# 2019 Technology Research Review

February 12, 2019, Wanapum Dam Visitor's Center

| Page No. | Time | Presenter | Project Title | Yrs |
|---|---|---|---|---|
| | | Willett | Introduction | |
| | | | Final Reports | |
| 1 | 9:30 | Kalcsits | Calibration development for nutrient analysis using a handheld XRF: *a show and tell* | 16-17 |
| | | | Continuing Reports | |
| 13 | 9:45 | Jones | Development and validation of a precision pollination model: *No-cost extension* | 16-18 |
| 21 | 10:00 | Jones | Developing and validating models for tree fruit | 17-19 |
| 28 | 10:15 | Whiting | Reducing cold damage with cellulose nanocrystals | 18-19 |
| 35 | 10:30 | Kahani | Multi-purpose robotic system for orchards | 18-20 |
| 43 | 10:45 | Karkee | Towards automated canopy management in tree fruit crops | 18-19 |
| 51 | 11:00 | Honaas | Enhancing reference genomes for cross-cultivar functional genomics: *No-cost extension* | 17-18 |
| 58 | 11:15 | Good | Development of economical wifi-connected open-source sap flux probes | 18-19 |

**FINAL REPORT**
**WTFRC Project Number:** TR-16-101

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

**PI**: Lee Kalcsits
**Organization**: Washington State University
**Telephone**: 509-663-8181 ext. 229
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**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**: $32,754 **Year 2**: $33,818

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

**Other funding sources: None**

**WTFRC Collaborative expenses: None**

**Budget 1**
**Organization Name:** WSU **Contract Administrator:** Kim Rains
**Telephone:** 509-335-4564/509-663-8181 **Email:** kim.rains@wsu.edu

| Item | 2016 | 2017 |
|---|---|---|
| **Salaries**[1] | 16,000 | 16,640 |
| **Benefits**[2] | 5,610 | 5,834 |
| **Wages**[1] | 4,800 | 4,992 |
| **Benefits**[2] | 115 | 120 |
| **Supplies**[3] | 5,840 | 5,840 |
| **Travel**[4] | 392 | 392 |
| **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.

## RECAP ORIGINAL 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.

## SIGNIFICANT FINDINGS

- Other research groups are working on using XRF for non-destructive analysis of leaf tissue, apple roots, stems, and fruit. This is a general trend for using this technology for making these types of measurements. This instrument will have its primary utility as a research tool but for larger operations or consulting, this could contribute to assigning risk assessment for bitter pit incidence for commercial Honeycrisp orchards

- Significant linear regressions were obtained for Honeycrisp, Pink Lady, Fuji, D'Anjou pear, Bartlett pear, Starkrimson pear and sweet cherry. These will be put into a calibration software in the instrument. The scientific support at Bruker has had a turnover of scientists in the last year. It is now in less flux and we are working to input the calibrations into our instruments using their calibration software. Testing in a commercial orchard returned estimates that agree with ranges expected for mineral analysis.

- Skin thickness was not related to measurements between cultivars. However, within cultivars, there was a weak correlation between skin thickness and calcium concentrations.

- A calibration for fruitlets and mature fruits was developed for Honeycrisp because of differences in flesh density and nutrient concentrations.

- Commercial orchards were tested in 2018 for the use of PXRF for bitter pit risk assessment. Measurements made six weeks before harvest and at harvest were significantly correlated to bitter pit incidence after storage. However, as expected the relationship was not perfect but is a useful tool to assign risk.

- Newer PXRF systems come with existing calibrations but they would need to be verified as they are not the same calibrations that I have developed. The calibrations developed within this project could be easily incorporated into the newer model instruments.

| 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 | Completed 2017 |
| **2** | Look at how lab sampling depth affects the relationship between PXRF and lab analysis | Completed 2017 |
| **2** | Calibration sampling of Honeycrisp and Pink Lady | Completed 2017 |
| **2** | Skin thickness measurements of Honeycrisp and Pink Lady | Completed 2017 |
| **2** | Calibration development for Honeycrisp and Pink Lady | Completed 2018 |
| **1** | Fruitlet and cherry sampling | Completed 2017 |
| **2** | Calibration sampling for Gala apple and Bartlett pear | Completed 2017 |
| **2** | Skin thickness measurements for Gala apple and Bartlett pear | Not necessary |
| **2** | Calibration sampling for Fuji | Completed 2017 |
| **2** | Skin thickness measurements for Fuji | Completed 2017 |
| **3** | Calibration input into PXRF device and open source for industry use | Incorporated into excel but not instrument |

## RESULTS & DISCUSSION

We have worked to develop calibrations that provide quantitative measurements of calcium concentrations in apple, pear and cherry. The measurements made appear to be independent of skin thickness within each cultivar and is not a covariate in our calibrations which simplified calibration development. Through either quantitative or semi-quantitative analysis, comparisons among orchard lots or individual fruit can be made for calcium and potassium to provide some avenue at assigning risk in terms of the development of calcium related disorders. These types of measurements can be used to measure many fruit in a single tree to better understand how variation in nutritional distribution is related to bitter pit incidence. These differences can be expressed as semi-quantitative like in Figure 1 or translated to quantitative values using calibrations described later in this report.

*Applications of PXRF Technology*

Figure 1. Relative calcium concentrations (top), potassium concentrations (middle), and potassium: calcium concentration ratios (bottom) of fruit with changing relative canopy vertical position (y-axis) and relative radial distance from the trunk (x-axis) acquired using a portable x-ray fluorometer.

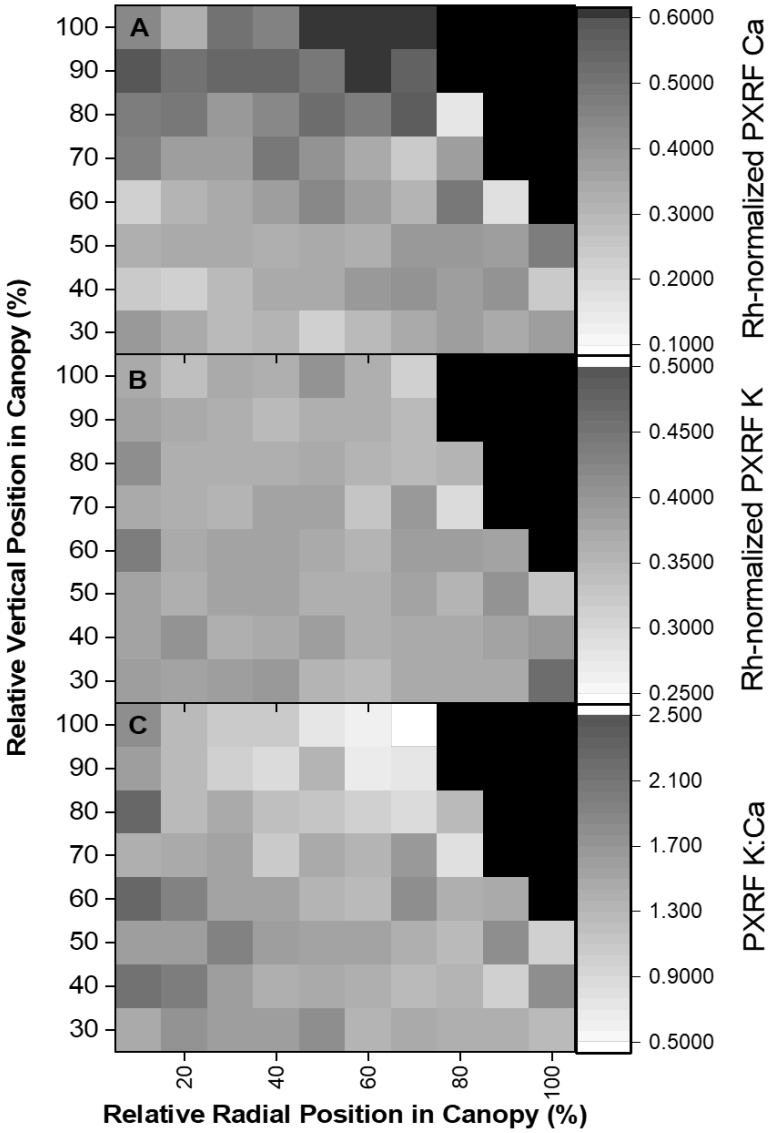

Figure 2. Two-dimensional distribution of bitter pit incidence (%) taken from different relative vertical and radial positions in the tree canopy of 78 ‘Honeycrisp’ apple trees from nine commercial orchards.

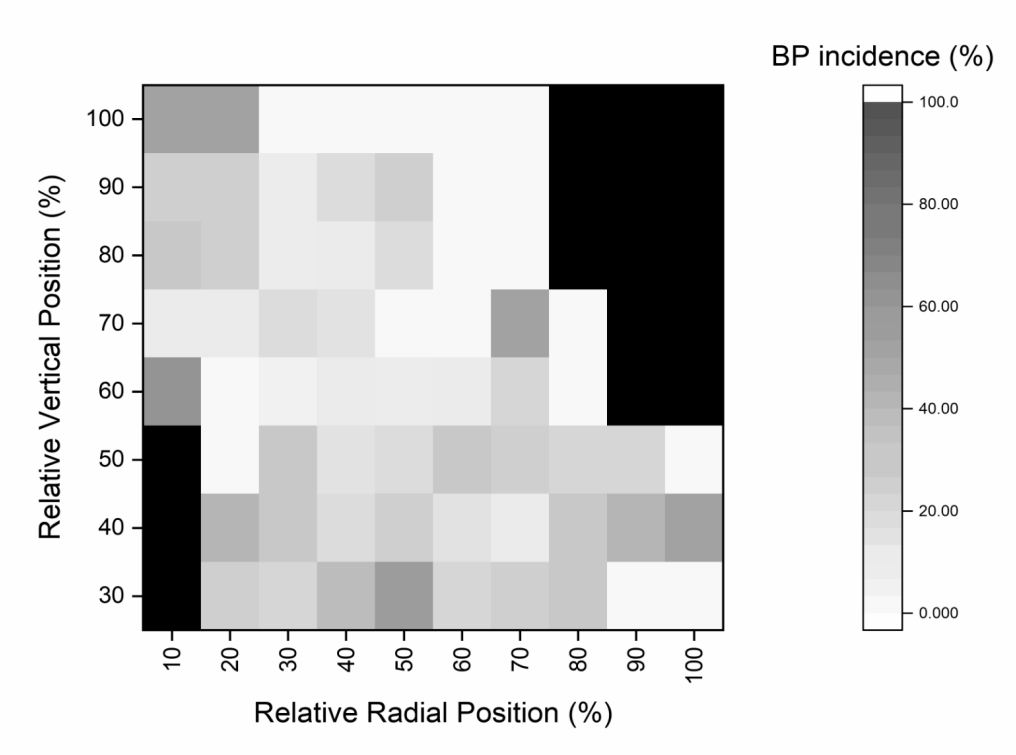


Figure 3. Different applications of PXRF measurements to work towards answering questions related to calcium and potassium in apple, pear, and cherry using top left: field measurements; top right: lab measurements of fruit; bottom left: matrix measurements; bottom right: pelletized ground tissue.

***Are cultivar specific calibrations enough?***

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. Additionally, skin thickness did not contribute to variability in measurements within individual cultivars. Across cultivars, changes in both peel and cortex density would likely contribute to variability in the readings that may or may not be related to bitter pit incidence. This would be a testable hypothesis for future work with segregating populations.

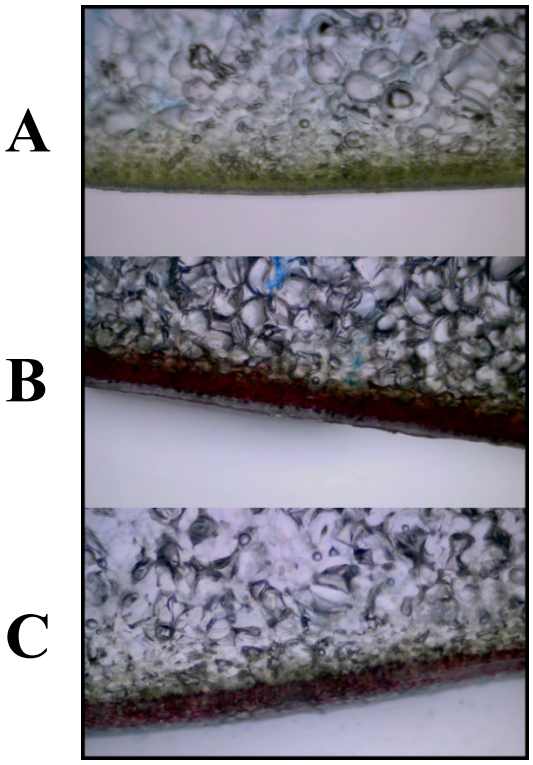


Figure 4. Images of peel thickness in Honeycrisp (A), Fuji (B), and Pink Lady apples harvested at maturity in 2016.

***Calibrations***

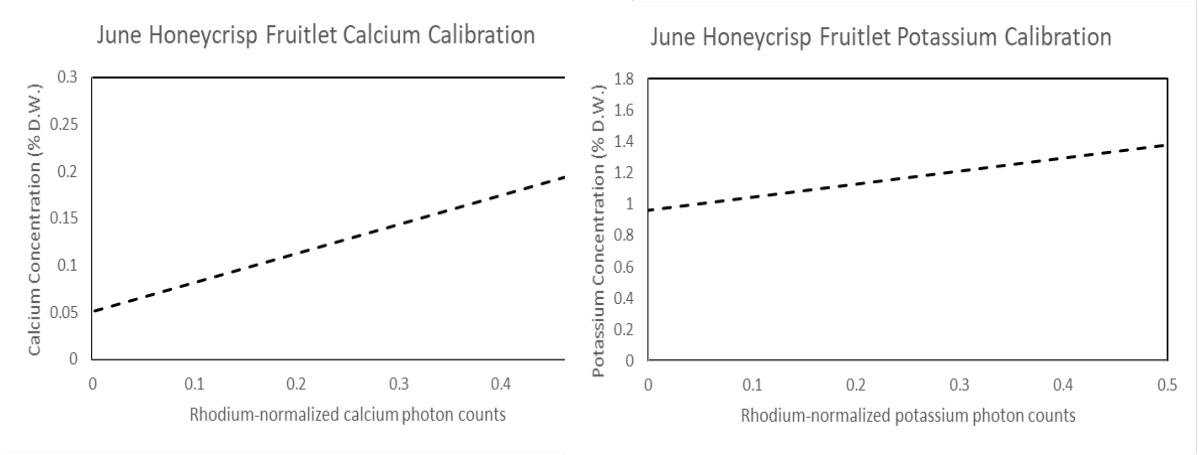


Figure 5. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruitlets analyzed in June. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w.

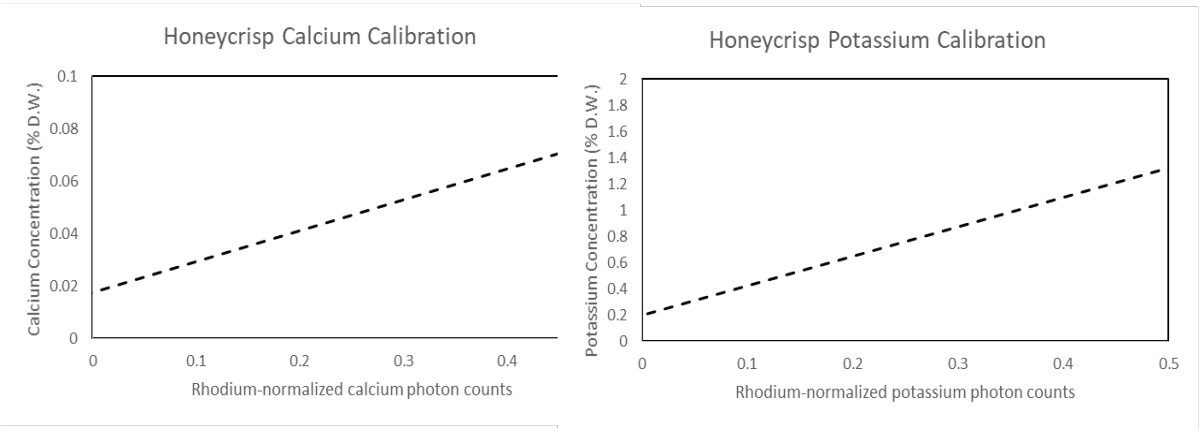


Figure 6. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

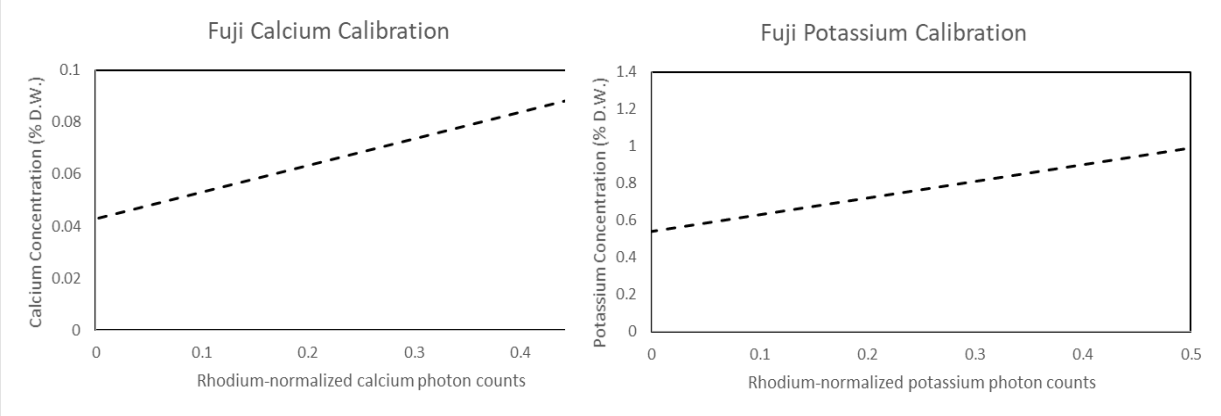


Figure 7. Linear regression for calcium (left) and potassium (right) in Fuji fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

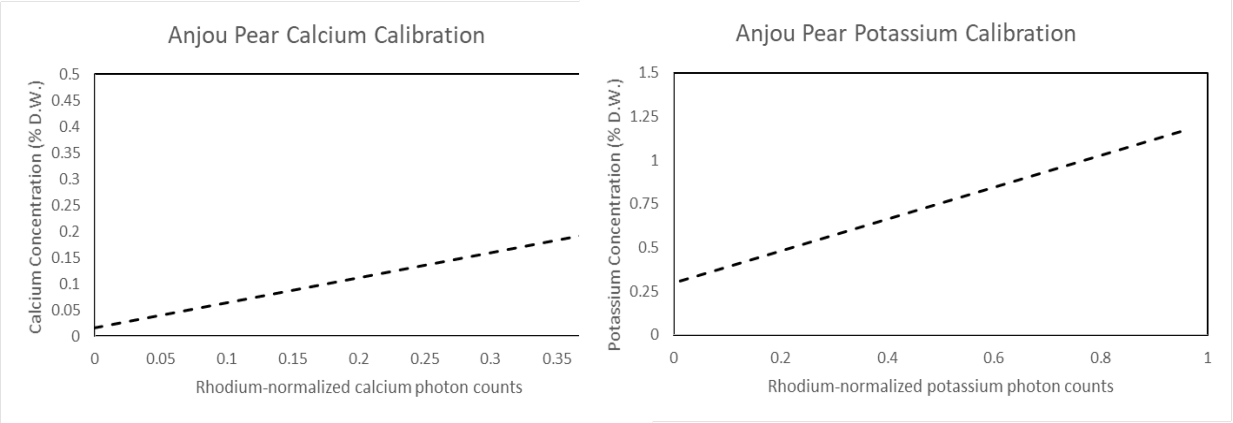


Figure 8. Linear regression for calcium (left) and potassium (right) in Anjou pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

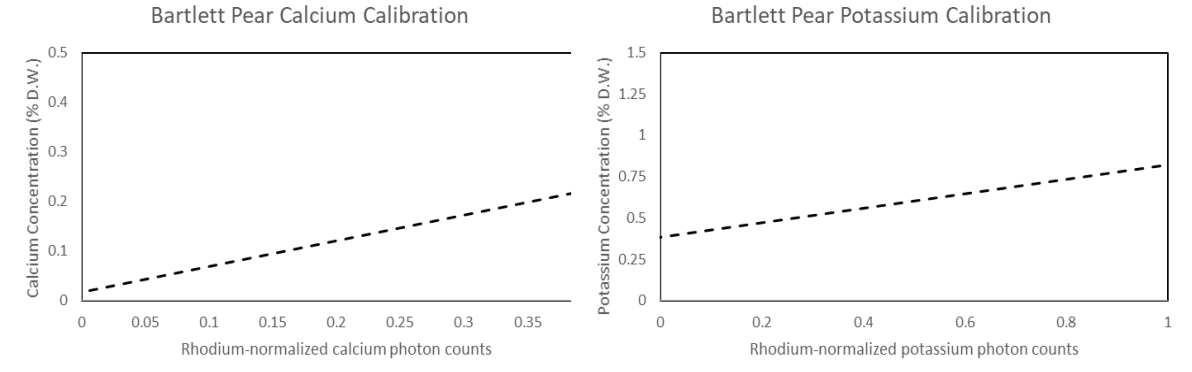


Figure 9. Linear regression for calcium (left) and potassium (right) in Bartlett pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

