2019 Technology Research Review
January 31 1, 2019
Wanapum Dam Visitor's Center, Vantage

CONTINUING PROJECT REPORT **YEAR**: 2 of 2 (NCE)
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
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

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

Total Project Request: Year 1: $32,754 **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:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 **Email:** arcgrants@wsu.edu/joni.cartwright@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.

OBJECTIVES

1. Identify how correlations between x-ray and lab analysis differ among apple and pear varieties with known differences in skin thickness.

2. Develop cultivar-specific and skin-thickness specific calibrations for non-destructive analysis of calcium and potassium in apple and pear.

3. Incorporate quantitative calibrations into the Bruker software for industry-friendly instrument use.

This project has the goal of looking at how surface measurements using a portable x-ray fluorometer relates with traditional lab analysis. In the previously funded project, the focus was to validate that the instrument measurements agree with traditional lab analysis. In the current project, we are seeking to develop calibrations that can be inserted into the commercially available unit for measurements of fruitlet, fruit at harvest or fruit in storage. However, there is evidence that each cultivar might behave differently with the instrument.

		Calibration Developed
Apple - June	**Honeycrisp**	**X**
Apple	**Honeycrisp**	**X**
	Pink Lady	**X**
	Fuji	**X**
	Gala	
Pear	**Anjou**	**X**
	Bartlett	**X**
	Starkrimson	**X**
Cherry	**Sweet Cherry**	**X**

SIGNIFICANT FINDINGS

- 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.

- Other research groups are working on using XRF for non-destructive analysis of leaf tissue. This is a general trend for using this technology for making these types of measurements.

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

- 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.
- A calibration for fruitlets and mature fruits was developed for Honeycrisp because of differences in flesh density and nutrient concentrations. This calibration for June fruitlet measurements could likely be used for other cultivars but will be tested out in 2018.
- The PXRF can be used for apples (fruitlets and harvested fruit), pears, and cherries with individual calibrations developed for each cultivar. See below for slopes of lines. **Calibration information is available upon request.**

METHODS

We measured three skin thickness of three apple cultivars (figure 1): Honeycrisp, Fuji, and Pink Lady to assess whether skin thickness impacted the PXRF measurements. Skin thickness averaged only 50 μM compared to 90 and 100 μM for Pink Lady and Fuji apples, respectively. We sampled for skin thickness from the exact same location as the PXRF measurements so that a direct relationship between the two measurements could be assessed. In 2017, calibrations were developed for three apple and three pear cultivars. There was also an additional calibration developed for June fruitlets and sweet cherry. Shortly, these calibrations will be input into the calibration instrument in the Kalcsits lab and will be available for demonstration by April 2018.

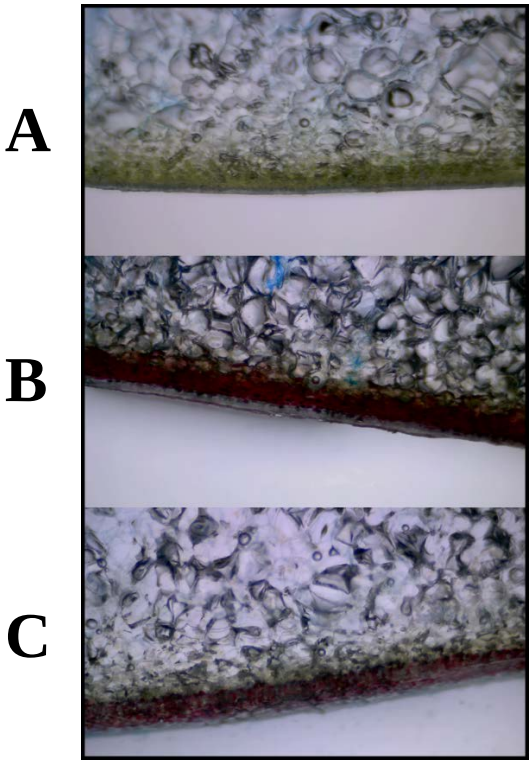


Figure 1. Images of peel thickness in Honeycrisp (A), Fuji (B), and Pink Lady apples harvested at maturity in 2016.

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

Objective	Activity	Completed or Anticipated Completion Date
1	Looked at how peel and flesh differ in nutrient concentrations in Honeycrisp	Completed 2016
1	Looked at how the relationship between lab analysis and PXRF differs between fruitlets and fruit at harvest	Completed 2016
1	Analyzed groups of apples, pears and cherries using PXRF and then lab analysis	Completed 2016
2	Calibration sampling for Anjou pear	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	January 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	February 2018

RESULTS & DISCUSSION

Skin thickness averaged only 50 μM for Honeycrisp apples compared to 90 and 100 μM for Pink Lady and Fuji apples, respectively. There was no significant relationship between skin thickness and calcium concentrations between cultivars. However, within cultivars there was a weak relationship between skin thickness and calcium concentrations. This implies that skin thickness can impact PXRF measurements but not to the degree as we hypothesized in the original proposal. The calibrations that were developed were from multiple fields and plots and were representative. In general, the y-intercept for calcium calibrations approached 0 whereas the intercept for potassium ranged from 0.2 to 1, depending on the sample being analyzed. The intercept of the best-fit line strayed from 0 for potassium because of the possible interference between the rhodium photon peak (the x-ray tube is made of rhodium). The slopes of lines for the calibrations varied by cultivar and highlighted the need for cultivar-specific calibrations. Correlation coefficients were always significant between PXRF measurements and destructive lab analysis and correlations improved when only the

peel and a small amount of flesh was used for the destructive sample. Following recommendations from Cornell and Penn State, we have started doing peel-only nutrient analysis as a better indicator of calcium balance in the fruit and better sensitivity to differences in nutrient concentrations between samples.

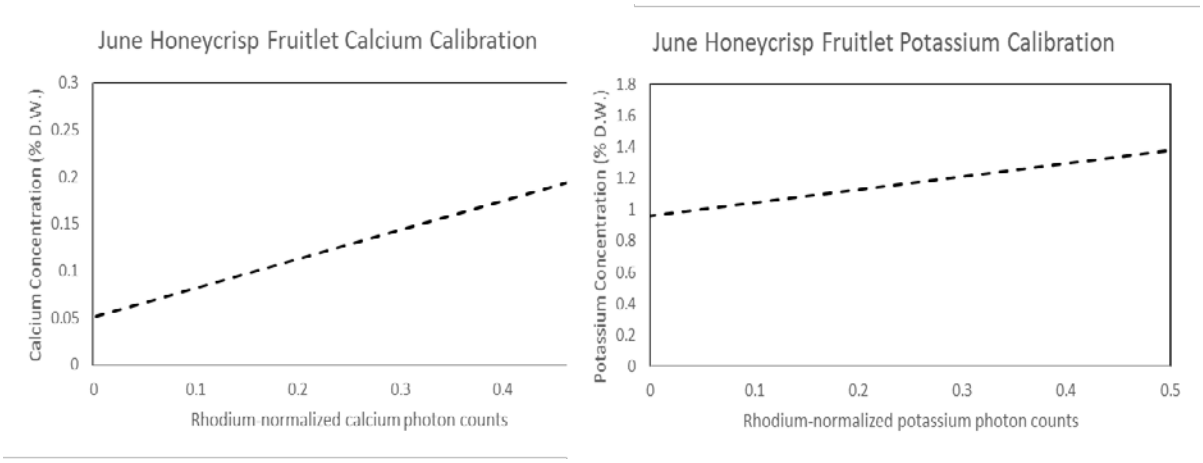


Figure 2. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruitlets analyzed in June. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w.

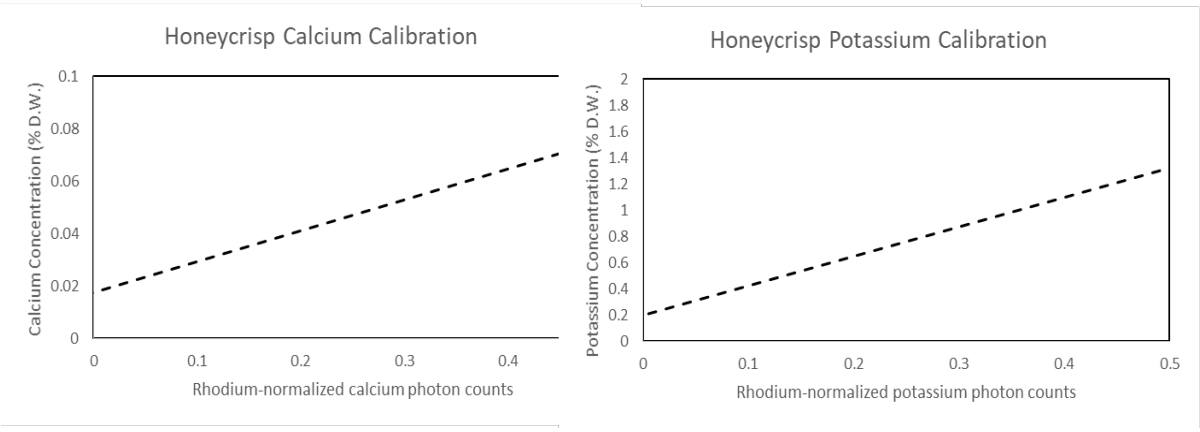


Figure 3. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

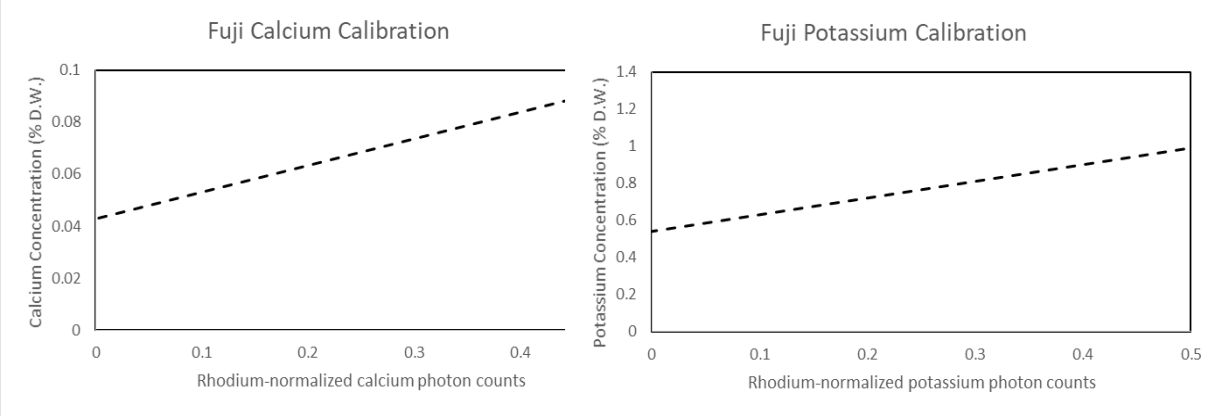


Figure 4. Linear regression for calcium (left) and potassium (right) in Fuji fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

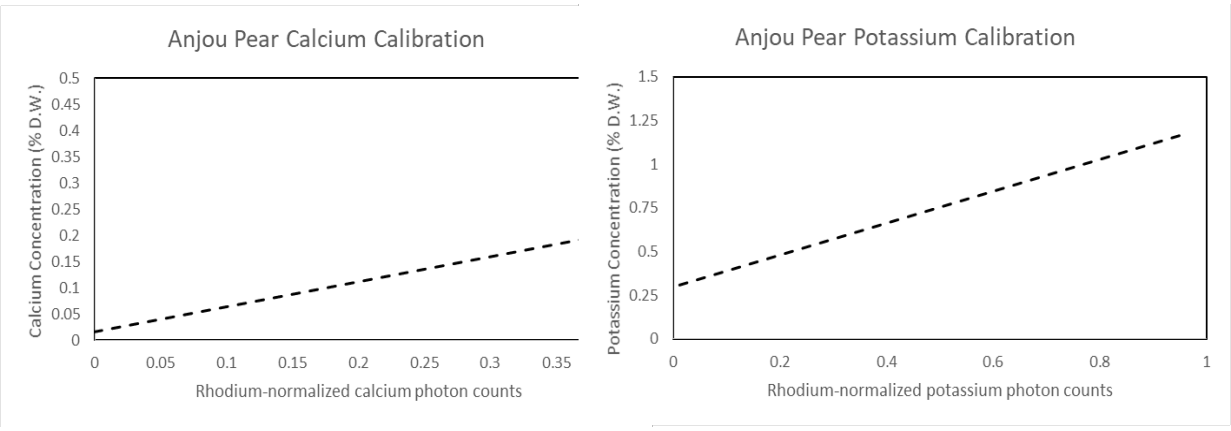


Figure 5. Linear regression for calcium (left) and potassium (right) in Anjou pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

