2019 Stone Fruit Research Review

Tuesday, November 26, 201

WSU-Prosser, large conference room

Time	PI	Project Title	YRS
10:00	Doornink	**Introduction**	
10:15	Beers	Spotted wing drosophila management in stone fruit: ***No-cost extension***	16-18
10:30	Harper	Understanding decline in peach trees infected by multiple phytoplasmas	19-20
11:00		Committee discussion	

FINAL PROJECT REPORT
WTFRC Project Number: ST-16-100

Project Title: Spotted wing drosophila management in stone fruit

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Cooperators: stone fruit growers

Other funding sources: None

Total Project Funding: $86,225

Budget History:

Item	2016	2017	2018	2019
WTFRC expenses	0	0	0	0
Salaries	10,695	22,245	23,135	0
Benefits	4,128	8,587	8,930	0
Wages	0	0	0	0
Benefits	0	0	0	0
Equipment	0	0	0	0
Supplies	1,000	1,000	1,000	0
Travel	1,835	1,835	1,835	0
Plot Fees	0	0	0	0
Miscellaneous	0	0	0	0
Total	17,658	33,667	34,900	0

Objectives

1. *Determine skin penetration force and flesh firmness levels necessary to allow spotted wing drosophila (SWD) oviposition (completed years 1 & 2).* This project began with the assumption that SWD were attacking nectarines and had been responsible for a considerable amount of damage in at least one year in the past (2013). Previous work with peaches indicated low suitability, but nectarines had not been investigated thoroughly. The presumption was that as fruit approached maturity, they become more susceptible, and flesh firmness and skin penetration force would be appropriate indicators for spotted wing drosophila, which needs to pierce the skin with its ovipositor.
2. *Test the use of synthetic lures to predict damage by SWD (completed years 1 & 2).* Based on the assumption that damage would occur in some years but not others, we tested commercial lures and traps to correlate with damage.
3. *Determine the number of traps per unit area needed to provide accurate prediction of damage risk (completed years 1 & 2).* Monitoring is labor-intensive, and we were interested in finding the most efficient way to arrive at a threshold for treatment.
4. *Investigate the probability that brown rot could be transferred to a healthy fruit by a brown-rot contaminated SWD under field conditions (new objective for 2018; Repeated in 2019 using both standard and selective media).* This objective was added based on the observation from the affected producers that the year when nectarines had suffered high levels of damage from SWD had also been a high-pressure brown rot year, and the two appeared to be related.

Significant Findings

- The SWD oviposited in, and emerged from, ripening nectarines at very low levels compared to a known susceptible host, sweet cherry
- Fruit skin penetration and flesh firmness of nectarine decreased as fruit became more mature, but these parameters were unrelated to the ability of SWD to attack the fruit
- The liquid traps captured both more males and total SWD than the yellow sticky cards in 2016, but the reverse was true in 2017. The threshold was trigged at the same point in time for all trap types and densities (likely due to the low threshold)
- Damage due to SWD was found in harvest samples, but successful emergence occurred only in split fruit (one of three orchards only); a higher threshold may be more appropriate
- SWD contaminated with brown rot spores caused infection in ripe and unripe nectarines; the mechanism appears to that of contamination of body parts rather than oviposition wounds
- Brown rot selective media exposed to field-collected SWD, or other *Drosophila* species, did not develop brown rot.
- All selective media arenas exposed to lab-contaminated SWD developed brown rot, but none was caused by field-collected insects. Only airborne contamination caused brown rot in the selective media placed in the field (with insects excluded).

Results and Discussion

Background: these experiments were performed in the WSU-TFREC laboratory and three nectarine orchards near Mesa, WA. These orchards had experienced high levels of SWD damage in 2013, and thus were chosen as study sites. The first two years of the project focused on SWD trapping and damage, but shifted to a potential association with brown rot in the final two years. Studies in the 2018 and 2019 were in collaboration with Dr. Achour Amiri, plant pathologist, who has expertise in brown rot.

Objective 1: *Determine skin penetration force and flesh firmness levels necessary to allow SWD oviposition*

Previous research has shown that peaches are low risk host crops for SWD. However, this insect can oviposit in them if they are over-mature; thus there may be a point in fruit development when they become susceptible. Our goal was to determine where along this continuum this point lies in terms of fruit maturity characteristics.