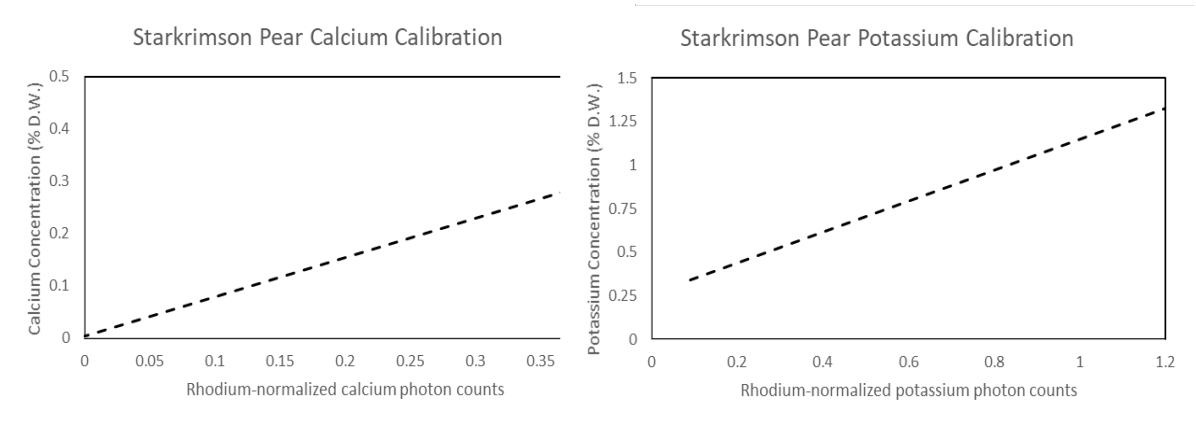


Figure 10. Linear regression for calcium (left) and potassium (right) in Starkrimson pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

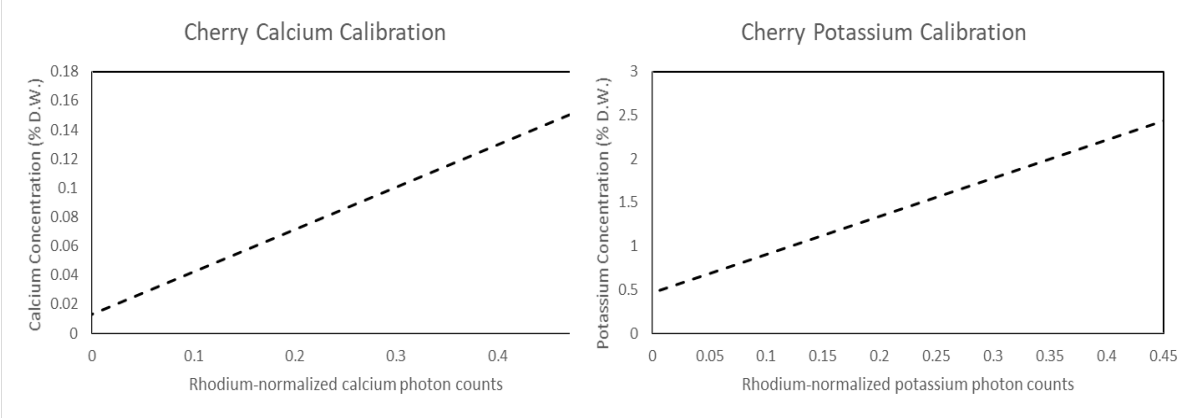


Figure 11. Linear regression for calcium (left) and potassium (right) in sweet cherry fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

When the PXRF was tested for risk assessment for commercial orchards, the calculated potassium and calcium concentrations fell within normal ranges observed for Honeycrisp fruit using destructive analysis. Calcium concentrations at harvest ranged from 0.014% to 0.06% dry weight whereas potassium concentrations ranged from 0.2 to 0.73% dry weight. These produced K:Ca ratios that ranged from as low as 5 to as high as 26 (Table 1). These ratios seems a bit lower than normal but were well correlated with bitter pit incidence (Figure 12). Calcium concentrations six weeks before harvest were greater than at harvest. In many cases, the concentrations had dropped by 50 to 80% during that time. This is a key time for rapid fruit growth as well as potassium influx into the developing fruit. Potassium concentrations did not decrease by nearly as much, even though this is a rapid period for fruit growth. This demonstrates that potassium transport to the fruit is much greater than calcium later in the season. There was not a complete agreement between the PXRF readings and bitter pit but that is equal to trends observed for traditional elemental analysis as well.

Table 1. Calibrated potassium: calcium ratios of Honeycrisp apple measured in fruit either six weeks before harvest or at harvest for 15 commercial orchards. Bitter pit incidence was counted after three months of storage and seven days at room temperature. Bitter pit risks were assigned pre storage to see how risk assessments that included vegetative growth related to bitter pit after storage.

| Orchard | BP risk Estimate | K/Ca Harvest | K/Ca 6WBH | BP% |
|---|---|---|---|---|
| A | High | 17.40 | 5.88 | 53 |
| B | Moderate | 12.61 | 2.57 | 27 |
| C | High | 18.61 | 5.68 | 27 |
| D | Moderate | 8.17 | 3.94 | 0 |
| E | Low | 8.79 | 3.38 | 26 |
| F | Low | 5.92 | 2.46 | 36 |
| G | Low | 4.24 | 1.72 | 30 |
| H | High | 23.71 | 6.74 | 70 |
| I | Low | 8.83 | 2.60 | 4 |
| J | High | 8.61 | 3.39 | 49 |
| K | High | 9.26 | 5.20 | 5 |
| L | High | 7.20 | 5.10 | 13 |
| M | High | 9.64 | 5.89 | 62 |
| N | Low | 8.95 | 3.68 | 11 |
| O | Low | 11.36 | 4.83 | 15 |

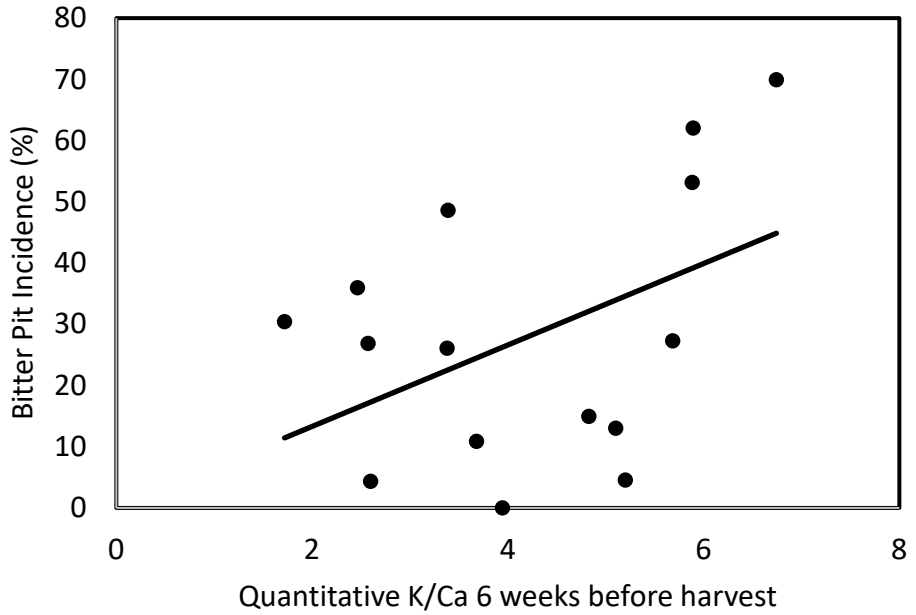


Figure 12. Calibrated potassium: calcium (K/Ca) ratios for Honeycrisp apple measured six weeks before harvest from 15 different commercial orchards related to bitter pit. Line represents best linear fit ($P<0.05$) for this relationship.

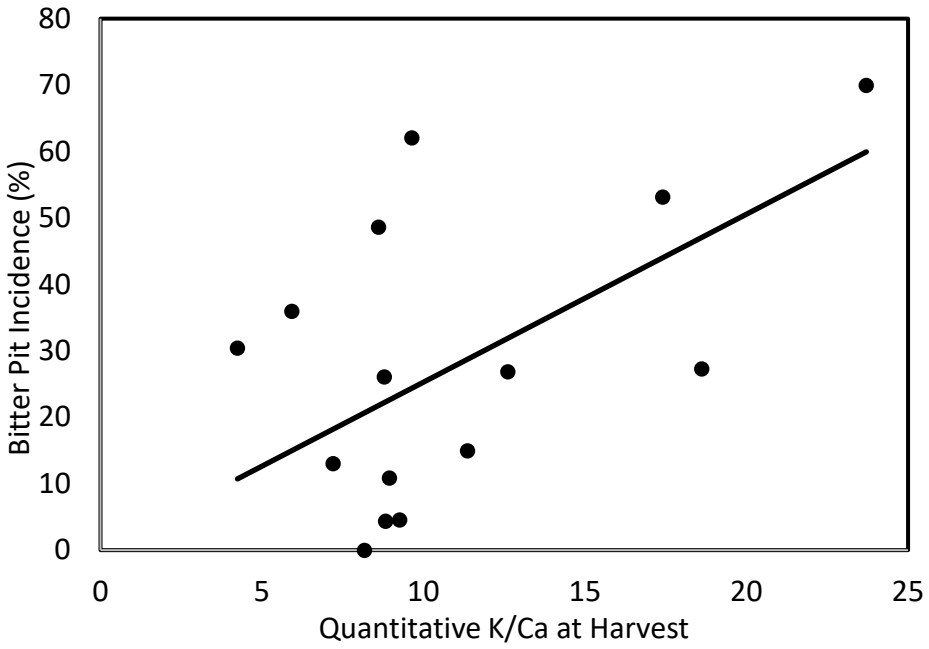


Figure 13. Calibrated potassium: calcium (K/Ca) ratios for Honeycrisp apple measured six weeks before harvest from 15 different commercial orchards related to bitter pit. Line represents best linear fit ($P<0.05$) for this relationship.

Since the start of this project, this information has been incorporated into two peer-reviewed publications and one more is in preparation that will use these approaches developed with this project. Furthermore, this work has been included in 10 state and regional talks, 4 national talks, and 5 international invited presentations. We are also in the process of testing its use for measuring strontium and rubidium uptake which act as tracers for calcium and potassium, respectively. We have found that the PXRF measurements are nicely related to analytical approaches for measuring these elements. This allows us to non-destructively sample the same tissue over the course of the season to measure fluxes into specific plant tissues. This instrument is being used in several labs in the US at least partially based on this project and funding from the WTFRC.

## EXECUTIVE SUMMARY

This project had the goal of developing some translation of the semi-quantitative measurements given using PXRF to quantitative measurements of calcium and potassium. Additionally, we sought to identify how surface measurements using a portable x-ray fluorometer related 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 sought 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.

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. Additionally, skin thickness did not contribute to variability in measurements within individual cultivars. Across cultivars, changes in both peel and cortex density would likely contribute to variability in the readings that may or may not be related to bitter pit incidence. This would be a testable hypothesis for future work with segregating populations from breeding material.

Since the start of this project, other research groups have started to integrate PXRF approaches into their research. This includes measurements of leaf tissue, apple roots, stems, and fruit. There is a general trend for using this technology for making these types of measurements since it provides rapid and immediate measurements that can be used non-destructively to track changes in the same sample over time or to measure many more replicates than would normally be feasible in research.

This instrument will have its primary utility as a research tool but for larger operations or consulting, this could contribute to assigning risk assessment for bitter pit incidence for commercial Honeycrisp orchards. There are several models of this instrument available but they only come with generalized calibrations that may not agree with measurements of fruit. Newer PXRF systems that come with existing calibrations would need to be verified as they are not the same calibrations that I have developed. The calibrations developed within this project could be easily incorporated into the newer model instruments and would be available if any industry members in Washington State wish to use this instrument in QC as part of their operations.

**THIRD YEAR REPORT** **YEAR**: Year 3 of 3 (No Cost Extension)
**WTFRC Project Number:** TR-16-102

**Project Title**: Development and validation of a precision pollination model


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**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)


**Total Project Request:** Year 1: $95,834 Year 2: $103,359 Year 3: $104,569

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

**WTFRC Collaborative Expenses:**

| **Item** | 2016 | 2017 | 2018 | **2019** |
|---|---|---|---|---|
| **Salaries** | 5000 | 5000 | 5000 | 0 |
| **Benefits** | 2000 | 2000 | 2000 | 0 |
| **Wages** | 8000 | 12,000 | 12,000 | 0 |
| **Benefits** | 2400 | 3600 | 3600 | 0 |
| **RCA Room Rental** | | | | |
| **Supplies** | | | | |
| **Travel** | 1800 | 2000 | 2000 | 0 |
| **Miscellaneous** | | | | |
| **Total** | 19,200 | 24,600 | 24,600 | **0** |

**Budget 1**
**Organization Name:** USDA-ARS **Contract Administrator:** Kathleen Vandebur
**Telephone:** 520-647-9160 **Email address:** Kathleen_Vanderbur@ars.usda.gov

| **Item** | | 2017 | 2018 | **2019** |
|---|---|---|---|---|
| **Salaries** | | $1000 | $1000 | 0 |
| **Benefits** | | | | |
| **Wages** | | | | |
| **Benefits** | | | | |
| **Equipment** | | | | |
| **Supplies** | | | | |
| **Travel** | | $4,000 | $4,000 | 0 |
| **Plot Fees** | | | | |
| **Miscellaneous** | | | | |
| **Total** | | | $5,000 | **0** |

**Footnotes:**

**Organization**: WSU-TFREC **Contract Administrator**: Katy Roberts/Kim Rains
**Telephone:** 509-335-2885/509-293-8803 **Email:** arcgrants@wsu.edu / joni_cartwright@wsu.edu

| **Item** | 2016 | 2017 | 2018 | **2019** |
|---|---|---|---|---|
| **Salaries**[1] | 35,000 | 45,000 | 46,800 | 0 |
| **Benefits**[1] | 15,120 | 11,493 | 11,953 | 0 |
| **Wages**[2] | 18,800 | 11,440 | 11,898 | 0 |
| **Benefits**[2] | 1,214 | 309 | 321 | 0 |
| **Equipment** | | | | |
| **Supplies** | 3,500 | 3,500 | 2,500 | 0 |
| **Travel**[3] | 3,000 | 1,500 | 1,560 | 0 |
| **Miscellaneous** | | | | |
| **Plot Fees** | | | | |
| **Total** | 76,634 | 73,242 | 75,032 | **0** |

**Footnotes:**
[1] Salaries and benefits are for a half-time grant manager
[2] Wages and benefits are for student temporary employees.

**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.

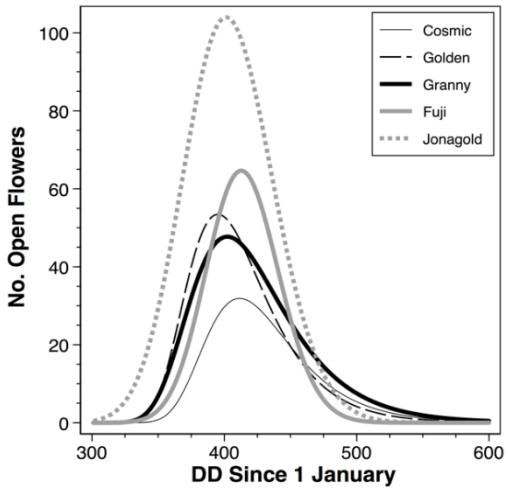
**Significant Findings:**

- Timing of full bloom is predictable for five new cultivars: Cosmic Crisp, Fuji, Gala, Granny Smith, Jonagold.
- Netting slows the progression of bloom compared to no nets, reduces the abundance of honeybees foraging, and results in lower and more variable fruit set.
- Honeybee foraging is driven by the number and relative abundance of open flowers on the mix of cultivars open at any time throughout the bloom period.
- Evaluation of the effect of climate change scenarios on honeybee foraging showed that temperatures during the bloom period will have a minor effect on foraging rates.
- The shorter daylength and lower intensity of sunlight occurring earlier in the year, when apples will bloom in Washington, will cause up to 20% reduction in foraging efficiency.

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

*Results*: The data were collected differently than previously done by Tory where he looked at the same blooms at each observation dates. Our data was taken by looking at flowering clusters so that we counted many more flowers, but individual blossoms could not be tracked. This means that our data had higher numbers of flowers blooming on each date, but that the same flower would be recorded multiple times. The number of flowers before and after peak were roughly equal. However, for the analysis, we just used the number of flowers as the weighting factor in fitting the distributions. The flowering data and the model fits showed that in our blocks Jonagold bloom was much more prolific and occurred earlier than the other five cultivars, and that Cosmic Crisp was the least prolific and occurred later in general than the other cultivars (Fig. 1). The flowering curve is extremely important in the prediction of fruit set and evaluating the honey bee foraging rates on the different cultivars as will be discussed in objective 3.

**Fig. 1.** Probability density functions for the different numbers of open flowers.




*Next Steps*: Our findings will require DAS to recalculate flower/bud phenology using the 15-minute accumulations instead of doing the single sine approach which generally had higher errors error rates. We will continue to collect bloom data next year to finish off the bloom models using the no cost extension.

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

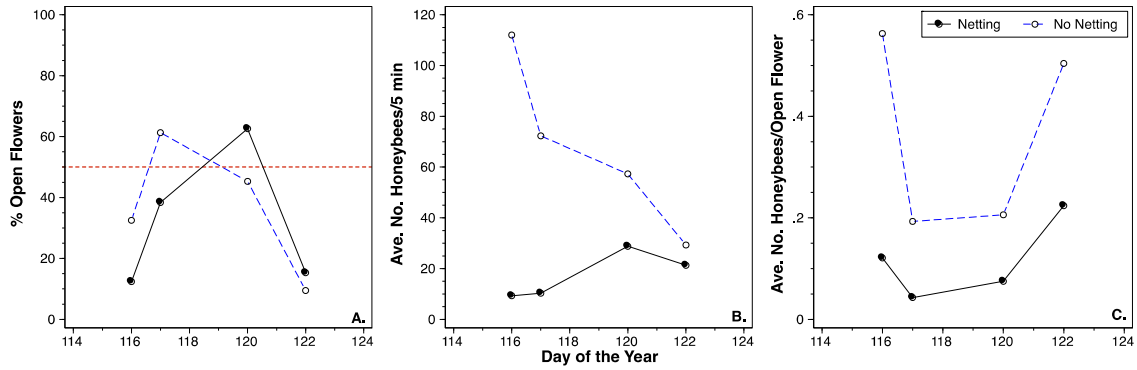
*Methods:* Two adjacent blocks of Fuji's were used for this non-replicated study. One block had overhead nets deployed before bloom while the other block was not covered with overhead netting throughout the study. The study utilized the entire 9 acre "no-net" block and ≈10 A of the 24 A block covered with netting. Trees in both blocks were trained on trellises. The net was a white 20% light-reducing netting that extended down over the top wires about two ft along the sides, 4 ft down along the front and all the way to down to the ground on the back (west side).

*Bloom progression assessment:* The blossoms were hand-thinned to about one flower per spur at the start of bloom in each block. In each block, three sections along the trellis were marked and each section contained ≈200 flower buds. The number of flowers observed for bloom progression was 618 and 610 flowers in the net and no-net blocks, respectively. The number of open flowers were recorded each time the flowers were observed, and these data was used to estimate % bloom.
*Honey bee abundance:* On April 4, four sets of four hives were placed under the nets next to the trees on the west side of the netted block and two sets of four hives were placed along the east edge of the block with no nets. The abundance of honey bees foraging in each block was assessed by slowly walking down a row and counting bees observed on or near apple trees during five-minute intervals. Three to six 5-min observations were made in each block on the days foraging bees were counted. All counts made within a block were averaged on a daily basis.

*Fruit set:* Transects were set up along the entire length of seven rows in each block and each transect contained five trees that were used for estimating fruit set (n = 35 trees per block). Distances for trees located along each transect were 40, 200, 360, 600 and 760 ft from the side of each block where the bee hives were located. The length of each tree row was ≈ 800 ft and the trees closest to the edges of the blocks were located 40 ft in from the edges.

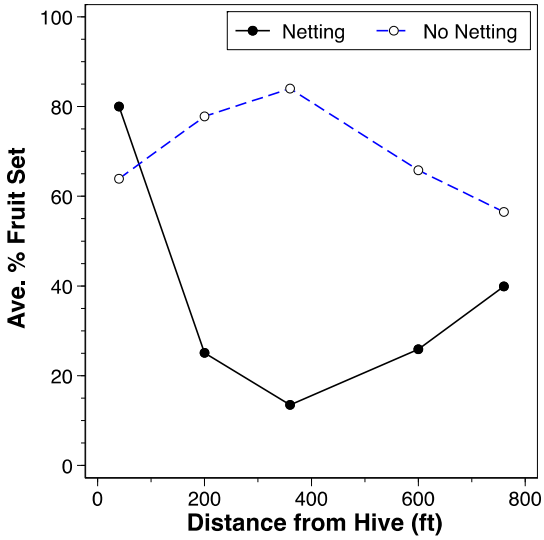
*Results:* We observed several differences between the net and no-net blocks. First was that bloom progressed earlier in the no-net block compared with bloom under netting (Fig. 2a). Fifty percent bloom occurred on 27 Apr in the no-net block compared with 29 Apr for 50% bloom under nets. We also observed considerably more honeybees foraging in apple trees without nets compared with the amount observed foraging under nets (Fig. 2b). When we standardized the number of bees per open blossom, we saw that the abundance of bees per open blossom was always higher in the block without nets (2c). Fruit set was more uniform (between 60-80%) along transects in the no-net block compared with the uniformity of fruit set observed under nets (15-80%) (Fig. 3). Fruit set in the block covered with nets decreased along the transects from the edges into the interior of the block.