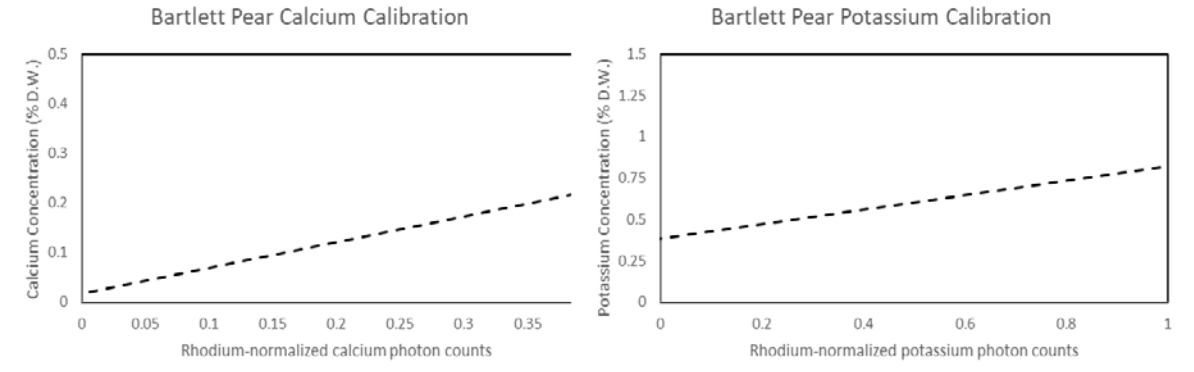


Figure 6. Linear regression for calcium (left) and potassium (right) in Bartlett pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

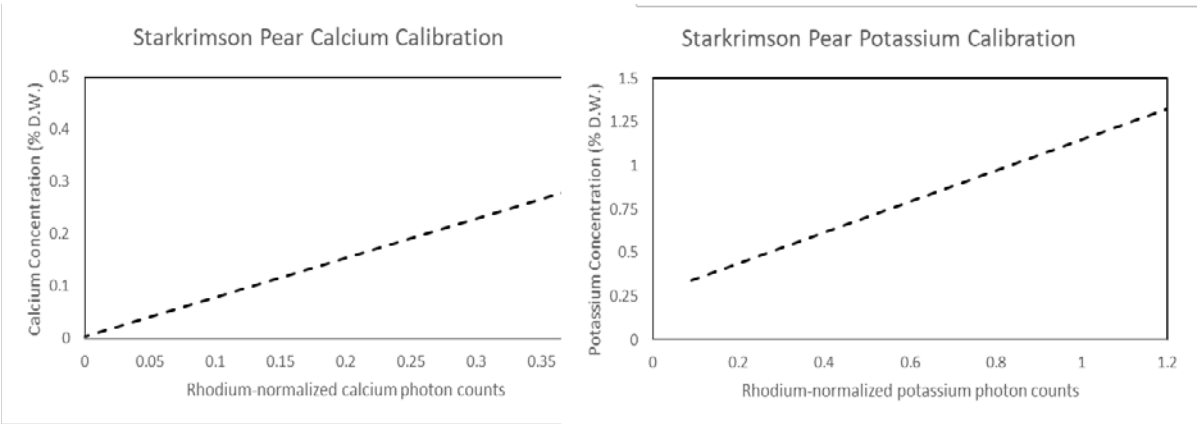


Figure 7. Linear regression for calcium (left) and potassium (right) in Starkrimson pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

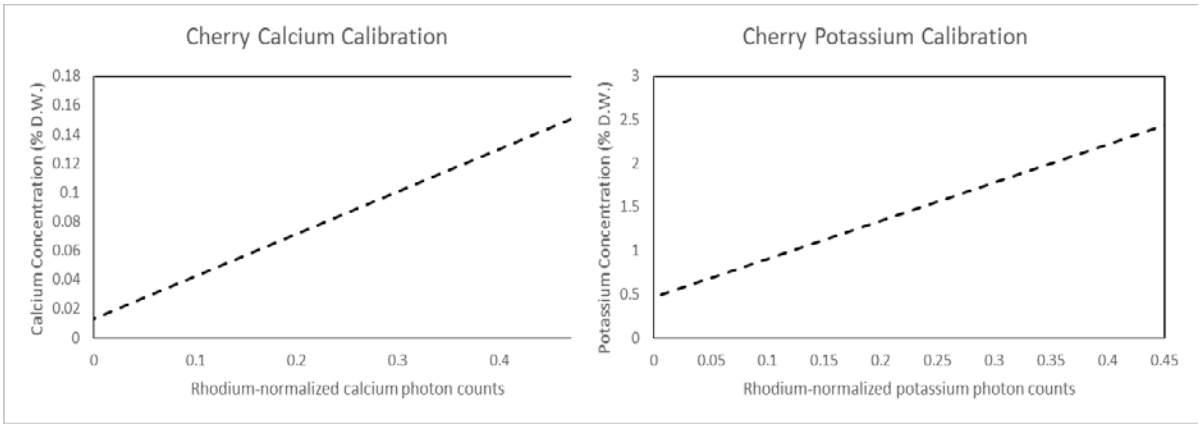


Figure 8. Linear regression for calcium (left) and potassium (right) in sweet cherry fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

Overall, the goal is to make this instrument more user friendly to the industry to provide accurate non-destructive information on the nutrient status of fruit. This will provide a more rapid decision platform than traditional lab analysis and has implications in horticultural and storage decisions. We are almost ready to input the calibrations into the PXRF instrument in the lab for testing in 2018.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: TR-17-101

Project Title: Tree stress monitoring with photochemical reflectance index sensors

PI: David J. Brown
Organization: Washington State University
Telephone: 509-335-1859
Email: dave.brown@wsu.edu
Address: Johnson Hall 405
City/State/Zip: Pullman, WA 99164-6420

Co-PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-663.8181 ext 229
Email: lee.kalscits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Jan Eitel, University of Idaho

Budget: **Year 1**: $42,552 **Year 2**: **$43,554**

Percentage time per crop: Apple: 66% Pear: 0% Cherry: 34% Stone Fruit: 0%

Other funding sources

Agency Name: USDA-SCRI
Amt. requested/awarded: $6.7 million (requested in 2018 SRS preproposal)
Notes: Dr. Brown is leading the effort, with the collaboration of Dr. Kalcsits and other co-PDs, to secure a large, national project focused on Precision Orchard Management for Apples with many different objectives and activities, including more basic research on photochemical reflectance index (PRI) sensors, looking at a wider range of factors influencing PRI response. Should we receive funding for both the SCRI project and this WSTFRC technology project, we would expand experiments.

Agency Name: WTFRC Apple Review
Amt. requested/awarded: $210,000
Notes: This is a complimentary project with different applied goals. This proposed project has horticultural and physiological objectives while the proposed WTFRC technology proposal is leveraging this planned experiment for validation of the PRI sensor.

WTFRC Collaborative expenses: None

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

Item	2017	**2018**
Salaries[1]	$18,200	$18,928
Benefits[2]	$6,852	$7,126
Supplies[3]	$14,000	$14,000
Travel[4]	$3,500	$3,500
Total	$42,552	**$43,554**

Footnotes:
[1] Salaries include two months of Postdoc time at $4,583.33/month in the Brown lab and three months of Research Assistant time at $3000/month in the Kalcsits lab.
[2] Benefit rates for the postdoc and research assistant are budgeted for 33.4% and 44.1%, respectively
[3] Supplies include sensor costs for purchase in year 1 and year 2 of the project, soil analysis costs and lab consumables and equipment maintenance
[4] Travel budgeted for travel to field sites, meetings with collaborators and presentation of results at industry winter meetings in Washington State

OBJECTIVES

1) Calibrate PRI canopy sensors to monitor soil moisture tension for high value apple and cherry varieties, taking into account potential complicating factors such as seasonality, meteorological conditions, soil texture, and nutrient status.
2) Deliver an online app and protocols that growers can use to monitor soil moisture and tree stress with PRI sensors in their orchards.
3) Develop protocols for determining the optimum number and placement of PRI sensors based the measured spatial variability of PRI sensor measurements and soil moisture tension in apple and cherry blocks.

While Year 2 of the project will be implemented largely as planned, we will make the following adjustments.

- Photochemical reflectance index (PRI) sensor noise

Project objectives have not changed, but substantial challenges were encountered in Year 1 of this project. In particular, PRI sensor data contained substantial noise when used to monitor relatively low-density apple canopies with larger leaves (relative to wheat). As PRI sensors are very sensitive to leaf angle and the fraction of non-photosynthetic surface exposed, even a slight breeze can substantially alter the signal. As a result, we were not able to significantly detect tree stress using PRI sensors in the 2017 drought experiment.

To address these problems, we plan to increase sensor data collection from every 30 minutes to every minute. This will allow us to average a large number (hundreds) of measurements both for the early morning "black reference" reading and the mid-day reading with the lowest PRI value. We can also develop algorithms to screen this data for outliers.

Secondly, we intend to place the sensors closer to the canopy to ensure the full field of view is focused on the canopy, and not potentially alley cover.

Thirdly, we will stockpile extra SRS sensors so that failed sensors can be rapidly replaced if necessary. We seemed to have purchased a poor batch of sensors that experienced substantial failure. Replacement sensors performed reliably, so we are hopeful of better 2018 results.

- Soil Water Potential (ψ) sensors

The METER MPS-6 soil water potential sensors (now marketed as Teros 21 sensors) proved both reliable and more responsive to irrigation deficits than volumetric water sensors. With funds from other sources, we will purchase and install additional sensors to address the spatial variability of water potential to augment activities and measurements addressing Objective 3. It may be that ceramic-based soil water potential sensors prove better for irrigation management than PRI sensors.

SIGNIFICANT FINDINGS

- There was too much noise in photochemical reflectance index (PRI) measurements recorded at 30-minute intervals to detect 'Honeycrisp' tree stress.
- Irrigation treatments have an effect on the leaf-level photosynthesis of apple trees, as indicated by stomatal conductance measurements.

METHODS

Experimental site and tree management

An experiment was set up at the WSU Sunrise Research Orchard using 240 Honeycrisp trees on M9-T337 that were planted in 2015 at a spacing of 3' x 12' (1210 trees/acre). The soil is an alluvial shallow sandy loam soil. The trees filled their canopy space in 2015 and 2016. The first year crop was in 2017. Using a randomized complete block design (See Figure 3), irrigation regimes were used that will withhold irrigation either early, middle or late in the season and compare it to a fully watered control.

Experimental design and irrigation treatments

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

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

Physiological Measurements

At end of each deficit irrigation period, physiological measurements were made including mid-day stem water potential and stomatal conductance. Plant water status, measured as Ψ_{md} was assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} were bagged for at least one hour in silver reflective bags to equalize the leaf and xylem water potential before readings were taken. Ψ_{md} was measured around solar noon. Stomatal conductance (mmol m^{-2} s^{-1}) was measured on mature, sun-exposed leaves on the upper half of the canopy using a decagon (Meter Inc, Pullman, WA) handheld porometer.

Sensor measurements

At one location within each treatment, METER MPS-6 (ψ in kPA and temperature) and ECH2O 5TE (volumetric water content, electrical conductivity, and temperature) were installed at a depth of 20 cm (within root zone). Monitoring locations were further equipped with PRI and NDVI Spectral Reflectance Sensors (SRS; METER, WA) to provide continuous spectral measurements. Downwelling irradiance was also be continuously measured using upward-pointing hemispherical PRI and NDVI METER SRS instruments. Measurements were recorded at 30-minute intervals. PRI and NDVI were computed using a band values of reflected over incident light. ΔPRI was computed as mid-day PRI (average of four measurements) minus early morning PRI (average of three measurements following sunrise.) Four NDVI mid-day measurements were averaged to compute daily values. Daily ψ and VWC measurements were computed by averaging measurements recorded at 30-min intervals over 24 hours.

