FINAL REPORT **PROPOSED DURATION**: 1 Year

Project Title: Towards identification of LCD linked volatile biomarkers

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Equipment cost match: $60,000 (FAIMS-Lonestar VOC Analyzer, Owlstone Medical, UK)

Total Project Request: **Year 1: $62,310** **Year 2:** **Year 3:**

Other funding sources: **None**
Amount:
Agency Name:
Notes:

WTFRC Budget: none

Organization Name: WSU-IAREC **Contract Administrator: Katy Roberts**
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Item	2021	2022
Salaries	37,800	
Benefits	13,212	
Wages		
Benefits		
Equipment		
Supplies	7,750	
Travel	3,548	
Miscellaneous		
Plot Fees		
Total	62,310	0

Footnotes: 9-month salary support ($37,800 plus $13,212 benefits) for a postdoctoral researcher is requested. Postdoc will work closely with the PIs in planning and conducting experiments, data analytics and reporting. $3,500 requested to procure scrubber(s) for existing FAIMS unit along with recalibration. $3,500 engineering plant volatile trapping system, and towards procurement of specialty jars for VOC trapping, Teflon caps and tubing, labels, gloves, zip ties, chem-wipes as well as analytical standards needed to confirm VOC biomarkers. $750 is requested towards procurement of N2 gas ($75/tank). $348 is requested for field sampling related travel (20 miles x 30 trip x $0.58/mile) and $1,000 towards extension outreach activities. $2,200 is requested for Postdoc to participate in regional/international (within US) conference to share project outcomes.

Objectives.
#1. This project aims to screen infected and uninfected cherry plant parts, i.e. limb, leaves, stem and fruit tissues of the highly susceptible 'Bing' cultivar at different growth stages to identify potential volatile biomarkers associated with X-disease and/or LCD infection. Once biomarkers are identified, the platform will be trialled in controlled and field environments.

#2. Pertinent technology and finding will be communicated to the industry by an array of outreach and extension methods; including a technology demonstration or video/webinars, grower meetings, and the tree fruit newsletter "fruit matters".

For this continuation report, we have focused reporting on following two specific aims:

1. To evaluate feasibility of portable FAIMS towards LCD symptoms detection; and
2. To identify the earliest pre-symptomatic growth stage where LCD symptoms detection is possible with FAIMS system.

Significant findings

- A portable FAIMS system could detect LCD symptoms from field samples of 'Bing' Cultivar starting shuck fall to the post-harvest growth stages. System could also detect the symptoms for *cv*. Benton, Tieton and Cristalina at post-harvest growth stage (field as well as greenhouse samples).
- The FAIMS also detected the LCD symptoms from root tissue samples collected at post-harvest stage (*cv*. Benton and Skeena).
- The third ion current peak (see fig. 3; in the CV-DF ranges of -0.72 to 0.51 V & 72 to 98%) was consistent distinguishing feature in the spectra for infected samples but not for the healthy samples.
- The ion current for the infected samples was consistently higher than the healthy samples for identified significant CV-DF combinations.

Industrial and economic significance. Findings of this study suggest that it would be possible to achieve high throughput detection of LCD symptoms using a portable FAIMS system starting pre-symptomatic growth stages. The FAIMS system could thus be useful as a complimentary LCD confirmation tool in the laboratory along with qPCR. Robust evaluation: for additional larger datasets at each of the growth stage for a given cultivar and 2) different susceptible cultivars needs to be performed, before industry considers using such system for high throughput and reliable LCD symptoms detection.

Methods
Sample preparation. **Aim 1**. The shoot limb samples of cherry trees were collected for the post-harvest growth stage from an orchard located in Buena, WA (*cv*. Benton). The samples sized approximately 15 cm in length and comprised of leaves and stems. The samples were collected from six trees of which three were confirmed with LCD infestation, and three with no detection (Healthy). These confirmations were provided by the WSU-Clean Plant Network (WSU-CPN) based on the molecular analysis (qPCR) in the previous growth season (2020).