**Fig. 2.** Effect of netting on flowering and honeybee foraging. A) Progression of flowering. B) Number of honeybees observed in 5 minute samples. C) Average number of honeybees per open flower.



We also saw additional ways that nets impacted pollinators. Bees often fly up and out when leaving an area. In this study, we observed that wild bees, bumble bees and honey bees became trapped in the upper corners where the nets were folded over the top wires. This resulted in exhaustion of the bees and an accumulation of dead and dying bees on the ground under the corner. It appears that having the edge of the net folded down over the top wire prevented some bees from leaving the netted area. One possible solution would be to install the nets so that they are flat. However, bees often were seen flying up and bouncing off the interior net ceiling indicating that nets inhibit upward flight of honey bees. Overall, orchard netting appears to negatively impact honey bee flight during bloom and subsequent fruit set.

**Fig.3.** Effect of netting on fruit set.




*Work next year:* This objective is completed.

***Obj. 3. Incorporate information on honeybee foraging and cross pollination rates into the pollen tube growth model to improve decision making and thinning practices. Evaluate foraging on cherry.***

*Methods Cultivar Choice and Fruit Set:* The evaluation of flowering is discussed in objective 1, but those data were also used to calculate the relative proportion of flowers that were open for the different cultivars throughout the bloom period (Fig. 4.) The proportion of open blossoms for each cultivar includes data from figure 1, and the relative abundance of the different flowers in our plots. That data was paired with the foraging rates (number of bees on each cultivar) that were taken every few hours throughout the foraging periods.

**Fig. 4.** The proportion of total open flowers each cultivar comprises throughout the bloom period. Based on data from figure 1

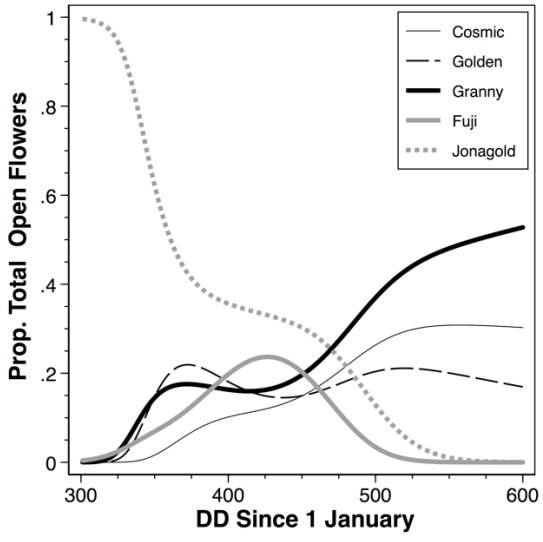
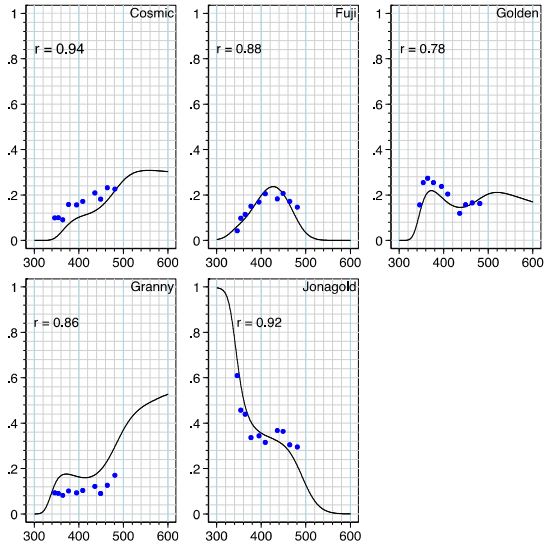



**Fig. 5**. Correlations between the relative number of open blooms (solid line) and the proportion of honeybees foraging each date on that cultivar.



We also did some studies on fruit set involving the four cultivars Fuji, Golden, Granny, and Jonagold; we were not allowed to do any thinning of the Cosmic Crisp in either the location at Sunrise or in Quincy, so we have fruit set if no thinning occurred, but nothing else for that cultivar. Hand thinning happened on April 24, 26, 28, and May 1st. These thinning dates corresponded to 337, 416, 459, and 504 DD. The "no thinning" count was done on 24 April, but assumption is that this happened at the end of the flowering period or ≈ 600 DD.

*Methods Cherry Flowering Time:* The cherry flowering was investigated for the first time this past year. We were able to determine the bloom phenology of Chelan cherries at a single location near Rock Island. The overlap of bloom in apples and cherries made it nearly impossible to sample both and the choice of apples as being more important was based on the larger amount of data that we had and felt that we could finish up this past year. We still intend to do more work on cherries this coming year on a no-cost extension to the grant.

*Results Cultivar Choice by Honeybees:* The data showed that the honeybee distribution on the different cultivars was highly correlated to the relative proportion of flowers that were in bloom (Fig. 4). The correlations were very good and support the idea that the honeybees do not actively discriminate among cultivars, instead their distribution is related to the numbers of flowers that are open on a particular cultivar at any given time. While there were some differences where the number of bees (dark dots) were higher than the proportion of flowers open on Cosmic Crisp (Fig. 5) – this is likely due to the Cosmic Crisp block being closest to the large bee yard. Similarly, the Granny area sampled for bees was the location that was farthest from any of the hives, whereas Fuji, Golden, and Jonagold were about the same distance from the hives and track the bloom density curve very well.

*Results Fruit Set:* The fruit set was highest early in the bloom period for all cultivars, then tended to flatten out by the second time the flowers were thinned (Fig. 6). The exception to this was Fuji whose flowering tends to start and peak later than the other cultivars – that cultivar didn't flatten out until the last hand thinning on 1 May. Jonagold, which starts blooming early and comprises the majority of the cultivars in our blocks showed very little variation in fruit set throughout the season. This is because early on, the majority of the flowers open were other Jonagold flowers, so that the cross-

**Fig. 6.** Bloom curve (solid line) and fruit set when all open flowers were removed at five different times of the season for four different apple cultivars

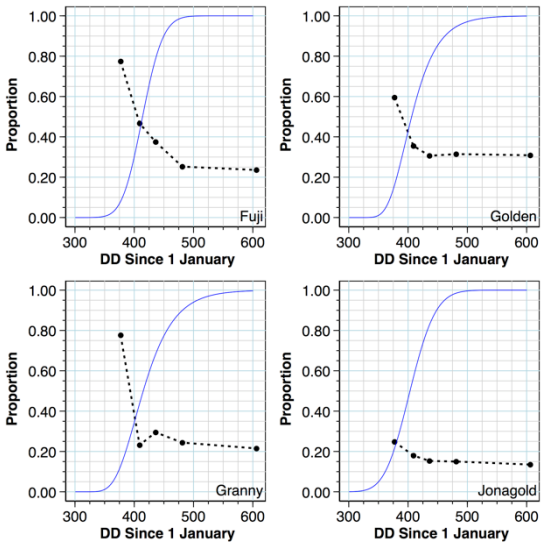
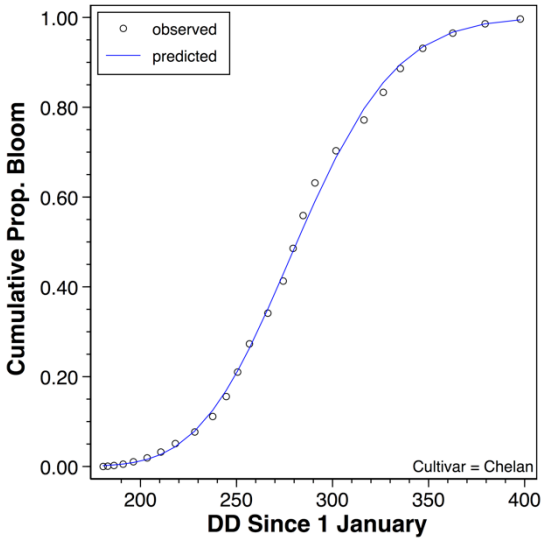



**Fig. 7**. Observed phenology of Chelan cherry at Rock Island in 2018. Predicted line comes from the gamma distribution.



pollination rates for that cultivar were very low initially, and throughout the majority of the flowering period (until about 460 DD).
*Work next year:* We currently have completed most of the fruit set model. We have shown that honeybees choose to forage on the cultivars with the greatest number of blooms at any point in time and that foraging levels track with the relative bloom curve (e.g., Fig. 6). We also have finished the proportion of cross-pollinating visits and are in the process of developing the portion where we can track the age class of each bloom and its probability of setting fruit. The work next year will focus on how bloom age affects probability of fruit set; this will require data collection on bloom longevity and the probability of fruit set at different bloom ages as well as modeling efforts to implement the data that we will be collecting. As we get into the prediction of fruit set, it appears that the primary use of the pollen tube growth model will be to give spray intervals for thinning and may be directly replaced by the flowering curves. However, until we finish work on the fruit set model, we cannot be sure of this.

*Results Cherry Bloom:* The cherry bloom was well predicted by the gamma distribution (Fig. 7) and showed bloom started around 200 DD peaked around 290 DD and was finished by 400 DD. These data are obviously preliminary but show that the approach used in apples can also be used in cherries.

*Work next year:* We hope to evaluate several more cherry orchards with a mixture of cultivars next year on a no-cost extension of this project.

***Obj. 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.***

Evaluation of bloom time was presented in last year's progress report and showed that at three representative locations (Richland, Wenatchee, Wapato) median bloom time (median is when half the years evaluated will be above and half below all bloom time values) will be changed by 21 days using the mild scenario (RCP 4.5 -up to 650 ppm $CO_2$ with stabilization after 2100) and 30 days using the increasingly more likely climate change scenario (RCP 8.5 – 1380 ppm $CO_2$ and still rising at 2100.

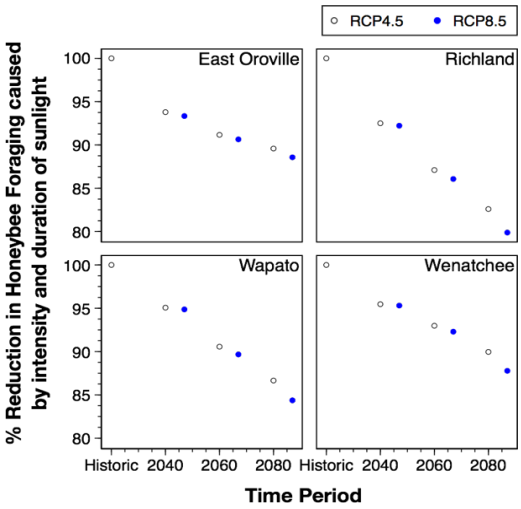
The earlier flowering time poses several possible problems for honeybee pollination. First, bloom starts earlier in the year which means that the day length occurring at those earlier dates will reduce honeybee foraging time (since they only forage during the day). Secondly, the sunlight is less intense early in the season, which also affects foraging rates. Third, the temperatures around the bloom period may be more variable with either higher or lower temperature profiles during the day. The temperature profile is also a key driver of honeybee foraging and could affect foraging either positively or negatively. The fourth potential issue is that Washington tree fruit is not the only crop that is affected by climate change and it is likely that crops like almonds that bloom earlier in the year will also be shifted. At first glance, it might seem the shift in almonds will not be a problem, because we use the same bees that pollinate California almonds. However, population growth in honeybee colonies is driven by day length. Egg laying by queens does not occur until day lengths reach 10-11 hours photoperiod. Thus, we might not have well developed colonies (with large amounts of brood from pollination of almonds) to the extent that we currently enjoy.

*Methods***:** To test the first two issues, we used data gathered the past two years for bloom timing of Cosmic Crisp (very late blooming) and Jonagold (very early blooming) and examined the period between 5% flowering of Jonagold and 95% flowering of Cosmic Crisp. We used the same climate change scenarios for the same three locations (Richland, Wenatchee, Wapato) as before and added another one (Oroville) to examine how much the temperatures and reduced sunlight might affect honeybee foraging rates across the north-south extent of Washington tree fruit production. As before, we used the periods for historical (1979-2005), 2040 (2025-2055), 2060 (2045-2075), and 2080 (2065-2095) using the two climate change scenarios.

*Results*: We found that the median temperature profile during the bloom period did not vary more than 1.2°F at any site with either climate change scenario. These relatively small temperature changes during the bloom period resulted in about the same performance in honeybees foraging, with only a slight change (max < 3.4%) in foraging rates related to temperature. Essentially, even though the bloom period occurs much earlier on a calendar date basis, the temperature profiles will not vary enough to effect honeybee foraging.

In contrast to the temperature effects, the median sunlight duration at the earlier dates of bloom vary from ≈1.1-1.6 hours less (RCP8.5) or 1-1.3 hours (RCP4.5) with the reduction increasing from Oroville to Richland. These values correspond to a reduction in foraging time of 7.5-10.9% or 6.4-9.5% for the more severe and less severe scenarios, respectively.

**Fig. 8**. Change in honeybee foraging rate caused by shift in time of bloom which causes foraging to occur under less intense sunlight and shorter days. RCP4.5 is mild climate change scenario, RCP8.5 is the more severe situation.




In addition to reduced foraging times because of the earlier flowering times, the sunlight intensity at any given time is also affected by day of the year and is reduced early in the year compared to the historical foraging time. The differences in sunlight intensity from bloom occurring earlier in the year causes about a 4-10% reduction in foraging rates compared to the historical normal bloom period (this is based on the clear sky radiation, so it doesn't consider any change in cloud cover that is not predictable). Overall, the reduction in foraging caused by changes in both sunlight duration and intensity amounts to roughly 10.4-17.4 and 11.4- 20.1% reduction (RCP4.5 and RCP8.5, respectively) compared to the historical time of bloom. All of these effects are smallest in the more northerly locations and increase going south and as time goes on.

The changes in honeybee foraging related to climate change appear to be primarily a result of the shorter day length and lower intensity of sunlight earlier in the season. The climate change scenarios do not provide any indication of cloudiness, so our study assumes the clear sky radiation value and how that changes over the year. If there are any differences that are systematic (*e.g.,* earlier days are cloudier as the climate changes), then the effects may be greater or lesser than what our study suggests. Regardless, the changes should occur relatively slowly, but the expectation should be that the bees will be less efficient (up to ≈20%) which would require more bees to achieve the same results as we have currently. Another way to view this is from the perspective of "climate analogs", where we look at a location in the future and compare it a current location. In this sense, the flowering time in Oroville will be similar to the Richland location in 2080 under the RCP4.5 and in 2060 under RCP8.5. Similarly, in 2040 Wenatchee will have the same median flowering time as Richland currently does under either climate change scenario.

The more pressing problem for honeybees may be the "downstream" effect of moving California's almond bloom to earlier in the year, which would result in smaller colonies coming out of California because of the limitation on honey bee population growth that occurs when day lengths are in the order of 10-11 hours. If this occurs, we can expect more problems managing the honey bee population and growing colonies that can be used for both honey bee production and to pollinate a wide range of crops (not just tree fruit in the Pacific Northwest).

*Work next year*: We will use the no-cost extension to evaluate how much earlier the bloom of almonds could be expected and use Gloria's honeybee population growth model (already existing) to evaluate the effect of moving the bloom period of almonds and apples earlier in the season on honeybee colony strength at the time of pollination of tree fruit in Washington state.

**CONTINUING PROJECT REPORT** **YEAR**: 2 of 3
**WTFRC Project Number: TR-17-102**

**Project Title:** Developing and validating models for tree fruit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**Cooperators**: None

**Total Project Request: Year 1:** $90,878 **Year 2:** $94,832 **Year 3: $99,695**

**Percentage time per crop:** Apple: 40% Pear: 50% Cherry: 10% Stone Fruit: 0%

**Other funding sources**

**Agency Name:** WSU Extension
**Amt. awarded: $** 198,268
**Notes:** This is the funding WSU Extension has committed to support maintenance of WSU DAS and implementation of new models.

**WTFRC Collaborative expenses:**

| **Item** | 2017 | 2018 | **2019** |
|---|---|---|---|
| **Salaries** | 6,000 | 4,000 | 4,000 |
| **Benefits** | 2,000 | 1,200 | 1,200 |
| **Wages/Benefits**[1] | 14,000 | 18,000 | 20,000 |
| **Supplies** | 0 | 0 | 0 |
| **Travel**[2] | 2,500 | 2,600 | 2,700 |
| **Miscellaneous** | 0 | 0 | 0 |
| **Total** | 24,500 | 25,800 | **27,900** |

**Footnotes:**
[1] Wages/benefits adjusted in years 2 and 3 to reflect new WA minimum wage schedule.
[2] In-state travel to research plots.

**Budget 1**
**Organization:** WSU-TFREC **Contract Administrator:** Susan Cao/Kim Rains
**Telephone:** 509-335-4564/509-293-8803 **Email:** bentjen@wsu.edu / kim.rains@wsu.edu

| Item | 2017 | 2018 | 2019 |
|---|---|---|---|
| **Salaries**[1] | 34,020 | 35,380 | **36,796** |
| **Benefits**[2] | 13,442 | 13,979 | **14,539** |
| **Wages**[3] | 8,000 | 8,320 | **8,653** |
| **Benefits**[4] | 216 | 225 | **234** |
| **Equipment** | 0 | 0 | **0** |
| **Supplies**[5] | 2,500 | 2,600 | **2,704** |
| **Travel**[6] | 4,000 | 4,160 | **4,326** |
| **Miscellaneous** | 0 | 0 | **0** |
| **Plot Fees** | 4,200 | 4,368 | **4,543** |
| **Total** | 66,378 | 69,032 | **71,795** |

**Footnotes:**
[1] Matt Jones (0.25FTE, T. Melton 0.45 FTE).
[2] 34.1% (Matt Jones); 48.3% (Melton).
[3] Student 40 hr/wk for 16 wks.
[4]2.7%.
[5]Includes lab and field supplies.
[6]In state travel.

**Objectives**:

1. Develop and validate a demographic model for pear psylla to assess pesticide effects on population management.
2. Continue to collect validation data for demographic models for mites and aphids.
3. Development new fruit growth models for Honeycrisp, Fuji, and Golden Delicious.

**Significant Findings:**

- Psylla phenology appeared similar to last year but will still require at least another year's data collection.
- The psylla phenology will be incorporated into the pesticide-effects model for psylla
- The phenology of pear bloom was collected at the pear sites sampled for psylla this year and provided good information for Bartlett and D'Anjou, but the phenology data for Bosc was limited compared to the other two.
- A demographic woolly apple aphid (WAA) model was developed that allowed us to investigate the effect of temperature alone on WAA population dynamics. We found that when hourly temperatures started to exceed 92°F for more than 2-3 hours, that population crashes tended to occur. This was also seen in our field plot data.
- Analysis of rosy apple aphid and apple grain aphid phenology is complete and shows their populations are generally restricted to two different windows in the season. These models will begin to be incorporated into DAS this coming year.
- Work on the fruit growth models for Cosmic Crisp, Fuji, Jonagold, and Honeycrisp continued this year and the data is consistent with that collected last year. We hope to have preliminary models for our beta users for Fuji, Jonagold and Honeycrisp this year, but the Cosmic Crisp data is limited in geographic distribution and will require more data collection.

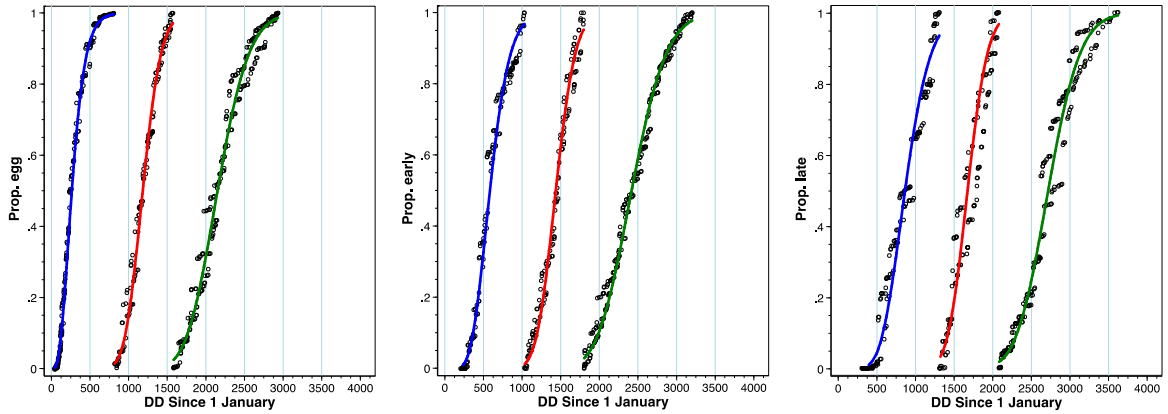
***Obj. 1. Develop and validate a demographic model for pear psylla to assess pesticide effects on population management.***

*Methods Pear Psylla.* Phenology data for pear psylla were collected at five locations with low-intensity management; samples were taken twice a week from February until the end of October. The number of adults (winterform and summerform), eggs, and immature stages (instars 1-3 and instars 4-5) was determined from beat samples and shoot samples. Shoot samples were visually inspected before leaves were developed, and subsequently processed through the mite brushing machine as the leaves became close to full size. In addition, unbaited sticky yellow cards were placed in each orchard (8/site) to catch more adults as well as the pear psylla parasitoid, *Trechnites psyllae*.

Weather data came from the high-resolution historical data provided by darksky.net which provides data at 0.6 x 0.6-mile resolution as well as from data loggers placed in the orchard from the period 2016-2018. Data was fit by maximum likelihood to five different statistical distributions and examined for the best overall fit across the range.

