FINAL PROJECT REPORT

Project Title: Canine Detection of Western X Disease in Controlled and Field Settings

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Cooperators: Washington Tree Fruit Research Commission (WTFRC); Scott Harper, Washington State University (WSU); Teah Smith, Zirkle Fruit Company; Hannah Walters, Stemilt Growers; Garrett Bishop, G.S. Long Company

Other funding sources:
Amount: $5,053.57
Agency Name: Oregon Sweet Cherry Commission (OSCC)
Notes:

Total Project Funding:
Budget History
Organization Name: Rogue Detection Teams (RDT)
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Item	**Year 1:**	**Year 2:**	**Year 3:**
Salaries		--	
Benefits		--	
Wages	$4,460	$2700 ($25k)	$29,700
Benefits		--	
Equipment			
Supplies		$392	$500
Travel	$377	$1900	$5,500
Miscellaneous			$5,300
Plot Fees			
Total	$4,837	$29,872	$41,164

OVERVIEW

Little Cherry Disease (LCD) is an emerging problem for Pacific Northwest cherry growers and has been found statewide in Washington since 2010. Trees infected with any of the three diseases must be removed, but detection of symptoms is challenging for human surveyors and not evident until 2-3 weeks before harvest. Molecular detection methods are neither cost-effective nor practical on an orchard-wide scale. Rapid detection methods are needed, and trained detection dog teams (DDTs) may provide a quick screening method for identifying diseased trees for targeted molecular verification.

Rogue Detection Teams (RDT) began utilizing DDTs, a combination of a bounder (commonly referred to as a handler) and a detection dog, on a preliminary pilot study in October of 2019 with Little Cherry Disease 2 (LCD-2). Due to the aggressive nature of Western-X, RDT switched the focus from LCD-2 to Western-X after that initial pilot study.

For the field component, RDT deployed two DDTs in January 2021, during the pruning season to determine if winter is an optimal time of year to survey orchards for LCD. RDT supplemented field deployments with indoor leaf trials and began developing a systematic process for searching unknown leaves and collecting statistics on DDTs' accuracy.

While DDTs have had success detecting viruses both in controlled environments and field visits to orchards, there still remain challenges in determining the best method of deploying detection teams.

RDT requested funding to explore the following objectives in 2021.

- Visit 3 to 4 orchards during January 2021 when trees are being pruned.
- Visit orchards/nurseries to test nursery stock for infections.
- Visit orchards in March/April 2021 just prior to planting new stock.
- Continue testing and developing an efficient method of collection for use in a controlled environment.

Based on current results (see Table 1), RDT is confident that DDTs are able to detect Western-X disease and Little Cherry Virus 2 infected samples in a controlled setting and have completed trials to determine a protocol to systematically test unknown samples with a high level of accuracy.

DDTs tested 540 samples with an overall accuracy (accuracy on healthy and infected samples) of 0.9870 (range = 0.9741 to 1) with a true positive rate (accuracy of dog correctly alerting to infected samples) of 0.95 (range = 0.9048 to 1) and a true negative rate (accuracy of dog correctly passing healthy samples) of 0.9955 (range = 0.9894 to 1).

Timeline of Events: Received samples, Facility training, Field visits.

Date	**Type**	**Notes**
7/18/2019	Samples	Received infected and non-infected LCD-2 samples from Teah Smith, Rob Curtiss, Scott Harper
10/21/2019	HQ Trails	First run-through on samples using the new box apparatus, **successful identification of infected LCD-2 samples.**
10/30/2019	Report	Submitted the first progress report
11/12/2019	Presentation	Presentation on the first pilot study
11/21/2019	Samples	Received samples from Teah Smith of healthy and Western-X disease-inoculated leaves, twigs, bark, and roots
December 2019	HQ Work	Training at RDT Headquarters (HQ), Heath Smith with Pips
January 2020	HQ Work	Training at RDT HQ, Jake Lammi, Suzie Marlow, Heath Smith, Collette Yee with Skye, Zilly, Ranger, Pips, and Dio. **Successful detection of samples infected with WESTERN-X.**
02/26/2020	Field Visit	Field visit Othello, Jake, Suzie, and Heath with Skye, Ranger, Zilly, and Pips
03/06/2020	Field Visit	Field visit Rockport, Jake, and Suzie with Skye, Ranger, and Zilly
March 2020	Samples	Received Samples from Zirkle, Teah Smith
March 2020	HQ Work	Training at RDT HQ, Jake, and Suzie with Skye, Zilly, and Ranger
05/17/2020	Report	Follow up report on progress submitted
May-June 2020	HQ Work	Training at HQ
06/02/2020	Funding Request	Out of cycle funding request submitted
06/05/2020	Funding Awarded	Funding award granted ($18,102)
06/08/2020	Field Visit	Field visit at Zirkle - CRO Ranch, Jake and Suzie with Skye, Ranger, Zilly
06/08/2020	Samples	Obtained samples from Zirkle - CRO Ranch, RDT brought to HQ
06/20-21/2020	Field Visit	Field visit at Zirkle - CRO Ranch, Jake and Suzie with Ranger, Zilly, Skye, and Dio
06/24-27/2020	HQ Work	Training at HQ with Ranger, Skye, Dio
06/28-29/2020	Field Visit	Field visit at Zirkle - CRO Ranch, Jake with Ranger and Skye

