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# Presidio Phytophthora Management Recommendations



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# Presidio Phytophthora Management Recommendations (modified)

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## Executive Summary

The genus *Phytophthora* includes a diverse group of ecologically and economically important plant pathogens, containing more than 120 known species (Lévesque 2011, Kroon et al. 2012, Martin et al. 2012, Yang et al. 2014). This includes some of the world's most destructive plant pathogen species (Brasier 1996). Unfortunately, the movement of plants, soil, and planting materials enable *Phytophthora* pathogens to move around with them (Jung and Blaschke 2004), and thereby threaten plant biodiversity. Maintaining plant biodiversity is very important in California's unique ecosystems, which contains many species found nowhere else in the world (Myers et al. 2000).

In this recommendation manual, "best practices" emphasize managing nurseries, ecosystems, and other landscape areas to prevent the introduction and amplification of *Phytophthora*. These recommendations are systematic yet simple, providing a template for common procedures.

Recommendations are also provided to monitor *Phytophthora* in nurseries and landscapes. Monitoring crops in nurseries and plants in landscapes is the best available approach to understand when/where infestations occur, and give insight as to which areas and crops are free of pathogens. The nursery monitoring recommendations given are paired with sampling protocols that are intended for the Presidio, but may be adopted throughout the Golden Gate Natural Recreation Area nurseries. These protocols outline how to clear potting mix, propagation beds and furrows, and plant species that are at risk to *Phytophthora* species. The

landscape monitoring included in the recommendations explains the process of baseline sampling (to evaluate sites prior to restoration), and other necessary sampling procedures.

Recommendations are given to help prevent the movement of *Phytophthora* into the Presidio during importation of materials and plants. Importing plants and landscape materials including soil, into the Presidio brings with it the added risk of introducing new organisms into the Presidio; this includes *Phytophthora* pathogens (Griesbach et al. 2012). Exotic invasive species can more easily move in with the importation of plants from outside sources and should be evaluated with that in mind. Recommendations are given on how to evaluate plants and materials brought in from outside the Presidio.

Treatment recommendations are provided for containers, plant propagules, imported soils, high value trees, sites, and for Presidio compost and soil. Treatments like these can aid in the prevention of spreading disease causing organisms, including *Phytophthora*.

The issue of *Phytophthora* is a real threat to plant species diversity and it is exceedingly important to prevent the introduction and spread of these insidious organisms in order to preserve plant biodiversity within the Presidio. This document provides the baseline recommendations necessary to help prevent new *Phytophthora* issues. The recommendations should be used in combination with each other in order to create an integrated strategy for effectively managing *Phytophthora*.

## Introduction to the *Phytophthora* Issue

### *Phytophthora* Issues Around the World

*Phytophthora* are increasingly becoming known as the genus of the worst plant pathogens to natural ecosystems around the world, and problems are mostly due to human related dispersal. For example, the restoration of riparian habitat across Europe with *Alnus glutinosa* and *Alnus incana* nursery stock contaminated with the deadly hybrid alder *Phytophthora: P. alni* subsp. *alni* (Brasier 2003, Jung and Blaschke 2004, Figure 1. A) resulted in dieback of *Alnus* species across many parts of Europe (especially north and central Europe). It can affect as much as 80% of the *Alnus* species trees in a stand (Jung and Blaschke 2004) and can lead to stream bank failure (Černý K. and Strnadová V. 2010). Another example includes the introduction of *Phytophthora cinnamomi* which has led to unwanted and irreversible changes in ecosystems including the unique and important Jarrah Forests (Figure 1. B) of Western Australia. *P. cinnamomi* was introduced into Western Australia during road building and logging operations (Podger 1972) and since has resulted in frequent restoration failure. In Spain *P. cinnamomi* was also unintentionally introduced during restoration projects with *Quercus ilex* (Figure 1. C), and *Quercus suber*, and in many areas restoration has not worked due to introductions of the pathogen along with the host (Brasier 1996, Sanchez et al. 2001, Robin et al. 2002, Plieninger et al. 2003). *Phytophthora* species in nursery stock and soil for restoration provides an opportunity for these pathogens to cause disease.

Figure 1. *Phytophthora* species introduced to natural ecosystems around the world



A The Alder *Phytophthora*, a deadly hybrid, was introduced via the restoration pathway in North and Central Europe especially. Trees of the genus *Alnus* become diseased and die from the invasive alder *Phytophthora*, which is now widespread across much of Europe. This pathogen was introduced during restoration planting with contaminated *Alnus* species nursery stock. The pathogen causes root and stem disease (upper left), stream bank failure due to the resulting disease is not uncommon (upper right); in some areas as much as 80% of the *Alnus* plants are affected (lower). Photo credit: Thomas Jung; Amelie Rak; Thomas Cech respectively.



B. Jarrah Dieback in Western Australia. The deadly introduced plant disease caused by *Phytophthora cinnamomi* kills *Eucalyptus marginata* trees and affects nearly 2,000 different plant species. The pathogen was most likely introduced to the region during logging and road building, restoration attempts, which have failed over many areas due to this pathogen and other *Phytophthora* species. *P. cinnamomi* has now spread into several forest areas changing and degrading ecosystems. Photo Credit: E. Hansen



C. Phytophthora disease killed *Q. ilex* in Spain. *P. cinnamomi* was introduced during attempts to restore areas with *Q. ilex* and *Q. suber*, it kills these trees often after they are well established by causing a root disease. Photo credit: Laura Sims.

## *Phytophthora* Issues in California

California forests too, are suffering from the introduction of *Phytophthora* species. In the bay area of California especially, *Quercus agrifolia* and *Notholithcarpus densiflorus* die from the disease sudden oak death (SOD, Figure 2. A), which is caused by *P. ramorum* (Rizzo et al. 2002), and the pathogen has a remarkably wide host range of woody plants in general (Grünwald et al. 2008). The pathogen spreads to *Q. agrifolia* from infected *Umbellularia californica* that occur nearby in the same ecosystem, and from one *N. densiflorus* to another. The *U. californica* suffer only minor symptoms mainly on their leaf tips (DiLeo et al. 2009) but are very important in spreading the disease to *Quercus* species and *N. densiflorus* (Figure 2. A, inset). Human-mediated movement of infected plant material or soil resulted in the dispersal of *P. ramorum* into natural areas and nurseries (Cushman and Meentemeyer 2008, Grünwald et al. 2012). National and Global trade of infested nursery stock contributed significantly to the spread of the pathogen betwixt nurseries (Goss et al. 2009, Grünwald et al. 2012), and started the SOD epidemic in California (Meetenmyer et al. 2004, Mascheretti et al. 2008, Grünwald et al. 2012).

**Figure 2. Introduced *Phytophthora* in natural areas of California**



A. *Phytophthora ramorum* and sudden oak death (SOD). *Phytophthora ramorum* introduced to California forests kills *Q. agrifolia* and spreads uncontrolled from infected *U. californica* foliage (inset). In coastal California especially, *Q. agrifolia*, and *N. densiflorus* die from the disease called SOD. Photo credit: Laura Sims.

Another example where *Phytophthora* are causing dieback of serious concern is in Lone California, *Arctostaphylos* species are dying from most likely a repeated introduction (Garbelotto, M. personal comm. 2015) of *Phytophthora cinnamomi* and other *Phytophthora* species (Swiecki et al. 2003, Figure 2. B), to natural stands of *Arctostaphylos myrtifolia* and *Arctostaphylos pallida*. There, the dieback is slowly spreading and now affecting relatively large areas in this very sensitive and unique habitat.



B. *Phytophthora* killed *Arctostaphylos* species. In Lone California, *Arctostaphylos* species are dying from the most likely repeated introduction of *Phytophthora cinnamomi* and other *Phytophthora* species. Photo Credit: Ted Swiecki.

The most recent evaluations of restoration sites and nurseries in California suggest that several different *Phytophthora* species are being introduced via the restoration pathway on several different plant species grown in nurseries (Latham et al. In Press, Table 1). These introductions pose a serious threat to California's unique biodiversity. Allowing *Phytophthora* species in nursery stock provides novel conditions for the pathogen that can end up resulting in epidemics, threaten endemics important to conservation, and result in restoration failure.

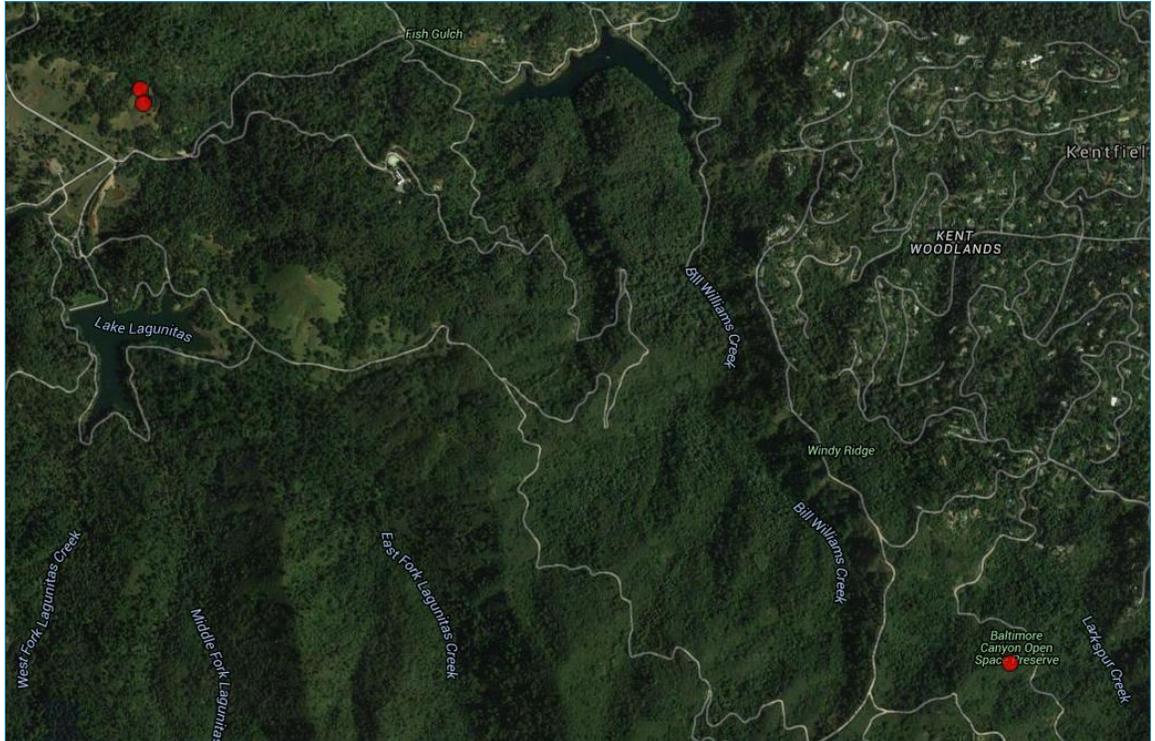
## *Phytophthora* Issues in the Presidio and Golden Gate Natural Recreation Area

Surveys within the Golden Gate Natural Recreation Area (GGNRA) including the Presidio (Figure 3 A) have found widespread (Figure 3 B) as well as more confined areas, infested with *Phytophthora* (Figure 3 C); and very recent surveys of nursery plants are coming up positive for *Phytophthora* (Figure 3 D-F). The Sudden Oak Death pathogen *Phytophthora ramorum* is now widespread throughout most of the coastal bay area including on Mt. Tamalpais (Figure 3 B) and on Bolinas Ridge. *Phytophthora* dieback of sites has changed stand structure, resulting in difficult to manage areas (Figure 3 G), and areas with high fuel loads (Figure 3 H-I). At a few sites on Mt. Tamalpais, another species, *Phytophthora cinnamomi*, is present, (Figure 3 C) affecting *Arbutus menziesii*, and probably more plant species. Due to the known issues of *Phytophthora*, GGNRA nurseries began to evaluate their plants in the nursery, plants from the nursery moved into restoration sites, and remnant areas. These evaluations have found *Phytophthora* species from different plant species including (but not limited to): *Frangula californica* (syn. *Rhamnus californica*) *Heteromeles arbutifolia*, and *Ceanothus thyrsiflorus* (Figure 3 D-F).

**Figure 3. Surveys in the Golden Gate Natural Recreation Area (GGNRA) and including the Presidio have found sites in the area, and plants in the nursery infested with *Phytophthora***



A. Widespread dieback of *Quercus* species and *Notholithocarpus densiflorus* from Sudden Oak Death caused by *Phytophthora* on Mount Tamalpais in the Golden Gate Recreation Area. Photo Credit: Janet Klein.



B. Specific sites where *Phytophthora cinnamomi* has been recovered from Mount Tamalpais (red dots), local plant species this *Phytophthora* can infect and kill are *Arbutus menziesii*, *Umbellularia californica*, *Quercus* species, and *Arctostaphylos* species. The pathogen does not appear to be widespread yet, and so it is very important not to spread it further. Data for map from: Ted Sweicki.



C. Presidio *Phytophthora* detection map. Several different *Phytophthora* species have been found thus far in the Presidio, the list is not yet comprehensive. Map by: Christa Conforti.

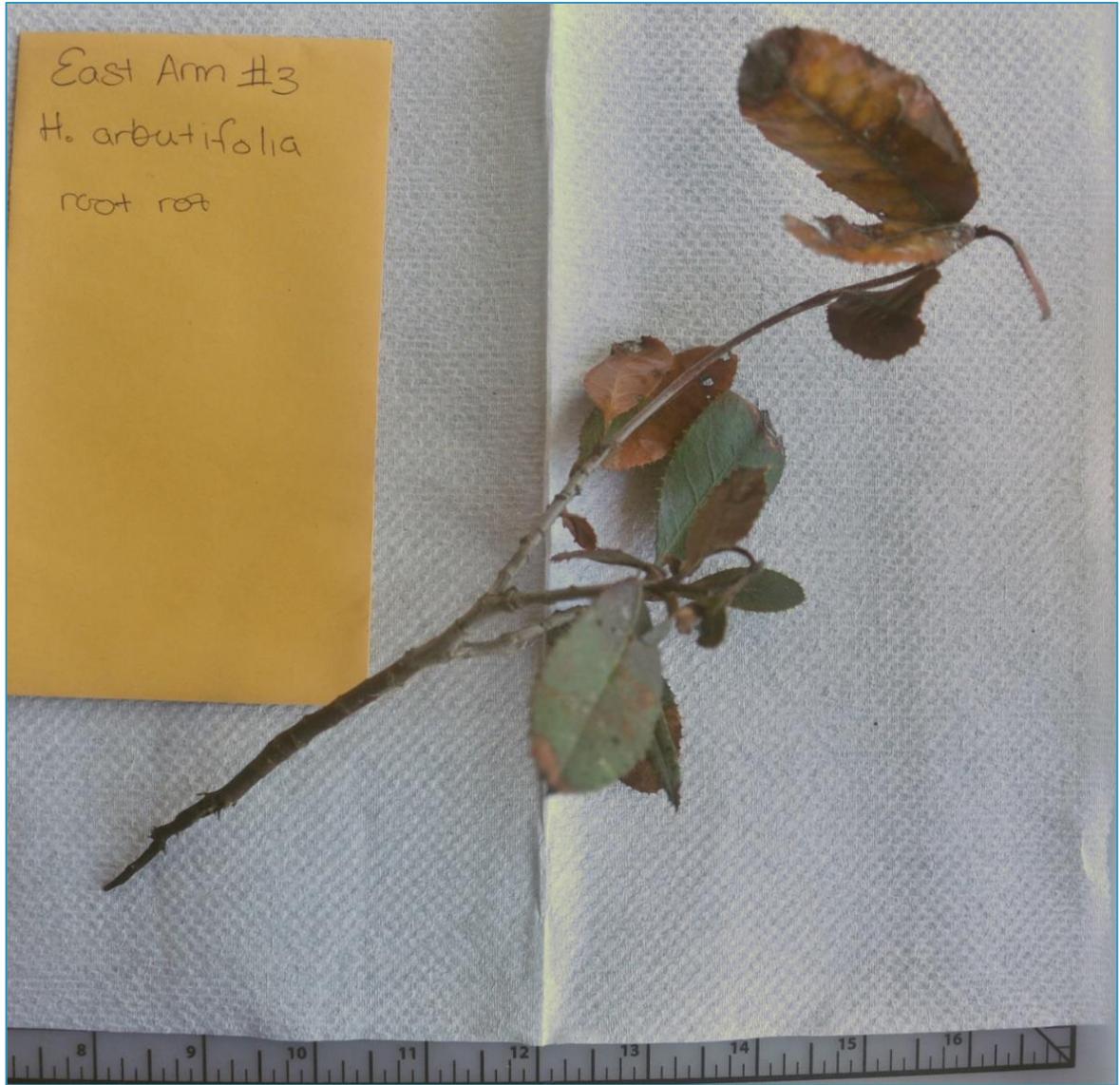


D. Unhealthy top growth of *Ceanothus thyrsiflorus* prompted testing, which revealed the presence of *Phytophthora* at the Presidio nursery in 2015. Photo Credit: Laura Sims.



E. Some of the crops of *Frangula californica* plants from the Presidio nursery appeared to be severely affected by *Phytophthora* and following testing it was found they were in fact positive.

Photo Credit: Laura Sims.



F. Some nursery grown *Heteromeles arbutifolia* that were outplanted at various sites in the GGNRA in 2015 were later sampled and found to be positive for *Phytophthora*. Photo Credit: Laura Sims.



G. Phytophthora dieback of sites within the GGNRA has changed stand structure, resulting in difficult to manage sites. Photo credit: Janet Klein.



H. *Phytophthora* infested areas with high fuel loads. Photo Credit: Richard Cobb.



I. *Phytophthora ramorum* and other *Phytophthora* species kill trees and plants in a way that has resulted in areas with high fuel loads in Marin County. Photo Credit: Richard Cobb

The Presidio Trust, GGNRA, and GGNPC have begun to address the *Phytophthora* issue by forming a tri-agency task force to develop this document, initiate its use, and improve it when it is necessary to do so. The recommendations within this document will be used to help produce cleaner planting materials, improve sanitation, monitor for pathogens, evaluate imports, and treat materials where appropriate. As our understanding of the issue improves, this document will be modified and updated to reflect necessary changes.

## Table of Key Points

What are key points? Key points are ‘cheat sheets’ to help remember the really important details of appropriately managing *Phytophthora*. These points cannot replace proper training nor do they act as a replacement for the full text, however, once you have a strong grasp of the issue, they will help you to remember the essentials.

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# **Chapter 1.**

## **Presidio Best Practices**



## Introduction to Best Practices

Pests and pathogens including *Phytophthora* can move into a nursery or landscape by riding aboard incoming materials be it from the field, nursery, or other location. To avoid bringing in unwanted pathogens, nursery and landscape managers, employees, contractors, and volunteers should utilize best practices to avoid harm to all areas in and around the Presidio. Key points are provided at the beginning of each section to make remembering the essentials easier.

# Presidio Native Plant Nursery Best Practices: (Part I) operations, and (Part II) propagule collections

## Presidio Native Plant Nursery Best Practices Part I: operations

### **Key points:**

- ✓ **Proper watering is essential. Group plants based on their watering needs.**
- ✓ **Proper sanitation is key to growing healthy plants.**
- ✓ **You must sanitize your shoes upon entry and exit of the nursery.**
- ✓ **The ground at the nursery is considered dirty, do not allow plants to touch the ground. If plants are placed on the ground, they must be discarded.**
- ✓ **Growing benches are considered clean, do not put any dirty tools (gloves, buckets, trowels etc.) onto a growing bench.**
- ✓ **Keep areas between buildings tidy.**
- ✓ **Do not allow hose nozzles to lay on the ground, and if they do, they must be sanitized before use.**
- ✓ **Remove and dispose of leaf and soil debris from plant production areas ASAP.**
- ✓ **After every crop rotation, disinfest propagation areas in greenhouses, and shadehouses.**
- ✓ **Only use new or clean and properly sanitized containers for plant production.**
- ✓ **Seed are preferred for propagation, only make collections from healthy plants in healthy nursery beds, collect seed away from the**

**ground and contamination sources wherever possible and chemically treat low growing seeds and all cuttings according to the treatment recommendations.**

- ✓ **Pasteurize the potting mix and store it in enclosed sanitized or sterile containers.**
- ✓ **Control weeds.**
- ✓ **Routinely inspect the nursery plants for symptoms of infection.**
- ✓ **Routinely inspect the surrounding landscape for symptoms of infection.**
- ✓ **Divert soil and water movement from adjacent properties.**
- ✓ **Avoid the accumulation of standing water in your nursery.**
- ✓ **Keep culled material in a containment bin for disposal or recycling.**
- ✓ **Ensure there is no run off from the cull pile bins towards the nursery or media components.**
- ✓ **Document procedures.**
- ✓ **All personnel must attend routine trainings.**
- ✓ **As this protocol is updated and refined, we will share it with you.**

**Please stay tuned.**

*Water management.* Proper watering is essential, and there are several ways to manage watering plants. Start watering early (before dawn 2-3 am) to avoid prolonged leaf wetness and avoid overhead irrigation where possible. Irrigation should always be managed so that plants are not watered inappropriately.

Inappropriate watering includes (1) drought and drench irrigation, (2) watering late (extends leaf wetness time), and (3) allowing plant bases to sit in pooling water.

Improved irrigation can be achieved by grouping plants on benches based on their watering needs, and then watering each bench of plants for the appropriate amount of time. Use of tensiometers or soil moisture probes may be useful to determine the appropriate amount of time, where it is difficult to gauge otherwise. Do not allow plant bases to sit in pooling water. Increase plant spacing and install fans helps to improve airflow and reduce leaf wetness time.