Each sample contained four limb units collected randomly from the trees and four replicate samples were collected per tree. As a reference to the field samples, samples were collected from two confirmed negative (Healthy) trees of the same cultivar managed in a green house facility.

Aim 2. The shoot limb samples of cherry trees (*cv*. Bing, size: same as above) were collected at the flowering, shuck fall, pit hardening, first straw, and harvest growth stages from an orchard located in Wapato, WA. The limbs included flowers at flowering stage, some flower petals at shuck fall stage, light green fruits at pit hardening stage, yellowish fruits at first straw, and matured fruits at the harvest stage. Leaves and stems were present in all above samples at all the stages. Total nine trees were selected in the orchard; of which, six were confirmed with LCD infestation, and three with no detection. As in objective 1, these confirmations were provided by the WSU-CPN. Three replicate samples were collected randomly from each selected tree. Similar to objective 1, the reference samples were collected from confirmed negative trees of the same cultivar managed in a green house facility of the CPN. The samples considered for Aim#1 were also included in this objective for postharvest growth stage analysis.

The collected samples were kept in sanitized glass jars of 1 gal and sealed with a cling film wrap to allow aerobic respiration. The sealed jars were then stored for a duration of 3 hours for volatile headspace accumulation. Post the storage period, the volatile headspace of the jars was sampled using a portable FAIMS system.

Volatile sampling. Post the storage duration of each sample, the cling wrap was removed, and the jar was immediately covered with a Teflon lid (fig. 1). The lid had two openings of which one was connected to the carrier gas cylinder (inlet) and the other connected to the ionization chamber of the FAIMS (outlet) through Teflon tubes. Nitrogen was used as a carrier gas that streamed at a flow rate of 1.5 L/min and pressure of 50 kPa inside the jar through the inlet to push the accumulated headspace with volatiles through the outlet into the ionization chamber (fig. 1). These volatiles gain charge in the ionization chamber and then move and deflect in proportion to their mass, under the influence of a dispersion field (DF or electric field) and compensation voltage (CV or electric potential). Such movement/deflection creates ion current spectra. Total six ion current spectra were collected for each sample jar and pertinent ion current spectra files were saved in the FAIMS computer. These files comprise of ion currents for a total of 26,112 CV-DF (512×51) combinations.

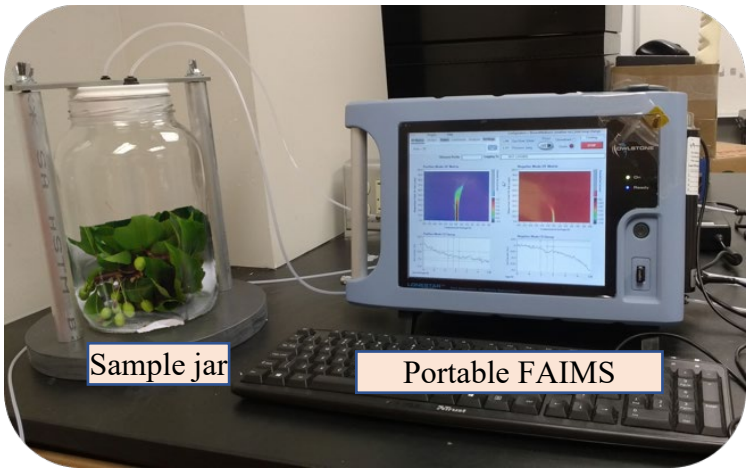


Figure 1. Volatile headspace sampling of cherry samples using a portable FAIMS system.

Data analysis. The data analysis steps are summarized in figure 2. The ion current spectra files were extracted into "*csv" format for further analysis. For each sample, two middle ion current spectra were used during the analysis. The current patterns were initially evaluated to identify the distinctness between the LCD positive (Infected) and non-positive samples (Healthy). Based on initial visual observations, a consistent threshold filter was applied to extract the ion current peaks for the two sample types. A region of interest (ROI) was then fixed for a range of CV and DF for all the samples. The ion current feature in this ROI was extracted for each ion current spectra and statistical difference in their magnitudes was evaluated between the infected and healthy samples. Next, a principal component analysis (PCA) was conducted to recognize the differences between the two sample types. All such analyses were first conducted for the field samples and were then contrasted with the green house samples.

