# *Sustaining healthy restoration habitat: Phytophthora research for the San Francisco Public Utility Commission (SFPUC)*

FS Agreement No. 20-JV-1127239-005

## FINAL PROGRESS REPORT: CONTINUED WORK


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## Training dogs to detect *Phytophthoras* and *Phytophthora*-infected plants: Phase 4

In collaboration with USDA Forest Service, Dr. Matteo Garbelotto, Director, Forest Pathology and Mycology Lab Extension Specialist & Professor, Department of ESPM, U.C. Berkeley, Tina Popenuck, UC Berkeley affiliate and under the training direction of Lauralea Oliver of k9inSCENTive, LLC, Phase 4 of training and testing for accurate detection of *Phytophthoras* and *Phytophthora*-infected plants with two dogs, Bolt and Banshee, concluded on May 6, 2022. An additional two days of testing were conducted with the remaining project funding. Testing focused on a study of the ability of SCENTdogs Bolt and Banshee to detect pathogenic *Phytophthora* in decreasing quantity from composite soils. Though dilutions testing had been conducted in the previous portion of this phase, this additional work presented the dogs with even more diluted preparation of the target pathogen. This final progress report summarizes the work completed and the results of testing during this additional phase of work.

The scent racks are continually being used to train and test both dogs at the Berkeley Lab. The scent racks in use are pictured below.

## 1 – How sensitive are trained scent detection dogs to the presence of pathogenic *Phytophthora*?

The additional dilutions training was a way to further ascertain any difference in the dogs' alerts depending on the amount of *Phytophthora* (inoculum) present in a sample. Two *Phytophthoras* were used in the trial – *P. cinnamomi* and *P. cactorum*. Two types of soil were used – SFPUC Nursery mix (WZ) and Sunshine Mix 4 (M4).

Various dilutions of *Phytophthoras* were tested, this time at a much lower disease incidence value than previous tests. Beginning with a jar containing 40gr of soil, ten pieces of inoculum were added. This was then grown out for four days. After the incubation period, the inoculum was thoroughly mixed. Then, 4gr of this inoculum was added to a glass jar holding 36gr of clean soil. This smaller sample was then carefully mixed. The "mother" inoculum was dispersed in the same manner, taking 4gr from it and adding it to new jars of 36gr clean soil. This process was continued until all the original inoculum was used and was performed in triplicate. All sample jars for testing consisted of 40gr diluted inoculum.

The dilution samples were then labeled as follows:
Jar a = 1:1
Jar b = 1:10
Jar c = 1:100
Jar d = 1:1,000
Jar e = 1:10,000
Jar f = 1:100,000
Jar g = 1:1,000,000
Jar h = 1:10,000,000

Single-blind trials were set up. Each soil type and inoculum were tested in series before moving on to the next group of soil types. During each run, the dogs were presented with ten samples: three diluted *Phytophthora* positive samples, one *Phytophthora* positive control sample and six negative control samples of the same soil type. Testing was conducted over two days with every sample being searched by both dogs once. There was a total of 96 duplicate samples in the trial.

## Below is an excerpt representing overall results from the dilutions trial.

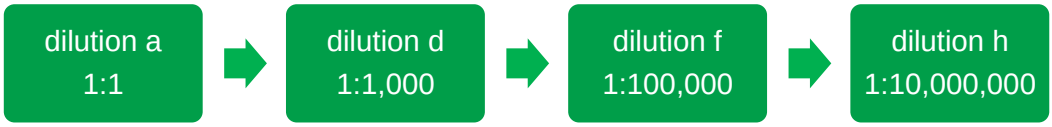
| *M4.1 P. cact* | Dilution | Agdia Positive? | Pear Positive? | Dog Positive? |
|---|---|---|---|---|
| P-1 a | 1:1 | Yes | No | Yes |
| P-2 b | 1:10 | Yes | Unknown | Yes |
| P-3 c | 1:100 | Yes extremely low | Unknown | Yes |
| P-4 d | 1:1,000 | Yes very low | Unknown | Yes |
| P-5 e | 1:10,000 | Yes very low | Unknown | Yes |
| P-6 f | 1:100,000 | Yes very low | Unknown | Yes |
| P-7 g | 1:1,000,000 | Yes very low | Unknown | Yes |
| P-8 h | 1:10,000,000 | Yes very low | Unknown | Yes |

