Project Title: Real-time Detection of Little Cherry Disease using Detector Canines

Report Type: Final Project Report

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Project Duration: 2 years + 1 year, no-cost extension
Total Project Request for Year 1 Funding: $74,267
Total Project Request for Year 2 Funding: $116,045
Other related/associated funding sources: None

Budget 1
Primary PI: Jessica Kohntopp
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Item	2023	2024
Salaries	$40,000.00	$40,000.00
Benefits	$13,600.00	$13,600.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$2,640.00	$500.00
Travel	$8,338.00	$12,276.00
Plot Fees		
Miscellaneous		
Total	$64,578	$66,376

Footnotes: Year-1: PI Jessica Kohntopp requests salary based on below median detection dog trainer salary survey conducted by Highland Canine Training LLC School for Dog Trainers. Supplies for boxes/tins, harnesses, booties, canine training supplies, camera for videos, tripod, mini freezer etc. Travel to cover 5 trips of 5 days each to bring the canines to WA for blind studies, mock orchards, demonstration and outreach days. **Year-2** travel costs were doubled because canines will be in WA more times for in depth studies.

Budget 2
Co-PI: Scott Harper
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
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Contract administrator email address: arcgrants@wsu.edu

Item	2023	2024
Salaries		
Benefits		
Wages	$6,233.00	$43,631.00
Benefits		
RCA Room Rental		
Shipping		
Supplies	$955.80	$3,538.20
Travel		
Plot Fees		
Miscellaneous		
Total	$7,189	$47,169

Footnotes: Year-2: Co-PI Harper requests allocation of $30,996 in salary (0.5 FTE) and 11,159 in benefits, with 12,203 in supplies and consumables for the generation of peptides for detector dog training.

Budget 3
Co-PI: Corina F. Serban
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: (509)-335-2885
Contract administrator email address: arcgrants@wsu.edu

Item	2023	2024
Salaries		
Benefits		
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$500.00	$500.00
Travel	$1,809.00	$1,809.00
Plot Fees		
Miscellaneous	$191.00	$191.00
Total	$2,500	$2,500

Footnotes: Year-1: Co-PI Serban requests $500 towards extension and outreach activities; $1,809 for travel and accommodation; and $191 for miscellaneous items such shipping costs. **Year-2:** request is similar to Year-1.

Objectives:
1. Train canines to detect little cherry virus-2 (LChV-2), and the X-disease phytoplasma (XDP) by using infected plant samples (Year 1).
2. Validate the accuracy, sensitivity, and specificity of the canines to detect LCD pathogens in young cherry trees in a single-blind greenhouse/potted plant studies (Year 1) and single-blind studies in commercial orchards (Year 2).
3. Examine the effects of dormancy and temporal infection cycles on canine detection (Year 2)
4. Extension and Outreach – monthly meetings, demonstration at field day and technical factsheet (Years 1 & 2)

Given the challenges associated with sampling for this disease, particularly due to its uneven distribution *in planta*, it was determined that continued qPCR testing of the trees in the absence of complete dissection was not an efficient use of resources. A no-cost extension was filed - and accepted - to allow continued monitoring of these trees with canines as well as visual assessment for symptoms over the next couple years.

Significant Findings:
- Canines are capable of detecting LChV-2 and XDP infection in trees in advance of detection by qPCR.
- Canines have shown 99.72% accuracy, 96% sensitivity, and 100% specificity for the detection of LChV2 and XDP in a blind controlled study and successfully displayed odor discrimination from

other diseases and stressors.
- Canines alerted on sap/phloem extracts, and then on pathogen specific proteins, suggesting at least one of the targets imprinted on were components of the pathogens themselves
- Low temperatures inhibited detection of low concentration positives, while close tree spacing limited pinpointing of trees, suggesting environmental and spatial considerations for field deployment.
- Overall, canine detection shows potential as a rapid, non-destructive, whole-tree screening tool for little cherry pathogens.