Fruit maturity was measured on a sample of 10 fruit/block on multiple dates (2016-2017). The timing was based on projected harvest, which was nearly a month earlier in 2016. Fruit maturity measurement included weight, flesh firmness, and skin penetration force (Fig. 1). On the final 2-3 dates closest to harvest, an additional 10 fruit were used to bioassay the ability of female SWD from a lab culture to oviposit and successfully develop to the adult stage. Fruit was picked in the morning, transferred directly into individual plastic containers, and exposed to females the same day. Mated female SWD (five per arena, 10 days old) were deprived of an oviposition substrate for 24 h, then exposed to a single nectarine fruit for 16 h. For comparison, a known susceptible host (sweet cherry) was assayed at the same time, using 12-14 cherries to provide an equivalent weight to the single nectarine (Fig. 2). At the end of the exposure period, females were removed, and the fruit was examined for oviposition punctures with breathing filaments (internal egg deposition) or eggs laid on the surface of the fruit (external oviposition); the latter is an indication of poorer host acceptance.

Fig 1. Fruit texture analyzer used to measure skin penetration force

Fig. 2. Bioassay arrenas for nectarines, cherries, and drosophila medium (left to right)

Both skin penetration force and flesh firmness decreased over time as the fruit matured (Fig. 3) in all three orchards. Oviposition in nectarine fruits was negligible on all both years (Fig. 4a, b) with many ovipositions external, compared to the high level of internal ovipositions in the known susceptible host, sweet cherry. Total ovipositions in cherries were variable, but about 34-fold higher overall than in nectarines.

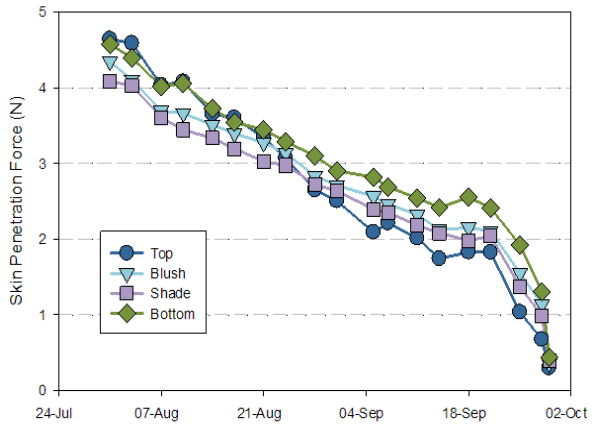


Fig. 3. Fruit skin penetration force over time, 2017.

The average skin penetration force for each orchard and date was regressed against the average oviposition and adult emergence of SWD in lab bioassays. There was no relationship between skin penetration force and resulting oviposition and emergence of SWD from nectarine fruit (data not shown), essentially because there was almost zero attack by SWD on the nectarine fruit.

Objective 2: *Test the use of synthetic lures to predict damage by SWD*

The first synthetic lure was available for testing in 2013, based on the Cha-Landolt blend of acetic acid, ethanol, methianol, and acetoin. Three commercial lures are now available, generally providing higher capture than apple cider vinegar. Several seasons of tests indicate that the Scentry lure consistently captures more SWD, and thus offers the best opportunity for early detection of adult activity in an orchard, and to base a spray threshold on trap capture. The use of traps for spray thresholds was tested in three nectarine orchards, cv 'Summer Blush' in eastern Washington in 2016-2017. Traps were deployed in late July-mid-August and checked twice per week during the preharvest and harvest season. A 1-acre section of trees was designated as the study area, and six traps were deployed near the center (3 per row with one buffer row between). Three of the traps were a liquid-based (lure + drowning fluid) jar trap (Scentry trap) baited with the Scentry synthetic lure. The drowning fluid was 300 ml of water with a surfactant (liquid dish soap) and a preservative (sodium benzoate) added. The second set of three traps had Scentry lures backfolded in AlphaScents yellow sticky traps (Fig. 5). The drowning fluid in the liquid traps was collected and replaced at each visit, and the contents counted in the laboratory with the aid of a microscope. The AlphaScents sticky traps were counted *in situ*, scanning only for males, which were removed after counting. The trap positions were rotated between rows at each visit. A provisional threshold of five SWD in any of the six traps per block was the trigger to begin protective pesticide applications, to be continued through harvest at 7-10 day intervals at the grower's discretion.