Hang up hoses so that they are not laying on the ground. Do not allow the hose nozzle or spray wand to sit on the ground or become soiled. If the hose nozzle or spray wand becomes soiled, wipe it off, spray it down with isopropyl alcohol before using. Then always hang it up after use.

Monitor, and at minimum bi-annually test for waterborne pathogens, irrigation water from any source other than a clean well (regularly tested and potable), or a municipal water supply. Testing should be to confirm that the water is free from pathogens prior to use on plants or material for planting.

*Cleaning and Sanitation.* Be sure to remove and dispose of leaf debris from plant production areas. After every crop rotation, disinfect propagation areas, greenhouses, and shadehouses. Use a power washer to remove all visible soil and debris. Then spray with isopropyl alcohol to disinfect the surfaces that are exposed to plant material, and amendments.

Keep plants elevated (on a table 3-4' above the ground) to avoid ground contaminates. If plants are placed on the ground, they must be discarded.

Keep your planting medium and plants free from pathogens. Pasteurize the potting mix and store it in sanitized or sterile containers. Clean and sanitize containers and tools. Use new or clean and properly sanitized containers for plant production. Inspect 'sanitized' pots for debris accumulation. Discard or clean and sanitize again all containers that have not been properly cleaned and sanitized. After each use, clean tools to minimize the spread or introduction of pathogens. Hang up hoses and keep nozzles off the ground (see water management for details on hose maintenance).

*Nursery layout and upkeep.* Nursery layout and upkeep is important for healthy plants. This is especially important in regards to water movement, culled material, and potential hosts.

Divert soil and water movement from adjacent properties to prevent contamination of the nursery. Avoid or minimize accumulation of standing water on the shadehouse and greenhouse floors and on pathways within, leading to and away from nursery facilities. Repair or replace damaged areas on floors or benches that allow standing water to accumulate.

Place all culled material in a containment bin for disposal or recycling. If the infested plants are found to be contaminated with a quarantine pest the cull pile must be quarantined and treated, or disposed of, according to the most current regulatory requirements. Contact the CDFA for procedure requirements if this

occurs. Ensure there is no run off from the cull pile bins or from the nursery to media components. Any runoff from the nursery should be directed away from media components, media mixing area, and growing beds to prevent contamination. Ensure the cull pile is clearly separated from media mix components, clean growing material, and plants. In fact, the cull pile should be down and away from your nursery. Keep a ten-foot area cleaned and sanitized around the cull pile bin. Water should not be allowed to accumulate near cull piles and must be managed.

Reduce potential inoculum dispersal from known host plants (Appendix 1). Group plants on benches based on their watering requirements (see water management above). Allow adequate spacing in and around these plants, avoiding long monocultures of hosts. Increase spacing between plants and plant species.

Adequately control plants that may harbor disease and insect pests. Weeds on the nursery site must be controlled, as they may harbor pathogens and insects. Remove known host plants from the nursery landscape or monitor regularly for disease (three times a year, spring, summer and winter), and keep these plants away (100 feet or more) from greenhouse entries and shade house perimeters.

*Inspect, test, sanitize, dispose, and document.* Routinely inspect the nursery plants for symptoms of infection. Routinely inspect the surrounding landscape for symptoms of infection. If suspicious symptoms are observed on plants, dispose of heavily symptomatic plants not needed for diagnosis, send in what is needed for

testing, move any remaining plants and plants immediately surrounding, away from other plants, and clean and sanitize the area immediately. Keep records of diseased, symptomatic, or dead plants, and dispose of these plants assuming the worst-case scenario for proper disposal. Mark and record the location where symptomatic plants were located. Clean and sanitize all surfaces in and around where the symptomatic plants were. Keep records of symptomatic (or dead) material (assume the worst-case scenario for proper disposal), and dispose of these plants. Take action to resolve issues.

*Training.* All personnel must attend routine trainings on prevention, monitoring, testing, and proper growing techniques. All personnel must be trained to recognize symptoms and signs of disease, and characteristics of common pest infestations. Symptoms and signs of disease, and common or unusual pest infestations will not be allowed to go unattended.

*Documentation of Program Procedures.* Provide proof that the nursery best practices were implemented by keeping records. Refer to the tracking and reporting guidelines for your nursery and work with your Plant Pathogen Coordinator and a Plant Pathologist on documentation. Keep a copy of sampling records on the appropriate field sheets. Along with this nursery manual on best practices keep: employee training records, internal and external review procedures and events, copies of datasheets for monitoring field sites (related to your nursery for propagule collections) and the nursery. Keep a list of the implemented

procedures from this manual, and adapted changes that were necessary to best fit your nursery.

## Presidio Native Plant Nursery Best Practices Part II: propagule collections

### **Key points:**

- ✓ **You should not be carrying green debris in your truck when you are carrying propagative materials.**
- ✓ **Clean your truck bed before transporting propagative materials.**
- ✓ **Clean vehicle, clothes, boots, and tools before entering the nursery.**  
**You must sanitize your shoes upon entry and exit of the nursery and only use clean and sanitized tools at the nursery.**
- ✓ **Please be mindful of where you park your vehicles and how you are bringing dirty seed and propagule materials into a clean nursery.**  
**Contain propagules; the nursery staff will give you site-specific direction on these details.**
- ✓ **Once shoes are clean and sanitized, clothes are clean and propagules are contained, workers coming from the field may bring in propagative materials, and enter and exit areas for propagation.**
- ✓ **Use clean gloves when at the nursery (no dirty field gloves).**
- ✓ **The ground at the nursery is considered dirty. Never allow propagative materials to touch the ground at the nursery and do not use material that does -discard it.**
- ✓ **Never leave field collected materials laying about.**
- ✓ **Shoes and tools must be cleaned and sanitized upon departing the nursery.**

**For propagation material from the field to the nursery:**

- ✓ **Seed are preferred for propagation.**
- ✓ **Only make collections from healthy plants in healthy field sites.**
- ✓ **Collect seed away from the ground and contamination sources wherever possible.**
- ✓ **Treat low growing seeds and all cuttings according to the treatment recommendations.**

*For vehicles and people entering the nursery.* All people coming from the field will be cleaned-up before entering the nursery facilities. Ensure that your clothes do not harbor obvious debris, which may carry insects or pathogens. Clean and sanitize your boots and tools. Vehicle must be cleaned of debris regularly and washed at the designated wash stations. Once vehicle arrive at the nursery they should be parked away from plants and planting materials. You should not be carrying green debris in your truck at any time that you are transporting propagative materials, or when you are going to the nursery. Dispose of the debris first, before making collections, then clean the vehicle by removing all visible debris, visit a wash station if the vehicle is muddy or excessively dusty (use good judgement). Clean the truck bed before going to the field to make propagule collections to bring back to the nursery (see Chapter 1 page 14 for details on cleaning your truck bed).

Always and only ever collect from healthy plants in healthy sites, and seeds are preferred. Collect seeds well away from the ground (far enough away that they are not becoming muddy or soiled usu. 3-5'), and if they are from low growing

plants or plants immersed in water, then treat the seed following propagule treatment recommendations in this document. If necessary, cuttings may be used, but should be collected and propagated in the cleanest possible way, and then must be treated following treatment recommendations in this document. Do not collect propagules from areas with known disease problems or with visible damage. All propagules will be inspected and rejected if damaged before introducing them to the nursery stock. Remember, nursery plants should only be propagated from healthy field plants, and healthy plant parts.

A treatment that is the most appropriate based on the potential for pathogen or other pest infestation issues will be used to clean the propagules and cuttings prior to planting (please see the treatment section for details). Propagule treatment is not used here to rid propagules of disease but to disinfest (see glossary for definition of disinfest and disinfect) surfaces! Therefore, it is appropriate for treating healthy, low growing seeds and all healthy cuttings according to the treatment recommendations (again see treatment section for details).

Tools used to remove plant material will be cleaned and sanitized between sites (if you get in your truck and move to a new location, this is a new site, for more on this see field best practices). All propagules, seeds, cuttings, divisions etc. from the field will be as clean as possible before moving them away from the site to the nursery, and will be contained for transport.

Field Best Practices: (Part I) picking up plants from the nursery, and  
(Part II) planting, and other activities

## Field Best Practices Part I: picking up plants from the nursery

### Key points:

- ✓ **Plants cannot be returned to the nursery once they have been taken to the field.**
- ✓ **Check that plants are cleared for planting before taking them.**
- ✓ **Wear clean clothes to pick up plants and you must sanitize your shoes upon entry and exit of the nursery.**
- ✓ **The ground at the nursery is considered dirty; therefore never allow the plants to touch the ground at the nursery.**
- ✓ **Do not use your field tools in the nursery.**
- ✓ **Regularly clean your vehicle, to avoid bringing contaminants to the nursery.**
- ✓ **Truck beds should always be maintained and thoroughly cleaned out before transporting clean materials.**

*Planning.* Be very thoughtful about the quantity of plants you pick up. Only take the number of plants that you can reasonably manage. Plants removed from the nursery cannot be brought back. No plants will be accepted back at the nursery once they have been checked-out by field staff. If you anticipate needing to hold plants at a field site, please work with the nursery staff to strategize how to care for and protect them (shadecloth, water, etc.) until planting. Be sure the plant species you are picking up are cleared for planting and ask the nursery manager if you are unsure.

*Sanitation.* Shoes must be cleaned prior to entering field sites. A separate set of tools is recommended for nursery and field; and tools must be cleaned regularly. Gloves should be clean, and clean gloves should be used in the field and in the nursery. Brush dirt off shoes/tools, and spray tools/soles of shoes down to wet with 70% isopropyl alcohol. If you are unsure of the protocol, please inquire about training. Note that contractors and volunteers should follow these measures as well.

Regularly clean your vehicle, to avoid bringing contaminants to the nursery. On regular intervals, clean your vehicle at the designated wash stations to remove soil and debris that may occur on the vehicle from working in the field. Pay special attention to accumulation of material on tires and in wheel wells. This is especially important following exposure to muddy and/or dusty conditions.

Truck beds should be maintained and thoroughly cleaned out before transporting clean materials (plants, clean tools, and supplies). To maintain truck beds keep a dustpan and broom in the vehicle and sweep out any remaining debris after completing tasks. Simply remove and properly dispose of all coarse debris, then sweep out the bed of the truck. Before transporting clean materials (plants, clean tools and supplies) spray the bed with water to loosen up the dirt, then use a push broom and a bucket of soapy water to scrub it down. Make sure to clean out areas that may collect debris. Finally hose it off and let it dry. If you cannot remove visible soil/debris, it should be repaired. Truck beds should always be maintained.



## Field Best Practices Part II: planting, and other activities

### Key Points:

- ✓ **Start clean-stay clean. Sanitize shoes and tools at the start of each workday and between sites.**
- ✓ **Tarps should be kept clean and thoroughly washed before using them in a new area.**
- ✓ **Weeds should be removed, and collection bags for weeds should be cleaned between sites.**
- ✓ **Irrigation should always be well managed.**
- ✓ **Reduce runoff and water according to plant species needs.**
- ✓ **Ensure that plants used for revegetation are free of pathogens.**
- ✓ **Ensure that the site has been tested for, and is free of contamination threats.**
- ✓ **Consider an avoidance treatment strategy for contaminated sites that must be revegetated.**
- ✓ **Only import clean soil/planting materials.**
- ✓ **Organize your work schedule to minimize clean-up time.**
- ✓ **Minimize movement between sites: as much work as possible in as few sites as possible per day, will minimize movement between sites.**
- ✓ **Start your workday with clean gear, then visit healthy sites first, and contaminated sites last.**

*Sanitation.* Sanitize shoes and tools at the start of each workday. Care should be taken not to transport dirt or mud between sites. Shoes and all planting tools must be sanitized prior to entering the site. Brush dirt off shoes/tools, and spray tools/soles of shoes down to wet with 70% isopropyl alcohol. If you move between sites, the sanitation procedures above should be repeated before leaving each site (a new site is a location you must get into your vehicle and drive to).

Under dry and wet conditions if an infested area (or potentially infested area, this is an area that displays obvious symptoms of decline or disease that has not yet been identified) has been visited, clean vehicles, also clean and sanitize, shoes and tools. In dry conditions, vehicles that may have contacted contaminated soils before traveling to disease-free areas are to be cleaned first, by brushing off dirt before entering a site. During the dry season water or an approved polymer can be used to help dust suppression. In wet and muddy conditions, tires and wheel wells can accumulate large amounts of soil and debris, and during this time, it is especially important to stay on roads to avoid tracking soil. Soiled vehicles must be washed at a designated wash station prior to entering a new site. It is advised to park vehicles on roads and to avoid travel through muddy areas.

Tarps should be cleaned between uses at different locations. First, shake it out to remove loose dirt, and spread it out over a large flat surface. Then, spray it with water and let the water set on it for 5-10 minutes to loosen up the dirt. Follow by scrubbing with soapy water and a push broom. Pay special attention to

grommets and edges that may collect debris and soil, make sure to clean out all areas that may collect debris. Finally hose it off and let it dry. Special cleaners are available for tarps and these may help to preserve the tarp, if not considered environmentally safe, then a general cleaner: simple green®, or dish soap: Dawn® is also acceptable.

*Watering.* Similar to in the nursery, avoid overhead irrigation if possible and/or start watering early (before dawn) to avoid prolonged leaf wetness; irrigation should always be well managed. Do not use untreated irrigation water from any source other than a known clean well (potable) or a municipal water supply. Water should not be allowed to accumulate in open spaces due to factors such as neglect of faulty irrigation equipment. These water sources if exposed to untreated soil or plant debris may harbor large accumulations of inoculum. Divert soil and water movement from adjacent areas to prevent contamination of the planted site.

Do not allow hose nozzles for watering multiple areas to become soiled. Keep nozzles off the ground. Do not drag around excessively muddy hoses. It should not be necessary to water in areas with mud (as they are already wet) and so it should be possible to water without hoses becoming muddy. Muddy hoses can be avoided by watering the furthest points from the bib first and working back toward the water source.

Avoid and minimize the accumulation of standing water around planted

areas and on pathways leading to and away from planted areas. Repair and replace damaged irrigation equipment that would allow standing water to accumulate. Do not allow irrigation water to flow from one area to another from excessive irrigation because the soil and water itself can move pathogen propagules from contaminated areas to uncontaminated areas. Reduce runoff and water according to plant species needs. Where appropriate turn down the water, water deeply, and less frequently.

*Weeding.* It is important to keep up with weeding as weeds can harbor pests, pathogens, and are a nuisance themselves. It is best to pull weeds prior to seed head production to help minimize seed release during the weeding process. It will also help improve effective composting (if you decide to go that route) as some weed seed tolerates very high temperatures.

Reusable nylon collection bags for weeds could carry pathogens, pests, and weed seed from one site to another if they are dirty. Clean collection bags between sites. First, make sure to remove all large debris, second, thoroughly wash it in hot soapy water until no visible debris remains, then rinse, and allow it to dry. If the inside of the nylon bag is lined with a contractor bag and only the outside of the nylon bag is in contact with debris and soil, it is only necessary to clean the outside of the nylon collection bag. Clean, reusable nylon collection bags can be used at the next site. For an alternative to cleaning, see the next paragraph.

If you do not have time to clean reusable nylon collection bags, or if you prefer, place weed material in a paper lawn/leaf bag, and dispose of the bag and weeds. If you are composting weeds, ensure that your composting facility accepts this material, pull weeds prior to seed head production, place them in the paper lawn/leaf bag, seal the bag, and compost the whole thing.

*Revegetation.* Ensure that plants used for revegetation are free of pathogens before moving them to sites (check with your nursery grower), have a healthy appearance, and are free of weeds. Ensure that the site has been tested for contamination threats well in advance of moving plants to a site (see monitoring section for details). For field sites that are found to be contaminated with low levels of a non-quarantine pathogen, plant with non-host plant species. Using plant species that are outside of the host species genera can help to avoid disease (see treatment section for more details). For heavily contaminated field sites, it may be possible to attempt to pasteurize an area (see treatment section for details); these procedures are currently being examined, and tested.

Use plant species that are appropriate for the site. Planted sites should reflect a healthy remnant site of the same habitat type. In addition, using seed from adjacent, healthy, remnant habitat can help to reduce the chance of offsite issues.

Do not import contaminated materials to your site. Ensure imported materials from another location within the Presidio, or from outside the Presidio,

have been adequately tested and/or treated before being moved to your site. See the treatment section for details. Keep informed regarding changes in these procedures as we develop improved methods in this area.

*Organization.* Organize your work schedule to minimize clean-up time. To be most efficient, as much work should be done in as few sites as possible per day (as opposed to less work in more sites over several days). That way you can minimize the amount of time needed to clean up between sites. In addition, if you know that you are visiting a contaminated site (or sites), visit that site (those sites) last and visit healthy field sites first, or only visit one type, or the other in a single day.

## Best Practices for Projects Involving Earth Moving Equipment

### Key points:

- ✓ **Maintain clean heavy equipment at sites. Rumble strips and pressure washers should be used to easily and regularly remove soil and debris.**
- ✓ **Thoroughly clean heavy equipment between sites and jobs at designated cleaning stations.**
- ✓ **Runoff from washing dirty equipment or from soil displacement will be contained and will not be allowed to enter roads or streams.**
- ✓ **Soil will not be tracked between areas or across walkways and roads.**
- ✓ **Repair road and pathways that contain potholes that could accumulate standing water, ASAP.**

Maintain clean heavy equipment at sites and between sites. Maintaining clean heavy equipment at sites can be achieved by using rumble strips and a pressure washer to easily and regularly remove soil and debris from the equipment.

Thoroughly clean heavy equipment between sites and jobs at designated cleaning stations, following the guidelines posted at the cleaning station.

Disturbed soil and water running through construction areas will be controlled. Runoff from dirty equipment or from soil displacement will be contained and will not be allowed to enter roads or streams. Soil will not be tracked between areas or across walkways and roads. Water will not be allowed to accumulate due

to site disrepair. Repair road and pathways that contain potholes that could accumulate standing water, ASAP.

# **Chapter 2.**

## **Presidio Monitoring and Sampling**

## Introduction to Monitoring and Sampling

Monitoring will help identify *Phytophthora* in nurseries and landscapes. The nursery monitoring recommendations and sampling protocols hereinafter are for the Presidio, and can be used and modified for use throughout the Golden Gate Natural Recreation Area nurseries to clear potting mix, compost, propagation beds and furrows, and plant species that are at risk to *Phytophthora* species. For monitoring in landscapes, information on how to sample, and sanitize is included. It is important to remember that for potting mix, compost, and soil -false negatives are possible and monitoring will usually only be able to identify heavily infested material. Furthermore, even with monitoring and sampling in place, it is imperative to be diligent from the start and maintain best practices throughout the production process. Integrate monitoring and sampling with best practices, and importation recommendations.

## Presidio Native Plant Nursery Monitoring and Sampling

### Key Points:

- ✓ Follow the sampling protocols.
- ✓ Refer to Appendix 1 to determine plant species at risk.
- ✓ Potting mix should be made from pathogen free components.
- ✓ The potting mix should be made and stored away from contamination threats in sanitized or sterile containers.
- ✓ Sample early to allow time for sample testing and treatment.
- ✓ Inspect nursery plants weekly for diseases and/or pests, and then record your observations and take action.
- ✓ Decide whether you are monitoring a plant species for *Phytophthora* contamination or detecting the disease agent by using the Monitoring and Detecting Decision Chart.
- ✓ If another problem is suspected, manage appropriately and expediently.
- ✓ If foliar *Phytophthora* is a concern, test symptomatic plants using the Agdia *Phytophthora* ImmunoStrip® tests.

### In the case of *Phytophthora* detection:

- ✓ If a quarantine-level *Phytophthora* species is detected contact the CDFA for instructions.

- ✓ **Photograph and document any visible symptoms and document the event.**
- ✓ **As soon as possible, discard all the contaminated crop plants into a contained (landfill bound) bin.**
- ✓ **Sweep and dispose of any loose soil or debris on benches.**
- ✓ **Pressure wash/ or thoroughly clean benches ensuring no splash of crops on nearby benches.**
- ✓ **Sanitize surfaces with 70% isopropyl alcohol.**
- ✓ **Take notes on surrounding plant crops and proceed with testing these if there was the potential for cross contamination.**

The sampling protocols herein describe the methods for evaluating potting mix and compost, beds and furrows, as well as plant species at risk to *Phytophthora* in the GGNRA nurseries. Refer to Appendix 1 for plant species that are considered at risk based on the plant species available for the GGNRA nurseries specifically (this list is not for all plant species or all plant species in California). The list (Appendix 1) includes plant species that are considered moderate to high risk species currently used in the GGNRA nurseries. This list should be updated as new information becomes available, or when new plant species are added.

*Potting mix and compost.* As an initial evaluation, to help ensure that your potting mix has not become contaminated it is important to evaluate it and it is component

parts. This process is important because, if the contamination is identified, then the problem can be treated accordingly and issue resolved. First, prior to distribution to your nursery, ensure the compost part of the potting mix tested negative at the compost yard. Then, to protect the compost during delivery, ensure the truck bed holding your compost is cleaned and sanitized before it is filled. In addition, make sure each amendment that is added to the potting mix is sterile, pasteurized or known to be free of contaminants before adding it to the compost. The potting mix should be made and stored away from contamination threats including: untreated mud, soil, gravel, cull material, dirty containers, dirty tools, unhealthy plants, and green debris. Remember to use only new or properly cleaned and sanitized containers. The procedure below will be used on the potting mix at each nursery in July. This procedure will also be used on the compost at each stage of the composting process. For compost, recomposting can be used for piles that test positive for non-quarantine level *Phytophthora* species.