08/17/2020	Funding Awarded	Additional funding awarded ($25,000)
08/27/2020	Samples	Received samples from Stemlit Growers, Hannah Walters
August 2020	Construction	Obtaining materials for and building the apparatus needed to work through numerous samples
09/28/2020	Samples	Received samples from Zirkle, Teah Smith
09/29/2020	Samples	Received samples from Mattawa Nursery, Andrew Hunsperger
October 2020	Report	Compiled findings into a report
December 2020	Teaching	Continued teaching of samples received from Marrawa and Zirkle
January 2021	Field Visit	Winter field visit to Mattawa, Zirkle - CRO Ranch, Wapato, Jake, and Suzie with Ranger, Zilly, Skye
January 2021	Nursery Tour	Winter nursery tour, with Jake and Suzie
February-March 2021	Testing Methods	Testing of methods to systematically search leaves in a controlled setting, Jake, Suzie, and Heath
March-April 2021	Leaf Trials	**Conducted leaf trials with a combined accuracy of 0.9870**
April 2021	Report	Compile findings into a report

Objectives and Significant Findings:

First Objective: Visit three to four orchards during January 2021 when trees are being pruned.

In January 2021, RDT sent two DDTs to visit three cherry orchards (Mattawa, Zirkle - CRO Ranch, and Wapato) in central Washington one week after pruning. DDTs started at the Mattawa orchard, as it was the only orchard of the three that had lab-confirmed healthy and infected trees. Bounders introduced detection dogs to the infected trees, providing instruction to sit followed with a reward. Throughout the day, both DDTs repeated the process on the infected trees in the orchard.

On the second day of orchard visits, RDT visited Zirkle - CRO Ranch. DDTs presented prunings from healthy trees and one infected tree to the detection dogs. After each odor introduction, the prunings were randomized and the process was repeated. Once the detection dogs were independently alerting to the infected pruning, new prunings were added. Both detection dogs were able to correctly identify the infected prunings the first time they were introduced to them. The bounder then introduced detection dogs to live infected trees to further assist them in understanding the connection between prunings and live trees.

On day three, RDT visited the Wapato orchard. Upon arrival, RDT was informed there were no reliable healthy trees at the orchard. Bounders took the detection dogs to the confirmed infected trees and rewarded them once they alerted at the tree, however DDTs were limited by the lack of healthy trees at the orchard. After visiting the three orchards, RDT decided to spend another day working with the detection dogs at the Mattawa orchard where confirmed healthy and infected trees were present.

Second Objective: Visit nurseries to test nursery stock for infections.

During the January orchard visits, RDT visited two different nurseries to evaluate if it would be practical to use DDTs for the detection of infected nursery stock. RDT met with nursery representatives and were given a detailed tour of the nursery grounds.

Third Objective: Visit orchards in March/April just prior to planting new stock

Washington Tree Fruit Research Commission (WTFRC) decided to forgo the March/April orchard visits. RDT instead focused on continued research and development of a method to detect LCV in a controlled setting.

Fourth Objective: Continue testing and developing an efficient method of collection for use in a controlled environment.

- **Teaching:** Expand on the development of the teaching process by introducing the dogs to new lab-confirmed healthy and infected samples.