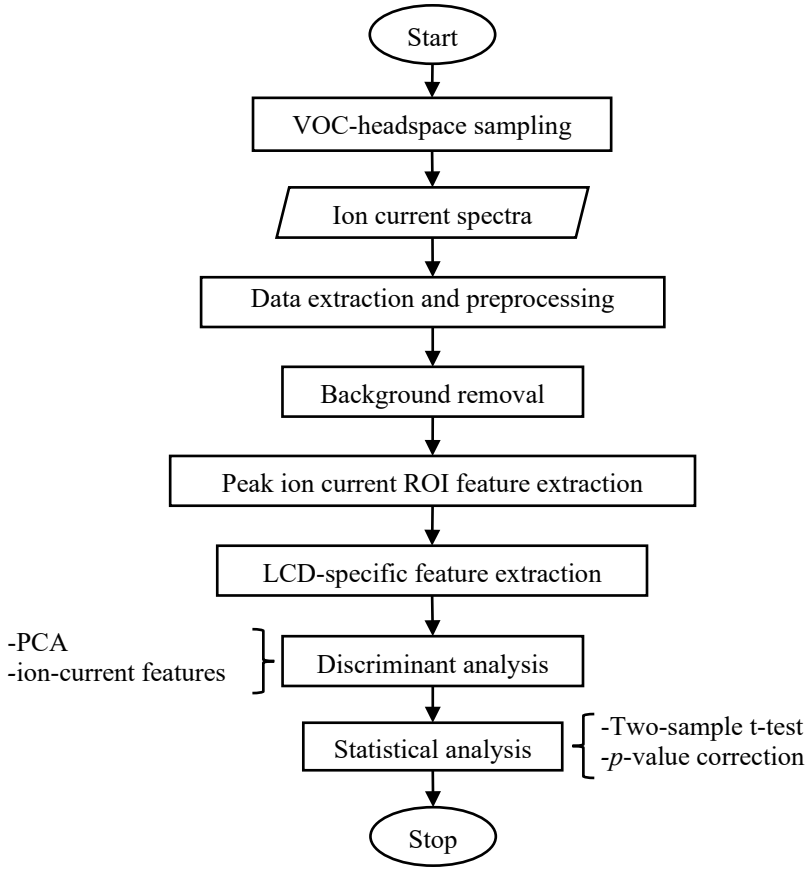


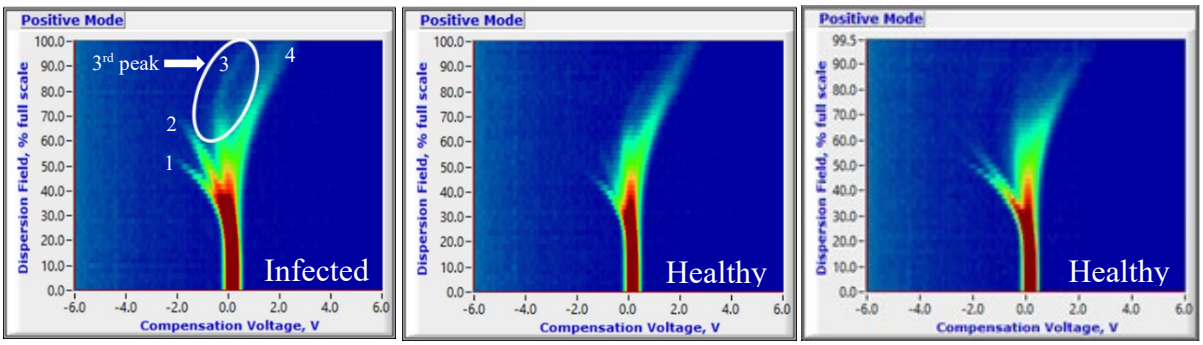
Figure 2. Data analysis pipeline for evaluating FAIMS for LCD detection.