The amount of potting mix sampled depends on the size of the potting mix pile (Table 1). For a pile of potting mix of one - four cubic yards, take a sample equivalent of four heaping standard trowel scoops (hsts). For a pile between five - 30 cubic yards, use one (hsts) per cubic yard of soil up to 30 cubic yards. For piles that are, very large 31–50 cubic yards (compost yard) take a sample equivalent to 30 cubic yards (or 30 hsts). If there are two or more piles made at the same time and treated the same in the compost yard, then they can be considered one pile. Mix the total sampled soil (compost or potting mix) in a clean container, divide the

sample into the number of divisions suggested in Table 1 and then place each divided sample into a separate bait container. When you are sampling ensure that your sample is representative of the whole pile by collecting soil at several points across and around the pile. Clean up and proceed with sampling, keeping in mind not to tread on the pile with dirty gear. False negative are possible and so monitoring is not considered a replacement for best practices.

Potting Mix or Compost Sample			
Size of Potting Mix or Compost			
Pile: (cubic yards (yd <sup>3</sup> )	1-4	5-30	31–50
Number of heaping standard			
trowel scoops	4	1 /yd <sup>3</sup>	30
Number of divisions	2	3-5	6

**Table 1.** The amount sampled depends on the total size of the pile.

*Propagation beds and furrows.* *Phytophthora* species are sometimes temporally ephemeral and so it is necessary to sample more than once a year. Sample at least two months prior to use in order to allow time for sample testing and treatment (if necessary) before planting, then twice a year during periods of use to ensure they are not contaminated with *Phytophthora* species at some point during the process. The sampling protocol for beds and furrows differs somewhat due to

the shape differences between them. However, the same amount of soil will be used for both beds and furrows: 2 L /30 ft<sup>2</sup>. Samples from both groups should contain soil from locations across the bed or furrow instead of being clumped in one area. The soil should be collected starting about ½ to 1 inch below the surface, in the root zone. Each bed and each furrow should be given a number, which should be used in your sampling number scheme to keep track of the history of each furrow and each bed. Use a trowel to dig up the soil; the sampler should use a shovel instead if using the trowel is too difficult. Clean your tools between sampling each furrow and each bed, placing the collected soil into one gallon sized clean plastic bags. Be sure to seal the plastic bags and check for holes to eliminate leakage.

*Furrows.* Take three 2L samples for a 3' x 30' furrow. Each 2L sample will be composed of two 1L sub-samples. Stagger the subsamples, starting at one end and moving toward the other. Take the first sub-sample (left-hand x in S1 box, Figure 1) at the furrow edge and then take the second sub-sample cross width over the furrow and lengthwise down 6 feet. Place the subsample from 0 feet and 6 feet into the sample bag S1. Move lengthwise down the furrow and repeat this twice, for samples S2 and S3. If there are symptomatic plants within the furrow and the sampling scheme does not adequately sample these plants, then change the location of your sampling to correspond to the location of the symptomatic

plants, make notes of the changes, and place labeled flags where the samples were taken for later reference. Make note of the plant species in the furrow.

**Figure 1.** Sampling scheme for a 3' x 30' furrow



*Beds.* Take one 2L sample for every 30 ft<sup>2</sup> of garden bed area. For each sample (S1–3), collect soil from around the bed, preferably all around a single plant species, making note of the plant species in the sample. Each 2L sample will be composed of targeted sub-samples from around two or three symptomatic looking plants within the 30-ft<sup>2</sup> area. If it proves difficult to tell whether the plants are symptomatic, collect subsamples from near one central, and two edge plants, and combine them in the sample bag.

*Routine symptom inspection.* As part of a routine plant health assessment, inspect nursery plants weekly for symptoms and signs of diseases and/or pests, and then record your observations. Known host plants of concern (Appendix 1) should be inspected for Phytophthora disease as a part of the assessment as well. In order

to check plants for symptoms that may be the result of Phytophthora disease, look for whole plant/leaf blight, root rot, stem necrosis or dieback, branch dieback, chlorosis, bleeding lesions, root collar canker, wilting, stunted growth or the appearance of water stress. In the absence of more blatant symptoms, carefully remove a few plants from pots and look at the outer roots. Look for necrotic flecking, dead, broken roots, and water soaked lesions. Only remove plants from containers if they are established to avoid damage. Potential leaf symptoms include foliar blight and leaf tip necrosis.

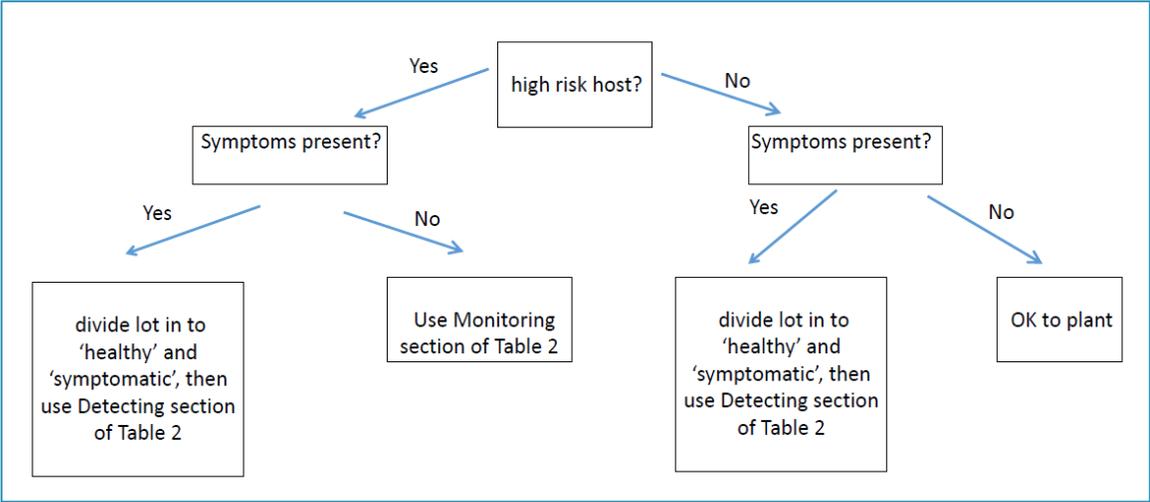
If a plant lot is determined to be symptomatic, be sure to describe and record all of its symptoms. Move the surrounding plants one row past symptomatic plants, (unless plants are overgrown or overlapping each other, then move the whole rack) to a designated area where the risk of spread is lower, away from the healthy plants. Mark and clean the area following the management protocol best befitting the situation (see page 8 Chapter 1). Your plants are now split into healthy and symptomatic groups. Note that a *healthy group* is not a *healthy plant lot*.

*Sampling plant species.* See the appended table (Appendix 1) for a list of plant species that should be included in nursery sampling. Plants only need to be sampled once in the growing season, when they are well established in pots but as soon as possible following establishment. For some plant species foliar

*Phytophthora* species infection is a concern, so these plant species should be tested using the Agdia Phytophthora ImmunoStrip® tests when they are symptomatic (Appendix 1). If a plant species in your nursery is not included in the list but displays advanced Phytophthora-type symptoms (see definition) then proceed with sampling as if that plant species were a known host and report the incident. Appendix 1 should be updated regularly to reflect new findings. If the potting mix was found positive for *Phytophthora* species, then sample all plants growing in the infested potting mix as if they were symptomatic hosts.

*Monitoring and detecting.* Decide whether you are monitoring a plant species for *Phytophthora* contamination or detecting the disease agent (Figure 2). This can be done by answering the question: Are all the plants in the plant lot healthy, displaying no Phytophthora- type symptoms? If the answer is *yes* then sample based on the Table 2 guidelines for sampling under “Monitoring”, if the answer is *no* sample based on “Detecting”.

Figure 2. Monitoring and Detecting Decision Chart



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Monitoring:

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**Sampling plant species from a healthy plant lot**

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plant lot size	healthy	symptomatic
10-75	3	na
76-300	4	na
301-750	4 + 1 composite of 25	na
751-1500	5 + 1 composite of 25	na
1501-2000	5 + 2 composite of 25	na

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Detecting:

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**Sampling plant species from a symptomatic plant lot**

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plant lot size	healthy group†	symptomatic group
10-30	discard lot	discard lot
31-75	3	3
76-300	3	5
301-750	3 + 1 composite of 25	4 + 1 composite of 25
751-1500	3 + 2 composite of 25	4 + 2 composite of 25
1501-2000	3 + 3 composite of 25	4 + 3 composite of 25

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† If there are not enough plants in the healthy group to take the suggested amount then the lot should be discarded

**Table 2.** The size of a plant lot (plants of one plant species grown in a nursery and propagated together) and the number of plants that should be sampled and tested based on whether the plant lot is healthy (upper, monitoring) or symptomatic (lower, detecting). Symptomatic refer to plants with *Phytophthora*-type symptoms (see definition). If the plant lot is symptomatic, be sure to describe and record the symptoms and move all symptomatic and surrounding plants away from healthy plants immediately, to a designated area where the risk of spread is lower. Mark and clean the area the plants came from following the best management protocol. Sample the healthy group and the symptomatic group separately, according to the table guidelines under 'detecting'. To ensure this group of plants is free from *Phytophthora* and is adequately evaluated. Plants that were closest to the contaminated area should be sampled.

*Soil sampling plants.* Make sure plants are watered prior to sampling. Know the plant species you are intending to sample and the approximate number of plants of each species (Use Table 2). It will not be necessary to take a composite sample if there are 300 or less plants, instead, take the number of whole plants listed (Table 2).

*Plants to sample.* Gauge the number of plants that should be sampled (Use Table 2). If the plant lot is symptomatic, be sure to describe and record the symptoms.

Move all symptomatic plants and surrounding plants away from the healthy plants immediately, to a designated area where the risk of spread is lower. Afterwards, mark and clean the area the plants came from following the best management protocol. Your plants are now split into healthy and symptomatic groups. Note that *a healthy group is not a healthy plant lot*. Sample the healthy group and the symptomatic group separately (Table 2, Detecting) place a marker in each sampled pot. Label the sample bags/buckets clearly, so it can be determined from the label which were from the healthy group and which samples were from the symptomatic group. To ensure plants in the healthy group are uncontaminated, plants from the healthy group should be sampled such that areas closest to the potentially contaminated area are sampled.

If the plant species is high risk host within the GGNRA (Appendix 1, yellow rows) and your plant lot is healthy, take the minimal sample possible for the size of the plant lot (*Healthy plant lot* and *healthy* column, Table 2). For example, if you have 130 known host plants that are all healthy, then take four whole plants for a sample. Keep records of all results and for composite samples place a marker in each sampled pot. If the plant lot is healthy and a moderate (Appendix1, white rows) to low risk species (species not in Appendix 1, but part of the GGNRA nursery plant species list) then you do not need to sample the plant lot. Remember Appendix 1 is expected to change as we increase our understanding of *Phytophthora* on California native plant species so make sure you are using the most up-to-date list.

Overall, the purpose of these sampling methods is to sample for *Phytophthora*. If a different problem were suspected, the correct procedure would be to sample for the issue that is suspected. For example, if plants have symptoms of leaf chewing insects only, root systems appear healthy, and there are no other symptoms of *Phytophthora*, the lot would be considered healthy for the purposes here. Manage any other problems appropriately and expediently. If the plant species is not high risk on the list (Appendix 1, GGNRA nurseries only) and you do not observe any *Phytophthora*-type symptoms, (see definition) you do not need to sample the plants for *Phytophthora* species. If the plant species is on the list (Appendix 1, GGNRA nurseries only) and is a moderate risk host then examine the plant closely for symptoms, checking with the Agdia *Phytophthora* ImmunoStrip® tests before clearing the plant lot. It is unnecessary to sample the moderate risk plants (and plant species not included on the list, these are low risk) that do not have symptoms of disease. Risk ratings are subject to change with the growing knowledge base. In other words, risk status of a plant species may change as our knowledge and understanding of *Phytophthora* in California native plant nurseries and ecosystems increases, so always refer to the most up to date list.

*Measurement for composite sample.* Be sure soil is of uniform wetness (before sampling (Water in the morning, and sample in the afternoon, for example). To

calibrate the stainless steel Fisherbrand™ Scoopula™ for measurement, measure out one full heaping tablespoon of potting material (of the same wetness and type as the plant lot, for simplicity use one of the plants from the plant lot). Fill a Scoopula™ spatula with the contents of the tablespoon from the scoop end up. Mark the spatula to the calibrated fill line. Clean with 70% alcohol, dry, and do not wipe away your mark. Then, mark clearly across with a permanent marker. Be prepared to re mark as 'permanent' mark may fade after cleaning with alcohol several times. This should give a relatively precise measurement from each of the sampled plants, within the plant lot. If the soil is very similar across different plant lots, it may not be necessary to calibrate again.

*Supplies.* 70% alcohol, paper towels, Fisherbrand™ Scoopula™ spatula, tablespoon, heavy duty plastic bags appropriate (currently used) for baiting, permanent marker, pencil, clean gloves, data sheet, and plant tags.

*Sampling technique.* Using the calibrated Scoopula™ spatula, take the measured amount out of the container twice, once from each side of the container. Place the removed soil in the bag used for baiting. Clean the spatula between soil removal from each plant and between samples. Cleaning between plants is very important to reduce the risk of spreading soil-borne disease issues. This can be done quickly and easily with an alcohol wipe (70% isopropyl alcohol wetted paper towel).

Take a sample by running your tool down the inside of the pot at its edge. A composite sample (see definition) is taken from several, in this case 25, plants of the same species (Table 2). Mark the sampled plants and place the removed soil from each of the plants into the same bag that will be used for baiting. Close and carefully label each sample bag so the sample can be identified to nursery, plant species, composite sample number, plant lot health status and plant group health status. The plant sample ID system is already in place; use the known ID system. Do not repeat ID names. Contact your Plant Pathogen Coordinator for details.

*Transporting sampled material.* Make sure bags/buckets are sealed and upright so that the soil remains in the bottom. Place samples in cooler with an icepack for transporting to the baiting location, if not baiting on site. Samples can be kept in a cold room or cooler with an ice pack until it is time to bait them or no longer than 72 hours later. Do not freeze the samples or allow them to heat above 80 °F.

*In the case of recovering (non-quarantine) Phytophthora species from potting mix or compost.* The following instructions are suggested only for *Phytophthora* that is non-quarantine level pest. If a quarantine-level *Phytophthora* species is detected it is necessary to contact the CDFA and to follow their methods.

1. *During the composting process.* If compost tests positive prior to being added to potting mix, run the compost back through the composting process.
2. *Prior to planting.* If potting mix tests positive for *Phytophthora* species prior to planting, then steam pasteurize to eliminate the problem, re-test to confirm negative, and then proceed with planting.
3. *Following planting.* If potting mix tests positive for *Phytophthora* species following planting or in the midst of planting, steam pasteurize the remaining mix to eliminate the problem before proceeding with any further planting. Then sample *all* plants grown in the infested soil as if they were symptomatic hosts (Table 2).

*In the case of recovering (non-quarantine level) Phytophthora species from plants in the nursery.* The following instructions are suggested only for *Phytophthora* that is non-quarantine level pest. If a quarantine-level *Phytophthora* species is detected it is necessary to contact the CDFA. Remember you have divided your plants from the crop (lot) into symptomatic and healthy groups. If the healthy group tests positive it must be discarded along with the unhealthy group of plants. If the healthy group tests negative discard only the unhealthy group and re-test the healthy group, before out-planting.

1. Photograph and document any visible symptoms, and document the event.
2. As soon as possible, discard all plants from the unhealthy group (or the entire crop if the 'healthy' group tested positive) of the contaminated crop into the landfill bin. Even if plants in the unhealthy group are *Phytophthora* negative, unthrifty plants should be discarded.
3. Sweep and dispose of any loose soil or debris on benches of contaminated crops.
4. Pressure wash/ or thoroughly clean benches ensuring there is no splash onto crops on nearby benches.
5. Sanitize surfaces with 70% isopropyl alcohol.
6. If the crop tested positive, take notes on surrounding plant crops and proceed with testing these, if the contaminated plants were intermingled or nearby on the same bench, identify and test plants with potential for cross contamination.
7. If the 'healthy' group tested negative initially and was saved, retest as if it were a healthy crop to ensure that it is in fact *Phytophthora* negative. If it tests positive or develops symptoms later discard it and follow the above instructions for cleaning and sanitizing.

*Leaf symptom sampling.* Certain plant species are considered foliar hosts that are grown within the GGNRA. These plant species (Appendix 1, refer to Agdia Foliage Test column 'Yes') should be evaluated for foliar symptoms. If the plants are symptomatic, choose ten plants of the same species, and then test each of the ten plants by using a test strip for each plant. If any of the plants are positive then move all ten to a clean work area and remove six symptomatic leaves from one of the ten plants. With a disinfected hole-punch, remove one leaf piece from the disease-healthy-tissue margin of each of the six leaves. Plate each hole-punched leaf piece from each of the six leaves onto one *Phytophthora* selective media plate. Repeat for the other nine plants and submit plates to the UC Berkeley Forest Pathology and Mycology Lab. For Baiting Techniques details see the Appendix B6-1.

## Monitoring and Sampling in Presidio Landscapes

### Key Points:

- ✓ **Sanitize boots immediately prior to entering site.**
- ✓ **Walk the entire site to identify potential plants infected with Phytophthora-type symptoms, and then select 10 plants of one species or 5-10 each of several plant species.**
- ✓ **For extraction of plants, use a clean hand pick, pin flag, and trowel. Mark the pin flag with the species code and collection number, then install the flag.**
- ✓ **Retrieve GPS coordinates for each plant, and record the information on a field sheet.**
- ✓ **Using a trowel, extract the plant from the ground and immediately place it into a clean and labeled Ziploc® bag.**
- ✓ **Take (1) picture of the plant within the bag.**
- ✓ **Fill the hole and disinfect your tools before leaving the site, allowing the drip to runoff into the disturbed soil.**
- ✓ **Sanitize boots/tools upon leaving the site.**
- ✓ **Pack your sample in a cooler.**
- ✓ **Return samples to the Plant Pathogen Coordinator at a designated location.**
- ✓ **Sampling in sites prior to a new landscape project is important.**
- ✓ **Do not plant contaminated nursery stock.**

✓ **Get involved with the Presidio SOD Blitz sampling.**

Monitor field sites to determine if the site is contaminated with *Phytophthora*. A site is an area that you are working. When you get in a vehicle and move, your next location is a new site. Furthermore, if a location changes drastically, from one area to another that can be a way to determine that you are looking at two (or more) sites and not one. Monitoring is best in the spring following rains, in the early summer, and again in the fall when the rains begin again. Do not go out when the conditions are excessively wet, to avoid spread. Taking several samples from a site will help ensure a correct diagnosis. For smaller sites (1/4 acre or less) it is possible to take less samples, five samples, but for most sites take ten (assuming one plant species sampling). Work with your Plant Pathogen Coordinator for additional details beyond the information provided below.

*Sampling Process.* Sanitize boots immediately prior to entering site. Walk the entire site to identify potential plants infected with *Phytophthora*, looking for *Phytophthora*-type symptoms. Select plants. If sampling symptomatic individual plants for the detection of *Phytophthora*, select 10 plants of one species per site, and sample whole plants or roots or leaves from individual plants. If sampling a variety of species for monitoring purposes, select five-ten individuals of each species, and collect root samples from each individual (unless you are sampling

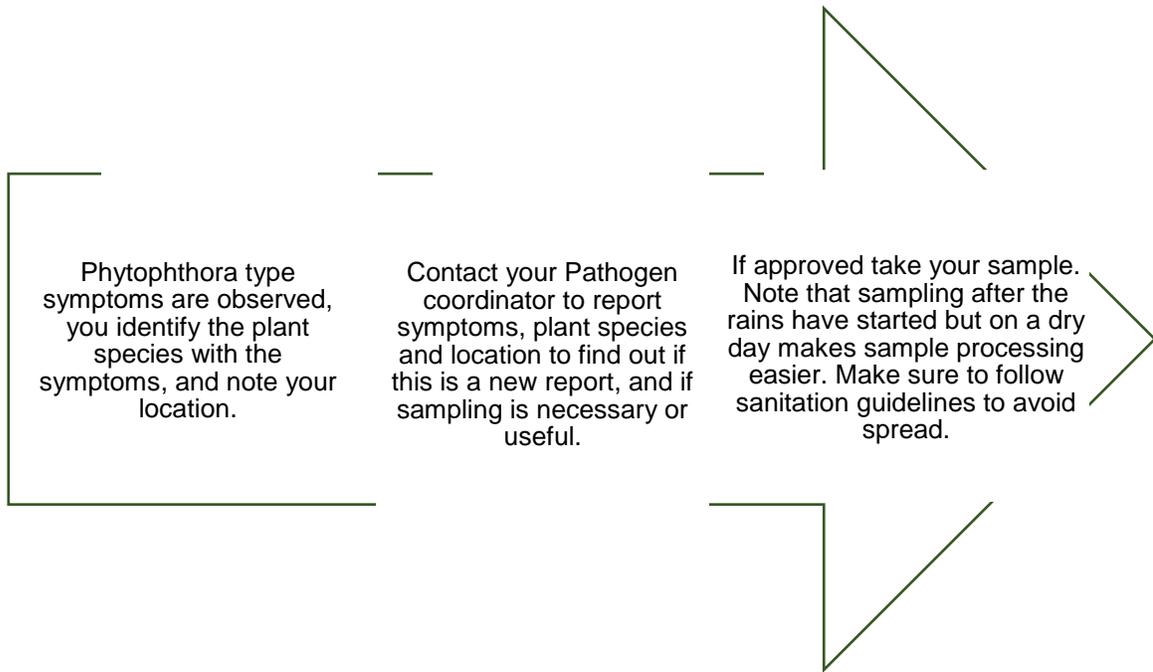
for foliar *Phytophthora*). For extraction of plants: use a clean hand pick, pin flag, and trowel; mark the pin flag with the species code and collection nom., and install the flag. Then take two pictures of plant in situ: landscape context, and close up. Retrieve GPS coordinates and record. Wearing gloves clean the area around the plant. Using a hand pick, loosen soil around plant while not disturbing root ball and stock. Using a trowel, extract the plant from the ground and immediately place it into a clean and labeled Ziploc ® bag. Take a picture of the plant within the bag. Fill the hole and disinfect your tools before leaving the site, allowing the drip to runoff into the disturbed soil.