  DDTs have the ability to both generalize on an odor or to specialize on specific odors. As such, introducing detection dogs to an assortment of at least 20 laboratory-confirmed healthy and infected samples assists them in learning what is being asked of them, i.e., to generalize on the infected leaf odor and not become specialized on only a small number of samples. Twenty-nine healthy and 20 infected samples, confirmed by Washington State University (WSU) researchers, were utilized for the teaching process. To start, bounders placed one infected sample into a wall apparatus with several holes for a detection dog to sniff, hereinafter referred to as the ring (figure 1) and placed healthy samples in the remaining locations. Bounders instructed the detection dog to search the ring. If the detection dog had a change of behavior at a healthy sample the bounders would give a soft verbal correction and encourage them to continue searching the ring. When the detection dog smelled an infected sample, the bounder instructed them to sit, followed by giving the ball as a reward. This process was repeated with a small subset of healthy and infected leaves until detection dogs were able to correctly identify the infected sample on their own (without a verbal "sit" command).

- **Development:** Develop a systematic process for searching unknown leaves and collect statistics on the DDTs' accuracy.

  Detection dogs are capable of learning patterns. As such, it was important that RDT develop a systematic process for collecting data that ensures the only constant pattern is the infected versus healthy odor. To start, a random, blind scenario was set up in the ring with a mix of healthy and infected samples. A random number generator was used to determine the simulated infection rate (range 0% to 25%) and the location of the infected samples within the ring. In addition to the randomly infected samples that were placed in the ring, one additional infected sample was placed in a location that was known to the bounder. This ensured that if the random infection rate was 0%, the detection dog could still be rewarded. This served three purposes: 1) Since the ring is a circle, detection dogs will continue searching until they find a sample or are removed from the search. Removing a detection dog from the search can cause confusion and the dog may be more inclined to indicate at healthy samples. Having one infected sample where the location is known ensures that the detection dog always has an infected sample to find. 2) If the random infection rate is 0% for multiple sessions in a row a detection dog may become confused at the lack of rewards and indicate at healthy samples. Always keeping one known infected sample in the ring ensures the dog always has a play reward. 3) RDT bounders noticed that within a session, if a detection dog had one error, they are more likely to have two, creating a ripple effect. Keeping one known infected in the ring informs the bounder whether the detection dog is still indicating at infected samples. Next, the bounder chose a starting location and encouraged the detection dog to search the ring. A session ends after a DDT completes searching the ring or has an error. If a detection dog indicates at a blind sample and is rewarded, that sample is pulled, and the DDT continues searching the ring until the DDT detects the known infected sample. As mentioned previously, if a detection dog has one error within a session it can create a ripple effect leading to more errors in the same session. Keeping the same subset of samples in the ring allows detection dogs to quickly cross-examine one another and limit the magnification of errors. The starting and ending point of the search was noted and all samples the detection dog tested were recorded. After each round, every sample was taken out of the ring and a new setup was created.

- **Cost:** Determine how many samples could be done per day and estimate a cost per sample.

  Utilizing the statistics gathered from the leaf trials RDT extrapolated the samples per hour to estimate a cost per sample.

Results:

First Objective: Of the three orchards that RDT visited during the pruning season in January 2021, with the aim to evaluate if the winter season would be optimal for surveys, only one had both healthy and infected trees for the DDTs to work. To truly test whether this season would have ideal working conditions, more orchards with both infected and healthy samples would have been ideal. As such, DDTs were unable to distinguish between healthy and infected trees in the orchard. More research is required to determine if winter is a viable season to conduct orchard surveys.

Second Objective: Also, in January 2021 RDT visited two nurseries to evaluate whether it would be practical to use DDTs to detect infected nursery stock. RDT observed that the nursery warehouses were too densely packed with young trees for DDTs to be able to reliably detect infected trees. When odor becomes as concentrated as it was in the nurseries, DDTs become unable to locate the source of the target odor.

Third Objective: WTFRC decided to forgo the March/April orchard visits to focus on continued leaf testing in a controlled setting.

Fourth objective:

- **Teaching:** During the teaching phase, the DDTs were able to alert to the new infected leaf samples while ignoring the healthy ones. We did not observe detection dogs consistently alerting to any of the new healthy samples. However, there was one infected sample that all the dogs initially had a weak response at during the teaching phase, sample "X", which was a lab-confirmed infected sample ("weak response" defined here refers to a detection dog having a change of behavior more subtle than their alert behavior).

