Towards the identification of Little cherry disease linked volatile biomarkers

Report Type: Final Report

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Item	2022	2023	2024/2025
Salaries	46,200	48,048	
Benefits			
Wages			
Benefits	16,014	15,120	
Equipment			
Supplies	15,120	16,655	
Travel	2,697	2,697	
Miscellaneous			
Plot Fees			
Total	80,031	82,520	0

Footnotes: Year-1: 11-month salary support ($46,200 plus $16,014 benefits) for a postdoctoral researcher is requested. Postdoc will work closely with the PIs in planning and conducting experiments, data analytics and reporting. $1,500 requested to procure FAIMS sampling experiment lab consumables such as PTFE tubing, sampling glass jars, gloves, chem-wipes, headspace trapping cling films and N2 carrier gas. $7,120 requested to procure Tedlar bags ($178/ pack of 10 bags x 40 pack) for plant volatile trapping, and $5,000 towards PI-Ruddell's GC/MS consumables and maintenance. $2,697 is requested for field sampling related travel (150 miles /trip x 20 trips) as well as GC/MS analysis travel from Prosser to USDA-ARS Wenatchee, WA (5 nights per diem + 270 miles/trip). PI Serban requests $1,500 towards extension outreach activities. **Year-2**: request is similar to Year 1 expect salary rate is adjusted by 4% per WSU policies. **Year-3.** Approved No Cost Extension until 12/31/2025.

Objectives 1. Volatile biomarker-based early X-disease and LCD infection detection for 'Bing' and 'Skeena' cultivars using Field Asymmetric Ion Mobile Spectrometry (FAIMS) technique, 2. Develop a comprehensive understanding of associated volatile biomarkers release using GC/MS technique, and 3. Conduct pertinent extension education and technology demonstrations.

Significant Findings. This project evaluated FAIMS technique (objective 1) for early detection of LCD/X-disease in 'Bing' and 'Skeena' cherry cultivars by analyzing volatile profiles across seasons and different growth stages. The pit hardening stage consistently showed significant differences in volatile emissions between symptomatic (S) and asymptomatic (AS) samples, with AS samples having higher ion current values in both cultivars. Temporal variations were also observed at other stages, though less consistently, indicating the importance of the pit hardening stage for early detection of X-disease. Cultivar-specific differences and environmental conditions across seasons also influenced volatile emissions. Project also identified key volatile biomarkers, using GC-MS static and dynamic sampling technique (objective 2), associated with the X-disease infestation. Potential biomarkers include, Ethanol, (E)-2-hexenal, propanoic acid, and acetone, ethyl acetate, (Z)-2-penten-1-ol, (Z)-3-hexen-1-ol*, 2-hexenal, acetaldehyde, acetoin. *Practical Implications*: 1) *Early Detection*: Identification of the pit hardening stage as critical for detecting X-disease, 2) *Targeted Monitoring*: Growers can focus on non-destructive (e.g., Canine based) scouting efforts during the pit hardening stage, 3) *Biomarker Utilization*: The volatile biomarkers identified in this project can be used to develop more precise diagnostic tools, enhancing the accuracy of X-disease detection, 4) *Cultivar-Specific Strategies*: Understanding cultivar-specific differences in volatile emissions can help tailor disease management practices to specific cherry varieties, improving overall crop health, and 5) *Seasonal Adaptation*: Recognizing the influence of environmental conditions on volatile emissions enables growers to adapt their monitoring and intervention strategies according to seasonal variations.

Methods

Field sampling and preparation. Table 1 summarizes details about field sites and objective/analysis technique specific sampling during the three-year (2021-2023) project period. In each case, samples from the lower canopy zone of sweet cherry trees were collected from orchard sites located in Central Washington. The samples were typically collected at the key growth stages: flowering, shuck fall, pit hardening, first straw, harvest, and post-harvest for the commercially grown cultivars (Table 1). Trees in the experiment were selected based on the preliminary molecular analysis done by PI-Harper's lab (detailed below) and symptomology. Trees and branches were labeled with colored tape and labels for consistent data collection throughout the experiment duration. Sub-sections below detail methods specific to each of the volatile analysis technique.

Molecular analysis (qPCR). Stem samples were collected for conducting qPCR-based molecular analysis as a validation of the volatile sensing approaches. For all sites, samples were collected within a similar time frame (see table 1). Post-sample collection, a small section of stem from each branch was used for phloem tissue extraction. These extracted tissues were chopped using a razor blade and stored in a bead-beating tube. For further processing, 0.1 g tissue sample was scaled in labeled tubes. Similarly, the plant tissue stored at -80 °C was also weighed in labeled tubes (GC/MS samples). The Nucleic acids from these samples were extracted using the cetyltrimethylammonium bromide (CTAB) extraction method. Resulting samples were stored at -20 °C. The amplified samples were used as the template for the qPCR reaction. All the samples were diagnosed with X-disease phytoplasma (XDP), Little Cherry Virus-2 (LChV-2), Prunus Necrotic Ringspot Virus (PNRSV) and Prunus Dwarf Virus (PDV). The bacterial canker symptoms were

observed based on visual scouting of the trees at the time of sampling. The results of the molecular analysis were used for comparative analysis.