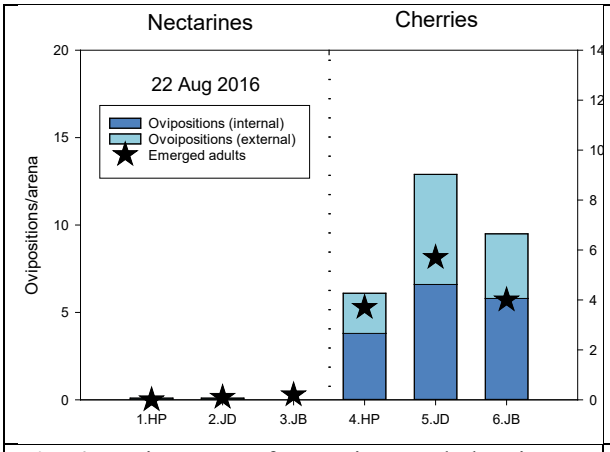


Fig. 4a. Bioassays of nectarines and cherries, 2016

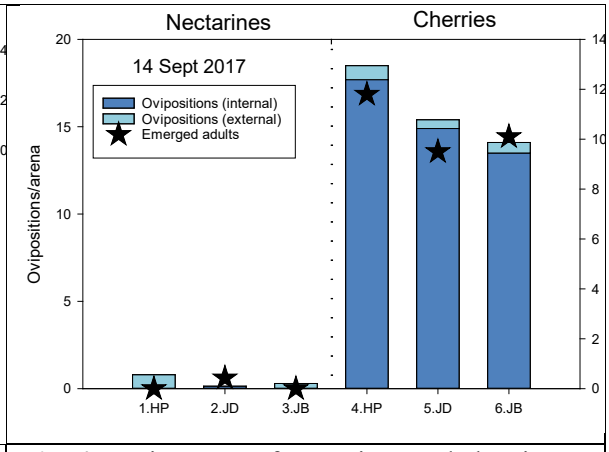
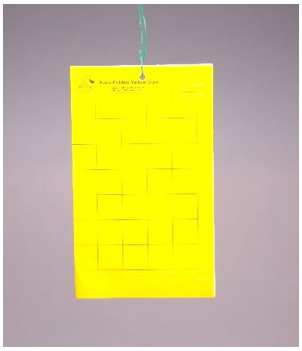


Fig. 4b. Bioassays of nectarines and cherries, 2017

The success of the threshold was determined by examining *in situ* 1,000 fruit in each plot. All damaged fruit were collected and returned to the lab to rear out any arthropods found in the fruit. Two fruit damage assessments were made (1,000 fruit/plot) on two harvest dates in the six study plots. In 2016, the first sample yielded no SWD or other *Drosophila*. In the second sample, a total of 21 damaged fruit were found, 2 of which contained SWD (total of 10 adults). All of the fruit with SWD were categorized as 'split', indicating that a prior physical or physiologically induced wound may have been responsible for the entry point. Other *Drosophila* species were also found in the damaged fruit (*n*=24), some of which overlapped with fruit in which SWD were found. In 2017, a single SWD male emerged from a damaged fruit collected on the last harvest date.

A - Scentry trap

B - Scentry lure



C - AlphaScents yellow sticky trap

Fig. 5. Traps and lures used in Obj. 2.

In 2016, an additional sample of damaged nectarine fruits from the same growing region was taken on 26 August. Each fruit was photographed to record the appearance the damage, and then incubated for 16 days to determine if any *Drosophila* spp. were present. Of the 11 fruit, 9 were infested with *Drosophila* (SWD or other species), and 5 with SWD. While these results confirm that SWD infestation is occurring in the field, it is unknown whether the infestation with SWD occurred in injured or uninjured fruit.

