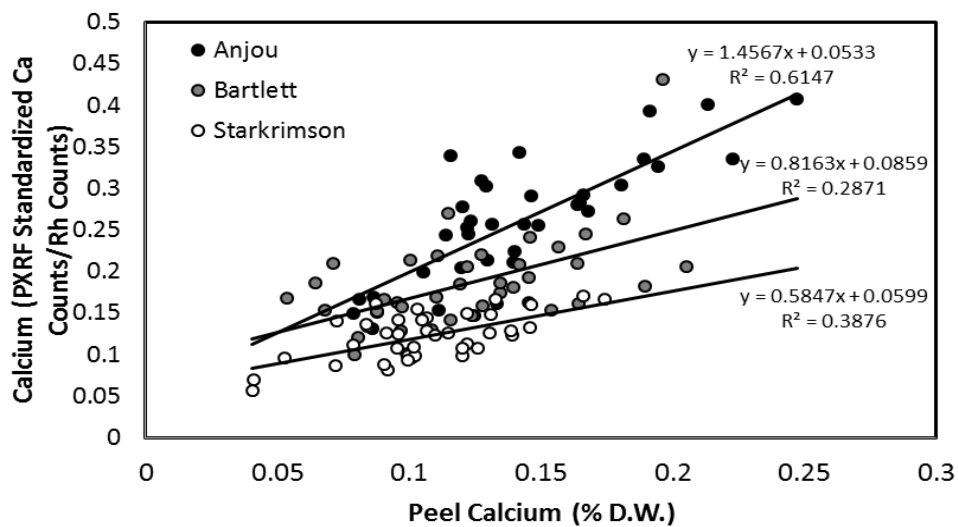


Figure 4. Non-destructive PXRF calcium measurements compared with lab analysis for Anjou (black circles), Bartlett (grey circles), and Starkrimson (white circles) pear sampled at harvest.

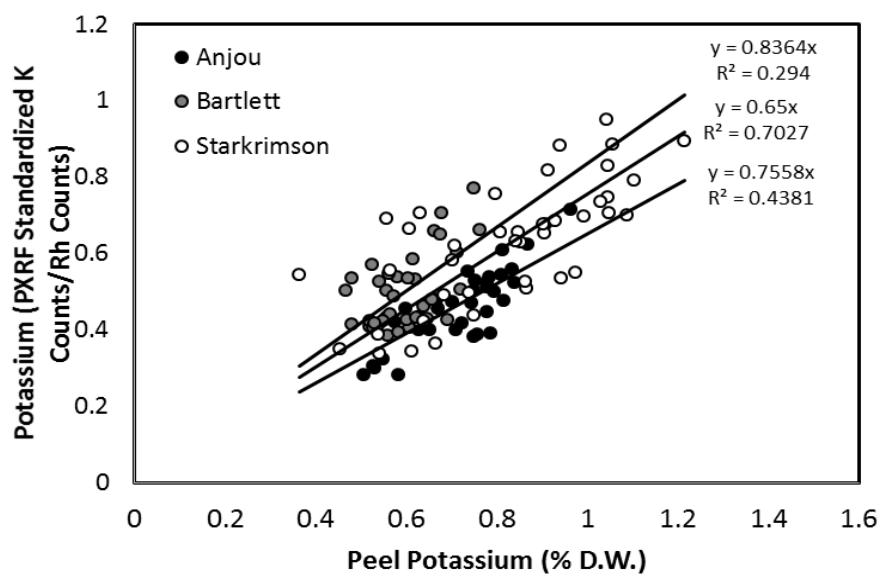


Figure 5. Non-destructive PXRF potassium measurements compared with lab analysis for Anjou (black circles), Bartlett (grey circles), and Starkrimson (white circles) pear sampled at harvest.

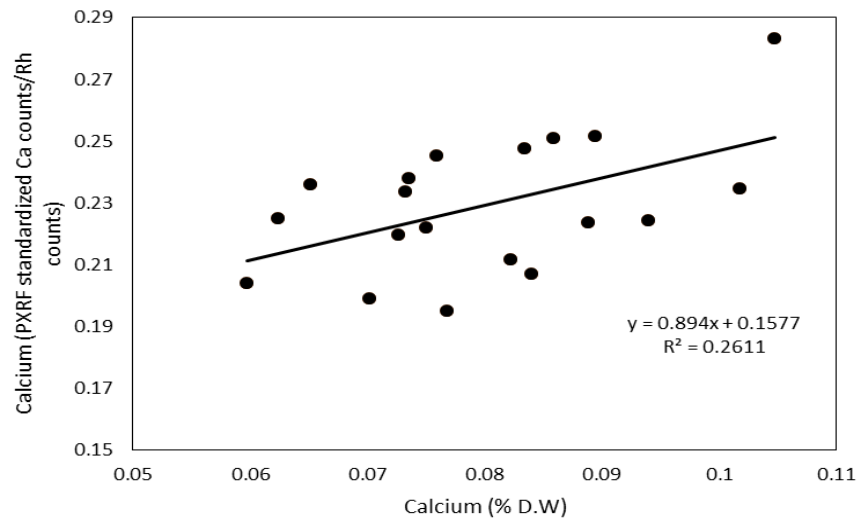


Figure 6. Non-destructive PXRF calcium measurements compared with lab analysis for cherries harvested from June to July 2016

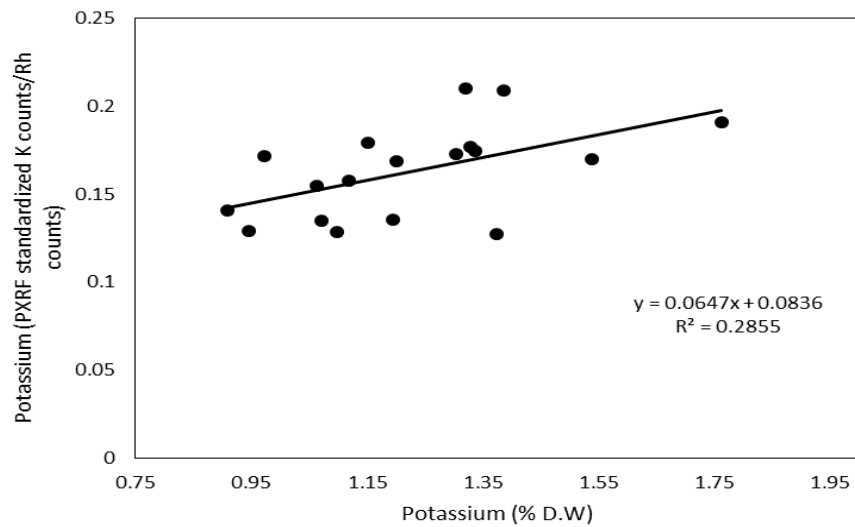


Figure 7. Non-destructive PXRF potassium measurements compared with lab analysis for cherries harvested from June to July 2016

Overall, the goal is to make this instrument more user friendly to the industry to provide accurate non-destructive information on the nutrient status of fruit. This will provide a more rapid decision platform than traditional lab analysis and has implications in horticultural and storage decisions. However, we still need to understand the reasons behind variable slopes in the calibrations and the contributions of peel and flesh analysis ratios to the overall PXRF value. When this is complete, it will make it easier to compare different cultivars using this approach and increase the confidence in adopting it for nutrient analysis in the industry.