Methods

Initial training

Two canines were selected for this project and trained to detect the three pathogens associated with Little Cherry Disease (LCD). Known LCD positive and negative potted trees were used as training aids for the canines. Canines underwent a 1-week acclimation period followed by 8–10 weeks of odor and obedience training. Training consisted of 7–10 sessions per day, 4–5 days per week, in a controlled environment. Canines were positively reinforced with verbal praise and brief toy play when they correctly identified the target odor through self-discovery; incorrect alerts were not reinforced. Training continued until canines reliably alert by sitting and remaining at the target odor until released. Once the canines were confirmed to be responding to the target odors, they were evaluated through a series of single-blind greenhouse/potted plant and field-based trials.

Single-blind studies

Two types of single-blind studies were conducted to assess a) Accuracy: The total amount of correct alerts on positive trees and non-alerts on negative trees, b) Sensitivity: The total amount of correct alerts on positive trees, and c) Specificity: The total amount of correct non-alerts on negative trees. The first trial ran the dogs through a greenhouse containing approximately 200 potted negative plants in which seven potted positive plants were randomly placed. The second trial involved placement of 60 positive and negative plants in rows in an open, outdoor location, with randomized replicates in which the observers changed the placement of the positives while the handler was unaware of the locations. Replicates were performed 23 times, giving a total number of 1380 plants scouted.

This process was repeated in a commercial orchard where the observers knew the location of trees that had previously tested positive by standard sampling collection and qPCR testing protocols. However, in practice it was found that the canines alerted on additional trees that had tested qPCR negative. To confirm the canine detection trees were dug out of the ground and dissected, with tissue samples collected vertically down the stem every 4” (~10 cm), with larger diameter pieces divided into two or four samples to capture spatial distribution. These were extracted and tested by qPCR.

Training aid development

To investigate the ability of the canines to detect the LCD-associated pathogens, we first macerated XDP infected leaf and stem tissue in phosphate-buffered saline (PBS) pH 7.4 at a 1:10 (w/v) ratio in a mortar & pestle and removed the sediment by centrifugation. From this we prepared a serial 1:10 dilution series down 10^{-9} and placed these on cotton pads in small cages for scouting by the canines, along with pathogen negative and buffer controls. Following successful indication on these extracts we then performed phloem sap extraction from XDP infected plants by high-speed centrifugation of stem sections and again created a serial dilution panel down to 10^{-9}, using pathogen negative sap as the diluent. Finally, to better identify the target(s) that the canines had imprinted on, we used the strategy of a) performing LC-MS on sap extracts from XDP, LChV2 and PDV positive plants to see if there were significant differences that could be attributed to pathogen activity, and b) expressed the structural coat proteins of LChV-1, LChV-2 and immunodominant membrane proteins of XDP, with PDV and PNRSV coat proteins, and a highly expressed *Pseudomonas syringae* pv. *syringae* protein

as nontarget controls. These proteins were used for the preparation of dilution series as above, pipetted on cotton pads in small cages for scouting by the canines, along with nontarget proteins and buffer controls.

Environmental, temporal and spatial effects on detection
Additional field studies were used to evaluate potential factors that could affect detection, including environmental conditions, tree dormancy, tree spacing, and the presence of nearby infected trees. Previously identified positive trees were evaluated alongside their corresponding qPCR results to examine the effects of tree age and infection level on canine detection. Tree spacing and proximity to neighboring infected trees were also assessed to determine whether adjacent trees or potential odor transfer could influence canine responses. Seasonal and environmental conditions, including dormant trees and frozen ground, were evaluated during searches conducted at different times of the year. Previously searched blocks were re-evaluated using a modified search approach, with each canine sit alert recorded during the search. .These trials were designed to evaluate both the capabilities and practical limitations of using canines as a tool for LCD detection.