- **Development:** DDTs tested a total of 540 leaf samples to gather statistics and test the systematic process for searching unknown leaves. The DDTs had a combined overall accuracy (accuracy on healthy and infected samples) of 0.9870 (range = 0.9741 to 1) with a true positive rate (accuracy of dog correctly alerting to infected samples) of 0.95 (range - 0.9048 to 1) and a true negative rate (accuracy of detection dog correctly avoiding healthy samples) of 0.9955 (range = 0.9894 to 1) (Table 1). It is worth noting that the known infected sample included in every session was only calculated into the results if the DDTs failed to detect it. If the DDT successfully alerted to the known sample, it was not included as it was not a true blind sample. If the known infected sample was included in the results, the true positive of all four DDTs would increase. More error occurred when DDTs failed to detect infected samples (false negatives) rather than alerting at healthy samples (false positives). There was a total of five false negatives and two false positives. There was no consensus among DDTs on any of the false negatives, or false positives showing the importance of having multiple detection dogs cross-examine one another (Table 2, Table 3).

Table 1: *Detection Dog Team Accuracy*

	True Positive	True Negative	Total Accuracy
Nelson	0.9091 (20/22)	0.9894 (93/94)	0.9741 (113/116)
Ranger	0.9655 (28/29)	1.00 (131/131)	0.9938 (159/160)
Skye	1.00 (28/28)	1.00 (119/119)	1.00 (148/148)
Zilly	0.9048 (19/21)	0.9896 (95/96)	0.9744 (114/117)
Total	0.95 (95/100)	0.9955 (438/440)	0.9870 (533/540)

Over a combined 540 samples, DDTs had a total overall accuracy (accuracy on healthy and infected samples) of 0.9870 (range = 0.9741 to 1) with a true positive rate (accuracy of dog correctly alerting to infected samples) of 0.95 (range = 0.9048 to 1) and a true negative rate (accuracy of dog correctly avoiding healthy samples) of 0.9955 (range = 0.9894 to 1).

Table 2: *Instances of False Negatives*

a: ***Sample T632***

	Sample T632	Failure to detect sample	Successful Detection	Percent Detected
Dog	Nelson	0	1	100%
	Ranger	0	1	100%
	Skye	0	2	100%
	Zilly	1	1	50%
	Total	0	2	100%

b: ***Sample T635***

	Sample T635	Failure to detect sample	Successful Detection	Percent Detected
Dog	Nelson	0	1	100%
	Ranger	0	1	100%
	Skye	0	1	100%
	Zilly	1	1	50%
	Total	0	2	100%

c: ***Sample T751***

	Sample T751	Failure to detect sample	Successful Detection	Percent Detected
Dog	Nelson	0	1	100%
	Ranger	1	1	50%
	Skye	0	1	100%
	Zilly	0	1	100%
	Total	1	4	80%

d: ***Sample T946***

	Sample T946	Failure to detect sample	Successful Detection	Percent Detected
Dog	Nelson	1	0	0%
	Ranger	0	1	100%
	Skye	0	1	100%
	Zilly	0	1	100%
	Total	3	9	75%

e: ***Sample T796***

	Sample T796	Failure to detect sample	Successful Detection	Percent Detected
Dog	Nelson	1	0	0%
	Ranger	0	1	100%
	Skye	0	1	100%
	Zilly	0	1	100%
	Total	0	1	100%

Over a combined 540 samples tested, DDTs had a total of five instances of false negatives (samples T632, T751, T946, T796 and T635). There was no consensus among DDTs on any of the false negatives, showing the importance of having multiple detection dogs cross-examine one another.

Table 3: *Instances of False Positives*

a: ***Sample 266***

Dog	Sample 266	Incorrect alert to healthy sample	Successful pass of healthy sample	Percent Detected
	Nelson	1	2	100%
	Ranger	0	2	100%
	Skye	0	2	100%
	Zilly	0	1	100%
	Total	0	2	100%

b: ***Sample 245***

Dog	Sample 245	Incorrect alert to healthy sample	Successful pass of healthy sample	Percent Detected
	Nelson	0	2	100%
	Ranger	0	3	100%
	Skye	0	1	100%
	Zilly	1	2	100%
	Total	0	5	100%

Over a combined 540 samples tested, DDTs had a total of two instances of false positives (samples 245 and 266). There was no consensus among DDTs on any of the false negatives, showing the importance of having multiple detection dogs cross examine one another.