Results and Discussion

<u>Aim 1</u>. To evaluate feasibility of portable FAIMS towards LCD symptoms detection.

The raw ion current spectra derived as an output of the volatile-headspace sampling by FAIMS system were distinct for infected and healthy samples at postharvest stage. Herein, a third ion current peak was consistently dominant for the infected samples (See fig. 3a for CV (x-axis) range of -0.72–0.51 V, and for DF (y-axis) range of 72–98%). However, such peak was not observed in the ion current spectra for the healthy samples (fig. 3b). This observation suggests that the healthy samples may not display a third peak in the ion current spectra as was also observed by the ion current spectra for healthy reference samples from greenhouse (fig. 3c).

The processed ion current spectra obtained after noise removal from the raw spectra is shown in figure 4. Herein, the presence of ion currents in the fixed ROI (CV-DF ranges of -0.72–0.51, and 72–98%) confirms the above observations for LCD infected samples (fig. 4a). The absence or negligible ion currents in ROI for healthy samples also confirms the above observation for healthy samples (figs. 4b and 4c).



(a) (b) (c)

Figure 3. Ion current spectra for (a) infected and (b) healthy samples from the orchard and (c) healthy samples from green house (Postharvest growth stage).

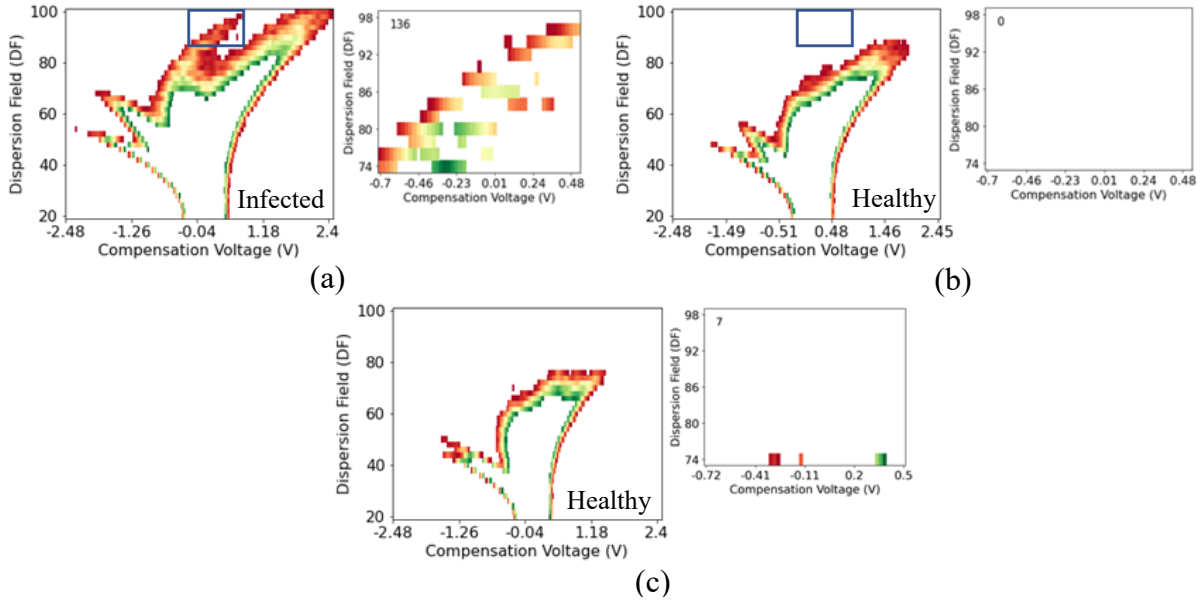


Figure 4. Filtered ion current spectra and features in the fixed region of interest for (a) infected and (b) healthy samples from field and (c) healthy samples from the greenhouse.