RESULTS & DISCUSSION

Sensor monitoring

Processed sensor data are reported in Figures 1-4 below. Soil water potential (ψ) and volumetric water content (VWC) measurements were highly correlated. However, ψ measurements proved more responsive to water stress conditions than volumetric water content (VWC) measurements. Measuring ψ also has the advantage of directly providing information on water-related plant stress, whereas VWC has to be converted to ψ using pedotransfer functions. The ΔPRI measurements, even with multiple readings average, proved too noisy for meaningful interpretation. Normalized difference vegetation index (NDVI) values changed little over the course of the season for all but the early treatment. In Year 2, we intend to more closely examine the relationship between measures of ψ and tree stress.

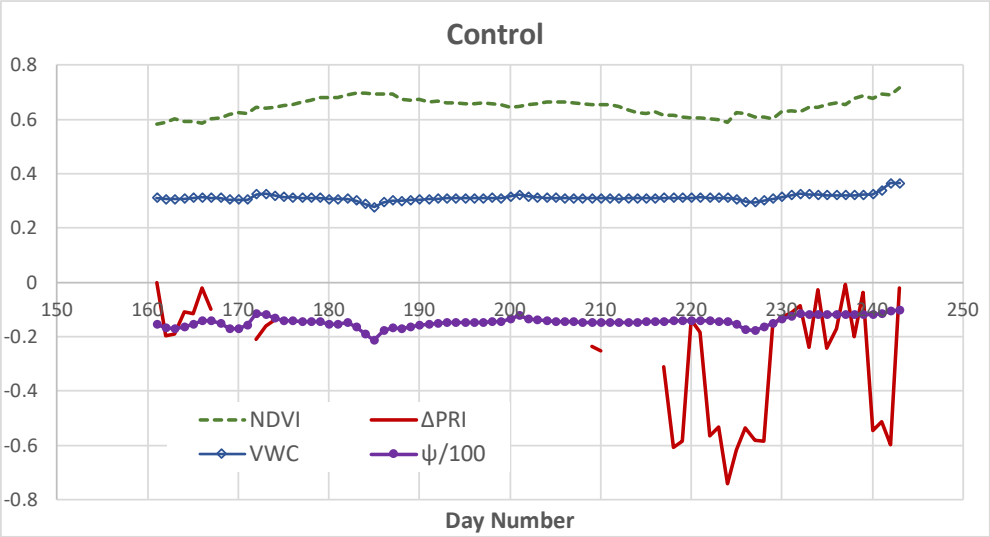


Figure 1. Monitoring sensor data for 'Honeycrisp' apple experiment with no water limitations (control). Data plotted include mid-day canopy normalized difference vegetation index (NDVI), volumetric water content (VWC, m^3-H_2O m^{-3}-soil), delta photochemical reflectance index (ΔPRI), and soil water potential (ψ, kPa).

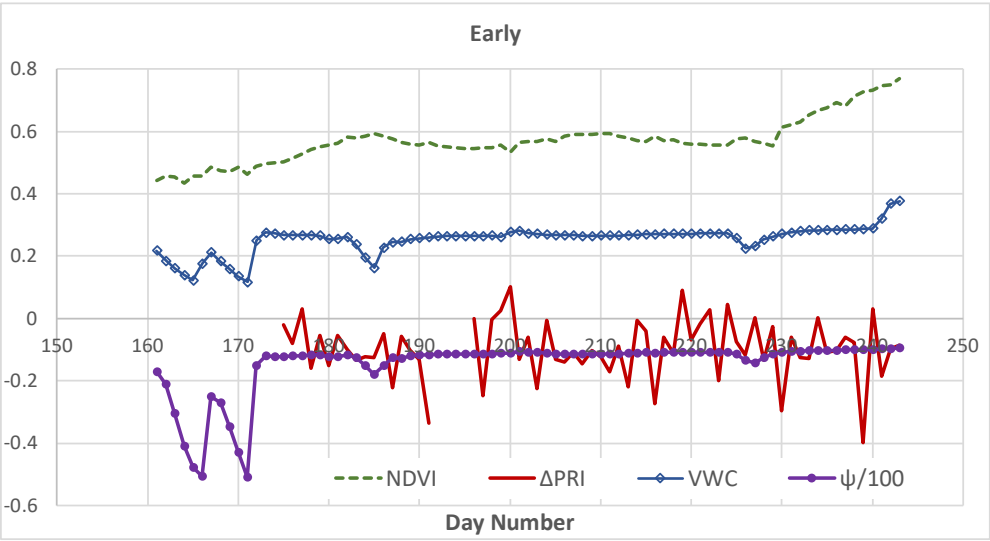


Figure 2. Monitoring sensor data for 'Honeycrisp' apple experiment with middle water deficit period (30 days ending day 171, 2017). Data plotted include mid-day canopy normalized difference vegetation index (NDVI), volumetric water content (VWC, m^3-H_2O m^{-3}-soil), delta photochemical reflectance index (ΔPRI), and soil water potential (ψ, kPa).

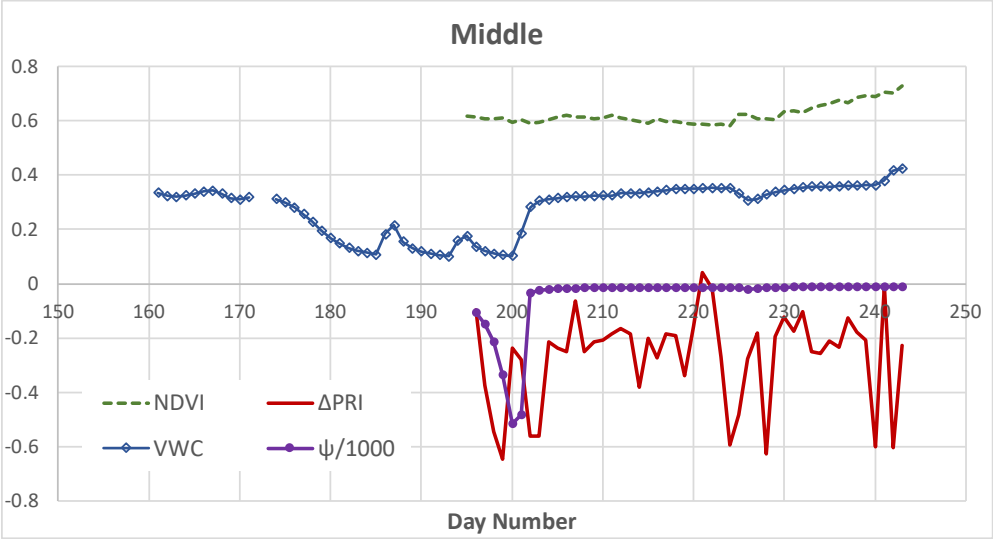


Figure 3. Monitoring sensor data for 'Honeycrisp' apple experiment with middle water deficit period (30 days ending day 200, 2017). Data plotted include mid-day canopy normalized difference vegetation index (NDVI), volumetric water content (VWC, m^3-H_2O m^{-3}-soil), delta photochemical reflectance index (ΔPRI), and soil water potential (ψ, kPa).

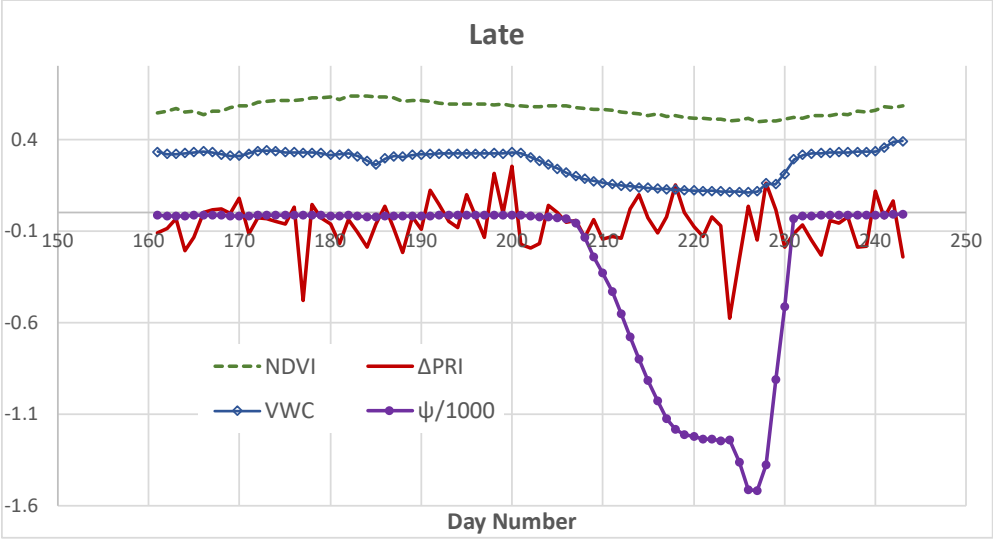


Figure 4. Monitoring sensor data for 'Honeycrisp' apple experiment with late water deficit period (29 days ending on day 229, 2017). Data plotted include mid-day canopy normalized difference vegetation index (NDVI), volumetric water content (VWC, m^3-H_2O m^{-3}-soil), delta photochemical reflectance index (ΔPRI), and soil water potential (ψ, kPa).

The results of this experiment cannot support the use of canopy spectral reflectance sensors to monitor tree stress for irrigation management. However, further research is needed on the ability to reduce ΔPRI noise by collecting and processing temporally dense PRI measurements.

Physiology

At the end of each deficit irrigation period, midday leaf water potential was greater in the deficit irrigation treatment compared to the well-watered control (Figure 5). The midday stem water potential increased, even in the control as the season progressed due to increased vapor pressure deficit as air and soil temperatures increased and the relative humidity decreased. For the middle season deficit treatment, midday leaf water potential didn't fully recover and returning to well-watered conditions and was still significantly greater than the well-watered control on August 17th. These changes in midday leaf water potential stimulated changes in stomatal conductance (Figure 6) which are closely linked to plant photosynthesis and water-use. Stomatal conductance was significantly lower at the end of each deficit irrigation treatment compared to the well-watered control. This demonstrates that the irrigation treatments are having an effect on the leaf-level photosynthesis of the trees.

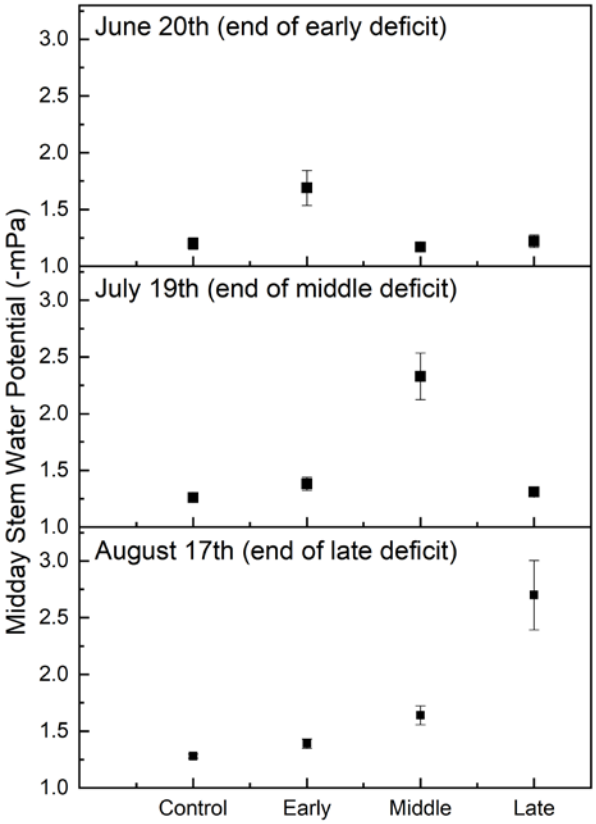


Figure 5. Midday leaf water potential (-mPa) on June 20th, July 19th, and August 17th for leaves on 'Honeycrisp' apples that were irrigated with no water limitations (control), an early water deficit period (30 days ending June 20, 2017), a middle water deficit period (30 days ending July 19th, 2017), or a late water deficit period (29 days ending August 17th).

