


Sampling and Diagnostic Guide for Soil-borne *Phytophthora* species on California Native Plants

Part 1. Selecting Plants to Sample

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Introduction. Over the past several years, *Phytophthora* has emerged as a significant problem on California native plants in nurseries and restoration areas. Here we provide guidance for *Phytophthora* sampling and survey design. The tips may be applied to determine *Phytophthora* incidence in natural ecosystems and restoration sites, and they are presented with the goal of reducing the risk of unintentional introduction of exotic pathogens and improving restoration plant health. In **Part 1. "Selecting Plants to Sample"** we provide direction for where and how to sample. **Note.** To avoid spreading pathogens, good sanitation is required when sampling so this guidance should be used along with instructions for cleaning tools, boots, hands (gloves), and with care to not inadvertently transport incidental debris or soil.

Additional sections will be forthcoming with instructions for sampling handling, baiting and other procedures. For sample identification, the soil or plant material may be sent to the California Department of Food and Agriculture laboratory for identification. Information on fees and how to deliver samples is available at https://www.cdfa.ca.gov/plant/PPD/plantpath.html. For questions, contact Janice Alexander, Phytophthoras in Native Habitats Work Group at jalexander@ucanr.edu.

How to select specific areas to sample. When managing *Phytophthora* in restoration sites, one of the first steps is to determine whether the pathogen is present on the site, both pre- or post-planting. Survey design and sampling is site-specific, but we have assembled a few pointers to help identify the most likely places to find infected plants based on symptoms and site characteristics. Caution is warranted, it is very difficult to prove *Phytophthora* is not present. At times pathogens can be present with no visible symptoms -- these microbes don't always express themselves in ways that people can see.

In most cases when sampling a planned or in-progress restoration area, site selection is predetermined by restoration goals. A primary decision revolves around the tradeoffs between sampling width (the number of sites) and depth (the number of samples per site). In contrast, for landscape-level surveys, site selection is critical. Proper site and sample selection maximizes the amount of information gained from each sample. (We use "site" to mean one localized, continuous area.)

Although much about the natural distribution of *Phytophthora* is unknown, the distribution of soil-borne *Phytophthora* species on a landscape is not typically continuous, nor is it random. Therefore, surveys should focus sampling on areas with the highest likelihood of finding *Phytophthora*. This not only increases the chances of finding the pathogen but allows for a stronger interpretation of negative results.

Land use history. When embarking on a survey, review the site's previous use. While California has native pathogens, their occurrence is typically rare to uncommon, and it appears that human activities are ultimately responsible for most of the damaging infestations of *Phytophthora*. Many ecosystems are not pristine, having previously been used for agriculture or horticulture, both of which facilitate establishment of *Phytophthora* species. Roads and trails, even those no longer in use, enhance soil movement. Past restoration, or other plantings are possible *Phytophthora* introduction pathways.

Moving with soil. One of the most important ways *Phytophthora* can be spread is by movement of soil, either anthropogenically or naturally (Fig. 1). *Phytophthora* moves *through* soil unassisted, but this movement is limited, typically a few meters a year, or less. This movement is much faster downhill than uphill and is greatly facilitated by contact with roots.

Fig. 1. A site experiencing anthropogenic soil movement. Potentially infested soil is being moved in along a closed road via unauthorized off-road vehicle use. The closed road was selected for potential restoration and managers wanted to assess if it was already infested by *Phytophthora*. The sampling focused on the area where the people are standing, since it is closest to the source of the potentially infested soil. *Phytophthora crassamura* was later baited from soil along this closed road.

Areas that are likely to have had soil moved into them should be prioritized for sampling. When surveying these disturbed sites, consider the source of the soil and target that location for initial sampling. For example, former roads or trails are commonly slated for restoration. When evaluating these project areas, surveys should target the trailhead or road origin, where soil movement is most likely. If *Phytophthora* is detected, then subsequent surveys can focus on assessing the extent of the infestation.

Note. A primary consideration during sampling is to make sure proper sanitation is practiced so that soil or pathogens are not unintentionally spread.

Fig. 2. A restoration outplanting within the flood plain of an urban river. The California mugwort being sampled likely encounters many swimming spores during times of flooding. Several *Phytophthora* species were baited from roots and soil, and directly from the river water.

