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Cooperating State Departments of Agriculture

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New Pest Response Guidelines

Phytophthora species in the Environment and Nursery Settings

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Phytophthora species in the Environment and Nursery Settings

This document is not intended to be complete and exhaustive. It provides a foundation based upon available literature to assist in the development of appropriate and relevant regulatory activities. Some key publications were not available at the time of writing, and not all specialists and members of the research community were consulted in the preparation of this document.

References to commercial suppliers or products should not be construed as an endorsement of the company or product by the USDA.

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Cover Photo: Courtesy of Alina Greslebin and Carlos Baroll "Phytophtora austrocedrae"

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INTRODUCTION

Purpose

This New Pest Response Guideline (NPRG) presents available information for designing a site specific action plan implementing detection, diagnosis, containment and control, or eradication of *Phytophthora* species from a forest or nursery setting. Specific emergency program activity should be based on information available at that time. Any new detection may require the establishment of an Incident Command System to facilitate management of personnel associated with the emergency. This document is meant to provide the necessary information to launch a response to a detection of an exotic *Phytophthora* in a nursery or forest setting.

> Please note: Detections of *Phytophthora ramorum* are to follow protocols set forth by the USDA *P. ramorum* National Program. This NPRG is not to supersede any current protocols of this program, as the national program has the most recent and up-to-date information on survey and detection methods for this specific pest. For more information regarding the *P. ramorum* National Program, please visit http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml.

The document provides background information on the diseases, their hosts, and the causal pathogens. This management approach is an amalgam of methods employed by USDA programs in this country and

programs in other countries. It is intended to provide a starting point for a control/eradication or management program, with modifications to be made as the program develops.

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) agency developed these guidelines through discussion, consultation, or agreement with other APHIS staff, the Agricultural Research Service (ARS), Forest Service (FS), university advisors, states, tribal groups, and industry. It is to be used in conjunction with other agency regulations, guidelines, and manuals when conducting program activities. The information contained in these guidelines is based on the best scientific information available at the time of writing. Specific emergency program actions should be based on the best information available at the time of the incident.

Disclaimers

Document Comprehensiveness: This document is not intended to be complete and exhaustive, but provides a basic foundation, based upon the literature available, to assist further work.

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Pest Status

The genus *Phytophthora* encompasses approximately 100 phytopathogenic species. *Phytophthora* species negatively impact ornamental nurseries and forests throughout the world. In addition to causing large losses of nursery stock, exotic *Phytophthora* species also threaten natural ecosystems, where they can cause catastrophic damage. Crop and forage species can also be impacted by *Phytophthora* species. Leaf spots and lesions, dieback, cankers, wilt, small leaves, chlorosis, necrosis, and root rot are examples of symptoms that develop as a result of *Phytophthora* infection.

Some important, well-known *Phytophthora* species include *P. infestans, P. sojae,* and *P. ramorum*. *Phytophthora infestans* caused the Irish potato famine in the 19th century and is considered the most serious disease of potatoes. *Phytophthora sojae* costs the soybean industry millions of dollars annually. *Phytophthora ramorum* is the causal agent of sudden oak death (SOD), a disease that has been damaging important nursery and forest plants in North America and Europe (Rizzo *et al.*, 2005). Full genome sequences are available for *P. infestans*, *P. ramorum, P. sojae* (Tyler *et al.*, 2006; Haas *et al.*, 2009); given the large investment of time and money necessary to sequence a complete genome, the availability of this wealth of data highlights the importance of this group of pathogens.

Phytophthora species are Oomycetes, also called water molds (Erwin and Ribeiro, 1996). These fungal-like organisms can produce motile zoospores, sporangia and chlamydospores (asexual structures), and oogonia and antheridia (sexual structures) (Erwin and Ribeiro, 1996). Oospores (from oogonia and antheridia) and chlamydospores are tolerant of a wide range of climatic conditions and can provide long-term survival for some *Phytophthora* species. In addition, mycelia, sporangia, and encysted zoopores have been noted to survive in wood and other host tissues (Erwin and Ribeiro, 1996). If conducive climate and susceptible host material are available, sporangia and zoospores can be produced multiple times during a season and promote the disease to epidemic levels (Erwin and Ribeiro, 1996).

Several 'new' species of *Phytophthora* have been described, indicating that taxonomic knowledge of this genus is potentially incomplete. Additionally the discovery of species hybrids, such as *P. alni* subsp. *alni*, indicates that new *Phytophthora* species are evolving, possibly in nursery locations, where related but geographically isolated species come in contact. This New Pest Response Guideline will focus on species of *Phytophthora* that can impact either forests or nurseries and are exotic to the conterminous United States (Table 1-1). Species that impact crops are not covered in this guideline unless they also impact forest or nursery species. Species with considerable taxonomic confusion are also not covered in this guideline (Table 1-2).

Exotic *Phytophthora* Species Prevention

Federal and state regulatory officers must conduct inspections and apply prescribed measures to ensure that the disease or pathogen does not spread within or between properties. Federal and state regulatory officers conducting inspections should follow the sanitation guidelines in the beginning of the Survey section to prevent spreading contaminated plant material or tools to other facilities before entering and upon leaving each property.

Program Safety

The safety of the public as well as the program personnel is a priority consideration in preprogram planning and training, and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Contacts

When an emergency program for the pest has been implemented, its success depends on the cooperation, assistance, and understanding of other groups. The appropriate liaison and information officers should distribute news of program progress and developments to interested groups, including:

- Other Federal, county, and municipal agricultural and forestry officials

- Grower groups (such as specific commodity or industry groups)
- Commercial interests
- Academic entities with agricultural/forestry interests
- Land-grant universities with Cooperative Extension Services
- State and local law enforcement officials
- Public health agencies
- Native American Tribal Governments
- Foreign agricultural interests
- National and local news media, and
- The public

Initiating an Emergency Pest Response Program

An emergency pest response program consists of detection and delimitation, and may be followed by programs in regulation, containment, eradication, and control. The New Pest Advisory Group (NPAG) will evaluate the pest. After assessing the risk to U.S. plant health, and consulting with experts and regulatory personnel, NPAG will recommend a course of action to PPQ management.

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported.

2. The pest is examined and pre-identified by regional or area identifier.

3. The pests identity is confirmed by a national taxonomic authority recognized by USDA–APHIS–PPQ–National Identification System.

4. Existing *New Pest Response Guidelines* are consulted or a new NPAG is assembled in order to evaluate the pest.

5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators, and trading partners.

6. A delimiting survey is conducted at the site of detection.

7. An Incident Assessment Team may be sent to evaluate the site.

8. A recommendation is made, based on the assessment of surveys, other data, and recommendation of the Incident Assessment Team or an NPAG, as follows:

- Take no action
- Regulate the pest
- Contain the pest
- Suppress the pest
- Eradicate the pest

9. State Departments of Agriculture are consulted.

10. If appropriate, a control strategy is selected.

11. A PPQ Deputy Administrator authorizes a response.

12. A command post is selected and the Incident Command System is implemented.

13. State Departments of Agriculture cooperate with parallel actions using a unified command.

14. Traceback and trace-forward investigations are conducted.

15. Field identification procedures are standardized.

16. Data reporting is standardized.

17. Regulatory actions are taken.

18. Environmental Assessments are completed as necessary.

19. Treatment is applied for required pest generational time.

20. Environmental monitoring is conducted, if appropriate.

21. Pest monitoring surveys are conducted to evaluate program success.

22. Programs are designed for eradication, containment, or long-term use.

Support for Program Decision-Making

The USDA/APHIS/PPQ Center for Plant Health, Science and Technology (CPHST) provides technical support, in consultation with other scientists and technical working groups when appropriate, to emergency pest response program directors concerning risk assessments, survey methods, control strategies, and other aspects of

pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policy for pest response programs.

Table 1-1. Exotic *Phytophthora* species included in this New Pest Response Guideline (NPRG).

***Phytophthora* species**	**Why is pest included in the NPRG?**
*Phytophthora alni (*subsp. *alni, multiformis,* and *uniformis)*	Forest pathogen – An aggressive root and collar rot of riparian, plantation, and shelterbelt alders
Phytophthora alticola	Potential nursery and wildland pathogen – *Eucalyptus*, the primary host, is a major landscape taxon (mostly in California).
Phytophthora austrocedrae	Forest pathogen in South America – Aggressive species causing withering, defoliation, and death.
Phytophthora boehmeriae	Pathogen of *Pinus* spp.
Phytophthora captiosa	Potential nursery and wildland pathogen – *Eucalyptus*, the primary host, is a major landscape taxon (mostly in California).
Phytophthora colocasiae	The number of hosts of this pathogen and countries where the pathogen occurs makes this species important as a potential risk for nurseries. Reported to occur in the United States (CA, NC, HI), but reports from conterminous United States have not been verified.
Phytophthora fallax	Potential nursery and wildland pathogen – *Eucalyptus*, the primary host, is a major landscape taxon (mostly in California).
Phytophthora frigida	Potential nursery and wildland pathogen – *Eucalyptus*, the primary host, is a major landscape taxon (mostly in California).
Phytophthora gallica	Tree pathogen
Phytophthora idaei	Potentially present in raspberry cultivars
Phytophthora iranica	Occurs on solanaceous plants, including tomato, which can be sold in nurseries, or planted in gardens.
Phytophthora italica	*Myrtus communis* (true myrtle) – A tree species – is the reported host.
Phytophthora kernoviae	Forest and nursery pathogen. *Rhododendron* is a host.
Phytophthora melonis	Crop and garden species are hosts; threat to nurseries.
Phytophthora multivesiculata	Ornamental hosts (*Cymbidium*, boat orchids)

Phytophthora multivora	Potential nursery and wildland pathogen – *Eucalyptus*, the primary host, is a major landscape taxon (mostly in California).
Phytophthora pinifolia	Forest pathogen
Phytophthora polonica	Isolated from soil associated with *Alnus glutinosa*. Potential forest pathogen.
Phytophthora porri	Nursery and crop species are potential hosts.
Phytophthora primulae	Nursery/ornamental hosts (*Primula*)
Phytophthora psychrophila	Isolated from soil below *Quercus robur*, *Q. ilex*, *Q. pubescens*, and *Q. petraea.* Potential forest pathogen.
Phytophthora quercina	Forest pathogen
Phytophthora tentaculata	Nursery/ornamental hosts
Phytophthora uliginosa	Isolated from soil below *Quercus robur* and *Q. petraea.* Potential forest pathogen.

Table 1-2. Exotic *Phytophthora* species <u>not</u> included in this New Pest Response Guideline.

***Phytophthora* species**	**Why pest is not included in the NPRG?**
Phytophthora andina	Taxonomic confusion. Initial assessment is that this species is not different from *P. infestans*; most experts feel this is not a valid species.
Phytophthora botyrosa	Hosts are rare under cultivation as landscape plantings or as ornamentals.
Phytophthora x *cactorum-nicotianae*	A hybrid of *P. cactorum* and *P. nicotianae.* Both parents are present in the United States.
Phytophthora cajani	Crop host (minor crop – pigeon pea)
Phytophthora canavaliae	There is a single report of this species – Waterhouse (1970) included a translation of the description by Hara. This species designation requires validation.
Phytophthora cinnamomi var. *parvispora*	Taxonomic confusion. Variety designation requires validation by molecular techniques. Only reported host is a minor landscape plant (*Beaucarnea* spp.) in tropical areas.
Phytophthora cinnamomi var. *robiniae*	Appears to be a single report from China; Molecular research is necessary to clarify the status of 'variety'.
Phytophthora clandestina	Crop (alfalfa) and forage (clover) hosts only.
Phytophthora cyperi-bulbosi	Single observation in 1953 on *Cyperus bulbosus* in India. Not reported on any other host or from any other country and has not been cultured (Erwin and Ribeiro, 1996).
Phytophthora humicola	Natural host is unknown.
Phytophthora ipomoeae	Taxonomic confusion. Reported as indistinguishable from *P. infestans* and *P. mirabilis* on ITS-1 rDNA; only morphology and host specificity is used to differentiate. More research is needed using sequences of additional isolates and recent collections.
Phytophthora japonica	Crop host (rice) only

Phytophthora leersiae	Known only from a herbarium specimen. No cultures are available for study. Sequencing analysis is needed to validate the species.
Phytophthora lepironiae	Known only from a herbarium specimen. No cultures are available for study. Sequencing analysis is needed to validate the species.
Phytophthora macrochlamydospora	Crop host (soybean) only
Phytophthora megakarya	Hosts (*Theobroma cacao* and *Irvingia* spp.) are rare under cultivation as landscape plantings or as ornamentals.
Phytophthora mexicana	Taxonomic confusion. *P. mexicana* is very similar to *P. palmivora* or *P. capsici* but appears to be phylogenetically distinct from *P. capsici*.
Phytophthora mirabilis	Taxonomic confusion. There is a strong indication that it is a synonym of *P. infestans*.
Phytophthora morindae	Outside of Hawaii, where species originally described (Nelson and Abad, 2010), the host (noni- *Morinda citrifolia*) is rare under cultivation as landscape plantings or as ornamentals.
Phytophthora oryzo-bladis	Crop host (rice) only
Phytophthora palmivora var. *heterocystica*	Taxonomic confusion. Unclear whether this variety is morphologically distinct. No cultures are available and it is reported only from the initial publication.
Phytophthora parsiana	Taxonomic confusion. New species reported for high temperature isolates that had been determined to be *P. cryptogea* or *P. drechsleri.* Some sequences cited in paper of *P. parsiana* correspond to other species. One isolate from study was from the United States.
Phytophthora plurivora	Sequence data from United States indicates that it is already present in the United States but was misclassified as *P. citricola.*
Phytophthora polygoni	Weedy host plants (*Polygonum* spp. and *Rumex dentatus*)

Phytophthora quininea	Hosts (*Cinchona officinalis* and *C. pubescens*) are only grown in Hawaii – introduced there.
Phytophthora verrucosa	Species requires validation.
Phytophthora vignae	Minor crop host – *Vigna* spp. (cowpea)

CONTENTS

PEST INFORMATION

Nomenclature

Phylum: Oomycota
Class: Oomycetes
Order: Pythiales
Family: Pythiaceae
Genus: *Phytophthora*

Synonyms: Vary with the specific pathogen.

Preferred Common Name: Varies with the specific pathogen.

Phytophthora alni

Background Information for *Phytophthora alni*

Phytophthora alni is a hybrid pathogen of alder (*Alnus* spp.). Because this *Phytophthora* hybrid does not consist of a single entity but comprises a range of phenotypically diverse allopolyploid genotypes, *P. alni* was split into three subspecies: *P. alni* subsp. *alni* (*Paa*), *P. alni* subsp. *uniformis* (*Pau*), and *P. alni* subsp. *multiformis* (*Pam*) (Brasier *et al.*, 2004). The variants appear to range in their virulence and pathogenicity on European alders. *Phytophthora alni* subspecies *alni* appears to be the most aggressive and pathogenic to European alder species (Brasier and Kirk, 2001). The two other variants, *Pau*

and *Pam*, appear to be significantly less aggressive than *Paa*, though still considered pathogenic. The *Paa* variant is considered the primary agent killing alders in Europe, and is the most frequent subspecies observed in Europe at this time (Brasier, 2003).

History and Distribution for *Phytophthora alni*

In 1993, a previously unknown and lethal disease of alder was described in southern Britain (Gibbs, 1995). Initially it was thought to be caused by *Phytophthora cambivora*, a fungus well-known as a pathogen of broad-leaved trees but not previously reported from alder. Even though the alder pathogen exhibited female gametangia (oogonia) with distinctive surface ornamentation and two-celled amphigynous (collar-like) male gametangia (antheridia), it quickly became clear that the pathogen was an entirely new species. The pathogen had several unusual properties and was suggested to be a new species hybrid, which had probably originated relatively recently (Brasier *et al.*, 1995). The pathogen was self-fertile rather than outcrossing, had submerged instead of an aerial colony type, and had markedly lower cardinal temperatures for growth. It also exhibited an unusually high level of zygotic abortion (Brasier *et al.*, 1995).

Brasier *et al.* (1999) used molecular analysis to show that the alder fungus was a hybrid most likely between *P. cambivora* and a fungus close to *P. fragariae* (a pathogen of strawberry). Researchers originally thought that *Pam* and *Pau* were genetic breakdowns of *Paa* (Brasier *et al.*, 1999). More recently, Ioos *et al.* (2006) demonstrated that *P. cambivora* and *P. fragariae* are not the progenitors of *P. alni*. They showed that *Paa* is actually a hybrid of *Pam* and *Pau* (Ioos *et al.*, 2006; Ioos *et al.*, 2007a, b).

The disease is widespread in southern England and in Europe in general. The disease has been reported from 17 countries: Austria, Belgium, Czech Republic, England, Estonia, France, Germany, Hungary, Ireland, Italy, Lithuania, The Netherlands, Poland, Scotland, Slovakia, Slovenia, Spain, and Sweden (Webber *et al.*, 2004; Solla *et al.*, 2009). Reports from Denmark do not now appear to have involved the alder Phytophthora (Gibbs *et al.*, 2003).