Results and Discussion
Initial training
Initial training began with two XDP-positive and 10 negative trees. However, substantial differences in pot size, plant age, foliage, soil, fertilizer, and tagging made the plants unsuitable for reliable disease-specific training. Canines were initially trained using potted plants enclosed in boxes to minimize visual cues and continued training on this material for approximately six weeks while awaiting more suitable training material. The second set consisted of eight positive and 22 negative plants with standardized potting media, fertilizer, tagging, and other characteristics. The canines were re-initialized on the new material before transitioning to an outdoor 24-plant training grid, where they progressed well despite environmental distractions. Distractor training incorporated plants affected by other diseases and environmental stressors to assess the canines' ability to distinguish the target odor from non-target odors. Distractor training was successful, with both canines demonstrating reliable odor discrimination and no apparent difficulty distinguishing the target odor from distractors. Once proficiency was established, a blind study was conducted.

Single-blind studies
In the greenhouse trial, both canines correctly identified all seven positive plants, as well as an additional plant that was later sampled and confirmed positive for XDP. It should be noted that this was the first time the canines had searched inside a greenhouse, the handler was unaware of the positive plant locations, and the canines had never encountered positive plants other of the eight plants used in their training material.

In the outdoor study both canines were ill (due to unfortunate uncontrollable circumstances the day before) although Aika was affected more. Humma remained focused and continued to perform, producing results closer to those observed during the mini study. Aika, however, was frequently distracted by eating grass, resulting in 12 misses. After the canines recovered, the mini study was conducted, and Aika was substantially more focused and her performance reflected that. During the mini blind study, both canines missed two positive plants. It should be noted, in both blind studies, the canines never missed the same positive plant. Missed plants occurred during different runs or at different positive plant locations. Neither canine has ever produced a false alert; although they occasionally missed positive plants, they did not alert on any negative plants, reflected by the 100% specificity. Overall, the number of positive plants missed, excluding Aika's initial run while ill, was relatively low. Under controlled conditions, the mini blind study demonstrated that the canines achieved **99.72% accuracy, 96% sensitivity, and 100% specificity**, demonstrating their ability to reliably distinguish LCD-positive plants from negative plants.

***Table 1**: Blind Study*

	Initial Blind Study		Mini Study
Canine	**Humma**	**Aika**	**Both Canines**
Sensitivity	**95.05%** (96/101)	**88.12%** (89/101)	**96%** (48/50)
Total Positives Missed	**5**	**12**	**2**
Specificity	**100%** (1279/1279)	**100%** (1279/1279)	**100%** (670/670)
Total False Alerts	**0**	**0**	**0**
Positive Predictive Value	**100%**	**100%**	**100%**
Negative Predictive Value	**99.61%**	**99.07%**	**99.70%**
False Positive Rate	**0.00%**	**0.00%**	**0.00%**
False Negative Rate	**4.95%**	**11.88%**	**4.00%**
False Discovery Rate	**0.00%**	**0.00%**	**0.00%**
Accuracy	**99.64%** (1375/1380)	**99.13%** (1368/1380)	**99.72%** (718/720)

Footnotes: *Accuracy: The total amount of correct alerts on positive trees and non-alerts on negative trees. Sensitivity: The total amount of correct alerts on positive trees. Specificity: The total amount of correct non-alerts on negative trees*

During in-orchard testing, the canines alerted on several trees had tested negative by qPCR. These results raised the possibility that standard sample collection methods (i.e. a pooled sample based on a minimum of tissue from collected from four compass points around the tree) were failing to capture infected tissue. Five of the canine-alerted trees that had previously tested PCR-negative were selected for removal and sequential dissection. Between 76 to 112 samples were collected from each tree, and qPCR testing indicated that each of the five trees had at least one positive result, confirming the canines' alerts. The Ct values for these positives ranged between 36 – 38 cycles, or approximately <5 phytoplasma cells per section. The positive tissue samples were unevenly distributed around the trees, with no consistency in location between the five trees dissected.