*Sanitizing Process.* Sanitize boots and tools when entering, and exiting sites. Sanitize tools within a site if healthy plants are being examined following the sampling of diseased plants, or remove these (healthy) first or use a different set of clean and sanitized tools, for healthy and diseased looking plants. To clean tools, first clean by physically removing and wiping away all gross material. Then, irrigate with 70% Isopropyl alcohol starting from top-down

*Transporting samples.* Place the samples in a cooler with frozen ice packs. The intent is not to freeze the samples but to keep them at around room temperature (70°F) or slightly lower, to avoid samples reaching higher temperatures (80°F or higher) and to keep the samples out of direct light.



## Flow chart for when to sample and test in the field



*Baseline sampling.* Sampling in sites prior to a new landscape project is important. It can be used to help gauge the *Phytophthora* species in project sites. There are challenges to this type of sampling. If there are no plants at the site and it is vacant soil, there may still be resting structures of *Phytophthora* that would be difficult to detect and so it would be very possible to get a negative result from sampling and testing even with the pathogen there. At these sites plant susceptible (pathogen free!) sentinel plants prior to planting and/or immediately test the nearest surrounding vegetation. Be considerate of the fact that simply testing the nearest surrounding plants would not be adequate in a case where the site to be planted

differs in some significant way from the nearby vegetated areas, for example, the site has imported soil. If the site already has vegetation, existing vegetation can be evaluated by taking samples of it and testing it for *Phytophthora*. Potential host plants displaying symptoms are the best choice but if unavailable, select any symptomatic non-host or asymptomatic host plants available, if that is not available either, then haphazardly select whichever non-host plants are available. This information can then be used to gauge which plants from clean plant stock could be planted without harm (non-hosts) to a site or the landscape and/ or whether another treatment may be necessary or a change in management for the site should be considered.

Baseline sampling data should not be used to plant contaminated nursery stock. Adding more *Phytophthora* could provide added (intra and/or inter) species genetic diversity to the site and/or increase the abundance of the pathogen. Added abundance and/or genetic diversity could be just what was needed to create disease (Mitchell & Kannwischer-Mitchell 1983, Fraedrich et al. 1989, Neher, & Duniway 1991, Goodwin et al. 1998, Anderson et al. 2004, Judelson 2005, Fisher et al. 2012, Brasier 2013). If the idea is to gauge how damaging a particular *Phytophthora* has been in a given landscape thus far, it could be interesting but misleading if applied to how a pathogen is expected to behave when the managing strategy, and pathogen pool has changed. If *Phytophthora* is collected from a site without disease this could simply be suppression due to lack of intraspecies genetic diversity. By adding contaminated plants, you could be helping the

pathogen, for example if the sexual cycle was limiting prior to the introduction of new pathogen genetic stock, helping the pathogen by increasing the genetic diversity of the pathogen, potentially adding virulence (disease causing ability) to the species at the site. By adding contaminated plants, you are also increasing the abundance of the pathogen. Adding contaminated stock is playing a dangerous numbers game that has much greater potential for an outbreak. If a site is contaminated an avoidance approach is a safer bet (see treatment section).

Every year the Presidio Trust and the Forest Pathology and Mycology Lab at UC Berkeley work together to sample for *P. ramorum* in the landscape as a part of the SOD (Sudden Oak Death) Blitz. Get involved and help identify the location of *P. ramorum* in the Presidio landscape by being involved in SOD Blitz.

**Chapter 3.**

**Importing Plants and Materials into the**

**Presidio**

## Introduction to Importation

Imported materials such as commercial nursery plants and planting materials such as soil have the potential to carry plant pathogens including exotic pathogens and pests like *Phytophthora* into landscaping. Once in, the exotics may move into adjacent forests and natural area, or infest nearby conservation nursery plants. Conservation nursery plants are often grown to increase populations of rare or endangered plants and unintentional planting of, or cross contaminating conservation nursery plants through infested imports, can destroy the endemic habitat that cannot be replaced. Therefore, it is very important to, as best as is possible, ensure that imported materials and plants are free from pathogens and pests. Herein follows a guide to importing plants, and planting materials.

Importing Plants: Evaluating commercial nursery plants, using Agdia ImmunoStrip® testing and/or baiting for *Phytophthora*

**Key Points:**

- ✓ **At this time, there is not a way to drop imports from testing.**
- ✓ **Only inspect and test plants that did not have a fungicide application within 45 days.**
- ✓ **Plant lots with obvious Phytophthora-type symptoms should be rejected.**
- ✓ **Plant lots that do not exhibit obvious Phytophthora-type symptoms should be examined, sampled and tested.**
- ✓ **Plants must have fully developed root systems before testing.**
- ✓ **Sample 20% of plants in each lot to create one composite sample for the Agdia ImmunoStrip® testing.**
- ✓ **Sample according to the guide below for baiting.**
- ✓ **A single positive is grounds for rejecting the whole lot.**

*Guidelines.* Only inspect plants that did not have a fungicide application within 45 days. Plants that have not undergone the wait time of 45 days must be held without spray and then inspected. Plant lots with obvious Phytophthora-type symptoms should be rejected. These symptoms include:

- a. Whole plant or leaf blight
- b. Root rot

- c. Stem necrosis or dieback
- d. Branch dieback
- e. Chlorosis
- f. Bleeding lesions
- g. Root collar canker
- h. Wilting
- i. Stunted growth
- j. Appearance of water stress

Plant lots that do not exhibit obvious *Phytophthora*-type symptoms should be examined, sampled and tested with (1) Agdia ImmunoStrip® tests, or (2) by baiting. To take samples, plants must have fully developed root systems that reach the edge or near the edge of the container so that they can be safely removed from the container without damaging the plant and at the same time be able to sample the root system. Wear latex gloves, and sanitize gloves and sampling tools between composite samples. Sampled plant lots that test positive for *Phytophthora* should be rejected. A single positive is grounds for rejecting the whole lot.

*Eliminating some plants from testing.* There is not a good way to drop imports from testing at this time. The issue is without adequate background information on where these plants are coming from procedures for eliminating some plants from

testing may allow in contaminated plants. The problem with imports is that in most cases you will not know the conditions the plants were grown under. Since this is the case, at this point in time, we can not eliminate plants from the testing procedure.

*Sampling for the ImmunoStrip® tests.* Sample 20% of plants in each lot if there are 25 or more plants to create one composite sample per lot. For lots with less than 25 plants take at-least 5 samples. Place samples in a clean Ziploc® bag. Close and carefully label each sample bag so that the sample can be identified to date, nursery, and plant species. There are some difference for sampling smaller and larger sized plants.

Herein is a description of how to sample plants that are up to one gallon in container size and for plants in containers larger than one gallon. Know the plant species you are evaluating. Know what the roots should look like for that particular plant species. Remove the plant from the container. Inspect root system and look for symptomatic roots. If the root system is symptomatic, reject the plant lot. For all others, capture any discolored root or water soaked lesions in the sample. If this is not present, take a sample from the bottom of container and the lower half of root zone. For one gallon sized containers or smaller plants, take approximately one tablespoon of root. For larger plants, take approximately two tablespoons of root.

*Troubleshooting.* From any sample, if you are unable to remove the plant from

the container, expand drainage holes in the bottom of the pot using a utility knife (make sure it has a new or sanitized blade). Take approximately one tablespoon of root. Do this at two drainage holes per pot. If unable to expand drainage holes at bottom of pot, use a trowel or soil probe to expose roots at least half way down the edge of the container, and take approximately one tablespoon of root. Do this in two places per container.

*Testing with Agdia-brand Phytophthora ImmunoStrip® tests.* Use Agdia-brand Phytophthora ImmunoStrip® tests and the SEB1 buffer in the mesh bag. Make sure the tests are not expired, have been stored refrigerated at 4 °C between uses, and tightly sealed in a desiccated container at all times. Test strips and sample extraction bags (buffer bags) should be warmed to room temperature prior to use. Do not expose test strips to moisture and use tests at room temperature. For each lot, use a minimum of five tests. For larger lots use the number equal to 15% of the number of plants in the lot (0.15 times the number of plants in a lot (single plant species all from one nursery and propagated together). Examine roots in each composite sample. Use the root pieces that have both living and dead material along the root piece, if this is difficult to see use the worst looking roots in the composite sample. Use a 0.15g to 0.30g sample of roots for each ImmunoStrip® test in order to make roughly a 1:20 dilution of root to buffer.

Cut off the top of the buffer bag with sanitized scissors. Insert the 0.15g

root sample between the mesh linings in the bag and place the sample near the bottom of the sample extraction bag with sanitized forceps. Extract the sample by rubbing it gently and firmly between the mesh linings with a blunt object such as a pen, permanent marker or preferred tool handle of choice. The color of the buffer/root solution will turn light brown as it becomes fully extracted. Place the ImmunoStrip arrow down into the extraction bag on the side without the mesh (in the channel). Place the strip in the buffer so that the buffer is not above the white line at the base of the arrows. Record results of each test after 30 minutes in the buffer.

*Sanitizing gloves and tools.* Brush any large chunks of soil off tools and gloves, removing all of the visible soil then spray-drench parts of tool that have come in contact with soil, and spray-drench appropriate gloves, with 70% isopropyl alcohol or Lysol ®. Wipe with clean cloth, or let air-dry.

*Steps for bait testing commercial plants before delivery.* Start the process eight weeks before the delivery date. Inspect project plants for the symptoms listed above (make sure to document all symptoms observed), and reject plant lots containing plants with these symptoms. Return or discard the plants; clean and sanitize the area they were removed from. For plant lots that appear healthy refer to Table 1 below, to determine the sample size based on the size of the plant lot. Once the quantity of plants to be sampled has been determined, use the protocol

from Chapter 2 page 13 section “*Measurement for composite sample*” for instructions on how to take a composite sample. The reference to “whole plants” in the table (Table 1.), means that you remove and bait the whole plant. For details on the baiting procedure, refer to “*Appendix 5. Baiting Technique*”. Use the information above and the table below (Table 1.) to produce a baited sample for lab analysis.

Table 1. Sampling plant species from a healthy ornamental plant lot for baiting

plant lot size†	healthy plants to sample + size of composite sample
10‡	3 whole plants
11-30	4 whole plants
31-75	5 whole plants
76-100	4 whole plants + 1 composite of 25 plants
101-200	5 whole plants + 1 composite of 25 plants
201-300	5 whole plants + 2 composite of 25 plants each
301-750	5 whole plants + 3 composite of 25 plants each
751-1500	5 whole plants + 4 composite of 25 plants each
1501-2000	5 whole plants + 5 composite of 25 plants each

† A Plant lot is a group of plants of the same species grown at one nursery from a single crop at the same time.

‡ Use the Agdia-brand Phytophthora ImmunoStrip® tests if there are less than 10 plants for samples.

## Importing Planting Materials and Soil

### Key points:

- ✓ **All materials- planting materials and soil that is imported presents a real threat of contamination for the Presidio.**
- ✓ **If it is necessary to import materials there is a risk that you will be importing in pests, diseases, and weeds that are new to the Presidio.**
- ✓ **Only import if it is necessary to complete a project.**
- ✓ **Use a source that has a good reputation and develop a trusting relationship with this source that is mutually beneficial.**
- ✓ **Steam pasteurize soil, mulch and compost before it is imported.**
- ✓ **Protect all materials from contamination during transport, in a clean and enclosed container.**
- ✓ **Make sure to store imported material in a location where it can not become contaminated once it has arrived.**
- ✓ **Be wary of any source that suggests shortcuts around getting the job done right.**
- ✓ **Ensure that compost, soil, and mulch is of the highest quality, certified and tested for weeds, pests and pathogens. If there is no certification process, start one!**

*Presidio-wide.* All materials- planting materials and soil that is imported presents a real threat of contamination for the Presidio. Before importing planting materials and soil, determine if it is truly necessary to do this. Consider if there other options to importing foreign materials. If it is necessary to import materials there is a risk that you will be importing in pests, diseases, and weeds that are new to the Presidio and the risk cannot be eliminated, you have to face the fact that you are taking a chance and that risk is yours.

To reduce the risk:

Find a source within the Presidio, worst case scenario you are moving around what is already here, then follow the guidelines on treating materials within the Presidio, and then protect your materials during movement. If the procedure is followed carefully this is the lowest risk. For more details, see the guidelines for treatment of materials within the Presidio.

If the above source in the Presidio, is not available use a source that has a good reputation and develop a trusting relationship with this source. Evaluate whether the jobs they have done have been successful in the long term. This entails evaluating your sources. You should have many reliable experiences importing materials from your source. One good experience does not mean you should let down your guard -businesses change all of the time. If all sources are considered contaminated from experience or it is simply unknown, then start by working with a source that is willing to steam pasteurize the planting materials and soil that is imported, this is a good start (refer to treatment section for steam

pasteurization temperature information). Then protect the planting materials and/or soil from contamination during transport, which simply means transporting it in a clean and enclosed container, and then make sure that once it arrives, to store it in a location where contamination will not be an issue.

Make sure that the pathogen free planting materials and soil is transported in a clean bin that is sealed during transport. Make sure, if the soil is for planting that it is a high quality soil for planting. Pathogens are not the only problem, fill dirt cannot grow healthy plants. Do not move soil from infested areas into the Presidio. Have the materials tested for pests, pathogens, and weeds before it is moved in, a negative does not guarantee the material is clean but does give some measure of confidence. Once in, do not use infested areas for storing clean material.

When importing planting materials such as compost, soil, and mulch it should be of the highest quality, certified and tested for weeds, pests, and pathogens. Be wary of any source that suggests shortcuts or complains about the level of work necessary to get the job done right. If there are, no sources that currently certify and test for weeds, pests, and pathogens start a certification process! Work with your source to develop a system that will work to benefit both parties. Once a system is in place it will work better, be more efficient, and produce better products than what is currently available.

## **Chapter 4.**

# **Treatment Recommendations for the Presidio**

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## Treatment Introduction

The treatment recommendations section describes how to manage and treat contaminated materials and locations. Once *Phytophthora* has been introduced into a natural area, it can be very difficult, if not impossible to eliminate, and very costly to manage. However, treatment of materials used prior to planting greatly reduce the chance of introduction and can serve as methods to help protect natural areas. The methods outlined hereinafter for field situations are very rudimentary, under development, and likely to be updated. On the other hand, most treatments for nursery propagation and container sanitation are relatively well developed, still the chemistry (for chemical treatments) may change, and solarization methods, are still in development.

## Treatments for Containers

### **Key Points:**

- ✓ **Always begin with clean planting materials, including clean containers.**
- ✓ **If reusing containers for plants or clean planting materials, assume they are contaminated, and these containers must be cleaned and disinfested.**
- ✓ **Cleaning and using a disinfectant or heat is a disinfestation process.**
- ✓ **Proper disinfestation of containers can be the difference between success and failure of containerized plants.**
- ✓ **First wash containers then complete the disinfestation process by using a sodium hypochlorite solution, or heat.**
- ✓ **Carefully follow the instructions described in this section (key points should not be used alone for treatments).**
- ✓ **Refer to the MSDS when using chemicals for safety and proper handling instructions.**
- ✓ **Be careful and use common sense when handling hot objects and chemical materials.**

In the Presidio native plant nursery, plants are grown for out planting into the Presidio. Therefore, it is extremely important to begin with clean planting materials, including clean containers. How to treat any used plant containers that are to be reused is detailed below.

If containers for plants are to be reused, assume they are contaminated. Proper cleaning and use of a disinfectant/heat requires only a minimum amount of effort, and the effort taken can be the difference between success and failure of containerized plants. Cleaning and using a disinfectant or heat is a disinfestation process. Physically cleaning removes most of the debris that harbors pathogens, pests and weed seed, and the disinfectants (or another disinfestation process) can kill *Phytophthora* and other microbial pathogens that remain. Take the extra effort to help ensure the success of your plants. All used containers must be physically cleaned, and further disinfested.

*Washing containers.* All soil and debris must be removed from pots prior to using a chemical disinfectant or other disinfestation process (like steam or hot water). This is because soil and debris remaining on pots makes the disinfestation process less effective. The soil and debris can be removed from pots, first by physically removing any large chunks, then by scrubbing them with soap and water or by using a pressure washer. Make sure that if you are washing them in a sink that the sink has the proper type of plumbing (such as a p-trap) and a floor drain strainer to handle the remaining soil and debris.

Once the soil and debris has been removed from the containers they can be disinfested using one of several options outlined below. Each disinfestation option is gone over briefly in the following paragraphs.

### *Completing the Disinfestation process for containers*

*Sodium hypochlorite.* Sodium hypochlorite is the active ingredient in standard household bleach. After containers have been thoroughly cleaned to remove all debris and soil they can be disinfested with a bleach solution. One part standard household bleach (5-6% sodium hypochlorite) can be added to nine parts water to make a 10% bleach solution. Containers are soaked in the 10% bleach solution for 30 minutes, then removed and allowed to dry. Following the bleach treatment, the containers are clean, disinfested, and ready for use.

*Aerated steam.* Another method for disinfesting containers after they have been washed is with aerated steam. The amount of heat a container can handle depends on the type of plastic they are made of. Aerated steam heat temperatures can be controlled by adding more air to reduce the heat of the steam. Then the appropriate temperature is achieved to avoid melting containers. Test a few containers first at a particular temperature to see how they handle the heat, or check with the manufacturer before steaming a large number of containers. Be sure to heat your containers to at-least 140-180° F for 30 minutes.

*Hot water.* Following cleaning, another method to finish disinfesting containers after they have been washed is with hot water. Containers can be held in a

180° F water bath for 30 minutes. This hot water treatment will kill most pathogens, pests and weed seeds that remained after washing. Following the hot water bath, they are ready for use.

*Solarization.* Disinfesting pots with solarization is a newer method and will need to be tested at your facility to see if the methods works (high enough temperatures are reached) at your location. After containers have been washed to remove all soil and debris, containers are moistened and stacked with a temperature gauge in the center of the stack. The stack is then covered with a double layer of anti-condensation clear plastic so that no air can escape. The stack is placed in an empty greenhouse during the hottest summer months. Temperatures should reach at-least 158° F for 30 minutes or 140° F for an hour.

## Treatments for Plant Propagules

### Key Points:

- ✓ Propagules should only be collected from healthy plants and healthy plant parts.
- ✓ Treatment described herein is not to 'cure' diseased propagules.
- ✓ Treatment is to remove/kill pathogens on the surfaces of healthy plant propagules.
- ✓ Cuttings and divisions should always be treated.
- ✓ Seeds collected from the ground, that are soiled, that are from low growing plants, and/or from low growing plant parts must be treated.
- ✓ Carefully follow the instructions described in this section (key points should not be used alone for treatments).
- ✓ Refer to the MSDS when using chemicals for safety and proper handling instructions.
- ✓ Always refer to the manufacturers instructions when using fungicides.
- ✓ Only use fungicides if you have the required approval.

*Seed and cutting treatment.* Propagules should only be collected from healthy plants. Two in-depth options are given for plant propagule treatments. One is

based on the commercial instructions of the fungicide ZeroTol 2.0, and the other was developed for treatment of Lupines to control *Fusarium* by Tom Gordon at UC Davis, and may be effectively used on other plant species. Cuttings and divisions should always be treated as well as seeds that must be collected from the ground, that are soiled, that are from low growing plants or from low growing plant parts.

As a pre-plant dip treatment, there are several options such as a bleach dip, or other commercially available fungicides. Two options are given herein. The first one given here: use ZeroTol 2.0 or a similar product for the control of damping-off, root and stem rot diseases such as *Phytophthora* and *Pythium*. This particular product currently also, controls *Rhizoctonia*, *Fusarium*, *Penicillium*, or *Thielaviopsis* on ornamental and nursery plants. Herein the product is suggested for use on healthy seeds, or cuttings to control surface infestations only.

*ZeroTol 2.0 protocol.* Always follow the product guidelines, agricultural use requirements, and safety procedures including wearing the appropriate PPE (personal protective equipment). Always refer to the SDS (MSDS) for specifics on safety requirements for example: <http://www.biosafesystems.com/assets/sds-zerotol-2.0.pdf>. Check for updates and make sure the safety information you are referring to aligns with the product that you are using and is up-to-date.

Follow the instruction on the product label. If using ZeroTol 2.0. Refer to the label for example: <http://www.biosafesystems.com/assets/zerotol-2.0-specimen-label.pdf>. Follow the instructions (according to the label) for example from the Zeritol 2.0 specimen label:

- Use a dilution of 1:100.
- Immerse the seeds or cuttings, then remove them and allow them to dry.

Do not rinse away the product.

- Always follow the label for the particular product in use.