Until 2006, the alder *Phytophthora* had not been found outside of Europe. A report suggests that a similar pathogen may have been found in a nursery in Minnesota, infecting woody ornamental species (Schwingle *et al.*, 2007a; Schwingle and Blanchette, 2008). Additionally, *Phytophthora alni* subsp. *uniformis* was found in Alaska in 2007 (Adams *et al.*, 2008). *P. alni* is not known to occur in the conterminous United States.

Damage to Hosts by *Phytophthora alni*

P. alni causes a serious disease of alder (*Alnus* spp.), including lower stem bark lesions, root and collar necrosis, and crown dieback typical of other *Phytophthora* diseases. Diseased trees do not show crown symptoms until most of the bark at the base of the tree has been killed.

From a distance, diseased alders attract attention in mid-to-late summer, because the leaves are abnormally small, yellow, and sparse. They often fall prematurely, leaving the branches bare. In a tree with severe crown symptoms, the lower part of the stem is often marked with a black or rusty colored exudate known as 'tarry spots' that can occur up to 2-3 meters from the ground. The spots indicate that the underlying bark is necrotic or dead (Thorain *et al.*, 2007). Over the next few years, the fine twig structure, the bark, and eventually the trunk will break up. It is quite common for narrow strips of bark to remain alive and to support a limited growth of new shoots from the trunk and major branches (Webber *et al.*, 2004). Adventitous roots may be seen on the stems of trees as a result of prolonged flooding of the root system, in response to death of the bark, or as a result of *Phytophthora* disease (Gibbs *et al.*, 2003). Although not a specific symptom, the development of adventitious roots can be a useful indication of the presence of a bark lesion further down the stem. Early and often excessive fructification with unusually small cones is also observed (Jung and Blaschke, 2004).

Economic Impact and Ecological Range of *Phytophthora alni*

There are four alder species native to Europe: the common alder (*Alnus glutinosa*), the gray alder (*A. incana*), the Italian alder (*A. cordata*), and the green alder (*A. viridis*). In general, members of the genus *Alnus* are pioneer species, able to colonize bare, open ground rapidly and with a great ability to tolerate wet sites (Webber *et al.*, 2004). The roots have specialized nodules that fix atmospheric nitrogen as a result of a symbiotic association with the actinomycete *Frankia*. Common alder in particular has considerable landscape value along waterways; it plays a vital role in riparian ecosystems and the root system helps to stabilize riverbanks (Webber *et al.*, 2004).

Very high losses have occurred in some localities (parts of France and Germany); while in others the disease impact has been relatively small (Webber *et al.*, 2004). The susceptibility of North American *Alnus* species is currently unknown, so it is difficult to assess the economic and ecological range of *P. alni*. Cech (1998), however, reports that strains of the alder *Phytophthora* were pathogenic to *Alnus rubra* (red alder), which is present in the United States and is one of the few commercial hardwood species in the western United States (Cree, 2006). Jung *et al.* (2007) developed a model to predict the potential distribution of *P. alni* in Bavaria, Germany in order to have a tool for assessing the potential hazard posted by *P. alni* to forests in other regions of the globe. Preliminary results of an application of the multi-criteria model (drainage, streams, climate, distribution of alder species, distribution of wholesale and retail nurseries, and urban settlements) to the United States, indicates that there are regions with susceptible alder forests. Heavy loss of alders due to *Phytophthora* infection could result in significant ecological effects including changes in forest composition, soil composition, wildlife food and habitat, and increased soil erosion (Cree, 2006).

Life Cycle and Biology of *Phytophthora alni*

Little is known about the biology of the pathogen under field conditions. Most species of *Phytophthora* infect their hosts mainly by motile spores (zoospores) that are dispersed through water and in the soil. This could explain the high incidence of the disease on alder in the riparian zone, although the disease may also occur in sites remote from waterways (orchard shelter belts and woodland plantations). According to Brasier (2003), it is rare to isolate the pathogen from river water or from soil around infected alder trees. Ioos *et al.* (2005), however, detected *P. alni* in a river using a PCR test, which confirms that *P. alni* can spread naturally in surface water.

Zoospores of the pathogen have been shown to be attracted to fine roots of alder, but it is not known whether such roots are infected in the field. On the contrary, it appears that infection may take place through the bark near the root collar (Webber *et al.*, 2004). Mechanical injury may not be necessary for infection. Foliar and crown symptoms do not occur until the root collar has been largely girdled. Thus, many years may elapse between infection and the appearance of visible disease in the crown of affected trees (Cree, 2006).

Baiting tests in Germany showed that the alder *Phytophthora* was present in rootstocks of alders from three out of four nurseries that regularly brought in alder plants for re-sale, but not in rootstocks from four nurseries that grew their own alder from seed (Jung and Blaschke, 2004). This suggests that one avenue of spread for *P. alni* is via the planting of nursery stock.

Little is known about the role of oospores in the biology of the pathogen. The alder *Phytophthora* is homothallic, and produces oospores in culture, but viability as determined by the tetrazolium bromide method, is very low (26 to 31 percent) and no germination was observed in more than 4,000 oospores analyzed (Delcan and Brasier, 2001).

Optimum temperature of the pathogen in culture is 22.5-25.0^{o}C with the upper temperature limit about 30^{o}C (Brasier *et al.*, 1995; Santini, 2001).

Plant Hosts for *Phytophthora alni*

Phytophthora alni causes disease of alder (*Alnus cordata*, *A. glutinosa*, *A. incana*, and *A. viridis*).

In greenhouse inoculation trials, *Castanea sativa* (chestnut), *Juglans regia* (walnut), and *Prunus avium* (sweet cherry) were shown to be experimental hosts (Santini *et al.*, 2003; Santini et al, 2006).

Phytophthora disease of alders

Phytophthora alni Brasier & S.A. Kirk

Taxonomic Rank: Oomycetes: Peronosporales: Peronosporaceae

NAPIS: FICBPUT
Bayer code:

Selected Images from Invasive.org

View All Images at Invasive.org

Feature(s); Smooth-walled oogonium of P. alni (Swedish variant) with oospore and amphigynous antheridium.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Symptoms; Common alder (A. glutinosa) in a non-flooded forest plantation with root and collar rot caused by P. alni.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Symptoms; Grey alder (A. incana) with collar rot caused by P. alni; note the typical tarry spots at the outer bark and the tongue-shaped orange-brown necrosis of the inner bark.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Symptoms; Root system of a 2-year-old nursery grown common alder (A. glutinosa) with necrotic lesions caused by P. alni.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Symptoms; 2-yr-old sprouts of of an 8-yr-old coppiced Alnus glutinosa showing wilting due to root and collar rot caused by P. alni.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Symptoms; Alder plantation on former agricultural land; note infected grey alder (A. incana) with sparse, chlorotic and small-sized foliage.
Thomas Jung, , Bugwood.org
Additional Resolutions & Image Usage

Taxonomic References:

Index Fungorum. Paul Kirk. CABI, CBS and Landcare Research. http://www.indexfungorum.org/

Invasive Listing Sources:

National Cooperative Agricultural Pest Survey Target Species 2006, 2007, 2009

North American Forest Commission Exotic Forest Pest Information System

Partners

Invasive.org: Center for Invasive Species and Ecosystem Health
The University of Georgia - Warnell School of Forestry and Natural Resources and
College of Agricultural and Environmental Sciences - Dept. of Entomology
Last updated on Tuesday, February 02, 2010 at 10:08 AM

Phytophthora alticola and *P. frigida*

Background Information for *Phytophthora alticola, P. frigida*

Cold-tolerant *Eucalyptus* spp. are grown extensively for pulp-wood production in summer rainfall areas of South Africa with an altitude above 1150 m. During the mid-1980's, an increased demand for pulpwood led to the expansion of cold-tolerant *Eucalyptus* plantations, the establishment of a breeding program for cold-tolerant species, and the introduction of several alternative *Eucalyptus* spp. from seeds collected in natural stands in Australia.

Phytophthora collar and root rot is a widespread disease affecting a number of cold-tolerant *Eucalyptus* spp. in South Africa. This disease hampers progress towards introducing alternative *Eucalyptus* spp. yielding high pulp volumes. *Phytophthora* spp. known to be associated with collar and root rot of *Eucalyptus* spp. in South Africa include *P. boehmeriae*, *P. cinnamomi*, and *P. nicotianae*. In 2001, *P. nicotianae* caused disease outbreaks on several cold-resistant *Eucalyptus* spp. in South Africa. This was particularly interesting as *P. cinnamomi*, rather than *P. nicotianae*, has typically been associated with mortality of cold-tolerant *Eucalyptus* spp.

History and Distribution for *Phytophthora alticola, P. frigida*

Maseko *et al.* (2007) conducted a survey to assess the presence of *P. nicotianae* and other invasive *Phytophthora* spp. in *Eucalyptus* spp. in South Africa. The study resulted in the isolation of two putative new *Phytophthora* species, *P. alticola* and *P. frigida* associated with symptoms of *Phytophthora* collar and root rot.

Both species, *P. alticola* and *P. frigida*, are reported from South Africa. *P. alticola* has also been reported from Swaziland.

Damage to Hosts by *Phytophthora alticola, P. frigida*

The most common disease symptom of *Phytophthora* collar and root rot is progressive wilting of the leaves due to girdling of the root collars. When the bark is removed, brown lesions extending from the roots are typically observed. Other disease symptoms include root disease, bleeding lesions from diseased stem tissues, and the formation of epicormic shoots on the stems of dying trees. Dying trees are usually present in small patches throughout the plantations, especially in areas prone to water-logging during the rainy seasons.

Economic Impact and Ecological Range of *Phytophthora alticola, P. frigida*

Phytophthora collar and root rot disease hampers progress towards introducing alternative *Eucalyptus* spp. in South Africa, potentially yielding high pulp volumes.

Although *P. frigida* and *P. alticola* were proven to be pathogenic on *Eucalyptus dunnii*, they were substantially less pathogenic than *P. cinnamomi*. Their relative importance as tree pathogens and in the *Phytophthora* complex associated with collar and root rot will need to be determined.

Life Cycle and Biology of *Phytophthora alticola, P. frigida*

Due to the recent descriptions of *P. alticola* and *P. frigida,* little is known about their lifecycles and biology.

P. alticola is homothallic in culture and thus, likely to be an inbreeding species. The cardinal temperatures for *P. alticola* were 15^{o}C and 30^{o}C. None of the isolates examined grew below 10^{o}C or above 30^{o}C. The optimal temperature was 25-30^{o}C for growth. The pathogen is sensitive to hymexazol.

The distinctive morphological features of *P. alticola*, which include papillate and caducous sporangia, indicate that it is adapted for wind or splash dispersal.

P. frigida, in contrast, is a heterothallic species and has predominantly been found on *E. smithii*, planted in areas with an altitude above 1150m in South Africa.

The cardinal temperatures for *P. frigida* isolates examined were 10^{o}C and 30^{o}C. None of the *P. frigida* isolates grew at 5^{o}C or 3^{o}C. The ability to grow at temperatures lower than 15^{o}C indicates adaptation to a cool temperate climate. It has not been associated, however, with shoot dieback of forest tree species examined. Distinctive morphological characteristics include a stellate to petalloid growth pattern, and the ability to utilize L-asparagine better than nitrate as sole nitrogen source. The pathogen is tolerant to hymexazol.

Plant Hosts for *Phytophthora alticola, P. frigida*

Eucalyptus badjensis, *E. dunnii*, *E. macarthurii* are reported hosts for *P. alticola*.

Acacia decurrens, *A. mernsii, Eucalyptus dunnii*, and *E. smithii* were reported as a host for *P. frigida*.

The pathogens were isolated from rhizosphere soil around diseased plants.

Aboveground and belowground disease symptoms of *Phytopthhora* root and collar rot in *Eucalyptus* spp. (A) Young *E. badjensis* killed by *P. alticola* in Midillovo progeny trial. (B–D) Collar rot and formation of epicormic shoots in *E. badjensis*. (E) Root rot of *E. macarthurii* caused by *P. alticola*. (F) Root rot of *E. saligna* caused by *P. frigida*. (G) Discolouration of the inner collar and kino exudation of *E. smithii* infected by *P. frigida*.>

Pictures courtesy of Michael J. Wingfield

Phytophthora austrocedrae

Background Information for *Phytophthora austrocedrae*

Austrocedrus chilensis (Cordilleran cypress) is an endemic tree in the Cupressaceae found in southern Argentina and Chile. It forms pure and mixed stands with *Nothofagus* spp. and, among the few conifers inhabiting southern Argentina, it has the largest distribution, covering approximately 160,000 hectares.

Mortality of *Austrocedrus chilensis*, termed 'Mal del Cipres' or 'cypress wither', in Argentina was first detected in 1948. The mortality had been studied for many years but its cause had remained unclear. Several studies of biotic and abiotic factors have tried to elucidate the origin and causes of this disease, but in spite of the amount of information gathered, a satisfactory etiology had not emerged.

Pythiaceous fungi have been suggested by several authors as a possible causal agent. For this reason, a survey of *Phytophthora* spp. of *Austrocedrus* forests was conducted. Five *Phytophthora* species were detected inhabiting soil of declining *A. chilensis* forests but none of them showed a clear relationship with the decline (Greslebin *et al.*, 2005).

History and Distribution for *Phytophthora austrocedrae*

Greslebin *et al.* (2007) isolated a new *Phytophthora* species, *P. austrocedrae*, from necrotic lesions of stem and roots of *Austrocedrus chilensis*.

The disease is confirmed to be present in Argentina. According to Filip and Rosso (1999), the disease possibly occurs in south-eastern Chile.

Damage to Hosts by *Phytophthora austrocedrae*

The main symptom of the disease is progressive withering and subsequent defoliation of the tree, which finally dies while standing. The first visible symptom in individual trees is chlorotic foliage. Trees may die rapidly, in which case foliage changes from chlorotic to red (Filip and Rosso, 1999). On the rootlets of affected trees, dead tissue can be observed. This becomes severe during dry summers. Basal resinous exudates and red-brown necrotic lesions are seen in the inner bark extending up the bole from killed roots. Brown cubic rots and sapwood caused by wood-decomposer fungi are frequently, but not always, associated with dead or dying trees (Greslebin *et al.*, 2007). Trees of all sizes are affected.

Economic Impact and Ecological Range of *Phytophthora austrocedrae*

A. chilensis is economically valuable because of the high quality of its wood used for construction and woodworking, and it has great tourist and scenic appeal (Greslebin *et al.*, 2005).

The disease affects tourism, recreation, and commercial forestry in Argentina (Greslebin *et al.*, 2005; Manna *et al.*, 2008b). *Austrocedrus* forests surround most of the tourist cities and villages. It is nearly impossible to carry out appropriate silvicultural management of affected stands because the

appearance and evolution of the disease cannot be predicted. Another serious consequence of the disease is the replacement of native forests with exotic introduced species. This is due mainly to the lack of effective control leading the public institutions in charge of forest management to authorize land owners with affected forests to fell the dead trees and replace them with other species.

Life Cycle and Biology of *Phytophthora austrocedrae*

'Mal del cipres' begins in the root system. The distribution and pattern of spread of mortality in a stand (patchy) is consistent with a soil-borne pathogen. The disease appears to be associated with areas of poor drainage, fine-textured soils, and areas of higher precipitation (Manna *et al.*, 2008b). Allophanic volcanic soils do not appear to favor disease development (Manna and Rajchenberg, 2004). The disease has been associated with low canopy cover, organic horizon thickness, elevated pH, and high understory cover (Manna *et al.*, 2008a).

P. austrocedrae is a homothallic species characterized by semipapillate sporangia, oogonia with amphigynous antheridia, and very slow growth (1-2 mm/day on V-8 agar at 17.5^{o}C optimum temperature). Growth was favored by cool temperatures. Isolation from diseased trees was successful when plates were incubated at 17^{o}C but unsuccessful at temperatures of 20^{o}C or above.

Plant Hosts for *Phytophthora austrocedrae*

Austrocedrus chilensis

P. austrocedrae, aboveground symptoms

Pictures courtesy of Alina Greslebin and Carlos Baroll

P. austrocedrae, aboveground symptoms (2)

Pictures courtesy of Alina Greslebin and Carlos Baroll

P. austrocedrae, inner bark red-brown necrotic lesions

Pictures courtesy of Alina Greslebin and Carlos Baroll

P. austrocedrae, inner bark red-brown necrotic lesions

Phytophthora boehmeriae

Background Information for *Phytophthora boehmeriae*

Phytophthora boehmeriae was first isolated from *Boehmeria nivea* (ramie, or Chinese silk tree), by Sawada (1927) in Taiwan. It has not been reported in Taiwan since then (Ho, 1990), but has been reported from multiple other countries.

History and Distribution for *Phytophthora boehmeriae*

The disease is present in Argentina, Australia, Brazil, China, Greece, India, Japan, Korea, Mexico, Papua New Guinea, South Africa, Taiwan, and New Zealand.

There is a herbarium record of *P. boehmeriae* from the United States in 1946, however, no locality or host information is provided. (http://194.203.77.76/herbIMI/DisplayResults.asp?strName=Phytophthora+boehmeriae). *P. boehmeriae* is not believed to be present in the United States.