Fig. 6. High and low density trapping plots, Mesa, WA

Objective 3. *Determine the number of traps per unit area needed to provide accurate prediction of damage risk*

Little is known about the source of SWD occurring in blocks, specifically whether the major source comes from habitat surrounding the block, or from within the block itself. This makes the number and position of traps used for action thresholds difficult to determine. Observations to date indicate that the older ACV traps have a limited range of attraction, but newer lures are untested.

To address this question, the same blocks used in Obj. 2 were used in 2016-2017, locating a second 1-acre plot next to the Obj. 2 plot (Fig. 6). In contrast to the low trap density used in Obj. 2, and the second plot had a high trap density, using only the Scentry lure/yellow

AlphaScents sticky trap combination. Traps were laid out in a grid pattern throughout the block, 5 traps in each of 4 rows, or 20 traps per 1-acre plot. Traps were checked twice weekly *in situ*, without changing the lure or trap, and removing males after counting. The same threshold of 5 SWD (males) in any trap used in Obj. 2 was used, as well as the same method of determining success of the threshold.

In 2016, the liquid traps caught the highest numbers of SWD, including males (Fig. 7). The high-density and low-density yellow sticky cards generally stayed below the threshold of 5/trap. In contrast, the trends for 2017 were the reverse: the low density sticky cards had the highest captures, and the lowest numbers of males were caught in the liquid traps. Both the 4-week difference in harvest maturity, and the difference in trapping period make these numbers difficult to compare, but the lack of consistency in trap performance was unexpected. In light of the lack of evidence of SWD attack on nectarines, the point is probably moot.

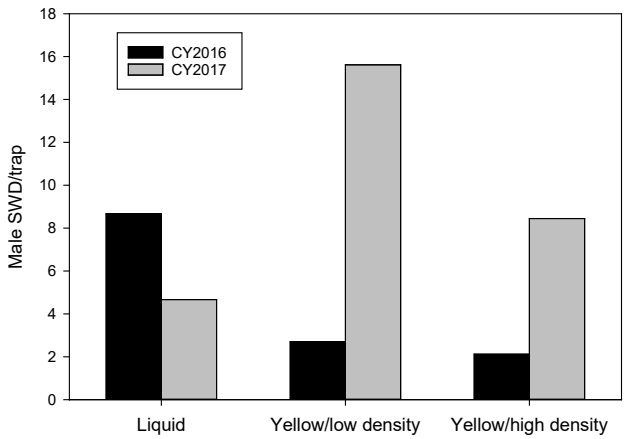


Fig. 7. Average male SWD/trap in three trap types and two trapping densities, 2016-2017.

Objective 4: *Investigate the probability that brown rot could be transferred to a healthy fruit by a brown-rot contaminated SWD under field conditions.*

This objective was based on observations from producers that during a year when SWD damage in nectarines was prominent (2013), that this season was also a high-pressure year for brown rot. This raised the question of a possible association between the two pests, with the proposed hypothesis that SWD was infecting fruit with brown rot.

A preliminary experiment was done in the laboratory in 2018 to investigate two possible mechanisms for brown rot caused by SWD: 1) oviposition wounds made by females as a point of entry for the pathogen, and 2) the 'dirty feet' hypothesis), that surface contamination of SWD (regardless of sex) could transfer the infection. The latter was carried out with males to avoid the possibility of oviposition wounds (even though previous research indicated these were relatively rare in nectarines).

Fig. 8. Petri dish with sporulating brown rot and adult spotted wing drosophila.

To test the 'dirty feet' hypothesis, we grew culture of sporulating brown rot, and placed 10 males and 10 females on the surface (Fig. 8). We removed the flies after 1 min exposure, and placed them individually on standard (potato-dextrose agar) media and brown-rot selective media in Petri dishes. The flies were removed after 24 h, and the media incubated for 7 days. All of the standard media produced active brown rot cultures, while the selective media did not. This was an indication of

the failure of the selective media, although it did demonstrate that surface contamination of flies could transfer brown rot to media.

