FINAL PROJECT REPORT YEAR: 1

Project Title: Canine LCD Detection Skills Applied to Nursery and Orchard Settings

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Cooperators: Michael Barclay and Janice Barclay (WKC instructors); WKC board members; WKC dog handlers (volunteers); Nathan J. Hall and Mallory DeChant (Texas Tech University), Hallie McMullen (Scentsational Detection Dogs); Scott Harper (WSU); Lav Khot and Gajanan Kothawade (WSU); Jeffery Bullock (Wenatchee Valley College); Teah Smith (Zirkle Fruit); Hannah Walters (Stemilt Growers)

Total Project Request: $ 45,500

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
PI: Lynda Pheasant
Organization Name: Wenatchee Kennel Club (WKC)
Contract Administrator: Lynda Pheasant
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Item	2022
Salaries	
Benefits	
Equipment	$8,170
Supplies	$14,830
Travel	$12,000
Miscellaneous	$9,000
Plot Fees	
Total	$44,000

Footnotes: WKC budget consists in the following: Equipment: goes towards training equipment, technology equipment and freezer; Supplies: training aids and nursery stock; Travel: mileage reimbursement to participants and cooperators; Miscellaneous: cost for sample analysis.

Budget 2
Co PI: Corina F. Serban
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy

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Contract administrator email address: arcgrants@wsu.edu

Item	2022
Salaries	
Benefits	
Equipment	
Supplies	$593
Travel	$907
Miscellaneous	
Plot Fees	
Total	$1,500

Footnotes: Co PI Serban requests $593 towards extension outreach activities and $907 for travel.

OVERVIEW:

Little Cherry Disease (LCD) is an umbrella term used for two different pathogens – Little Cherry Virus-2 (LChV-2) and X-disease phytoplasma (XDP), that cause similar symptoms in cherries. LCD has reached an epidemic level of infection in Washington sweet cherry orchards, causing the removal of ca. 1,000 acres in the past five years according to a survey conducted by WSU/OSU Extension. Once a tree is infected, there is no cure for it, and the only control measure is the tree removal. Several factors are preventing the sweet cherry industry to stop the spread of the LCD infection. Among the most important, is the lack of optimized or new screening methods to quickly identify LCD infections. Canine detection skills project could provide an early detection tool for LCD identification, control, and eradication. The next step toward industry adoption and implementation is to conduct the structured integration of LCD detection dogs into nursery and orchard settings for further "live" training and assessment of skills. This project is designed to increase detection proficiencies and facilitate transition of canine detection skills from controlled settings to field environments.

The WKC is a non-profit (501c3) all-volunteer organization that was established in 1963. The WKC dogs that participated in this project varied in size, age, gender, breed, and training experience. The dogs are scent-sport competitor dogs trained by their owner/handler to perform a variety of canine sports such as obedience, rally, agility, hunting, lure coursing, barn hunt and competitive scent work. WKC trained consistently 5-7 LCD detection dog/handler teams to increase proficiencies to facilitate transition to field environments. Canine participants in this project include pedigreed dogs (some with champion/grand champion titles), dogs of nondescript "All American" breeding, and rescue dogs. The breeds that participated were: Rhodesian Ridgeback, Standard Poodle, German Shepherd, Rescued Mix-breed, Wheaten Terrier, Entlebucher Mountain Dog, German Shorthair, and Labrador Retriever. WKC dogs are not "purpose bred" for detection work, nor are the handlers paid professionals. Participants volunteered their expertise as dogs' handlers along with their dogs to the development and success of this LCD detection dog project. Geographically, participants represent all four counties of North-Central Washington: Chelan, Douglas, Grant, and Okanogan – counties having substantial economic investment in the cherry industry.