*Lupine Germination Experiment – Bleach Protocols.* Author: Tom Gordon, UC Davis, for *Fusarium* surface contamination

- Rinse seeds with running water.
- Gently stir to help remove soil from seeds.
- Rinse seeds 1-2 minutes in 1% sodium hypochlorite (bleach) solution
  - Dilution conversion
    - Bleach is 8%
    - 7 parts water per 1 part bleach, to reach 1% solution. If bleach was 5% it would be 4 parts water per 1 part bleach, etc.
- Dip seeds in distilled water to remove sodium hypochlorite.
- Put in a bowl of just-boiled water and let sit in the same water overnight.

*Other treatment ideas (must be tested).* As for seed treatments, my first choice would be 30% hydrogen peroxide, which tends to be more penetrating than bleach.

Heat treatment could be effective but that would require knowing what temperatures can be used without reducing seed viability.

*Details.* We find 1% sodium hypochlorite to be reasonably effective in removing superficial microbes from plant surfaces. This can be obtained by dilution of commercial bleach, in which the concentration of sodium hypochlorite is typically in the range of 5-6%.

One limitation on the effectiveness of a bleach treatment is contact with the surface being treated, particularly those that are hydrophobic and/or have a textured surface such that microbes may be protected within depressions into which the liquid does not penetrate. You can minimize this problem by including a surfactant in the bleach and/or pre-treating with 70% ethanol or isopropyl alcohol.

The sequence we usually use for plant material is to first rinse with running water to remove soil particles, then immerse briefly in water containing a surfactant (we use 0.1% tween 20 but other surfactants can serve the same purpose). We would extend the time period in water, and perhaps include stirring, as needed to remove soil. Next is 10-20 seconds in 70% ethanol or isopropyl alcohol followed by 1-2 minutes in 1% sodium hypochlorite. If seeds are air dried, hypochlorite will volatilize as chlorine gas. Alternatively, the bleach treatment can be followed by a dip in sterile water to remove the hypochlorite more quickly.

## Presidio Compost Handling and Yard Plan

### Key Points:

- ✓ **The green debris pile should be placed down and away from the finished compost.**
- ✓ **Each stage of the compost should be placed in succession per existing guidelines for managing compost.**
- ✓ **Wear clean clothes and use clean tools and machinery when working with the final stages (sanitary stage through to final stage) and the final compost.**
- ✓ **Heavy equipment should be regularly cleaned at the designated wash stations.**
- ✓ **Compost from the sanitary stage forward should only be moved with a clean loader.**
- ✓ **Curbs or barriers should be placed at the outer edge of compost piles where vehicle traffic is a hazard.**
- ✓ **All compost should be kept on the asphalt and should not be mixed with native soil or incoming soil except at the earliest stages prior to the sanitary stage.**

- ✓ **Always follow the best possible composting standards, not the minimal acceptable, and include regular monitoring for heat and pathogens.**
- ✓ **Heat is especially important for the inactivation of pathogens in compost.**
- ✓ **The outer edges of windrow piles are more likely to be unable to reach high enough temperatures to kill pathogens.**
- ✓ **Managing heterogeneity of piles is critical.**
- ✓ **Monitor temperatures at several locations throughout the pile including the outer areas.**
- ✓ **Compost must be allowed to stabilize before use. Fresh compost has been shown to support regrowth of pathogens.**
- ✓ **Finished compost should be stored away from contamination threats. Finished compost can be bagged and stored in enclosed new/ properly disinfested container.**
- ✓ **Completed compost should be transported, in a clean and enclosed container.**
- ✓ **Regularly monitor for plant pathogens at the compost yard.**

The green debris pile should be placed down and away from the finished compost. Each stage of the compost should be placed in succession per existing guidelines for managing compost. Wear clean clothes and use clean tools and machinery

when working with the final stages (sanitary stage through to final stage) and the final compost.

Curbs or barriers should be placed at the outer edge of piles or adequate space should be placed between incoming traffic and composting piles. It is very important that vehicle traffic and foot traffic is not crossing the compost across the edges as these are potential sources of contamination. All compost should be kept on the asphalt and should not be mixed with native soil or incoming soil except at the earliest stages prior to the sanitary stage. Incoming vehicle traffic should not travel past the final stages of compost. Any material that leaves the pile (from a spill, drift, etc.) is to be composted again.

Always follow the best possible composting standards, not the minimal acceptable, and include regular monitoring. Heat is especially important for the inactivation of pathogens in compost in general (Christensen et al. 2001, 2002). The outer edges of windrow piles are more likely to be unable to reach high enough temperatures to kill pathogens, and due to this pile conditions may be very heterogeneous. Managing the heterogeneity by properly turning the piles is critical. For monitoring it is important to check the temperatures at several locations throughout the pile including the outer areas. Do not bias temperature results, make sure to account for the different temperature conditions that exist in the pile. In addition, the sanitary through final compost should be monitored using the sampling recommendations presented in 2-2 of this document. Therefore, no

compost will leave the facility without first undergoing at least two rounds of testing.

Compost must be allowed to stabilize before use. Fresh compost has been shown to support regrowth of pathogens (Russ and Yanko 1981; De Bertoldi et al. 1991, Christensen et al. 2002). The ability to support regrowth of pathogens is not acceptable for the Presidio.

Use sentinel plants i.e., plants that are very susceptible to *Phytophthora* along the edge of composting facility site for monitoring purposes. Plant sentinel plants where they will be near yard but need little maintenance. In regular intervals, once the plants are established, incorporate these plants into the compost-monitoring schedule. The intention of using sentinel plants is to monitor the landscape that may influence the final stages of the compost. Keep this in mind when considering sentinel plant location.

Prevent soil/debris on the loader from bringing *Phytophthora* to the compost yard and contaminating the final stages of compost (sanitary phase forward). If the loader is used outside of the compost yard it should be washed and inspected for soil and debris prior to re-entering the yard. Tires, undercarriage and loading bucket and any other part of the vehicle that will come into contact with the final stages of compost should be thoroughly cleaned. In general, the equipment should be well maintained, which includes keeping heavy machinery reasonably free of dirt and debris between uses. Compost from the sanitary stage forward should

only be moved with a clean loader. Wash the loader in the designated wash station prior to moving compost from the sanitary stage forward.

In the case that the compost test positive for *Phytophthora* or *Pythium*, it will be necessary to compost again. Another option would be to pasteurize the material following composting (see next section). In the case that sentinel plants test positive, it will be necessary to pasteurize the material before use.

It is important that the completed compost does not become contaminated. Finished composted should be stored away from contamination threats. It can be bagged, and stored in an enclosed container. Completed compost should be transported, in a clean and enclosed container.

## Treating Imported and Local Planting Materials: soil, mulch, and compost

### Key Points:

- ✓ **Planting materials considered herein include soil, plant based mulch, and compost.**
- ✓ **Planting materials originating within the Presidio that are to be transported should be treated using pasteurization techniques prior to use.**
- ✓ **Imported planting materials originating outside the Presidio should be treated using pasteurization techniques prior to use.**

Currently planting materials locally within the Presidio that are to be transported to a new location and imported planting materials from outside the Presidio including: soil, plant based mulch, and compost, should be treated using pasteurization techniques. Locally, within the Presidio, it is known that there are areas that contain infestations of *Phytophthora* as highlighted in the “*Introduction to the Issue*” under the section “*Phytophthora Issues in the Golden Gate Natural Recreation Area and the Presidio*” starting on page 15 of this document.

Therefore, it is important to pasteurize the planting materials that are to be moved to a new location in order to prevent moving *Phytophthora*, and potentially other pathogens around. There are exceptions, if the starting material is considered pathogen free (for example after the development of the much anticipated

certification program) and has been transported and stored in a manner that keeps it protected from contaminants, such as in an enclosed, clean and undamaged container, there is no reason to apply additional treatments to this material. In addition, following the “*Presidio Compost Yard Plan*”, the finished and stable Presidio compost that tests negative for *Phytophthora* and *Pythium*, transported and stored properly, would be a source that should not require further treatment further treatment.

There are methods that have been developed and adapted locally that could be used to steam pasteurize planting materials and help to handle the issue. SF Rec and Park (GG Park Nursery) has developed a method for pasteurizing bulk planting materials such as compost. It is done by filling a dump truck bed or detachable trailer full of soil. The truck bed/trailer is fitted with pipes for pumping steam into the soil. Then the pipes are attached to a steam generator that steams heats the soil for the necessary time. The truck bed is covered during the steaming process to avoid heat loss. The temperature can be measured several times in several locations during and following the heating process to ensure adequate heating. A similar system should be developed for use in the Presidio.

## Treatments for Sites

### Key Points:

- ✓ **Treatments for sites are in development.**
- ✓ **Treatments will work best if cultural controls are also used especially managing irrigation.**
- ✓ **Mulching if properly applied and maintained can help to reduce the spread of disease causing organisms in some cases.**
- ✓ **Disease can be avoided by using plant genera that are non-hosts, and/or potentially plants with known resistance to disease.**
- ✓ **Trials can be used to establish, where in the Presidio the solarization, and/or other treatment methods may be feasible.**

This part of the document covers potential site treatment options as well as some cultural controls for sites. If a site has tested positive for *Phytophthora* and it is considered worthwhile to attempt field treatments herein are some potential options. Treatments should not occur when the pathogen is actively spreading (usually this is during wet periods or following heavy watering) because moving soil and equipment during this time may unintentionally lead to additional spread. Avoid additional spread by treating outside of the rainy season and not attempting to treat in muddy or wet conditions. Please reference the TRC memo dated

August 31, 2015, (or the most current document available) for potential treatments for pasteurizing soil in fields:

<https://drive.google.com/open?id=0BzPGsfCRAg-8VUluSFIFa1NhXzQ0Sy1LclFwTmo1Q3VOZfV3>

*Pasteurizing soil in fields.* If an area is thought to have a relatively confined or new outbreak, then it may be feasible to try pasteurizing soil in those areas. This type of method is currently under consideration for use at MacArthur Meadow, which was contaminated with *Phytophthora cinnamomi*. See the above attached document for details.

Of the methods described in the attached document, “Alternative treatment 3” was considered the best because one it uses steam heat, and dry heat may require considerably higher temperatures to achieve the same results and for improving soil. The second reason is that, in the process of moving the soil, large chunks are broken down in “Alternative treatment 3” making the soil medium more uniform. It is important to ensure that all of the soil reaches the appropriate temperature and the fact that the soil becomes more homogeneous in the process should improve even heat distribution. An excerpt from the method:

“Ex-situ Steam/Hot Air Treatment using Engineered Soil Piles. This treatment alternative involves constructing engineered soil piles, which would be designed similar to bio piles or compost piles. The engineered soil piles would be constructed in “lifts”, where soil would be mounded to a depth

of no more than a few feet. Slotted or perforated piping would be installed in the soil piles. Steam and/or hot air would be generated onsite using a mobile unit and would be injected into the pipe network placed within the soil pile. To minimize heat loss, a tarpaulin cover or other insulating material would be used to cover the soil pile. If steam were used, soils would likely need to dry prior to reuse. For this approach, it is anticipated that the void space between soil particles in the soil pile would be larger and more homogeneous than an in-situ alternative, thereby decreasing the likelihood of insufficient and non-uniform heating. The design parameters of the engineered soil piles (e.g., pipe spacing, pile size, energy requirements) would be modeled after bio piles or compost piles; however, a robust, site-specific pilot study would need to be performed in order to confirm the efficacy of this approach and refine system design elements. Although there are too many unknowns currently to estimate treatment costs for this alternative prior to pilot testing, this alternative is considered feasible for stockpiled soil from MacArthur Meadow and/or other Presidio sites”.

The other treatment method that may also be acceptable was alternative treatment 1. This method may work especially if the soil can remain moist during the procedure and achieve the heat levels described. This method used the On-Site Evaporative Desorption Unit (EDU). This method was the most well developed of the treatments outlined. However, dry heat is usually not used. For steam heat, the target temperature should be set to 140 °F in order to target an increasingly broad spectrum of pathogenic organisms and pests besides *Phytophthora*. However, it is unclear that dry heat at this temperature would achieve the same or necessary result, but may work if the soil is moistened for the process.

*Controlling irrigation-* Appropriate irrigation is extremely important for managing plant health and reducing the risk of developing root rot from adjacent or nearby diseased areas/within areas containing low levels of the pathogen. It will be necessary to replace irrigation emitters and faulty systems to avoid waterlogging the soil. Do not water soil that is already wet because waterlogged soil can accelerate disease development, and do not place emitters so that water sprays directly onto tree stems or plant foliage. Schedule irrigation based on local evapotranspiration or by installing soil moisture monitoring tensiometers. Good irrigation management is especially important when used to reduce the risk of spreading disease to trees near the margins of root-rot diseased areas in order to reduce the risk of spreading disease into these areas and slow the spread if it is present.

*Mulching-* Mulching if properly applied and maintained can help to reduce the spread of disease causing organisms in some cases. Mulches have the added benefit of reducing the amount of water used (decreasing the chance of splash dispersal), and mulch has been shown to reduce some forms of disease transmission of root-rotting *Phytophthora* species. Although mulching itself generally will not eliminate the chances of disease development if the pathogen is already there, it can still be useful. For example, if the pathogen is in a nearby location but not at the site of concern, mulch can slow the spread because it has

been shown that active spore dispersal is reduced when mulch is utilized. Mulches conserve soil moisture by reducing the amount of evaporation that occurs from the soil's surface, and by increasing the water holding capacity of some soils. Therefore, to properly maintain a mulched area it is important to reduce the amount of water used because diseases favored by wet soil conditions, such as those caused by *Phytophthora* can be exacerbated if mulched soils are overwatered or watered at the same levels as they were before mulching. If mulches are improperly applied then they can introduce pathogens for example if they are contaminated or if they are applied directly to the tree base. However, disease control has been achieved if mulches are applied properly (kept away from the bases of trees near the root crown and stem), irrigation is appropriate (reduced based on lower water needs), and the source of the mulch is pathogen free or that mulch has been properly treated.

*Treating mulches made of plant material.* Mulch made from plant materials should be from a certified clean source, or steam pasteurized. The temperature that is needed to inactivate pathogens will vary based on the composition of the mulch and the ability of the heat to reach the cooler areas of the mulch pile. However, whatever the mulch type is, making sure the cooler parts of the pile reach 140° F for one hour is a general guideline and a good starting point that can be optimized with further use once the mulch types are chosen. Although it is true that

*Phytophthora* has been shown to be killed at slightly lower temperatures this temperature can help to manage other common pathogens as well. Mulch that has been stored in a contaminated area needs to be decontaminated prior to use. Clean mulches (free of disease propagules), properly applied, can help to suppress root disease development, and reduces the amount of watering necessary.

To apply mulch, first, fix irrigation system problems in locations within and near contaminated areas, then apply clean mulch away from the stems and root collars of trees and established plants. Monitor the level of water needed after installing mulch, based on local evapotranspiration, or by installing soil moisture monitoring tensiometers. A useful guideline for how to use tensiometers is provided here: <http://ucanr.edu/files/47946.pdf>. After applying mulch, schedule irrigation frequency based on the results of monitoring.

*Avoidance.* Disease can be avoided by using plant genera that are non-hosts and/or plants with known resistance to disease. If a plant is not susceptible to a pathogen then it cannot develop disease. One approach to treating an area that is contaminated with *Phytophthora* is to use non-host plant species. Many monocots and grasses are not hosts of *Phytophthora*, but the species-host combination can be evaluated for the site of concern. Avoidance may be especially useful when dealing with *Phytophthora* species with a limited host list.

A technique for planting in areas containing *Phytophthora* with a wide host range or with highly desirable plant species is to use resistant plant species to avoid disease development. In some cases, there are resistant plant types, for example, several western Australian native plants have been found that are resistant to *Phytophthora cinnamomi*:

[http://www.cpsm-phytophthora.org/downloads/natives\\_resistant.pdf](http://www.cpsm-phytophthora.org/downloads/natives_resistant.pdf).

*Phytophthora cinnamomi* resistant non-native plants may be a possibility for planting in the Presidio, if they are from certified clean stock and are planted into areas where non-native plants are considered acceptable, such as in the Presidio Landscape Zone, and in locations where *Phytophthora cinnamomi* is already established.

*Solarization*. Trials can be used to establish, where in the Presidio the solarization method is a feasible option. The Presidio is within the 'fog-belt', which is not usually considered an area where solarization is a viable option; however, there may be micro-locations that are hot enough. The best time to attempt solarization is during the warmest months of the year- usually July, August and September in the Presidio. The depth of soil that needs to be heated to optimal depths for high temperatures ranges from two to 12 inches below the soil surface. If an area herein is being considered for solarization, heat it to 12 inches. An appropriate site is one that has been demonstrated that high enough temperatures are reached at

that site. Next, remove the plants, including the root systems and weeds from the site. Sites that already are without plants may be easier to solarize (new construction sites, etc.). Solarization should be used in open spaces without a lot of vegetation.

First, create a pile of the soil to be solarized. Second, lay down an impermeable layer, add a windrow of soil, moisten it, and cover with clear anti-condensation plastic film. The film should be thick enough that it cannot be punctured during the solarization process. Run drip irrigation underneath the anti-condensation plastic. Maintaining soil moisture is a very important factor in soil solarization. Seal the edges with sand worms or gravel. Do not allow water to collect on the upper surface of the plastic. Put a data logger in the pile one near the surface two inches below, and one near the bottom center. If necessary, turn the pile during the process to expose lower soil layers to higher temperatures. It may take 6-8 weeks to achieve high enough temperatures in the fog belt, even in microclimate areas where it has been shown to be warm enough. During soil solarization, temperatures can reach 95°F to 140°F (35-60°C) depending on factors such as soil type, season, location, and soil depth. High temperatures induce changes in soil volatile compounds that are toxic to organisms already weakened by high temperature. Soil solarization is effective against many fungal root disease causing pathogens including *Verticillium*, *Fusarium*, *Pythium*, and *Phytophthora*. It can also help control bacterial pathogens and reduce soil populations of different plant parasitic nematodes. The longer the soil is heated,

the better and deeper the control of all soil pathogens. Thus, long, hot, and sunny days work best to kill soil borne pathogens. *Phytophthora ramorum* has been shown to be killed following 30 minutes of exposure to 122 °F by Oregon State University Researchers. Other researchers at UC Davis have shown that it was killed by three days at 104 °F. These types of experiments thus far with solarization have been completed in well-controlled environments that are typical of nursery systems and cropping systems, higher temperatures for longer periods are suggested here for less controlled environments where even temperature distribution is likely to be a very real issue.

Here are five links to helpful information on soil solarization:

(1) <http://www.ext.colostate.edu/pubs/crops/00505.html>

(2)

[http://www.apsnet.org/publications/plantdisease/backissues/Documents/1991Articles/PlantDisease75n11\\_1160.PDF](http://www.apsnet.org/publications/plantdisease/backissues/Documents/1991Articles/PlantDisease75n11_1160.PDF)

(3) <https://edis.ifas.ufl.edu/in824>

(4)

[http://c.ymcdn.com/sites/www.oan.org/resource/resmgr/imported/digger2/Digger\\_201306\\_pp33-36\\_OSU.pdf](http://c.ymcdn.com/sites/www.oan.org/resource/resmgr/imported/digger2/Digger_201306_pp33-36_OSU.pdf)

(5) <http://www.ipm.ucdavis.edu/PMG/PESTNOTES/pn74145.html>



## Treatments for Individual High Value Trees in the Presidio Landscape Zone

### Key Points:

- ✓ **Individual, uninfected high value trees near contaminated areas may be treated with a phosphonate injection treatment prior to infection in order to help keep them from becoming infected.**
- ✓ **Injections may be beneficial if not phytotoxic.**
- ✓ **Mulching can be used together with phosphonate injection to slow the spread of pathogens into the treated area from neighboring areas that are contaminated.**

Individual, high value trees adjacent to or are amidst a contaminated area may be treated with a phosphonate injection treatment prior to infection in order to help keep them from becoming infected. These types of trees will most likely occur in the Presidio Landscape Zone. Phosphonates cannot eradicate *Phytophthora* from an area, and ongoing management is required throughout the life of the treated trees. Some plants suffer from the phosphonate injection itself and it may be necessary to test plants prior to treatment if no information is available regarding the phytotoxic reaction of the plant species. Follow the applicator instructions and dose guidelines. If the trees are treated as the plants are beginning to leaf out, the phosphonates will be translocated into leaves, which will be ineffective in

controlling root rot. In general it is best to treat after trees have leafed out in the early summer, or in early fall prior to rain to avoid spreading disease-causing propagules. Understanding a trees physiology will be important to know when to treat. Treat them during periods of active root growth to ensure that the fungicides are translocated to the roots (for root rot control). When the best time is will vary by plant species based on the plant species phenology within the area that plants are located. Mulching can be used together with phosphonate injection to slow the spread of pathogens into the treated area from neighboring areas that are contaminated. Best outcomes for treatment of sudden oak death (SOD) are in susceptible trees treated prophylactically. When treating for SOD it has been shown that it is best to treat in fall to minimize the risk of spreading the infection, and making sure to disinfect drill bits and injection needles between trees.

General guidelines on how to inject a phosphonate treatment into *Quercus agrifolia* for sudden oak death are given in the following presentation:

<http://nature.berkeley.edu/garbelottowp/wp-content/uploads/PhosphonateApplicationv5.pdf>

Videos are also available to learn how to inject trees:

[http://arborjet.com/how\\_to\\_use/training\\_videos/](http://arborjet.com/how_to_use/training_videos/)

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Baker, K. F., ed., 1957. The U.C. system for producing healthy container-

grown plants. Manual 23. Calif. Agri. Expt. Sta. Ext. Service. 332 pp.

Parke, J. Managing Phytophthora

<http://www.oregon.gov/ODA/shared/Documents/Publications/NurseryChristmasTree/ManagingPhytophthora.pdf>

Parke, J. Lewis, C. Protecting Container Grown Plants. Digger 2011.