Table 1. Field sites and volatile headspace analysis sampling details.

Season	Site	Cultivar	Growth Stage	Analysis Method*	Trees Sampled** S	Trees Sampled** AS
2021	Site 1	Bing	Flowering	1,4	6	3
			Shuck fall	1		
			Pit hardening	1		
			First straw	1		
			Harvest	1,4		
			Post-harvest	-		
	Site 2	Benton	Post-harvest	1,4	12	12
	Site 3	Cristalina	Post-harvest	1,4	3	3
		Tieton	Post-harvest	1,4	3	3
2022-23	Site 4	Bing	Flowering	1,3,4	5	4
			Shuck fall	1,4		
			Pit hardening	1,4		
			First straw	1,2,3,4		
			Harvest	1,2,3,4		
			Post-harvest	1,4		
	Site 5	Skeena	Flowering	1,3,4	4	4
			Shuck fall	1,4		
			Pit hardening	1,4		
			First straw	1,3,4		
			Harvest	1,2,3,4		
			Post-harvest	1,4		

*1: FAIMS; 2: GC-MS-static; 3: GC-MS-dynamic; 4: qPCR technique; S: Symptomatic; AS: Asymptomatic

**1 replicate/tree for FAIMS and GC-MS dynamic sampling technique, 5 replicate/tree for GC-MS static sampling technique.

Field Asymmetric Ion Mobile Spectrometry (FAIMS). Field collected samples were stored in 1-gallon glass (sterilized) jars. Each sample jar was covered with a food-grade cling film for aerobic storage conditions (Fig. 1). Samples were then analyzed using a portable FAIMS system using a custom-developed unit (Arasaradnam et al., 2016; Kothawade et al., 2021). The glass jars were covered by a Teflon lid having two stoppers with two holes, one as an inlet for the carrier gas source (nitrogen air) and another to flush out the volatiles to the FAIMS ionization chamber. A total of six scans were collected for each sample jar at the optimized operation parameters (flow rate: 1.5 L min^{-1} and pressure: 60 kPa). The FAIMS unit takes ~4 minutes to collect these six scans (i.e., 60 s/scan). Blank jar was scanned as a reference in data analysis for each sampling day.

Figure 1. *Portable FAIMS system analyzing cherry leaves volatile profile.*

The FAIMS scans output is an ion current spectrum that is proportional to the mass of distinct volatile organic compounds (VOCs) under a range of dispersion fields (DF: 0 to 100%) and compensation voltages (CV: -6 to 6V). The ion current spectrums from all scans generate three-dimensional data consisting of 51 DFs, 512 CVs, and resultant ion currents (I_c, arbitrary units, AU). The system needed to be purged for about 40 minutes using nitrogen air before scanning the next sample jar to remove the residues from the previous sample. The purge time can be shortened in future, commercial use, by reducing sample size, i.e., introducing a lesser amount of VOC headspace to the unit.

Data analysis. Raw FAIMS scans from the volatile's headspace analysis were extracted to *.csv file format. Noise removal and feature engineering was then performed to identify the important features. A threshold value (I_c> 0.02) was, determined using histogram-based thresholding, used to remove background noise. Subsequently, a total of four generalized regions of interest (ROIs) were defined based on the I_c clusters and were used to extract the sum of I_c (I_{sum}) at all CV-DF combinations. I_{sum_all} as sum of entire spectra was also computed as a feature. Additional features such as, maximum ion current (I_{max}) and area under the curve (I_{AUC}) were extracted at each DF within a given range of CV for the DF specific analysis. A scanline at a given DF value and CV range is a 2D representation of the spectra. The I_{AUC} was calculated for each DF value and compressed along with the sample names as rows and DF values in columns and respective I_{AUC} value. The I_{AUC_sum} feature was then used for temporal analysis of the VOC profiles over the growth stages. The number of phytoplasma copies in the sample were calculated using a calibration curve and quantification cycles (Cq) values (Wright et al., 2022) from qPCR results. Correlation analysis was performed between important features and the number of phytoplasma copies in a sample. All the analyses and visualizations were conducted in Python software (version 3.6, Python Software Foundation, Wilmington, DE, USA) packages using libraries, 'NumPy', 'pandas', 'seaborn', 'sklearn' and 'matplotlib'.

Gas Chromatography-Mass Spectrometry (GC-MS). Experiments were conducted during 2022 and 2023 growing seasons. For the 'Bing' cultivar, samples were collected from a commercial orchard located near Sunnyside, WA. For the 'Skeena' cultivar samples were collected from the orchard at the WSU Roza experiments block in Prosser, WA. All samples were collected from the lower canopy zone. From each tree, five leaves were collected per replicate, with five replicates per tree for both symptomatic and asymptomatic trees.