Damage to Hosts by *Phytophthora boehmeriae*

In nature, *P. boehmeriae* can infect, damage, and blight the seedlings and bolls of cotton, the leaves and stems of ramie, the leaves of paper mulberry (*Broussonetia papyrifera*), the fruits of citrus, and the roots of pine.

Cotton: On seedlings, *P. boehmeriae* produces round or irregular, water-soaked, dark-green spots or lesions on cotyledons or true leaves, resulting in premature leaf fall or wilting of some or all leaves under cold, humid conditions. On the roots and root-stem transition zones, the disease first appears as brown streaks and then as a brown rot, resulting in wilting and death of whole seedlings.

On bolls, dark-green, water-soaked lesions generally form at basal cracks or tips, causing rotting of the tissues within, with a layer of fungal growth on the surface under humid conditions. Sporangia may be present on the surface of rotten bolls and oospores may be present on the cotton lint and internal carpel surface of infected bolls. As the spots develop, whole diseased bolls may rot away. Infected tissues may turn almost black. Infection is restricted to the lower two-thirds of the cotton plant (Elena and Paplomatas, 1998).

Ramie: The pathogen attacks leaves and stems. The leaf spots are round or irregular, water-soaked and light green at first, then dark brown or dark green. At later stages, the spots turn yellowish brown or gray at the center, with a brown margin. Diseased leaves tend to fall early. Elliptical, dark-brown stem lesions are present, mainly at the bases, resulting in rotting of whole bases.

Paper mulberry and Chinese wingnut: Leaf spots similar to those found on cotyledons or leaves of cotton are observed. Root rot is also observed on

Chinese wingnut.

Citrus: The pathogen causes brown fruit rot and root stock gummosis.

Pine: The pathogen causes root rot on pine (Oxenham and Winks, 1963).

Geraldton wax plant: Plants show progressive yellowing of the branches from the base to the top of the stems. Leaves of diseased plants become grayish green, then yellow, and finally straw-colored. Leaves remain attached to the branches after the plants die. Root and stem discoloration was observed and the root cortex sloughed off (Wolcan and Lori, 2001).

Black wattle: Disease caused by *P. boehmeriae* is evident by dark lesions at the trunk base without gum exudation up to 10 m in height (Dos Santos *et al.*, 2006).

Economic Impact and Ecological Range of *Phytophthora boehmeriae*

P. boehmeriae has been reported on multiple hosts in multiple studies. Economic and ecological information is lacking on most hosts with the exception of cotton in China.

In China, *P. boehmeriae* is the principal agent causing cotton blight and ramie blight. Cotton blight, including cotton seedling blight and cotton boll blight, is one of the main diseases in cotton in mainland China. A severe attack can kill 30-50 percent of seedlings in the field in cool, wet weather. Seedling blight mortality up to 60 percent has been recorded in fields (Tang, 1990). Cotton boll blight injures bolls, resulting in rotting. The rotting rate of bolls is 10 to 30 percent in ordinary years, with a maximum over 50 percent in rainy years. In addition to yield loss, the disease affects cotton quality, resulting in reduced fiber length and decreased ginning outrun.

In Greece, *P. boehmeriae* boll rot has been reported as a new threat to cotton cultivation. In Australia, it has damaged citrus and pine, causing rot of fruits and roots, respectively.

P. boehmeriae was reported as one of the causal agents of the gummosis complex in black wattle in Brazil and is now of quarantine importance for the citrus industry. This was the first report of the involvement of *P. boehmeriae* in the etiology of the gummosis complex of black wattle in Brazil (Dos Santos *et al.*, 2006).

Life Cycle and Biology of *Phytophthora boehmeriae*

P. boehmeriae is homothallic and oospores form abundantly in host tissues. When the tissues (leaves, bolls, etc.) infected by *P. boehmeriae* decompose, oospores formed in the diseased tissue are released into the soil. Under suitable conditions, they germinate by germ tubes to form sporangia or mycelia, which may produce sporangia. The germination mode of sporangia is affected mostly by temperature. It has been observed that at 18 to 20^{o}C, all

sporangia germinated indirectly to produce zoospores, whereas at 22 to 24°C most sporangia germinated directly and terminated with secondary sporangia capable of releasing zoospores (CABI, 2006).

All spore forms may be carried long distances by water or soil and all germinate in water. When the spores lodge (encyst) against a stem or root of a certain host, such as ramie or pine, or splash onto a leaf or fruit of a suitable host plant, such as cotton, they germinate to produce appressoria or mycelia that infect the plant through the cuticle or via stomata or wounds. The role of the chlamydospore in the lifecycle is not clear. Some researchers suggest that the fungus may overwinter as chlamydospores; others suggest that chlamydospores are seldom present in *P. boehmeriae*, and oospores can overwinter and survive in the soil (CABI, 2006).

The pathogen is favored by high humidity and warm temperatures; therefore, it frequently occurs in low, wet areas in rainy years. The optimum temperature for mycelial growth in culture is 25 to 30°C, minimum 9°C, and maximum 34.5°C. The minimum temperatures for sporangium and oospore formation are 25°C and 25 to 28°C, respectively. Darkness stimulates mycelial growth and oospore formation, whereas illumination inhibits both (CABI, 2006).

There is evidence that *P. boehmeriae* is seedborne in cotton and can be disseminated by seeds (CABI, 2006).

Antheridia are amphigynous and almost spherical in shape. Often with a residual oil globule. Antheridia have been shown to change to paragynous on media with low nutrient levels (Gao *et al.*, 1998).

P. boehmeriae is one of the most sensitive to metalaxyl of all *Phytophthora* species and has been used as a bioassay for metalaxyl in soil.

Plant Hosts for *Phytophthora boehmeriae*

Acacia mearnsii (black wattle), *Ailanthus altissima* (tree-of-heaven), *Araucaria hetrophila* (Norfolk Island Pine), *Avicennia* spp. (mangrove), *Boehmeria frutescens* var. *concolor* (nakai), *Boehmeria nivea* (ramie), *Broussonetia papyrifera* (paper mulberry), *Cedrus deodara* (Deodar cedar), *Chamelaucium uncinatum* (Geraldton waxplant), *Citrus sinensis* (orange), *Citrus* spp., *Eucalyptus dunnii*, *Eucalyptus grandis*, *Eucalyptus macarthurii*, *Eucalyptus pilularis*, *Eucalyptus smithii*, *Ficus* spp., *Gossypium hirsutum*, *Gossypium* spp., *Malus domestica* (apple), *Malus sylvestris* (apple), *Persea americana* (avocado), *Persoonia longifolia* (long-leaf Personnia), *Pinus patula* (Mexican yellow pine), *Pinus* spp., *Pterocarya stenoptera* (Chinese wingnut), and *Solanum melogena* (eggplant) are hosts for *P. boehmeriae*.

Experimental hosts for *P. boehmeriae*: *Allium fistulosum* (Japanese bunching onion), *Benincasa hispida* (Chinese waxgourd), *Capsicum annuum* (bell

pepper), *Cephalonoplos segetum* (common cephalanoplos), *Chenopodium album* (fathen), *Convolvulus arvensis* (field bindweed), *Corchorus capsularis* (white jute), *Cucumis sativus* (cucumber), *Cucurbita moschata* (butternut squash), *Ipomoea batatas* (sweet potato), *Ixeris denticulata* (stebbins), *Ixeris laevignata* (stebbins), *Lycopersicon esculentum* (tomato), *Nicotiana rustica* (tobacco), *Nicotiana tabacum* (tobacco), *Phaseolus vulgaris* (bean), *Portulaca oleracea* (little hogweed), *Pyrus* spp. (pear), *Rehmannia glutinosa* (Chinese-foxglove), *Ricinis communis* (castorbean), *Solanum tuberosum* (potato), *Taraxacum mongolicum* (Mongolian dandelion), *Vicia bungei* (vetch), and *Xanthium sibericum* (Siberian cocklebur).

P. boehmeriae symptoms on Tree of Heaven (*Ailanthus altissiman)*

Pictures courtesy of Byung-Soo Kim

Phytophthora captiosa and *P. fallax*

Background Information for *Phytophthora captiosa, P. fallax*

A locally severe crown disease of Eucalyptus trees has been recorded since 1986 in New Zealand. The main species affected are *Eucalyptus saligna, E. botryoides, E. regnans, E. delegatensis,* and *E. fastigata*. Dieback has been recorded in the central North and in Southland. The organisms responsible are two species of *Phytophthora,* which have recently been described as *Phytophthora captiosa* and *P.fallax* (Dick *et al.*, 2006). This disorder, in contrast to most *Phytophthora* diseases, can occur high in the canopy of susceptible trees.

History and Distribution for *Phytophthora captiosa, P. fallax*

The two fungi (*Phytophthora captiosa* and *P. fallax*) are the first *Phytophthora* species to be described only from New Zealand and the first to be closely associated with the foliage of *Eucalyptus* spp. The majority of *Phytophthora* spp. are present in the soil and cause root disease; those species affecting aerial plant parts usually occurring on small plants. Canopies of *Eucalyptus* can be as high as 20 meters. Their modes of infection and dispersal are unknown.

Phytophthora captiosa and *P. fallax* are only known to occur in New Zealand.

Damage to Hosts by *Phytophthora captiosa, P. fallax*

Leaves, petioles, seed capsules, peduncles, and twigs may become infected. The effects of the disease range from minor leaf spots to major foliage loss and sometimes dieback.

The disease is associated with crown disease and causes premature leaf drop and twig dieback upon inoculation.

Economic Impact and Ecological Range of *Phytophthora captiosa, P. fallax*

At this time, no information is available on the economic impact and ecological range of these species.

Life Cycle and Biology of *Phytophthora captiosa, P. fallax*

P. captiosa and *P. fallax* have non-papillate, non-caducous sporangia and both are self-fertile (homothallic). Non-papillate, non-caducous sporangia are usually associated with soil-and root-inhabiting *Phytophthora* species rather than aerially dispersed species. Additionally, sporulation of both species has not been observed for either species in the field. The mode of infection and spread of these non-caducous *Phytophthora* species in the *Eucalyptus* tree canopy remains unknown. Studies have been proposed to assess whether invertebrates might be acting as vectors from foliage.

Plant Hosts for *Phytophthora captiosa, P. fallax*	*Eucalyptus botryoides* and *E. saligna* are reported hosts for *Phytophthora captiosa*. *E. delegatensis*, *E. fastigata*, *E. nitens*, and *E. regnans* are reported hosts for *Phytophthora fallax*.

P. Fallax symptoms on leaves of *E. fastigata*

Picture courtesy of Margaret Dick

Phytophthora colocasiae

Background Information for *Phytophthora colocasiae*

The causal organism of leaf spot on taro (*Colocasia esculenta*) was first described as *Phytophthora colocasiae* in Java (Indonesia) (Raciborsky, 1900). There is little information on the origin of *P. colocasiae*, but there are indications of an Asiatic origin (Zentmyer, 1988). Only the A1 mating type of *P. colocasiae* occurrs in Hawaii and only the A2 mating type is reported from Taiwan, indicating that they are not likely from the center of origin of the pathogen (Ko, 1979; Ann *et al.*, 1986). Zhang *et al.* (1994) showed that Hainan Island, an offshore island in the tropical region of southern China, is inside the center of origin of *P. colocasiae*. *P. colocasiae* has been distributed in vegetatively propagated material and by most likely via soil.

History and Distribution for *Phytophthora colocasiae*

The disease is currently present in the following countries/territories: American Samoa, Argentina, Bangladesh, Belau, Borneo, Brazil, Brunei, Burma, Ceylon, China, Dominican Republic, Equatorial Guinea (Bioko), Ethiopia, Federated States of Micronesia, Guam, India, Indonesia, Japan, Korea, Madagascar, Malaysia, Mauritius, Myanmar, Nepal, Northern Mariana Islands, Pakistan, Palau, Papua New Guinea, Puerto Rico, Philippines, Reunion, Samoa, Seychelles Islands, Solomon Islands, Sri Lanka, Taiwan, Thailand, and Trinidad and Tobago.

United States: There are numerous reports from Hawaii, where the pathogen has a widespread distribution. Reports from continental United States (North Carolina and California), however, need to be confirmed. Abad *et al.* (1994) report *P. colocasiae* on American ginseng in North Carolina, but according to Abad (personal communication) the isolate needs to be confirmed via molecular methods.

Damage to Hosts by *Phytophthora colocasiae*

Affected leaves initially show small dark brown to olive-green spots (often water-soaked), which enlarge rapidly and turn purplish brown with yellowish margins. The lesions frequently form concentric zones (zonate) and exude drops of yellowish liquid. The exudate and leaf spot symptoms are best viewed at night or in the early morning hours. Some of the diseased tissues may be covered with a whitish fuzz of sporangia. When the petiole or leaf stalk becomes infected, rots are usually long, brown, and occur anywhere on the stalk. When the rot expands, the stalk becomes soft, is often unable to support the weight of the leaf, and breaks (Brooks, 2005; CABI, 2006).

As the disease progresses, the lesions (mostly along the leaf margin) continue to expand and frequently coalesce. Diseased tissues disintegrate, forming holes of irregular size, and affected leaves collapse within 20 days of unfurling, compared to 40 days for healthy leaves (Jackson and Gollifer, 1975). Exudates associated with diseased tissue become yellow to brown and form small crusts. The normal six to seven leaves per plant can be reduced to three or four leaves per plant by severe disease incidence, which reduces net

photosynthesis and corm yield. If weather conditions are favorable, the entire field can be blighted in 7 to10 days (Trujillo, 1965). Highly susceptible cultivars appear to be 'melting' in the field, producing smaller and smaller leaves on shorter petioles (Brooks, 2005; CABI, 2006).

In both tolerant and resistant cultivars, diseased tissue falls away from spots, forming holes (often referred to as 'shot-holes') in the leaf (Brooks, 2005; CABI, 2006).

After harvest, gray-brown to dark-blue lesions occur on undamaged corms. These lesions enlarge rapidly and coalesce. The boundary between healthy and diseased tissues is usually indistinct and soft. Affected corms are almost completely decayed 8 days after harvest in wet conditions.

Economic Impact and Ecological Range of *Phytophthora colocasiae*

P. colocasiae is a limiting factor in the production of taro in Southeast Asia and the Pacific Islands, where it is consumed as a staple food crop on the farms in developing countries (Brooks, 2008; Gallegy and Hong, 2008). A reduction in yield of 30 to 50 percent may occur if conditions are favorable for disease development (Erwin and Ribeiro, 1996).

Taro leaf blight was reported in the Samoan Archipelago in 1993 (Brooks, 2008). 'Niue' was the favored cultivar at the time, comprising over 75 percent of an estimated US $10 million in annual exports for Western Samoa and most of the 357,000 kg grown for local use in American Samoa. By 1995, the epidemic had decimated the susceptible cultivar 'Niue', reducing Samoa's export market to $60,750 (US) and American Samoan production to a reported 5,000 kg (Brooks, 2008).

Life Cycle and Biology of *Phytophthora colocasiae*

The mycelium of *P. colocasiae* is hyaline, coenocytic, inter- or intra-cellular. The haustoria are slender, long, and unbranched (Thankappan, 1985). The sporangiophores are very slender, unbranched, extremely narrow at the tip, and measure up to 50 µm in length. The sporangia are elongated, lemon- or pear-shaped, and measure 38-60 x 18-26 µm. They germinate directly or indirectly depending on weather conditions. When indirect germination occurs, as many 12 reniform, biflagellate zoospores are released. Thick-walled, round, hyaline chlamydospores are also formed sometimes.

The oogonium is spherical and yellow. Amphigynous antheridium persists at the base of the oogonium for a considerable period after the oospores are formed. The oospores are spherical, have a 20-28 µm diameter, and lie free in the oogonium.

Leaf blight of taro is mainly a foliar disease and occurs under warm conditions. *P. colocasiae* grows at 15 to 35^{o}C; with an optimum of 27 to 30^{o}C. Epidemics are likely when zoospore release is maximized by repeated night time temperatures close to 20^{o}C and relative humidity of 90 to 100

percent (Trujillo, 1965; Narula and Mehrotra, 1984). Visible lesions appear two to four days after inoculation and, under humid conditions, can destroy large taro leaves with a 30- to 40-day lifespan in 5 to 10 days. Yield losses may reach 50 to 60 percent under severe blight conditions and susceptible taro cultivars can be destroyed completely (Brooks, 2008).

Inoculum is always available in some areas due to continuous cropping, but not in all areas where the crop is seasonal. Survival of *P. colocasiae* in the wild is poorly understood. Neither chlamydospores nor oospores have been reported under field conditions, although they form readily in agar culture. Additionally, the pathogen is heterothallic, requiring two mating types to form oospores. Mycelium within stored corms used in propagating is a possibility for pathogen survival. Quintugua and Trujillo (1998) showed that spores can remain viable for three months (as resting zoosporangia and/or chlamydospores); however, they also predicted that survival was less than one year in the absence of a host, due to the poor saprophytic ability of the pathogen.

Long distance dispersal occurs by means of vegetatively propagated material and probably through soil. Local dissemination is via rain splash or wind-driven rain. In wetland (flooded) taro production, sporangia and zoospores are spread between plants and fields by paddy water (Brooks, 2005).