| M4.2 *P. cinn* | Dilution | Agdia Positive? | Pear Positive? | Dog Positive? |
|---|---|---|---|---|
| P-25 a | 1:1 | Yes | No | Yes |
| P-26 b | 1:10 | Yes | No | Yes |
| P-27 c | 1:100 | Yes very low | No | Yes |
| P-28 d | 1:1,000 | Yes extremely low | Unknown | Yes |
| P-29 e | 1:10,000 | Yes extremely low | Unknown | Yes |
| P-30 f | 1:100,000 | Yes extremely low | Unknown | Yes |
| P-31 g | 1:1,000,000 | Yes extremely low | Unknown | Yes |
| P-32 h | 1:10,000,000 | Yes extremely low | Unknown | Yes |

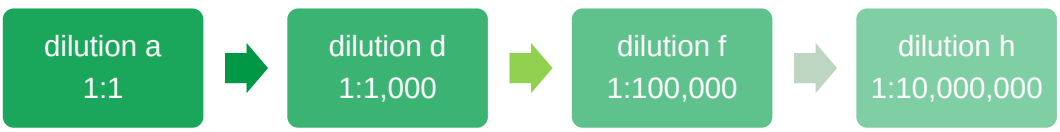
| M4.2 *P. cinn* | Dilution | Agdia Positive? | Pear Positive? | Dog Positive? |
|---|---|---|---|---|
| M4.2 *P. cact* | Dilution | Agdia Positive? | Pear Positive? | Dog Positive? |
| P-41 a | 1:1 | Yes very low | Unknown | Yes |
| P-42 b | 1:10 | Yes extremely low | Unknown | Yes |
| P-43 c | 1:100 | Yes extremely low | Unknown | Yes |
| P-44 d | 1:1,000 | Yes very low | Unknown | Yes |
| P-45 e | 1:10,000 | Yes extremely low | Unknown | Yes |
| P-46 f | 1:100,000 | No | Unknown | Yes |
| P-47 g | 1:1,000,000 | No | Unknown | Yes |
| P-48 h | 1:10,000,000 | Yes very low | Unknown | Yes |

M4 = Sunshine Mix 4 ex; M4.2 *P. cact* = Sunshine Mix 4, replicate two of *P. cactorum*

## Visual of dog detections over dilutions




## Visual of Agdia detections over dilutions



The photos shown below are of the Agdia strips revealing results from testing all the *Phytophthora* positive dilutions and a handful of the known *Phytophthora* negative controls. It can be observed that as the concentration of the pathogen declined in the samples, so too did the ability of the immuno strips to detect it. The dogs, however, seemed to show no decline in response to the presence of the pathogen despite the concentration. Samples are currently in the process of being pear baited. Results are still being recorded but as of this report, none of the diluted inoculates have produced a positive pear bait result.

## Summary and Future Work

The results of the continued work in Phase 4 are supportive of continued testing on composite soil samples acquired from collaborating nurseries to obtain more data. Only when we have more robust data will we have any true detection rates. The additional methodologies recommended for testing include nursery leaf testing, combined dogs' results, and further exploration of the dogs' capacity to detect the pathogen at very low incidence values.

The dilutions study results support the notion that the dogs may be more sensitive to the presence of pathogenic *Phytophthora* than current testing methods. These results have strong implications in how scent detection canines might serve as an effective tool in screening composite soils of nursery plants. Though the dogs will not completely replace current testing methods, they may help to significantly reduce the amount of time and labor required in testing by limiting sample sizes to those plants which are strongly suspect.

Continued testing with host nursery soil samples would be advantageous. Worth exploring is the dogs' ability to detect the pathogen on leaves from suspect and control plants from local nurseries, as the soil tests were conducted during this recent phase. The dogs have shown strong recognition to the pathogen on leaves. This option has not yet been explored in a nursery setting such as the Presidio Nursery.

To better our results regarding the positives we continue to suggest the use of the combined data from both dogs. The use of two dogs to cross-check samples is a strong method.

At the conclusion of Phase 4 training and testing, we feel that the dogs are proficient in determining which plants are *Phytophthora* free. This is a considerable achievement at this stage of the project, and is, we believe, the true power of the approach. We also believe that the dogs are more equipped to pick up the signature scent of the pathogen, thus indicating presence, at a rate that certainly exceeds that of immune strip testing.