In March 2021 WKC initiated an Agricultural Detection Dog program as a pilot project to explore the following objectives: 1) Can companion dogs be trained to detect LCD, and 2) Can WKC develop an appropriate Agricultural detection dog training program. PI Pheasant has served as the WKC Project Coordinator from the onset. Board members Mike and Janice Barclay served as instructors from March – November 2021. Throughout the project, Board members John and Helen Njus have served as

professional videographers for WKC. The Barclays brought in professional detection dog trainer Cooperator Hallie McMullen who offered training consultation and establishment of a control group in Idaho. As a proof of concept, WKC invited Collaborator Nathan Hall (Director of the Canine Olfaction Research Laboratory at Texas Tech University) to evaluate proficiencies of the LCD Detection dog programs, at WKC and in Idaho. Mid-August 2021 the Ag Dog program lost three handlers and had to re-build by adding three other new handlers. On August 23, 2021, PI Pheasant (WKC) met for the first time with Corina F. Serban (WSU Extension) to explore the continuance of the WKC's "proof of concept" pilot project into submitting a proposal project with the WTFRC. Based on that meeting and seeing the Ag Dog class in action, Pheasant and Serban agreed to be Co-PI's on the research project and submitted a new research proposal to the WTFRC in Oct 2021. The loss of the trainers for the Ag Dog class in Nov 2021, Mike and Janice Barclay, left the project with new challenges, creating a delay and reset of the project. In Jan 2022 the Canine LCD Detection research project was formally initiated. Class program was hampered by extreme weather and by lack of root and bark samples. In March, Cooperator Hallie McMullen conducted a workshop with focus on preparation for April 2022 evaluations. Cooperator Mallory DeChant replaced Cooperator Nathan Hall conducting the evaluations at WKC (none performed in Idaho). Two out of the six dogs passed the proficiency test with 90% accuracy. Moving forward, the dogs were given opportunities to work in the "field" environments even though several of them had zero experience in a commercial cherry orchard. Throughout 2022 the Ag Dogs have held nine instructional field experiences at local cherry orchards and research plots. In July 2022, due to numerous events such as extreme heat, and the need to increase the training proficiencies it was decided to hold the trainings and evaluations indoors at WKC Training Center in East Wenatchee. PI Pheasant stepped away from this project at the beginning of August 2022. Afterwards Co-PI Serban lead the trainings for the month of August while coordinating the weekly training plan with Cooperator Hallie McMullen. At the end of this project Cooperator Hallie McMullen held an evaluation on Sept 5, 2022.

OBJECTIVES:

1. *Increase LCD detection proficiencies in controlled indoor/outdoor settings*
   Three double blind tests/evaluations were run during this project. One prior to the start during proof of concept with Collaborator Nathan Hall (August 2021), one at the midpoint with Collaborator Mallory DeChant (April 2022), and one at the end with Collaborator Hallie McMullen (September 2022).

2. *Develop next-step training protocols to facilitate transition into field experience*
   The dogs were given introduction to the plant materials and orchard experiences. This objective was in development including developing of the protocols. As the dogs were reaching proficiency in Objective 1, they would be able to move forward to Objective 2. With the time and training delays created by the loss of Mike and Jan Barclay, the group was not able to achieve these goals in the time available.

3. *Provide introductory field experiences to enhance LCD detection dog confidence and skill*
   Throughout 2022 the Ag Dogs have held nine instructional field experiences at local cherry orchards and research plots. The group decided to not try to make it to the nurseries because of all the unknowns and the time it would take to test nursery stock, get the results back and then get the dogs in the field.

4. *Improve sample management and analysis*
   Critical to this project is the management and analysis of cherry plant material samples provided to LCD detection dog training classes. Cooperator Hannah Walters provided samples in 2021 and

Cooperator Teah Smith provided samples in 2021 and 2022 on-as needed basis. Cooperator Scott Harper provided some of the PCR tested samples in 2022 as well. Additionally, Cooperators Lav Khot and Gajanan Kothawade took samples from some of the same trees that the dogs used in their trainings to evaluate the volatile profile using FAIMS and CG/MS techniques.

5. *Provide education and extension on "Canine LCD Detection Skills"*
   In July 2021 WSU hired a new Little Cherry Disease (LCD), Information Technology Transfer (ITT) Extension, Corina F. Serban. Co-PI Serban provided education and extension opportunities for WKC, collaborators and industry wide outreach.