***Table 2:** 5 Tree Study Results*

	CT Value	Sample Size	PCR Positive Results	% Chance of Selecting Correct Tissue	Canine Success
Tree #1	38.54 38.53 37.45 37.41	**76**	**5**	**6.58%**	**100%**
Tree #2	37.46	**87**	**1**	**1.15%**	**100%**
Tree #3	37.69 36.91 36.89	**95**	**3**	**3.16**	**100%**
Tree #4	37.92	**112**	**1**	**0.89%**	**100%**
Tree #5	37.57 37.21 37.77	**94**	**3**	**3.19%**	**100%**
Total	**---**	**464**	**13**	**2.80%**	**100%**

These results highlight the difficulty of selecting the tissue for PCR testing at early stages of infection, a limitation shared by all methodologies that rely on destructive sample collection, and why repeated collection of individual samples from the same tree to confirm a previous positive is not a viable practice. It also underlines the superiority of the canine detection method, as the canines are not limited by in-planta distribution of the target, as well as being significantly faster and less expensive than full-tree dissection.

Training aid development

Canines successfully alerted on the positive samples of both sap from macerated tissues, and phloem tissue exudates at dilutions as low as 10^{-9}, while no indications were observed with known phytoplasma-negative samples or controls. As this dilution is considerably lower than had be detected by qPCR for either LChV2 or XDP, it may be suggested that the compound(s) the canines were imprinted on may be expressed either by the pathogen, or induced by the pathogen and expressed by the plant host, at concentrations higher than 1:1 with the pathogen itself (Table 4). This, combined with the known scattered *in planta* distribution of the pathogen early in the infection cycle, underlines the difficulty, if not futility, in attempting validating every positive canine alert using molecular methods: the canines appear have imprinted on a compound/or series of compounds that are present at greater concentration than the pathogen itself.

***Table 3**. Dilution series of sap exudates from a known X-disease positive plant, with number of canine alerts and approximate phytoplasma concentration as determined by qPCR.*

Dilution Step	Approximate phytoplasma concentration (cells)	Canine Alerts (Humma // Aika)
1	200,000	Alert // Alert
10^{-1}	20,000	Alert // Alert
10^{-2}	2,000	Alert // Alert
10^{-3}	200	Alert // Alert
10^{-4}	20	Alert // Alert
10^{-5}	2	Alert // Alert
10^{-6}	0.2	Alert // Alert
10^{-7}	0.02	Alert // Alert
10^{-8}	0.002	Alert // Alert
10^{-9}	0.0002	Started to struggle // Alert

Based on these results we next performed untargeted LC-MS from phloem sap exudates to determine if there was a compound or series of compounds that could be associated with X-disease or LChV2 infection, using PDV infected samples as a control. Unfortunately, this screening was inconclusive due to the small sample size, with no compounds found to be pathogen-associated (data not shown) and needs to be repeated in greater depth.

In parallel, the LChV-1 and LChV-2 coat proteins, as well as the XDP immunodominant membrane proteins were expressed *in vitro*, along with PDV, PNRSV coat proteins and cherry canker effector as negative controls. The canines alerted on dilutions of the LChV-2 and XDP proteins, down to femtomolar (-10^{12}) concentrations, while not reacting to the non-target proteins and controls (PBS buffer and bovine serum albumin). Interestingly, the while not specifically trained on LChV-1 due to the lack of singly infected positive control plants in their initial training, one of the canines (Humma) alerted on the LChV-1 coat protein whereas the other (Aika) passed it up as a negative. This behavior is consistent with the in-field screening of nursery plants performed in conjunction with WSDA in 2025, where Humma had alerted on LChV-1 positive trees whereas Aika did not. As the LChV-1 and LChV-2 proteins are structurally very different (data not shown) it suggests, that the Humma imprinted on LChV-1 in the mixed-infection training materials earlier whereas the Aika did not.

The expressed proteins provide us with a highly adaptable and reproducible training aid. While not representing the entire range of compounds that could be present in or emitted by an infected plant, they are exclusively specific to the individual pathogens unlike, for example, a host-produced volatile such as terpenoid and allows the canines to be trained down to specific targets first, then introducing host background. It also allows the stepping-down of concentrations to determine the limits of detection by the canines and will form the basis of training efforts for the the next generation of canines.