Post feature extraction, the magnitude of ion current for the infected samples was significantly and consistently higher than the healthy samples (fig. 5a). These ion currents for all the infected and healthy samples, when analyzed with PCA, showed distinct patterns (fig. 5b). Overall, FAIMS could be highly suitable for detection of LCD symptoms at postharvest stage. Also, about 40% of the total 26,112 CV-DF combinations (at 5% level) and 11% combinations (at 1% level) were critical and aided in distinguishing the healthy and infected samples.

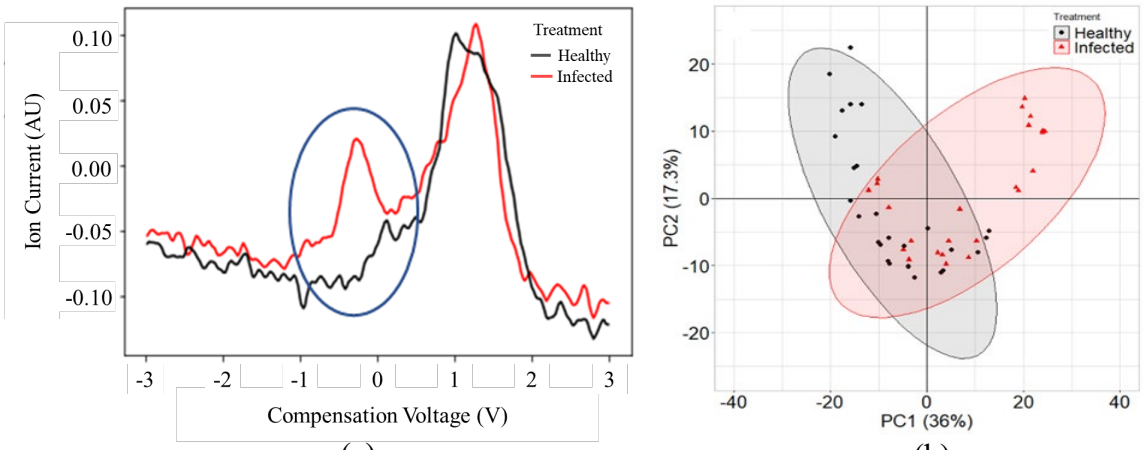


Figure 5. (a) Ion current magnitudes and (b) their pattern distinction for healthy and infected samples using principal component analysis.

<u>Aim 2</u>. To identify the earliest pre-symptomatic growth stage where LCD symptoms detection is possible with FAIMS. Similar to observations in objective 1, the third peak (as a dominant peak) was observed from shuck fall until postharvest growth stages (fig. 6). This peak initiated at shuck fall and strengthened in intensity with the crop growth stage. The peak was however inconsistent at the flowering stage (fig. 6a). With these preliminary observations, it can be inferred that LCD symptoms could be detected as early as at the shuck fall growth stage. Moreover, pertinent to the third peak, the ion current magnitudes were significantly higher for the infected samples compared to the healthy samples at all the growth stages (fig. 7).