Moving with water. *Phytophthora* species proliferate and spread in water, and *Phytophthora* spores are found in rivers, creeks, lakes, and ponds. (Bodies of water can be baited for *Phytophthora*, for an overview see https://ucanr.edu/sites/rizzolab/Research_Projects/Stream_Monitoring/.) Whether outplanted or naturally recruited, vegetation near watercourses, that are even sporadically subject to flooding, are at risk of *Phytophthora* infection (Fig. 2). Inundation risk is an important consideration

when selecting restoration site locations – sites that are flooded are more likely to yield *Phytophthora* than upland, dry sites. Plants that are more likely to be flooded should be prioritized for *Phytophthora* sampling. For previously outplanted sites, knowing which species are in a local waterway can help determine whether plants may have become infected onsite due to exposure to floodwater.

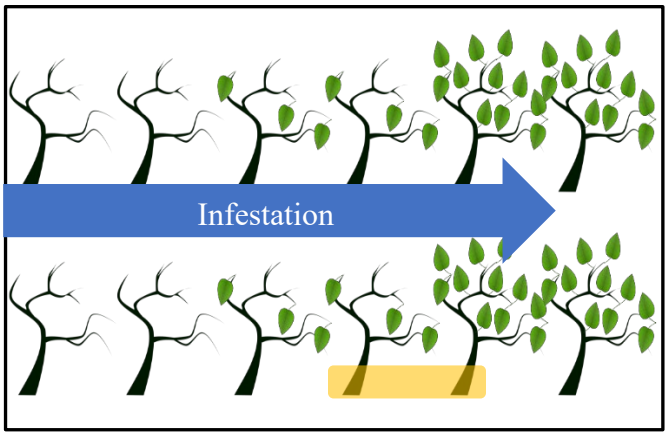

Fig. 3. Illustration of a disease front. In this schematic, the infested area grows from left to right, and above-ground symptoms lag behind root infections. If possible, samples should be collected in an area where symptoms first become visible (indicated in yellow), as the pathogen may no longer be present or viable in the areas where plants have died.

Topography, soil, and local vegetation. Sample in areas with woody plants, in low-lying areas since they hold the most water, and because *Phytophthora* is more common downhill. Consider local soil-type information when selecting sites. *Phytophthora* is more likely to be found in soils with high clay content and low organic matter, and in areas with greater moisture retention.

Although it is not always visible, *Phytophthora* root rot may form a "disease front" or root disease center with a gradation between declining or dead vegetation (Fig 3). As the infestation area expands, plants on the edge become infected and begin to decline then die. If the leading edge can be determined, sampling should be targeted around declining, rather than dead, plants.

Edges, or transitional areas between vegetation types, provide useful areas to sample. Edges pick-up a greater range of physical conditions, and a location with multiple vegetation types provides a greater diversity of potential *Phytophthora* hosts. Edges may represent historical disease fronts, and so sites containing edges may detect the causal agents of previous declines.

Fig. 4. At this active restoration site, downhill soil movement from the road and potential flooding from the currently dry creek increase risk of *Phytophthora* exposure.

Sample selection

Which plants to sample. *Phytophthora* populations are associated with their plant hosts. So, when sampling, collect soil beneath plants and include roots. A good sample choice is often the most symptomatic individual at a particular site. But the most symptomatic toyon at one site might show

slight stunting or leaf yellowing, while at another, the most symptomatic toyon may exhibit severe branch dieback and wilting. Note that some *Phytophthora* species make resistant spores than can last for long periods of time in dead plant material or soil, allowing them to survive while they are not infecting a plant.

Sample selection is not a precise science, but the following four factors can help target plants for sampling:

1. Check planting basins with weak plants. If a plant was produced in a nursery with poor phytosanitation, the plant may have become infected in the nursery.

2. Look for pronounced symptoms (stunted, off-color, blotchy, thin crown) on live plants. When multiple individuals of a plant species are present, the most symptomatic one is the most likely to test positive. Partially green plants are best, avoid decayed, older-dead plants.

3. Sample areas with flooding potential. Plants in areas that are prone to flooding or standing water should be targeted.

4. Evaluate disturbed areas. Disturbance, such as movement of soil from roads and trails, increases the likelihood of infection (Fig. 4).

Fig 5. In this restoration outplanting in a natural ecosystem, plants in low-lying areas were targeted for sampling as the most likely places to yield a *Phytophthora*-positive sample.

When sampling consider:

Microtopography. Plants growing in areas where water collects should be preferred over those on slopes (Fig. 5).

Soil type. Soil with higher clay content has a greater chance of harboring *Phytophthora*.