SIGNIFICANT FINDINGS:

- The desired percentage of proficiency was not reached by all the dogs. However, the performance of the dogs did support positive proof of concept that any dog can be trained to detect LCD. The group would need additional time to meet the levels of proficiency included in the proposal.
- Some of the factors that may have impacted the results include:
  - New dogs entered the program and a couple of veteran dogs left the program due to medical/other issues.
  - The loss of the trainers for the team, Mike and Janice Barclay, creating a delay and reset for the project.
- Environmental conditions contributed to the success or failure of the dogs accurately alerting on positive samples in the orchard. High temperatures physically limited the dog's ability to work in the field during the summer months. Handlers needed to take in the account the direction and force of the wind. There were also concerns about the presence of pesticides, weeds such as goat heads, cherry cannons, and poisons set out for vermin. These variables could only be learned by working in the orchards and not by working only in controlled environments.
- This work done in this project has created the opportunity for next steps in the process moving forward. There was a lot of learning done regarding LCD by the group which will increase performance timelines and improve methodology.
- The next steps for canine LCD work should include a small group of focused dogs that are trained on a regular basis, at least several times a week using a specifically developed training schedule including regular validation testing. The steps would include odor validation, proofing, and experiences in the appropriate field setting.

METHODS:

1. *Increase LCD detection proficiencies in controlled indoor/outdoor settings.*
   August 2021 evaluation. All plants materials were provided by Cooperators Hannah Walters and Teah Smith. Tree cuttings were placed in labeled Ziplock bags and held at refrigerated temperatures until use. All samples were coded as either samples obtained from positive trees or negative trees with a unique sample ID. Dogs were tested in a three alternative forced choice test. Unused mailer boxes were used to hold either a glass 8 oz mason jar with a screen/perforated lid (Wenatchee location) or a stainless-steel canister with a perforated lid (Idaho location). A hole in the cardboard box was made to hold the container with the sample upright on the floor, giving the dog access to sniff. All dogs completed 15 three alternative forced choice test experimental trials. For each trial, one diseased cutting and two non-diseased cutting were presented. Individual dogs were tested with the same three samples for all 15 trials, but different clipping samples were given to each dog. Some dogs were tested with the same positive source sample (but different samples within the source).

April 2022 evaluation. Prior to the start of the testing period, the handlers were allowed 5 training trials so they could become familiar with the search pattern and calling out an alert. The test period consisted of each dog searching 6 canisters for a total of 10 trials. The odorants utilized were positive cutting, negative cutting, grass, gravel, and a blank canister. One out of the six cannisters had a positive cutting. The position of each odorant in the canisters was pseudo-randomized so the target odor was not in the same position for more than 3 trials in a row. Handlers were blind to the location of the target odor (positive cutting) and were asked to call out the number of the canister their dog was alerting to, the experimenter then indicated if dog was correct or incorrect. As typical practice for WKC training, handlers searched the cannisters until dog located the correct target cannister or the handler called an all clear.

RESULTS AND DISCUSSION:

1. *Increase LCD detection proficiencies in controlled indoor/outdoor settings.*

Three double blind tests/evaluations were run to test the LCD detection proficiencies. One prior to the start during proof of concept with Collaborator Nathan Hall (August 2021), one at the midpoint with Collaborator Mallory DeChant (April 2022), and one at the end with Collaborator Hallie McMullen (September 2022). In addition, Co-PI Serban collected data from the training classes held during August 2022 to track the proficiencies of each dog indoors. The dogs performed at different levels during these tests.

- August 2021 evaluation. The overall results from this project showed that two of the eleven dogs showed proficient detection of the LCD infected samples from the healthy samples by showing immediate detection during warm-ups and detection above change levels. An additional 4 dogs reached above chance levels with reinforced experience with the target material, but 5 dogs did not show detection above chance levels. Together, these results provide a positive proof-of-concept that citizen science trained dogs can successfully discriminate LCD infected cherry samples from healthy cherry samples, but not all dogs meet proficiency to demonstrate above chance performance.
- April 2022 evaluation. The objective of this evaluation at the WKC was to evaluate the accuracy of six dogs that have previously been trained to detect LCD. The results below show that two dogs out of six passed the proficiency evaluation with 90% accuracy.