*Methods Pear Bloom*. Pear bloom phenology was evaluated at four locations with the cultivars Bartlett (3 locations), Bosc (1 location), and D'Anjou (2 locations). At each location we evaluated 60 fruiting buds (4 buds per tree from 15 randomly chosen trees – one bud per quadrant of each tree) and classified them as dormant, swollen bud, bud burst, green cluster, white bud, bloom, and petal fall. We visited each location twice per week to evaluate the clusters. Data analysis was done for each cultivar by using a maximum likelihood fit to one of three statistical distributions: (gumbel, gamma, Weibull).

**Fig. 1.** Phenology of pear psylla egg-laying (below left), early instars (1-3) (below center) and late instars (4-5) (below right) over the period of 2016-2018 using 14 orchard-years of data.

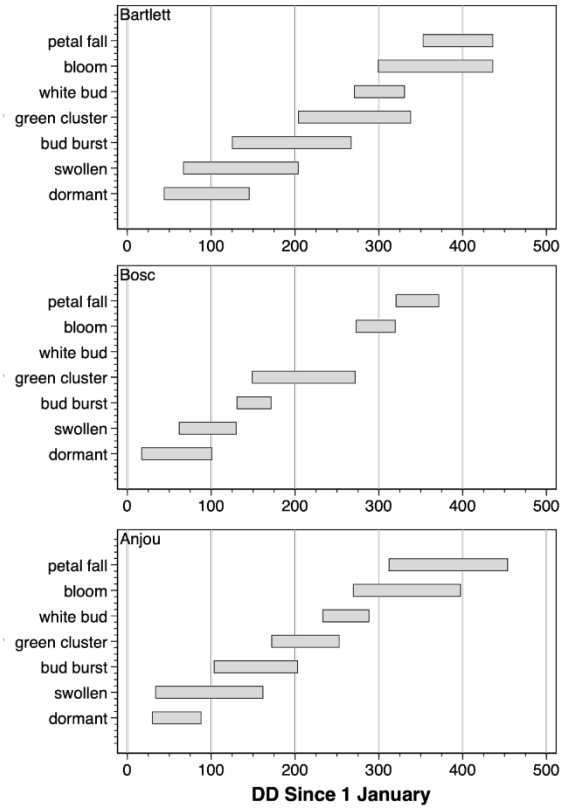



*Pear Psylla Phenology.* We evaluated the beat tray samples over the entire three years of data we have collected (one year before this project started). Because the orchards were treated, we needed to combine data from the different orchards to prevent us from just seeing changes in phenology related to pesticide application. We split the data into two different groups based on their locations (the locations were randomly chosen to be in each group). The phenology of the eggs, early instars (instars 1-3) and late instars (4-5) were tracked separately. The data fit the gamma distribution well for each stage and generation (Fig. 1). We also tracked the phenology of a partial fourth generation, but because the generation was not completed, it cannot be modelled.

As with last year's data, the summerform adult stage never disappeared at the end of the season and those individuals all die as colder temperatures occur. Winterform adults appeared starting at 10 September vs 5 September last year.

*Pear Bloom.* Examination of the model fits showed little variation between them, with the gumbel distribution being slightly better than the other two. The Gumbel distribution was used to estimate each of the bloom stages for the Bartlett and D`Anjou (Fig. 2). The Bosc dataset (fig. 2, middle) showed very little variation after green cluster and reflects the single location and our sampling timing which as affected after sprays were applied which prevented us from getting some of the data during the bloom period.

**Fig. 2**. Bloom timing for three different pear cultivars taken at orchard locations where pear psylla was monitored during 2018.




*Work next year.* We will gather another year of data on pear psylla phenology and pear bloom phenology at the same sites. We have developed a preliminary version of the pesticide effect model for pear psylla and will update that with this year's information on phenology as well as refine some of the output and options that are available. The pesticide effects model will be incorporated into a web site which we should have online this winter; it will allow users to add their spray records to

evaluate how the different life stages were covered as well as the effect on population levels compared to an untreated control. If the pear data for bloom next year is consistent with this year's data, we will incorporate those into DAS as well. Analysis of the *Trechnites* data will be performed in late winter.

***Obj. 2. Continue to collect validation data for demographic models for mites and aphids.***

*Methods*. Phenology data were collected for woolly apple aphid (WAA), green apple aphid (GAA), two-spotted spider mite (TSSM), European red mite (ERM), and brown mite (BM). For GAA and WAA, four apple orchards were sampled twice a week from the end of March to mid-October. We sampled 100 shoots early in the year and later 100 leaves (10 randomly chosen per 10 randomly chosen trees. The number of nymphs, nymphs w/ wing buds, wingless adults, and winged adults was recorded for each aphid species.

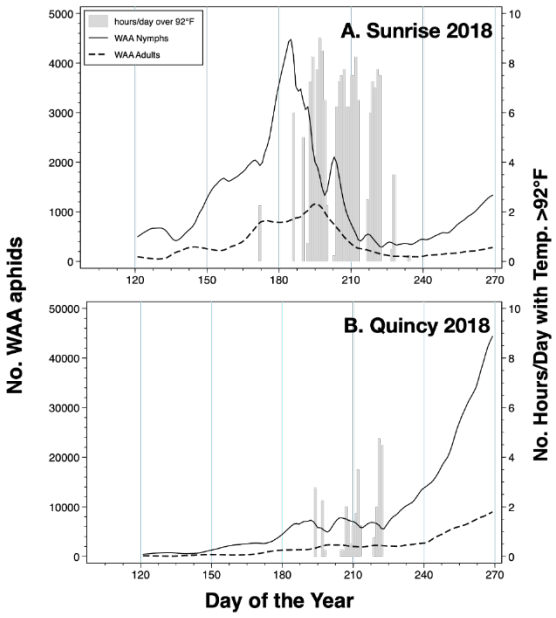
Phenology data for ERM and BM were collected from six apple orchards, twice a week from start of April until late-October. Initially, when eggs started to hatch, double-sided sticky tape was placed tightly around 50 branches per site (1 per tree) to detect mobile immature stages. After leaves expanded, a total of 100 leaves from 20 trees per site were collected and run through the mite brushing machine. Mite numbers were recorded by species and stage. In addition to the canopy samples, we also collected mites from the ground cover. Our results from last year showed that common mallow (button weed) consistently had high numbers of TSSM, so all the ground samples focused on that plant.

*Results and Discussion*

*Woolly apple aphid*. The phenology of woolly apple aphid has been extremely difficult to define. This is partially because it can be decimated by generalist predators and has a highly effective parasitoid, *Aphelinus mali*. Perhaps even more confounding than the effect and sometimes inconsistent activity of biological control, is that there appears to be a temperature component that is not extremely consistent. To address this issue, we completed a survey of the literature, synthesized the information, and developed a demographic model that adjusts mortality and reproduction every 5 DD based on the average temperatures that occurred over that period, and tracks stage structure and population level over time. This model is intended not to provide exact estimates of the population in the field, but to allow us to quantify the effect of temperature alone on WAA population dynamics. The model helps make sense of how the temperature affects WAA, because there are delays in when the temperature affects the different stages and even comparatively small adult population allows population growth to happen quickly when conditions are suitable.

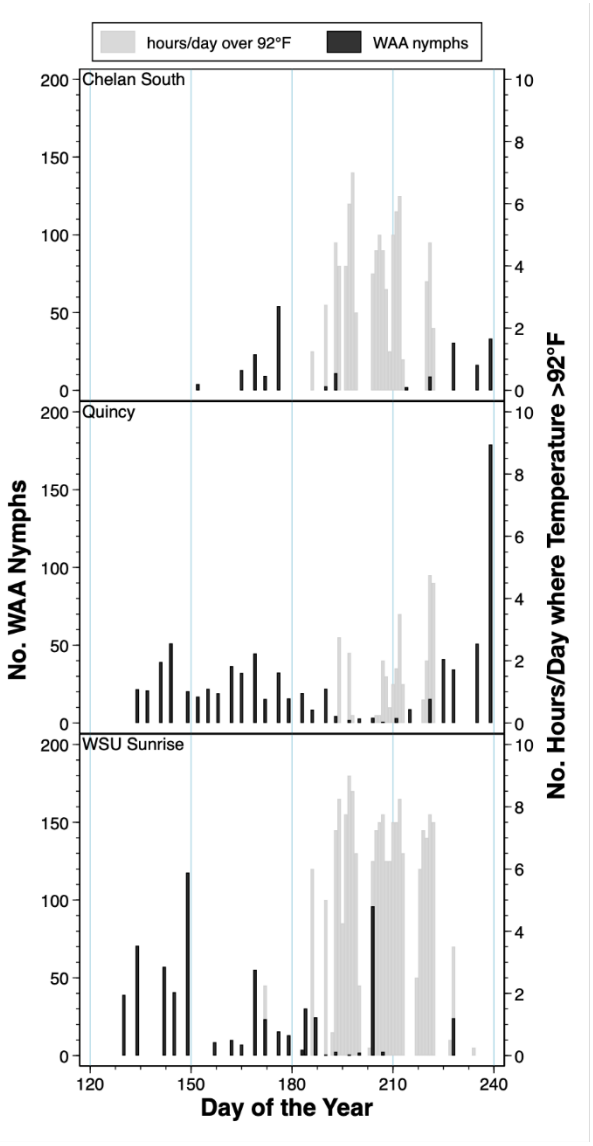
In running the model and evaluating our field data, one thing that jumped out at us was that when the number of hours per day that had temperatures over 92°F started to occur, WAA populations would crash (although with a delay) and after the temperatures dropped below 92°F, a rapid increase would occur (Fig. 3,4).

**Fig. 3.** Examples of population trends of WAA based on the demographic model. A. Warm site (Sunrise) and B. Cooler site (Quincy). Note the difference in y-axis.



The model shows that the population trends are highly responsive to temperature and that temperature alone can cause quick and significant drops in populations that mimic what we see in our orchard plots (if there is no significant biological control). Evaluation of the model run with data from different locations and years, shows we have two different population trends that tend to occur: (1) at warm sites and years, once the temperatures increases and the number of hours per day over 92°F increases, the populations crash (generally in July) and rebound when the temperatures decrease (generally mid-August) and increase throughout the fall (Fig. 3A); (2) at cooler sites or years, the population growth is not restricted to significant degrees and overall seasonal population levels are significantly higher (Fig. 3B). During the cooler years, there is a reduction in growth rate during mid-summer, but it is not enough to cause the population levels to crash, and only a leveling off of the population during the warm period occurs. This provides a possible window that could allow biological control agents to "catch up" with the WAA population, but at the cost of a population level that is much higher in general than at the warm areas where peak population density is constrained by the temperature levels.

**Fig. 4.** Population level of WAA nymphs at 3 different locations in 2018 overlaid on the number of hours/day where the temperature was >=92°F. A. Chelan South. B. Quincy. C. WSU Sunrise.




*Rosy apple aphid & apple grain aphid*. The phenology of rosy apple aphid and apple grain aphid from 2015-2017 were quantified and a model was developed that allows us to narrow the windows of when the aphids are in the orchard. That was shown in last year's progress report.

*Work next year.* We have not yet had time to perform more analysis of this year's spider mite data, but previously have found that we can predict when TSSM females emerge from diapause. We will start the analysis of brown mite, and complete the analysis of TSSM, ERM and brown mite in late-February and modify any of our field experiments as suggested by the analysis.

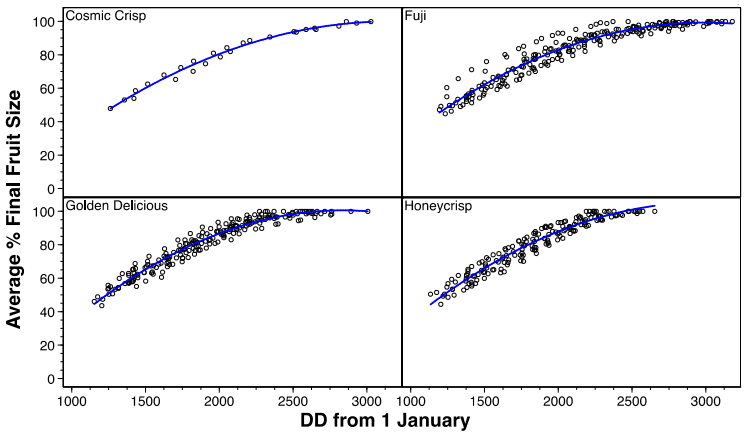
We currently have enough information on rosy and apple grain aphids to begin implementing models for both species on DAS and hope to have a preliminary version for our beta testers this year.

***Obj. 3. Development of new fruit growth models for Honeycrisp, Fuji, and Golden Delicious***

*Methods.* We collected data from 11 geographic areas representing the topographic and climatic diversity of Central Washington production areas from Brewster Heights to North Pasco. We concentrated on Golden Delicious, Fuji and Honeycrisp at each node, but also collected data on

Cosmic Crisp at the WSU Sunrise location. After early drop was completed, we tagged fruit and then measured the same fruit each week until harvest. Each fruit measurement was recorded separately, so that we could assess how the individual fruit size changed over the course of the season. We analyzed the data as the proportion of the final fruit size for each fruit, so that we don't have to worry about the effects of thinning, fruit load, or return bloom size. This method allows us to predict when the fruit reaches a given percentage of the final fruit size. The fruit size data was paired with temperature data, and degree days from 1 January (base temperature 40.1, upper threshold 75.7).

**Fig. 5**. Average growth rate for four apple cultivars 2017-2018.




*Results*. Our average fruit growth data showed good agreement for most sites and cultivars. The one variable site was in south Orondo (near Baker Flat) where the orchard was on a south facing slope that probably affected the Fuji grow size estimates by being warmer. Even with that caveat, the fruit size for the different cultivars seems to be consistent over the past two years at all the sites (Fig. 5).

*Work next year.* The data for Cosmic Crisp is definitely less extensive than what we need to implement on DAS, but next year we will also include a planting in Quincy, which will bring us to three sites (we had one site in 2017, two in 2018) which should increase our data collection on that cultivar. We will also collect another year's data on the other three cultivars.

**CONTINUING PROJECT REPORT** **YEAR**: 1 of 2 Years
**WTFRC Project Number:** TR-18-101

**Project Title**: Reducing cold damage with cellulose nanocrystals


| | | | |
|---|---|---|---|
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| | | | |
|---|---|---|---|
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| **City/State/Zip**: | Richland/WA/99354 | **City/State/Zip:** | Prosser/WA/99350 |


**Cooperators**: Olsen Brothers

**Total Project Request:** Year 1: 50,086 **Year 2: 42,650**

**Percentage time per crop:** Apple: 50% Pear: 0% Cherry: 50% Stone Fruit: 0%

**Other funding sources**

**Agency Name:** USDA NIFA AFRI Bioenergy
**Amt. requested/awarded:** $404,030
**Notes:** Funded for 2018-2021 to investigate cellulose nanocrystal suspension preparation, thermal properties, field trials with CNC

**Agency Name:** WSU Commercialization Gap Fund
**Amt. requested/awarded:** $50,000
**Notes:** this funding is to evaluate plant-based dispersions for reducing cold damage, protect the IP, and develop a business plan for the commercialization of the IP

**Budget 1**

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

| **Item** | 2018 | **2019** |
|---|---|---|
| **Salaries** | 31,471 | 32,730 |
| **Benefits** | 2,360 | 2,455 |
| **Wages** | 4,800 | 4,992 |
| **Benefits** | 455 | 473 |
| **Equipment** | | |
| **Supplies** | 10,000 | 1,000 |
| **Travel** | 1,000 | 1,000 |
| **Plot Fees** | | |
| **Miscellaneous** | | |
| **Total** | 50,086 | **42,650** |

**Footnotes:** salary for graduate research assistant to work out of WSU-IAREC; wages are to support hourly employees to assist with field trials and hardiness assessments; supplies in year 1 include materials to build 'polar pod' system (datalogger, thermocouples, heating elements, power supply) for hardiness evaluation in spring (i.e., when DTA is ineffective)

## OBJECTIVES

1. Evaluate the utility of cellulose nanocrystals (CNC) applications for reducing cold damage
2. Summarize and disseminate key findings with stakeholders

## SIGNIFICANT FINDINGS

- We have developed a reliable process for creating dispersions of cellulose nanocrystals (CNC)
- CNC treatments via handheld electrostatic sprayer provided resistance to cold damage in apple and sweet cherry
- CNC at 1 % was less effective than CNC at 2 %
- Cold-hardiness could be improved by 0 to 2 F at 1 % CNC treatment and by 3.6 to 7 F from 2 % CNC treatment
- CNC treatment may advance flowering in sweet cherry

## METHODS

We will conduct field trials over two years, evaluating the efficacy and practicality of CNC films for reducing cold damage in apple and sweet cherry. We hypothesize that there is potential to reduce cold damage via three mechanisms:
1) Protection to tissues during dormancy (i.e., mid-winter)
2) Protection to buds during anthesis (i.e., spring)
3) Manipulation (delay) of flowering timing from CNC applications in late winter/early spring

1*:* Protection to tissues during dormancy:
Typically, tree fruit buds and perennial woody limbs enter dormancy in the fall in response to key environmental cues, day-length and temperature. Tissues accumulate cold hardiness (i.e., the ability to withstand low temperatures) gradually, reaching maximum cold hardiness level in late fall generally. Hardiness follows a general sequence of acclimation and deacclimation. Low temperatures during mid-winter can be lethal, damaging buds, or shoot cambium tissue. In this research, sweet cherry and apple trees will be treated with two rates of CNC dispersions (1% or 2%) or water alone in late fall, following leaf drop using an electrostatic sprayer (provided in-kind by On Target Spray Systems, Mt. Angel, Oregon). Treatments will be made to five replicate 5-tree blocks, treating both sides of trees in a completely randomized design. To evaluate treatment effects on bud hardiness, 10 replicate 2-year-old limbs (2 limbs per block per sampling date) will be pruned and analyzed for cold hardiness at PI Whiting's lab in Prosser by exotherm analyses in a programmable freezer using established techniques (Andrews, et al., 1983). Both reproductive buds and young shoot tissue will be assessed for hardiness. Briefly, collected tissue will be placed on thermoelectric modules and placed inside the freezer where air temperature will decline at 1 °C/hr until tissue death is recorded on the data acquisition system. Exotherms will be analyzed by field treatment and we will determine the lethal temperature to kill 10, 50, and 90% of tissue – the $LT_{10}$, $LT_{50}$, and $LT_{90}$, respectively. Importantly, this method is effective regardless of orchard environmental conditions (i.e., we do not require a natural cold weather event to test treatment effects). In addition, tissue hardiness will be evaluated at two-week intervals to track treatment effects throughout dormancy. Initial samples will be made in November, and we will continue to evaluate hardiness via differential thermal analyses until this technique is no longer effective – this occurs in early spring as tissues deacclimate. Samples will be collected from within 100 meters of a weather station so that we can

monitor orchard climate. Should a natural cold event occur, we will collect buds from treated and untreated trees and assess damage in the laboratory by dissection after 24 hours at room temperature.

2*:* Protection to buds during anthesis:

The effectiveness of CNC at protecting apple and sweet cherry floral buds during anthesis will be investigated by comparing hardiness of buds treated with either CNC, or water alone. Treatments will be made as described above, in orchards using a commercial electrostatic sprayer. This trial will compare different CNC dispersion concentrations (0.5 wt.% to 2 wt.%) and the efficacy of multiple vs. single applications. We hypothesize that the thermal protection of CNC will be gradually lost as buds open and flowers emerge, exposing new, untreated tissues. The following treatments (5 total) will be made in the same orchards (1 sweet cherry and 1 apple) to five 5-tree blocks:

1) Water alone
2) CNC applied at 2% during:
    a. 'green tip' stage
    b. 'tight cluster' stage
    c. 'full white' stage, or
    d. fully open flowers

Again, 10 replicate limbs will be collected 24 hours after application and brought to the WSU hardiness lab. We propose to build a 'polar pod' system for evaluation of tissue hardiness when DTA no longer works. This system is comprised of small aluminum cylinders wrapped with heating elements and insulation. Each cylinder is fitted with a thermocouple to track intra-pod air temperature. The cluster of pods (10-12) are placed in a standard chest freezer. Using a programmable datalogger, each individual pod can be maintained at a predetermined temperature by activation/deactivation of the heating element. This system has been evaluated for grape and blueberry, proving reliable and robust. For our work, half of the pods will be loaded with treated material, half with control material. Sections of ca. 10 cm fruiting wood will be placed inside the programmable pods and the cluster will be kept in a chest freezer. We anticipate programming replicate pods to maintain temperatures of 1 °C to -5 °C at 1 C intervals. Replicate shoots will be removed and allowed to sit at room temperature for 24 hours before dissection and assessment of tissue damage. We will assess damage to the floral tissues, with particular interest in the pistil, and shoot cambium. Tissue will be rated as either dead or alive.

3*:* Manipulating flower timing:

We will also assess the potential for formulations of CNC to delay flowering in both sweet cherry and apple. We hypothesize that by creating a CNC treatment that is white, tissue temperature will be reduced, and flowering will be delayed. This delayed flowering may be beneficial by allowing buds to avoid natural spring frost events. Treatments will be made to five 10-tree blocks in commercial orchards using the same application system as above. A single application will be made prior to bud-break, and treated trees will be compared in their flowering stages with untreated trees. We will assess flowering on 2-year-old flowering wood, monitoring at least five limbs per replicate block (i.e., 25 limbs per treatment) at 2-3 day intervals until all floral buds have opened. We will also document the progression of bud-break and flowering by collecting digital images of representative limbs at the same 2-3 day intervals.

In addition, we will assess the role of application technology on efficacy of CNC. This work will compare the electrostatic application system with a standard airblast system. We will conduct this work at the WSU Roza farm in a 'Skeena' sweet cherry block. CNC dispersions will be applied at 15 gallons/ac (electrostatic sprayer) vs. 100 gallons/ac with the airblast sprayer. Each system will be applied to five 5-tree blocks, and hardiness will be assessed on replicate limbs as described above via DTA.