Giant African snails (*Achatina fulica*) have been shown to transmit *P. colocasiae* in taro (USDA APHIS, 2007).

Plant Hosts for *Phytophthora colocasiae*

Alocasia macrorhiza (giant taro), *Alocasia* spp. (taro), *Amorphophallus campanulatus* (elephant-foot yam), *Bougainvillea spectabilis* (bouganvilla), *Cantharanthus roseus* (periwinkle), *Colocasia antiquorum* (elephant's ear), *Colocasia esculenta* (taro), *Colocasia* spp., *Dracontium polyphyllum* (guapa),, *Hevea brasilensis* (rubber), *Panax quinquefolius* (American ginseng), *Piper betle* (betel), *Piper nigrum* (black pepper), *Ricinis communis* (castorbean), *Vinca rosea* (periwinkle), *Xanthosoma sagittifolium* (yautia), and *Xanthosoma violacea* (blue taro) are reported hosts of *P. colocasiae*.

Phytophthora gallica

Background Information for *Phytophthora gallica*

Phytophthora gallica was isolated from soil beneath a declining mature *Quercus robur* (English oak) and from the littoral zone of a lake with *Phragmites australis* (common reed) and *Salix alba* (white willow) as dominants. The pathogen was informally designated as *Phytophthora* taxon 'G'.

History and Distribution for *Phytophthora gallica*

The pathogen has been found in France and Germany (Jung and Nechwatal, 2008). Recently the pathogen has been identified in Alaska in surveys of alder stands (Trummer, 2009).

Damage to Hosts by *Phytophthora gallica*

In pathogenicity tests, *P. gallica* was only weakly aggressive to *Q. robur* and *S. alba*, and non-pathogenic to *Phragmites australis*. Therefore, its ecological niche, whether as a pathogen or saprotroph, remains uncertain. However, a saprophytic lifestyle seems unlikely given the much higher growth rates of the *Pythium* species ubiquitous in these wet ecosystems (Jung and Nechwatal, 2008).

P. gallica was also moderately aggressive to *Alnus glutinosa* and *Fagus sylvatica* and non-pathogenic to *Fraxinus excelsior* (Jung and Nechwatal, 2008).

Economic Impact and Ecological Range of *Phytophthora gallica*

The economic and ecological impact of *P. gallica* is unclear at this time.

Life Cycle and Biology of *Phytophthora gallica*

Because the species has only recently been described, there is little information available on the life cycle and biology of *P. gallica*. *P. gallica* is a non-papillate, slow growing *Phytophthora* species; *P. boehmeriae* and *P. kernoviae* are its closest relatives. *P. gallica* produces colonies with limited aerial mycelium and variable growth patterns. Gametangia are not formed in single or mixed cultures with tester strains of known mating types, indicating that *P. gallica* may be sexually sterile. *P. gallica* produces globose and elongated irregular chlamydospores, of which a high proportion are abortive. In water culture, irregular hyphal swellings and non-papillate persistent sporangia are formed abundantly (Jung and Nechwatal, 2008).

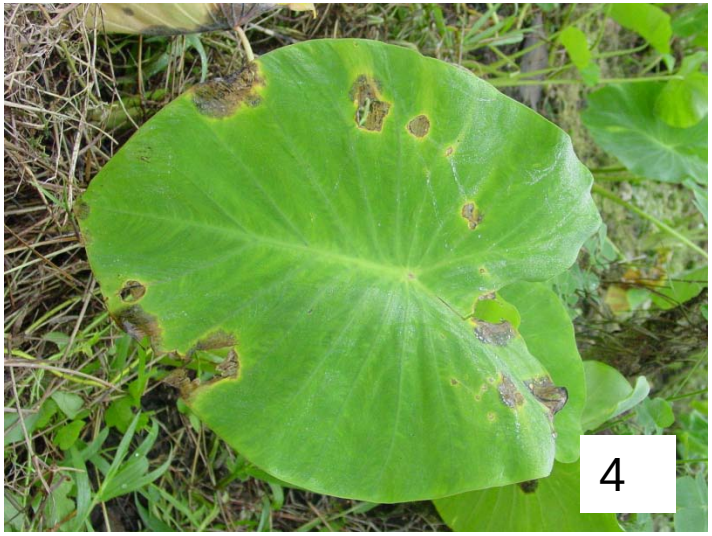
Plant Hosts for *Phytophthora gallica*	*Quercus robur* (English oak), *Salix alba* (white willow), *Alnus glutinosa* (alder), and *Fagus sylvatica* (European beech) are reported hosts for *P. gallica*.

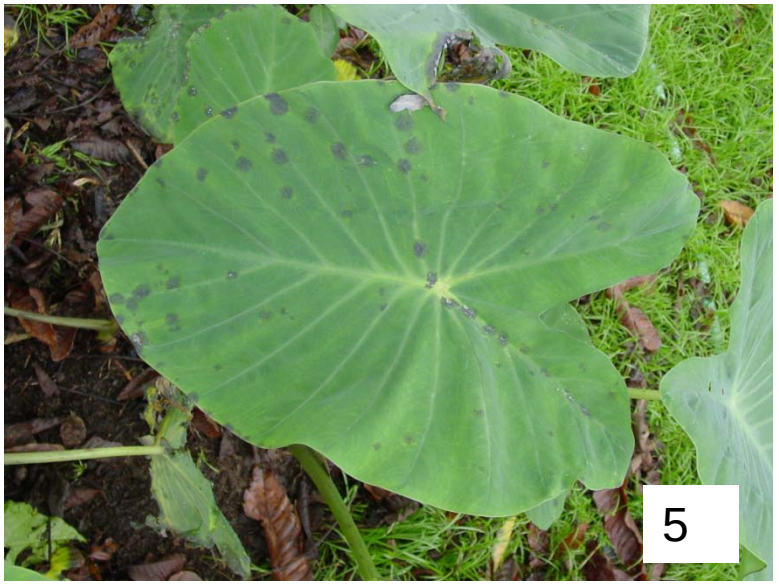
Taro leaf blight lesions caused by *P. colocasiae on* cultivars of *C. esculenta.*

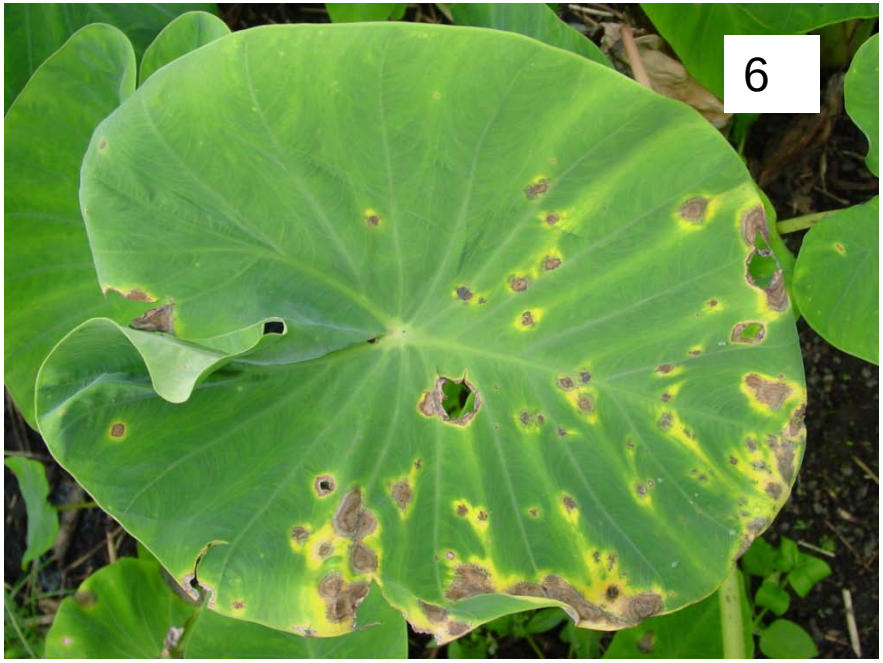
1. Taro leaf blight (TLB) lesion with silvery ring of sporangia
2. Target-like lesion
3. TLB lesions at 48 hours

Pictures courtesy of Fred Brooks

Taro leaf blight lesions caused by *P. colocasiae on* cultivars of *C. esculenta.*







4. Lesions with chlorotic halos
5. New lesions after a rain
6. Early lesion development

Pictures courtesy of Fred Brooks

Taro leaf blight lesions caused by *P. colocasiae on* cultivars of *C. esculenta.*

7. Plant exudate from young TLB lesion
8. Leaf damage

Pictures courtesy of Fred Brooks

Phytophthora idaei

Background Information for *Phytophthora idaei*

In 1987, raspberry plants from several cultivars raised from root cuttings at the Scottish Crop Research Institute (SCRI) were found to be infected with a *Phytophthora* spp. The plants had been used as uninoculated checks in a test to screen raspberry plants for resistance to *P. fragariae* var. *rubi* and had been placed under conditions suitable for the development of the disease. Although the check plants showed no aerial symptoms, the root systems of some of the plants showed up to 20 percent root rot, and numerous oospores were seen in the cortex of the root (Kennedy and Duncan, 1995). Papillate sporangia were observed after floating pieces of root for 2 to 3 days at 15^{o}C. Kennedy and Duncan (1995) formally described the species *Phytophthora idaei*.

History and Distribution for *Phytophthora idaei*

The pathogen is currently known only from England and Scotland.

Damage to Hosts by *Phytophthora idaei*

Root rot is observed with *P. idaei*, but no aerial symptoms are observed. Research at SCRI showed that a trend for reduced cane height was common to most cultivars for plants grown in the ground, not under greenhouse/ glasshouse conditions (Young *et al.*, no date).

Economic Impact and Ecological Range of *Phytophthora idaei*

Information is not available on the economic and ecological impact, but levels of root rot in stocks at the SCRI rarely exceeded 5 percent of the root system.

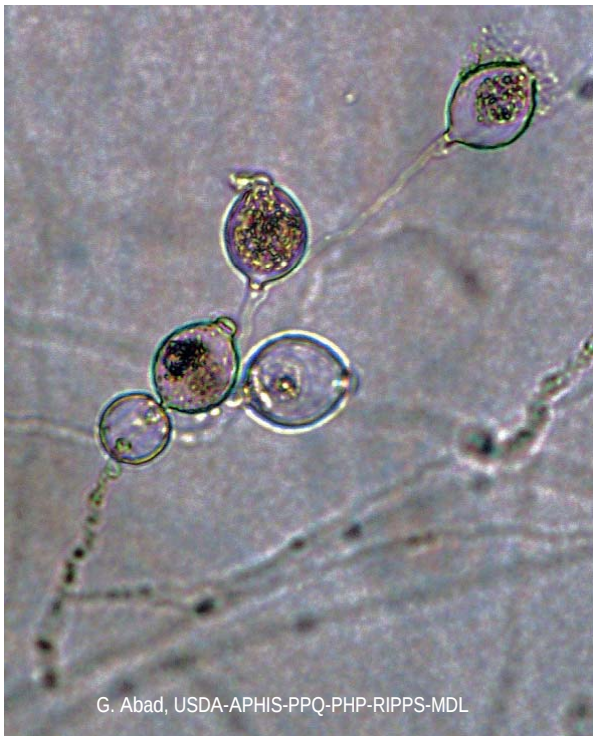
Life Cycle and Biology of *Phytophthora idaei*

The pathogen is potentially spread by cultivation of apparently healthy plants. The pathogen is homothallic with paragynous antheridia. Chlamydospores and hyphal swellings are not formed. Growth, sporangial formation, and oogonial and oospore formation are optimal at 20-22^{o}C.

Plant Hosts for *Phytophthora idaei*

Rubus idaeus (red raspberry) is the only known host for *P. idaei*.

Phytophthora idaei D.M. Kenn. 1995



Raspberry plants inoculated with *P.idaei* and *P. rubi*

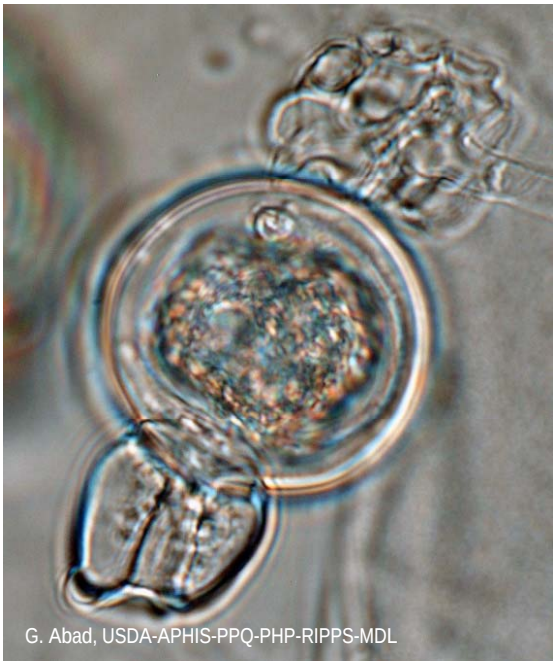
a. *P. idaei*
b. Control
c. *P. rubi*

Picture courtesy of David E.L.Cooke, Scottish Crop Research Institute

Phytophthora iranica

Background Information for *Phytophthora iranica*	*Phytophthora iranica* was first isolated and described in Varamin, Iran by Ershad (1971) from roots of eggplant. When artificially inoculated, tomato fruits, sugar beet roots, and potato tubers were susceptible, but eggplant, cucumber, sweet orange and apple fruits were not susceptible.
History and Distribution for *Phytophthora iranica*	Iran
Damage to Hosts by *Phytophthora iranica*	*P. iranica* was isolated from eggplant but was not pathogenic to eggplant by experimental inoculation (Ershad, 1971). *P. iranica* caused a soft rot of potato tubers, which turned pink when exposed to air. This symptom is also characteristic of pink rot of potatoes caused by *P. erthroseptica*.
Economic Impact and Ecological Range of *Phytophthora iranica*	Information is not available on the economic and ecological impact of *P. iranica.*
Life Cycle and Biology of *Phytophthora iranica*	The minimum temperature for growth is 10^{o}C, optimum 27.5^{o}C, and maximum 35^{o}C (Erwin and Ribeiro, 1996). Sporangia are papillate (sometimes with two papillae per sporangium); ovoid, obpyriform, ellipsoid to subspherical, 30 to 72 µm long x 22 to 51 µm wide (average 47.9 x 36.8 µm) (length-breadth ratio 1.3:1); and persistent on the stalk. Sporangiophores are short and sympodially branched (Ershad, 1971). Chlamydospores are rare; 17 to 41 µm in diameter, average 28.7 µm; mostly intercalary; and rarely terminal. Hyphal swellings are not formed. Oogonia are formed in a single culture (homothallic) and are subspherical and 21 to 45 µm in diameter, average 34 µm. Antheridia are mostly paragynous, but occasionally an amphigynous antheridium is found; oospores are aplerotic, 15 to 37 µm in diameter, average 29.3 µm; the oospore wall is 1 to 6 µm thick, average 3 µm (Ershad, 1971).
Plant Hosts for *Phytophthora iranica*	*Solanum melongena* (eggplant) (isolated from), *Solanum tuberosum* (potato), *Lycopersicon esculentum* (tomato), and *Beta vulgaris* (sugar beet) are reported hosts for *P. iranica*.

Phytophthora iranica Ershad 1971



Phytophthora italica

Background Information for *Phytophthora italica*

Phytophthora italica was originally described as *P. iranica*. It was isolated and proved to be pathogenic to roots of myrtle in Italy (Belisario *et al.*, 1993). Cacciola *et al.* (1996) compared the myrtle isolate with an authentic *P. iranica* isolate. The myrtle isolate could be distinguished from *P. iranica*, as well as *P. citricola* and *P. pseudotsugae* by morphological characteristics and by polyacrylamide gel electrophoresis of mycelial proteins and esterase.

History and Distribution for *Phytophthora italica*

Italy

Damage to Hosts by *Phytophthora italica*

P. italica causes root rot of myrtle.

Economic Impact and Ecological Range of *Phytophthora italica*

Information is not currently available on the economic and ecological impact of *P. iranica.*

Life Cycle and Biology of *Phytophthora italica*

Growth occurs between 10 and 35^{o}C, with optimum at 26^{o}C.

Sporangia are papillate (frequently bipapillate, sometimes with three papillae per sporangium); ovoid, obpyriform, subspherical or broadly ellipsoid, 14 to 56 µm long x 11 to 38 µm wide (average 39 x 29 µm) (length-breadth ratio 1.1:1.5); and persistent on the stalk. Sporangiophores are short and sympodially branched. Chlamydospores are rare. Oogonia are formed in a single culture (homothallic) and are subspherical, often slightly flattened or pyriform with short stalk and smooth walls. The diameter of oogonia is 15 to 29 µm, average 24 µm. Antheridia are paragynous and subspherical. Oospores are aplerotic, 11 to 23 µm in diameter, average 19 µm (Cacciola *et al.*, 1996).