Effects of environmental, spatial and temporal variables on detection

Older Cherry Block C: qPCR Results Samples were taken on Sept 3, 2024 from the nine trees in the table below with their corresponding dog responses and qPCR test results. Both canines alerted hard on Tree D running from both directions and did not alert on nor showed interest in the symptomatic Tree F that came back positive with a CT Value of 20.13 (~480k phytoplasma cells) for XDP. This could be due to the canines' odor threshold at the time. The canines have seen qPCR positive potted plants in those high ranges before, but the trees were much younger and smaller than in this block. It is believed the canines were within their 'normal reward threshold' when they were smelling the adjacent tree shown in the Figure 3 on the right. The handler was concerned the same sort of scenario could have happened with Tree G. Samples were retaken from that tree on Sept 24, 2024, and both trees on either side of it. When results came back, both the adjacent trees came back non-detect and this time Tree G did come back qPCR positive (XDP 33.9, ~54 cells). Tree J could have been missed by both canines as there are times they struggle finding back-to-back trees. Another explanation could be due to the canines alerting on a 33.58 (~66 cells) right before the 39.66 (<5 cells). Looking back to the diagram above, odor is continuously dispersing from the tree and the canines are constantly having to work through the residual rings. With such a weak infection beside a higher infected tree, the canines could have marked it as residual odor and kept searching.

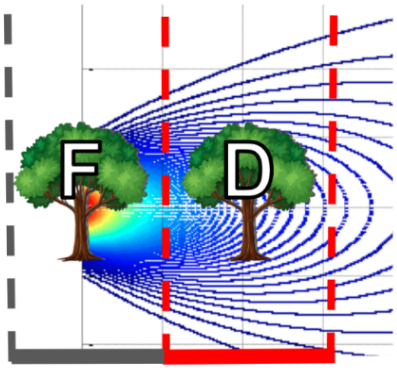


Figure 1: *Odor Threshold*

Table 4: *Block C qPCR Results*

Tree Sample	Humma Alert	Aika Alert	Humma Hesitate	Aika Hesitate	qPCR Result Sept. 3	Retested Sept. 24
Unknown Tree A	X	X			LChV-2 (37.89)	
Unknown Tree B	X	X			XDP (33.58)	
Unknown Tree J (Adjacent to Tree B)					XDP (39.66)	
Unknown Tree C		X	X		-	
Unknown Tree D (Adjacent to Tree F)	2x	2x			-	
Symptomatic Tree F					XDP (20.13)	
Symptomatic Tree E					-	
Unknown Tree G	X	X			-	XDP (33.9)
Unknown Tree H			X	X	-	

Dormancy Study:



Figure 2. *Trees pictured from each month respectively for the Dormancy Study*

In November and December, the canines consistently alerted on the same trees without difficulty. However, in January, their performance differed, with both canines experiencing difficulty detecting some trees presumed to have lower-level infections. In some cases, they alerted only on a second pass, while in others, they did not alert at all. When brought to Older Block A the following day, both canines had fewer difficulties detecting the positive trees. It should be noted that CT values in Younger Block B ranged from 37–39, while those in Older Block A ranged from 35–38.
In January, the canines were only run past eight previously alerted trees in Younger Block B after the handler observed that they were struggling to detect the trees. Continuing to work the dogs until they alerted on a second pass was not considered beneficial, as this could reinforce the behavior of requiring a second pass to make an alert. The January results are as follows:

Table 5: *Canine Dormancy Study Results for January*

January Results: Younger Block B				
Canine	**# of Previously alerted trees**	**Alerted first pass**	**Alerted second pass**	**Did not alert at all**
Humma	8	6	1	1
Aika	8	3	5	0
January Results: Older Block A				
Humma	9	7	2	0
Aika	9	8	0	1

We believe the frozen ground and the biology of this disease contributed to the canines' difficulty in detecting certain trees, particularly lower infected trees. This factor will be considered when determining the most effective seasonal timeframe for canine deployment.

