FINAL PROJECT REPORT

Project Title: Pilot Study: Canine Detection of Western-X disease

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Cooperators: Teah Smith, Zirkle Fruit Company

Other funding sources
Agency Name: Washington Tree Fruit Research Commission
Amount awarded: $6462
Total Project Funding: $6462

Budget History:

Item	**Year 1:**	**Year 2:**	**Year 3:**
WSU Harper:	$2,000 for salary, benefits, consumables, and shipping		
RDT:	$4,462 for five pilot training days at $892.40 per day		
Total	$6462		

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 virus strains must be removed, but detection of symptoms is difficult 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 detector canines may provide a quick screening method for identifying diseased trees for targeted molecular verification.

Rogue Detection Teams (RDT) began a preliminary pilot study with Little Cherry Disease 2 (LCD-2) but switched to target Western-X due to the aggressive nature of the disease. The Western-X pilot study took place in a similar fashion to the LCD-2 study, but also includes a field trial component. For the field component RDT deployed teams in orchards to determine if detection teams can distinguish between infected and healthy trees in an orchard setting.

Teah Smith with Zirkle Fruit Company provided disease-free and Western-X diseased plant materials (leaves, branches, seedlings, etc.) for testing at the RDT facility. Using clean and virus-infected plant materials, RDT began presenting odors to canines to evaluate their responses. Positive responses and the display of the desired behavior were rewarded as part of the operant conditioning program. The canine's proficiency was calculated and at least 80% proficiency was required to complete the pilot exercise. If canines are found to reliably detect infected cherry parts/plants, then the pilot program will be considered a success and will proceed to the next phase: seeking external funds for a full-scale program.

Samples of healthy and Western-X disease-inoculated leaves, twigs, bark and roots were sent by Teah Smith to RDT on November 21, 2019. Samples were shipped overnight and immediately frozen until they could be dehydrated to reduce the potential for mold development on the samples.

SIGNIFICANT FINDINGS

Indoor Trial 1
December, 2020
RDT Facility
Detection Dogs: Pips
Bounders: Heath Smith

Presentation: Building off of the work done with little cherry disease-2, an apparatus was constructed in order to introduce detection dogs to the Western-X samples. Six rectangular platforms were constructed to create an interchangeable design. For this work, platforms were arranged in a hexagonal structure as seen in Figure 1. Each section of the box is four feet in length and contains two 3.5" holes that hold a wide mouth mason jar. Depending on the substrate of the odor, jars can be either pint or half-pint sized. For this study, RDT used half-pint sized jars covered with a fine mesh screen to reduce contamination from the dog's nose as they sniff the jars. The platforms were arranged in a circle formation where a total of twelve samples could be placed in the six boxes. Of the twelve samples that are placed, one sample would always be a laboratory confirmed infected sample. Infected samples were denoted with a red sticker on the side of the jar for easy reference. Another 11 "healthy" samples were placed in remaining holes, all laboratory confirmed healthy samples were denoted with a green sticker on the side of the jar. It is worth noting that leaves were present in all confirmed infected samples.

Initial trials were conducted by Heath Smith and Pips. Pips, a 9 year old Australian cattle dog, was able to discern infected samples within a few hours of work. By mid-December, Pips had no problem identifying all infected samples. However, Pips continually had a false positive response to one “healthy” sample; Othello Block 2SH 7B. This either meant that 1) the sample had been contaminated, 2) we had taught Pips to alert to a different odor than expected, 3) the sample was infected with LCD-2, which Pips had also been taught to alert at, or 4) the sample was actually infected.

Figure 1. Box apparatus.

Indoor Trial 2
January, 2020
RDT Facility
Detection Dogs: Skye, Zilly, Ranger, Pips, Dio
Bounders: Jake Lammi, Suzie Marlow, Heath Smith, Collette Yee

Following the false positive alert by Pips, RDT decided to open the testing to a larger number of teams. None of the 4 dogs that joined the trials in January had been taught to alert at LCD-2 positive samples. The detection dogs included Pips as well as Skye, a 6 year old Kelpie mix, Zilly, an 8 year old Australian cattle dog, Ranger, a 7 year old black Labrador mix, and Dio, a 7 year old Australian cattle dog. The bounders included Heath Smith as well as Suzie Marlow, Collette Yee and Jake Lammi.

We prepared 18 healthy samples and five infected samples to work with during trials. Initially, 11 healthy and three infected samples were used for the first few rounds of odor introduction. Once

the dogs started to differentiate on their own between the infected and healthy samples, the other infected samples were included in the rotation. The dogs had not been exposed to these infected samples before but indicated with a sit that they were infected samples. All five dogs independently differentiated between the infected versus healthy samples.