Washington Organic Recycling Council, R., 2009. Best management practices: guidelines for pathogen control at organic material processing facilities.

[www.compostwashington.org](http://www.compostwashington.org).

*Koike S., Wilen CA. UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries;*

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Robinson, D.W. 1988. Mulches and herbicides in ornamental plantings.

*HortScience* 23:547–552.

# **Glossary**



**Chemical disinfectant**— a chemical with germicidal action used to aid in removing contaminating microorganisms from surfaces.

**Composite sample**— a combined sample, taken from several plants (in this case 25 plants, see Table 2) of the same plant lot (and plant species). All of the material for a composite sample is placed in one single bag and labeled appropriately.

**Disease**—an abnormal condition resulting in a disorder of structure or function of an organism that develops after some period. May affect one or more, or all of an organism. In many cases, disease can be interpreted as a condition associated with particular symptoms and (with biotic diseases) signs.

**Disinfect (plant pathology)**—to eliminate a pathogen from infected plant tissues. (<http://www.apsnet.org/edcenter/illglossary/Pages/A-D.aspx>)

**Disinfestation process**—kills or removes pathogens or other contaminating microorganisms that have not yet initiated disease, and are found on surfaces or in growing media. The *process* indicates there is more than one-step, for example, cleaning and then use of a chemical disinfectant, or cleaning and then heating.

**Disinfest**— to kill or remove pathogens that have not yet initiated disease, or other contaminating microorganisms, that occur on inanimate objects such as: tools, benches, floors, containers, on the surface of plant parts such as the seed surface, or in soil.

**Mycelium**— a mass of filamentous material (hyphae) that composes the ‘body’ of the fungus or fungal-like colony.

**Oospore**— the sexually produced resting spore of oomycetes (*Phytophthora* is a type of oomycete).

**Pasteurization**— partial sterilization of a substance at a temperature and for a period of exposure that destroys objectionable organisms, such as pathogens, weed seed, and/or insect pests, without radically changing the quality of the substance that is being pasteurized. The time and temperature that is used will depend on the substance that is pasteurized, the objectionable organism that is being targeted, and the type of heat that is used. *Compare with sterilization.*

***Phytophthora de Bary***— a cosmopolitan genus of important plant pathogens first described in 1876.

**Phytophthora-type symptoms**—Advanced symptoms: whole plant or leaf blight, root rot, serious stem necrosis, bleeding lesions, root collar and/or stem canker, wilting or the appearance of water stress. Minor symptoms: leaf tip dieback, small water soaked lesions and/or necrotic flecking on roots. Symptoms are often general and diagnostic testing is usually necessary.

**Plant lot**—the plants of one plant species that were propagated at the same time and at the same nursery, may be referred to as a crop.

**Scoopula**— an elongated stainless steel spoon-like spatula tool usually used to measure out chemical substances, used here to remove potting medium from along the edge of a containerized nursery plant below the surface while minimizing damage to the root system.

**Sign (plant pathology)** — the physical presence of the pathogen itself from the host. Signs and symptoms are used to help diagnose disease.

**Soil**— used loosely in this document to mean the potting material, soil, potting soil, soilless potting material, or compost.

**Spore (oomycetes)** — a minute reproductive unit produced as the result of a sexual or an asexual process that can give rise to an individual. Some types of spores are resistant to adverse conditions.

**Sporangium**— a specialized organ surrounding endogenously produced spores. In *Phytophthora*, the cytoplasm within the sporangium cleaves to produce several motile spores called zoospores.

**Sterilization**— the removal or complete destruction of all pathogens and other microorganisms by treatment with heat, chemistry, or other means. Sterilization can usually be accomplished with wet steam under pressure at 121 °C (250 °F) for 20 minutes, but times and temperature may vary depending on the characteristics of the object being sterilized or how it is contained. Dry heat is another means of sterilizing, but the temperature and times used may drastically differ from wet steam under pressure. *Compare with pasteurization.*

**Symptom**—the resulting physical response of the host to the pathogen once disease has developed.

**Zoospore**— a motile spore that has flagella.

## **Appendix 0. *Phytophthora* 101**

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The genus *Phytophthora* contains some of the most destructive pathogens of forest trees, and plants especially woody species in natural areas. Over the past two years in California, awareness of the issue has increased drastically and business as usual for restoration is now considered unsustainable for creating healthy ecosystems.

*Phytophthora* species are plant pathogens, which are organisms that cause disease in plants (Agrios 2005). The Latin name *Phytophthora* means plant destroyer for good reason, as a species within the genus can cause a wide range of diseases on plants from annual vegetables, to seedlings, ornamentals, established forest trees, and other woody perennials (Erwin and Ribeiro 1996). Yet these organisms remain somewhat elusive, and the native origin of only a very few species is known for the genus *Phytophthora*.

Forests and natural areas have evolved with many herbivorous organisms. However when conditions change, such as through the movement of contaminated plants or trees into new environments; change in the environment itself, or management that results in new host/ pathogen combinations including new variants of a pathogen being moved in where the species already is, these changes can lead to detrimental outcomes and unwanted landscape changes. *Phytophthora* species are plant pathogens that decrease the success of planted natural areas and are capable of infesting nurseries (Parke et al. 2014). They can cause surprising damage by moving from plant production materials and from

infested material, such as nursery stock, to pristine forests and woodland areas (Jung and Blaschke 2004, Goss et al. 2009). When more species, different species, or more pathogenically virulent strains, or isolates are brought into natural areas with infested material it can vastly increase problems with attempting to restore the area.

The plant parasite *Phytophthora* like other pathogens infects plants by becoming intimately associated with them, then multiplying and growing at the expense of the plant (Agrios 2005). Being in a relationship with *Phytophthora* is a cost to the infected plant's health over time. Symptoms develop due to the removal of nutrients and water from plant growth as the plant instead produces a suite of chemicals in attempts to defend itself. Symptoms can also result from enzymes that the pathogen produces to break down plant cells. As plants become weakened they may look like they are starving or that they have suffered from drought issues or other horticultural problems but are, in fact, diseased.

Phytophthora disease is a type of infectious plant disease. An infectious plant disease is one that is caused by a biotic (living) organism within a plant. The pathogen in many cases reproduce on the infected plant and then spread to another in order to cause disease there. The infectious disease initiates on contact with a susceptible part of the plant, and when the infection process is successful, it is followed by the pathogen growing and multiplying on the diseased plant, then spreading from diseased to healthy plants. This results in small to very large

epidemics, depending on pathogen virulence, environmental factors, host availability, and host susceptibility.

In the case of *Phytophthora* disease, a plant becomes infected when it is attacked by the pathogen. Both the pathogen and the plant must occur together at the same time in the same location and must interact with one another. If the conditions are too extreme at the time the pathogen encounters the plant for the pathogen to grow or reproduce, it may not be able to attack the plant and so can be present without the development of disease (or the plant may simply be able to resist the attack). As the environment changes the conditions for the pathogen, also change, with new opportunities for disease development possibly arising again later.

Infectious disease results from a series of events that occur in succession and start with inoculation (Agrios 2005). The first event in disease is inoculation, which is where the pathogen is allowed to contact the plant in a location where infection is possible. If zoospores (swimming spores of *Phytophthora*) are the inoculated material, then inoculation is followed by encystment, and then germination. During the encystment process, the zoospore loses its flagella (used for movement), forms a thickened outer wall and excretes an adhesive material to secure the encysted spore to the plant root (Sing and Bartnicki-Garcia 1975). Zoospores can do this and germinate without an external food supply because they contain stored energy supplies similar to plant seeds. After the cyst is attached to the root, it can germinate. Many *Phytophthora* species cause root and

stem rots, so it is necessary for the pathogens to be exposed to the stem or the root of the plant for disease development to initiate. Keeping *Phytophthora* out of soil and off containers can reduce the chance the pathogen will be exposed to the roots and stems of your plants that you are trying to keep healthy, and reduces the chance that infectious disease will occur. Likewise, in a foliar *Phytophthora* it would be important to keep the pathogen away from foliage tissue.

### ***So what is the risk?***

There is empirical evidence that Northern California in particular is vulnerable to disease epidemics caused by *Phytophthora* species. Out of four of the most devastating *Phytophthora* species in natural ecosystems around the world: *ramorum*, *lateralis*, *cinnamomi*, and *alni* subsp. *alni*, Northern California has suffered from the first three listed. *P. ramorum* is an invasive species that was introduced and became established in Northern California and southern Oregon; it has devastated *Notholithocarpus densiflorus* and *Quercus agrifolia* in many locations throughout the region. Once introduced it may be possible to slow the spread but only at great expense (management costs), and all attempts at eradication have been futile. *P. cinnamomi* had its worst effects in Western Australia but has caused serious epidemic issues in southern Europe from Spain across much of the Mediterranean region, and in California, one of the issues is that it has devastated the rare Lone Manzanita in Lone California.

The risk of disease developing into an epidemic at any given site is the probability that a certain intensity of incidence or severity of a particular disease will be reached. Numerous host, pathogen and environmental factors must be accounted for in the assessment of risk for particular plant diseases at a particular site. If there is concern regarding disease, development at a particular site several factors should be considered. These factors include the history of the site, for example, the site was the location of a greenhouse or nursery facility in the past, as well as drainage and irrigation conditions. The relationship between the host and pathogen that are under evaluation should also be known, and accounted for. In addition, the presence of inoculum already on the site, and prevailing weather conditions during periods of susceptibility for each *Phytophthora* species, and plant host species present on site. Answer questions like: Does the site have poor/inadequate drainage and/or irrigation? Is there the high potential of vector contamination for the particular site of interest? What was the plant source (in planted areas)? Where did the materials that make up the site come from? What is the history of disease development, and in which plant species? Knowing all of these factors for a site can help to determine whether or not a site is appropriate for planting, and what the relative risk that a disease epidemic will occur at the particular site is.

### **What are the sources of *Phytophthora* infestation?**

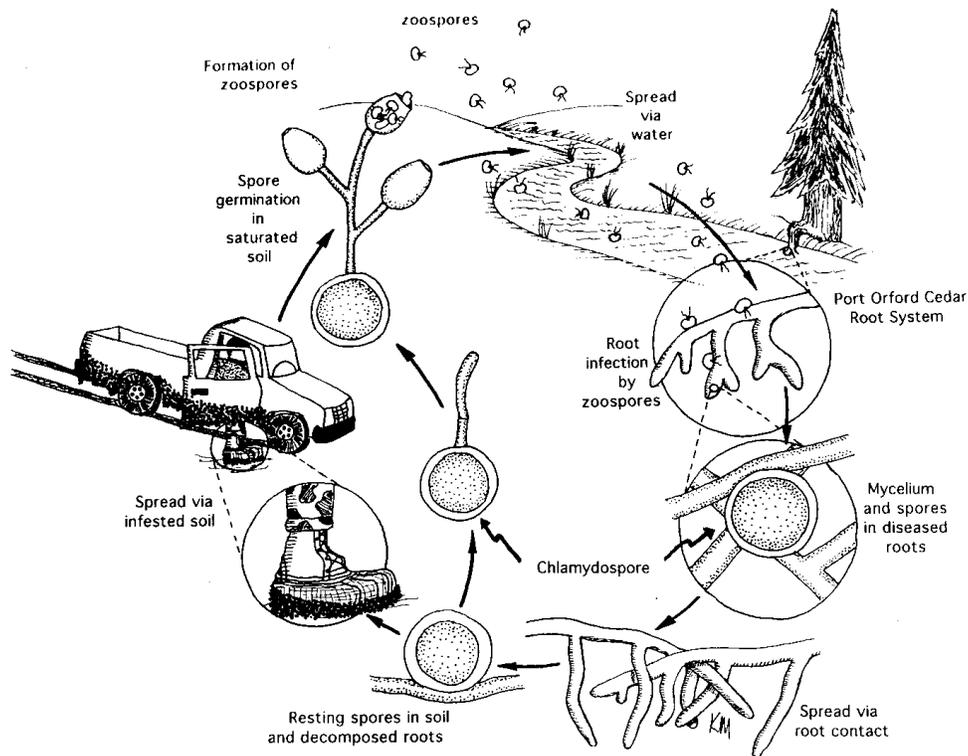
The most important movement of *Phytophthora* from place to place in the Presidio is suspected to be on infested plant stock. Other very important sources include

bringing in contaminated soil and gravel (or moving contaminated soil around within the Presidio), sharing dirty pots, allowing plants to stay in a nursery for a longer time than takes to establish them for the out-planting (which increases their likelihood of exposure and of becoming a source). People with dirty clothes, and especially shoes, containing soil and plant debris and their vehicles (including heavy equipment) can move inoculum sources around, including into the nursery and into field sites and natural locations.

### **How does it move?**

Once *Phytophthora* is in a location it can move passively around via the flow of water, and can actively swim in particles and small streams of water in the soil.

Some species that have detachable propagules called dehiscent sporangia can move in air currents. Once a root-rotting *Phytophthora* has infected the plant, root-to-root contact is important for spread. For a specific disease in Argentina, there is good evidence that cattle are important in the *Phytophthora* disease Mal del ciprés. A specific example of how a *Phytophthora* species can move around once it is introduced into a natural landscape is given in the lifecycle diagram below. It is important to remember that the Genus *Phytophthora* is composed of over 100 different species and each of these may vary in how they are dispersed, move around, and establish infection. For most species of *Phytophthora*, the spread pathways are not well documented.



**Lifecycle Diagram from:** Managing Port-Orford-cedar and the Introduced Pathogen *Phytophthora lateralis*. Everett M. Hansen, Donald J. Goheen, Erik S. Jules, and Barbara Ullian. Plant Disease 2000 84:1, 4-14

Some pathogens such as viruses and fastidious bacteria are injected directly into their host by insects that move from an infected plant to an uninfected plant. As the insect feeds, it injects the pathogen into the host tissue immediately surrounding the pathogen with host tissue. However, *Phytophthora* diseases are only very rarely suggested as being transmitted by insects and so in general, they are probably moving to host tissue by other means. A few insect vectors that should be controlled in greenhouse situations for oomycete spread are fungus

gnats, shore flies and snails. Both fungus gnats and shore flies are not usually a problem except in cases where plants are over watered, or water is allowed to accumulate. These insect pests can move fungal spores from one container to another. It has been documented for the movement of *Pythium* species. It should be noted the same conditions (high moisture) that are the best conditions for promoting *Phytophthora* growth initially are those that would be good for the nursery pests such as fungus gnats, whether or not *Phytophthora* is moved in this way, these conditions should be avoided. *Phytophthora* propagules (parts of the fungi that can move to another area) can move through air currents, soil and water to a host-- examples of which are sporangia and zoospores.

It is important to keep in mind that *Phytophthora* does not necessarily move around like other pathogens. *Phytophthora* species in general are not moved around by insects like many other pathogens are, and the few possible ways they could be moved by insects were mentioned earlier. However, having additional plant health issues from insect-vectored viruses, bacteria, fungal pathogens and the insects themselves exasperates problems and makes disease diagnosis much more complex. Beyond *Phytophthora*, it is very important to limit and control insects that damage plants and/or vector disease, and weeds that harbor insects and pathogens. There has not been convincing evidence that small animals move *Phytophthora* from one area to another, although this area of research is ongoing.

Water can move *Phytophthora* from one area to another. There is some evidence that suggests this is more important for some species than it is for

others. A great example where it is important is in the movement of *Phytophthora alni* from one riparian location to another by stream flow, followed by flooding events, which moves the pathogen into the root systems of susceptible *Alnus* trees in nearby riparian habitats that were previously uninfected before the flooding event. The alder *Phytophthora* disease system in Europe is one that resulted from attempts to restore stream banks in Europe with nursery grown *Alnus* species. This resulted in widespread dieback of *Alnus* in southern Britain especially (Brasier et al. 1995, Gibbs et al. 1995, Gibbs 1999). It then caused disease in other areas of Europe (Cech 1998, Streito et al. 2002, Streito 2003, Webber et al. 2004, Jung and Blaschke 2004). It causes a serious lethal root and collar disease especially of *Alnus glutinosa* (Brasier 2003) but also *Alnus incana*.

Some plant species act as carriers, but do not suffer a physiological disturbance (disease). An example is *Umbellularia californica*, which is a carrier of *P. ramorum*, but is not killed or physiologically damaged by the pathogen (DiLeo et al. 2009). The *U. californica* acts as a carrier/source of inoculum because during conducive times spores are produced prolifically on the leaf surface. The spores on the leaves can then be transmitted to susceptible hosts such as *Quercus agrifolia*. The pathogen can move short distances in rain splash and wind to the nearby susceptible *Quercus* species. The pathogen occasionally can move longer distances from *U. californica* to oak in turbulent airflow during severe weather events, and in air currents along river channels in coastal areas where severe air currents prevail.

## ***Phytophthora* species currently of concern to native plant nurseries and restorations in California**

The genus *Phytophthora* is very large and contains nearly 130 different plant pathogen species. Genetically the species are grouped into ten clades with evolutionary and practical significance. A limited number of these, from six clades (1, 2, 4, 6, 7, and 8), have shown up in recent surveys and/or have impacts in natural areas in California already and of course as we study and research, the list will grow. Many Clade 1 species have contributed to major problem in nurseries, agriculture and planted landscapes, of these, two have shown up in recent surveys and have been known in California for some time *P. cactorum* and *P. nicotianae*, and two other from this clade have only recently been documented in California: *P. hedraiandra* and *P. tentaculata*. Clade 2 species contain some mainly canker causing pathogens of woody hosts that show up in nursery and irrigation systems around the world: *P. citricola*, *P. plurivora*, and *P. multivora*; they have shown up in recent surveys in California. The later two were only described in the last ten years and prior to that were most likely grouped with the former listed *P. citricola*. Clade 6 species, which are often associated with riparian ecosystems in forests, are occasionally found acting as saprophytes near disease centers, and occasionally found killing trees, even away from riparian areas. Two of the species that have shown up in recent surveys *P. gonapodyides* and *P. chlamydospora* can cause root disease in *Alnus rubra* in western Oregon. Outside of riparian ecosystems, these are generally of minor concern but one species from

this group was the causal agent of a serious epidemic of pines in Chile:

*Phytophthora pinifolia*. This is the first *Phytophthora* known to be associated with needles and shoots of a *Pinus* sp., and its aerial habit is unique in clade 6, it has not been found in California, but it is important to keep in mind this group may seem relatively harmless but has the potential to be very destructive. The second to last clade of interest here, clade 7 contains three species of concern *P.*

*cambivora*, *P. cinnamomi*, and *P. niederhauserii*. The first two cause serious forest and natural area diseases around the world, and show up in nurseries around the world. The later *P. niederhauserii* was only recently described (Abad et al. 2014) but in its description several authors from around the world came together because it has been found causing nursery disease around the world. The last clade of species of recent concern in California is clade 8. This clade contains the notorious *P. ramorum* causal agent of sudden oak death as well as three other species that have come up in recent surveys: *P. cryptogea*, *P. dreschleri*, and *P. lateralis*. In the following paragraphs is a more detailed description of the *Phytophthora* species currently of concern to native plant nurseries and restorations in California. This list is likely to grow quickly, as research in this area is currently being conducted.

A research study needs to be done in California to help understand the ecology of these species already in planted and natural systems. The pathogenicity of each species depends on the hosts they are found on, and since we are currently putting together the lists of host/pathogen associations, we do not

know the impacts they will have. Still many characteristics about each are known and so are grouped and described below. The descriptions are broken down by clade, for some clades host amplitude (> 10 = broad, or < 10 = narrow), and species.

*Phytophthora* Clade 1. Broad host range-

***Phytophthora cactorum***. Documented cases of *Phytophthora cactorum* go back to 1870, under the synonym *Peronospora cactorum*. It was first reported from rotting cactus plants (*Cereus giganteus*) in central Eastern Europe. *P. cactorum* occurs worldwide but is most common in regions with temperate climates. *P. cactorum* parasitizes over 200 different plant species across many different plant families, from weeds to full-grown trees. In California, it causes very serious aggressive diseases of strawberry and nut crops such as almond, agricultural systems are more studied with *P. cactorum* than natural setting studies. There can be a quite a bit of pathogenic variability in different individuals (isolates) of *P. cactorum* in California, with some very aggressive to certain hosts and others less aggressive (Bhat et al. 2006). It is a real problem for woody plant species in particular, and is also quite pervasive and may exist for a long time without obvious symptoms. Well-managed landscapes that contain *P. cactorum* may not exhibit disease (Garbelotto personal communication regarding *Quercus* species on the UC Berkeley campus infected with *P. cactorum*). Although common *P. cactorum* is known as a troublesome root-rotting pathogen of woody plants around the world (Erwin and Ribeiro 1996). Very recent findings suggest that it may in fact

be hybridizing with a closely related species in California (Sims unpublished); it is unknown what the impacts these hybrids will have. It has most recently found associated with several native plant nurseries and restorations sites from plant species with disease including *Ceanothus ferrisiae* and *Ceanothus thyrisflorus*, *Heteromeles arbutifolia*, *Diplacus (Mimulus) aurantiacus*, *Arbutus menziesii*, *Actostaphylos* species, *Frangula californica*, *Lonicera hispidula*, and *Quercus agrifolia*. It is most likely to be the major causal agent of disease for at-least some of the above species.