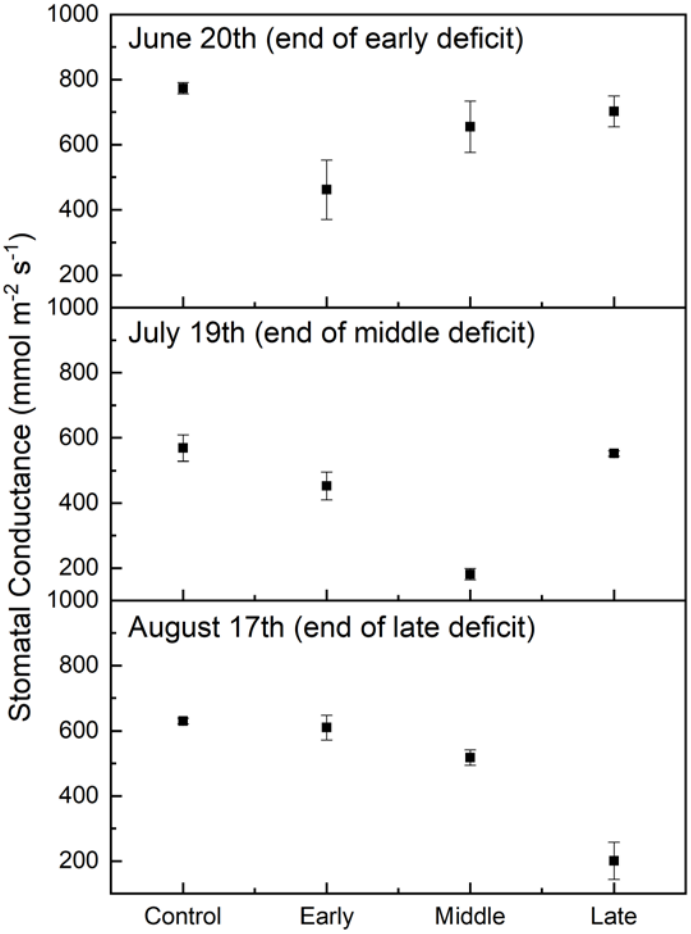


Figure 6. Leaf stomatal conductance (mmol m^{-2} s^{-1}) on June 20th, July 19th, and August 17th at 12:00 p.m. for leaves on 'Honeycrisp' apples that were irrigated with no water limitations (control), an early water deficit period (30 days ending June 20, 2017), a middle water deficit period (30 days ending July 19th, 2017), or a late water deficit period (29 days ending August 17th).

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

Project Title: Development and validation of a precision pollination model

PI: Gloria DeGrandi-Hoffman
Organization: USDA-ARS Tucson
Telephone: 520-647-9187
Email: Gloria.Hoffman@ARS.USDA.GOV
Address: Carl Hayden Bee Research Center
Address 2: 2000 East Allen Rd
City/State/Zip: Tucson, AZ 85719

Co-PI (2): Vincent P. Jones
Organization: WSU-TFREC
Telephone: 509-663-8181 x291
Email: vpjones@wsu.edu
Address: Dept. Entomology/TFREC
Address 2: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI (3): Tory Schmidt
Organization: WTFRC
Telephone: 509-665-8271 x4
Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave
City/State/Zip: Wenatchee, WA 98801

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

Total Project Request: Year 1: $95,834 Year 2: $103,359 Year 3: **$104,632**

Percentage time per crop: Apple: 80 Pear: 0 Cherry:20 Stone Fruit: 0
(Whole % only)

Other funding sources: None

WTFRC Collaborative Expenses:

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

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

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

Footnotes:

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

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

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:

- Bloom phenology data were collected for five new apple cultivars. Analysis showed that the majority of cultivars have similar bloom timing, thus providing excellent bloom overlap and available compatible pollen for cross-pollination. An exception was Cosmic Crisp that began blooming just prior to full bloom on the other cultivars. The bloom timing of Cosmic Crisp relative to other commercial cultivars should insure sufficient compatible pollen for blossoms that open prior to the full bloom period. Compatible pollen may be limited after full bloom, as other cultivars may have too few blossoms to attract foraging honey bees.
- Predictions of the daily honey bee foraging population generated from weather and bloom data accurately estimated the number of bees foraging on each apple cultivar. Additionally, predictions of honey bee foraging activity from the DAS-Honey Bee Foraging Model were highly correlated with actual foraging activity during apple bloom.
- Preliminary assessment of over-tree shade netting on honey bee foraging indicated that foraging activity was not affected by the shade cloth. However, it appears that the shade cloth may affect the bees' ability to return to their colonies.
- Hive Genie™ are electronic devices that enable us to record honey bee activity at the hive entrance. They will be used to validate the foraging model.
- Evaluation of different climate change scenarios at Wenatchee, Richland, and Wapato suggest that flowering will occur on average 21-32 days earlier than the historical average. Effects on honey bees will be examined next year.

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

Flower Phenology. This year, we collected bloom phenology data at the WSU Sunrise orchard from six cultivars (Fuji, Gala, Golden, Granny Smith, Jonagold, and Cosmic Crisp), and also analyzed data collected in 2016 from Honeycrisp and Gala (Honeycrisp were collected from two locations, Gala from one). Data were collected using the same methods

Table 1. Degree-days required for bloom of nine different apple cultivars. 2008-2013 is from previous analysis of Tree Fruit Extension Team data; 2017 and 2016 are data from this proposal.

Cultivar/year	DD for Bloom	
	mean	**stdev**
2008-2013		
Cripps Pink	505.97	61.85
Gala	548.64	57.13
Red Delicious	544.68	60.38
2017		
Fuji	544.9	70.79
Gala	540.18	68.26
Golden Delicious	540.18	68.28
Granny Smith	536.01	65.55
Jonagold	538.82	67.43
Cosmic Crisp	584.62	96.20
2016		
Honeycrisp	553.00	43.50

reported last year from work by the AWN, WTFRC and the Tree Fruit Extension team. Data collected for the last two years focused only on the bloom period and not all the different stages of the flower bud development. By evaluating previous data sets, and focusing on the bloom period (we merged the first bloom category and the full bloom category), we described bloom progression as a function of accumulated heat units for six additional cultivars, so that we now have bloom descriptions for nine cultivars. Since Gala was common to all the data sets, we are able to validate previously derived functions describing bloom progression for this cultivar.

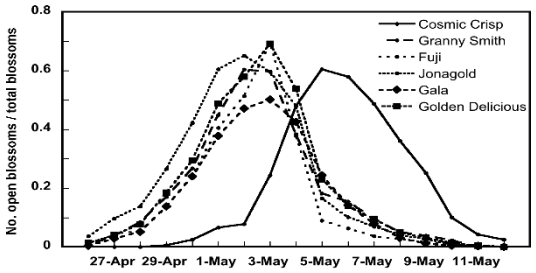


Fig. 1. Apple bloom phenology by date (2017).

*Results Flower Phenology***:** Analysis of the nine cultivars showed that bloom timing was very similar for most of the cultivars, with the mean bloom period being about 544 DD. Variance was similar among the cultivars and all fit the normal distribution (Table 1, Fig. 1). However, two cultivars, Cosmic Crisp (mean =585 DD), and Cripps Pink (mean = 506 DD) differed from the others in the timing for the start of bloom, and the length of the bloom period. Cosmic Crisp had a variability that was about a 1.3-fold higher than the other cultivars. This may be due to the age of the trees, inherent variability in the cultivar or conditions specific to that block where data were collected. Regardless, we appear to have bracketed the high and low of most of the bloom times and with additional validation data collected this coming year, should have the ability to add six new cultivars to the models on DAS in 2019.

Work next year: We will collect bloom data from additional sites, and validate existing bloom equations for the six varieties.

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

A shade house structure was erected at WSU TFREC to study the impact of shade netting on honey bee foraging. The 30’ x 14’ rectangular structure consisted of two rows of three 12’ posts sunk into the ground 2-3’deep with approx. 15’ long 2x4’ crossbars. The poles within a row were 15’ apart. The top of each cross bar was 7’ high. A strand of 0.25” airplane cable was stretched along each row of poles and stapled in place 7’ above the ground. One 15’ edge of a 16x 15’piece of white shade cloth (ChromatiNet 20% shade factor, color: Pearl Leno, Green-tek, Visalia, CA) was fastened to the middle crossbar while the opposite edge was fastened to a 15’ length of 1” PVC pipe. The shade cloth could be pulled over to cover either the northern or southern half of the structure with the end attached to the PVC pipe draped over a distal crossbar and the sides of the net draped over the airplane cable (Fig. 2). The netting was rolled up and fastened to the middle crossbar with cord when it was not deployed.


Fig. 2. Shade structure with potted sentinel flowering plants.

Sentinel potted flowering plants were placed on the ground in the middle of both the northern and southern sections. Each section had two planters with flowering plants that are attractive to honey bees including a planter of *Caryopteris x clandonesis* ‘Dark Knight’ and another with *Salvia rusa*

'Russian sage', *Echinacea* hybrid 'Sunseekers' and *Lavanda angustifolia* 'English lavender'. The plants were watered daily and fertilized weekly.

To assess whether this shade cloth structure influenced honey bee foraging on the sentinel flowering plants. We moved the shade cloth to the northern or southern half of the structure half and compared foraging activity on the plants to periods when the shade cloth was completely removed. Whether the sky was clear or overcast and how the shade net was deployed were noted during each honey bee observation.

Thirty-nine sets of honey bee foraging observations on the sentinel potted plants were made; 16 when the sky was overcast and 13 when it was sunny. There were no differences in the average number of honey bees foraging on the flowering plants during sunny periods in uncovered (mean ± SEM: 7.9 ± 0.7) or covered (8.8 ± 0.6) areas of the structure ($t_{24df} = 0.88$; $p = 0.39$). Foraging honey bee were lower on cloudy days in both uncovered (1.8 ± 0.5) or covered (1.6 ± 0.4) areas of the structure ($t_{34df} = -0.29$; $p = 0.78$). There was no difference in the average number of honey bees observed on flowers when the net was covering either the northern or southern section (north 7.5 ± 1.1; south 6.8 ± 1.5 honey bees per observation) ($t_{34df} = -0.41$; $p = 0.69$).

In addition to counting honey bee visits on flowers, other behaviors were noted. Honey bees were seen moving between the potted plants numerous times. The honey bees were obviously able to see the plants regardless of the shade cloth. Additionally, honey bees that were leaving the plants to presumably return to the hive tended to fly upwards while orienting themselves for the return flight. Honey bees that left uncovered, net-free flowers were able to fly up and depart while honey bees leaving the plants covered by nets flew up and bounced off the bottom of the net 2-3 times or more before they were able to escape the overhead net. While others have observed "disoriented" honey bees under shade netting in orchards, it is possible that the honey bees were able to visit flowers but then had a difficult time returning to their hives. Similar behavior was observed in some caged tree experiments at WSU TFREC's Sunrise orchard. In this case, small beehives were placed inside these cages to facilitate pollination of apple blossoms. While some honey bees were seen visiting the apple blossoms, others were observed bouncing off the bottom of the overhead netting, apparently trying to leave the area. Other honey bees tried to gain entrance into the cages because they were seen repeatedly trying to fly into the netting along the sides of the cages. It may be that orchard netting is more of a barrier that inhibits honey bee flight rather than serving to disorient honey bees by reflecting filtered light. A study will be conducted next year using larger areas under nets.

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

This past year, we deployed a modified version of Dr. DeGrandi-Hoffman's honey bee foraging model for beta users on DAS. The DAS-Honey Bee Foraging Model (DAS-HBFM) incorporates the effects of temperature, wind speed, rainfall, and solar radiation on honey bee foraging activity and generates a daily foraging score. A score of 100 is a perfect foraging day (i.e., cloudless, no wind or rain, and temperatures >75ºF), while a score of 0 is a day when bees did not forage (e.g., rain all day). Weather conditions for DAS-HBFM are provided by WSUAgWeatherNet, and are specific for the orchard site. The model provides the foraging score at the end of the day, and also reports scores from the previous three days. In addition, scores for the next four days also are projected based on weather predictions. The honey bee foraging model can be used to assess the foraging activity during bloom of any crop, and is a major component for predicting fruit set in apples, cherries and other deciduous fruit crops.

We compared daily foraging score predictions generated by the DAS-Honey Bee Foraging Model (DAS-HBFM) during apple bloom with actual foraging activity recorded in the field. If the foraging scores accurately reflect actual foraging activity, then the scores should be highly correlated with the foraging activity we measured in orchards. We indeed found that the DAS-HBFM scores generated during apple bloom were highly correlated with actual foraging activity (Pearson correlation of foraging score and actual foraging activity = 0.932 p-value < 0.0001) (Fig. 3).