Plant species. Woody shrubs and trees, with their extensive root systems are considered most susceptible to soil-borne *Phytophthora*. However, rush (*Juncus* spp.) and herbaceous plants can also become infected. Priorities for sampling should match the value of the plant in the landscape and knowledge about species' susceptibility.

When to sample. *Phytophthora* activity syncs with active root growth and is correlated with moisture, making recovery most likely during the growing season and rainy season. In California's Mediterranean climate, in many areas, the growing season and best time to sample extends from the late autumn

through winter and into early spring but depends on elevation and latitude. As molecular detection techniques become increasingly available, the need for *Phytophthora* to be viable in the soil sample will be alleviated, but the pathogen will always be easiest to detect during periods when it is actively sporulating. Tools and more guidance are provided in Figs. 6 – 9.

Fig. 6. A metal spade is a good choice for soil sampling in softer soils. Metal spades have few crevices which makes them easier to sterilize between samples. In this planting basin, the plant had recently died but the soil was tested.

Fig. 7. A trenching shovel maybe used for sampling harder soils. Its narrow profile facilitates sampling soil associated with restoration plants without harming too many roots, and it can be applied forcefully.

Fig. 8. This blue oak sapling is stunted or declining but still alive, which makes it a good choice for sampling.

Fig. 9. While this tree is still green, it has long internodes and relatively sparse foliage compared to other similar individuals in the site, making it a good choice for sampling.

Appendix – Background

Introduction to *Phytophthora*. *Phytophthora* (ancient Greek: "plant destroyer") is a genus of plant-pathogenic, microscopic organisms that have a long history of killing plants in agriculture (e.g. late blight of potato, *Phytophthora infestans*, cause of the Irish potato famine) and in natural ecosystems (e.g. sudden oak death, caused by *P. ramorum*). In restoration ecology, *Phytophthora* species that encounter plants, either in a nursery or in the field can pose major obstacles to project success. Unintentional introductions of *Phytophthora* into natural ecosystems during restoration activities may turn planting into a means of habitat degradation rather than rehabilitation.

***Phytophthora* & *Pythium*: the "oomycetes".** *Phytophthora* and its relative *Pythium* are oomycetes that resemble the molds of the Kingdom Fungi, but are actually more closely related to brown algae. Many species of *Phytophthora* and *Pythium* (as well as *Phytopythium*, a group recently split from *Pythium*) have broad host ranges, attacking the roots, stems, or foliage of a wide variety of woody shrubs and trees, and to a lesser extent, herbaceous plants. Their broad host range facilitates the infestation of novel habitats by increasing the chance of encountering susceptible hosts, both in nurseries or restoration sites, and greatly increases the risk of an accidental introduction leading to a disease epidemic in an invaded ecosystem, such as sudden oak death (*P. ramorum*).

Unlike most members of the Kingdom Fungi ("true fungi"), oomycetes usually have swimming spores (zoospores) that enable them to locate and infect plants. This aspect of oomycete biology is exploited in the detection technique known as "baiting" where a sample is flooded with water and living plants or plant parts (the bait) are floated so that it is likely to be encountered by zoospores. The technique can be done without access to a laboratory, and its sensitivity allows for the assessment of larger quantities of soil as compared to other methods. Green (partially ripe) pears are a widely available

and common bait, that develop dark and relatively distinctive lesions following infection by *Phytophthora* or *Pythium.*

False negatives vs. false positives

An important consideration when interpreting the results of a *Phytophthora* survey, is the likelihood of **false negative** results -- failing to detect *Phytophthora* that is actually present. If isolates baited from samples are morphologically analyzed by a diagnostician, or genetic (DNA) sequences are obtained from isolates, the likelihood of a **false positive** (detection of a species of *Phytophthora* when it actually was not present) is low. However, in general, the risk of a **false negative** for any given sample or survey is significant for a variety of reasons. In this guide we provide only general pointers.

For more information

California Oak Mortality Task Force, www.suddenoakdeath.org.

Instructions for using green pears to bait for *Phytophthora* in soil / root samples:
http://phytosphere.com/soilphytophthora/pearbaitingPhytophthora.htm.

Best management practices – Phytophthoras in Native Habitats Work Group
https://www.suddenoakdeath.org/welcome-to-calphytos-org-phytophthoras-in-native-habitats/resources/. BMPs for restoration and field work, nurseries, and other guidance.

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Figure on cover page. (Left) Sticky monkey-flower (*Diplacus aurantiacus*) and (Right) California rose (*Rosa californica*) showing dieback associated with *Phytophthora*.

Photo credits: UC-Davis, Department of Plant Pathology.
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