Static headspace volatile analysis was conducted at the first straw and harvest stages. Figure 2 depicts the typical sampling and analysis protocols. For the sample collection, 50 ml centrifuge tubes were used to store the samples (5 leaf/replicate). Liquid nitrogen was used to flash freeze the samples. These samples were then stored in dry ice until moving them to the -80 °C facility. Prior to volatile analysis, scaling of ground plant tissue (0.5g) in 20 ml glass vials was performed in a box filled with liquid nitrogen to avoid sample thawing at room temperature.

HPLC water, 5-Hexen-1-ol, and Isopropyl butyrate were used to prepare an internal standard (ISTD) during further extraction for volatile headspace analysis using GC-MS technique. A polystyrene box filled with liquid nitrogen (1" covering the bottom) was then used to store an aluminum block with samples. Next, 10 µl ISTD and 1 ml NaCl saturated solution were added to the samples, and all samples were incubated at room temperature, followed by sonication and agitation. Volatile headspace was adsorbed onto glass traps packed with Tenax TA porous polymer (TDU tubes, Gerstel, Linthicum, MD, USA) (Lee et al., 2002; Hewavitharana et al., 2019). Thermally desorbed analyte was injected and analyzed using Agilent (Santa Clara, CA, USA) 6890/5975 GC/MS equipped with a Gerstel (Baltimore, MD, USA)

Multipurpose Sampler (MPS), Dynamic Headspace Sampler (DHS), and Thermal Desorption Unit (TDU) (Rudell et al., 2009; Hewavitharana et al., 2019).

In case of *dynamic volatile headspace analysis*, samples including leaf tissue were collected from the same sites as reported above and stored in the 1-gallon glass jars. The volatile headspace from the sample jars was trapped on the adsorbent Tenax TA packed in glass collectors using a vacuum pump. The sorbent tubes were then analyzed using the GC-MS system as reported above.

Data analysis. Deconvolution and peak identification of volatile biomarkers was performed using quantitative analysis software (Quant. 10.1, Agilent Technologies, Palo Alto, CA, USA). Compounds were identified using custom libraries containing retention indices and mass spectra. The data was compared with standard spectral catalogs (NIST05) and authentic standards. Compounds without definitive matches were labeled as 'tentative' or 'unknown' along with their spectral information. Raw data of VOCs relative abundances were preprocessed, including normalization and missing value imputation. Samples were then classified as asymptomatic (AS) or symptomatic (S) based on qPCR results. Partial Least Squares-Discriminant Analysis (PLS-DA) was used for classification, evaluating model performance with cross-validation and R2/Q2 metrics. To identify influential VOCs, used was Variable Importance in Projection (VIP) scores, considering compounds with VIP scores >1 as significant. Further validation was performed using these findings using ANOVA and Tukey's Honest Significant Difference test ($p < 0.05$). The relationship between significant VOC abundances and pathogen infection levels were analyzed using Pearson's correlation analysis, with significance levels set at $p < 0.05$ and $p < 0.1$. All the analysis were performed in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/), R (version 3.6.1, R Core Team 2022, Vienna, Austria, and R Studio Inc., Boston, MA, USA).

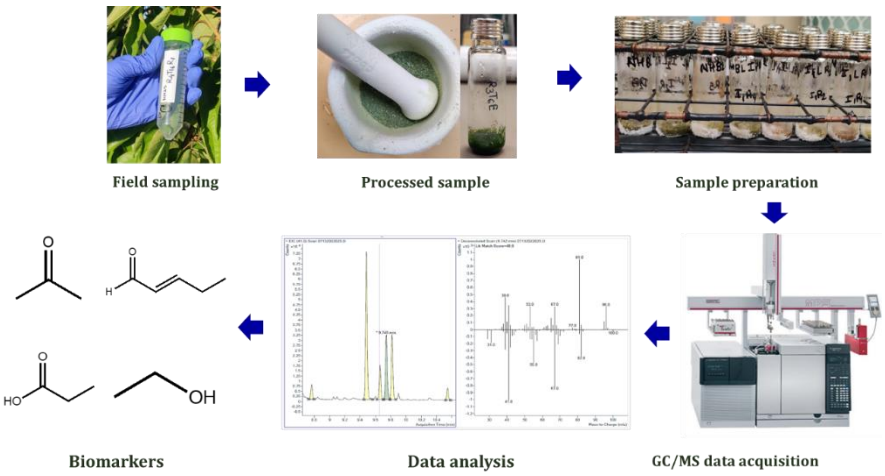

Figure 2. *Workflow of destructive headspace sampling combined with GC/MS analysis for volatile biomarkers identification.*

Results and Discussion

Objective 1. Volatile biomarker-based early X-disease and LCD infection detection for 'Bing' and 'Skeena' cultivars using the FAIMS technique.