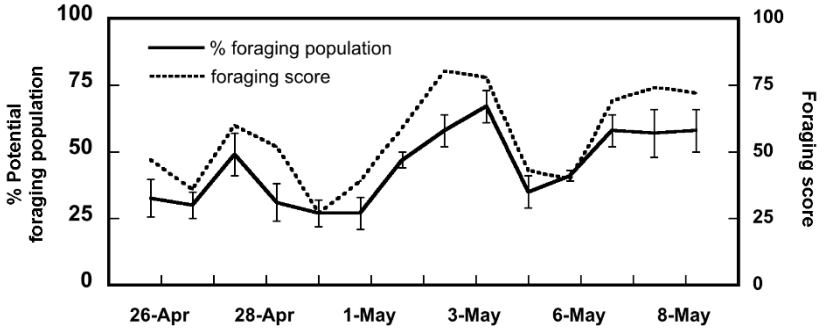


Fig. 3 Comparison of daily percentage of the potential foraging population in the orchard that are actively foraging based on actual counts of honey bees foraging on apple trees and the daily foraging score generated by the DAS-Honey Bee Foraging Model.

In addition to predicting foraging bees leaving hives, we derived equations to predict the proportion that will forage on apple trees. These predictions are based on the number of open blossoms on trees of each cultivar. We validated foraging predictions by counting bees foraging on six apple cultivars. We found that predictions of honey bees foraging on Jonagold, Granny Smith, Gala, Golden Delicious and Fuji trees based on weather and bloom condition were within the 95% confidence interval for each day during bloom except May 1 (Fig. 4). Similarly, predictions of foraging activity on Cosmic Crisp were accurate except for May 6 where actual foraging activity was significantly lower than predicted.

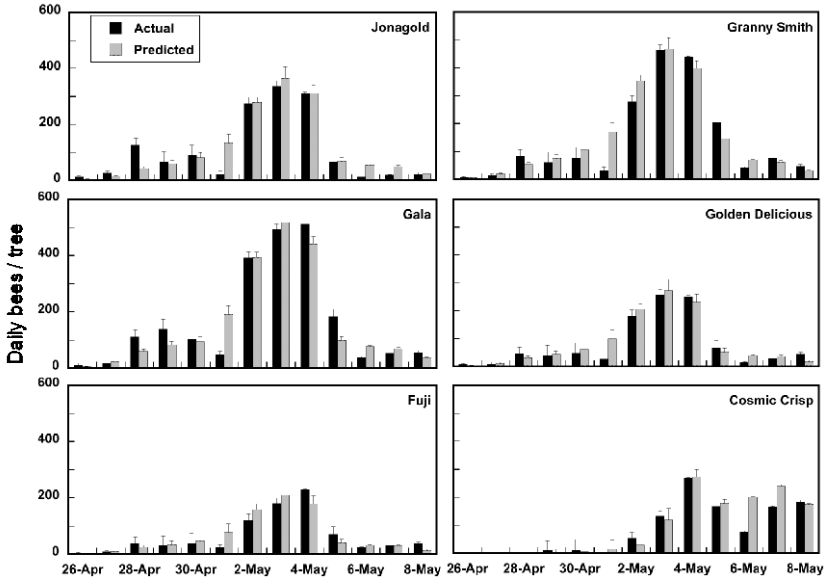


Fig. 4. Actual and predicted numbers honey bees foraging on apple cultivars. Predicted values are based on daily weather conditions and the number of open blossoms on trees of each cultivar.

Evaluation of Electronic Hive Monitors.

Observing honey bee activity at the hive entrance allows us to update and validate impacts of weather on honey bee activity including foraging. This is typically done by observers counting honeybees leaving the hive. Another way to collect these data is to set up video cameras near the entrance of bee hives to record activity. Observations of activity are then related to key weather parameters (temperature, rainfall, wind speed and solar radiation) that impact foraging. These two methods are time consuming, and it can be extremely difficult to count bees leaving the hive during optimal foraging conditions when activity is high.

To help us overcome time constraints involved with counting honey bees, we purchased three Hive Genie™ units (Montgomery, TX) that were marketed as devices that can count honeybees leaving and entering the hive (Fig. 5). Briefly, these are solar-powered electronic devices that have eight openings with sensors that record the number and direction of honey bees as they pass through gates. Data is then transmitted to a wireless network and transmitted over the internet to a site that tabulates and arranges the data. Two of the units worked periodically during the summer-fall assessment period while the third unit failed and was returned. Preliminary trials this summer showed we can used these devices to monitor honey bee activity in relation to key environmental parameters (Fig. 6).

Fig. 5. Hive Genie's monitoring honeybee activity at hive entrance.

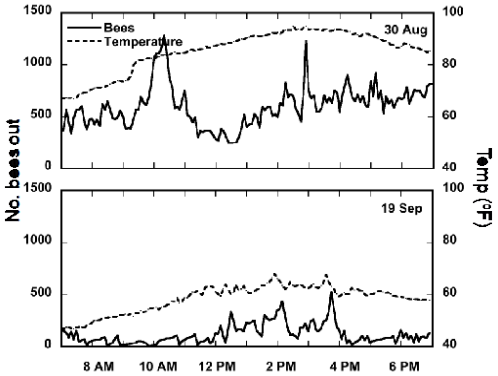


Fig. 6. Monitoring honey bee foraging behavior at hive entrance with Hive Genie™. Cool temperatures reduce the number of bees leaving the hive.

Work this coming year. We will install the Hive Genie™ units on hives located near apple and cherry sites during the 2018 pollination season to obtain data on foraging activity. The objective is to correlate honey bee activity at the entrances of hives to foraging scores generated from the DAS-HBFM during apple and cherry bloom.

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

We obtained climate change data from 10 different climate models using two different scenarios: RCP4.5 (up to 650 ppm CO_2 with stabilization after 2100) and RCP8.5 (1380 ppm CO_2 rising still at 2100). These are two of four possible Representative Concentration Pathways (RCP) that are used by each of the 10 different climate models and the median of the 10 different model outputs is used to give the "best" representation of what is expected over time. In reality, there is a "cloud" of data points around each line that reflects the differences from year to year and between the different climate change models. However, plotting that variability obscures the overall trends that we are trying to show.

We obtained the data from the University of Idaho's applied Climatology lab for three different locations: Wenatchee, Wapato, and Richland. The data cover the period from 1950-2005 (historic data checking the accuracy of the climate models), and 2005-2099 and include the two climate change scenarios discussed above. The data were down sampled to provide daily maximum and minimum data over the entire dataset.

We started with evaluating how the bloom period will change during the periods (1979-2005), 2040 (2025-2055), 2060 (2045-2075), and 2080 (2065-2095). We present the average behavior of the flowering over each period and focused on two cultivars, Fuji and Cosmic Crisp. Fuji tends to occur at the average time for a broad range of cultivars (see objective 1), while Cosmic Crisp bloom is the latest cultivar we found in objective 1.

The data for the Wenatchee site using the RCP4.5 scenario showed the largest displacement between the historical and 2040's data, and the change in latter continued to occur, but with very little

difference between 2060 and the 2080 projection. This is because the RCP4.5 scenario is based on the idea that the CO_2 concentrations will stabilize at a higher level. Even with this mild scenario, the offsets between time ranges are significant with 10% flowering shifting about 21 days between the historical and 2080's projections, so the new "normal" will be flowering around April first (Fig. 7A, B).

The RCP8.5 scenario shows roughly equal spacing between the different time periods; this is because the CO_2 concentrations are not reaching equilibrium, but instead increasing over time. In this scenario, the changes for 10% flowering occur about 30 days earlier by the 2080's time period; the average change from 2040's to 2060's to 2080's is about 8 days per time period (Fig. 7C, D).

Evaluation of the other 2 sites shows the similar trends as seen at the Wenatchee site, with the same 8-day shift per time period for the RCP8.5 and virtually no difference between the 2060's and 2080's projections in RCP4.5. In the case of Richland, 10% flowering will occur about 1 month earlier than the historical data using the RCP8.5 scenario.

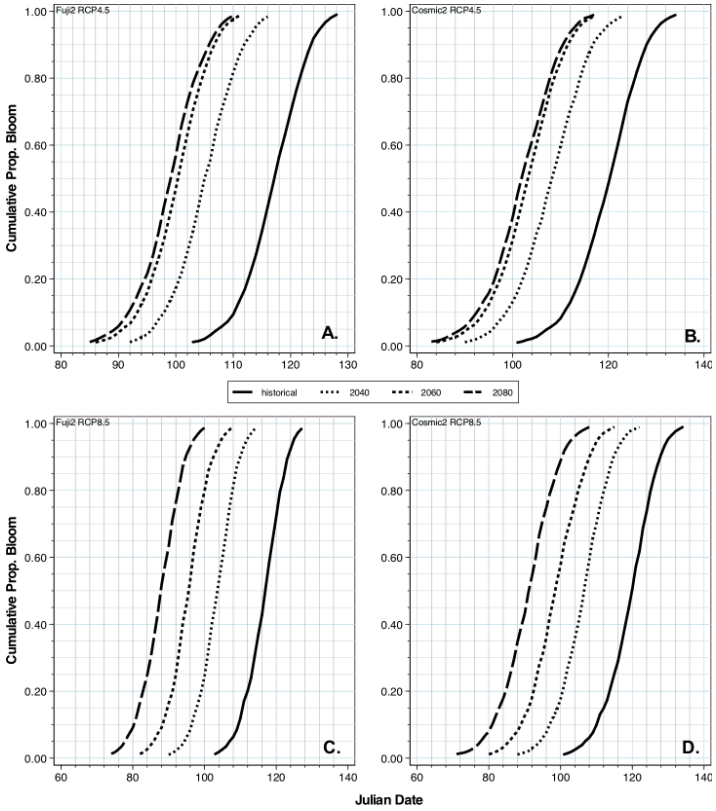


Fig. 7. Shift in flowering time for Fuji and Cosmic Crisp apples using different climate scenarios. A. Fuji with RCP4.5 (stabilized CO2 at lower level by 2100). B. Cosmic Crisp at RCP4.5. C. Fuji at RCP8.5 (high CO2 without equilibrium by 2100). D. Cosmi.

Although we have completed the evaluation of flowering, we have not yet finished the potential effects on honey bee flight. The trees have a much lower threshold for development (≈40°F) than the lower threshold for honey bee flight (≈50°F), so the shift of flowering to earlier in the year do not directly address the question of whether the honey bees will be as efficient as pollinators at different periods in the future; that will come as we work on the honey bee flight portion of the projections.

Work next year: The key part will be to examine how the shorter day length and temperature changes early in the year affect honey bee foraging rates. The foraging rates depend on rainfall, wind speed, solar radiation, and temperature. The solar radiation is easily estimated over the day for each day of the year, and we can get the temperature effects from the climate projections. The climate projections for wind speed and rainfall are not likely to be very useful, but by concentrating on the solar radiation and especially temperature effects, we can estimate how foraging will be affected during the key period when bloom

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

WTFRC Project Number: TR-17-100

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

PI: Loren Honaas
Organization: USDA, ARS
Telephone: (509)664-2280
Email: loren.honaas@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Co-PI: Joshua Der
Organization: Cal State University, Fullerton
Telephone: (657)278-4115
Email: jder@fullerton.edu
Address: 800 North State College Blvd.
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**

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
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**

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
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**

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 2 goals:
Early in year 2 we will continue to assess genetic differences between cultivars of interest, based partly existing analyses, but also on new 3rd generation (PacBio) sequencing (step 1). Once we have a confident assessment of genetic differences, we will shift efforts to strategy development (step 2) so we can leverage this information to attempt to improve digital gene expression measurements. The 3rd step will be to examine our improved method by comparing to the baseline measurements completed in year 1.