Dog ID	Training period Accuracy	Testing period Accuracy	Testing period miss rate	Testing period False Alerts
1	20%	50%	10%	11 times
2	0%	40%	10%	6 times
3	40%	40%	0%	7 times
4	30%	30%	20%	10 times
5	100%	**90%**	0%	1 time
6	30%	**90%**	0%	1 time

- Classes held indoors in August 2022 to track the proficiencies of each dog over time.

Note: N.A means the dog was not present.

Dog ID	Testing period Accuracy (%)			
	Aug 8	Aug 15	Aug 22	Aug 29
A	71%	60%	**83%**	40%
B	57%	60%	67%	60%
C	57%	60%	67%	40%
D	43%	N.A	33%	60%

E	71%	40%	N.A	40%
F	57%	**80%**	33%	N.A
G	43%	N.A	N.A	N.A

- September 2022 evaluation. The overall results show that all participants have been working on their handling skills. The dogs were working more independently. It also appeared that the dogs were more definitive in their answers. There were still some issues with dogs choosing the negative samples, but there was progress in that area as the dogs made choices sooner and weren't waiting for handler to help. There are several factors that would be next steps for resolving these things, such as changing out canisters, so the dogs aren't influenced by odors left by other dogs and so on. There weren't obvious consistencies in errors, for instance the dogs didn't show a propensity to false alert if the negative came before the positive or vice versa. However, there was an increase in false indications in the last 4 runs. There are several different factors that could play into that result, such as search endurance, odor influence from not replacing canisters, or others. Results are shown in table below:

Dog ID	Testing period Accuracy	Testing period miss rate	Testing period False Alerts
A	50%	0%	5 times
B	70%	0%	3 times
C	40%	0%	6 times
D	60%	0%	4 times
E	**80%**	0%	2 times

2 *Develop next-step training protocols to facilitate transition into field experience.*

The dogs were given introduction to the plant materials and an orchard experience. This objective was in development including developing of the protocols to facilitate transition from controlled indoor/outdoor setting to field experience in nurseries and orchards. As the dogs were reaching proficiency in Objective 1, they would be able to move forward to Objective 2. With the time and training delays created by the loss of Mike and Jan Barclay, the group was not able to achieve these goals in the time available.

3 *Provide introductory field experiences to enhance LCD detection dog confidence and skill.*
The first introductory field experience in a cherry orchard was on May 23, where several of the dog/handler teams had never had exposure inside a cherry orchard. The dogs found little connection between LCD odors in sample cans and LCD odors in trees. WKC developed a program for carefully staging the introduction of LCD detection in orchard settings. Over the next several weeks field classes were held at local orchards where the dogs learned orchard search techniques, trying to find positive samples, and ignoring the negative samples contained in a variety of packaging materials placed at various elevations within the orchard setting. On June 27th, Ag Dogs returned to a different orchard where dogs invested time canvassing the ground cover plants rather than indicating the positive source trees. On July 25 three dog/handlers teams had the opportunity to travel to WSU research orchards in Prosser for additional field training. The group quickly learned that the site raised far more questions than it provided support for LCD detection dog training because of different inconsistent factors such as smaller inoculated "rootstocks" type trees, tall ground cover, cannon noises and extremely hot weather.

4 *Improve sample management and analysis.*
In addition to samples being provided by Cooperator Hannah Walters (2021) and Cooperator Teah Smith (2021 and 2022), the samples also had corresponding information such as titer levels, collection date and orchard location. WKC had a designated secured refrigerator where the samples were stored for dog training use. The WKC handlers developed their own training record sheets that they used in the field introductions to collect observations tracking each dog's progress.

5 *Provide education and extension on "Canine LCD Detection Skills"*

Education and Extension opportunities provided to WKC and collaborators

- Co-PI Serban hosted monthly 1 h virtual meetings 'Canine LCD Detection Collaborators Meeting' in February, March, April, May, July, and August 2022, where the progress of this project was shared with all the collaborators, and everyone was invited to provide feedback (15 participants).
- Dr. Tobin Northfield (WSU Entomology) was invited to give a presentation on Little Cherry Disease topic to the WKC handlers, to learn more about the topic and provide them opportunity to ask questions. August 8, 2022 (12 participants).
- PI Pheasant hosted 'Ag Dogs semi-annual meeting' on July 25, 2022 (10 participants).