Feasibility of the volatile-based detection of LCD/X-disease was evaluated on Benton cultivar samples at post-harvest growth stage of 2021 season. Typical spectra (positive polarity) for a representative LCD/X-disease symptomatic (S) and asymptomatic (AS) samples is shown in Figure 3. Three I_c peaks (clusters) were observed in AS samples while an additional peak curving to the left and then top (labelled as signature peak; CV: -0.11 to 0.51 V and DF: 64–84 %) was present in S samples (Fig. 3a). FAIMS spectral dataset, combined for all seasons for given cultivar and given growth stage, was processed to extract ion current sum (I_{sum}), maximum ion current (I_{max}) and area under the curve (I_{AUC}). Higher ion currents were observed at selective regions in the FAIMS spectra for S samples compared to AS samples ($p < 0.05$). Such

discrimination was prominent at ion current features at different CV- DF ranges of -3 to 3 V and 20–98 %. The ion current plots at the identified DF ranges also confirmed a clear distinction between the symptomatic and asymptomatic samples. Cultivar-specific variation in the ion current spectra was observed in all the samples.

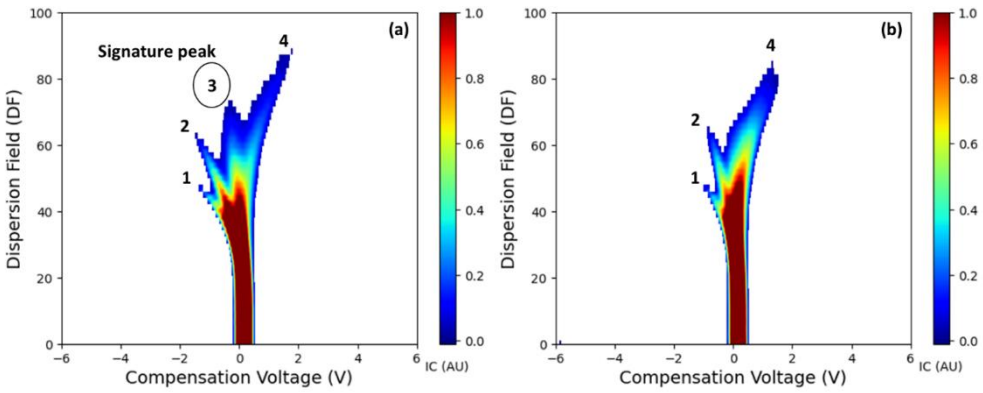

Figure 3. *FAIMS spectra with volatile headspace signature specific to (a) LCD/X-disease symptomatic, and (b) asymptomatic samples for 'Benton' cultivar. The potential signature peak observed in infected samples is highlighted with a circle.*

Validation with other host species of LCD/X-disease. The FAIMS spectra in the LCD/X-disease infected samples were observed distinct from the AS samples for the other host species, such as peach and nectarines. While LCD/X-disease infection in peaches and nectarines exhibits visual symptoms on both leaves and fruits, and trees may exhibit decline with time and eventually die. The FAIMS spectra obtained from these hosts showed distinct characteristics compared to the spectra of sweet cherry samples. The maximum I_c values in both peaches and nectarines were lower than those observed in sweet cherry samples. Specific to peach samples, a distinct peak was observed in S samples and could be a signature peak for LCD/X-disease. This confirms the diagnostic potential of FAIMS-based detection in other host species of these diseases. While similar observations could be inferred from the nectarine samples, the limited number of samples prevented further conclusions from being drawn.

Early detection feasibility

Bing Cultivar. The FAIMS spectra for the S and AS samples varied between the growth stages and across the seasons. FAIMS derived features I_{AUC_sum} for the respective ROIs were used for the temporal analysis of the volatile profile of the cherry stem samples. The Box–whisker plot (Fig. 4) shows the comparison between the S and AS samples. It illustrates the temporal variations in FAIMS-derived I_{AUC_sum} features for ROIs 3 and 4 across the three growing seasons (2021, 2022, and 2023). The data indicates that the volatile profile varies throughout the different growth stages. Significant differences between the S and AS samples were more prominent during the pit hardening stage across multiple years, where AS samples tend to have higher I_{AUC_sum} values. At the flowering and shuck fall stage, variations were observed but with less consistency across the years. This variability could potentially be linked with the titer level, symptom expression and dynamic nature of the disease. Overall, both the 2022 and 2023 seasons were not environmentally favorable for XDP symptom development in *Prunus* species. Specifically, 2022 was characterized by cold/freeze in early spring and 2023 season had high temperatures in late June. This might have impacted on the season-specific variation of volatiles release.