Significant Findings:
- Cultivar-specific gene *coding sequences* are polymorphic
- Polymorphisms contribute to altered gene activity measurements in targeted analysis
- A majority of 35,770 detected Granny Smith transcripts are polymorphic
- Custom, cultivar-specific read mapping reference brought in more data

Methods:
We selected 29 candidate genes (15 from Granny Smith (GS) and 14 from Honey Crisp (HC)) to explore genetic differences between cultivars of interest and the reference cultivar, Golden Delicious. After creating cultivar specific gene predictions from raw gene activity data, we searched for matches to the Golden Delicious candidate genes. We took the matching genes and analyzed them on a case-by-case basis to examine genetic differences. We designed validation experiments for the cultivar specific predictions and developed gene activity assays for each candidate. Gene activity measurements for each candidate were correlated to digital estimates (RNA-Seq) using version 1 of the Golden Delicious genome. We then improved the correlation analysis by reciprocal searches between cultivar predictions and known Golden Delicious genes.

Results and Discussion:
Granny Smith and Honey Crisp gene coding sequences are polymorphic compared to Golden Delicious genes We confirmed the gene prediction for each candidate by subjecting them to custom gene tests. This test found that the predictions were by-and-large highly accurate, producing expected polymerase chain reaction (PCR) results (Figure 1). After validation, the next step was a case-by-case analysis of cultivar-specific genes. This showed >70% of the candidate genes were polymorphic (Table 1). The polymorphisms included Single Nucleotide Polymorphisms (SNPs), insertions, and deletions. Of those with polymorphisms, >70% had altered protein-coding sequences, presenting the possibility of altered biological activity. We used the cultivar specific gene sequences to develop gene activity assays (via quantitative real time Polymerase Chain Reaction - qPCR) with >95% success, while adhering to strict community guidelines (high efficiency, high specificity, rigorous controls).

Polymorphisms cause reduced or altered gene activity measurements With gene-specific assays in hand, we tested a number of biological samples that are part of ongoing experiments to examine gene activity in the postharvest period in Granny Smith and Honey Crisp apple. We used the transcriptome data from the parallel work to estimate gene activity for our candidate genes. A majority of Granny Smith candidates showed good agreement (mean R^2=0.80), yet most of the Honey Crisp candidates showed poorer correlations (mean R^2=0.37). This is possibly due to the gene selection method. Granny Smith candidates were chosen from a transcriptome analysis, so they were essentially pre-validated for success in a digital gene activity analysis. Honey Crisp candidates were chosen from published studies that lacked digital gene activity measurements. We hypothesize polymorphisms and annotation errors are the basis for the disagreement.

We were able to improve the correlation between the two gene activity measurements by searching for better matches to our candidate genes (Table 1). This improvement illustrates a key point – Golden Delicious reference genes that are genetically closer to other cultivar specific genes provide a more accurate gene activity measurement. This preliminary result supports the hypothesis that correcting polymorphisms in reference Golden Delicious gene sequences will improve RNA-Seq based gene activity measurements across cultivars.

A majority of detected Granny Smith transcripts are polymorphic

In addition to estimating gene activity with our RNA-Seq data, we can also estimate polymorphism levels in cultivar specific genes based the low level of mismatches allowed in the read mapping step. This analysis shows that ~70% of Granny Smith genes have polymorphisms, and ~3.2% of the average gene is polymorphic. A preliminary analysis shows that while most are not highly polymorphic, several transcripts are (Figure 2).

de novo *transcriptomes bring more data into experiments*

Initial mapping experiments in apple showed that we could map 84% of our apple transcriptome data to the Golden Delicious reference genome (consistent with other published work). However, qPCR validation showed substantial disagreement among our candidate genes, suggesting errors in the RNA-Seq data. Therefore, we refined our approach by limiting the analysis to known genes, rather than the entire genome. This reduced the number of reads included in the analysis to roughly 60%, but showed improved agreement with our validation tests (see Table 1). We also generated a custom set of gene predictions generated from our raw data for Granny Smith and Honey Crisp. Using this, we were able to confidently map >80% of our data. This lends support to our hypothesis that reference sequences that more closely match the raw data improve data use efficiency in digital gene activity measurements, possibly improving measurement fidelity. A key limitation to custom gene predictions from raw data is that the gene models, while highly accurate, are often fragmented. We anticipate that we can use the apple genome to resolve fragmentation issues, while simultaneously using the custom gene predictions to correct SNPs in the genome towards construction of an enhanced RNA-Seq reference.

Figures and Tables:

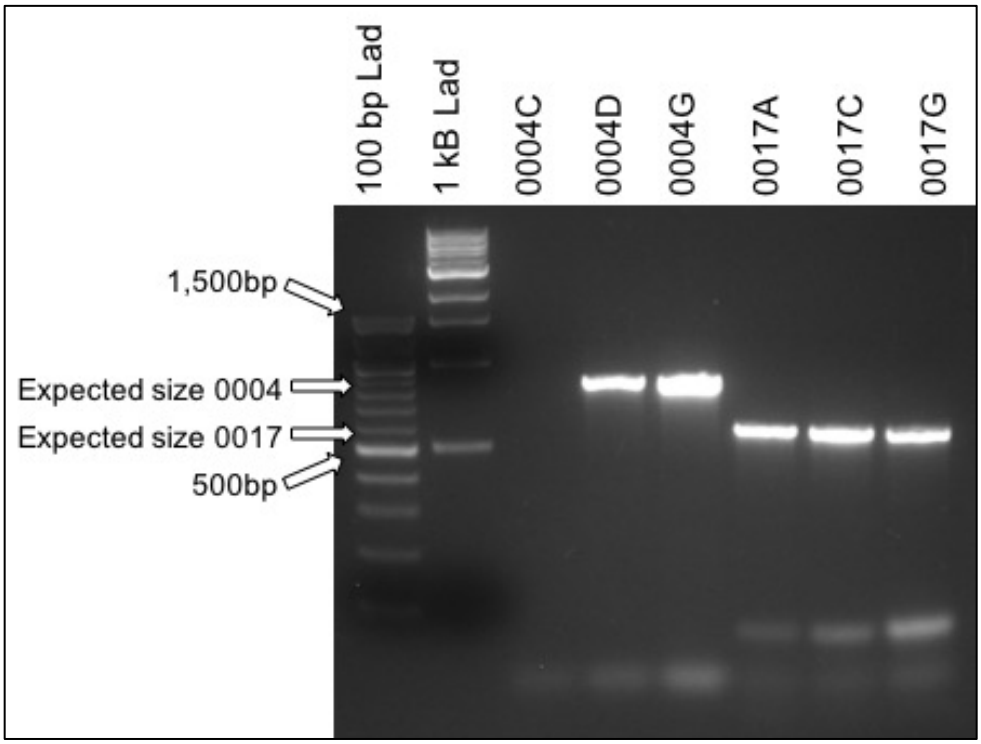


Figure 1. **Gene predictions were validated with PCR** For each *de novo* gene prediction called a contig (*de novo* assembly fragments are called contigs for "contiguous sequence"), we developed a PCR assay that would validate the prediction. The assay should produce a PCR product of the expected size if the gene prediction was accurate. This validation was a strict criterion for each candidate.

Gene ID	*Identity (%)*	*Polymorphic (Y or N)*	*Polymorphic Protein (Y or N)*	*Protein identity (%)*	*Polymorphic Primer (Y or N)*	*qPCR Efficiency (%)*	*qPCR vs RNA-seq Correlation*	*NewBlast Transcriptome Correlation*	*Pairwise Identity change*
GS_01	0.94	**Yes**	**Yes**	0.71	No	98.0	0.6734	0.6660	-6.70%
GS_02	0.99	No	NA	0.97	No	92.3	0.9198	NA	NA
GS_03	1.00	No	NA	0.98	No	94.8	0.8774	NA	NA
GS_04	0.94	**Yes**	No	0.92	**Yes**	97.6	0.9730	NA	NA
GS_05	0.96	**Yes**	**Yes**	0.91	**Yes**	96.4	0.8947	NA	NA
GS_06	0.82	**Yes**	**Yes**	0.97	**Yes**	93.2	0.0062	0.3527	5.80%
GS_07	1.00	No	NA	1.00	No	94.6	0.8713	NA	NA
GS_08	0.97	**Yes**	**Yes**	0.96	**Yes**	96.2	0.1067	0.5441	2.30%
GS_09	1.00	No	NA	0.99	No	109.7	0.6856	0.7900	0.50%
GS_10	0.90	**Yes**	**Yes**	0.97	**Yes**	106.5	0.4897	0.9401	7.20%
GS_11	0.98	**Yes**	**Yes**	0.98	**Yes**	96.2	0.7996	0.4079	-5.30%
GS_12	0.97	**Yes**	**Yes**	0.95	**Yes**	96.3	0.8614	NA	NA
GS_13	0.99	**Yes**	No	0.97	No	108.5	0.9570	NA	NA
GS_14	0.98	**Yes**	**Yes**	0.97	**Yes**	100.3	0.7818	NA	NA
GS_15	0.99	**Yes**	**Yes**	0.78	**Yes**	92.3	0.8279	NA	NA
HC_01	0.99	**Yes**	**Yes**	0.99	No	108.0	NA	NA	NA
HC_02	0.99	**Yes**	**Yes**	0.99	No	98.1	NA	NA	NA
HC_03	0.99	**Yes**	No	1.00	No	97.8	NA	NA	NA
HC_04	0.99	**Yes**	No	1.00	**Yes**	108.0	NA	NA	NA
HC_05	0.99	**Yes**	No	1.00	**Yes**	98.6	0.6283	0.3455	-5.26%
HC_06	0.93	**Yes**	**Yes**	0.92	**Yes**	113.4	0.7486	0.8189	4.00%
HC_07	0.93	**Yes**	**Yes**	0.93	**Yes**	102.1	0.3474	0.5117	6.35%
HC_08	0.97	**Yes**	**Yes**	0.92	**Yes**	97.3	0.5655	0.1647	-19.16%
HC_09	0.93	**Yes**	**Yes**	0.99	**Yes**	94.2	0.0315	0.3013	3.30%
HC_10	0.96	**Yes**	No	1.00	**Yes**	113.5	0.7059	0.8261	3.60%
HC_11	0.98	**Yes**	**Yes**	0.97	**Yes**	106.6	0.6678	0.3771	-3.58%
HC_12	0.89	**Yes**	**Yes**	0.98	**Yes**	103.3	0.1931	0.2451	6.05%
HC_13	0.82	**Yes**	**Yes**	0.96	**Yes**	105.3	0.5659	0.6950	14.94%
HC_14	0.92	**Yes**	**Yes**	0.77	**Yes**	102.9	0.5573	0.337	-5.00%

Table 1. **Cultivar specific gene forms are highly polymorphic, and polymorphisms alter gene activity measurements.** (GS = Granny Smith, HC = Honey Crisp). **Identity** & **Protein Identity** = percentage of identical sites. **Polymorphic** & **Polymorphic Protein** = custom gene model identical to the reference? **qPCR efficiency** = 80-120% is acceptable, 90-110% is desired. **qPCR vs RNA-Seq correlation** = correlation analysis between methods of measuring gene activity. **New BLAST Transcriptome correlation =** same as **qPCR vs RNA-Seq correlation** except with better matches. **Pairwise identity change =** difference between next best match and original match (sometimes a negative change).