***Phytophthora nicotianae***. First described from tobacco plants in 1896, this species until recently was not reported from forest at all, it is synonymous with *P. parasitica*. This is a common and serious pathogen in the nursery and agricultural trades and has very recently begun to be recovered from forest surveys, but thus far, recovery from forests and natural areas has been ephemeral. It has most recently been recovered in California from native plant nurseries and restorations from sticky monkey flower (*Diplacus (syn. Mimulus) aurantiacus*), and arroyo willow (*Salix lasiolepis*). This species can hybridize with *P. cactorum* and the hybrids are also being recovered from recent surveys in California from arroyo willow (*Salix lasiolepis*) and toyon (*H. arbutifolia*).

***Phytophthora hedraiandra***. First described in 2004 from isolates collected from *Viburnum* species leaf spots in the Netherlands, at the time, nothing was known about geographic distribution or pathogenicity. It is genetically and morphologically very similar to *Phytophthora cactorum* but with enough differences to be

considered its own species. Since its description in 2004, it has been described on *Viburnum* in Spain and Italy, and from *Rhododendron* in the United States. In 2015 in California *P. hedraiandra* was recovered from toyon *Heteromeles arbutifolia* and *Frangula californica*. In the recent past has been recovered from *Arctostaphylos* species grown in nurseries.

***Phytophthora tentaculata***. This species was described in 1993 in Germany after being recovered from greenhouse ornamental plants: *Delphinium* species, *Chrysanthemum* hybrid species and *Verbena* hybrid species. At the time it was described the pathogenicity was not evaluated. It was later reported in Europe and China causing crown, root, and stock rot of ornamental in nurseries China and Europe. *P. tentaculata* was first discovered in the United States in California from sticky monkey flower (*Diplacus* (*Mimulus*) *aurantiacus*) in California and was reported in 2014. It has also been recovered in California from toyon (*Heteromeles arbutifolia*) and *Salvia* species.

#### Clade 2.

***Phytophthora citricola***. This species was first isolated from orange fruit with brown rot symptoms in Taiwan in 1927. This species was found to be a species complex with the advent of molecular techniques and the closely related species are difficult to differentiated based on morphology alone. Before molecular techniques this 'species' was considered to have a very broad host range. In the loosest sense, this species causes very similar symptoms to *P. cinnamomi*, but generally somewhat less severe, and has a wide host range. In the strictest sense,

much of the disease that was previously attributed to *P. citricola* actually belongs to other species: *P. plurivora*, *P. pini*, and *P. multivora* are a few. Much of this has yet to be sorted out. At-least in Europe, much of the woody plant disease in forests that was previously attributed to *P. citricola* now belongs to *P. plurivora*. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from *Frangula californica*.

***Phytophthora multivora*** was first described in 2009, it is morphologically similar to *P. citricola*, and probably was grouped with it prior to molecular techniques. *P. multivora* is pathogenic to bark and cambium of *E. gomphocephala* and *E. marginata* and is believed to be involved in the decline syndrome of both these eucalypt species in south-west Western Australia. Since its description, it is recognized as being present in surveys of *Phytophthora* species. Its role in disease here is not yet understood. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from *Ceanothus thyrsiflorus*, *Baccharis* species, *Mimulus* species, *Arctostaphylos* species, *Frangula californica*, and *Aesculus californica*.

***Phytophthora plurivora*** was first described in 2009. It was described based on a re-designation of *Phytophthora citricola* isolates from woody host in European forests. It also shows up in west coast surveys of *Phytophthora* species. In the most recent California surveys of *Phytophthora* species from native plant nurseries and restorations in California, it has been recovered from *Carex barbarae*, *Cornus serricae*, *Corylus cornuta*, and *Rhododendron occidentale*.

#### Clade 4. Narrow host range-

***Phytophthora quercetorum*** was first described in 2008 after isolates belonging to an undescribed *Phytophthora* species were frequently recovered during a *Quercus* species forest soil survey of *Phytophthora* species in eastern and north-central USA in 2004. At that time, the species was recovered from the east coast oaks: *Quercus rubra*, *Q. macrocarpa*, and *Q. phellos*. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from the west coast oak species: *Q. agrifolia*.

#### Clade 6.

**“*Phytophthora* pgChlamydo” = *Phytophthora chlamydospora*** was first isolated from waterlogged roots of a planted ornamental *Prunus* species in Gloucestershire, UK in 1971. It is commonly found in riparian soils and streams worldwide and is a pathogen of riparian plant species in these systems including *Alnus rubra*, this does not mean it is native to the United States, it is not known where it is native. It is very closely related to *Phytophthora gonapodyides* but differs in that it produces long-lived asexual resting structures called chlamydospores. It has increasingly been found to hybridize with other species within its taxonomic clade. It is one of few clade 6 *Phytophthora* species that shows up in nursery surveys. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from *Quercus agrifolia*. As far as its impact, *P. chlamydospora* occasionally is recovered from cankers on trees and roots (Reeser et al. 2008, Navarro et al. 2014, Sims et

al. 2014, Hansen et al. 2015) and foliage of horticultural nursery stock (Jung and Blaschke 2004, Yakabe et al. 2009, Hansen et al. 2015). It has been associated with root rot of Port-Orford-cedar (*Chamaecyparis lawsoniana*) in German nurseries (Hansen et al. 1999, Hansen et al. 2015), and with root rot and stem cankers in nurseries and Christmas tree plantations of fir (*Abies* species) (Brasier et al. 1993, Hansen et al. 2015).

***Phytophthora gonapodyides*** was first described in 1909 on *Alnus* species in Denmark causing twig blight. This species may be either native or more likely naturalized throughout much of the west coast of the United States. It can cause a moderate level of disease of *Alnus rubra* in the United States reported in Oregon. It has been reported from plant debris in forests and is occasionally found as a pathogen of individual trees and other species of trees besides *Alnus*. In many cases *P. gonapodyides* is found along with other *Phytophthora* and the symptoms caused by *P. gonapodyides* in general are milder when compared to other *Phytophthora* species. A better look at riparian tree and plant disease, which are for the most part understudied, may in fact reveal a different story. This species may stimulate sexual reproduction of other *Phytophthora* species in natural systems, which may aggravate outbreaks. Restoration planting in wetland or riparian locations where this species persists may prove to be difficult. On the west coast of the United States, it has been reported as a pathogen to very few plant species, but may be more prevalent in riparian habitats. At this time, it is not considered an important pest in nurseries and if it causes problems for

restorations, it would most likely be from planting in areas where this species already is. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has only been recovered once from one grass species: *Hordeum brachyantherum*.

***Phytophthora megasperma*** was first described in 1931 after recovery from the rotted root system of hollyhock (*Althea rosea*). It is closely related to both *P. gonapodyides* and *P. chlamydospora*. It differs from these two species in that a single individual produces long-lived sexual resting structures called oospores. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from *Myrica californica*, *Juncus* species, *Bacharis douglasii*, *Platanus racemosa* and *Euthamia occidentalis*.

#### Clade 7. Broad host range-

***Phytophthora cambivora*** was first described as the synonym of *Blepharospora cambivora* in 1917. It is best known for causing ink disease of chestnut in the United States and Britain. *P. cambivora* causes root rot of several woody plant species. On the west coast of the United States it is known for causing canker disease, and in Oregon mortality of golden chinquapin trees (*Chrysolepis chrysophylla*), it appears to be spread by movement along roads in this particular disease system (Saavedra et al. 2007). *Phytophthora cambivora* is heterothallic, meaning that there are two different mating types necessary for sexual reproduction to occur. Both mating types are documented on the west coast of the United States (Saavedra et al. 2007). In 2015 in California *P. cambivora* was

documented as being recovered, from Manzanita (*Arctostaphylos* species, unspecified), toyon (*Heteromeles arbutifolia*), and coast live oak (*Quercus agrifolia*). It has been documented in California from Amador and San Joaquin Counties (Mircetich & Matheron 1976). In these accounts, it was causing disease of cherry trees. In accounts that are more recent it was found in Sonoma County on madrone and *U. californica*, and others counties have not yet been listed.

***Phytophthora cinnamomi*** was first described as the causal agent of stripe canker for *Cinnamomum burmanii* in Burma in 1922. Since that time, it has been described as a serious root rotting pathogen of numerous woody plant species. By 1980, the host list for *P. cinnamomi* around the world was up to 950 plant species and the list has grown since. A list for California is given below in table 1. It is the causal agent of Jarrah Dieback in Western Australia, and there it has caused landscape level changes to ecosystems. In Australia, *P. cinnamomi* is considered to be in the top five environmental threats to the continent. *P. cinnamomi* is a soil borne pathogen and requires warm, wet soils to infect roots. Since 1900, it has caused major epidemics on native chestnuts in the United States and Europe, along with threatening the stability of entire forest and the heath community ecosystems in some parts of Australia. Together with drought, it may be a major predisposing factor in the Iberian oak decline. It has been shown that with climate warming in the Mediterranean climates of Northwestern Europe that disease is expected to get worse especially in the coastal areas but that the disease will not be in areas with cold winters such as central and Eastern Europe (Brasier 1996).

***Phytophthora cinnamomi* host list for California from the Systematic Mycology and Microbiology Laboratory Fungus-Host Database :**  
**Listed are host name, (disease if specified), State, and reference number for the database**

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Abies concolor (Root rot.): California - 25284,  
Arctostaphylos densiflora California - 25284,  
Arctostaphylos myrtifolia California - 38194,  
Arctostaphylos viscida California - 38194,  
Calocedrus decurrens California - card,  
Camellia japonica California - 773, 2615, 25284, 25787, 42827,  
Camellia sp. California - 25787,  
Castanea dentata California - 25284,  
Castanea sp. California - 39227,  
Ceanothus griseus var. horizontalis California - 25284,  
Cedrus deodara (Root rot.) California - card, 773, 2615, 25284,  
Cedrus sp. California - 25284,  
Chamaecyparis lawsoniana (Root rot.) California - 25284,  
Chamaecyparis lawsoniana cv. Elwoodii (Root rot.) California - 2615,  
Citrus sp. (Root rot.) California - 94, 3929, 25284,  
Cupressus sempervirens (Root rot.) California - 773,  
Cupressus sempervirens cv. Glauca (Root rot.) California - 2615,  
Daphne sp. California - 25284,  
Erica regerminans (Root rot.) California - 773, 2615,  
Erica sp. (Collar rot.) California - 94, 25284,  
Eucalyptus sp. California - 9927,  
Iberis sp. California - 25284,  
Juglans regia California - 2883, 25284, 44189,  
Juglans sp. California - 25786, 25787, 39227,  
Juniperus chinensis California - 25284,  
Juniperus sabina California - 25284,  
Juniperus virginiana cv. Canaertii California - 2337,  
Leucadendron argenteum California - 38938,  
Leucadendron discolor California - 38938,  
Leucadendron rubrum California - 38938,  
Leucospermum reflexum California - 38938,  
Libocedrus decurrens (Root rot.) California - 773, 2615,  
Macadamia integrifolia California - 1701, 3929, 25284,  
Macadamia tetraphylla (Root rot.) California - 3929, 25284,  
Myrtus compacta (Root rot.) California - 773, 2615,  
Persea americana California - card, card, card, card, 699, 773, 3893, 3929, 13027, 25284, 25787, 39071,  
Persea borbonia (Root rot.) California - 94,  
Picea sp. (Seedling blight.) California - 25284,  
Pinus canariensis (Root rot.) California - 773, 2615,  
Pinus mugo California - 25284,  
Pinus radiata (Root rot; damping off and root rot; monterey pine, insignis pine; root rot) California - 773, 2615, 25284, 25787,  
Pinus sp. (rootlet disease, decline) California - 25284,  
Platanus racemosa California - 25284,  
Polystichum munitum (Root rot.) California - 2475, 25284,  
Protea neriifolia California - 38938,  
Protea obtusifolia California - 38938,  
Prunus americana (Crown and root rot.) California - 3929, 25284,  
Prunus armeniaca (Crown and root rot.) California - 3929, 25284,  
Pseudotsuga menziesii (Root rot.) California - 25284,  
Quercus agrifolia (Trunk canker.) California - 1688, 25284, 40566, 43975,  
Quercus sp. (Root rot.) California - 773, 25284,  
Quercus suber (Trunk canker.) California - 1688, 25284,  
Rhododendron sp. (Wilt; wilt of rhododendron) California - 25284, 25787,  
Thuja compacta (Root rot.) California - 2615,  
Thuja occidentalis cv. Compacta California - 773,  
Vaccinium ovatum California - 3929, 25284,

In California the recorded presence of *P. cinnamomi* goes back to 1942. *P. cinnamomi* has more recently been reported from *Arctostaphylos* species, Madrone, and oak in California. It was reported in 2006 (Garbelotto et al.) that two *Quercus* species: *Q. engelmannii* and *Q. agrifolia*, dealt with the same pathogen quite differently, even in the same environment. The decline of *Quercus* species infected by *P. cinnamomi* was more serious for *Quercus agrifolia* than for *Q. engelmannii*, and the disease was only observed in conjunction with other factors on the latter: in particular, with the presence of the oak twig girdler, *Agrilus angelicus*, an insect favored by stress conditions such as drought. In and near Lone California, *P. cinnamomi* has caused landscape level mortality of *Arctostaphylos* species. In 2003 it was reported that (*Arctostaphylos myrtifolia*) a rare endemic evergreen shrub restricted to lone formation soils in the foothills of the Sierra Nevada and the widely distributed *A. viscida* (whiteleaf manzanita) had an extensive mortality within two mixed stands of *A. myrtifolia* and *A. viscida* near Lone, CA. At one site, nearly all plants of both species in a 0.25-ha area had died. At a second site, most of the *A. myrtifolia* and *A. viscida* plants on several hectares died at least 5 years earlier. Plants of all age classes were affected and *Phytophthora* species were recovered from both species. *P. cinnamomi* was found to be the causal agent of severe disease in both plant species, however other disease causing *Phytophthora* species on site contributed to the issue. Before 2003, *P. cinnamomi* had not been reported to cause significant mortality in natural stands of California native species. In Lone, the disease most likely resulted from

the repeated introductions of *P. cinnamomi*. In a presentation at the Forest Pest Council Meeting in 2014, Dr. Sweicki reported on *P. cinnamomi* killing *Arctostaphylos pallida* in the Huckleberry Botanical Regional Preserve and Joaquin Miller Park; Madrone from China Camp State Park, A location in San Mateo County, Mt. Tamalpias Watershed, and Jack London State Park. Most recently, it was found in plant debris in the Presidio at MacArthur meadow, it was suggested that because the area used to be a nursery site, it was probably introduced then.

Across southern Europe, *P. cinnamomi* causes widespread collapse to *Quercus suber* and other Mediterranean *Quercus* tree species. The effects in parts of California with Mediterranean climate could end up being similar to those the Mediterranean region of southern Europe, if efforts are not made to limit introductions. In Spain and California *P. cinnamomi* has been reported to infect and kill *Q. suber* that are native to southwest Europe including Spain. *P. cinnamomi* is thought to be limited from spreading into much of central Europe due to colder temperatures and the drought in the south promotes disease expression (Brasier 1996). It is probable that in parts of California it is too cold and/or too wet to support disease and that the current drought here, will promote disease expression in diseased host species.

*Phytophthora cinnamomi* has had major impacts on trees in the southeast of the United States. Old records suggest that *P. cinnamomi* devastated chestnut forests in the southern United States long before it attracted attention in other

parts of the world, and preceded the onset of Chestnut blight (<http://www.acf.org/pdfs/summit/Jeffers.pdf>). It is also the causation of littleleaf disease in the South East of the United States, which is a decline of *Pinus echinata* (Erwin and Ribiero 1996, Mistretta 1984).

***Phytophthora niederhauserii***. Described in 2014, but before it was described as an official species it had been described unofficially as a taxon dating back to 2001. In its 2014, description is was described from 13 ornamental plant species in nurseries around the world. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from sticky monkey flower *Mimulus (Diplacus) aurantiacus* and *Salvia* species.

#### Clade 8.

***Phytophthora cryptogea*** was first described in 1919 in Ireland as causing tomato foot rot. Worldwide it is now recognized as a pathogen of ornamental plants especially. In nursery type situations, it can destroy susceptible crops very quickly because it can move from plant to plant easily. In California, 2015, in association with native plant nurseries and restoration sites *P. cryptogea* was recovered from *Anaphalis margaritacea*, *Heteromeles arbutifolia*, *Eriophyllum staechadifolium* and *Diplacus (Mimulus) aurantiacus*.

***Phytophthora dreschleri*** was first described in 1931 from rotting potatoes. It has a wide host range and is mainly a root rotter but also causes disease of fruits. It may be indigenous to southeast Alaska. It is difficult to untangle its history because prior to molecular diagnostics it was most likely synthetically grouped

along with many other *Phytophthora* including the more recently described *P. niederhauserii*. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations, it has been recovered from *Mimulus* species.

***Phytophthora ramorum*** was first described in 2000. Before *P. ramorum*, the biggest *Phytophthora* threats to forests and natural areas in California was the soil borne *Phytophthora* species *P. lateralis* only a real problem on a single host. *P. ramorum* is spread (mostly short distance) through air currents and rain splash, and does not affect the root systems in the way other soilborne *Phytophthora* species do in forests and natural ecosystems. Although *P. ramorum* does have an important soil phase in nursery situations. It has a broad host range but cause serious disease in the United States on *Quercus agrifolia*, and *N. densiflorus*. The disease can go virtually unnoticed and is somewhat easy to manage in the nursery trade. However, once it spread from the nursery trade into coastal forests in northern California and southern Oregon *Quercus agrifolia* and *N. densiflorus* die-offs occurred in alarming numbers. Both of these plant species are very important to California's unique biodiversity, and the affected *N. densiflorus* stands after they have been decimated by *Phytophthora ramorum* become a great fuel source, which is devastating for forest management. The broad host range is one factor, which makes *Phytophthora ramorum* impossible to eradicate once it has been introduced. This species was moved into forests through the commercial, not the native plant nursery trade, but can make restoring natural landscapes challenging.

#### Clade 8. Narrow host range-

***Phytophthora lateralis***. This species is found in landscape plantings throughout the Pacific Northwest (British Columbia to northern California) and, increasingly, in Europe. In the forest, it has now spread throughout the native range of Port-Orford-cedar, which spans from southwest Oregon into Northern California. *P. lateralis* is practically host specific, having less severe disease occurring, occasionally, on yews and cypresses. A few other cases have been reported on other plant species but they were most likely misidentified and were never reported again. The best strategy for dealing with this disease is to avoid planting Port-Orford-cedar. In the recent evaluation of *Phytophthora* species in California native plant nurseries and restorations it has not been recovered most likely due to the fact the known host has not been evaluated.

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**Appendix 1. Plant list for inclusion in nursery sampling.**

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Priority is based on risk and indicated by row color; yellow rows indicate high and white indicate moderate risk. All High priority plant species are sampled regardless of their health status. Moderate priority plant species are sampled if they are symptomatic (test first with the Agdia test strips).