Plant Hosts for *Phytophthora italica*

Myrtle communis is the primary host of *P. italica*. The pathogen is reported to be weakly pathogenic to tomato seedlings and apple fruit (brown discoloration results).

Phytophthora kernoviae

Background Information for *Phytophthora kernoviae*

Phytophthora kernoviae was first discovered in Cornwall in October 2003 during detailed surveys for *P. ramorum*. An unknown *Phytophthora* was isolated from a large (>1m^2) aerial bleeding lesion on a mature European beech, *Fagus sylvatica*, in a woodland in Cornwall, southwest England (Braiser *et al.*, 2005). The same *Phytophthora* was isolated concurrently from *Rhododendron ponticum* at another woodland site in the same area. The new *Phytophthora* was informally designated as "*Phytophthora* taxon C" and was officially named *P. kernoviae* by Brasier *et al.* (2005). The name is derived from Kernow, the Cornish name for Cornwall where the organism was first observed. A nursery in the northwest of England (Cheshire) was found to be contaminated with *P. kernoviae*, but all infected plants were destroyed, and the outbreak is considered eradicated.

The pathogen was first detected in 2006 in New Zealand (North Island), in two samples (soil and *Annona* spp. (cherimoya, custard apple). When collections and earlier studies of *Phytophthora* species were revisited, it was found that *P. kernoviae* was already occurring in stands in the 1950s to 1960s. At present, *P. kernoviae* has been detected at several sites in soil samples in the North Island has not caused extensive damage.

History and Distribution for *Phytophthora kernoviae*

England, Scotland, Ireland, and New Zealand

Damage to Hosts by *Phytophthora kernoviae*

Note: The full host range of *P. kernoviae* is not known and needs to be further investigated.

Symptoms of *P. kernoviae* include bleeding trunk cankers on beech that eventually girdle and kill the tree. Leaf spots, blight, and shoot dieback develop on foliage hosts where the pathogen sporulates. The pathogen is dispersed by rain to bole hosts, such as beech and oak, where cankers develop.

Tree hosts:

<u>*Fagus sylvatica* (beech):</u> Initial symptoms are bleeding lesions on the trunk found anywhere from ground level up to 12 meters. The bleeding is usually dark brown to blue-black, and similar to symptoms caused by *P. ramorum*. Underneath, orange-pink to pinkish-brown active lesions in the inner bark are visible. Sometimes girdling of the entire tree can occur. Older lesions appear sunken.

<u>*Quercus robur* (English oak):</u> Bleeding lesions on the trunk are similar to

those on *F. sylvatica* but may be more difficult to see both internally and externally due to the thick outer bark ridges and outer bark plates of oak. Bleeding can occur from cracks between the bark ridges. Older cankers may not become sunken as with beech.

Quercus ilex (Holm oak): Symptoms are entirely limited to severe necrotic leaf lesions and dieback of epicormic shoots (new shoots arising from near the base of the plant). There has been no evidence of sunken bleeding cankers on the stems of infected trees.

Liridodendron tulipifera (tulip tree): Disease symptoms occur on foliage, shoots and trunk. Multiple bleeding lesions are formed on the trunk from ground level up to 9 meters, and the bark becomes highly corrugated as a result. Internal lesions range in color from pale chocolate to dark chocolate to blue-black. Lesions tend to be limited in size (approximately 15 x 20 cm). Lesions can also develop on leaves; these are fairly restricted to leaf-tips (approximately 10-15 mm in length) and on the leaf margins. The necrotic tissue dries out to a dark black color. Shoot dieback also occurs, and infected shoots are defoliated.

Magnolia spp.: Distinctive symptoms are found on infected foliage with infection occurring anywhere on the leaf surface. Multiple infections are evident as numerous dark brown necrotic patches, giving leaves a spotty appearance. There is a tendency for these necrotic spots to merge and develop towards the center of the leaf (midrib), and as lesions become well developed, leaves are noticeably mottled. The mottling may have angular edges, and uninfected tissue between necrotic areas becomes chlorotic (yellowing). Infections that take place at the leaf margin and cause it to collapse and form a hard, dry rim. The petioles (leaf stalk) can be infected and disease often progresses along the base following petiole infection. Buds can also become diseased, turning light khaki-gray.

Shrub hosts:
Rhododendron spp: The majority of the findings of *P. kernoviae* have been on *R. ponticum* and rhododendron hybrids. Early leaf symptoms include a blackening of the leaf petiole that often extends into the base of the leaf. This necrotic (dead and dying) lesion may progress farther into the leaf tissue and in extreme cases affect the whole leaf. Occasionally, however, only blackening of the leaf tip is observed. Both old and young leaves appear to be affected equally. Leaves infected with *P. kernoviae* fall within a few weeks of infection, which is unusual for a *Phytophthora* infection of rhododendron. Shoot dieback and cankers can occur, which may girdle the stem tissue and cause leaves above the lesion to wilt. In severe infections, the whole bush may be killed. Leaf and stem infections can be found at any height or position on a rhododendron bush (Beales *et al.*, 2006).

Pieris spp. and *Michelia doltsopa*: Leaf blight symptoms similar to rhododendron are seen, with no obvious dieback or cankers. Infection on *Michelia doltsopa* is characterized by necrotic leaf lesions on the leaves that progress along the leaf margin and into the tissue of the leaf blade. Necrotic leaf tissue is characteristically a dark black-brown color. Lesions on leaves of *Pieris* spp. are typically a light tan to rusty brown color. Necrosis progresses directly towards the central (midrib) vein and along the vein, causing a visually striking leaf blight (Beales *et al.*, 2006).

Differences in pathogenicity have been observed between New Zealand and United Kingdom isolates of *P. kernoviae* (Widmer, 2008). On *Magnolia stellata* and *Rhododendron* 'Cunningham's White', New Zealand isolates showed higher necrosis than isolates from the United Kingdom.

Economic Impact and Ecological Range of *Phytophthora kernoviae*

Like *Phytophthora ramorum*, *P. kernoviae* presents a risk to both the forestry and nursery industries. In Europe, *Fagus sylvatica* is an important forest tree and is planted for amenity purposes. Rhododendrons are commonly grown ornamentals, but *R. ponticum* is considered an invasive plant in woodlands. Other ornamental hosts are valuable trees and shrubs. Comparative inoculations of *P. kernoviae* and *P. ramorum* on beech stems suggest that lesion development and tissue colonization are significantly more rapid for *P. kernoviae* than for *P. ramorum* (Brasier *et al.*, 2005). Thus, the rate of spread and mortality in woodlands is potentially greater for *P. kernoviae*, given that the more slowly colonizing *P. ramorum* has already proven to be a serious threat to invaded forests (Benson *et al.*, 2008). Potential hosts for *P. kernoviae* occur in both east and west coast forests of the United States.

Life Cycle and Biology of *Phytophthora kernoviae*

Further studies are needed on the biology and epidemiology of *P. kernoviae* since this is a newly described species of *Phytophthora.* Like *P. ramorum*, *P. kernoviae* causes widespread foliar necrosis and shoot dieback of the often dense understory rhododendrons. Also like *P. ramorum*, *P. kernoviae* has caducous sporangia (containing zoospores) and is most likely aerially or splash dispersed.

Under suitable conditions, asexual reproduction takes place, producing new sporangia. Numerous sporangia develop on the surface of leaf lesions during periods of leaf wetness. During periods of rain, sporangia are splashed or blown by the wind to other hosts, where motile zoospores are liberated. Zoospores presumably infect through the bark of beech and other tree hosts in a manner similar to those of *P. ramorum*. Long-distance spread can be accomplished by the movement of infected rhododendron, beech, and other hosts. *P. kernoviae* is apparently not a root pathogen, but it can be isolated from soil. Movement of soil, litter, or plant debris can also spread the disease. Spread of *P. kernoviae* within a woodland or drainage basin also can occur when propagules of the pathogen are introduced into the runoff water either directly or when associated host debris enters streams.

Sporangia are not formed on bleeding stem cankers, but are found on infected leaves, shoots, and fruit (Denman *et al.*, 2006). Canker hosts are, therefore, not considered important for subsequent pathogen dispersal, but may be important for pathogen survival. Additionally, *P. kernoviae* has been found to produce sporangia on asymptomatic foliage (Denman *et al.*, 2008).

Since the pathogen is homothallic, the possibility exists for the production of oospores on mycelium colonizing the phloem and xylem tissue in trunk canker lesions. Oospore production might provide a potential pathway for long-term survival when trees die and bark is shed; however, oospore production has yet to be documented in nature (Benson *et al.*, 2008). Chlamydospores have not been observed in the laboratory or under field conditions.

P. kernoviae has papillate, caducous to mouse-shaped sporangia (average size 39.6 x 25.6 µm, range 30 to 50 x 20 to 32 µm) and is homothallic with amphigynous antheridia approximately 11 x 10 µm and oogonia with an average diameter of 25 to 27 µm.

Plant Hosts for *Phytophthora kernoviae*

Annona cherimola (cherimoya/custard apple), *Castanea sativa* (European chestnut), *Drimys winteri* (winter's bark), *Fagus sylvatica* (beech), *Gevuina avellana* (Chilean hazelnut), *Hedera helix* (ivy), *Ilex aquifolium* 'Variegata' (variegated holly), *Liriodendron tulipfera* (tulip tree), *Lomatia myricoides* (river lomatia), *Magnolia* spp. (*M. amoena, brooklynensis, cylindrica, delavayi, kobus, liliflora, mollicomata, salicifolia, sargentiana, sprengeri, stellata,* and *wilsonii*), *Magnolia* Gresham hybrid 'Joe McDaniel', *Magnolia* Gresham hybrid 'Sayonara', *Magnolia* Leonard Messel, *Magnolia* x *soulangeana*, *Michelia doltsopa* (sweet michelia), *Pieris Formosa* (Wakehurst pieris), *Pieris japonica* (Japanese pieris), *Podocarpus salignus* (willow podocarp), *Prunus laurocerasus* (cherry laurel), *Quercus ilex* (Holm oak), *Quercus robur* (English oak), *Rhododendron* spp., *Rhododendron ponticum* (pontic rhododendron), *Sesquiadendron giganteum* (giant sequoia), and *Vaccinium myrtillus* (bilberry) are reported hosts of *P. kernoviae* (Defra, 2009).

A finding in New Zealand in the 1950s from below a stand of *Pinus radiata* has been shown by molecular analysis to be *Phytophthora kernoviae* (Defra, 2009).

Denman *et al.* (2006) list susceptibility of detached leaves to infection by *P. kernoviae. Liriodendron tulipfera* (tulip tree), *Magnolia solangeana*, and *Rhododendron catawabiense* showed high susceptibility.

Phytophthora melonis

Background Information for *Phytophthora melonis*

Phytophthora melonis was first isolated from Katsura (1968, 1976) from diseased cucumber (*Cucumis sativus*) plants in Japan. The new species was characterized by homothallism, amphigynous antheridia, semipapillate sporangia, and the production of chlamydospores. A similar disease was reported on cucumber and other cucurbits in mainland China, Taiwan, Iran, Egypt, Turkey, Korea, and India. Isolates from all of these countries were similar to *P. melonis* except the sporangia were non-papillate and proliferated internally; also, chlamydospores were not found. Isolates were considered heterothallic. The latter isolates have been identified as *P. melonis* or as *P. drechsleri*. One isolate causing cucumber blight in China was treated as a new species, *P. sinensis* (Yu and Zhang, 1982).

The original description by Katsura is erroneous (Erwin and Ribeiro, 1996). Ho *et al.* (2007) published a redescription of *Phytophthora melonis* to correct errors in the original description.

Ho and Jong (1986) considered *Phytophthora melonis* a synonym of *P. drechsleri* but this was not supported by molecular analysis (Cook *et al.*, 2000). Ho (1986) examined isolates of *P. melonis, P. sinensis*, and *P.drechsleri* from *Cucumis* spp. and suggested they were synonymous. Despite confusion, it is now clear that whilst *P. melonis* and *P. sinensis* may be synonymous, they are unrelated to *P. drechsleri* (Mills *et al.*, 1991; Cooke *et al.*, 2000).

History and Distribution for *Phytophthora melonis*

The current distribution for *P. melonis* includes: China, India, Iran, Japan, Korea, Taiwan

Damage to Hosts by *Phytophthora melonis*

Cucumber: *P. melonis* causes a late blight of leaves, stems, and fruits. Soon after heavy rains, infection is initiated in the collar region, which turns greenish brown and becomes soft. The stem eventually shrinks, and the plant falls over. Lesions on leaves become darker green than the rest of the leaf and slowly progresses toward the pedicel if moist conditions prevail. Infection on fruit is usually initiated at the tip, resulting in deeply depressed, dark green, water-soaked lesions. A soft rot of the fruit follows (Erwin and Ribeiro, 1996). Rapid wilting and death of the plants is also common.

Pointed Gourd: A fruit and vine rot is caused by *P. melonis*.

Pistachio: Gummosis, characterized by the exudation of gum from the crown, is observed. The tissues under the bark of the crowns, lower trunk of affected trees, and roots turn brown or dark brown and rot, often resulting in death of

the tree.

Economic Impact and Ecological Range of *Phytophthora melonis*

Little information is available on the ecological and economic impact of *P. melonis.* Since 1983, mortality was estimated as averaging 10-12 percent in pistachio in Iran (Mirabolfathy *et al.*, 2001). Losses have been on the increase. Due to rotting of cucurbit fruit, considerable yield loss would be expected.

Life Cycle and Biology of *Phytophthora melonis*

Phytophthora melonis was easily dispersed by zoospores and sporangia carried in irrigation water and via wind-blown rain splash in Taiwan (Lin and Wu, 1985). *P. melonis* is most active after heavy rains. Rain water accumulating around the stem of the plant results in invasion of the collar region. In Japan, the disease is most severe from the middle of May to the end of June (Erwin and Ribeiro, 1996).

Lin and Wu (1985) showed that soil moisture strongly influenced the survival of *P. melonis*. The fungus persisted only for four weeks in flooded soil. In soil with 6 percent or 20 percent moisture content, a condition that permitted soil aeration, however, the fungus could be detected from the infested soil for a period of 20 and 5 weeks, respectively.

Plant Hosts for *Phytophthora melonis*

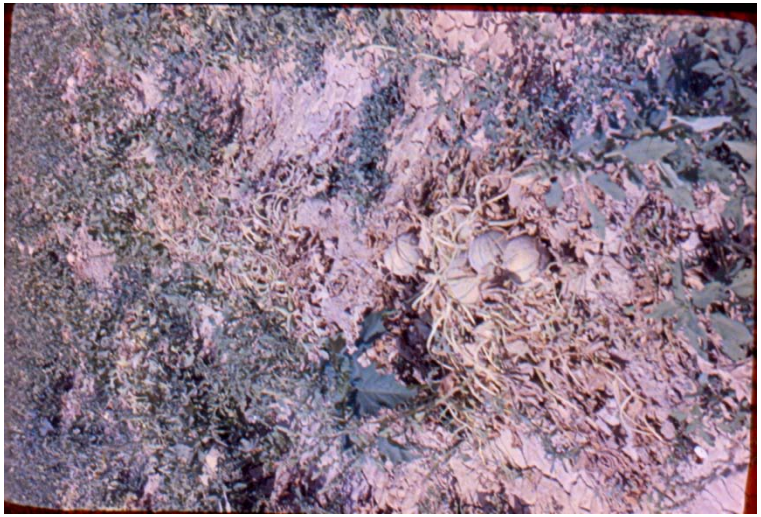
Cucumis sativus (cucumber), *Cucumis melo* (cantaloupe), *Citrullus lanatus* (watermelon), *Pistacia vera* (pistachio), *Trichosanthes dioica* (pointed gourd) are hosts for *P. melonis* (Guharoy *et al.*, 2006). *Lens culinaris* (lentil) was also identified as a host (Esmaili-Shirazifard and Banihashemi, 2008).

Banihashemi and Mirtalebi (2008) used safflower seedlings as a selective host to discriminate between the morphologically similar *P. melonis* and *P. drechsleri*. *P. melonis* could not infect safflower seedlings; while *P. drechsleri* from various hosts attacked safflower within a short period. At least six other *Phytophthora* species (*P. asparagi*, *cactorum*, *cryptogea*, *erythroseptica*, *palmivora*, and *quercina*) are capable of infecting safflower. This method should only be used once a morphological identity has been established. Additionally *P. melonis* does not induce pink rot symptoms in potato tubers unlike *P. drechsleri* (Mostowfizadeh-Ghalamfarsa *et al.*, 2005; Esmaili-Shirazifard and Banihashemi, 2008).

Symptoms of the disease caused by *P. melonis* in cantaloupes *Cucumis melo)*

Pictures courtesy of Somnath Bhattacharyya

Symptoms of the disease caused by *P. melonis* in Pointed gourd (*Trichutsanthes diocia* Roxb.)



Pictures courtesy of Somnath Bhattacharyya

Phytophthora multivesiculata

Background Information for *Phytophthora multivesiculata*

Ilieva *et al.* (1998) examined *Phytophthora* isolations from diseased *Cymbidium* orchid plants samples received from growers by the Dutch Plant Protection Service since 1991 that could not be assigned to a known species of the genus. The authors named a new species, *Phytophthora multivesiculata*, as the causal agent based on pathogen morphology and isozyme analysis.