## RESULTS AND DISCUSSION

In 2018 we conducted several trials evaluating the ability of CNC treatments to improve bud hardiness. All treatments were made with a single-nozzle electrostatic sprayer, courtesy of On Target Spray Systems (Figure 1). Pressure was provided from a portable air compressor. For each experiment the CNC dispersion was prepared in PI X. Zhang's lab in WSU Tricities campus. The necessary volume (typically ca. 1 L) was loaded into a plastic reservoir secured above the sprayer. Application volume was ca. 50 gal/acre and calibrated by collecting sprayer output for 30 sec intervals and determining the volume sprayed at a constant pressure (ca. 14 PSI). Applications were made by holding the sprayer about 2 – 3 feet from the target trees and applying the treatment by moving the sprayer in a Z-pattern from the top to the bottom of each tree. Several untreated guard trees were skipped between treatments in each experiment.

**Figure 1.** Single-nozzle electrostatic sprayer used for CNC applications to sweet cherry and apple trees. The black arrow indicates the 'tank' containing the CNC dispersion. The white arrow indicates the nozzle.

In a trial in a mature ***'Scifresh' apple*** block northeast of Prosser we compared two concentrations of CNC (1% and 2%) with untreated control. This trial was conducted on 18 April when trees were at about 20% full bloom (i.e., all king flowers in lower half of trees were open). CNC treatments improved hardiness of 'Scifresh' apple flower buds (Figure 2). CNC at 1% was marginally effective at improving hardiness, and it did not reduce the lethal temperature required to kill 10% of buds (LT10). In fact, clusters treated with 1% CNC exhibited greater pistil death than untreated at 26.6 and 21.2 F. In contrast, treatment with 2% CNC improved hardiness, reducing pistil death at every temperature. LT10 was 30.2 for untreated flowers and 26.6 for flowers treated with 2% CNC (an improvement of 3.6 F). The greatest

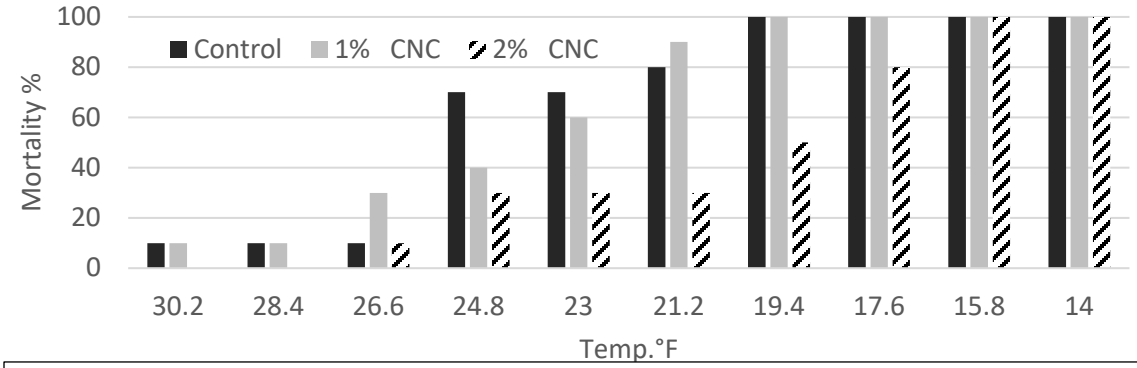


**Figure 2.** The effect of field-applied dispersions of CNC at 1% and 2% on the incidence of pistil mortality in 'Scifresh' apples. Treatments were applied 18 April and hardiness was assessed on randomly selected clusters 24 hr after treatment.

improvement in flower hardiness was observed at 21.2 F, a temperature at which 80% pistil death was recorded for control, 90% for 1% CNC, and only 30% for 2% CNC (Figure 2). The protective effect of 1% CNC was variable, and lost by ca. 21 F. In contrast, the protective effect of 2% CNC was significant, and not lost until ca. 16 F. Based on these results, untreated trees would have complete crop loss at about 19 – 20 F, and trees treated with 2% CNC would have ca. 50% crop remaining, not losing the entire crop until ca.16 F.

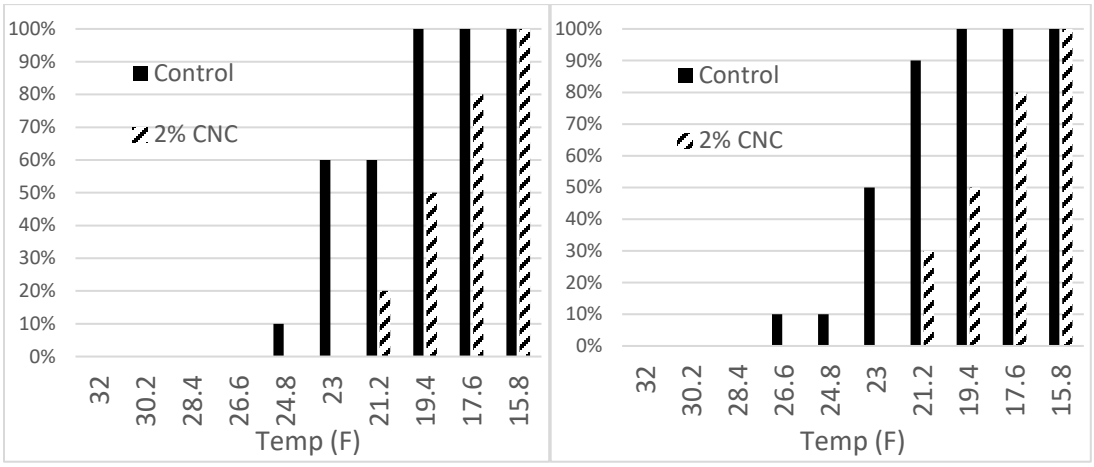


**Figure 3**. Pistil death (% mortality) in 'Skeena' (left) and 'Selah' (right) sweet cherry trees treated with 2% CNC on 26 March, at 'side green' stage of bud development. Assessments made 24 hr after field treatment.

In a ***sweet cherry trial on 'Skeena' and 'Selah'***, we similarly found significant improvements in flower hardiness with applications of 2% CNC (Figure 3). Treatments were made on 26 March in a block at the WSU-Roza experimental orchard north of Prosser. Average bud development was similar for 'Skeena' and 'Selah' at side green. In 'Skeena', LT10 was 24.8 F for untreated and ca. 22 F for trees treated with 2% CNC, an improvement of about 3 F. In 'Selah', LT 10 for untreated flowers was 26.6 F and ca. 22.3 F for trees treated with 2% CNC, again, an improvement of about 4.3 F. In 'Skeena' the greatest protective effect occurred near 23 F where untreated flowers exhibited 60% death and treated flowers were 100% viable. Complete crop loss would have occurred in untreated trees at ca. 19.4 F whereas treated trees exhibited only 40% pistil mortality at this same temperature. The degree of mortality for 'Selah' was similar, with 100% pistil mortality at 19.4 F – in contrast, buds from trees treated with 2% CNC exhibited only 50% pistil mortality at this temperature.

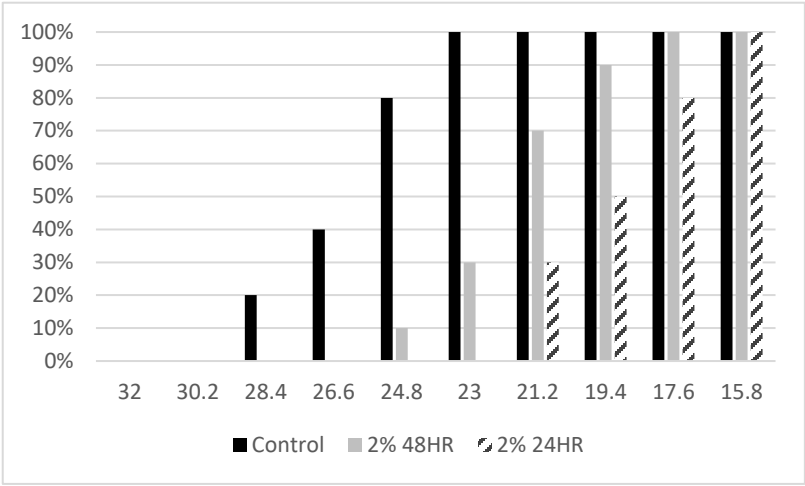


**Figure 4.** 'Selah' pistil mortality assessed 24 and 48 HR following 2% CNC treatment at side green.

In ‘Selah’, we also investigated the longevity of a 2% CNC treatment by sampling buds 24 and 48 HR after CNC application in the field. Buds sampled 48 HR following treatment were less hardy than those sampled 24 HR after treatment (Figure 4). Interestingly, the untreated control samples were also less hardy (compare control in Figure 4 to control in Figure 3 for ‘Selah’), suggesting that there was an overall loss of hardiness rather than a loss of the protective treatment of the CNC. The CNC-treated flowers exhibited improved hardiness compared to untreated control flowers at the 48 HR sampling time. The longevity of the improved hardiness with CNC treatment will be assessed in greater detail in 2019. This will be important for determining when reapplication may be necessary, as the treatment effect is lost.

**CONTINUING PROJECT REPORT** **YEAR**: 2

**Project Title**: Multi-purpose robotic system for orchards


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**Cooperators**: Columbia Fruit Packers, Auvil Fruits Inc.

**Total Project Request:** **Year 1:** 248,058 **Year 2: 250,780** **Year 3:** 255,692

**Percentage time per crop:** Apple: 100%

**Other funding sources: None**

**Budget 1**
**Organization Name: FFRobotics** **Contract Administrator: Avi Kahani**
**Telephone: +972 545615020** **Email address: avikahani@ffrobotics.com**

| Item | 2018 | 2019 | 2020 |
|---|---|---|---|
| **Salaries** | $59,400 | $63,000 | $66,150 |
| **Benefits** | $5,940 | $6,300 | $6,615 |
| **Wages** | $30,450 | $31,500 | $33,075 |
| **Benefits** | $3,045 | $3,150 | $3,308 |
| **Equipment** | $25,000 | | |
| **Shipping (**)** | | $10,000 | $10,000 |
| **Supplies** | $12,000 | $8,000 | $6,000 |
| **Travel (*)** | $20,000 | $21,000 | $22,000 |
| **Miscellaneous (***)** | $10,000 | $25,000 | $25,000 |
| **Total** | $167,950 | **$167,950** | $172,148 |

**Footnotes: (*) Travel budget is requested to cover the travel and accommodation (Travel from Israel)**
**(**) Shipping product to field experiments (***) Equipment**

**Budget 2**
**Organization Name:** Washington State University **Contract Administrator:** Katy Roberts
**Telephone:** (509) 335-4564 **Email address:** katy.roberts@wsu.edu

| Item | 2018 | 2019 | 2020 |
|---|---|---|---|
| **Salaries** | $53,522 | $55,662 | $57,889 |
| **Benefits** | $5,101 | $5,304 | $5,516 |
| **Wages** | $6,000 | $6,240 | $6,490 |
| **Benefits** | $600 | $624 | $649 |
| **Equipment** | | | |
| **Supplies** | $12,000 | $10,000 | $8,000 |
| **Travel *** | $5,000 | $5,000 | $5,000 |
| **Plot Fees** | | | |
| **Miscellaneous** | | | |
| **Total** | $82,223 | **$82,830** | $83,544 |

**Footnotes: *Travel budget is requested to cover the mileage for field experiments and to visit collaborators/co-PIs**

## 1. OBJECTIVES

The following are the project objectives that remained same as the ones proposed originally.

*1) Optimize camera configuration for multi-arm operation of our robotic harvesting machine*
*2) Integrate and demonstrate multi-arm harvesting robot to cover entire tree height*
*3) Evaluate the performance of the harvesting robot while in motion*
*4) Demonstrate integration of the harvesting robot with fruit conveying and bin filling system*
*5) Investigate machine vision and robotic end-effectors for blossom and green fruit thinning*

### 1.1 Timeline of the Project Activities

| Objectives# | Research Activities | Time | | | | | |
|---|---|---|---|---|---|---|---|
| | | Year 1 | | Year 2 | | Year 3 | |
| 1 | Develop a robotic system with multiple cameras | | | | | | |
| | Optimize camera locations and create fruit map for harvesting based on accessibility | | | | (1) | | |
| 2 | Develop a robotic system with multiple arms | | | | | | |
| | Evaluate the machine for harvesting entire trees | | | | | | |
| 3 | Develop a control system for automated forward motion control | | | | | | |
| | Evaluate the machine for automated operation during motion | | | | | | |
| 4 | Integrate multi-arm robot with a harvest aid platform | | | | | | |
| | Evaluate the performance of the machine for harvesting, conveying and bin filling | | | | | | |
| 5 | Develop machine vision system for flower and green fruit detection | | | | | | |
| | Preliminary evaluation of a robotic system for flower and green fruit thinning | | | | | | |

There is a minor variability in the schedule projected at this time. In the table above, **gray** cells represent the original schedule while **green** cell added at the end of second activity for objective 1 shows a minor change this time.

Production/manufacturing issues in North America caused a slight delay in readiness of the FFRobot to be tested during the harvesting season in Washington State. The extra efforts in Israel allow us to run initial tests with 6 arm system in Israel. First set of field experiments in Washington will now be in 2019 harvest season.

## 2. SIGNIFICANT FINDINGS

- The most important accomplishment this year is that we were able to build a full-scale system and evaluate (preliminary) it in Israel, which shows that the system works. A youtube video showing the machine working in the field can be found at https://youtu.be/rpPHR-mZEOQ.

- The fruit detection algorithm developed based on a deep learning technique works properly. The technique also showed promise for detecting obstacles such as branches and trellis wire.
- The multi arm system is working properly with minimum effects between the different arms.
- The current system is taller than many commercial orchards; we need to build a dynamic system to support different orchard structure including infrastructure for netting.
- We also learned that the picking mechanism need to be further optimized through:
    - Incorporating improved vision, path planning and navigation algorithms
    - Improving the mechanical design

Preliminary results with blossom detection algorithm showed great promise for accurately detecting blossom in orchard environment.

## 3. METHODS

**Harvesting Objectives 1 to 4:**

*3.1 Obj.# 1: Optimize camera configuration for multi-arm operation of robotic harvesting machine*

*Introduction:* Our team has been developing and evaluating a robotic apple harvesting machine over the past several years (www.ffrobotics.com). So far, field tests have been conducted with one robotic arm (simple, linear actuation) with a picking hand attached. The FFRobot arm with a camera fixed on the platform to which the robotic arm is attached can be seen in action in https://www.youtube.com/watch?v=Dfu6jm6AHFQ. This system has provided good visual data acquisition and access to the fruit with one robotic arm. Work done by both of our teams (FFRobotics and WSU) has shown that, in a modern fruiting wall orchard, more than 95% of apples can be detected using a camera system like this (e.g. Silwal, 2016). Adding additional robotic arms makes it necessary to evaluate whether the location of the camera on the platform will yield the same results, and investigate the alternative of fixing the camera to the base of the robotic arm to achieve best data acquisition results. WSU team has been leading this objective in collaboration with FFRobotics team.

*Materials:* The current vision system has been modified to facilitate placement of the optical hardware on the base of the robotic arm attached to the platform frame on which the arm is mounted. A set of field data was collected to conduct a detection study in order to determine what percentage of apples are actually detected by the vision system from different locations. The system will be evaluated in different kinds of orchards including.

(A) An orchard with fruit thinning to singles and pruning tree growth to approximately 10 inches beyond the trellis wires.

(B) An orchard with mechanical pruning

(C) Different canopy architectures including V-shape and Tall Spindle system.

*Procedure:* The entire image acquisition process will begin by scanning the canopy directly in front of the initial multi-arms robot position. Based on previous research and our experience in orchards, some apples are blocked by other apples, leaves, branches, trunks and trellis wire, which will be difficult to be accessed and picked using a robotic hand. A method is necessary to detect different canopy parts and other objects in apple canopies so that the machine can identify completely visible and accessible apples. A deep learning-based image processing technique is being used to identify different parts of the canopy and other objects as potential obstruction to apples for robotic picking. Using this image processing technique, we can detect apples that are not blocked by objects like other fruit, branches, trellis wire and trunk. These fruits will be identified as completely visible and accessible fruit, which will be picked by robotic hands. After the initial picking cycle is completed, we will scan the same section to see if more fruit are exposed with desired level of visibility and accessibility. The process will be repeated until no accessible fruit are available in the canopy. The picking system will then move down the row and the process will be repeated (as discussed in the following sub-sections). Missed apples will be hand counted and compared to the number of detected apples. For vertical trees, this process can be repeated from other side of the canopies to maximize the

fruit harvesting percentage. The technique has been also extended to process videos collected by moving machine, which allows understanding the potential improvement in fruit detection through different viewing angles.

*3.2 Obj.# 2: Integrate and demonstrate multi-arm harvesting robot to cover entire tree height*

***Fig. 1: Multiple robotic arms supported by one frame.***

*Introduction:* As discussed in Obj. #1, our prior prototypes were based on one arm which limited the ability of the robot to pick the entire tree sections. It was proposed to investigate and introduce hardware and software changes to enable the dynamic structure of several robotic arms to gain the full range of 3 feet width, 3 feet depth, and 12 feet height canopies. We built such a system and evaluated (preliminary) in Israel during 2018 harvest season (See Fig. 3 in the results and discussion section). FFRobotics team has been leading this research activity in collaboration with WSU team.

*Materials:* Hardware and software will be modified to support the multi robot arms (4-6 robotic arms) in the same frame allowing dynamic movements along the height axis of the tree (Fig. 1.). The new software algorithms control the entire system to allow best performance with dynamic coordination between arms in term of their work-space.

*Procedure:* The image acquisition and processing system (described in Obj.#1) provided coordinates of linearly accessible fruit in the entire work space of the machine (which is roughly 3ftx3ftx12ft). Optimization techniques were employed in the same spot to provide sequence of fruit to be picked by each arm of the multi-arm robotic system. To optimize the system, more experiments will be carried out by sending the robotic arms to the desired fruit locations but will not pick the fruits. This experiment will allow evaluating several techniques of sequencing fruit picking pattern in the same location. Time taken to harvest individual fruit will be measured for each such technique.

*3.3 Obj.# 3: Evaluate the performance of the harvesting robot while in motion*

*Introduction:* Our current picking system is stationary during both image acquisition and picking, requiring manual movement of the system. We are introducing both hardware and software changes to enable the system to automatically move down the row in optimal steps as per the progress in fruit picking estimated by the camera system. FFRobotics team is leading this research activity in collaboration with WSU team.

*Materials:* We have been modifying the hardware and the software to support the optimized forward movement of the integration system. The integrated system has been described in Obj. #4.

*Procedure:* The entire system will begin by scanning the canopy to detect the fruits, which then will start the picking process and automatically move to the next stop. During the field evaluation, we will collect the capacity, the percentages of picked and bruises apples, the time between the consecutive locations and the time to stabilize the robotic frame to be ready for the next picking session. The picking system will then move down the row by certain distance (e.g. 1 meter; based on the frame structure) and the process will be repeated.

*3.4 Obj.# 4: Demonstrate integration of harvesting robot with fruit conveying and bin filling system*
*Introduction:* In prior field trials, the picking system was mounted on a harvesting aid system without full integration. In this work, it was proposed to integrate the picking system and the Harvesting Aid system to demonstrate bruise-free end-to-end, fully functional harvesting solution.

*Materials:* We have been modifying hardware and software to support the integration between the two systems. We will have 6 robotic arms in the same frame allowing dynamic movements along the height of the tree as an add-on for an existing Harvesting Aid System (Littau Harvester), or similar . The integrated system with the Harvesting Aid machine and our multi-robot conveyer system with combined control system of the two units will present the end-to-end solution from fruit harvesting from the trees through to conveyance all the way to the bin. FFRobotics and WSU teams are co-leading this research activity.

*Procedure:* As discussed before, the entire system will begin by scanning the canopy to detect the fruits, which will then start the picking process and automatically move to the next stop. The same type of data discussed in previous objective (e.g. harvester capacity, percentages of picked and bruised fruit, and the time between harvesting spots) will be collected for the end-to-end system (picking location to bins). The picking system will then move down the row as described before and the process will be repeated.

**Blossom and Green Fruit Thinning Objectives 5:**

*3.5 Obj. #5: Investigate machine vision and robotic end-effectors for blossom and green fruit thinning*

*Introduction:* Fruit harvesting is the major operation in apple orchards requiring a lot of seasonal labor. Once harvesting is automated, blossom and green fruit thinning will be another crucial step requiring automation or robotic solution. In this project, while fully developing and evaluating an integrated robotic harvesting system for harvesting, some efforts is being placed on robotic blossom and green fruit thinning. Our hypothesis is that, in the long term, all the manual operations in the field need to be automated and the machines need to be multi-functional with plug and play capability. Our robotic machine so far has been tested only for harvesting. But, this effort in blossom and green fruit thinning will ensure that we have basic technology ready for further development and integration with a robotic harvester in the next phase. WSU team is leading this in collaboration with FFRobotics.

*Materials:* Our teams (both FFRobotics and WSU) have developed and used camera system and image processing system for detecting apples and other objects in orchard environment. A multi-camera system was developed and used in Obj.#1 of this proposal for detecting accessible fruit for harvesting. We are using the same cameras and sensors to collect images from apple orchards during bloom and green fruit stages. The images are being analyzed to detect and localize flower and green fruit and a robotic system (using the same system described in Obj. #2) will be used to approach targeted flower and green fruit cluster for removing desired amount of flower and green fruit.