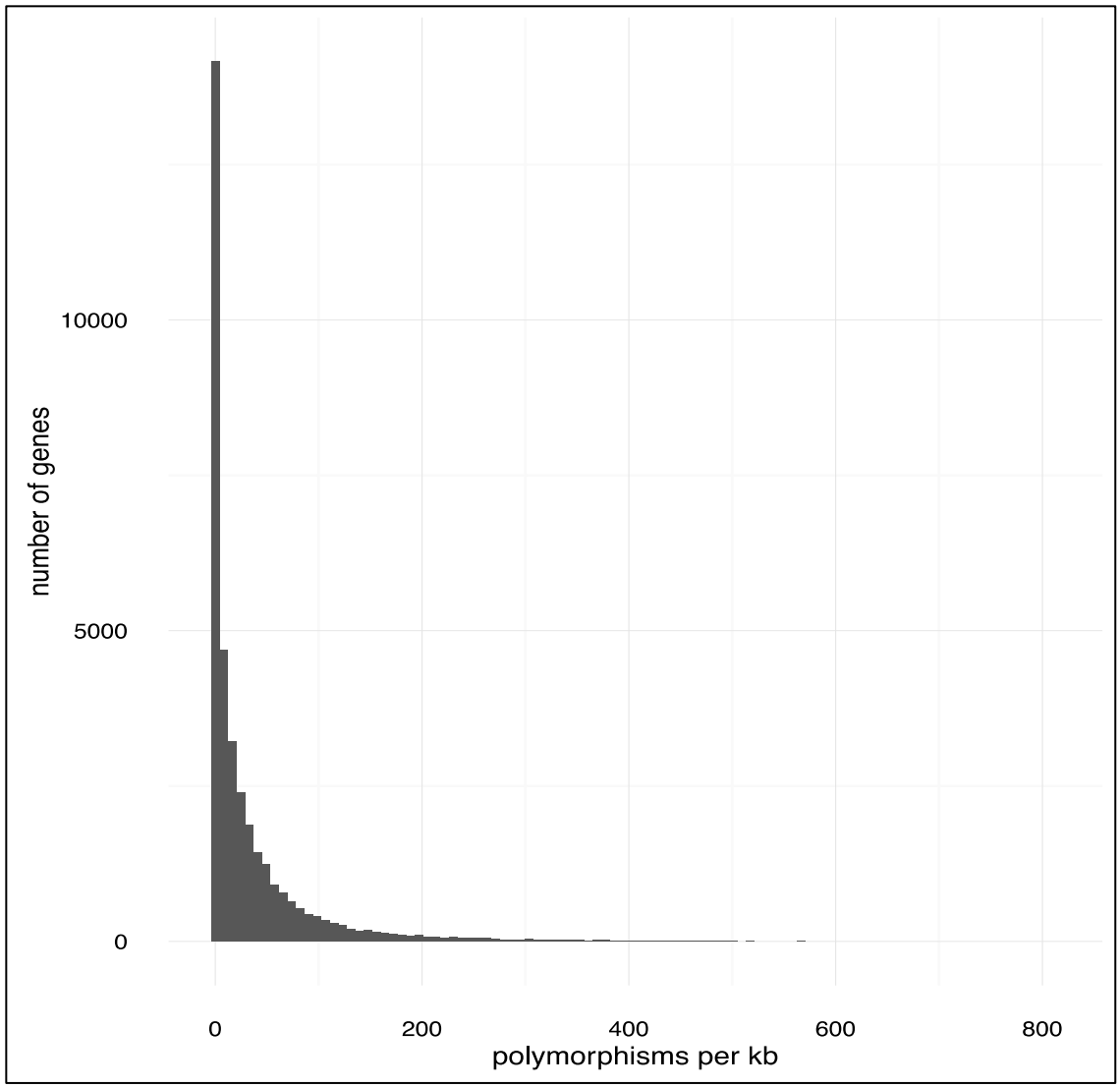


Figure 2. **Some Granny Smith genes are highly polymorphic.** This histogram shows the distribution of polymorphic genes in this analysis. Most show low levels of polymorphism, 0-50 polymorphisms per kb, but some genes have very high possible levels of polymorphism, up to 500 polymorphisms per kb (50%).

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

Project Title: Developing and validating models for tree fruit

PI: Vincent Jones
Organization: WSU-TFREC
Telephone: 509-663-8181x291
Email: vpjones@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI: Ute Chambers
Organization: WSU-TFREC
Telephone: 509-663-8181x290
Email: uchambers@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee, WA 98801

Co-PI: Tory Schmidt
Organization: WTFRC
Telephone: 665-8271 x4
Email: tory@treefruitresearch.com
Address: 1719 Springwater
City/State/Zip: Wenatchee, WA 98801

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:** Katy Roberts/Joni Cartwright
Telephone: 509-335-2885/509-663-8181 x221Email: arcgrants@wsu.edu / joni_cartwright@wsu.edu

Item	2017	**2018**	2018
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] Ute Chambers (0.25FTE, T. Melton 0.45 FTE).
[2] 34.1% (Chambers); 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

- Peak migration into the orchard by psylla winterform adults happens at ≈90 DD, which coincides with the start of egg laying (12.4 hours photoperiod). The first nymphs were found at 310 DD, with the first summerform adults found at an average of 655 DD.
- We have a preliminary model for pear psylla describing egg-laying, occurrence of instars 1-3, instars 4-5, and summerform adults.
- The first occurrence of new winterform adult psylla happens when daylength in the fall drops below 14.5 hours (last week in August to first part of Sept depending on the location).
- 95% of rosy apple aphids were collected between 800 and 1600 DD, and collections after that period were generally restricted to just a few trees in the orchards. RAA winged adults returned to the orchard ≈ 2500 DD, but were in low numbers that are hard to detect.
- Apple grain aphids build up early and are mostly gone by 1400 DD, and return late in the fall at ≈2700 DD.
- Initial results indicate that photoperiod may play a role in diapause termination of overwintering aphid and mite eggs and that degree-day accumulations using estimated bark temperature instead of air temperature may be more accurate in predicting spring egg hatch.
- Data for fruit growth models of Cosmic Crisp, Golden Delicious, Fuji, and Honeycrisp were collected and preliminary analysis shows the fruit growth is easily predicted using degree-days since 1 January.
- Fruit growth models for Red Delicious, Cripps Pink, and Gala were available on WSU-DAS for beta user feedback this past year. After collecting feedback from the beta users, we will release those models to all DAS users in 2018.

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

Methods. Phenology data for pear psylla were collected at six locations with low-intensity management, twice a week from February until October 2017. 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. 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*. Data from the yellow cards have not been completely processed or analyzed. The following results are based on the beat and shoot samples, including data collected in 2016.

Results and Discussion. The winterform adults reached the highest levels at about 90 DD, which probably reflects the peak migration into the orchard (Fig. 1). Egg production was first found about the same time (at 12.4 hours photoperiod), and younger nymphs (instars 1-3) were found starting at 310 DD, with older nymphs (instars 4-5) first being found at 510 DD. The first summer form adults were found on average at 655 DD. There appear to be three full generations (counting the winterform generation) and a partial fourth generation depending on the year and location.

The number of eggs appeared to be highest in the first generation (Fig. 2), but this might be an artifact of the sampling methods: in early season, the eggs are restricted to the new twigs and buds, and we used visual examination of twigs; whereas once the leaves start to expand, there is a much larger surface area over which females can lay eggs, and we switched to using the mite brushing machine to

evaluate the different stages at that time. As most of our sampling orchards were treated and tend to target overwintering eggs, sprays would tend to reduce the egg populations after leaf expansion starts.

We used the data from 2015-2016 to develop a model for all stages of the pear psylla. Because our data were collected in sprayed orchards (except for one), the differences in population levels between orchards are considerable and spray programs result in some orchard/years locations having significant reductions at different times. The only way to overcome this is to pool the data and use the composite data set to develop the model. Our data show that the phenology of all of the stages are predictable, with only a few situations showing significant deviations (*e.g.*, the winterform adults when the proportion found>0.8) (Fig. 3). Validation of the predictions will be the goal of the next two years, when we will refine some of the different parts of the model, as well as begin the construction of the demographic model that will enable us to predict how the population will respond to sprays at different times in the life cycle.

The development of the new winterform adults was first found in all the locations right around the 28th of August (≈ 14.5 hours day length). This is a bit sooner than reported in the literature, and may be in part a result of our more frequent data collections which allowed us to detect fairly low numbers when they first occurred. In most of the orchards, the adult population never was 100% winterforms at our last collections in early November, which indicates that some of the individuals escaped diapause induction and would die as the cold weather hit.

Fig. 1. Probability of collecting winterform adults in the spring. Dotted line indicates time of peak capture at 90 DD.

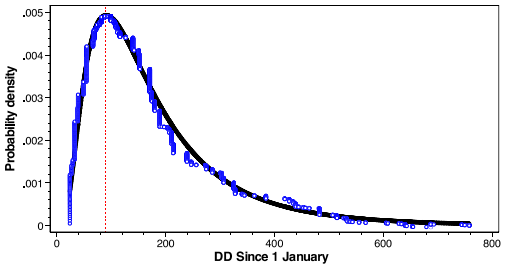


Fig. 2. Timing of eggs over all locations 2016-2017. Dotted line is 650 DD when the first summerform adults occur on average.

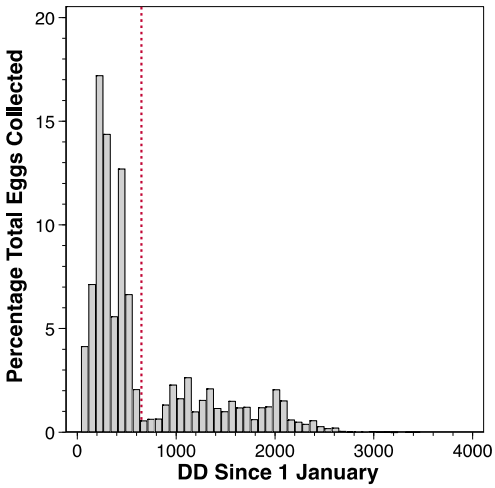
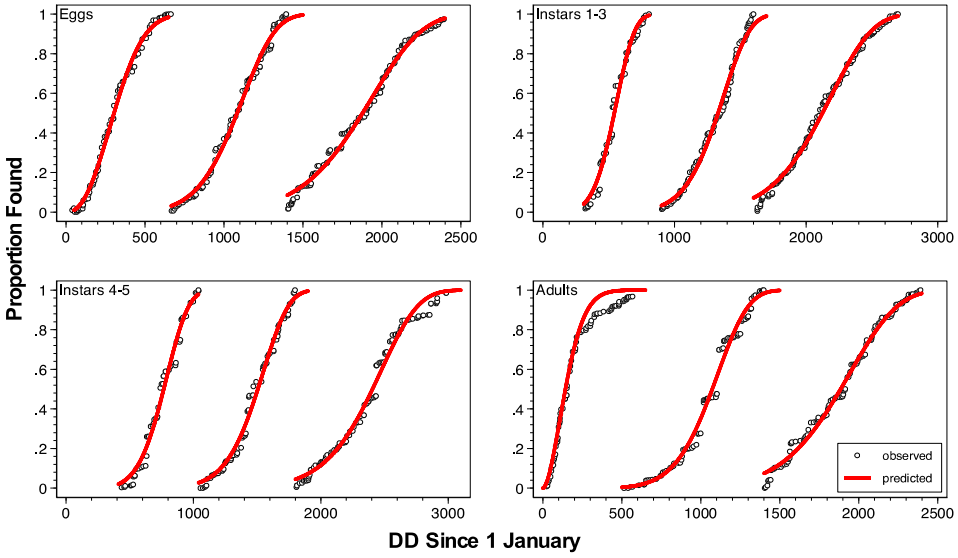


Fig. 3. Relationship between model predictions and observed field data for four different stages of pear psylla.



Work next year: We will continue to refine the psylla model by collecting more validation data in different situations. In addition, we will be processing the yellow panel data collected this year to evaluate adult phenology in comparison to the beating tray data. We will begin constructing the demographic model (*i.e.*, the pesticide effects model) and use the phenology data to make sure the demographic model accurately reflects the phenology observed in the field.

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), rosy apple aphid (RAA), and apple grain aphid (AGA), as well as two-spotted spider mite (TSSM), European red mite (ERM), and brown mite (BM). For GAA, AGA and RAA, five apple orchards were sampled twice a week from April 4 until November 7. Through September, 20-25 infested apple buds or, later, leaves were taken and the number of nymphs, nymphs w/ wing buds, wingless adults, and winged adults was recorded for each aphid species. During October and beginning of November, 40 leaf samples were taken once a week. For WAA, three apple orchards were sampled twice a week from May 30 until November 7, but data analysis has not been completed.

Phenology data for ERM and BM were collected from five apple orchards, twice a week from April 18 until November 1. 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.

TSSM phenology data were collected from the tree canopy in six pear orchards as well as from the ground cover in four pear, four apple, and one cherry orchard. Initial ground cover samples included all broadleaf weeds present. However, common mallow had consistently high numbers of TSSM, so all subsequent samples focused only on that plant. At each site, 30-40 common mallow leaves and/or 60-100 pear leaves were collected twice a week from May 9 until November 7. The pear leaves were processed through the mite brushing machine, and mite numbers and stages recorded. The mallow leaves needed to be visually inspected, as they were more fragile. Mite numbers and stages were recorded. The mite phenology data have been compiled, but not yet analyzed.