History and Distribution for *Phytophthora multivesiculata*

P. multivesiculata is reported from the Netherlands, New Zealand (North Island), and Australia.

Note: The specimen from Australia is from a single herbarium specimen originally identified as *Phytophthora syringae*, which has now been re-identified as *P. multivesiculata* (Cunnington *et al.*, 2009).

Damage to Hosts by *Phytophthora multivesiculata*

The leafy parts of the plants and the pseuduobulbs (modified stems) are attacked. Infection appears on the leaves after prolonged periods of rain or on indoor plants after they have been irrigated. According to Hill (2004), the disease first appears as dark green lesions that expand rapidly under humid conditions to form large irregular patches of water-soaked tissue on mature leaves.

The appearance of leaf lesions often signals the presence of a more damaging form of the disease. Infectious propagules that reach the foliage may be washed down to the base of the plant and, if immature leafy shoots are present, down into the center of these growths. There, the immature leaf tissues are attacked and a severe internal rot rapidly develops. Initially the rot is not visible and, often, the first indication that infection is present is a change in the color of the leaves. Infected shoots become gray-green and rapidly lose turgor. Often by this stage, the infection has already spread through the attachment point of the shoot into the adjacent pseudobulb. If the plants are young and have only one or two pseuduobulbs, the infection may prove fatal. Infected young pseudobulbs have a distinctive internal, blue-black or purplish-brown discoloration and a sour odor. The roots do not become infected and remain grayish-white apparently unaffected even after the rest of the plant has turned brown (Hill, 2004).

According to Ilieva *et al.* (1998), dry rot of leaves (with a somewhat waxy looking surface) with a change of color to brown with typical horizontal zebra-like stripes, about 0.5 cm wide with lighter discoloration in the middle and a dark brown to black margin are observed on *Cymbidium* plants infected with *P. multivesiculata*. The base of the *Cymbidium* bulbs showed wet brown-black discolored tissue.

Economic Impact and Ecological Range of *Phytophthora multivesiculata*

Information on the economic and ecological impact is not currently available.

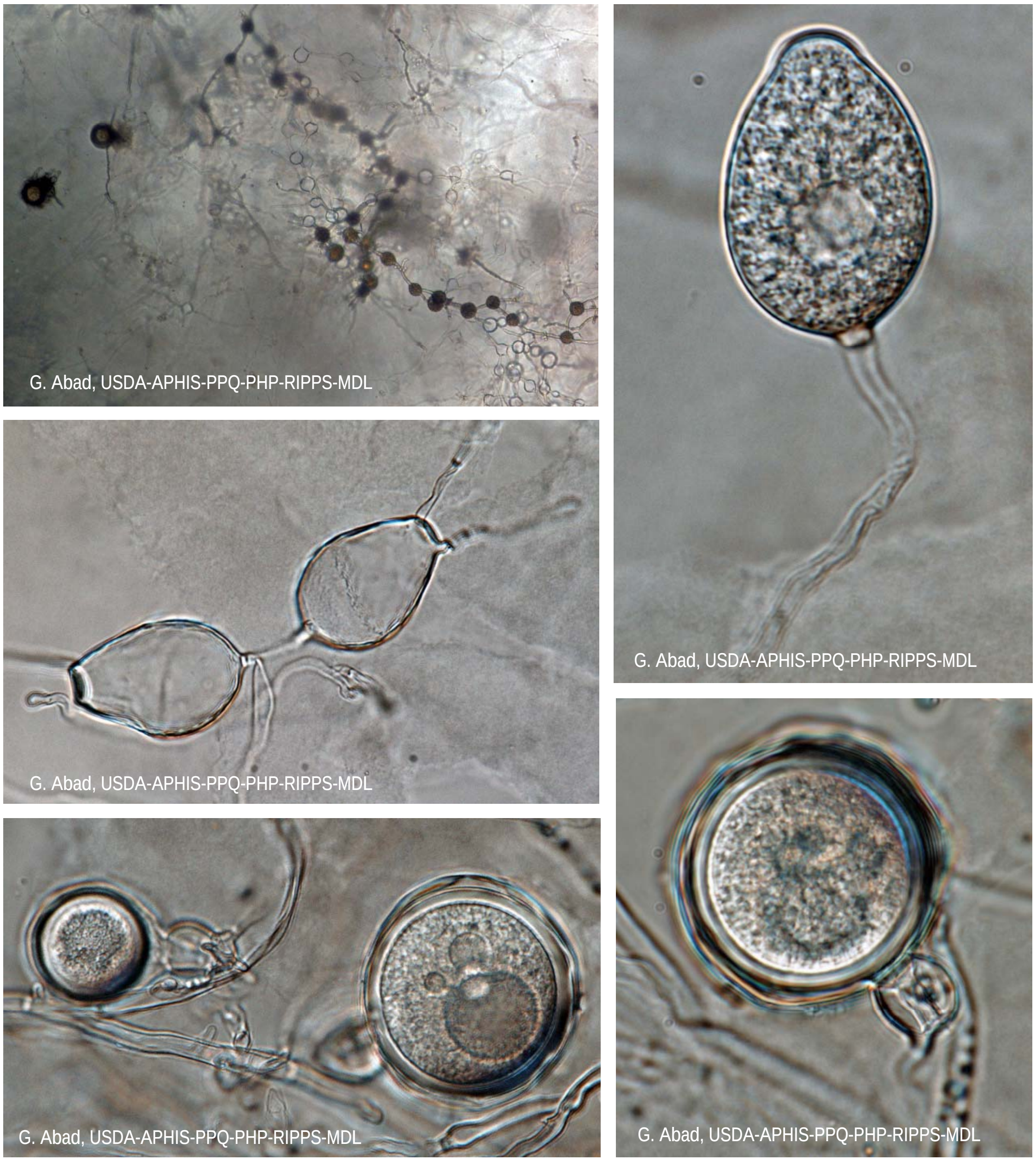
Life Cycle and Biology of *Phytophthora multivesiculata*

Hill (1982) observed that the *Phytophthora* spp. attacking *Cymbidium* plants in New Zealand tended to be more active and damaging in the late summer and autumn, when temperatures were at their seasonal highs. Ilieva *et al.* (1998) found that *P. multivesiculata* has a relatively fast growth rate at 20^{o}C and a high temperature for growth of 35^{o}C, the highest of any of the presently known Group IV *Phytophthora* species (Hill, 2004).

Plant Hosts for *Phytophthora multivesiculata*

Cymbidium spp. are the only reported hosts for *P. multivesiculata*.

Phytophthora multivesiculata Ilieva, Man in ‘t Veld, Veenbaas-Rijks et Pieters. (1998)



Phytophthora multivora

Background Information for *Phytophthora multivora*

As a result of wide scale forest quarantine and management of *Phytophthora cinnamomi* (an introduced species causing jarrah (*Eucalyptus marginata)* dieback) in Western Australia, extensive and regular testing of soil and plant tissue samples for *P. cinnamomi* at the Vegetation Health Service (VHS) laboratory of the Department of Environment and Conservation has led to the isolation of a large range of *Phytophthora* spp. and undescribed *Phytophthora* taxa.

In May and June 2007, *Phytophthora* isolates were recovered from the rhizosphere of declining *Eucalyptus gomphocephala*, *E. marginata*, and *Agonis flexuosa* in Yalgorup National Park. These isolates morphologically resembled *P. citricola* and had similar growth rates at 25^{o}C. Recent evaluation of the VHS culture collection using molecular techniques has identified most of these isolates as a new taxon (*Phytophthora* spp. 4) in the *P. citricola* complex (Burgess *et al.*, 2009). Phylogenetic analyses of the ITS and *cox*1 gene show that *Phytophthora* spp. 4 is unique and comprises a discrete cluster within the major ITS clade 2 of Cooke *et al.* (2000) with its present closest relative being *P. citricola.* Scott *et al.*, (2009a) formally described *Phytophthora* spp. 4 as the new species *Phytophthora multivora*.

History and Distribution for *Phytophthora multivora*

P. multivora is widespread in southwest Western Australia.

In GenBank, 11 ITS sequences, designated as *P. citricola*, are identical to *P. multivora*. Seven are from unpublished studies in Hungary, Canada, Switzerland, Korea, Japan, and three isolates are from Spain (Zea-Bonilla *et al.*, 2007; Moralejo *et al.*, 2009). In addition, an isolate designated as *P. sojae* in a study from Japan almost has an identical sequence (Villa *et al.*, 2006). Due to the widespread distribution of *P. multivora* across natural ecosystems in Western Australia, it is likely that Western Australia may be the source of dispersal, possibly via the nursery trade (Scott *et al.*, 2009a).

Damage to Hosts by *Phytophthora multivora*

Phytophthora multivora causes dieback symptoms and an overall decline.

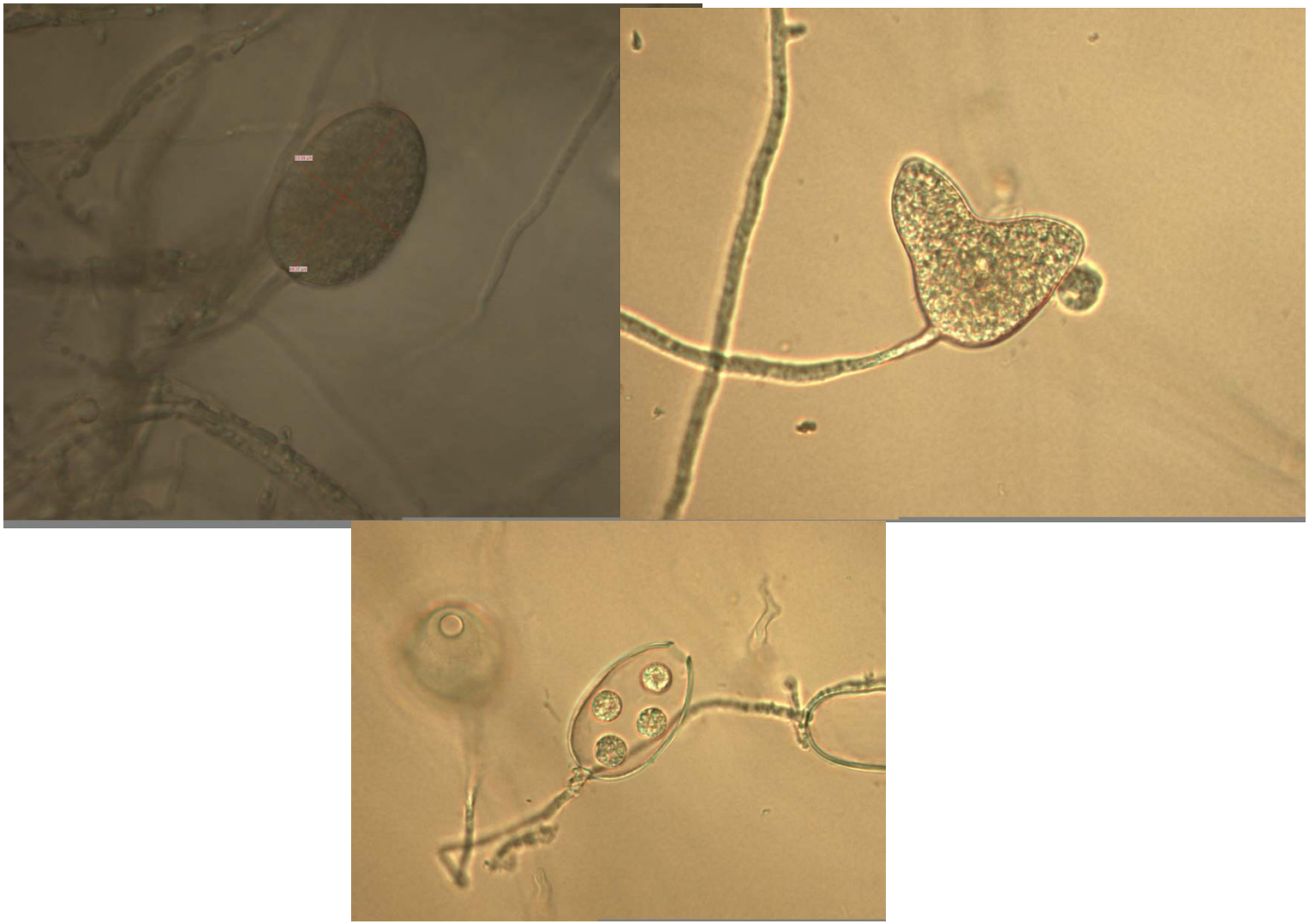
<u>*Eucaplyptus gomphocephala* and *E. marginata*</u>: Severe dieback and mortality are associated with *P. multivora*. Crown symptoms include thinning, clustering of leaves, and dieback of branches and parts of the crown.

<u>*Banksia attenuata*</u>: Sudden wilting and death due to the girdling of the collar. Tongue-shaped, orange-brown necrosis of the inner bark can be observed.

Economic Impact and Ecological Range of

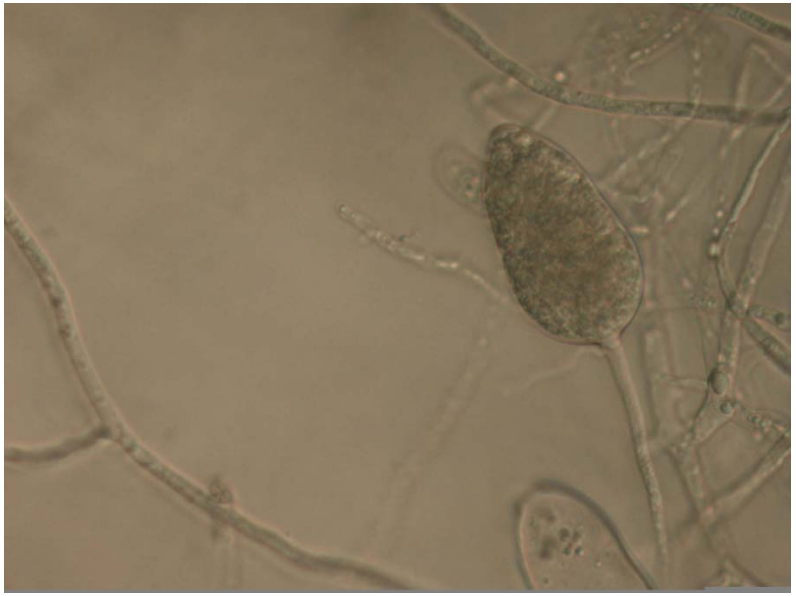
Phytophthora multivora, as its name indicates, can affect a wide range of species. Experts believe this pathogen could kill up to half of the plant species in Western Australia, equating to more than 3000 plant varieties (Mandurah Mail, 2009). Information on the economic impact is not currently

Images of *P. multivora*, sporangia



Pictures courtesy of Dr. Peter Scott

Images of *P. multivora*, sporangia



Pictures courtesy of Dr. Peter Scott

Phytophthora multivora available.

Life Cycle and Biology of *Phytophthora multivora*

Phytophthora multivora is the first record of a *Phytophthora* species associated with *Eucalyptus gomphocephala* decline and has been shown to proliferate on calcareous soils, believed to be suppressive to other *Phytophthora* species, including *P. cinnamomi*.

Initial results indicate that *P. multivora* attacks the fine roots of *E. gomphocephala* and may not colonize the stem collar under normal conditions. The high oospore wall index (ratio between the volume of the oospore wall and the volume of the whole oospore) indicates that *P. multivora* may be adapted to tolerate periods of prolonged drought (Scott *et al.*, 2009b).

Plant Hosts for *Phytophthora multivora*

Phytophthora multivora is pathogenic to *Eucalyptus gomphocephala* and *E. marginata* (jarrah) and is believed to be involved with the decline syndrome of both *Eucalyptus* species within the tuart woodland in south-west Western Australia.

Other (mostly native) Australian hosts include: *Agonis flexuosa* (peppermint tree), *Banksia attenuata* (candlestick banksia), *B. grandis* (giant banksia), *B. littoralis* (swamp banksia), *B. menziesii* (firewood banksia), *B. prionotes* (acorn banksia), *Bossiaea* spp., *Conospermum* spp., *Gastrolobium spinosum* (prickly poisonbush), *Leucopogon verticillatus* (tassle flower), *Patersonia* spp., *Pinus radiata* (Monterey pine - plantation), *Podocarpus drouyniana* (Drouyn's podocarpus), and *Xanthorrhoea gracilis* (graceful grass tree).

Based on sequence data, isolates from Spain on mango (*Mangifera indica*) are *P. multivora* (Zea-Bonilla *et al.*, 2007).

Phytophthora pinifolia

Background Information for *Phytophthora pinifolia*

Since 2004, a new disease of *Pinus radiata*, referred to 'Daño Foliar del Pino' (DFP) has appeared in the Arauco province of Chile and subsequently spread to other areas. Duran *et al.* (2008) officially name the pathogen *Phytophthora pinifolia*.