*Procedure:* In objective one, we have been developing a machine vision system to identify different canopy parts like leaves, fruit (matured), branches and trunks using a powerful algorithm called deep learning technique. In this work, the same algorithm has been revised and improved to detect flowers during the bloom stage, which will also be extended \to detect green fruit as early as possible. Flower and green fruit locations will be estimated using a stereo-vision system, which consists of two cameras installed slightly offset to each other. To add the capability, artificial lights will be installed in the robotic machine and the functionality of the image processing system will be evaluated both in day and night time. The locations of flower or green fruit in the given work space will be provided to a robotic machine (the same machine as in objective #2, with new end-effectors/hands developed for thinning) for reaching and killing desired flowers or green fruit. The multi-arm collaboration and optimization will be similar to the techniques discussed in Obj. 3 and 4. Various end-effector technologies will be evaluated for precision and effectiveness in removing desired amount of flower or green fruit from target canopy regions, which include brushing mechanisms, mechanical impact, precision chemical spray and air-stream.

**4. RESULTS & DISCUSSION**

*4.1 Obj.# 1: Optimize camera configuration for multi-arm operation*

Images and videos have been collected and are being processed for improved detection and localization of apples for fruit harvesting. Data were collected using an Intel RealSense 435 camera (Intel, USA) mounted on top of a robotic arm moving across its workspace. Video processing has a potential to provide additional visual perceptions for detecting occluded apples and those in clusters through a sequence of frames that provide varying viewing direction to the overlapping canopy areas. In addition, the machine vision system, developed using a Mask RCNN (Fig. 2; one of the latest deep


***Fig. 2: Understanding apple orchard images using deep learning; these sample images show the detection of fruits at pixel level along with branches, fruit calyx and occluded apples. This information will be used to improve the apple picking strategy in the future.***

learning techniques), was expanded to detect additional parts of tree canopies including branches, and leaves, along with fruits, so that important orchard characteristics such as branch obstruction, occlusion and pseudo-pendulum effects can be detected. Using a sequence of frames in the video stream collected, detected fruits were tracked over time to obtain optimal view to the fruit and optimal direction of picking. The proposed method detected fruit parts with an F1-score of 0.75 on a test dataset**.** As shown in Fig. 3, some apples were more clearly visible from one viewing angle than the other. For example, apple with ID 1 is more visible in the left image than in the right.


***Fig. 3: Apple detection with changing viewing angle/direction. Some apples are more visible in the left image while others are so in the right image.***

*Objective 2,3 and 4: Full-scale, integrated robotic system development and evaluation*

As discussed before, this year, we designed and fabricated two full scale robotic harvesting systems (Fig. 4 and 5). One machine was build and evaluated (preliminary) it in Israel, which shows that the system works, though there are a few aspects we need to improve as discussed in the significant finding section. A youtube video showing the machine working in the field can be found at https://youtu.be/rpPHR-mZEOQ.

The robotic picking system was integrated with a Tecnofruit conveying system for evaluating complete (end-to-end) harvesting process. The conveying system was not tested in the field as we were missing conveying components that transfer the fruits from the Harvesting unit into the main Tecnofruit conveying system. We are working now to complete this part and test it in our lab.

Originally, we intended to work with two harvest aid systems for full scale system integration. However, due to the complexity of integration, we decided to work with only one Harvesting Aid System. At this point in time, we are working with Tecnofruit CF105 harvest aid system (Fig. 5). The harvesting unit is a "add on" and in the future we will be able to integrate the system to other Harvesting Aids like Littau Harvester.

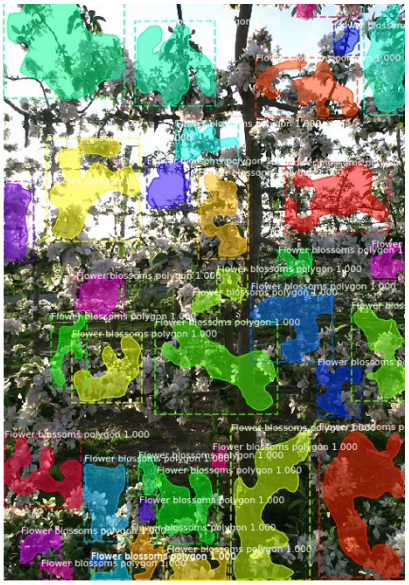

***Fig. 4: One of the two full scale robotic harvester developed this year. This machine was fabricated in Israel and tested in 2018 harvest season.***

From initial field experiments, we learned that the mechanical design of the multi-arm system could be further optimized to increase the overall speed of the machine (we were operating at only 20% of the designed speed of the motors in this experiment). In addition, we learned that path planning algorithm could expanded to optimize the allocation of individual arms to corresponding fruit. These improvements are ongoing, and with those, we estimate we can reach the target of 10K fruit per hour with the machine, which needs to be validate through further field experiments in the future.

*Obj. #5: Investigate machine vision and robotic end-effectors for blossom and green fruit thinning*

We collected over 125 images before and after thinning procedure in a commercial apple orchard in Washington. Apple trees were trained in vertical wall architecture. The images collected include 3D location of objects. The color images collected were fed to a Mask RCNN (a deep learning technique). Fig. 6 shows the detection of apple blossoms with this technique. The images contained sky in the background, which looked similar to blossom leading to some false detection of sky as apple blossoms. We will continue to improve the algorithms to detect blossom more accurately. First, the deep learning network was trained with limited dataset (50 images). Accuracy can be improved by including higher number of images in training the network. It can further be improved by implementing multi-class object detection in Mask RCNN. Furthermore, inaccurate detection of sky as blossom can be minimized by filtering out the background using depth information. With this approach objects detected beyond certain threshold will be considered as background and removed from detection process. We have also collected a image dataset for green fruit thinning. The images were acquired before and after the thinning operation. These images will be processed in near future for green fruit detection and localization.

***Fig. 6: Sample result for detecting blossom in apple orchards using deep l earning.***



**CONTINUING PROJECT REPORT** **YEAR**: 1 of 2 Years
**WTFRC Project Number:** TR-18-103

**Project Title:** Towards automated canopy management in tree fruit crops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**City/State/Zip:** Prosser, WA 99350

**Cooperators**: Curt Salisbury, Abundant Robotics Inc.; Dave Allan, Allan Brothers Fruits; Karen Lewis, Washington State University


**Total Project Request:** Year 1: $115,904 **Year 2: $80,740**

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

**Other funding sources**

*Carnegie Mellon University have a project scientist working in this project. Only a small fraction of the salary has been requested as per the budget 2 below. Remaining fund for the scientist's involvement will be covered by the university through other funding sources.*

**WTFRC Collaborative expenses: None**

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

| **Item** | 2018 | **2019** |
|---|---|---|
| **Salaries**[1] | $27,653 | $28,759 |
| **Benefits**[1] | $ 2,303 | $ 2,395 |
| **Wages** | $13,500 | $14,040 |
| **Benefits** | $2,448 | $2,546 |
| **Equipment** | | |
| **Supplies**[2] | $3,000 | $3,000 |
| **Travel**[3] | $1,000 | $1,000 |
| **Total** | $49,904 | **$51,740** |

**Footnotes:**
[1]Salary and benefit for a PhD student
[2]Cost to purchase sensors, metals, and other supplies for lab and field tests
[3]Travel cost for field data collection, and testing; and travel cost for cooperative meetings

**Budget 2**

**Organization Name:** Carnegie Mellon University **Contract Administrator:** Patricia Clark

**Telephone:** 412-268-3483 **Email address:** pclark@andrew.cmu.edu

| **Item** | 2018 | **2019** |
|---|---|---|
| **Salaries**[1] | $0 | **$5,000** |
| **Benefits**[1] | $0 | **$1,170** |
| **Wages** | $0 | **$0** |
| **Benefits** | $0 | **$0** |
| **Equipment** | $66,000 | **$18,454** |
| **Supplies**[2] | $0 | **$1,300** |
| **Travel**[3] | $0 | **$3,000** |
| **Miscellaneous** | $0 | **$76** |
| **Plot Fees** | $0 | **$0** |
| **Total** | $66,000 | **$29,000** |

**Footnotes:**

[1]A part of salary and benefit for a project scientist

[2]Cost to purchase sensors, metals, and other supplies for lab and field tests

[3]Travel cost for field data collection, and testing; and travel cost of cooperators

## 1. OBJECTIVES

The following are the project objectives, which remain the same as originally proposed.

1. Formulate objective pruning rules by integrating pruning strategy desirable for robotic/automated harvesting and the strategy currently used by growers in fruiting wall apple (e.g. formally trained) and cherry (e.g. UFO) orchards;
2. Develop a machine vision system to locate pruning branches in those two crop architectures.
3. Integrate and evaluate a robotic pruning machine.

***Timeline of the Project Activities (no deviation from the original proposal)***

| Objectives# | Research Activities | Time (Calendar Years and Quarters) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2018 | | 2019 | | | | 2020 | |
| | | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 |
| 1 | Develop pruning rules | | | | | | | | |
| | Identify pruning branches | | | | | | | | |
| 2 | Acquire canopy images and create 3D structure of trees | | | | | | | | |
| | Investigate potential for dead branch and flower bud detection | | | | | | | | |
| 3 | Evaluated integrated pruning robot | | | | | | | | |

## 2. SIGNIFICANT FINDINGS

1. For two apple canopy architectures observed, professional pruners followed similar strategies to select the branches to be pruned, but there was some variation in how they implement the strategies.
2. Field observation of commercial pruning operation and analysis of images captured before and after the pruning can lead to objective pruning rules.
3. Deep learning techniques show promise for analyzing canopy images to estimating various parameters such as size and location of branches and trunks.
3. There is a linear relationship between the cutting force necessary to prune apple tree branches and their diameter. Commercially available robot like the UR-5e (Universal Robots) can provide sufficient force to shear most of the branches in fruiting wall apple orchards.

## 3. METHODS

### 3.1 Objective#1: Pruning Rules and Pruning Branch Identification (Carnegie Mellon – Lead; WSU Participant; Abundant Robotics Collaborator)

In the past, it was found that, for the tall spindle tree architecture, the pruning process can be captured by four basic rules (Karkee et al., 2014); *i) remove diseased or dead woods/branches; ii) remove branches longer than a specified length; iii) remove branches larger than a specified diameter; and iv) remove branches to maintain a specified spacing.* Lehnert (2015) proposed eight rules for pruning tall spindle apple trees, some of which were similar to four different rules proposed by Karkee and Adhikari (2014). It was also claimed that two major rules; i) remove two to four largest limbs, and; ii) remove all vertically growing limbs (40 degree or less); will cover more than 90% of pruning job in tall spindle apple orchards. These rules developed in the past are essential for automated pruning of tall spindle apple orchards.

However, more work is necessary to develop pruning rules and apply those rules in identifying pruning branches in other tree architectures including formally trained apple architecture. Engineers, horticulturists and growers have been working together to explore pruning methods for the proposed canopy architectures. Special consideration will be given to the desired limb and fruit distribution for robotic apple harvesting that include the need of presenting fruit individually and without any obstruction by the branches, trunks or trellis system.

Similar to Karkee et al. (2014), the pruning rule formulation process included observation and analysis of the work of experienced pruners and supervisors. Experienced pruners were and will be selected from commercial orchard crews. They were asked to individually tag pruning branches on randomly selected fruit trees using unique color tags. To keep tagging independent between workers, tags were removed from the tree before another worker was asked to tag the pruning points on the same tree. Video and color images of each tagged tree were captured.

Pruning branches identified by workers as well as the total number of branches will be located and counted for each tree. Videos and still images will be analyzed to look for the pruning patterns and process each worker follows. A set of objective pruning rules will be defined using; i) expert's knowledge captured from engineering team based on their need for robotic harvesting; ii) horticulturists and growers based on their understanding of training practices, tree architectures and physiology; and iii) from experienced workers based on pruning processes they follow. We have been visiting with different collaborators to get their input on the pruning strategies to support the process of pruning rule identification.

After objective pruning rules are defined, the 3D tree structure created in Objective 2 and pruning rules will be used to identify branches for pruning. For this task, a novel deep learning-based method will be used to distinguish trunk, main branches and sub-branches or laterals of a tree. Geometric parameters of tree canopies including branch size (diameter), branch length and branch spacing will be estimated using the 3D measurements and corresponding color images. Once all the topological and geometric parameters of trunks and branches are estimated, decision can be made, using pruning rules, which branches need to be pruned out.

### 3.2. Objective#2: Machine Vision System (WSU and Carnegie Mellon – Co-Lead)

Under this objective, we have been focusing on creating 3D structures of apple and cherry trees trained in modern fruiting wall architecture (e.g. formal training for apples, UFO architecture for cherries). Five major steps are involved in the generation of 3D structures of fruit trees and identifying pruning points; i) image acquisition; ii) Faster Region-based Convolutional Networks (F-RCNN); (iii) Generative Adversarial Network (GAN); iv) point cloud generation; and v) combining (ii), (iii), and (iv). A stereo-vision camera, a laser sensor, and a Kinect 2 sensor have been used to capture 3D information of trees. Stereo-vision system will provide high-resolution 3D information, whereas laser sensor will provide more accurate 3D location. Kinect sensor also has the potential to provide equally accurate 3D information at lower cost. Comparison and fusion (when necessary) of 3D information from these sensors will lead to improved resolution and accuracy of 3D information of the trees. Stereo-vision camera will also provide complimentary color images.

After image acquisition and 3D point cloud generation, a novel deep learning-based system will be used to generate 3D structure of apple trees. A state-of-the-art object detection algorithm (F-RCNN) proposed by Ren S., et al. (2015) will be used to identity branching points from color images. These branching points are strong visual ques that will be used to detect branch occlusion that would be necessary to segregate individual branches as a whole. The link between the detected branching points will be associated using skeleton image generated by the Generative Adversarial Network (GAN).

The output of the GAN will be a binary image with an array of connected binary pixels that will trace the mid-section of branches in the color images. GAN is a semi-supervised machine learning technique that learns to generate synthetic images based on training dataset. A multi-channel GAN will be used to generate skeleton image for branches and main trunk. Once the skeleton has been identified, the curvature of the branches and trunk will be warped using the depth information obtained in the previous steps to reconstruct the 3D models. The length and size (diameter) of each branch will be estimated. Length can be estimated using the starting and end points of the branches. To estimate the diameter, skeleton of the tree will be overplayed on top of the color images taken from the same perspective. Then number of pixel in the orthogonal direction of the branch skeleton will be counted at the base of the branch (2 to 5 cm from the branch-trunk junction). Resulting 3D skeleton and geometric parameters will be used in identifying pruning branches as discussed in Objective 1. Preliminary results from Co-PI Kantor's lab have shown promising results in detecting dormant buds and cane structures in grape vineyards using the technique described above.

In addition to geometric parameters such as size and length, productiveness of a branch is another consideration for pruning. If a branch is dead or otherwise unproductive (without enough fruiting locations), it needs to be pruned out. Detecting if a branch is dead and estimating number of flower buds in live branches would be valuable information to make automated pruning more effective. This work will investigate the potential of using spectral signature (using a hyperspectral camera) to differentiate dead and live branches in the dormant season. When a dead branch is detected, the attribute of the branch will be updated in the 3D skeleton to indicate that the corresponding branch needs to be pruned out. Another task in this objective will be to investigate the potential of a machine vision system for flower bud detection and counting. This information will be important for both automated as well as manual pruning. Currently, many growers make decisions on pruning strategies based on number of flower buds before and after pruning so that desired level of crop-load can be achieved. Because the color, shape and size of flower buds will be similar to other parts of branches, automated detection will be challenging. We will explore the use of spectral signature in addition to geometric parameters to investigate its potential to address these challenges. We will also evaluate image resolution, viewing angle and computational power desired in detecting flower buds, which will help establish the potential for automated flower bud detection in dormant season. *This objective is being co-led by Carnegie Mellon and WSU teams; Carnegie Mellon team will lead the pruning branch identification task whereas WSU team will lead the flower bud detection task. However, both teams will work very closely to exploit the expertise and experience of the entire team.*

### 3.3. Objective#3: Integrated Robotic System Evaluation (WSU–Lead; CMU Participant; Abundant Robotics Collaborator)

In the proposed work, various end-effector mechanisms are being developed, which will be attached to a robotic manipulator (Fig. 1) to carry out various specific tasks. Machine vision and pruning branch identification system will also be integrated to the hardware system for complete system development and evaluation in field environment. The first end-effector will be a round cutter that can close around a branch. If everything around a branch needs to be removed (e.g. lateral branches in UFO cherries), this end-effector mechanism can be guided along the branch using the 3D structure of the trees developed earlier (Objective 2). A short saw-bar will also be developed and evaluated for removing all the secondary branches growing in a certain section of the primary branches. For example, everything growing longer than 6 inches below a horizontal

*Fig. 1: A robotic system available to PI Karkee to carry out field evaluation of the pruning system*

limb in a formal apple orchard can be removed with this mechanism. Another end-effector to be evaluated will be a scissor. This type of end-effector will be evaluated in cutting individual branches. Pruning rules identified in Objective 1 will guide the use of specific end-effector from the ones discussed in this paragraph.

## 4. RESULTS & DISCUSSION

We started this project in Summer 2018 and have made good progress in each of three objectives proposed for the project. The following, we will present and discuss major results obtained so far.

### 4.1 Objective#1: Pruning Rules and Pruning Branch Identification.

As a part of a planned field trip organized by WSU, Co-PI Abhisesh Silwal from CMU traveled to Prosser, Washington during late November 2018 to collect field data of dormant apple trees. Two major activities planned for this field trip included imaging the dormant canopies and observing pruning strategies practiced by professional pruners.

***Image Data Collection:*** The focus was to collect images before and after pruning dormant apple tree canopies as described under section 3.2, which will be used to associate the pruning decisions made by professional pruners through analyzing the differences between the prior and the posterior images. To image the trees, an active lighting-based camera system developed at CMU was used in broad daylight condition. This camera system generated high resolution color stereo images of the scene without having any effect from varying ambient lighting. Fig. 2, shows the experimental setup. During this trip we collected data on two different canopy architectures (V-trellis and vertical spindle, formally trained) at three different orchards (Vantage, Quincy, and Prosser). Altogether, the dataset now contains before/after images from forty-two apples trees that were randomly selected and manually pruned by professional pruners.

*Fig. 2: Experimental setup during the field data collection. The active lighting camera was mounted on the back of the Gator.*

As seen in Fig. 2, each target tree was marked with QR tags indicating the start and end of the canopy. The collected ground truth data included total number of dormant buds (also referred to as fruiting zones) on the third trellis wire, and width & length of branches of varying diameters. These ground truth data points will help to validate the performance of the vision algorithm as described under objective (Sec 3.2 and 4.2).

***Observing Current Pruning Strategies:*** The second important task during the field trip was the observation of the current practice in professional pruning. In order to include the observation and analysis of professional pruners, we selected pruners working on commercial apple orchards. We asked each pruner to tag the pruning points (using marking tapes) on branches of ten different trees. After imaging the marked tree, tags were removed and another pruner tagged the same tree branches using similar pruning rule. Video and still images were captured before and after each professional pruner tags tree branches for pruning. Data from this small-scale study will be used to analyze the consistency amongst pruners following identical pruning rules. Some of the major observations are listed below.

- Use of tools to set crop-load level based on branch cross-section area (BCA) was emphasized by some growers. A rule of thumb was used to estimate number of fruits for a given branch size. An arbitrary example could be '*6 apples per unit BCA'*. Different variations of tools to estimate BCA were found (e.g. Fig. 3).

**Fig. 3: Two different types of tools that could be used for measuring Branch Cross-sectional Area.**

- In practice, branch diameters were assessed visually by pruners, hardly any workers used the BCA tool when performing pruning task in the field. In formally trained orchards, most of the workers were guided by a given number of fruits per unit length of the lateral branches trained to trellis wires.
- Fruit spacing was considered an important parameter. The idea in general here was to distribute fruits evenly for higher quality. A minimum fruit spacing of ~3.5" was considered to be acceptable,anything closer might lead to clusters of fruit during harvest season.
- To achieve minimum fruit spacing, pruning ideally should consider bud spacing and prune branches such that the flower buds are not too close to each other. It was also evident that buds too close to the trellis wire needed to be minimized.
- Length of fruiting laterals was also an important factor. Laterals longer than ~8 inches were often trimmed back or pruned out.
- Considerations were also made to remove vertical fruiting sites and those right over or under the horizontal branches

*These observations provide a basis for developing pruning rules and identify pruning branches, which will be then an input to the robotic pruning machine.*

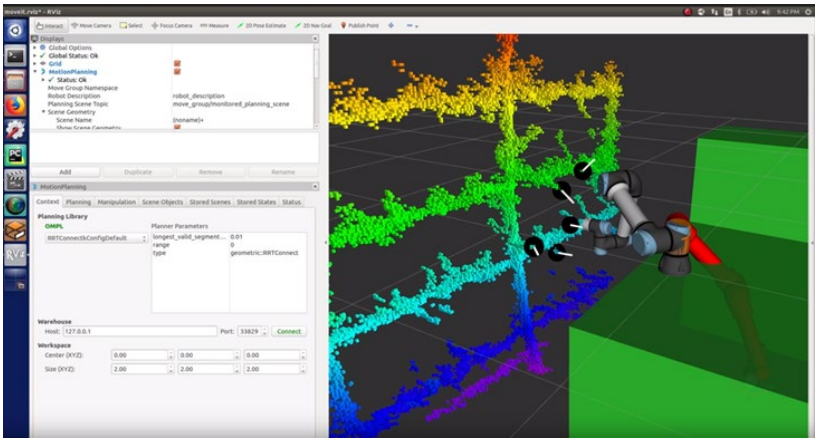
**4.2 Objective#2: Machine Vision System**

We plan to implement a machine learning-based approach to objectively extract knowledge from the images captured before and after pruning in the commercial fields, which will also be used to formulate suitable pruning rules for robotic pruning. Before such technique can be implemented, the vision system should be able to estimate key aspects of tree canopies such as branch and trunk structures and sizes, and number and location of buds. Currently, we have implemented a deep learning technique for image processing and are generating hand-labeled image dataset to supervise the learning process. Once the data labeling is completed, we will work on 3D reconstruction of the tree canopies, as well as pruning point identification and localization, which will be provided as an input to the integrated robotic pruning system.