In addition to phenology in the field, we examined egg hatch of field-collected aphid, ERM, and BM eggs in the lab. Twigs and spurs with aphid and mite eggs were collected at weekly intervals from February 14 until March 31 from four orchards for each pest. The twigs were placed in ventilated plastic cups and kept in a growth chamber at 60ºF with the photoperiod being adjusted weekly to outside conditions. The number of hatched ERM and BM larvae and aphid nymphs was recorded daily. Because newly hatched aphid nymphs cannot be easily distinguished by species, they needed to be reared until they were old enough to be identified. Therefore, a subset of the daily hatched aphids was transferred to fresh, untreated apple leaves (from 2-months old apple seedlings with 4-5 leaves in the greenhouse). The leaves were kept fresh in plastic cups on wet cotton balls, and aphids were checked twice a week and identified when old enough.

In the orchard, aphid and mite eggs can experience warmer micro-habitat temperatures as solar radiation heats up the leafless tree branches where the eggs are present. Therefore, bark temperature was estimated using linear regression between daily maximum air temperature and the sum of the hourly solar radiation derived from local bark temperature data from previous years.

Results and Discussion

Rosy apple aphid: We found that 95% of the aphids were collected between 800 and 1600 DD over the period from 2015-2017 (Fig. 4). Winged aphids return to the orchard in the fall starting around 2500 DD, but the numbers are very low and can be hard to detect (<0.3% of 91,424 aphids counted). While there is a well-known switch of host plants (generally to plantain) associated with RAA during

Fig. 4. Percentage of Rosy apple aphid versus DD over the period of 2015-2017. 95% of all collections were made between 800 and 1600 DD.

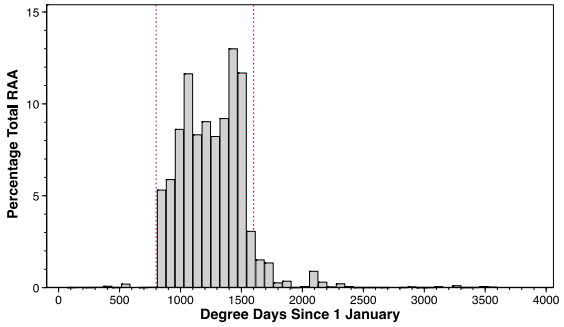
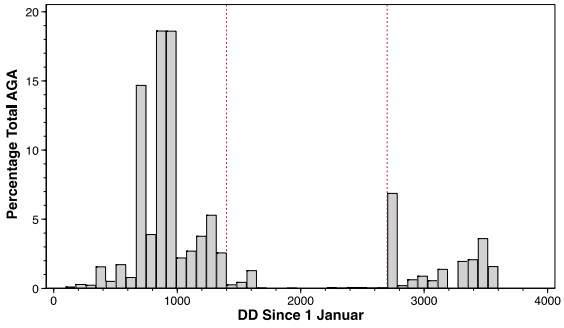


Fig. 5. Percentage of Apple Grain Aphid versus DD over the period 2015-2017. Populations are very rare over the period of 1400-2700 DD.

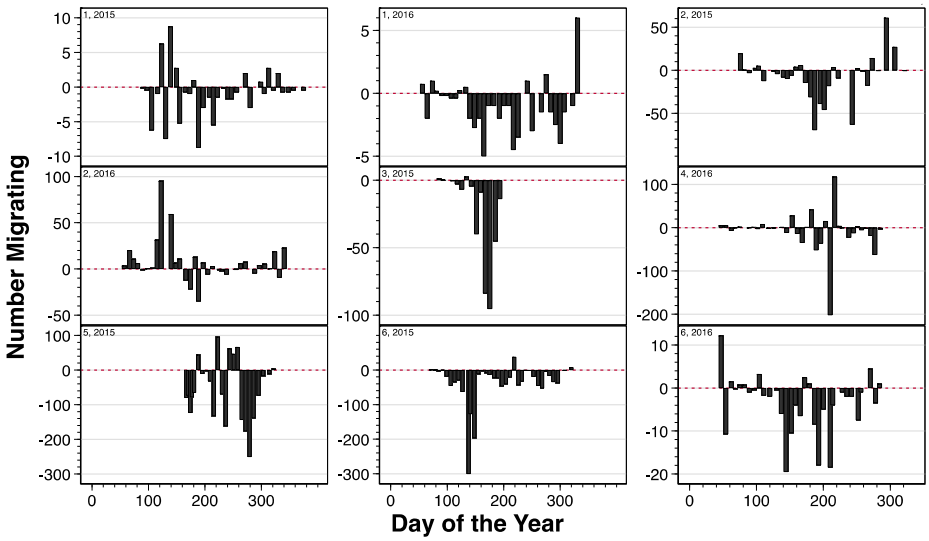


the summer, we also noticed that the average maximum temperatures in the periods during and after peak population levels increased between 12.5 and 8.9°F (average 10.3°F). In general, temperatures were close to 90.9°F after the peak, whereas during the peak period, they were closer to 80.5°F. Given that the upper threshold for RAA is around 75°F, it is likely that at least some of the reduction within the orchard during the summer is related to high temperatures, with the majority of the drop associated with the development of wings and movement by the adults.

Apple grain aphid: Apple grain aphid populations build up early season and migrate from the orchard with about 95% being gone by 1400 DD (Fig. 5). They then return late in the season, by about 2700 DD. The number coming back to the orchard is quite variable from year to year and orchard to orchard, with warmer years having more aphids returning than in cooler.

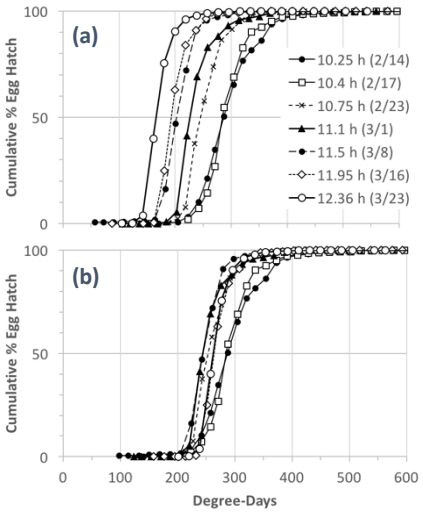
Woolly apple aphid: Although we collected the data, as of early December, we are still processing the samples and preparing the 2017 data for analysis. However, looking at data for the last two years, when WAA trunk migration was monitored using double-sided sticky tape (20 trees/orchard), we found that the majority of the movement was down towards the roots (Fig. 6) and did not occur in relation to high temperatures. Further evaluation of the temperature data showed that high population levels only occurred when maximum temperatures were under 90°F. However, population decreases were not always related to high temperatures and could occur at any time, likely because of a combination of temperature and natural enemy abundance.

Fig. 6. Net migration of WAA on the tree trunk up (positive numbers) or down (negative numbers) at 9 different orchard/year combinations.



Green apple aphid: Only one location yielded sufficient numbers of GAA eggs to evaluate egg hatch. Therefore, results are preliminary. The weekly sampling regime revealed that median egg hatch (50%) shifted with each collection date from 350 DD (Feb. 14, 10.2 hours day length) to 170 DD (Mar. 23, 12.3 hours day length), when using air temperature to compute the degree-days (Fig. 7a). Eggs were already hatching in the field on March 30, therefore, this sampling date was excluded from analysis.

Fig. 7. Green apple aphid egg hatch vs. accumulated degree-days after Feb. 20th using air temperature (a) and estimated bark temperature (b) by day length on sampling date.



Plotting egg hatch against DD calculated from estimated bark temperatures also shows a shift in median egg hatch to lower DD, but this shift only occurs for the first two collection dates from 350 DD to 250 DD. After Feb. 23 (10.75 hours day length), the DD for mean egg hatch remain relatively constant (Fig. 7b). This suggests that photoperiod plays a role in breaking the eggs' diapause, after which development resumes on a temperature basis. Photoperiod might act like a switch that turns on the eggs' response to DD, comparable to the effect of photoperiod on the diapause termination in TSSM females. A similar trend was seen with the RAA and AGA eggs collected from other locations, although not as clear due to smaller sample sizes. More data from multiple sites and years is needed to confirm this initial finding.

Mites: The timing and duration of ERM egg hatch changed considerably with the date of collection. Average egg hatch shifted from 1200 DD to 500 DD when using air temperature for DD accumulation. When using estimated bark temperature, average egg hatch was observed around 650-700 DD after mid-March (11.9 hours day length). This indicates that, on one hand, considerable warming of the eggs occurs in early spring due to solar radiation and, therefore, using estimated bark temperature results in more accurate DD accumulations. On the other hand, it appears that day length has an effect on the diapause termination of the ERM eggs similar to that described above for GAA.

Similar to ERM, brown mite egg hatch shifted with the date of collection (Feb. 17 – Mar. 30), although not as much, from an average of 700 to 400 DD, when using air temperature, or 690 to 600 DD when using estimated bark temperature. Mid-March appears to be the time again when degree-days from estimated bark temperatures for average egg hatch remain constant, indicating a controlling impact of day length on diapause termination in BM eggs. We also observed that, in contrast to European red mite, the egg hatch of brown mites was more synchronized, occurring over a shorter window of time.

Work next year: The phenology of the aphids is fairly well defined in terms of when the different stages are present in the orchard (Rosy and apple grain aphid). We will concentrate on the phenology of green apple aphid and WAA and evaluate whether we can use the stage distribution in a demographic model. Evaluation of the WAA data for 2017 will be completed late winter and will further inform direction of work on that aphid. For the mites, the stage distribution and phenology data will be analyzed in winter.

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

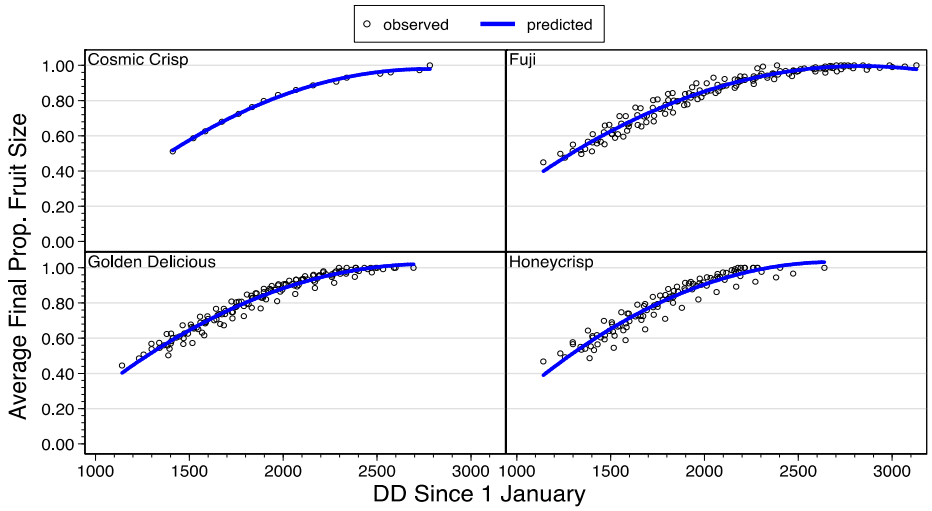
Methods. We collected data from 11 geographic nodes representing the topographic and climatic diversity of Central Washington production areas from Brewster Heights to North Pasco. We concentrated on Golden Delicious, Fuji and Honeycrisp at each node, but also collected data on Cosmic Crisp at the WSU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

number so 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).

Results and Discussion. Similar to the work reported last year, the growth rate of the different cultivars was predictable using a simple quadratic equation (Fig. 8). Honeycrisp had the greatest amount of variability, but even so, the regression equation predicted >93% of the variability in the data.

We also allowed our beta testers to evaluate the fruit growth model on WSU-DAS that had the cultivars Red Delicious, Cripps' Pink, and Gala. We currently have a survey out to our beta testers asking them for feedback and potential modifications they would like to see. We expect that those three models will be released to all DAS users this coming year.

Fig. 8. Average fruit growth rate at the different locations for four different cultivars 2017.



Work Next Year. As this data for all cultivars is only from a single year's data, we need to replicate it and in particular the Cosmic Crisp data needs the greatest amount of replication because it was only sampled at the WSU-Sunrise orchard. We also need to start sampling earlier in the season, as our data collection this year started at about 40% full fruit size; while there is a tradeoff in loss of small fruits through abortion, it would help us get a better set of predictions that would "turn on" before the 40% period – the regression equations are not accurate when extrapolated to lower numbers than the lowest observed data.