History and Distribution for *Phytophthora pinifolia*

In February 2004, unusual tree mortality appeared in a 6-year old *P. radiata* stand of about 70 ha on the Arauco coast of Chile. In October 2004 and in the same area, a serious needle blight disease was observed and associated with this mortality. The damage increased dramatically expanding to approximately 60,000 ha by the end of 2006 (Duran *et al.*, 2008).

Attempts to isolate the pathogen that caused the needle blight led to several

fungi, but upon inoculation these fungi did not reproduce the symptoms of the disease and were thought to represent opportunistic endophytes or secondary inhabitants.

Isolations from diseased needles in July 2007 on *Phytophthora* selective media consistently yielded a *Phytophthora* species. Pathogenicity of this *Phytophthora* species was confirmed and symptoms were reproduced on *Pinus radiata* seedlings (Duran *et al.*, 2008).

Phytophthora pinifolia is presently only known from Chile.

Damage to Hosts by *Phytophthora pinifolia*

The pathogen causes serious needle blight.

Mature Trees (up to 18-year-old): Symptoms begin with a reddening of the past year's needles in early winter. The first needles to display symptoms are those on the lower sides of the branches. Needles die and assume a distinctly gray color and begin to fall. Initially, dead and dying needles are retained on the branches giving the trees the appearance of having been severely scorched. Needles then fall from the trees, which can be almost entirely defoliated. New needle growth is not affected and the trees appear to recover until infection re-occurs in the following season. After two or three years of defoliation, trees occasionally die and this appears to be hastened due to infection by *Diplodia pinea* (Diplodia blight of pine), which is a well-known opportunistic pathogen (Duran *et al.*, 2008).

One of the earliest symptoms on affected needles is the emergence of dark resinous bands on the green needles, which appear transparent when viewed with backlighting. These bands are found at various positions, either close to the bases or higher up on the needles. Drops of resin are often found at the base of the needles and the tissue within the papery brachyblasts is commonly collapsed and has a light gray color. Needles often collapse from their bases just above the branches and hang at right angles from the branches. When the bark is removed, a distinct brown or reddish brown discoloration can be seen in the phloem and cambium, particularly where it is associated with dying needles.

Young Trees (up to one and 4-years-old): Symptoms appear different to those on mature trees. Damage to young trees is most common and most severe where stands occur alongside larger trees affected by DFP. One of the first and most obvious symptoms to appear on young trees is where young growing terminal shoots wilt and die rapidly. Lesions are typically found on these shoots or on the needles associated with them, depending on the time of the year. The damage resulting in the wilted growing shoots is typically found on parts of branches or shoots a short distance below the obviously affected tissue. Close inspection reveals needles with symptoms similar to those on older trees with infected bases leading to girdling of the stems. Dark

resinous bands can be found on the needles in the early stages of symptom development and infection appears to be concentrated within the papery brachyblasts at the base of the needle fascicles. On younger trees, necrosis of the cambium is more pronounced than that on mature trees and the impact of infection is commonly more severe. Resin can be found exuding profusely from the brachyblasts or the bases of the needles. Branches can have large numbers of needles hanging at right angles, apparently from their bases, and infections begin on the lower sides of branches. Removal of the outer bark reveals distinct lesions below the needle bases and in many cases, due to multiple infections, they coalesce to form cankers in the phloem and outer cambium. These cankers result in girdling of the stems or branches and wilting of the needles and shoots proximal to them. Naturally regenerated plants and newly planted seedlings are equally affected by DFP, and appear to wilt and die rapidly due to their small size (Duran *et al.*, 2008).

Economic Impact and Ecological Range of *Phytophthora pinifolia*

Forestry companies plant species of *Pinus*, *Eucalyptus*, and *Acacia*, non-native trees, in forestry plantations. Using these species, forestry companies produce solid-wood products and pulp for paper and rayon production (Duran *et al.*, 2008). Growth of these non-native trees is in many cases exceptional and typically far beyond that associated with the trees in the natural environment, which leads to greater economic value for these species. Daño Foliar del Pino is generally accepted in Chile to be the most serious problem yet to have affected pine forestry in the country (Duran *et al.*, 2008).

The United States produces nearly $67 billion in pine annually (NPAG, 2008). The introduction of *P. pinifolia* into the United States could have a devastating effect on the pine industry. With the United States importing $327 million in lumber and wood in the rough from Chile and exporting over $340 million in pine products in 2006, the establishment of *P. pinifolia* in the United States would have a great impact on pine products and trade in the United States (NPAG, 2008).

The discovery of an aerial *Phytophthora* spp. causing a serious disease of *P. radiata* in Chile, adds to a number of new and serious tree-infecting aerial *Phytophthora* spp. that have recently been discovered. Newly planted seedlings and naturally regenerated plants die if infected in the first year of growth, making *P. pinifolia* a serious threat to reforestation.

Life Cycle and Biology of *Phytophthora pinifolia*

The pine needle blight in Chile has a distinctly seasonal pattern of occurrence. Trees begin to show symptoms in early winter from about July onwards when the temperatures begin to drop to between 6 and 12°C. This is also the start of the rainy season and there appears to be a very close association of the disease with rainfall. A spatial analysis has shown that the southern slopes (higher humidity, most free water, and low solar radiation) are most severely affected (Duran *et al.*, 2008).

While the symptoms of DFP in Chile are consistent with the biology of an aerial *Phytophthora* sp., there are many questions regarding the biology of the pathogen that remain to be answered. While it is assumed that the sporangia are the infective propagules, this has yet to be shown experimentally. These structures were not abundant in culture and more natural conditions under which to produce them will need to be developed. Likewise, infection studies with zoospores and the infection biology and life cycle of the pathogen remain to be understood.

Plant Hosts for *Phytophthora pinifolia*

Pinus radiata (Monterey pine) is the only known host for *P. pinifolia*.

Phytophthora polonica

Background Information for *Phytophthora polonica*

In a survey of *Phytophthora* associated with alder decline in Poland, several isolates of a homothallic *Phytophthora* spp., which could not be assigned to other taxa including *Phytophthora alni* subspecies, were consistently recovered from rhizosphere soil samples. Based on morphology and sequence data, the *Phytophthora* was found to be a new species. Belbahri *et al.* (2006) described *P. polonica* as a new species of *Phytophthora*.

History and Distribution for *Phytophthora polonica*

Poland

Damage to Hosts by *Phytophthora polonica*

Phytophthora polonica was slightly pathogenic to alder twigs with tissue discoloration progressing a few millimeters beyond the inoculation wound after 10 days. The pathogenic status of *P. polonica* remains unclear and further research is necessary.

Economic Impact and Ecological Range of *Phytophthora polonica*

Information on the economic and ecological impact is not currently available.

Life Cycle and Biology of *Phytophthora polonica*

Little information is currently available on the life cycle and biology of *P. polonica*. *P. polonica* is characterized by moderate to slow growth rate of its colony on carrot agar at 20°C, high optimal (30°C), and maximum (38°C) growth temperatures, formation of catenulate, often lateral, hyphal swellings, large chlamydospores in agar media and in soil extract, persistent, ovoid, to ellipsoid nonpapillate sporangia, and large oogonia with paragynous and

sometimes amphigynous arrangement.

Plant Hosts for *Phytophthora polonica*

Alnus glutinosa (alder) is the only known host for *P. polonica*.

Phytophthora porri

Background Information for *Phytophthora porri*

Phytophthora porri was originally described as a pathogen of leek (*Allium porrum*), causing waterlogged areas followed by a whitening of the tips of leaves and other affected parts of this plant (Foister, 1931). Later, the pathogen was isolated principally from leek and other members of the Liliaceae and a few dicotyledonous hosts.

History and Distribution for *Phytophthora porri*

The pathogen is reported from Australia, Belgium, Canada, China, England, Greece, Ireland, Japan, Netherlands, Norway, Scotland, and South Africa (Taylor, 1965; Von Maltitz and von Broembsen, 1984; Erwin and Ribeiro, 1996). The reports from Canada on carrot, however, may be a distinct species (Stelfox and Henry, 1978; Ho, 1983).

France, Germany, Italy, New Zealand, Switzerland, and United States are also reported in the distribution for *P. porri*. This distribution is reported for *P. porri* from *Brassica* spp. The *Phytophthora* species from *Brassica* spp., however, is now included in *P. brassicae* (Man In't Velt *et al.*, 2002).

Damage to Hosts by *Phytophthora porri*

Allium spp. – Initially, yellowing and dying of the tips of leaves is observed, followed by this area turning white. The white area may measure half an inch to six inches long, and while most bend backwards, becoming disfigured by saprophytic fungi, some turn crisp, curl, and do not bend. Sometimes the apical attack is replaced by a marginal infection at any place from near the tip to about half-way down the leaf. As this area dies, the tissues contract and the leaf becomes twisted. Usually associated with these symptoms is a water-logged area developed half-way down or at the base of the leaf (Foister, 1931).

Campanula spp.: The crown region is gray-brown to chocolate brown; the discoloration spreads upwards into the leaf bases and flowering shoots and downwards into the roots. The upper parts of the roots are pale pink to brown and later turn dark brown and disintegrate, freeing the leafy rosette from the roots (Legge, 1951).

Dianthus caryophyllas (carnation): Collar rot is the primary symptom association with *P. porri*. Dark, wet lesions that later became gray and dry appear on the stalks (Kouyeas, 1977).

Gladiolus spp: Wet rot of the bottom leaves is seen, which leads to plant death (Kouyeas, 1977).

Lactuca sativa (lettuce): Diseased plants wilt and collapse completely within 2 to 4 weeks. Examination of wilted plants shows a dark, firm rot of stems that extends to the soil level upwards while the roots appear healthy (Sitepu and Bumbieris, 1981).

Economic Impact and Ecological Range of *Phytophthora porri*

Phytophthora porri is a serious disease of winter leek in Europe in intensive vegetable growing areas. The disease results in lower yields and loss of quality (marketable yield) (Dobson and Clarkson, 1989). Epidemics destroy more than 50 percent of the crop before January to April, when winter leek is harvested (CABI, 2006). Griffen and Jones (1977) reported yield losses of 30-50 percent in autumn-sown salad onions in the United Kingdom due to *P. porri*.

Life Cycle and Biology of *Phytophthora porri*

Phytophthora porri is homothallic. Oospores, and possibly chlamydospores which are very similar in shape, form in infected leaves, may enter the soil with leaf debris and survive the crop-free period from February till July, and probably longer. In autumn, some oospores may germinate immediately to form sporangia, which may release zoospores. Infections via oospore occur only on above ground plant parts (Smilde *et al.*, 1996a). Sporangia are not discharged easily by wind. The zoospores may be transported to the leaves of hosts by rain splash, thus triggering a new epidemic (Smilde *et al.*, 1996a). Prolonged dew periods and rain events (free water) have been shown to often precede the development of disease (Taylor, 1965; Smilde *et al.*, 1996a). Smilde *et al.* (1996b) reported oospore germination *in vitro*. Natural secondary spread of *P. porri* to neighboring rows was observed after 3 weeks in a leek field (Smilde and van Ness, 1992). There is no infection of the roots.

Phytophthora porri is characterized by non-papillate to semi-papillate sporangia, both amphigynous and paragynous antheridia, slow growth, low maximum growth temperatures, and coiling growth of hyphae (Man In't Veld *et al.*, 2002). Cardinal temperatures for mycelial growth of *P. porri* were <5 (minimum), 15-20 (optimum), and just above 25°C (maximum) (Smilde *et al.*, 1996b). The number of infections after zoospore inoculation of young leaf plants were relatively low at supra-optimal temperatures, but was not affected by sub-optimal temperatures. Even at 0°C, plants were infected. The incubation periods needed for symptoms formation were 36-57 days at 0°C, 13-18 days at 5°C, and 4-11 days at > 11°C (Smilde *et al.*, 1996b). Optimum and maximum temperatures for the *P. porri* isolate from lettuce were about 8 to 10°C lower than those from other hosts (Sitepu and Bumbieris, 1981).

Disease is patchy and is usually most severe in depressions where water

accumulates after rain or irrigation.

Plant Hosts for *Phytophthora porri*

Allium ascalonicum (shallot), *A. bakeri* (Chinese onion), *Allium cepa* (onion, shallot), *A. chinense* (rakkyo), *A. fistulosum* (Japanese bunching onion), *A. geyeri* (Geyer's onion), *Allium nipponicum* (Japanese garlic), *Allium porrum* (leek), *Allium sativum* (garlic), *A. tuberosum* (Chinese chive), *A. wakegii, Campanula persicifolia* (peach-bells), *Chrysanthemum* spp., *Daucus carota* subsp. *sativus* (carrot), *Dianthus caryophyllus* (carnation), *Gladiolus* spp., *Hyacinthus orientalis* (hyacinth), *Lactuca sativa* (lettuce), *Parthenium argentatum (*guayule), *Pastinaca sativa* (parsnip), *Rosa* spp. (rose), and *Tulipa* spp. (tulips) are reported hosts for *P. porri*.

The reports from Canada on carrot, however, may be a distinct species (Stelfox and Henry, 1978; Ho, 1983).

Isolates from the *Brassica* spp. are now referred to as a new species *Phytophthora brassicae* (Man In't Veld *et al.*, 2002).

Isolates of *P. porri* appear to be host specific. Isolates from *Allium* were only weakly pathogenic to cabbage and vice versa (De Cock *et al.*, 1992). Isolates from *Campanula persicifolia* and leek failed to cross-infect (Legge, 1951). Also isolates from carnation did not infect leek and vice versa. The isolate from lettuce, described by Sitepu and Bumbieris (1981) was not pathogenic to either leek or cabbage. Tichelaar and von Kesteren (1967) found that an isolate from onion could not infect leek.

Symptoms of disease caused by P*. porri* in leek (*Allium porrum)*

Pictures courtesy of Dr. Diederik Smilde

Phytophthora primulae

Background Information for *Phytophthora primulae*

In May 1949, diseased specimens of polyanthus (*Primula polyantha*) and *Primula* of an unknown species were received at the Ministry of Agriculture and Fisheries' Plant Pathology Laboratory, Harpenden, from Lewes, Sussex (England). Later that same month, polyanthus plants showing the same symptoms were received from Cornwall. All plants were infected by a species of *Phytophthora* (Tomlinson, 1952). Tomlinson (1952) officially described *Phytophthora primulae* as a new species.

History and Distribution for *Phytophthora primulae*

England, Denmark, Germany, Greece, Netherlands, New Zealand, and Scotland (Elena and Grigoriou, 2008).

Damage to Hosts by *Phytophthora primulae*

Primula spp: The symptoms of the disease closely resemble those of the Red Core disease of strawberry. Diseased plants are characteristically dwarfed, and the leaves are wilted and collapsed in succession from the outside to the center of the rosette. The wilt and collapse symptoms are observed mainly in the late spring (late April to May) in England. There is a poorly developed root system with a conspicuous absence of fine lateral roots. Some of the main roots were decayed backwards from the tip, giving a 'rat-tail' effect and affected roots, when cut longitudinally, showed a complete discoloration of the vascular tissue as in strawberry Red Core, although the discoloration was brown. In earlier stages of root infection, some roots may be healthy except for partial or general watersoaking and decay of laterals; while other roots may show partial loss of laterals and partial discoloration of the stele. The steles of old infected roots contain large numbers of oospores (Tomlinson, 1952).

Parsley: Symptoms include root and crown rot, chlorosis, and wilting of leaves (Elena and Grigoriou, 2008). Patches of stunted plants with yellowing foliage may be the first indication of a crown rot problem (Bennison and Green, n.d.). When the roots and crown are cut open, a brown discoloration is present, which becomes darker as the infection progresses. The outer cortex of the root may easily slough away. Severe infection results in foliage collapse and eventual plant death.

Experimental Hosts: Pathogenicity to apple was demonstrated using wound inoculation and resulted in a brown, circular rot spreading from the wound (2.5 cm in diameter after 10 days at laboratory temperature). All plants of tomato developed a dark lesion on the stem around the site of inoculation and the part of the plant above it collapsed and died (Tomlinson, 1952).

Economic Impact and Ecological Range of *Phytophthora primulae*

Polyanthus is cultivated commercially out-of-doors in England, mainly in the southern counties, and for the cut-flower trade. In one nursery, Tomlinson (1952) noted that *P. primulae* was causing disease in several thousand plants and was 'becoming a menace'. Bennison and Green (n.d.) report that parsley root and crown rot caused by *P. primulae* is the most common *Phytophthora* disease of herbs in the United Kingdom. Specific information on the economic and ecological impact of *P. primulae*, however, is not currently available.

Life Cycle and Biology of *Phytophthora primulae*

The optimum, minimum, and maximum growth temperatures of the polyanthus fungus were respectively 15-20°C, slightly above freezing point, and slightly below 27°C. At 17°C, *P. primulae* produces smooth hyphae and abundant sexual organs, whereas at 24°C, hyphal swellings occur and sexual organs are absent. By comparison with other species of *Phytophthora* and *Pythium*, the fungus appears to be a 'slow-growing' organism with a preference for low temperatures (Tomlinson, 1952).

Tomlinson (1952) observed that the fine lateral roots became affected first after being dipped in a zoospore suspension of the *P. primulae* for 24 hours. Brown discolored steles were detected in the roots after 2-3 weeks, first near the tip of root and spreading towards the main root. It seemed unlikely that the fungus gains entry into the steles of the main roots via the cortical tissues, but it appeared to reach them only after infection and growth through the lateral roots.