Field experiments were carried out using the selective media before the results of the preliminary experiments were tabulated. This experiment was to investigate the proportion of flies contaminated with brown rot, and thus capable of transmitted infection. SWD (and other *Drosophila*) were collected live using a Trappit Dome baited with a Scentry lure (Fig. 9). The traps contained a 15 ml cup of drosophila medium (to keep the flies alive) and a 15-ml cup of the selective media. The flies were returned to the lab and placed on selective media, with appropriate positive and negative controls. However, because the selective media did not produce brown rot, even on the positive controls, little was gained from this experiment, and a no-cost extension was requested.

Fig. 9. Trappit dome used to live-trap SWD adults.

In 2019, a new batch of selective media was obtained, and pre-tested to prove it would successfully grow brown rot, to again test the proportion of field-collected flies that could transmit the disease. A single field deployment of traps that tested the hypothesis that field-collected flies could transmit brown rot. The first used field collected flies returned to the lab and placed individually on media, and the second placed selective media directly in the traps with or without flies. We used both positive and negative controls. The negative controls used dishes of selective media in a trap which contained not lure, and with the entry hole screened off with fine mesh to exclude SWD and other *Drosophila*. This treatment tested aerial contamination, as distinct from insect contamination. The positive controls used a Scentry lure in the Trappit Dome, and selective media. The flies collect in this treatment were transferred individually to Petri dishes with selective media.

When field-collected flies were transferred individually to selective media, none of the flies caused brown rot on selective media (n=30, 10 from each orchard). Only the lab-contaminated (positive control) flies caused brown rot in this test, indicating the media was capable of growing brown rot. All other positive and negative controls gave the expected results, confirming the validity of the test.

Brown rot developed only rarely in the selective media place in the traps, and not in the expected treatments (those with lures and flies). The only instances of brown rot occurred where there was no lure and the opening was screened to prevent fly entry: 10% of the selective media arenas, and 20% of the standard media arenas. This indicates that aerial contamination is a significant source of brown rot infection, which is the understood method of fruit infection to date.

Executive Summary

Project Title: Spotted wing drosophila management in stone fruit

Key words: *Drosophila suzukii*, brown rot, disease transmission

Abstract (50 words): SWD showed little tendency to attack uninjured nectarines up to and during harvest. Different trap types and densities yielded inconclusive results, but a threshold is unnecessary if no damage occurs. Flies contaminated with brown rot transferred the pathogen to fruit in the laboratory, with no evidence to support field transmission.

A severe infestation of SWD in nectarines in 2013 in the Mesa, WA area prompted an investigation of the pest damage potential of SWD on this crop. While sweet cherries, a known vulnerable host, were readily attacked by SWD, virtually no eggs were laid on nectarine, with few resulting adults. Previous research on peach corroborates this finding, and also that damaged fruit is a suitable host.

Two types of traps were tested to determine an appropriate trapping density and treatment threshold. The yellow sticky trap was more user-friendly than a liquid trap (both used a synthetic lure); in one year the liquid trap caught more flies, and in one year the yellow sticky traps caught more. Fruit damage samples showed little infestation of nectarines in the field, and were inconclusive since we could not rule out physical damage as the starting point for attack. In any event, absent the potential for damage, a treatment threshold is unnecessary.

Based on observational evidence from managers, an association between SWD and brown rot was suggested (again referring to the 2013 season). This association was investigated to see if SWD was causing brown rot infections. We demonstrated that SWD artificially contaminated with brown rot spores could transfer spores to uninjured fruit. In addition, we demonstrated that this was not likely due to an oviposition wound by the female, but rather simple surface contamination by either the male or female (the 'dirty feet' hypothesis). We attempted to determine what proportion of field-collected flies were capable of transmitting brown rot (to selective media), but limited evidence suggested aerial contamination was responsible.

One possible avenue for future research would be to explore the reverse of this phenomenon: that SWD are attracted to areas on the fruit that are infected with brown rot for oviposition, and that changes in the fruit surface make oviposition more successful when it co-occurs with infection.

CONTINUING REPORT **YEAR**: 1 of 2

Project Title: Understanding phytoplasmas infecting stone fruit trees in Washington state

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Cooperators: None

Total Project Request: $91,835 **Year 1:** $46,380 **Year 2: $45,455**

Other funding sources
None

WTFRC Budget:

Item	2019	**2020**
Salaries	19,370	20,145
Benefits	7,510	7,810
Wages	-	-
Benefits	-	-
Shipping	-	-
Supplies	19,000	17,000
Travel	500	500
Plot Fees	-	-
Miscellaneous	-	-
Total	46,380	**45,455**

Footnotes:
Salaries and benefits for one 0.4X FTE postdoctoral researcher.
Supplies include laboratory consumables and sequencing services.
Travel is estimated mileage for field sampling.