Skeena Cultivar. The box-whisker plots (Fig. 5) depict the temporal variation in the FAIMS-derived I_{AUC_sum} features for the ROIs 3 and 4 in Skeena samples across the 2022 and 2023 seasons. The volatile profiles of S and AS samples vary across different growth stages. The pit hardening stage consistently showed a significant difference between the S and AS classes, particularly in 2023, where AS samples

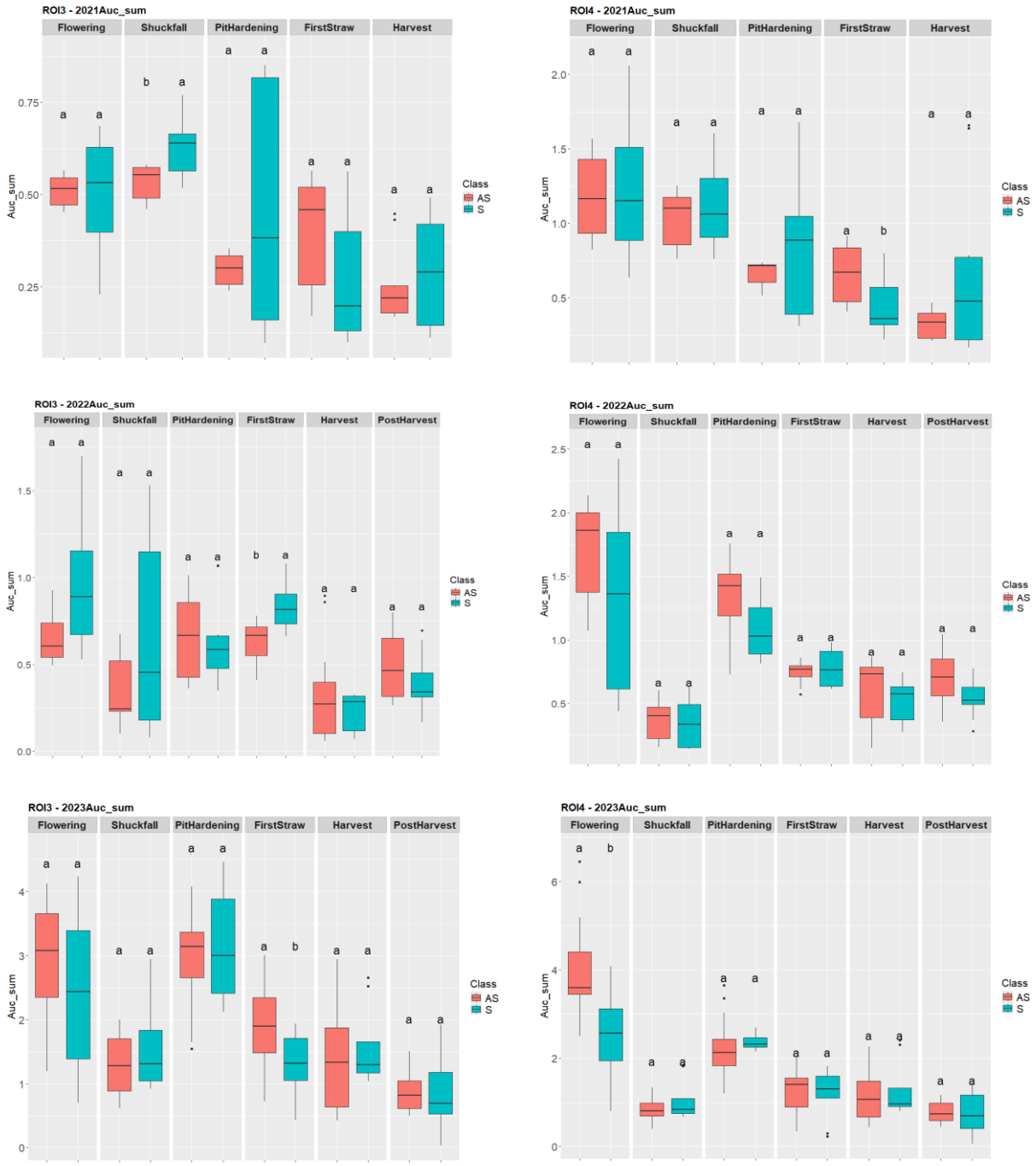

Figure 4. *Temporal variation in the FAIMS data derived* I_{AUC_sum} *feature for ROI's 3 and 4 (cultivar: Bing, combined dataset for 2021, 2022 and 2023 seasons).*

exhibit higher I_{AUC_sum} values. A similar pattern was observed for the Bing cultivar samples. This pattern suggests that pit hardening is a critical stage for detecting changes in volatile emissions linked to disease or stress responses. Similar to the previous analysis, the first straw and post-harvest stages show minimal differences between S and AS samples, indicating comparable metabolic activity during these periods. The flowering stage exhibits significant variability in both years but less consistency in terms of differentiation

between the S and AS classes. Overall, the results emphasize the importance of the pit hardening stage in detecting differences between S and AS Skeena samples, suggesting its potential for use in early disease detection or monitoring. *Correlation of FAIMS spectral features with titer level.* The correlation analysis between the I_{AUC_sum} for ROIs 3 and 4 with the Cq values was performed. For Bing cultivar and ROI 3 data, the correlation was weak and not statistically significant (r = 0.27). This indicates that there was no strong relationship between the titer level and I_{AUC_sum}. ROI 4 showed a moderate and statically significant correlation (r = 0.53) suggesting higher titer levels are associated with lower I_{AUC_sum} values. For the Skeena cultivar, a weak correlation (r = 0.28) between ROI 3 I_{AUC_sum} and Cq values was observed. ROI 4 I_{AUC_sum} showed moderate correlation (r = 0.38), indicating that as the titer increases, the AUC_sum values increase for the ROI4.