**4.3 Objective#3: Integrated Robotic System Evaluation**

Our team has recently started collaborating with Joseph Davidson (Assistant Professor, Oregon State University), who has purchased a UR-5e collaborative robot from Universal Robots for his research lab

at Oregon State University (OSU). This manipulator has shown good speed, reach and maneuverability to be used for an initial proof-of-concept demonstration of fruit tree pruning. PI Karkee will acquire the same or similar robotic system in recent future to speed up the system integration and preliminary evaluation within the first year of the project. Developing an integrated software stack for robot control is a key task required for a successful demonstration. Our approach to the problem includes the following sequential steps:



1) Create a probabilistic 3D map of the orchard environment using Octrees
2) Generate collision free paths to the identified pruning points using RRT*, an established algorithm designed to efficiently search nonconvex, high-dimensional spaces
3) Execute a controlled approach to the cut point using inverse kinematics support available in MoveIt, an open source tool that provides motion planning, kinematics, control and navigation support packages
4) Cut the identified branch

*Fig. 4. Simulation of branch pruning in Gazebo. A 3D OctoMap of an apple tree has been created from a Kinect scan. Five pruning points were selected randomly. The UR-5e traces a collision free path found with RRT*.*

Fig. 4 shows virtual demonstrations of the four steps described above using the Gazebo simulation environment. A capstone design group at Oregon State University (OSU) has designed a pneumatically operated shear that will be integrated with the UR-5e's controller. The end-effector tooling will be fabricated with standard manufacturing equipment like a CNC mill, bandsaw, waterjet, etc.

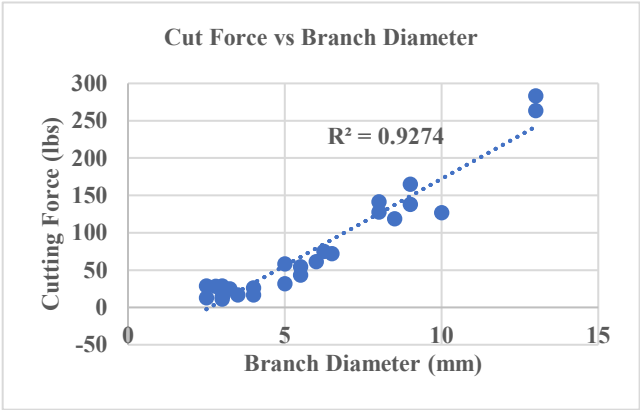



*Fig. 5: Regression analysis shows a linear fit between cutting force and branch diameter.*

*Fig. 6. Lab tests to determine branch cutting forces. Branches were collected from a commercial orchard in Prosser, WA in November 2018. Cutting tests used handheld shears and a force gauge.*

We also collected data to estimate force required to shear branches with different diameters. It was found that there is a linear relationship between cutting force and branch diameter (Fig. 5). Based on our analysis, the force required to shear a branch with mean diameter of ~ 7 mm is less than 100 lbs. We have a designed a pneumatically operated four-bar linkage that will provide a maximum of ~200 lbs cutting force (Fig. 6). In the next a few months, latest progress in developing the vision system, pruning rules, pruning branch identification method, and end-effectors will be integrated with a robotic manipulator and a preliminary field evaluation will be completed during this dormant season in apple orchards.

**CONTINUING PROJECT REPORT** **No-Cost Extension**
**WTFRC Project Number:** TR-17-100

**Project Title**: Enhancing reference genomes for cross-cultivar functional genomics


| | | | |
|---|---|---|---|
| **PI:** | Loren Honaas | **Co-PI:** | Joshua Der |
| **Organization**: | USDA, ARS | **Organization**: | CA State University, Fullerton |
| **Telephone:** | (509)664-2280 | **Telephone**: | (657)278-4115 |
| **Email**: | loren.honaas@ars.usda.gov | **Email**: | jder@fullerton.edu |
| **Address**: | 1104 N. Western Ave. | **Address:** | 800 North State College Blvd. |
| **City/State/Zip**: | Wenatchee, WA 98801 | **City/State/Zip**: | Fullerton, CA 92831 |


**Cooperators:** Stefano Musacchi & Sara Serra (WSU-TFREC), Claude dePamphilis (PennState)

**Total Project Request:** **Year 1:** $48,832 **Year 2:** $35,207 **Year 3:** NA

**Percentage time per crop:** Apple: 60% Pear: 40% Cherry: NA Stone Fruit: NA

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

| Item | 2017 | 2018 | 2019 |
|---|---|---|---|
| **Wages[1]** | $12,500 | $12,500 | |
| **Equipment** | $3,750 | $750 | |
| **Supplies** | $3,000 | $3,722 | |
| **Miscellaneous[2]** | $11,664 | NA | |
| **Total** | $30,914 | $16,972 | **0** |

**Footnotes:**
**[1]Data analysis including Research Support Agreements to cooperators**
**[2]Cooperative Agreement for PacBio library prep + sequencing to Penn State Group**

**Budget 2**
**Organization Name: CSU Fullerton** **Contract Administrator: Alison Nguyen**
**Telephone: 657-278-7621** **Email address: allisonnguyen@fullerton.edu**

| Item | 2017 | 2018 | 2019 |
|---|---|---|---|
| **Salaries[1]** | $8,922 | $9,234 | |
| **Benefits[1]** | $129 | $134 | |
| **Wages[2]** | $8,526 | $8,526 | |
| **Benefits[2]** | $341 | $341 | |
| **Total** | $17,918 | $18,235 | **0** |

**Footnotes:**
**[1]Salary and benefits for Joshua Der – 1 month**
**[2]Salary and benefits for Der lab student – 2 semesters**

**Objectives:**
**Enhance discovery of genetic factors associated with fruit quality differences** using existing and in-progress RNA-seq data, along with publicly available genomic resources:
*Step 1)* **identify genetic differences** between reference genomes and genomes of interest ('Golden Delicious' vs 'Granny Smith' & 'Bartlett' vs. 'D'Anjou').
*Step 2)* use bioinformatic approaches to **update the reference genomes** to reflect these differences creating custom, polished references for analysis of gene expression in each of the genomes of interest.
*Step 3)* **compare gene expression results** from the original and polished versions to calculate changes in read mapping rates focusing on total reads matched and changes in uniquely matched reads (both indicating changes in sensitivity for measuring gene activity) **to evaluate the efficacy of the genome polishing strategy.**

**Year 3 goals:**
In year 3 we will continue to assemble and assess genomes of 'Granny Smith' apple and 'D'Anjou' pear (See Objective 1 & 2). In addition to "from scratch" approaches, we will use the existing reference genomes to guide assembly, a strategy that may improve accuracy and reduce assembly effort (see Objective 2). We will repeat published (see Honaas et al. 2018 – https://doi.org/10.1016/j.postharvbio.2018.09.016) and ongoing gene activity analysis (partially funded by WTFRC award PR-17-104) to evaluate the effect on RNA-Seq analysis of matched genomes vs cross-cultivar approaches (see Objective 3).

**Significant Findings:**
- Modified genomic DNA protocol & successfully sequenced genomes
- Quantified differences genome-wide in 'Granny Smith' and 'D'Anjou'
- Validation and comparative analysis confirm cross-cultivar RNA-Seq issues

**Methods:**
To obtain genomic DNA from 'Granny Smith' apple and 'D'Anjou' pear, trees were obtained from Van Wells (Wenatchee, WA) and dormancy was broken in the USDA green house. Young leaves approximately 2 cm in length were harvested and flash frozen on liquid nitrogen. Frozen tissue was sent to cooperator dePamphilis for DNA extraction methods testing, genomic DNA quality evaluation, sequencing sample preparation tests, and then genome sequencing at Penn State's genomics core facility. Genome data were used to survey for genome differences between reference cultivars and cultivars of interest. Pilot genome assemblies are underway and are being tested in the first phases of the draft genome polishing workflow. A full suite of RNA-Seq analyses have been run for apple and pear, including validation, to use as a baseline for comparisons to similar analyses using the new genome references for 'Granny Smith' apple and 'D'Anjou' pear.

**Results and Discussion:**
***Streamlined genomic DNA prep led to successful genome sequencing in apple and pear*** Very high molecular weight DNA (HMW DNA - e.g. ~100,000bp pieces of DNA), is one essential component to accessing 3rd generation sequencing technology. Cooperator dePamphilis's group has successfully developed an extraction and purification protocol that results in good yields of HMW DNA (Figure 1). Long genomic fragments are extremely fragile such that mixing or shaking the sample creates shear forces that will break the very long and delicate strands. Other groups have used more expensive and elaborate methods to get DNA of sufficient quality (as in the recent double haploid 'Golden Delicious' genome). Our group, using specialized techniques like Pulsed Field Gel Electrophoresis (PFGE), has demonstrated that more rapid and cost-effective methods from flash-frozen young leaves yield suitable DNA for 3rd generation sequencing.

A second hurdle to getting valuable 3rd generation genome data is the unpredictable success rate during sequencing sample preparation. The only effective way to determine if HWM DNA samples

will work is to attempt test DNA preparations. At the Penn State genomics core we successfully generated data from multiple approaches and selected the highest performing strategy to sequence 'Granny Smith' and 'D'Anjou' - each yielding millions of reads that were >10,000bp (50x longer than 2nd gen technology). Using the same DNA we also generated 2nd generation genome data (shorter, but more numerous reads). These 2nd gen data are useful to quantify differences between reference and cultivar-specific genomes, as well as error-correct 3rd generation data during the iterative process of building and evaluating genome assemblies.

***Gene differences between cultivars present a hurdle to gene activity analysis***

We have continued to examine 'Granny Smith' and 'D'Anjou' data to identify polymorphisms that may be potentially problematic in RNA-Seq analysis. Using the 2nd generation genome data from 'Granny Smith' and 'D'Anjou' we counted polymorphisms as compared to the reference genomes ('Golden Delicious' and 'Bartlett', respectively). These polymorphisms are summarized in CIRCOS plots (Figure 2A & B). They show that both 'Granny Smith' and 'D'Anjou' have millions of polymorphisms that are distributed genome-wide as compared to the reference genomes.

We have used existing gene activity data to correct gene models, as well as build custom, cultivar-specific ones. By comparing results using corrected gene models (Figure 3A) vs. custom 'Granny Smith' gene models (Figure 3B) we have shown that error correction reduces noise in our gene activity measurements (compare Fig. 3A and 3B). We recently published a protocol for validating RNA-Seq data in cross cultivar experiments (Hargarten et al. 2018 - https://doi.org/10.1016/j.postharvbio.2018.09.016) that we used to examine the agreement between gene-specific tests and RNA-Seq estimates (Figure 4). The agreement between estimates of gene activity in apple is better than for pear, which is expected due to a poorer quality pear genome and more polymorphisms as compared to apple. The validation experiments support our original hypothesis showing a significant positive relationship ($P<0.001$) between genetic differences and discordant estimates of gene activity.

***Perspectives***

All together, these results show that millions of genetic differences in apple and pear are likely contributing to harmful noise in massive-scale gene activity measurements (RNA-Seq). Other sources of noise (biological + technical) besides single nucleotide polymorphisms are likely contributing to noisy gene activity measurements, so we fully sequence the genomes of 'Granny Smith' apple *and* 'D'Anjou' pear (now possible due to a decrease in cost for 3rd generation sequencing technology). These cultivar-specific draft genome assemblies will allow us to avoid noise from multiple sources (including polymorphisms) that arise during cross-cultivar RNA-Seq experiments. The end result, ***the main deliverable***, is improved gene activity measurements that enhance our ability to identify genes that play important roles in postharvest fruit quality. Knowledge of these genes allows us to search for biomarkers that may be useful to predict future fruit quality. As we use this information to learn about how fruit respond in the postharvest period we may also find patterns that suggest new or improved postharvest technology and practices.

Additionally, these genomic resources will be ready to use and freely available for the new pear genomics scientist at the ARS Tree Fruit Lab.

**Figures and Tables:**

**Figure 1.** Very long fragments of genomic DNA from our stream-lined protocol. 18 hour Pulsed Field Gel Electrophoresis (PFGE) of genomic DNA shows that our modified genomic DNA protocol was successful for both apple and pear and is comparable to high quality samples of *Theobroma cacao* (chocolate tree) DNA.

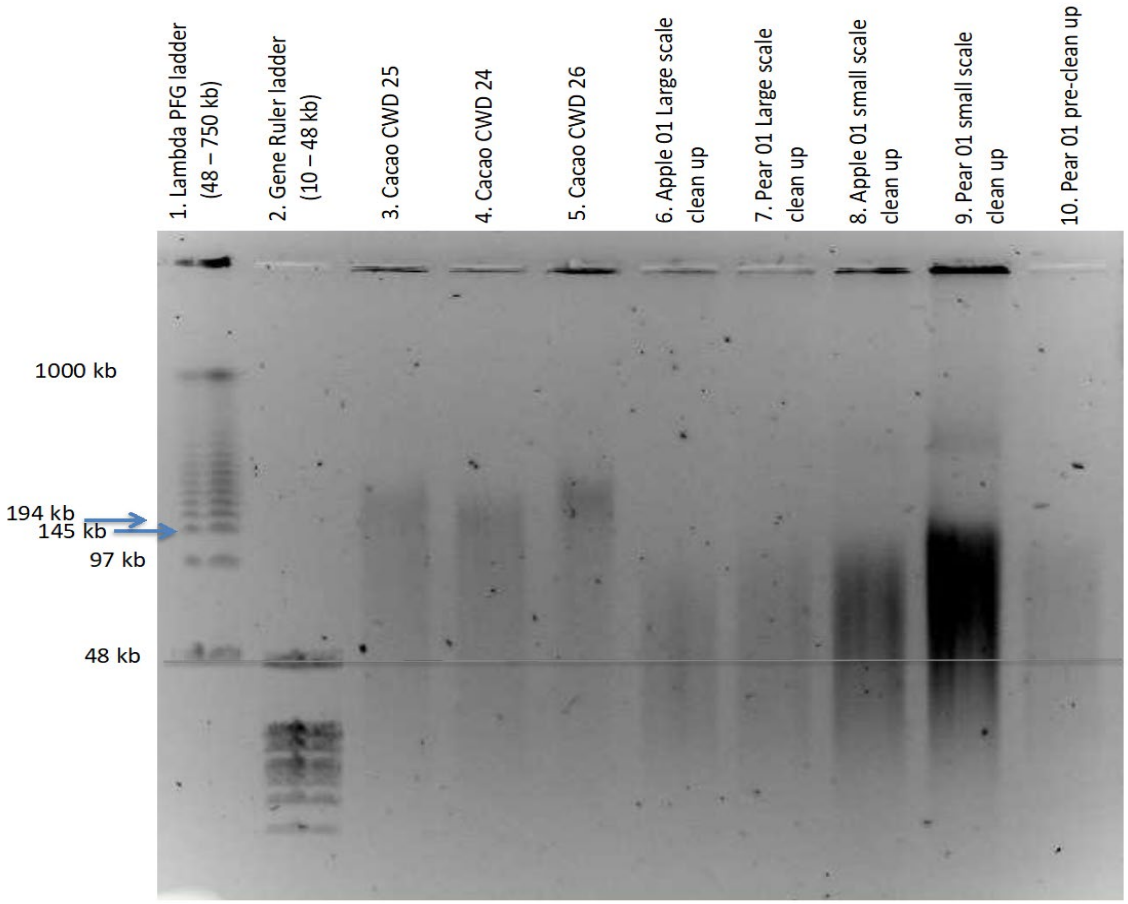


**Figure 2.** CIRCOS plots showing polymorphisms (outer trace) and genome data coverage (inner trace) in the A) 'Granny Smith' genome compared to the 'Golden Delicious' genome and B) 'D'Anjou' genome compared to the 'Bartlett' genome. Each block represents a large genomic fragment and the entire genomes of apple and pear are represented.

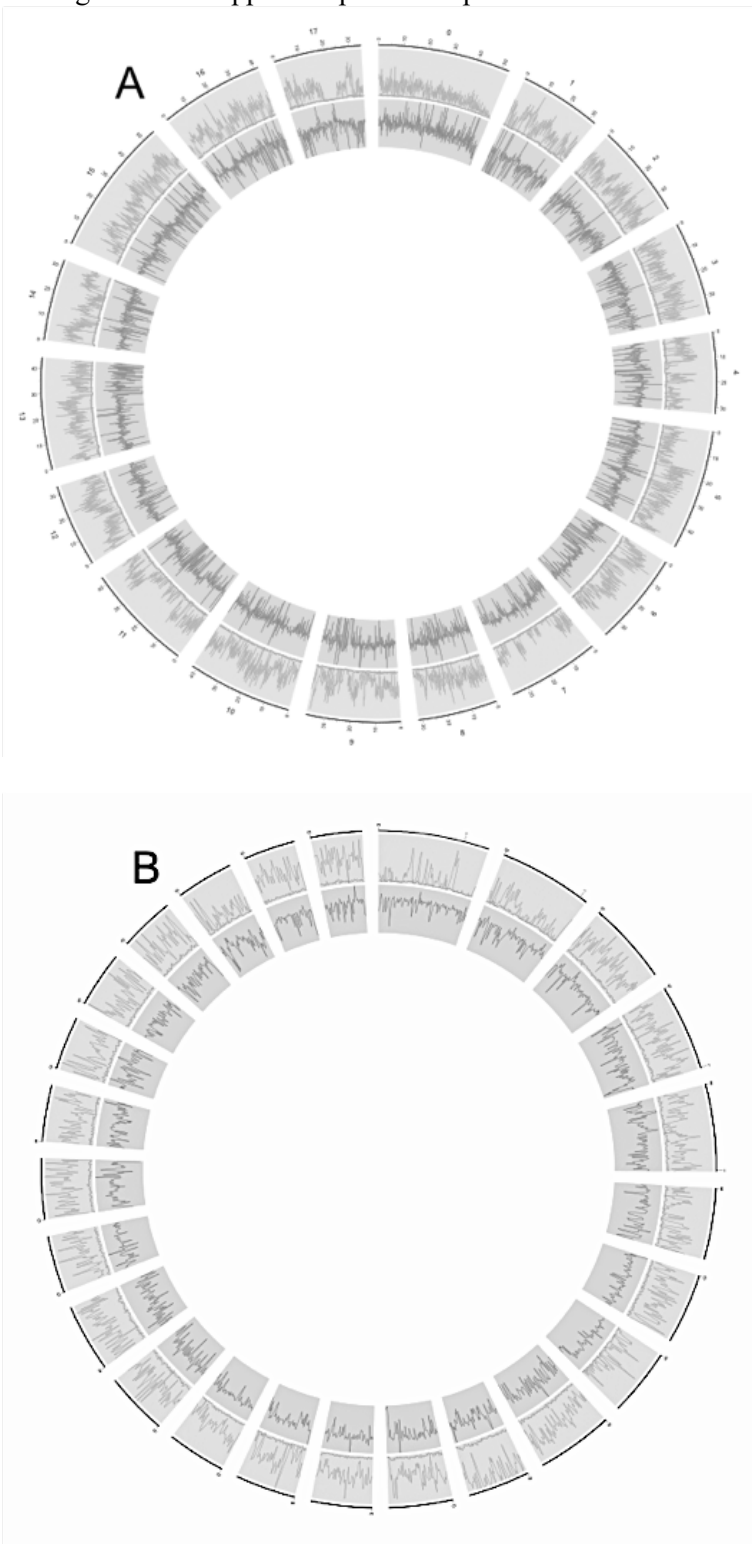


**Figure 3.** Mismatches between custom 'Granny Smith' gene predictions and the 'Golden Delicious' genome predictions reduce signal quality. Gene activity measurements using the 'Golden Delicious' reference genome (Phytozome) correlated with **A)** corrected 'Granny Smith' gene predictions (Stringtie) or **B)** *de novo* predictions using raw 'Granny Smith' data (Trinity). In **panel A**, 90-95% of the signal is explained by a linear relationship. In **panel B**, only 60-85% of the signal is explained by a linear relationship.