Plant species	Proximal Find		Phytophthora positive 2014–2015				USDA ARS Fungal Database Phytophthora Host	Phytophthora species				Agdia Foliage Test?	Notes
	West coast	CA	CDFA	Phytosphere	UCB	GGNRA (nurseries/field)		P. cactorum	P. hedriandra	P. multivora	P. cryptogea		
<i>Acer macrophyllum</i>	x	x					Yes	x				Yes	
<i>Achillea millefolium</i>	x	x			x	x	No						
<i>Aesculus californica</i>	x	x	x			x	Yes			x		Yes	
<i>Alnus rubra</i>	x	x					No	x					
<i>Anaphalis margaritacea</i>	x	x		x		x	No				x		
<i>Arbutus menziesii</i>	x	x	x				Yes	x				Yes	
<i>Arctostaphylos hookeri</i>	x	x	x				No		x				
<i>Arctostaphylos montereyensis</i>	x	x	x				No	x		x			
<i>Arctostaphylos pajaroensis</i>	x	x	x				No		x				
<i>Arctostaphylos pumila</i>	x	x	x				No	x	x	x			
<i>Baccharis obungasii</i>	x	x	x	x			No						
<i>Baccharis pilularis</i>	x	x	x				Yes	x		x	x		
<i>Carex barbarae</i>	x	x	x	x			No						
<i>Ceanothus ferrisiae</i>	x	x	x	x			No	x					
<i>Ceanothus rigidus</i>	x	x	x				Possibly ( <i>C. cuneatus</i> )	x					
<i>Ceanothus thrysiflorus</i>	x	x	x		x	x	Yes	x		x			
<i>Cornus sericea</i>	x	x				x	Yes						
<i>Corylus comuta</i>	x	x				x	Yes						
<i>Cupressus macrocarpa</i>	x	x					Yes	x					

Plant species	Proximal Find		Phytophthora positive 2014–2015				USDA ARS Fungal Database	Phytophthora species				Agdia Foliage Test?	Notes
	West coast	CA	CDFR	Phytosphere	UCB (nurseries/field)	Phytophthora Host		P. cactorum	P. hedriandra	P. multivora	P. cryptogea		
<i>Drymocallis (Potentilla) glandulosa</i>							Yes						
<i>Elymus glaucus</i>	x	x			x	x	No	x					
<i>Eriophyllum stacyifolium</i>	x	x	x			x	No				x		
<i>Euthamia occidentalis</i>	x	x			x		No						
<i>Fragaria vesca</i>							Yes	x					
<i>Fragaria chiloensis</i>	x	x					Yes						If symptomatic (stunted, dead plants, cut open longitudinally and look for red colored stele)
<i>Frangula (Rhamnus) californica</i>	x	x	x	x	x	x	Yes	x	x	x		Yes	
<i>Gautheria shallon</i>	x						Yes						
<i>Gilia capitata</i>							Possibly						
<i>Heteromeles arbutifolia</i>	x	x	x	x	x	x	Yes	x	x		x	Yes	
<i>Hordeum brachyantherum</i>							No						
<i>Juncus balticus</i>	x	x			x		No						
<i>Juncus effusus</i>	x	x			x		No						
<i>Juncus patens</i>	x	x	x	x			No						If <i>J. patens</i> is positive then test other <i>Juncus</i> species
<i>Lonicera hispidula</i>	x	x	x			x	Yes	x				Yes	
<i>Lupinus arboreus</i>	x	x					No						
<i>Lupinus charmissonis</i>	x	x					Yes						
<i>Lupinus formosus</i>							No						
<i>Lupinus varicolor</i>							No						
<i>Lycnothamnus floribundus</i>	x	x			x		No						
<i>Mimulus aurantiacus</i>	x	x	x	x	x	x	No	x		x	x		
<i>Myrica (Morella) californica</i>	x	x	x			x	No					Yes	

Plant species	Proximal Find		Phytophthora positive 2014–2015				USDA ARS Fungal Database	Phytophthora species				Agdia Foliage Test?	Notes
	West coast	CA	CDFR	Phytosphere	UCB (nurseries/field)	Phytophthora Host		P. cactorum	P. hedriandra	P. multivora	P. cryptogea		

B. Appendix 1. Plant list for inclusion in nursery sampling

<i>Pinus radiata</i>	x	x					Yes	x		x	x	Yes	
<i>Polystichum munitum</i>	x	x					Yes						
<i>Prunus ilicifolia</i>							Yes	x					
<i>Pseudotsuga menziesii</i>	x	x					Yes	x			x	Yes	
<i>Quercus agrifolia</i>	x	x	x	x	x	x	Yes	x		x			
<i>Rhododendron macrophyllum</i>	x	x					Yes					Yes	Carrier species for <i>P. ramorum</i> , may be very healthy with only minor leaf symptoms
<i>Rhododendron occidentale</i>	x	x	x			x	Yes	x				Yes	
<i>Rosa californica</i>	x	x					No					Yes	
<i>Rubus parviflorus</i>							Yes						Look for purple lesions at the base of the stem and canes that wilt soon after breaking bud or canes that do not break bud.
<i>Rubus ursinus</i>	x	x					Yes						
<i>Sambucus nigra</i>							Yes						
<i>Umbellularia californica</i>	x	x					Yes					Yes	Carrier species for <i>P. ramorum</i> , may be very healthy with only minor leaf symptoms
<i>Vaccinium ovatum</i>	x	x					Yes					Yes	

## **Appendix 2. Standard Operating Procedure (SOP) for Soil Pasteurization**

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Remember the end product is the point, which is high quality potting media (here referred to simply as soil), free of pathogens, the best medium possible for growing healthy plants! Work in a logical order to achieve success using the minimal amount of steps necessary, to produce the best product possible. Keep a clean and safe working environment. Handle all chemicals safely and with good judgement, consult the SDS when necessary or when there is concern. Quality control is very important. Immediately following pasteurization, check and record the temperature reached at several locations. If the pasteurized product does not read at-least 140°F at all points checked, then increase the temperature and continue pasteurizing. Be sure to store finished material away from potential contamination threats.

### Pasteurizing Soil

1. Place clean soil pasteurizer onto the clean footprint. Moving the equipment is a two-person job. At the Presidio, we are placing our equipment on the cement floor because it is strong enough to endure high temperatures and we can clean it.
2. Get a relatively clean (free of obvious debris to avoid soiling the floor as you move inside) wheelbarrow, and fill it with soil to pasteurize, wet the soil until it is as damp as a wrung out sponge. Wheel the soil to the soil pasteurizer; make sure to remove debris from the tires and the outside of the wheelbarrow, sanitizing the tires and your shoes before you enter the building. Dump enough damp soil into

the soil pasteurizer to fill it without compacting the soil, and place the lid back on the pasteurizer.

3. Wipe away any spilled debris from around the outside of the soil pasteurizer with a damp clean paper towel or cloth. Set the temperature to 140° F and plug in the pasteurizer. Maintain a temperature of 140° for two hours, checking several locations of the pile with a temperature reader to ensure the temperature has been reached, when completed unplug the pasteurizer. The red light should come on, indicating that the elements are heating up. When the light goes off that indicates that the temperature has been reached. Set an alarm to alert you regarding completion.

4. While machine is pasteurizing, sweep the ground around the soil pasteurizer to remove any spilled soil. Have a clean and sanitized wheelbarrow ready for transporting the pasteurized soil. A wheelbarrow can be washed with soapy water and brush to remove all dirt from the inside, outside, handles and tires or use some soap and a power washer if available.

5. Following cleaning, lightly spray the wheelbarrow where the soil will be held and the handles with rubbing alcohol or wipe down with an allowable disinfectant (check with the nursery manager). Quatrasan at the recommended dilution for cleaning surfaces is an available product that works but may or not be available for use in your nursery. If spraying with alcohol do so in a ventilated area, and avoid inhalation, wear safety goggles and gloves, refer to the SDS for details. Act

responsibly and use common sense when working with chemicals: Do not drink the chemicals or play with them, use practically and NOT excessively.

6. Leave your cleaned wheelbarrow next to the soil pasteurizer or in a clean area until ready to use. When pasteurization is complete, turn off (or unplug if there is no off switch) on your machine. Let the machine cool and make sure it is unplugged. Bring the clean wheelbarrow next to the soil pasteurizer, if it is not already there. Take the lid off the soil pasteurizer and set aside.

7. Sanitize two scoops by washing to remove all visible soil and debris, dry and spray with 70% Isopropyl alcohol (rubbing alcohol), set aside and let the alcohol dry on the surface. Then designate and label these as 'clean' scoops.

They can be stored in a clean tool drawer in a clean plastic bag. If you do not use them for anything but pasteurized soil and store them away from contamination threats, there is little to no chance they will become contaminated. If there is concern the scoops may have been contaminated, clean and sanitize the scoops again.

8. Put on clean gloves and scoop the upper layer of pasteurized soil into the clean wheelbarrow, stop when you get to the heating bars. At this point two people are needed to safely pick up the soil pasteurizer and lift it so that it is resting on the wheelbarrow. Some of the soil will remain on the ground, but most will stay in the soil pasteurizer. Use the sterile scoops to push the soil through the bottom of the soil pasteurizer into the wheelbarrow.

9. Label and cover the pasteurized soil in the wheelbarrow. Then place the soil pasteurizer back into its storage space.
10. Use the broom to sweep the remaining soil, place this in a bucket to be pasteurized again with the next batch or move it back outside, whichever is convenient. Clean up any remaining tools and debris.

## **Appendix 3. Safety**



## Chemical Safety:

When using chemicals like fungicides, always follow the product guidelines, agricultural use requirements, and safety procedures (which includes wearing the appropriate personal protective equipment, or 'PPE'). Always refer to the SDS for specifics on safety requirements of each product. Check for updates or changes on products that are being used to ensure that the safety information you are referring to is up-to-date and aligns with the product that you are using. Follow the instructions on each product label.

- Do not use any chemical if it requires certification for use and you are not certified.
- Do not use chemicals that you do not know how to use safely.
- Report any misuse of chemicals.
- Many chemicals require the use of gloves and safety goggles, some require respirators. Each chemical has a specific SDS that should be referred to before using and followed during use.
- Different dilutions of different chemicals may require different types of PPE and different levels of caution. Know the dilution!
- Store chemicals appropriately and do not store with chemicals that, when crossed, may react with each other.
- Know the shelf life of your chemical tools and dispose of them appropriately, when they are outdated.

- Seek the advice of a safety expert if/when information is lacking on a particular product.

#### Tool Safety:

Knives and other cutting tools with sharp or pointed parts are important tools that are used in plant pathology to help to assess disease by exposing and/or removing plant parts that can be used safely. The cutting tools used in plant pathology are similar or the same as those used at home for recreational projects like gardening, and home and field maintenance. There are many different types of cutting tools, so make sure that you have the right tool for the job.

Here is a short list of useful sharp and pointed tools that you may need in your plant pathology tool kit depending on the job

**Pulaski-** Large jobs and big trees with thick bark, remove thick outer bark, and pick side can be used for exposing roots of large trees with root disease

**Hatchet-** Medium size job, small or large trees or shrubs with thin bark.

**Pickaxe-** Removes hard soil on jobs that where a Pulaski is too large

**Pocket knife-** Various jobs, more focused removal of outer bark on roots or shrubs than is possible with tools with an axe blade. A pocketknife can be useful in many situations, but it, like any blade, should never be forced.

**Reinforced X-ACTO blade and knife handle-** Small jobs or big jobs with lots of small pieces, for excision of small, fine roots or cutting leaves or very fine stems.

**Loppers-** Removes medium size branches of trees and shrubs

**Clippers-** Removes small branches or leaves

**Shovels-** Removes large amount of soil by hand

**Trowel-** Removes small amounts of soil by hand

**Forceps-** Small and large jobs. There are forceps of many different sizes, which are used for removing tissue that has been excised with a blade.

Tools with blades come in many different forms, and each type of tool presents different hazards, although the primary hazard is the blade that can cause deep and severe lacerations. It is important to take these hazards seriously and follow safe work practices, including how to safely use, handle, carry, store, sharpen, clean, and maintain them for use in the workplace. The use of handheld tools with blades in the workplace is an obvious hazard and necessity so it is important to be aware of the dangers these tools present. Ultimately, you are responsible for your own safety. If you are inexperienced with a tool take a safety-training course, or follow the instructions and guidance of a safe and experienced work colleague if no training course exists.

Check the handles on your tools before use to make sure there are no cracks, and check the head of the tool if separable is on tight. For tools that you swing, establish a zone of safe movement before use by holding the sheathed head and making a 360-degree circle around you to make sure you can swing safely. Consider the size of a tool, for example, the safety area for a Pulaski will be larger than for a hatchet. Both hands are necessary to swing a Pulaski safely but only one hand is needed for swinging a hatchet. Keep the sheath covering your blade until you are ready to use it, and keep the blade sharp. Wear good boots to

protect your feet. Do not swing any sharp tool toward yourself, any part of your body, or anyone else.

Make sure that tool you are using is appropriate for the job and that you are comfortable using the tool. If any tool you are, using feels awkward it may not be the correct tool and could result in injuries. You should never need to force a knife or blade to do the job of a larger tool.

Here are some examples of using the right tool. For cutting small, fine small root pieces, a reinforced X-ACTO knife with a properly inserted blade is all you will need to excise the small pieces of roots, but even though it is reinforced, this blade is inappropriate and may break if used for larger jobs. On thin stems of young trees or shrubs, a pocketknife with a sharpened blade is very useful to remove the outer bark and assess inner tissues. Make sure the handle size of your pocketknife or any other tool is the right size to allow a firm grip, and make sure that the handle is clean so that it does not slip or stick. Pocketknives are useful for a variety of jobs. For shrubs, small trees, young trees or trees with thin bark a hatchet can be very useful for removing the outer bark and observing the inner bark. A larger axe or a Pulaski is very useful for large trees and trees with thick bark. These tools must be swung using both hands and are only appropriate for large jobs. A pickaxe or pick is very useful for removing hard soil around roots to expose root systems and evaluate root disease and to collect roots for sampling. For large trees, the pick side of a Pulaski can be used to expose roots in the same

way. A shovel and a trowel are hand tools used for soil removal of large and small jobs, respectively.

For any tools with blades, make sure that the blades are sharpened and clean before you use them. A rusty blade not only can transmit pathogens even after being wiped clean but it is also more likely to break while being used, and so presents a hazard. A dull blade is dangerous because it does not cut when and where you expect it to, it will slide first and then cut and by the time it cuts it may be cutting your finger or your hand instead of the intended object. Dull blades cause the user to apply more force in cutting, which may result in unsafe and awkward positioning and more opportunity for injuries to occur. To avoid accidents, learn how to sharpen your blade properly and maintain your blades.

High quality tools that are clean, sharp and properly maintained will make the job simpler, more efficient, and safe. You will be able to work safely, and will given time to clean and maintain your tools because, once you follow the cleaning procedure, the job itself will require less time and effort. This saves time not having to file injury reports and reduces/eliminates time spent in the emergency room. Sharp tools are especially important for properly cutting away bark, excising roots, removing shrub branches and even cutting leaves on herbaceous ornamentals, which is all a part of the assessment of plant health. These jobs can be done safely and efficiently with well-maintained tools and the right tools for the job.

## **Appendix 4. Soil Treatment under Review**

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Ms. Nina Larssen came up with a list of “Alternatives for *Phytophthora* Treatment”.

Of the methods Alternative 3 was considered the best because (1) it can use steam which is repeated in the literature as being better than dry heat for improving soil using pasteurization techniques. The second reason is that in the process of moving the soil large soil chunks be broken down making the soil medium more uniform which is important for all of the soil to reach the appropriate temperature. The method was described:

“Ex-situ Steam/Hot Air Treatment using Engineered Soil Piles This treatment alternative involves constructing engineered soil piles, which would be designed similar to bio piles or compost piles. The engineered soil piles would be constructed in “lifts”, where soil would be mounded to a depth of no more than a few feet. Slotted or perforated piping would be installed in the soil piles. Steam and/or hot air would be generated onsite using a mobile unit and would be injected into the pipe network placed within the soil pile. To minimize heat loss, a tarpaulin cover or other insulating material would be used to cover the soil pile. If steam were used, soils would likely need to dry prior to reuse. For this approach, it is anticipated that the void space between soil particles in the soil pile would be larger and more homogeneous than an in-situ alternative, thereby decreasing the likelihood of insufficient and non-uniform heating. The design parameters of the engineered soil piles (e.g., pipe spacing, pile size, energy requirements) would be modeled after bio piles or compost piles; however, a robust, site-specific pilot study would need to be performed in order to confirm the efficacy of this approach

and refine system design elements. Although there are too many unknowns currently to estimate treatment costs for this alternative prior to pilot testing, this alternative is considered feasible for stockpiled soil from MacArthur Meadow and/or other Presidio sites”.

Alternative treatment one may also be acceptable if the soil can remain moist during the procedure, which is important: On-Site Evaporative Desorption Unit (EDU). This method was the most well developed of the treatments outlined. However, dry heat is usually discouraged in pathogen abatement and it is recommended to keep the soil moist during treatment. Usually steam heat is preferred. Both treatments were considered potentially applicable to the treatment of larger projects like MacArthur Meadow and smaller projects within the Presidio.

Regarding the proposed treatments, we may want to increase the temperature slightly (target temperature 140 °F) to target a broader spectrum of pathogenic organisms and pests including true fungi and plant parasitic nematodes.

## **Appendix 5. Baiting Technique**

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This technique is a modified technique from a method used successfully to isolate multiple *Phytophthora* species from forest and later nursery soil. These baiting methods are loosely based on an original baiting method provided by W. Sutton. Particular days of the week were suggested in the original methods to help organize workflow and are left in, as a means of guidance only. This technique was developed and modified to help meet the long-term sampling/monitoring strategies of soil for the Presidio and GGNRA:

- a. Nursery
  - i. Potted plants soil
  - ii. soil environs
- b. Restoration sites soil throughout the Presidio and GGNRA
- c. the compost from the compost yard in the Presidio
- d. Forestry site soil in Presidio
- e. Landscape site soil in the Presidio

## Short Version

Thursday or Friday put all samples in order, load into pans 10 bags per pan. Keep in a cool location below 75° F but not freezing. Add enough Deionized water so that the soil is wet but not with a water line above soil. If the soil is already very moist, you do not need to add more water. Close bag check for leaks place inside another bag if leaking (or for large leaks get a new bag). If the sample is from a field site where the soil is dry, it is necessary to allow the sample to be presoaked for 5-7 days prior to baiting.

On Monday, prep the soil by adding another 100-500 mL of DI water. The amount should be modified so the water line is approximately one inch above the soil line. Assemble bait bags (see below for details). Make sure bait pieces are submerged in the water over the soil. Double check for proper orientation of all baits. After the baits are assembled, and in place, leave in place for 7 days ideally at cool room temperature (65° F - 75° F). You should move to cold storage if temperatures exceed 80° F for several hours. At the end of the incubation period (the following Monday), remove the contents of each bait bag onto a clean paper towel. Plate the contents of one bait bag onto a single oomycete selective plate.

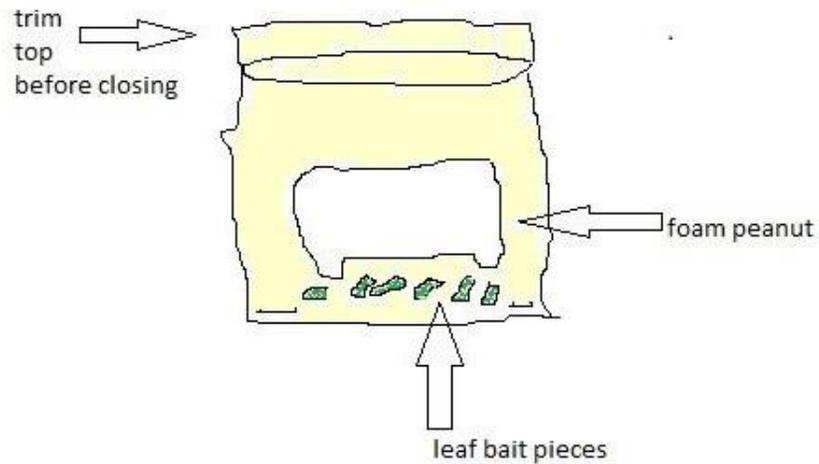
*Control-* For negative control prepare one sample bag with DI water and a bait bag of each type used. Every time baiting procedures are preformed a negative control should be included using the same materials (water, pear and bag type).

## **Additional Details**

*Bait bag assembly-* Use *Mellita® Super Premium Tea Filters*. Cut the tea filters in half, discard bottom half. Trim the top of the bag to leave about ½ and inch flap. Staple the bottom of the bag closed, by folding end over and placing a staple at each corner. Place the bait pieces in the bait bag. Place a foam-packing peanut in the bag with the bait pieces (Figure 1). Fold tea bag top over and staple shut. Float so that bait pieces are submerged in the water over the soil.

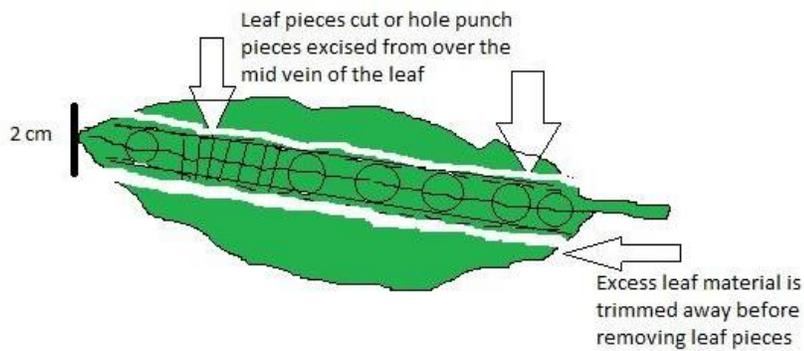
*Baits in a sample-* For all baits use healthy, clean plant material. If soap is used make sure to rinse away all soap, soap can kill zoospores. When the baits bags of each type (one rhododendron, one pear, one oregano) are completely assembled, place three bait bags in each sample bag. Float the bait bags with the bait pieces on the downside and the packing peanut on the upside. Double check for proper orientation of all baits.

**Figure 1.** For bait bag assembly trim the top of the bag to leave about ½ and inch flap. Staple the bottom of the bag closed, by folding end over and placing a staple at each corner. Place the bait pieces in the bait bag. Place a foam-packing peanut in the bag with the bait pieces.



### *Bait types*

*Rhododendron bait bag*- Using mature *R. macrophyllum* leaves cut away all leaf material except a 2 cm (across) strip with edges parallel to the midvein (Figure 2). It is important to prepare the baits with clean scissors. Cut across the midvein into 2 mm wide pieces (Figure 2). Each leaf piece should be 2 cm long and 2 mm wide. Place 6 leaf pieces in each bait bag add a foam-packing peanut and staple the top shut. Six hole-punched leaf pieces excised from along the midvein can be used in place of the six strips (Figure 2).



*Pear bait bag-* Using a green pear cut flat blocks 2-3 mm thick and ~2 cm in length placing 5 in a bait bag add a foam packing peanut and staple shut. You should expect these pieces to turn to mush during the baiting process.

*Oregano bait bag-* place six diced stem (about 2cm long) pieces (with leaves attached) in bait bag add a foam packing peanut and staple shut.

### *Plating baits*

Clean your work area with 70% alcohol. Process all sample on a clean work tray. Label plates as you go with sample number, date and bait type. Make a stack of cut paper towels (to reduce waste) cut in  $\frac{1}{2}$  and then make a stack cut into  $\frac{1}{3}$  sections.

Isolation tools:

Long Forceps

Short Forceps

Alcohol burner

Work tray

Autoclave bags

70% alcohol

Thin cutting blade with handle

Matches

At any one time only have one plate, one bait bag one flame cleaned pair of short forceps and one of each type (1/2 and 1/3) of paper towel on your clean tray.

Clean tools and tray in between samples. Have an autoclave bag set up next to you in a trashcan so you can discard materials as needed.

Place one bait bag (using long forceps) on the opened 1/2-paper towel. Pull open with short forceps. Blot baits on 1/3 sized paper towel. Blot dry, moving baits around on the paper towel until dry. For rhododendron baits, you can rub pieces dry. For pear baits cut mush pile into six sections for plating (using razor blade with handle). The leaves of the oregano may have separated from stems. Plate six sections three leaf, three stem if necessary. Check that plates are labeled before putting into storage container. Fill in the data sheet with corresponding plate information. Keep plates in a cool, dark location. Check daily for growth.

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