Monitoring Previously Ran Blocks: The canines have previously searched these blocks twice, with relatively consistent results between searches. and no more than 12 additional canine alerts identified over a one-year period. However, the search methodology used during these previous evaluations may have greatly influenced the results. These two blocks were the first "unknown" areas in which the

canines were deployed, and the handler was initially informed that it was expected to be a low-pressure block. As the canines began alerting on more trees than anticipated, the handler began challenging the alerts. When a canine alerted, the handler would pull the canine off the tree, return several trees back, and rerun the canine through the area. If the canine alerted again on the same tree, the response was recorded; if the canine did not re-alert, the search continued. This process may have resulted in some alerts not being recorded. This same search approach/pattern was used on both blocks during every reevaluation until 2026. This year, a greater number of trees than usual were exhibiting visible LCD symptoms, potentially due to environmental factors. The canines were brought back to the blocks and allowed to search without challenging their responses. Any tree on which the canine produced a sit alert was recorded as an alert. This change in search methodology resulted in a substantially greater number of recorded canine alerts compared with previous searches. Initial alerts on the two blocks were as follows: Block B Humma: 55 trees and Aika: 59 trees, these trees were left within the block. Block D 52 initial alerts by both canines were recorded and trees were removed from the block. After the new way of running, number of alerts jumped to 168 in block B (across 17 rows, omitting 16 rows) and 318 in Block D.

Overall, this project demonstrates the strong potential of canine detection as a whole-tree, nondestructive approach for identifying Little Cherry Disease. Under controlled conditions, the canines demonstrated high accuracy, sensitivity, and specificity, successfully discriminated LCD-associated odors from common disease distractors, and transferred their detection ability from potted plants to greenhouse, field, and commercial orchard settings. Their ability to detect positive sap and phloem exudates at extremely low dilutions further suggests that the canines may be responding to compounds associated with infection that occur at concentrations greater than the pathogen itself. The PCR and tree dissection results also highlighted an important limitation of using conventional molecular testing as the sole method for validating canine alerts. The highly patchy distribution of the pathogen within weakly infected trees means that even extensive tissue sampling may fail to capture infected material. The fact that all five dissected, canine-alerted trees contained PCR-positive tissue, while only 13 of 464 samples testing positive, demonstrates the challenge of confirming whole-tree detection through limited tissue subsampling and the need for a holistic approach. Field studies demonstrated that canine detection can be influenced by infection level, tree spacing, adjacent infections, season, ground conditions, and potentially the biology of the disease. While these factors may affect detection under certain conditions, the canines consistently demonstrated the ability to locate infected trees across a variety of environments. Continued research will focus on training new canines with the newly developed training aid, comparing their abilities to the two canines trained the conventional way, and evaluating the integration of canine screening into commercial nursery and orchard operations.

Literature Review

It has been proven repeatedly that the canine olfactory system is truly remarkable, and the depth of its full potential is not yet fully understood. The use of canines for odor detection is not a new phenomenon. Using canines for hunting dates back >12,000 years and the canine nose has since been used for a range of odor detection and tracking (Gottwald et al., 2020). The ‘traditionally’ accepted and more well-known application is police/military canines that have been trained for explosive and drug detection as well as human tracking. However, canines have also been used in medicine to find cancer, epileptic warnings, diabetic alert, and most recently COVID-19. In outdoor applications, conservation canines have become more prevalent, locating invasive species, endangered species, and wildlife scat. Other canines have been used to find bed bugs, cell phones in prisons, and cadavers (both human and animal). In the agricultural community, canines have been successfully trained to find a variety of plant diseases including citrus greening, citrus canker, and laurel wilt of avocado with accuracies as high as 99% (Gottwald et al., 2020). Canines have been

shown to be a practical tool for pathogen detection, in a quick, accurate, and non destructive manner.

F1K9 had HLB detection canines in orchards and residential properties with canines detection time being ~2s per tree, providing quicker results than any current diagnostic tool. SEIR grafts are models commonly used and accepted by the USDA APHIS to predict infectious disease. Whereas there is a susceptible group (S), exposed group (E), infected group (I), and resistant group (R). A variation of this model, SECIR, was used in the HLB paper adding in asymptomatic trees referred to as cryptic (C). This model accounted for 100+ different variables and the data gathered from the canines in the Temporal Study. This model demonstrates that pathogen control was possible when canine detection was followed by tree removal whereas current methods of PCR and visual detection failed to suppress the exponential trajectory which is shown in Figure 3.