OBJECTIVES

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present by high throughput sequencing.

   Preliminary work has shown that cherries and peaches in the Columbia basin are infected with X-disease phytoplasma, and that peaches and nectarines are also infected with peach yellow leaf roll phytoplasma (also known as pear decline). The incidence in peach and nectarine is unknown as they were recently detected in a brief survey in response to grower inquiries. Also, the number and type species of phytoplasmas in Washington is undetermined. This is a particular problem given the movement of material into and within the state. Therefore in this objective, we propose to survey stone fruit trees, including cherries, peaches, nectarines, apricots, and plums, in Washington to identify which phytoplasmas are present in the state. PacBio sequencing will be performed to obtain genomes for these phytoplasmas as little genomic information is available and are a necessary precursor to genotyping. Genotypic identification will be performed to see whether there is active movement of phytoplasmas from one stone fruit crop to another or from one county to another. These data will answer the questions of 'what' and 'where'.

2. Identify physiological markers associated with the disease by comparing fruit and phloem tissues of infected and healthy trees.

   Both of the presently identified phytoplasmas, X-disease and Peach yellow leaf roll, can affect the quality and quantity of infected stone fruit, yet previous research is limited to a few varieties or species, and, for peaches and nectarines, is primarily from California. Moreover, no data has been collected on the effects of infection by multiple phytoplasmas, as we have observed in both peaches and nectarines in the Columbia basin. Here we propose to examine symptoms in fruit and phloem tissue of infected trees, and by comparing these to healthy trees in the same location, determining type and severity of disease caused by endemic phytoplasmas. This will identify which phytoplasma species, aside from X-disease, are particularly problematic for the tree fruit industry in Washington.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.

   It is unknown how these phytoplasma species cause disease in infected stone fruit. Using transcriptomics, we will be able to determine which pathways have altered regulation in diseased trees and may be important to symptom development. Understanding which pathways are important to symptom development may one day help with breeding for tolerant trees. In year one, leaf tissue has been collected from symptomatic and asymptomatic trees for this purpose.

SIGNIFICANT FINDINGS

- Pear decline and X-disease phytoplasmas have been found to co-infect nectarines, peaches, and plums from Pasco to Wapato
- Apricots grown in the vicinity of phytoplasma infected cherries and peaches have not tested positive for phytoplasma
- Fruit in plums, peaches, and nectarines infected with both pear decline and X-disease phytoplasmas is misshapen, sometimes small, and exhibits delayed ripening

METHODS

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present by high throughput sequencing.

Samples are to be collected from stone fruit trees of representative species and cultivars throughout Washington, from both those that are symptomatic, or are in the vicinity of symptomatic trees. Trees will be screened for the presence of phytoplasmas by generic qPCR, with positives identified by species-specific PCR. A subset of samples with different tree species and phytoplasma combinations will be sequenced using PacBio and Illumina technologies. For PacBio sequencing, which will be used to sequence phytoplasma genomes, rolling circle amplification will be used to increase the amount of phytoplasma DNA, increasing the ratio of phytoplasma to host DNA that is sequenced. The genomes will be used as a map to identify strain specific differences. Cumulatively, the sequence data will provide information on which phytoplasma species are present in Washington state, which stone fruit trees they are present in, and how many genotypes of each phytoplasma are present. Using that genomic data, areas of the genome that diverge between isolates can be selected for the development of genotypic markers for that can be amplified and analyzed by Sanger sequencing or SSCP analysis. This will allow for the tracking of genotypes in fruit trees by species and geographic location, providing information on how widespread these pathogens are and where they are a problem.

2. Identify physiological markers associated with the disease by comparing fruit and phloem tissues of infected and healthy trees.