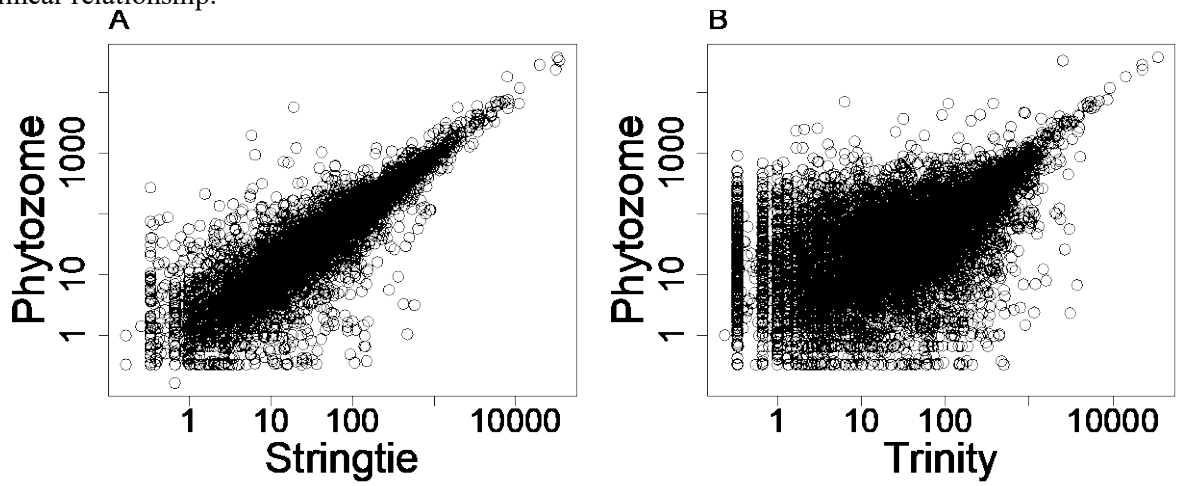
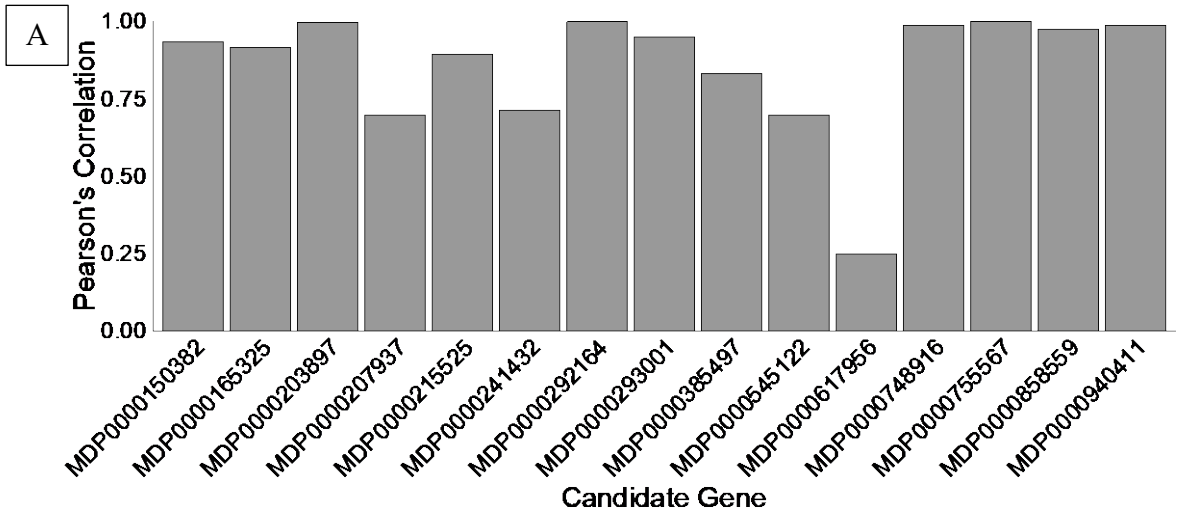
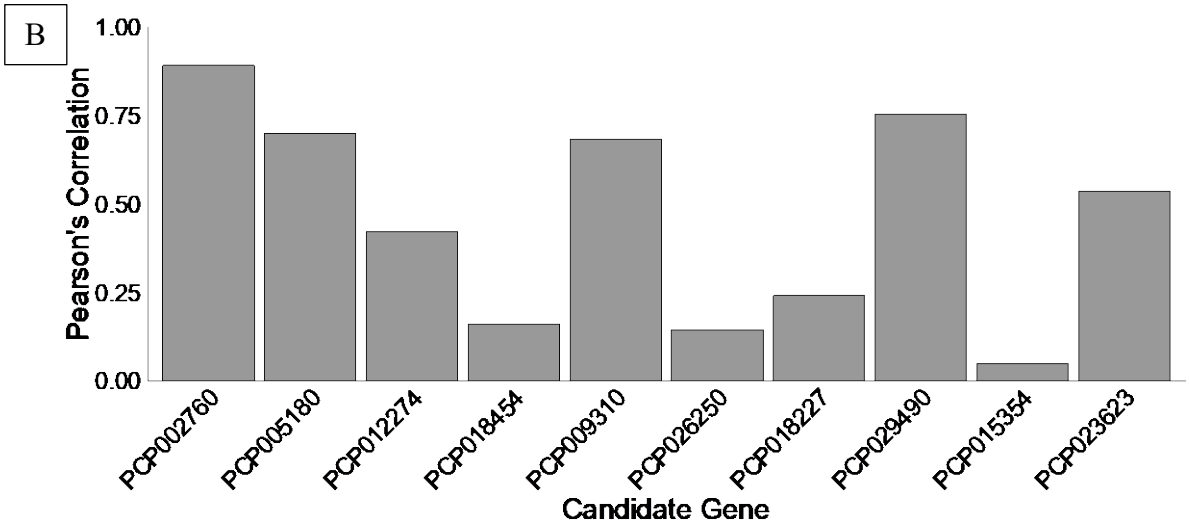



**Figure 4.** Validation of RNA-Seq (massive-scale gene activity measurements) using qPCR show A) moderate agreement (average $R^2$~0.8) for apple experiments and B) marginal agreement for pear experiments (average $R^2$~0.6). This is expected as the apple genome assembly is better, and genetic variation among pear cultivars is higher than among apple cultivars - both are hurdles to analyzing RNA-Seq data.




B
1.00
0.75
0.50
0.25
0.00
Pearson's Correlation
PCP002760
PCP005180
PCP012274
PCP018454
PCP009310
PCP026250
PCP018227
PCP029490
PCP015354
PCP023623
Candidate Gene

**CONTINUING PROJECT REPORT** **YEAR**: 1 (of 2)
**WTFRC Project Number:** TR-18-102

**Project Title:** Development of economical wifi-connected open-source sap flux probes

**PI**: Stephen Good
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**City/State/Zip**: Corvallis, OR 97331

**Co-PI(3):** Nik Wiman
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**Address 2**: Oregon State University
**City/State/Zip**: Corvallis, OR 97331

**Co-PI (4):**
**Organization:**
**Telephone:**
**Email:**
**Address:**
**Address 2:**
**City/State/Zip:**

**Cooperators**: Oregon State University Experiment Farms, grower cooperators

**Total Project Request:** $86,320 **Year 1:** $42,723 **Year 2: $43,597**

**Percentage time per crop:** Apple: 25% Pear: 25% Cherry: 25% Stone Fruit: 25%
(Whole % only)

**Other funding sources:** None

**WTFRC Collaborative expenses:** None

**Budget 1**

**Organization Name:** Oregon State University – Agricultural Research Foundation
**Contract Administrator:** Russell Karow
**Telephone:** (541) 737-4066 **Email address:** russell.karow@oregonstate.edu

| Item | 2018 | 2019 | 2020 |
|---|---|---|---|
| **Salaries** | $26,745 | $27,379 | |
| **Benefits** | $5,978 | $6,218 | |
| **Wages** | | | |
| **Benefits** | | | |
| **Equipment** | | | |
| **Supplies** | $7,500 | $7,500 | |
| **Travel** | $2,500 | $2,500 | |
| **Miscellaneous** | | | |
| **Plot Fees** | | | |
| **Total** | $42,723 | **$43,597** | |

**Footnotes:**

# DEVELOPMENT OF ECONOMICAL WIFI-CONNECTED OPEN-SOURCE SAP FLUX PROBES

Stephen Good[1], Chet Udell[1], & Nik Wiman[2]
(1) Department of Biological & Ecological Engineering, Oregon State University
(2) Department of Horticulture, Oregon State University

## Objectives

*Statement of Project Objectives:*

This project consists of three objectives:

(1) Develop low-cost alternatives to commercially available sap-flux monitoring systems. These probes will be based on published designs recently made available in academic/research literature that are not accessible to typical tree fruit producers.

(2) Develop wi-fi connectivity protocols that will allow these new sap-flux probes to be monitored remotely via the world-wide web. Measurements will be converted to tree and orchard level evapotranspiration measurements and placed online for end users.

(3) Make available, as extension publications and online, both the probe design and wi-fi connectivity protocols in a format where users with little technical experience can construct/create their own networks with minimal effort.

*Overall Goals and Relevance for Pacific Northwest Tree Fruit Producers*

This project directly addresses a number of key priorities for technology development in the WTFRC Technology Roadmap. Our objectives are designed in a manner so as to be directly beneficial to tree fruit growers in the Pacific Northwest.

It is expected that the direct, accurate, and low-cost monitoring of orchard-level water use obtained through this project will allow growers to reduce production costs while ensuring premium quality fruit is grown for the consumer. This is because accurate water use monitoring will allow for precision application of required water at the orchard block or individual tree level. Effectively, growers will be able to adjust irrigation rates to achieve desired transpiration rates.

Furthermore, accurate water use monitoring will allow for direct surveillance of orchard blocks, and fruit trees that are in danger of drought damage can be identified remotely. When individual or stand transpiration rates fall below critical thresholds, this signals that trees in these locations are not growing properly and should be investigated in person.

Finally, because transpiration, as directly measured in the sap flux probes, occurs only when leaf stomata are open during photosynthesis, transpiration rates can be related to biomass accumulation via photosynthesis. Sap flux measurements can be integrated as the growing season progresses to provide estimates of how much carbon has been assimilated by each individual tree or stand. These can then be translated into yield information about end of season harvest.

*Project Schedule for 2019-2020*

This project is proceeding on schedule. The planned activates for the second year of this project are:

| ***PROJECT TASK*** | **WINTER 2019** | **SPRING 2019** | **SUMMER 2019** | **FALL 2019** | **WINTER 2020** | **SPRING 2020** |
|---|---|---|---|---|---|---|
| *Direct tests heater and temperature sensors* | ■ | | | | | |
| *Lab based test of sap flux through a trunk section* | ■ | | | | | |
| *Finalize Design of sap flux probes for field deployment* | ■ | ■ | | | | |
| *Field deployment of sap flux probes in fruit orchards* | | ■ | ■ | ■ | | |
| *Refine data transfer and storage protocols* | | ■ | ■ | ■ | | |
| *Develop sap flux probe web-based interface for data display* | | | ■ | ■ | ■ | |
| *Disseminate project results via extension & journal publications* | | ■ | ■ | ■ | ■ | ■ |

**Significant Findings**

During the first year of this project we have made considerable advances. We have:

- Developed an initial prototype sap flux probe based on a simple printed circuit board (PCB) design. This is shown in figure 1A.
- Developed an initial prototype sap flux probe electronic controller, datalogger, and communication hardware. This is shown in figure 1B.
- Developed a prototype enclosure system. This is shown in figure 1C.
- Preformed an initial set of field tests in fruit trees in Summer 2018.
- Formalized a set of key engineering requirements for sap flux probe design.
- Formalized a plan for rigorous testing of different design to be implemented in the Winter/Spring/summer of 2019

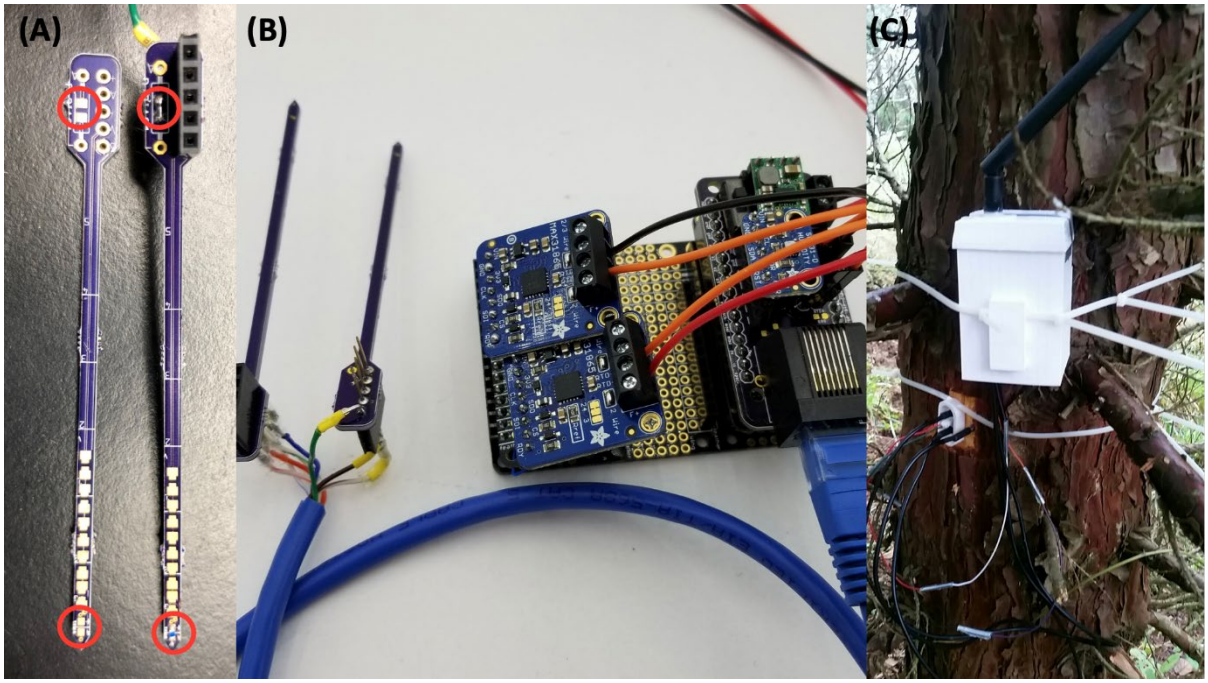


*Figure 1: (A) Sap flux probe printed circuit board prototype, (B) Arduino based electronic controller, data logger and communication hardware, (C) sap flux probe installed on a tree with electronics housing and antenna.*

**Methods**

This project is focused on development of printed circuit board (PCB) based sap flow probes. PCB designs have been created by our team using *EagleCAD*, with the current design shown in Figure 1A. The base PCB martials are being printed by OSHPARK, a popular PCB vendor which prints these at around 3 dollars per PCB. At the desired depth on the probe (see lower red circles in Figure 1A) a resistor or thermistor is soldered onto the PCB to either produce a heat pulse or measure its dissipation by flowing sap. Thermally conductive epoxy is then molded around the probe package.

The PCB is connected to a small microcontroller with build in analogue to digital conversion (upper red circles in Figure 1A). An Adafruit M0 Feather (Figure 1B), which costs around $35 dollars is working well in our current implementations. An SD card reader and card are connected to the microcontroller for internal storage of data. Because WiFi was not immediately available at the test site, we incorporated a 2G Fona GSM module to upload probe data online, which can then be accessed anywhere in the world with an internet connection. We are working on a LoRa (Long Range) model that connects to the system microcontroller for wireless transmission of data to an local internet hub. This will be a more cost-effective method as the number of probes per field site is scaled up. This package is housed in a small enclosure and connected to an external power source.

*Figure 2: Sap flow probe deployed in a cherry tree August 2018*

Tests were conducted in Summer 2018 on both Cherry and other test trees as shown in Figure 2. Tests conducted in September 2018 take advantage of a large set of instruments that were deploy by another research project, including an eddy covariance system to measure orchard evapotranspiration. This allowed our system to be compared to state-of-the-art instruments beyond the scope of our budget.

In the winter/spring of 2019 our team has prepared a number of detailed lab and field tests. First, different temperature sensors will be evaluated based on their accuracy (drift over time), reliability (waterproofness), and size (probe hole diameter). These tests will be conducted within the laboratory using a mineral oil bath on top of an electronically controlled heat plate. Once the preferred commercially available temperature sensor is selected, the completed sap flux probes will be calibrated in the laboratory. Liquid will be forced though cut -meter long sections of tree trunks at measurable rates. These sections will be outfitted with probes for absolute calibration of fluid flux. In the spring, summer, and fall of 2019

field trials will be conducted in Apple, Pear, Cherry, and Stone Fruit trees within the Willamette valley at the North Willamette Research and Extension Center (NWREC). During this period communication between the probe and the NWREC base station will be evaluated and de-bugged.

**Results and Discussion**

Tests conducted in August and September of 2018 were useful to our team for understanding the benefits and limitations to the current design. Figure 3 demonstrates estimated sap flux in a Cherry tree located on the Oregon State University campus. This test was allowed our team to evaluate the micro-controller, data storage and communication protocols, as well as our overall hardware. Sap flux was estimated based on calibration factors from commercially available Dynamax probes.

Figure 3 show the results of our PCB based sap flow probes along with a commercially available product (Dynamax TD30). Overall, good agreement was shown between our design and the Dynamax probes, however some discrepancies are also clear. The Dynamax probes were configured to measure the temperature difference ($\Delta T$) at much higher frequencies then our probes were programed to do. This resulted in our estimated temperature differences being much noisier than the Dynamax sensors when observations were averaged over 30-minute time blocks. Furthermore, the temperature sensors on the summer 2018 design demonstrated non-trivial drift and spiking behavior.

Because of the deficiencies identified in our previously selected temperature sensor, the Winter 2019 period will be used to identify a stable, robust, and economical replacement. Many viable options are available for purchase, and six selected models will be tested across a range of conditions. It is not necessarily true that the calibration coefficients published in literature are applicable to our newly developed probe package. For this reason, a number of tests will be conducted in the lab to estimate the coefficients required to translate a temperature difference into a sap velocity. 1-m long sections of different trees (Cherry, Apple, and Pear) will be obtained for these tests. Liquid will be forced though

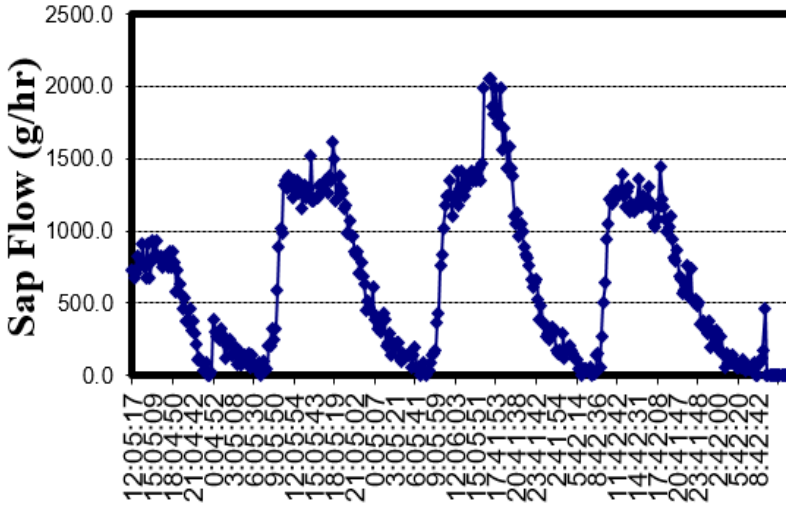


*Figure 3: Cherry Tree Sap Flow Test Result, August 2018*

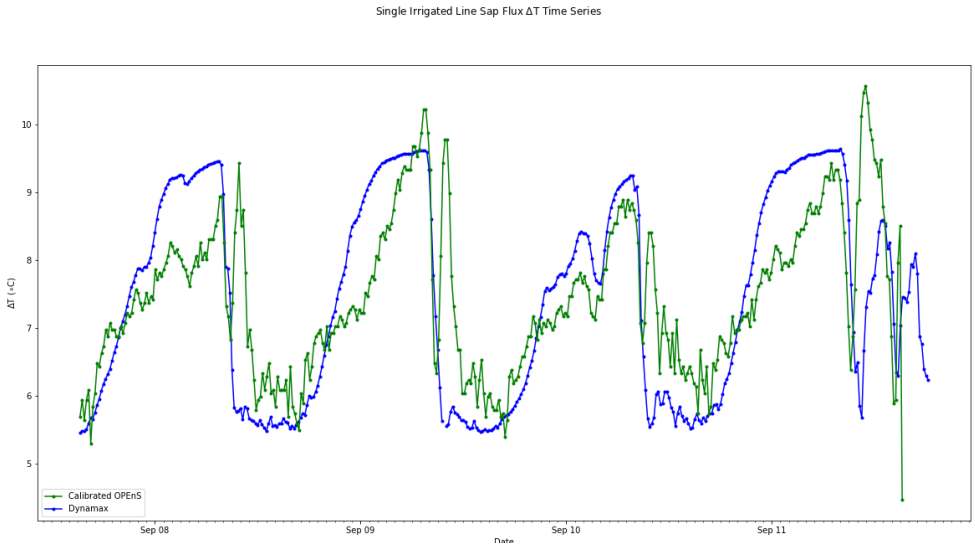


*Figure 4: Results of September 2018 field tests*

the trunks under pressure while our sap flux probes will be installed in the trunks. This will allow for absolute calibration of sap velocity estimates. By accurately measuring sap flux velocity and sapwood area we can then directly calculate the total water use per tree.

It is expected that in the upcoming months we will establish a viable, cost-effective, and easily constructed sap flow sensor. We expect that sap flux probes will provide a much better representation of actual orchard-level ET compared to ET estimates calculated from climatological data at remote weather stations. Data from the probes will improve irrigation programs and will lead to greater orchard productivity and may also promote water conservation.

We expect to publish the documentation of the build and programing details in an open access journal such as *Hardware X* (https://www.journals.elsevier.com/hardwarex). This will allow others to take our developed designs and either use time directly or further develop these sensors for their own use. This publication is expected to be completed in Spring and Summer of 2019. We also intend to publish in the Summer and Winter of 2019/2020 a comparison of our probes with a commercially available version such as the Dynamax probes. While we do not expect to beat these probes in total performance, we hope to provide reasonable estimates along with a variety of Internet-enabled communications capabilities at a fraction of the price. Finally, in Winter and Spring of 2020 we hope to summarize these two technical documents in a more approachable extension publication that provides information and best practices to the construction, installation, and operation of our sap flow probes.