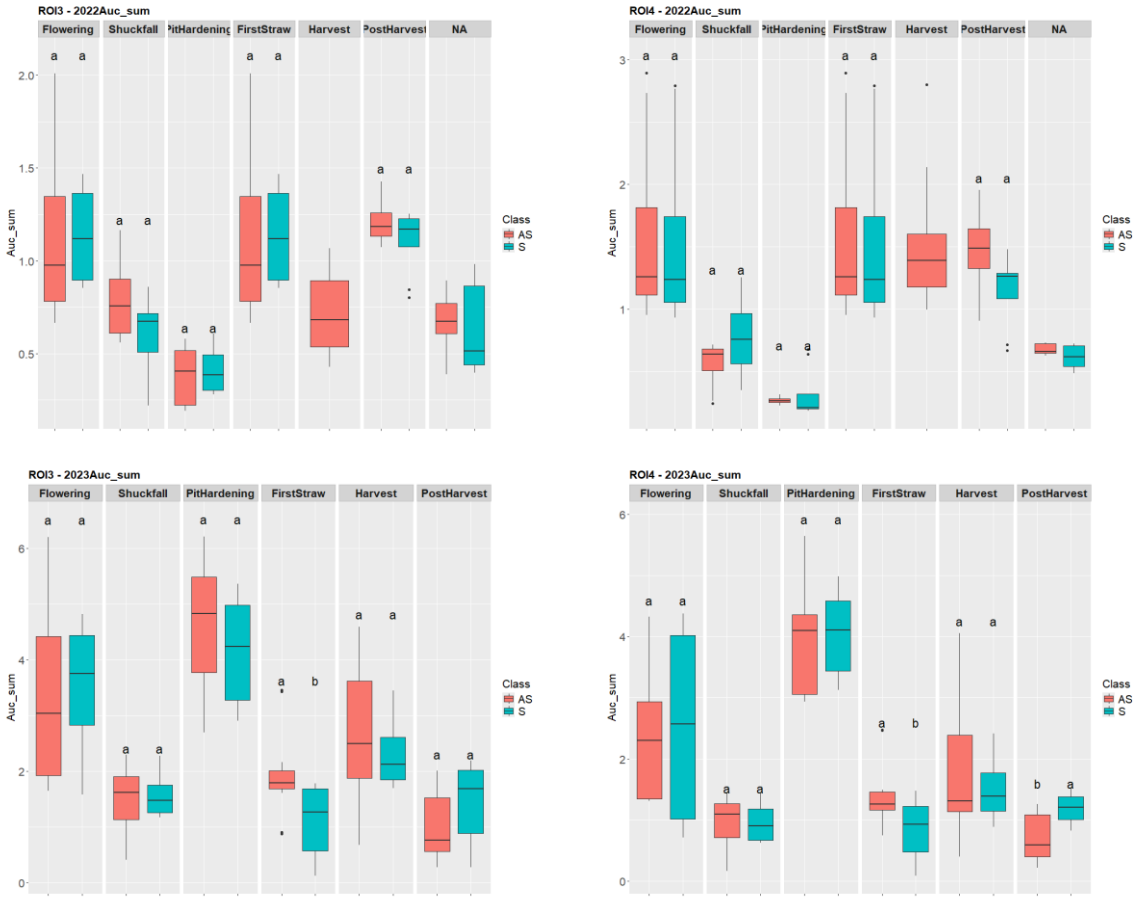

Figure 5. *Temporal variation in the FAIMS data derived I_{AUC_sum} feature for ROI's 3 and 4 (cultivar: Skeena, combined dataset for 2022 and 2023 seasons).*

Objective 2. Develop a comprehensive understanding of the associated volatile biomarkers release using the GC-MS technique.

The GC-MS analysis suggested that sweet cherry leaves release a broad range of primary and secondary metabolites. These metabolites were analyzed towards identification of XDP-linked volatile biomarkers (Fig. 6). The volatile compounds that significantly add importance to the class separation, for both first straw and harvest growth stages, were identified using the PLS-DA VIP scores. Compounds with high VIP scores in both years and growth stages (listed in Table 2) might be closely associated with the presence or absence of XDP infection in the leaf samples.

Static headspace sampling technique.