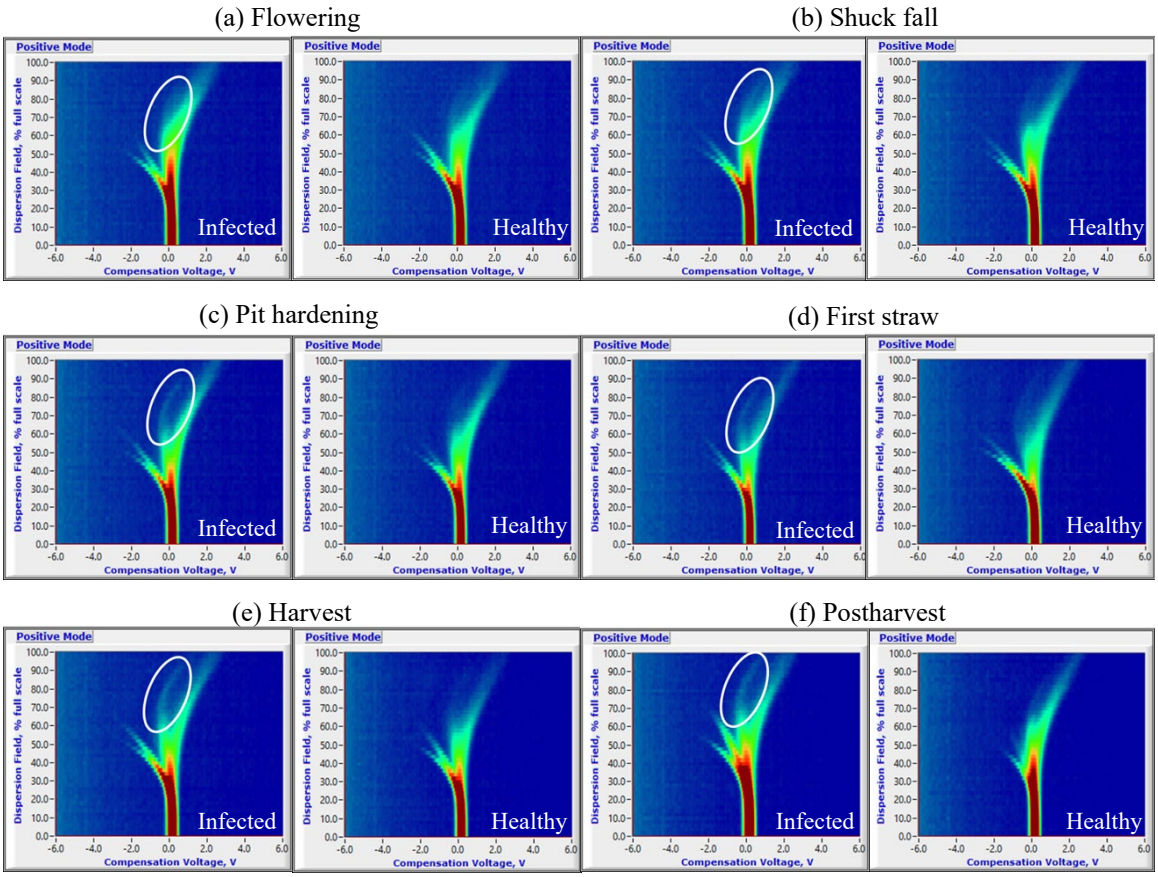


Figure 6. Raw ion current spectra plots for infected and healthy cherry samples collected from the orchard at (a) flowering, (b) shuck fall, (c) pit hardening, (d) first straw, (e) harvest, and (f) postharvest.