   The screening in objective one will allow for the identification of infected trees from which we will conduct observations to determine the effects of different phytoplasmas on tree growth and fruit development. Tree growth, vigor, leaf shape and time of leaf drop will be assessed throughout the growing season. Fruit size, shape, and color will be assessed by comparing fruit between healthy and diseased trees. Sugar content of fruit, which is often affected in phytoplasma infected plants, will be determined using a sucrose/D-fructose/D-glucose assay. Finally, phloem sections from healthy and diseased trees will be compared using microscopy, as phytoplasmas can cause physiological abnormalities in this tissue. Assessing these tissues will determine the pathogenicity and virulence of identified phytoplasma species, and in which tree species they are a problem.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.
   The role of multiple infections in disease development will be assessed using a transcriptomics approach and will be compared to single-infected trees and healthy trees. RNA will be isolated from the leaf and midrib tissue of healthy and infected trees, libraries prepared, and NGS performed. Differential gene expression analysis will identify genes that are upregulated or downregulated in infected trees. This will be paired with the physiological data collected during objective two to identify differentially expressed transcripts that may have a role in symptom development, allowing the future development of disease markers and/or disease tolerance in breeding programs.

RESULTS AND DISCUSSION

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present by high throughput sequencing.

A survey of stone fruit revealed two phytoplasmas in the area: X-disease and pear decline. X-disease phytoplasma is a problem in cherries, peaches, nectarines, and plums. The pear decline phytoplasma was found in peaches, nectarines, plums, and pears. These phytoplasmas have been found from Pasco to Yakima and as far north as Wenatchee. Apricots were also included in this survey, however, none, except for one tree exhibiting dieback symptoms, were positive for phytoplasma. A small number of Italian prunes were also screened and these were negative. Trees that exhibited disease symptoms (Figure 1) but did not test positive for phytoplasma will be sequenced. These samples may

have been negative because the phytoplasma was present at low titer or because existing assays do not detect these phytoplasmas.

Figure 1. Tree exhibiting phytoplasma symptoms such as witches' broom that did not test positive for the presence of a phytoplasma.

Sequencing of the phytoplasma genomes has begun. Nectarines that were positive for X-disease and/or pear decline were selected for sequencing. DNA sequencing allowed for the assemblies of large contigs for each genome. For X-disease, approximately 444 kb was obtained, spread out across five contigs. This built upon the existing genome, which is fragmented. For pear decline, no genomic sequence is available. A 103 kb sequence that did not match X-decline but did match other phytoplasmas is thought to be part of the pear decline genome. For both phytoplasmas, more genomic sequence must be obtained. To address this, we will use rolling circle amplification to preferentially amplify the phytoplasma DNA. These samples will be submitted for sequencing by PacBio, which generates long reads. These long reads will allow for the assembly of more complete genomes for both phytoplasmas. With genomes available, Illumina sequencing of additional samples will allow for genotyping and an estimate of the number of strains present in Washington for both phytoplasmas.

2. <u>Identify physiological markers associated with the disease by comparing fruit and phloem tissues of infected and healthy trees.</u>

X-disease and pear decline were the two phytoplasmas found in Washington state stone fruit this year. X-disease symptoms are well known in cherry, producing small cherries with poor taste. In peaches, nectarines, and plums, we found that X-disease and pear decline were often present in the same trees. In peaches and nectarines infected with both phytoplasmas, we observed yellowing of the leaves, shot-holing in leaves (characteristic of X-disease), swelling of midveins (characteristic of pear decline), and misshapen fruit that often exhibited delayed ripening or failed to ripen (Figure 2). In plums, we observed some leaf yellowing, swelling of midveins, and small, misshapen fruit (Figure 3).

Figure 2. Yellowing and shot-holing of leaves of nectarine trees infected with X-disease and pear decline phytoplasmas. Fruit appears misshapen, sometimes wrinkled, and does not ripen at the same time as healthy trees.

Figure 3. Plum tree infected with both X-disease and pear decline phytoplasmas. Leaves show yellowing and swelling of midveins. Fruit varies in size, sometime small and wrinkled.