Bing cultivar. A few compounds were consistently found across both seasons at specific growth stages. For example, (Z)-2-pentanal was present in harvest stage samples for both years. At the first straw stage, compounds such as hexanol, acetic acid, butanoic acid, propanoic acid, and n-hexane had a VIP score greater than 1 in both years. At harvest, significant compounds included (E)-2-hexenal, acetic acid, acetone, octane, and propanoic acid. Only acetic acid and propanoic acid were found in both stages over both years. At the first straw stage, five compounds, including hexanal (a stress-response aldehyde), were significantly different in abundance between AS and S samples. AS samples had higher levels of hexanal and Z-3-hexenal, while S samples showed a higher abundance of ethanol. Unknown compounds (1334, 71) and (1167, 57) also showed variations. Overall, volatile biomarkers associated with XDP infection were detected in Bing cultivar (leaves) at both the first straw and harvest stages. Ethanol levels were higher in symptomatic samples at the first straw stage, while propanoic acid, octane, n-hexane, and acetone were elevated in symptomatic samples at harvest. Some compounds, like vinyl aldehydes, GLVs (Z-3-Hexen-1-ol, (E)-2-hexenal), and unknowns (1967, 57) and (1418, 109), showed strong correlations with disease severity and titer levels.

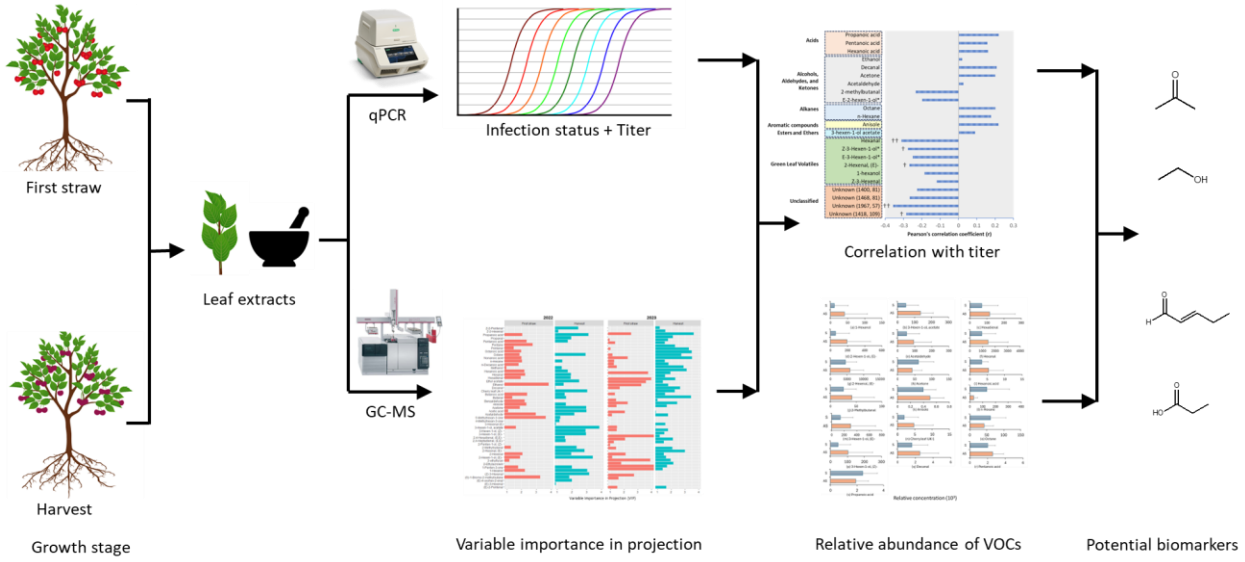

Figure 6*. XDP associated volatile biomarker identification workflow using GC-MS technique.*

Skeena cultivar. Volatile biomarkers associated with X-disease in the sweet cherry cultivar 'Skeena' were identified at the harvest growth stage. Several compounds were consistently significant across two seasons, including methanol, ethyl acetate, (Z)-2-penten-1-ol, and three unidentified compounds: unknown (1169, 83), unknown (1412, 81) and unknown (1468, 81). In 2022 season, E-2-Hexen-1-ol, E-3-Hexen-1-ol, acetaldehyde, and unknown (1412, 81) compound showed higher abundance in S trees compared to AS trees. The 2023 season data identified a broader range of compounds including several green leaf volatiles with higher abundance in S trees. Several GLVs exhibited strong positive correlations with infection levels. E-2-Hexenal, Z-3-Hexen-1-ol*, 2-Hexenal, acetaldehyde, acetoin, methanol and unidentified compounds, unknown (1334, 71) and unknown (1412, 81) showed positive correlations with the XDP titer levels (Table 2).

Dynamic headspace sampling technique.

A dynamic volatile headspace sampling method was evaluated to identify X-disease-linked biomarkers in stem samples of sweet cherries (cv. 'Bing' and 'Skeena'). Key compounds were identified using PLSDA based on variable importance in projection scores, with statistical significance was confirmed with the Mann Whitney U-test at $p < 0.05$. In case of Bing cultivar, key compounds identified were n-Hexane, methanol, unknown (1141, 41), E-3-Hexen-1-ol, 2-methylbutanal, Z-2-Hexenal with varying levels of relative abundance at different growth stages (Table 2). Whereas for Skeena, the significant compounds were, E-3-Hexenal, 1-Penten-3-one, unknown (1141, 41), pentane and butanal. A weak correlation was observed between the compound's relative abundance and pathogen level for both the cultivars and could be attributed to the type of sample and sampling technique. Overall, this project developed a dynamic volatile headspace analysis technique and assessed its applicability towards XDP detection. Identified biomarkers and variation between cultivar and growth stages, suggesting complex interaction between plant metabolism, stress responses and pathogen attack by insects.