Industry wide outreach

Project was mentioned in several oral presentations by Co-PI Serban at the:

- Tree Fruit Endowment Advisory Committee Meeting, March 15, 2022, Prosser WA *('Little Cherry Disease Extension & Outreach Program'; 30 participants)*
- LCD Northwest Horticultural Council -USDA Meeting, Feb 25, 2022 *('Extension Activities in Response to Little Cherry Disease'; 17 participants)*
- Cherry Institute Annual Meeting, Jan 7th, 2022, Yakima WA *('Latest Developments on Little Cherry Disease'; 100 participants)*
- WSDA Tree Fruit Technology Fall Tour, Oct 15, 2021, Prosser WA *('Little Cherry Disease Extension & Outreach'; 20 participants)*

Project details were shared by PI Pheasant (oral presentation) under 'New Experimental Research Projects: Early Detection' session at the Little Cherry Disease Day, on Feb 16, 2022, in Ellensburg WA (178 participants) organized by Co-PI Serban. Preliminary results/project details were shared by PI Pheasant (oral presentation) and a demonstration by Canine LCD detectors-in-training (two handler/dog teams) at the Little Cherry & X-disease Field Day on Jun 21, 2022, in Buena WA (57 participants) organized by Co-PI Serban. Following the Little Cherry & X-disease Field Day on Jun 21, 2022, Co-PI Serban had one interview in YaktriNews.com together with a field day summary (https://www.yaktrinews.com/researchers-use-dogs-to-sniff-out-infected-cherry-trees-in-eastern-washington/). Other Newspapers and Periodicals featuring project details:

- McClain, Sierra Dawn. "Pilot project uses dogs to sniff out little cherry disease". Capital Press, June 2, 2021. https://www.capitalpress.com/ag_sectors/orchards_nuts_vines/pilot-project-uses-dogs-to-sniff-out-little-cherry-disease/article_dd7230e0-c30c-11eb-9145-9baccaec1558.html
- Brown, Trent. "A nose for the orchard – Wenatchee Kennel Club trains 'ag dogs' to detect little cherry disease", June 15, 2022. https://www.wenatcheeworld.com/news/local/a-nose-for-the-orchard/article_63da7a4e-eb6f-11ec-aa9d-7fdcb8c20fb9.html

EXECUTIVE SUMMARY
Project title: Canine LCD Detection Skills Applied to Nursery and Orchard Settings

Keywords: Canine, Dog, Little Cherry Disease, Early LCD detection, Ag Dogs, Little Cherry Virus-2 (LChV-2), X-disease phytoplasma (XDP)

Little Cherry Disease (LCD) is an umbrella term used for two different pathogens – Little Cherry Virus-2 (LChV-2) and X-disease phytoplasma (XDP), that cause similar symptoms in cherries. Once a tree is infected, there is no cure for it, and the only control measure is the tree removal. Several factors are preventing the sweet cherry industry to stop the spread of the LCD infection. Among the most important, is the lack of optimized or new screening methods to quickly identify LCD infections. Canine detection skills project explored the possibility of providing an early detection tool for LCD identification, control, and eradication. This project was designed to increase detection proficiencies and facilitate transition of canine detection skills from controlled settings to field environments. The desired percentage of proficiency was not reached by all the dogs. However, the performance of the dogs did support positive proof of concept that any dog can be trained to detect LCD. The group would need additional time to meet the levels of proficiency included in the proposal. This work done in this project has created the opportunity for next steps in the process moving forward. There was a lot of learning done regarding LCD by the group which will increase performance timelines and improve methodology. The next steps for canine LCD work should include a small group of focused dogs that are trained on a regular basis, at least several times a week using a specifically developed training schedule including regular validation testing. The steps would include odor validation, proofing, and experiences in the appropriate field setting.