- **Cost:** To estimate samples per day and cost per sample, RDT utilized the statistics gathered from the leaf trials. We estimated that two or three DDTs cross-examining one another for consensus would average ~50 samples per hour. Based on a $65 per hour rate (current as to the writing of this report) and factoring in time for sample processing and proper storage, the estimated cost per sample ranged between $3 to $5 USD. The range in price is attributed to the variance in infection rate among orchards. The higher the infection rate, the fewer samples detection teams are able to work through per round. Each session ends when a detection dog receives a reward. If there is a higher infection rate, a DDT would work through fewer samples before giving a reward. In our leaf trial sessions, the simulated infected rate was randomly chosen (range 0% to 25%). RDT did not notice a drop in accuracy with a higher infection rate, but due to the ripple effect described previously, a higher infection rate has the potential for more errors.

Discussion: RDT obtained promising results in controlled setting experiments. A high success rate of 0.9870 highlights that when utilizing DDTs in a controlled setting, DDTs could become early detectors of diseased plants. In turn, this could serve to inform growers of plants requiring immediate removal in order to reduce the spread of infection. There was no consensus among DDTs on any of the false negatives, showing the importance of having multiple dogs cross-examine one another (Table 2, Table 3). False negatives occur for a variety of reasons ranging from the DDTs moving too quickly out of excitement, detection dogs breathing heavily and thus not smelling each sample effectively, or from detection dogs becoming mentally fatigued due to the intensity of the searches. As with any detection dog work, monitoring the detecting dog's energy is a key factor in success rates, and for this work specifically, towards keeping the occurrence of false negatives at a minimum.

In this study, there were two false positives. There was no consensus among DDTs on any of the false positives, once again highlighting the need for DDTs to cross-examine samples. A false positive may occur for a variety of reasons ranging from accidental influence from their bounder, contamination of the sample/apparatus, early detection of the odor from further up the line, or anxiety of missing a reward. Working in a controlled environment allows bounders to better manage and mitigate the potential for false positives. It is RDTs' professional opinion that the number of false positives would be much higher in a field environment.

The DDTs, Nelson and Zilly had lower overall accuracy than DDTs, Ranger and Skye. Nelson and Zilly accounted for six of the seven total errors. These errors can also account for the differences in total samples tested; Nelson and Zilly tested a combined 233 samples, while Ranger and Skye tested a combined 308 samples. In this study, each session ended following a successful alert, or an error. Nelson and Zilly had more total errors, and as such were able to test fewer samples. While Nelson and Zilly still had high overall accuracies, 0.9741 and 0.9744 respectively, one variable that may have led to lower accuracy is that overall, they have had fewer field deployments than both Ranger and Skye. Ranger and Skye were the primary detection dogs on the project and Zilly and Nelson were brought in to provide a variety of experience levels.

During RDT's previous field visits in summer, bounders observed several limitations to utilizing DDTs in orchards causing RDT to reexamine the efficacy of deploying DDTs in a field setting. Challenges and concerns include; 1) chemicals sprayed in orchards may be harmful to the health of the DDTs and add a layer of confusion for the detection dogs as to which odor bounders are asking them to detect (as an example, if infected plots are sprayed more frequently a detection dog may interpret that they should be detecting trees with fresh spray), 2) cheatgrass seeds (*Bromus tectorum*), as they pose a severe danger to being inhaled by the dogs, which could require expensive and emergency veterinary care, 3) puncture vine (*Tribulus terrestris*) which requires detection dogs to wear special boots to protect their feet during an already hot season (dogs sweat through their feet and when they become overheated they are significantly less efficient and effective), 4) limited movement between rows which would require the bounder to call a detection dog off an odor in order to walk around to enter the adjacent row, 5) limited ability for the DDTs to cross examine one another leading to a potential magnification of errors, and 6) an increased rate of false positives. These limitations are all extremely detrimental to the efficacy of the work, as it hinders the detection dog's ability to maintain focus which ultimately leads to a reduction in accuracy.

During the winter season, orchards do not employ a spray schedule and harmful invasive grasses have yet to emerge. While winter field visits did curb a few of the dangerous environmental conditions encountered in summer, there was still limited movement between rows and decreased ability for the DDTs to cross-examine one another. According to WSU researchers, LCD vacates the above-ground portion of the tree and is dormant in the root system during the winter months, which may pose detection challenges not yet explored.