As the dogs progressed, more healthy samples were added into the rotation. This provided more opportunities for the dogs to become accustomed to differentiating between infected and healthy samples. Of the seven other healthy samples added into the rotation, each dog indicated independently and consistently at one sample: Othello 7B. The four bounders conducting the trials worked independently of one another and discussed their results with one another only after the sessions were complete.

Othello 7B was re-tested at Scott Harper's laboratory at WSU and results continued to show the sample to be healthy. Possible explanations of all the dogs indicating on Othello 7B include: 1) Othello 7B may have an early stage of the disease that the laboratory did not detect, 2) Othello 7B may not be infected but may have a different stressor affecting it and is giving off stress signals (and odors) that are similar to those given off by trees infected with Western-X, or 3) The sample may have accidentally been contaminated with part of an infected sample during collection.

Field Introduction 1
February 26, 2020
Ranch: Othello
Duration: 1:00pm-5:00pm
Detection Dogs: Skye, Zilly, Ranger, Pips
Bounders: Jake Lammi, Suzie Marlow, Heath Smith

On February 26, 2020 three RDT bounders and four detection dogs visited the Othello Ranch in central Washington to conduct a field trial in an area with known infected trees. The purpose of the field trials were to take a further step in our communication with the dogs and how they can help us detect infected trees. It is important to understand our goal was not to test the dogs but rather to introduce them to infected trees in the field and gauge their reaction.

We were accompanied by Teah Smith and two employees from the Othello ranch during our first visit. We first toured the area without the dogs to get an idea of the layout and which trees were infected. We took care to not walk directly to the infected trees or touch any of the surrounding trees to avoid having any recent human odor at any particular tree. This block of trees had been recently pruned and mulched and there were no leaves present on the trees. At the time of this visit, Western-X had vacated the above-ground portion of the tree and was overwintering in the root system (Harper, Smith, & Curtis, 2019). However, our hope was that there may be residual odor from the disease still present in the above-ground portion of the tree that the dogs could detect, or that the tree as a whole takes on a unique odor after infection that persists through the winter.

Each dog was acclimated to the area for about five minutes before directed to begin searching. The search command RDT uses is “Find it”. Each team worked into the area from downwind to observe if or when the dog might detect the odor from infected trees. With no clear indication near the infected trees from the first dog, we had two freshly cut branches removed from the infected tree and set out in a clearing. The goal was to bridge the communication from the indoor sessions to the field setting. The dog was directed into the clippings and given an immediate reward when smelling the branches. We were also curious if freshly pruned branches possessed a stronger odor. With this type of detection it can be easier for the dog to recognize a stronger odor rather than a weaker odor masked by an array of novel odors from a new field setting. Using the freshly cut branches as a quick reward before entering the stand, the dogs were taken and directed to check various trees. We first directed the dogs to investigate healthy trees far from the infected area to reduce our bias directing the dog to only infected trees. We then moved toward the infected trees and directed the dogs to search areas where samples had been removed or scraped from the tree. Two of the dogs alerted where scrapes had been removed from the bark and where bits of mulch had been removed during the pruning near the infected trees. With the recent mulching, the amount of odor in the area may have been overwhelming to the dogs at first. In a situation like this, the longer the dogs are in an area the more they will be able to isolate particular sources of odor as they acclimate.

Field Introduction 2
March 06, 2020
Ranch: Rock Port
Duration: 1:00pm-5:00pm
Detection Dogs: Skye, Zilly, Ranger
Bounders: Jake Lammi, Suzie Marlow

On March 6, 2020, two bounders and three dogs visited another ranch, in Central Washington near Rock Port, for a second field trial. In order to build a clear communication bridge between indoor trials and field trials it is important to introduce the dogs to as many infected trees as possible. This allows the dogs to develop a broader understanding of the target odor. The first block had been freshly watered and had nine confirmed infected trees at the edge of the block. RDT followed the same procedure described in the Field Trial 1 section.

Indoor Trial 2
March, 2020
RDT Facility
Detection Dogs: Skye, Zilly, Ranger
Bounders: Jake Lammi, Suzie Marlow,

Following the second field trial, RDT was provided approximately 100 more samples. These samples included confirmed infected, confirmed healthy and unknown samples that had not been tested in a laboratory. Samples were collected in late February and consisted only of bark scrapings. Of those, 20 were infected, 20 were healthy and 60 were defined as “unknown”. All samples contained approximately five 2”x2” bark scrapings. They were collected with a new pair of latex gloves for each sample and clippers sanitized with alcohol swabs between clippings. They were stored in ziplock bags and kept in a cool and dark environment. Samples were not

frozen this time since there were no signs of molding and we were unsure whether freezing the sample would be beneficial or not to the odor signature. Samples were prepared by placing three 2”x2” pieces into a ½ pint ball jar, and the lid was replaced by a piece of mesh secured around the lip of the jar. Sample preparation was limited by the materials on hand and only six healthy and seven infected samples were processed.