Peaches, nectarines, and plums were collected from healthy and infected trees. The fruit was pureed in a blender, filtered twice through cheesecloth, and stored at -80°C. Sugar and metabolite analysis is ongoing. Glucose, fructose and sucrose concentrations have been determined for a small number of fruit collected from symptomatic and asymptomatic plums, peaches, and nectarines. In plums, there was no significant difference in sugar content between asymptomtic and symptomatic trees (Figure 4). In peaches and nectarines, two asymptomatic trees and three symptomatic trees were examined (Figure 4). While there was a drop in glucose and sucrose between symptomatic and asymptomatic Honeyhavens, this is too small a sample size to be conclusive. Next year a larger number of fruit will be collected for analysis. This analysis will include characterization of size and shape abnormalities as well as sugar and metabolite content. Analysis of the phloem tissue was not performed this year, but is planned for next year.

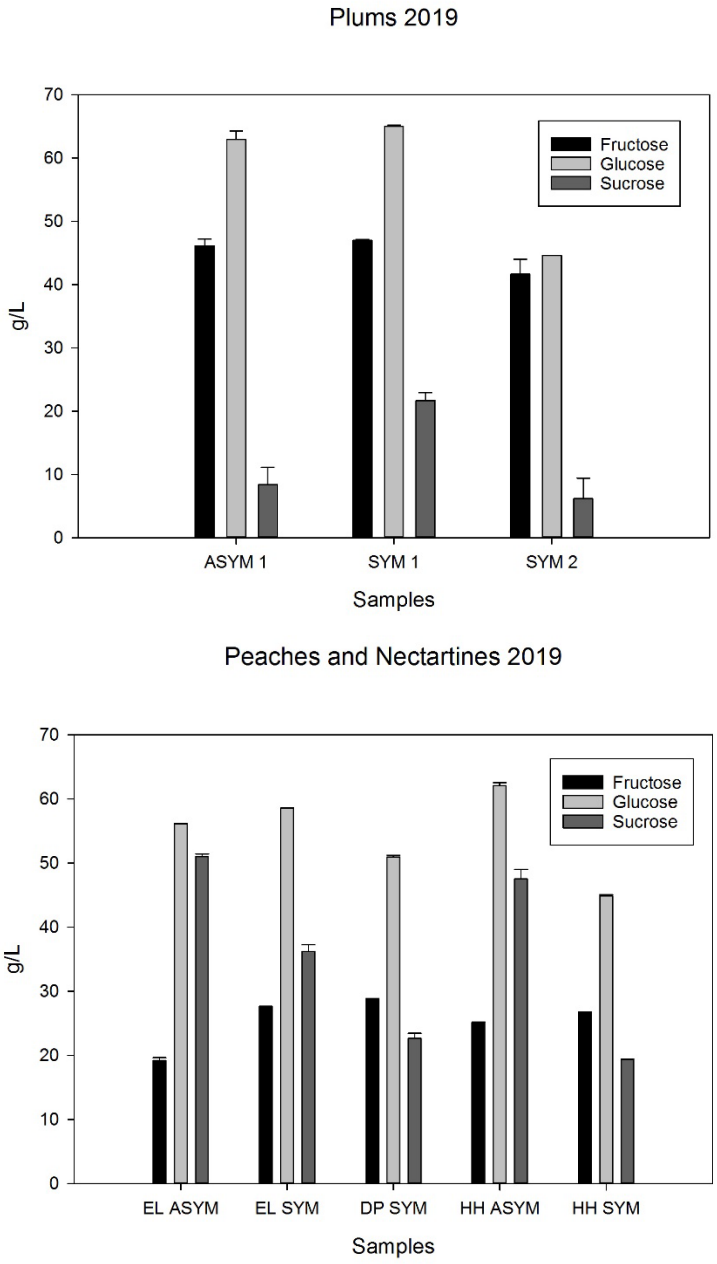

Figure 4. Fructose, glucose and sugar content in asymptomatic and symptomatic plums, peaches, and nectarines. Abbreviations are: Elegant Lady, EL; Diamond Princess, DP; Honeyhaven HH.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.

Tissue from healthy and infected trees was collected this summer and stored at -80°C. Over the winter, RNA will be extracted from leaf tissue and submitted for RNA-seq. Differential gene expression analysis will be performed to identify which pathways might be affected by the presence of the phytoplasma.