Working in a controlled setting mitigates all of the above limitations of deploying DDTs to a field setting while maximizing the DDT's work output. DDTs are able to continue working with preserved leaf samples throughout the entire year. The method RDT has developed creates a sustainable and repeatable process. Bypassing field deployments eliminates the dangers that working in the field poses, while simultaneously increasing the efficiency of DDTs and their ability to cross-examine one another. As such, this method could have a wider application as it could be easily taught to growers interested in learning how to have an in-house DDT or other local community-based groups that are interested in learning the methodology. RDT believes that collaboration between growers, research labs, and community dog groups, like a local Kennel Club, would be the ideal solution required for early detection and ultimately, for the eradication of LCD.

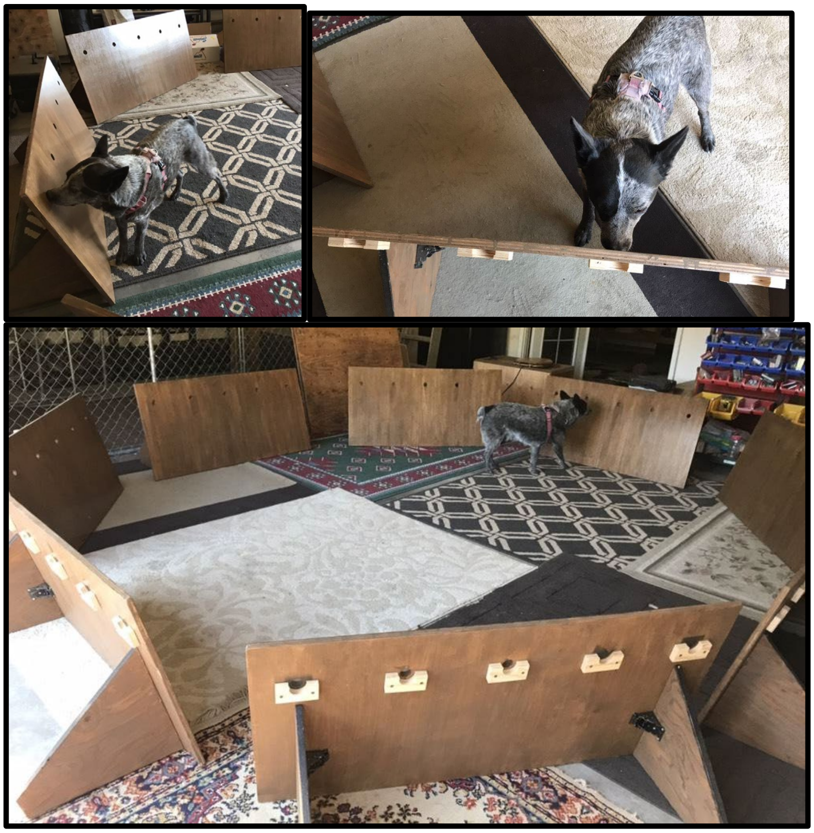

Figure 1. Wall apparatus with detection dog, Dio. Note: These photos were for demonstrating use of the wall apparatus, as such there are no jars with samples in the wall.

KEYWORDS, ABSTRACT AND EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

Project title: Canine Detection of Western X Disease in Controlled and Field Settings

Keywords: Detection, Dog, Detection Dog Team, Canine, Little Cherry Disease, Little cherry virus 2 (LChV2), Western-X phytoplasma (WX)

Abstract: Cherry trees can be detrimentally affected by small size, poor color, and bitter-tasting cherries via one or more of three pathogens in Washington state: Little cherry virus 1 (LChV1), Little cherry virus 2 (LChV2), or Western-X phytoplasma (WX). RDT is exploring the potential to use Detection Dog Teams (DDTs) in the early detection of these diseases before symptoms appear. RDT observed promising results in controlled setting experiments. Over the course of a combined 540 samples tested, DDTs had an overall accuracy of 0.9870. This high success rate highlights that when utilizing DDTs in a controlled setting, DDTs could become early detectors of diseased plants. This would serve to inform growers of plants requiring immediate removal to reduce the spread of infection. RDT believes that collaboration between growers, research labs, and community dog groups, like a local Kennel Club, would be the ideal solution required for the early detection and eradication of LCD.