Our goal with these new samples was to determine if the dogs could detect infected samples just from bark and if so did they find additional samples in the unknown category. Confirmation of whether the dog’s alert was accurate on an “unknown” would be determined by sending the samples to the laboratory at WSU.

RESULTS & DISCUSSION

Our goal is for dogs to successfully alert to infected plants while effectively and efficiently surveying an entire orchard. This would allow diseased plants to be removed that would not start showing signs of infection until 2-3 weeks prior to harvest. RDT’s ultimate goal will be to deploy detection teams at orchards that can evaluate the health of each tree in the orchard, with trees targeted by the dogs being further tested for confirmation.

Indoor trials

While our results are preliminary and with a limited number of samples it was clear that all five dogs were able to distinguish between infected and healthy samples when leaves were present in the samples. More work needs to be conducted to determine if the dogs can distinguish between infected and healthy samples when samples consist of only bark scrapings or if there is a seasonal difference with bark samples. It is important to bear in mind that we want to find a solution that works in a field setting, not just in a controlled environment.

Bearing this in mind, before progressing to the dogs alerting to potentially infected trees in unconfirmed samples we need to confirm that the dogs are able to alert to Western-X in late winter samples.

When we introduced the new healthy samples along with one of the original infected samples, the dogs quickly and successfully pinpointed the old infected samples with no reaction to the new healthy samples. As more healthy samples were rotated into the apparatus and a new infected sample was added into the rotation the dogs did not display an alert or reaction to the infected sample. This indicates that the dogs were unable to differentiate the unique odor of a new healthy sample from a new infected sample collected in February. At this time we are unable to report if that is because, 1) the disease is not present in the bark during the winter, 2) the samples need to be prepared in a different manner, perhaps being cut into smaller pieces for greater surface area, or 3) if there was a breakdown in communication with the dogs that resulted in them not understanding the question we were asking.

Indoor trials were put on a hiatus so that we could rethink the sampling process in order to lead to success.

Field trials

After two field days RDT believes that the dog and handler teams were making progress in detecting Western-X in live trees in an orchard. This work is still preliminary and more trials with a greater number of infected trees will need to be conducted to determine if the dogs are able to distinguish between infected and healthy trees in an orchard setting. Our ability is limited due to the need for orchards to spray infected trees before they transmit the disease to surrounding trees. We will need to work to coordinate a time that is safe and the odor of the tree has not been overlaid with a spray.

We also discussed the idea of having dogs work in nurseries where seedlings are potentially infected or on site prior to saplings being planted. This may work well in the late winter because the roots are exposed. In order for this to move forward we would need roots from diseased and healthy saplings.

Further considerations:

Indoor trials

- Utilize bark cuttings from the fall when Western-X is present throughout the entire tree
- Comparing leaves vs. bark cuttings in the same season to determine whether there is a stronger odor related to either material
- Utilizing infected saplings
- Continue experimenting with different methods of bark sample preparations
- If field trials prove unsuccessful, can dogs conduct vicinity and randomized indoor trials to help prevent spread?

Field trials

- Conduct field trials in the fall before spraying. Teams would conduct field trials at times when Western-X is thought to be in the visible portion of the tree
- Conduct field trials on nursery trees in early spring
- Greater repetition on a larger number of infected individuals is the best way for the dogs to lock onto the odor.

At this time RDT has conducted the agreed upon number of trials. It will need to be determined if it is beneficial to continue funding this work before further trials indoor and in the field continue.

KEYWORDS, ABSTRACT AND EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

Project title: Pilot Study: Canine Detection of Little Cherry Disease

Keywords: Detection, Dog, 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). The first step in this pilot study is to determine if canines can detect Little cherry virus 2 (LChV2), or Western-X phytoplasma (WX) in inoculated leaves. Although our results are preliminary, it does appear that canines can detect both LChV2 and WX in inoculated leaves. Further steps will include testing of bark samples and more field trials.

Bibliography

Harper, S., Smith, T., & Curtis, R. (2019, February). X Disease Phytoplasma (Western X). Retrieved May 6, 2020, from http://treefruit.wsu.edu/crop-protection/disease-management/western-x/