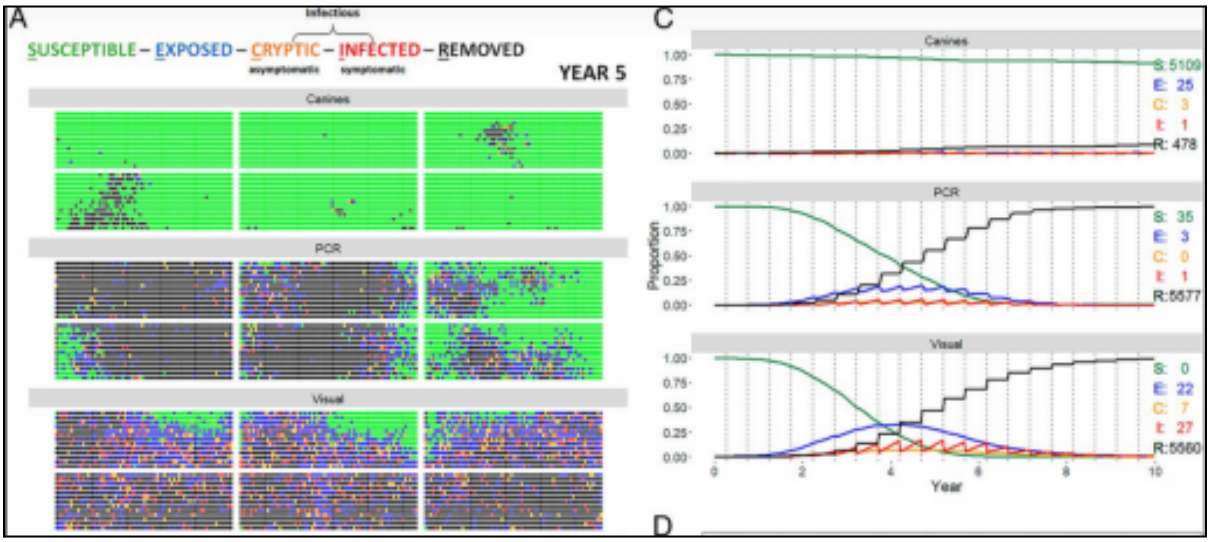
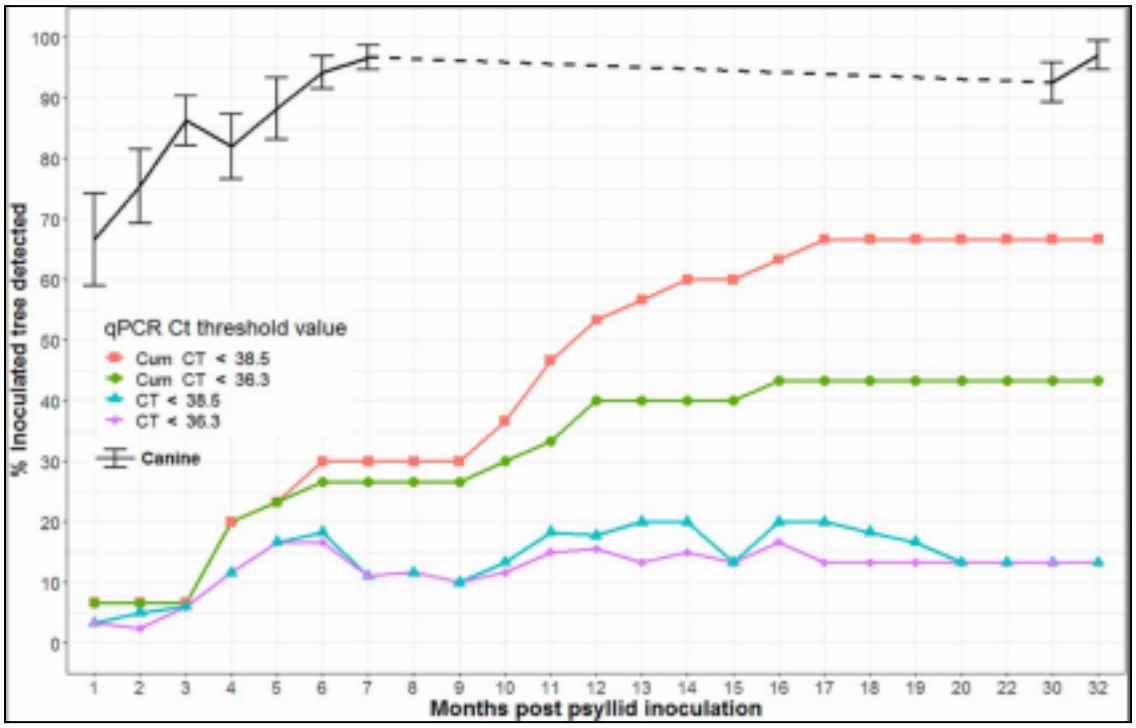