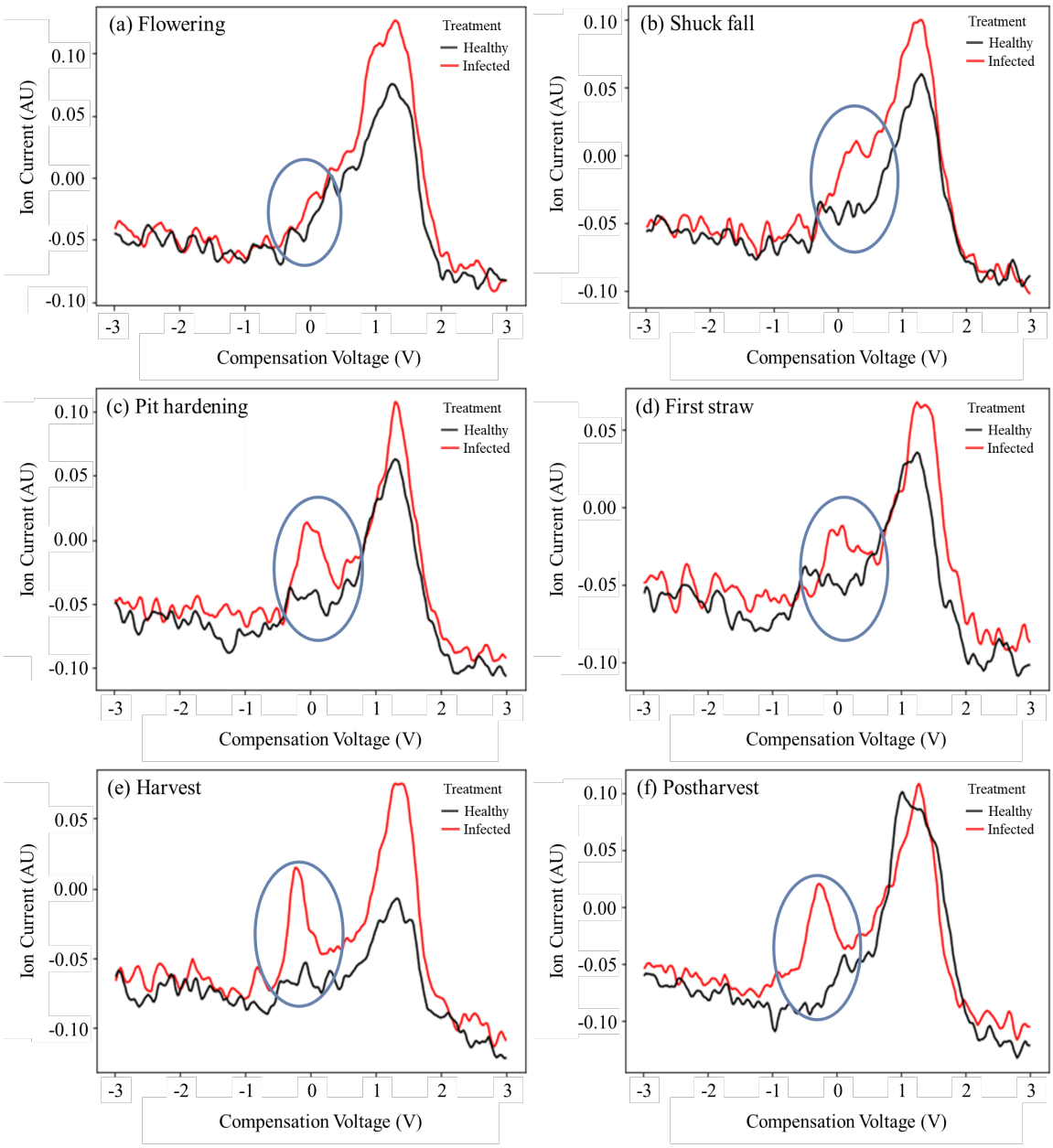


Figure 7. Ion current plots for the infected and healthy samples (differences highlighted in blue ellipse) at (a) flowering, (b) shuck fall, (c) pit hardening, (d) first straw, (e) harvest, and (f) postharvest.

Ongoing and future work:

Robust analysis of existing data, FAIMS system training w/identified CV-DF combinations: The FAIMS ion current data from the season 2020-21 identified the third ion current peak as a feature peak for the infected samples (*cv*. Bing). Additional data was collected to confirm the presence of this feature peak for cultivars: Skeena, Benton, Tieton and Crystalina. The feature peak was observed with different shape and intenisty in all the

tested cultivars. Our team is working on identifying the generalized range of CV-DF cominations that will be potentially used for early and rapid identification.

Collect new datasets: Through new two year project (2022-2024), plant samples including flowers, leaves, fruits and root tissue were collected from the field grown sweet cherry trees for 'Bing' and 'Skeena' cultivars. The FAIMS ion current data for the growing season 2022 confirmed that the signature peak is present in the infected samples.

Linkage with LCD detection dogs: Samples (stem) were collected for infected and healthy trees for the 'Skeena' cultivar at post-harvest stage in 2022 field season. These samples were analyzed using the FAIMS system as reported in the methods section. Samples were also collected from the same trees for LCD detection dogs. Our team is working on the data analysis and these efforts will be contrasted with the LCD detection dogs derived data.