Table 2. Potential biomarkers associated with XDP symptoms in sweet cherry cultivar 'Bing' and 'Skeena' for leaf tissue and stem samples.

Leaf tissue samples (GC-MS static)		**Stem samples (GC-MS dynamic)**	
Bing	**Skeena**	**Bing**	**Skeena**
Methanol	Methanol	n-Hexane	E-3-Hexenal
(E)-2-hexenal	Ethyl acetate	Methanol	1-Penten-3-one
Propanoic acid	(Z)-2-pentenol	Unknown (1141, 41)	Unknown (1141, 41)
Acetone	(Z)-3-hexenol*	E-3-Hexen-1-ol	Pentane
n-hexane	Unknown (1412, 81)	2-methybutanol	Butanal
(Z)-3-hexenol	Unknown (1468, 81)	Z-2-Hexenal	
Unknown (1967, 57)	E-2-Hexenal		
Z-3-Hexen-1-ol	Z-3-Hexen-1-ol*		
(E)-2-hexenal	2-Hexenal		
Unknown (1418, 109)	Acetaldehyde		
E-3-Hexen-1-ol	Acetoin		
1-hexanol	Unknown (1334, 71)		
Z-3-hexenal	Unknown (1412, 81)		
Unknown (1400, 81)			
Unknown (1468, 81)			
2-methylbutanal			

Objective 3. Conduct pertinent extension education and technology demonstrations.

Throughout the project period, we have actively disseminated pertinent technology and findings to stakeholders via various platforms. These include field days such as Little Cherry Disease Field Day (2022, 2023) and Little Cherry Disease Day (2022), research news flash talks at the WSTFA Annual Meeting & NW Hort Expo (2022, 2023), and a research update presentation at the Columbia Basin Tree Fruit Club (2023). Additionally, our work was highlighted at the 2024 Annual X-disease Research Summit and the X-disease Research Summit for Nurseries and Growers, both organized by USDA-ARS in Wapato, WA. Our

research story was featured in the February 2022 issue of Good Fruit Grower magazine (https://www.goodfruit.com/testing-the-testing-tools/), as well as in other electronic media such as the Yakima Herald-Republic News (2022) and a podcast (2023). The project outcomes were also presented to the research community during a session talk at ASABE-AIM 2022 in Houston, TX. The culmination of this work is a durable product: a PhD Dissertation by Gajanan Kothawade (August 2024), titled "Identifying Biomarkers of Little Cherry/X-Disease in Prunus avium Using Volatile Sensing Technologies" at Washington State University. Furthermore, four peer-reviewed research articles are currently being prepared for submission based on these efforts.

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Executive Summary

Project title: Towards the identification of Little cherry disease linked volatile biomarkers

Key words: Little Cherry Disease, Presymptomatic detection, Volatile Biomarkers, FAIMS, GC-MS

Abstract: This project evaluated FAIMS technique for early detection of LCD/X-disease in 'Bing' and 'Skeena' cherry cultivars by analyzing volatile profiles across seasons and different growth stages. The pit hardening stage consistently showed significant differences in volatile emissions between symptomatic (S) and asymptomatic (AS) samples, with AS samples having higher ion current values in both cultivars. Temporal variations were also observed at other stages, though less consistently, indicating the importance of the pit hardening stage for early detection of X-disease. Cultivar-specific differences and environmental conditions across seasons also influenced volatile emissions. Project also identified key volatile biomarkers, using GC-MS static and dynamic sampling technique, associated with the X-disease infestation. Potential biomarkers include, Ethanol, (E)-2-hexenal, propanoic acid, and acetone, ethyl acetate, (Z)-2-penten-1-ol, (Z)-3-hexen-1-ol*, 2-hexenal, acetaldehyde, acetoin. *Practical implications* of this research include: 1) Early Detection: Identification of the pit hardening stage as critical for detecting X-disease, 2) Targeted Monitoring: Growers can focus on non-destructive (e.g., Canine based) scouting efforts during the pit hardening stage, 3) Biomarker Utilization: The volatile biomarkers identified in this project can be used to develop more precise diagnostic tools, enhancing the accuracy of X-disease detection, 4) Cultivar-Specific Strategies: Understanding cultivar-specific differences in volatile emissions can help tailor disease management practices to specific cherry varieties, improving overall crop health, and 5) Seasonal Adaptation: Recognizing the influence of environmental conditions on volatile emissions enables growers to adapt their monitoring and intervention strategies according to seasonal variations.