Figure 3. *SECIR Model based simulation of CLas epidemic over 10-year period.*

A Temporal Study conducted within the HLB paper proved canines were able to find infection months to years prior to molecular methods of PCR shown in the table below. Plants in this study were artificially infected, kept track of via numbering system, and kept in a greenhouse to ensure no natural psyllid transmission took place. Every two weeks trees would be put in a grid in a computer-generated randomized order. The canines would run past the trees and data was collected on which trees were alerted on. Samples were taken for PCR at the same time. This test proved canines could be used as an early detection tool (Figure 4).

Figure 4. *Temporal assessment of canine vs qPCR detection of CLas infection.*



LCD is a widespread problem throughout Washington which has resulted in the removal of about 974 acres of sweet cherry trees which is more than 238,856 trees between 2015 and 2020 (Molnar et al., 2022). This has caused a large economic impact on growers of over $115 million (Molnar et al., 2022). Control is very difficult due to the short range of time that visual symptoms are present and the unfeasible cost of testing many trees through PCR. Canines could be the year-round, real-time, non-destructive diagnostic tool that is needed to help mitigate the spread of LCD.

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Executive Summary

Project Title: Real-time Detection of Little Cherry Disease using Detector Canines

Key words: Little Cherry Disease, Canine, Early Detection, Rapid Detection, Non-destructive Detection

Abstract
Little Cherry Disease (LCD) is a significant threat to the cherry industry, yet early detection remains difficult due to the short-lived and inconsistent nature of visible symptoms. While conventional PCR testing provides a diagnostic option, the patchy and uneven distribution of the pathogen throughout a tree makes it difficult to collect representative tissue and confirm low-level or early infections from limited subsamples. This project evaluated the use of canines as a potential year-round, real-time, nondestructive detection tool that provides a holistic, whole-tree sampling approach rather than relying on individual tissue subsamples.
Two canines were trained to detect the three pathogens that cause LCD and subsequently demonstrated discrimination between LCD and other common diseases and stressors. In blind, controlled testing, the canines achieved 99.72% overall accuracy, 96% sensitivity, and 100% specificity. They were successfully transitioned from potted trees to field and commercial orchard settings. Importantly, several trees alerted on by the canines initially tested non-detect by qPCR. Five of these trees were later removed and extensively dissected, and all five were confirmed positive, with only 13 of 464 tissue samples testing positive. This highlights the highly uneven distribution of pathogen within these trees and demonstrates why confirming early or weakly infected trees through qPCR is so difficult. Further expanding on the need for a method that can sample the whole tree.

Canines also successfully detected positive sap and phloem extracts at dilutions as low as 10^{-9}, suggesting they may be responding to one or more compounds associated with infection and may have learned multiple odor signatures associated with LCD. Field studies identified additional factors affecting detection, including tree spacing, adjacent infections, seasonal conditions, and low pathogen levels. Overall, these results demonstrate strong potential for canine detection as a tool for identifying LCD-infected trees and improving disease management. Continued research will focus on training new canines with the newly developed training aid, comparing their abilities to the two trained the conventional method, and evaluating the integration of canine screening into commercial nursery and orchard operations.

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