FINAL PROJECT REPORT

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

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Cooperator(s): Teah Smith, Zirkle Fruit Company

Total Project Funding: $6,462

Budget 1:
Organization Name: WSU **Contract Administrator:** Katy Roberts
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Item	2019
Salaries[1]	
Benefits	
Supplies[3]	
Travel[4]	
Miscellaneous	
Total	$2000.00

For salary, benefits, consumables and shipping

Budget 2:
Organization Name: C2AN Consulting **Contract Administrator:** Michael Lammi
Email address: mlammi@c2an.com

Item	2019
Miscellaneous	4,462
Total	$4,462

Footnotes: 5 pilot training days @ $892.40/day

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.

In order to determine if dogs can discern LCD-infected plants from clean plants, Rogue Detection Teams (RDT) based in Rice, Washington will provide one Research Scientist and detection dog team for a little cherry disease (LCD) pilot detection training project. Pilot study training will initially be 5 days with the option to extend up to an additional 10 days if needed. This preliminary work will occur prior to RDT fully developing methods and a training regimen to assess the feasibility of deploying detection dogs in the field to locate LCD. Preliminary investigations will not replace a full-scale 25-day training period but will provide some preliminary data on the ability of canines to detect virus-infected materials.

The Washington State University (WSU) Clean Plant Center Northwest will provide disease-free, little cherry disease-2 and western-X disease-inoculated plant materials (leaves, branches, seedlings, etc.) for testing at the RDT training facility. Using clean and virus-infected plant materials provided by Scott Harper (WSU), RDT will begin training by offering odors to canines to evaluate responses. Positive responses and the display of the desired behavior will be rewarded as part of the operant conditioning program. The canine's proficiency will be calculated and at least 80% proficiency must be achieved 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 disease-free, little cherry disease-2 and western-X disease-inoculated leaves were sent from WSU to RDT on July 18th. Samples were immediately refrigerated, per WSU's suggestion for the preservation of the training samples.

It is common for RDT's field teams to be deployed April through November on field studies, leaving the winter months for research and development projects. Samples arrived at a time when all field teams were deployed and the remaining Research Scientist was transitioning between a temporary field house and a newly purchased training facility and property for RDT. As of the writing of this report, while the initial introduction to the odor(s) has occurred, the full-scale effort will commence once RDT's support staff have returned from the field (~ November 18th) allowing for a much greater determination of potential success.

SIGNIFICANT FINDINGS

In an effort to have an idea of possible success before the deadline for this report of October 30th, temporary space was cleared at RDT's new facility and two sections of a box apparatus were available for use. A box apparatus is ideally divided into four or more sections, each section 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 was using half-pint sized jars covered with a fine window screen mesh to reduce contamination from the dog's nose as they sniff the jars. RDT currently has two sections of the box apparatus, both of which contain three rather than two holes. Four or more sections are preferred in that this allows a full circle/square to be arranged for training exercises. When a start and end are apparent to the dog it creates a biased

situation where the dog feels pressured to alert. To help reduce this in the current scenario the first hole was often left blank. Under the time constraints, RDT's goal was simply to determine if the dog was able to recognize any difference between diseased and healthy plant samples.

It is important to keep in mind that the dog will be introduced to six novel odors; five disease-free samples (incorrect) and one little cherry virus-2 (correct) sample. The instructor (handler) must clearly communicate to the dog what is expected at each sample. The dog is walked on a leash, quickly down the line of samples and given a calm "no" at each incorrect sample and a "sit" followed by an immediate reward at the correct sample. The idea being that the handler provides quick, confident rewards. RDT is not testing the dog during this phase, rather introducing the odor in a positive reward scenario. The odor is changed each time and repeated 6 times followed by a 10-minute break. After each break, a different "correct" sample is used and the process is repeated for 3 sessions.

Sessions are less than 30 minutes each and followed by at least a 10-minute break. Four sessions will be followed by a longer break of 30 minutes to 1 hour before beginning again.

The second step is to remove the instructor physically from the process and allow the dog to work independently with verbal corrections at "incorrect" samples. This is initially done as soon as the dog smells each hole with the instructor providing a calm "no" as soon as the dog smells the incorrect sample.

If the dog is able to alert correctly at detections the instructor will work to increase their confidence and the difficulty in the test providing less and less verbal instruction, until there is no verbal instruction given.

RDT is currently focusing on little cherry disease-2 (LDT-2). Once the dog has repeatedly shown success (or failure) at identifying LCD-2, RDT will replicate the experiments with Western-X. RDT does not want to confuse the dog or skew results by attempting both diseases at once.

Figure 1. Box apparatus. Photo credit: Jaymi Heimbuch

Figure 1 displays a box apparatus from an earlier model. Future models will be four feet in length and contain only two holes allowing for easier transport and placement in a circular setup.

RDT is currently using whole or halved leaves that are kept refrigerated in plastic bags when not being utilized in exercises. It is hypothesized that other conditions may provide easier detection by the canine but with the limited supply of leaves, it is not feasible to try these until each condition is tested. As there are a limited number of disease-free leaves, little cherry disease-2 and western-X disease-inoculated plants that were provided to RDT, in order to preserve the training samples, RDT will divide the samples into the following categories; whole, shredded, fresh/crushed, and dehydrated/crushed to ascertain if one category provides a better mechanism for virus detection by the dog. Samples will be handled with latex gloves to ensure that little to no human odor is introduced to the training samples. Odor disperses in the air in different ways and during the pilot phase of this project, instructors with RDT will be determining what method is the most ideal for odor recognition. As the early stages of the disease is not visible, were this method to be utilized in the field the handler will need to rely exclusively on the alert given to them by the dog(s). As such, it is crucial to test each category fully before moving to a different odor dispersal test. Initial samples were introduced whole.

RESULTS & DISCUSSION

If dogs can successfully alert to infected plants while effectively and efficiently surveying an entire orchard, diseased plants could be removed that would not normally show signs of infection until 2-3 weeks prior to harvest. RDT's ultimate goal will be to deploy detection teams at orchards and quickly evaluate the health of each tree in the orchard, with dogs alerting to diseased trees which can be further tested for confirmation.

There are numerous goals of this pilot study, the first of which is to determine can dogs detect a difference between diseased and healthy leaves. The initial answer to this question is, yes. At this point in time, three separate diseased samples have been correctly identified by one of RDT's

detection dogs. RDT stresses that these results are preliminary and do not guarantee success. Further tests will be used to confirm the following;

- Can the dog equally detect newly introduced diseased samples from various orchards?
- Does the detection accuracy increase at various stages of development, i.e. spring growth vs winter stems?
- Is the detection accuracy increased via sample presentation, i.e. dried and crushed leaves vs fresh leaves?
- Based on findings from indoor trials, how can this be best implemented in a field situation (outdoors) and at what time of year?
- Can these results be replicated over numerous dogs?

At this stage, RDT is focusing on increasing the dog's confidence in the odor and it is too early to provide figures on detection accuracy.

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.

Executive Summary:

If dogs can successfully alert to infected plants while effectively and efficiently surveying an entire orchard, diseased plants could be removed that would not normally show signs of infection until 2-3 weeks prior to harvest. RDT's ultimate goal will be to deploy detection teams at orchards and quickly evaluate the health of each tree in the orchard, with dogs alerting to diseased trees which can be further tested for confirmation.

The first goal of this pilot study is to determine if dogs can detect a difference between diseased and healthy leaves. Preliminary results show that three separate diseased leaves have been correctly identified by one dog. RDT stresses these results are preliminary and do not guarantee success. Further tests are required to replicate results.