Confirmation of volatile biomarkers release using GC-MS technique: Through new two year project (2022-2024), samples from the healthy and infected trees were collected for in-situ analysis using GC-MS system for the 2022 growing season ('Bing' and 'Skeena'). Results infers that Z-3-Hexenal and Z-2-pentenal are prominantly distiguishable and could be related to the LCD infection in 'Bing' cultivar. Analysis for 'Skeena' cultivar is on going along with additional method of volatile headspace sampling (partially destructive analysis method). These key volatile biomakers linked with the infected samples can be potentially used to 1) identify and develop a customized volatile sensing system, 2) develop FAIMS based detection alert system, and 3) to train the LCD detection dogs.

Executive summary

Little cherry disease (LCD) has been critically affecting the sweet cherry (Prunus avium) industry in the Pacific Northwest. Therefore, this study aimed at evaluating a high throughput field asymmetric ion mobility spectrometry (FAIMS) system towards early detection of the LCD infection of sweet cherry. Total fifteen trees were selected in two cherry orchards at Wapato, WA (*cv*. Bing) and Buena, WA (*cv*. Benton) which were confirmed as infected and healthy by the WSU Clean Plant Network. Shoot samples that included flowers, leaves, fruits, and stems were collected from the selected trees in each of the ***six*** growth stages: flowering, shuck fall, pit hardening, first straw, harvest and postharvest. Collected samples were stored in 1-gallon glass jar for three hours for volatile headspace accumulation. Post-storage period, accumulated headspace was sampled with FAIMS, and ion current spectra were acquired for each sample jar. A consistent presence of third ion current peak was observed in the spectra for infected samples but not for the healthy samples. Such infection-specific peak was observed from as early as shuck fall growth stage. Those peaks were present for compensation voltage (CV) range of −0.72–0.51 V and dispersion field (DF) range of 72–98% for all the growth stages. Pertinent to those peaks, the ion current for infected samples was significantly higher compared to healthy samples (Two-sample t-test, $p < 0.05$). Such observations were also supported by the healthy samples collected from greenhouse grown trees. A Principal component analysis showed the distinctness in the patterns formed by the infected and healthy

cherry samples. Similar findings were observed from the 2022 season data for infected and healthy samples from ‘Bing’ and ‘Skeena’ cultivar. The presence of third peak (CV: −0.66–0.55 V and DF: 70–98 %) was observed consistently in the ‘Bing’ cultivar whereas for ‘Skeena’ cultivar it was somewhat inconsistent. Overall, a portable FAIMS system was able to detect LCD infection symptoms at a high throughput rate and from pre-symptomatic growth stages. With robust databased investigation, portable FAIMS systems can be trained using the common features (e.g. third peak) for alarm-based alerts which could assist in timely identifying the LCD infestation in sweet cherry orchards.

Keywords: Little cherry disease, FAIMS, High throughput capacity, Ion current features, Alarm-based alerts.

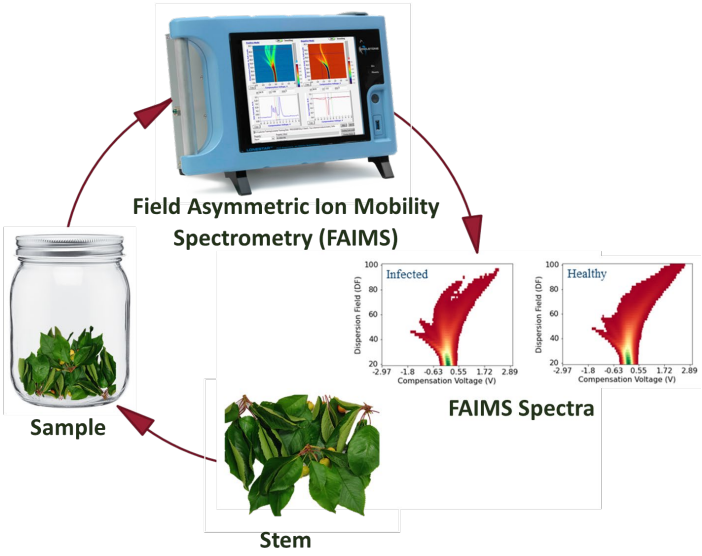


Figure 8. Schematic of little cherry disease detection using a portable FAIMS system.