In England, Tomlinson (1952) found that the source of inoculum of *P. primulae* in one nursery originated in the seedling boxes. The fungus was carried over during planting operations in soil adhering to the roots or in the roots of slightly affected young plants. The disease is usually associated with poor drainage, especially in overwintered crops (EPPO, 2000).

Plant Hosts for *Phytophthora primulae*

Primula spp. (polyanthus, primrose) and *Petroselinum crispum* (parsley) are reported hosts for *P. primulae.*

Elena and Grigoriou (2008) showed that the parsley isolate of *P. primulae* was fairly host specific to parsley. Inoculations of tomato, lettuce, cauliflower, broccoli, red cabbage, white cabbage, leek, Brussels sprout, carrot, potato, onion, and *Primula acaulis* with the parsley isolate of *P. primulae* did not result in disease development. Apple fruits developed a brown rot from the wound to the center.

Experimental hosts included: *Lycopersicon* spp. (tomato) and *Malus sylvestris* (crab apple). The pathogen was not pathogenic to potato tubers, onion bulbs, and cultivated or wild strawberries (Tomlinson, 1952).

Phytophthora psychrophila

Background Information for *Phytophthora psychrophila*

When a baiting procedure was preformed on soil collected beneath declining mature *Quercus robur*, *Q. petraea*, and *Q. ilex* from Germany and Southern France, an undescribed *Phytophthora* spp. was discovered. Jung *et al.* (2002) describe this species as *Phytophthora psychrophila* and two additional *Phytophthora* species from European oak forests.

History and Distribution for *Phytophthora psychrophila*

Phytophthora psychrophila is present in France and Germany

Damage to Hosts by *Phytophthora psychrophila*

The pathogen is associated with oak decline in Europe. Aboveground symptoms of oak decline include dieback of branches and parts of the crown, formation of epicormic shoots, high transparency of the crown, yellowing and wilting of leaves, and tarry exudates from the bark. All symptoms are indicative of water stress.

In soil infestation tests with *Quercus robur* seedlings, *P. psychrophila* was nearly non-pathogenic with damage not significantly different from the control inoculations (Jung *et al.*, 2002). The authors attributed this to incubation temperatures higher than optimum (18-22°C). In another soil infestation test with an incubation temperature of 12-20°C during the growing season and a 3-month winter period at 5°C, three isolates of *P. pyschrophila* were able to produce necrotic bark lesions on suberized tap roots of some *Q. robur* seedlings. *P. psychrophila* was unable to cause decay in apple fruits (Jung *et al.*, 2002).

Economic Impact and Ecological Range of *Phytophthora psychrophila*

The ecological role of *P. psychrophila* in the forest community remains unclear, but it is likely to be involved in the complex interactions between *Phytophthora* fine root damage, site conditions, climatic perturbations, anthropogenic nitrogen input into forest soils, insect defoliations, and secondary attacks by parasites and pathogens, which are considered to be responsible for the widespread decline of oaks throughout Europe. Specific information on the economic and ecological impact of *P. psychrophila* is not currently available (Jung *et al.*, 2002).

Life Cycle and Biology of *Phytophthora psychrophila*

Phytophthora psychrophila was isolated from non-hydromorphic soils. The optimum temperature for growth was 15-17°C. The maximum temperature for growth was slightly below 25°C. With its low cardinal temperatures and the limitation of successful isolations to the cool season, *P. psychrophila* is a typical low-temperature species, well-adapted to activity in the winter and springtime, and not active during the summertime (Jung *et al.*, 2002).

Plant Hosts for *Phytophthora psychrophila*

Quercus spp. are the only known hosts of *P. psychrophila.*

Phytophthora quercina

Background Information for *Phytophthora quercina*

European oaks (*Quercus robur* and *Q. patraea*) have experienced waves of mortality due to unknown causes since the 1980s. In the mid-1990s, a range of root infecting *Phytophthora* species were shown to be associated with classic oak dieback, including the newly described *Phytophthora quercina*. Several studies demonstrated a link between this newly detected pathogen and the dieback, including Jung *et al.* (1999) in Germany and Vetttraino *et al.* (2002) in Italy. Molecular evidence supports the designation of *P. quercina* as a distinct species (Cooke *et al.*, 1999).

Jung *et al.* (2000) showed that at least two different complex diseases are referred to under the name of 'oak decline'. On sites with a mean soil pH 3.5 or greater and sandy-loamy to clayey soil texture, *Phytophthora* species were commonly isolated from rhizosphere soil, and highly significant correlations existed between crown transparency and various root parameters. In contrast, in stands with sandy to sandy-loamy soils and a mean soil pH of 3.9 or less, *Phytophthora* species were not found. Usually a combination of abiotic stresses such as drought, frost, and soil compaction along with biotic factors such as *P. quercina* and several other *Phytophthora* spp. that have commonly been isolated from small necrotic roots of declining trees are involved in 'oak decline'.

History and Distribution for *Phytophthora quercina*

The current distribution of *P. quercina* includes: Austria, Belgium, England, France, Germany, Hungary, Italy, Luxemburg, Serbia and Montenegro, Scotland, Sweden, Turkey, and the United Kingdom (Hansen and Delatour, 1999; Jung *et al.*, 1999; Balcý and Halmschlager, 2002a,b; Jönsson *et al.*, 2003a).

Although there is a report of this species from Missouri (Schwingle *et al.*, 2007b), morphological and ecological differences separate these isolates from *P. quercina* in Europe. This species is not considered to be established in the conterminous United States.

Damage to Hosts by *Phytophthora quercina*

The pathogen is involved in oak decline in Europe. Aboveground symptoms of oak decline include dieback of branches and parts of the crown, formation of epicormic shoots, high transparency of the crown, yellowing and wilting of leaves, and tarry exudates from the bark. All symptoms are indicative of water stress.

The pathogen causes disease of the tree's fine feeder roots. Apart from dieback of nonsuberized and suberized roots, the pathogen has been shown to

cause abnormal root branching (Jung *et al.*, 1996b) and to produce elicitins (proteins that may cause necrosis of the leaves and induce yellowing and wilting of the infected host plant) (Heiser *et al.*, 1999; Brummer et al, 2002). Loss of roots leads to yellowing, crown thinning, wilting of leaves and eventual death of the trees. In pathogenicity tests under artificial conditions, *Q. robur* seedlings showed severe dieback, root necrosis, and leaf necrosis (Jung *et al.*, 1999). *P. quercina* has also been shown to cause substantial reductions in the fine-root length of mature *Q. robur* trees under natural conditions (Jönsson, 2004; Jönsson *et al.*, 2003b; Jönsson-Belyazio and Rosenbren, 2006).

Economic Impact and Ecological Range of *Phytophthora quercina*

The extent of economic damage caused by *P. quercina* and oak decline in Europe is not known. The organism is one of many possible biotic and abiotic contributors to an environmentally and economically damaging forest condition known to occur in Europe since the 1980s (Cree, 2005). *P. quercina* has been shown, however, to be pathogenic to at least some European *Quercus* species.

Life Cycle and Biology of *Phytophthora quercina*

Phytophthora quercina has been isolated from roots and from rhizosphere soil from oaks exhibiting symptoms of oak decline. The exact role of the fungus in oak decline has yet to be determined. *P. quercina* was described only recently by Jung *et al.* (1999) and very little is known about its biology. Inoculation of roots of oak seedlings with zoospores of the fungus resulted in dieback of unsuberized and suberized long roots, and distinct, sometimes girdling necroses on suberized roots, which mostly developed from infection of lateral roots (Jung *et al.*, 1999). Microscopic examination of infected roots revealed the presence of hyaline, non-septate, irregular to corraloid hyphae, and thick-walled oospores. The fungus is homothallic. Optimum growth in culture has reported to occur at 20°C (Jung *et al.*, 1999) and 25°C (Barzanti *et al.*, 2001), although it is able to grow at temperatures as high as 27.5°C (Barzanti *et al.*, 2001). The thick-walled oospores of *P. quercina* are capable of surviving unfavorable conditions for several years and not very sensitive to the fungicide metalaxyl (Jung, 2003a,b).

Phytophthora quercina is quite adaptable with regard to site conditions and can be found at dry sites that normally do not favor the survival of *Phytophthora* species (Balcý and Halmschlager, 2002b). Jönsson *et al.* (2003a) found that *P. quercina* was the most frequently found species in acidic soils (pH 3.5-5.5) in Sweden but did not occur in soils less than pH 3.5.

Phytophthora quercina has been found in nurseries in Germany and France, *P. quercina* is unlikely to be detected during regular nursery inspections since the period that oak plants for forestry use are growing in well-drained nursery beds (2-3 years) is too short for developing extensive fine root damage that would lead to above ground symptoms. Moreover, the fine root

symptoms caused by *P. quercina* are not readily distinguishable for those caused by other *Phytophthora* species. *P. quercina* is a soilborne pathogen and it is reasonable to conclude that soil, plant products contaminated with soil, or infected nursery stock could introduce *P. quercina* into new areas. Soil particles adhering to logging machines, machines for construction of forest roads, and trecking boots can also spread the pathogen. *P. quercina* spreads within infested stands via zoospores and surface water.

Cooke *et al.* (2005), after conducting AFLP analyses of European populations of *P. quercina*, suggested that the pathogen was recently introduced into Europe and has been most likely spread via forestry practices and plant trade due to the limited genetic diversity (within and between sites) and lack of genetic substructuring according to geographic origin or host species. The two subgroups that were distinguished may reflect an initial introduction of isolates with two different genetic backgrounds.

Plant Hosts for *Phytophthora quercina*

Oak (*Quercus cerris*, *Q. hartwissiana*, *Q. frainetto*, *Q. ilex*, *Q. robur*, *Q. petraea*, *Q. pubescens*, *Q. suber*, and *Q. vulcanica*) are reported hosts for *P. quercina*.

Phytophthora tentaculata

Background Information for *Phytophthora tentaculata*

Kröber and Marwitz (1993) first isolated *Phytophthora tentaculata* from the roots and stems of greenhouse-grown *Chrysanthemum frutescens*, C. *leucanthemum*, *Delphinium ajacis*, and *Verbena*. The pathogen was named as a new species based on morphological differences from established species.

History and Distribution for *Phytophthora tentaculata*

The current distribution of *P. tentaculata* includes: China, Germany, Italy, Netherlands, and Spain.

Damage to Hosts by *Phytophthora tentaculata*

African Daisy: The pathogen causes blighted leaves and crown and stem rot (Cristinzio *et al.*, 2006).

Aucklandia lappa: Symptoms of *P. tentaculata* include stalk rot, wilting, plant death (Meng and Wang, 2008).

Chrysanthemum spp., *Delphinium ajacis*, *Verbena* spp.: *P. tentaculata* causes root, collar, and stalk rot (Kröber and Marwitz, 1993; Moralejo *et al.*, 2004).

Oregano: Symptoms include leaf russeting and chlorosis, wilt, defoliation, and dieback of twigs, browning and rot of the basal stem, root rot, and subsequent collapse of the entire plant (Martini *et al.*, 2009).

Santolina chamaecyparissus: Root rot and brown, wilted, and dead foliage have been observed after infection by *P. tentaculata* (Alvarez *et al.*, 2006).

Economic Impact and Ecological Range of *Phytophthora tentaculata*

Little information is available on the economic impact and ecological range of *P. tentaculata*. The pathogen is a problem in nurseries and the host range continues to expand. Presence of *P. tentaculata* in the United States would likely cause severe economic impacts to the nursery trade, as well as environmental impacts on native species. In Italy, root and basal stem rot caused by *P. tentaculata* is the most serious soilborne disease of oregano reported (Martini *et al.*, 2009). Approximately 80 percent of infected plants die within 30 days after the appearance of symptoms in the canopy. Stock plants were also affected.

Life Cycle and Biology of *Phytophthora tentaculata*

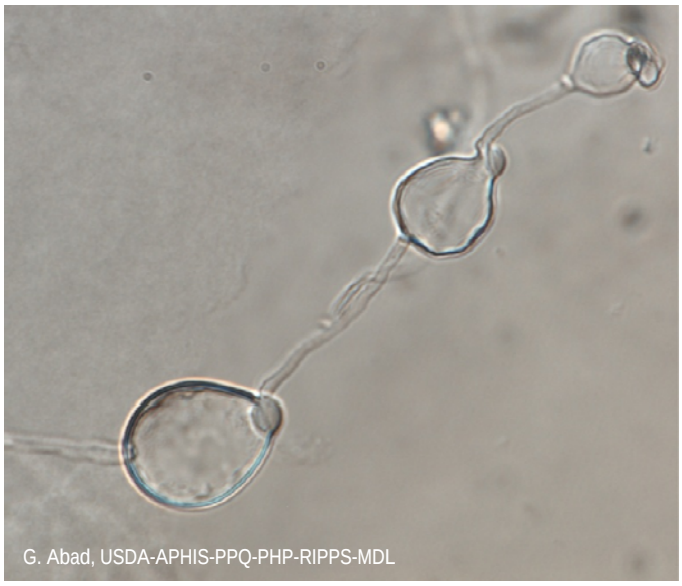
Little information about the lifecycle and biology of *P. tentaculata* is currently available outside of the original species description. The minimum temperature reported for growth is 7°C, optimum 15 to 25°C, and maximum 32°C. Mycelium of *P. tentaculata* is arachnoid (like a spider web), papillate sporangia are formed, chlamydospores and hyphal swellings are observed, antheridia are paragynous, and the pathogen is homothallic.

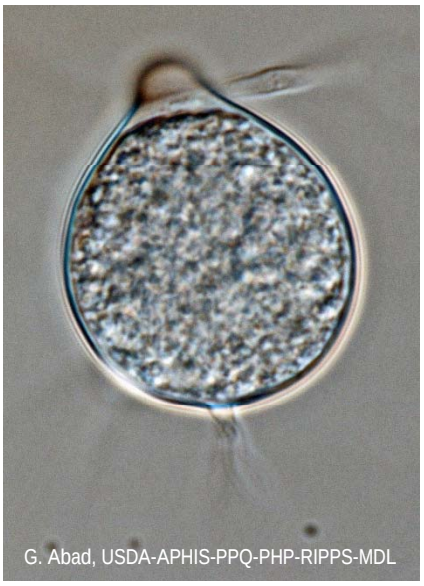
P. tentaculata may be moved via infected nursery stock. Meng and Wang (2008) speculate introduction of the pathogen into China via seed.

Plant Hosts for *Phytophthora tentaculata*

Aucklandia lappa, *Chrysanthemum* spp. (*frutescens*, *leucanthemum*), larkspur (*Delphinium ajacis)*, African daisy (*Gerbera jamesonii*), oregano (*Origanum* spp.), *Verbena*, and lavender cotton (*Santolina chamaecyparissus).*

Phytophthora tentaculata Kröber & Marwitz (1993)





Phytophthora uliginosa

Background Information for *Phytophthora uliginosa*

When a baiting procedure was performed on soil collected beneath declining mature *Quercus robur* and *Q. petraea* from Poland and Germany, an undescribed *Phytophthora* spp. was discovered. Jung *et al.* (2002) describe this species as *Phytophthora uliginosa* and two additional *Phytophthora* species from European oak forests. *P. uliginosa* is reported as "*Phytophthora* spp. 5" in other works.

History and Distribution for *Phytophthora uliginosa*

Phytophthora ulginosa has been reported from Poland and Germany.

Two unknown non-papillate homothallic *Phytophthora* isolates from oak forests in Austria showed all the characteristic features of *P. uliginosa* and the size of the oogonia and sporangia as well as their growth rates on V8 agar fell within the range of species as described; they can therefore be assigned to *P. uliginosa* (Jung *et al.*, 2002).

Damage to Hosts by *Phytophthora uliginosa*

The pathogen is associated with oak decline in Europe. Aboveground symptoms of oak decline include dieback of branches and parts of the crown, formation of epicormic shoots, high transparency of the crown, yellowing and wilting of leaves, and tarry exudates from the bark. All symptoms are indicative of water stress.

With a mean root rot of 43 percent, a mean reduction in fine root length and number of fine rot tips of 44 and 48 percent respectively, and dieback of the tap roots of most seedlings in a soil infestation test, *P. uliginosa* was pathogenic to *Quercus robur* (Jung *et al.*, 2002).

Economic Impact and Ecological Range of *Phytophthora uliginosa*

The extent of economic damage caused by *P. uliginosa* and oak decline in Europe is not known. The organism is one of many possible biotic and abiotic contributors to an environmentally and economically damaging forest condition known to occur in Europe since the 1980s. *P. uliginosa* has been shown, however, to be pathogenic to at least some European *Quercus* species.

Life Cycle and Biology of *Phytophthora uliginosa*

Phytophthora uliginosa is homothallic with paragynous antheridia and has non-papillate sporangia with a shallow apical thickening. The type isolate produced oogonia rarely after isolation from forest soil, but abundantly after reisolation from fine roots of artificially inoculated *Q. robur*.

The optimum temperature for growth was 17-18°C. The maximum temperature for growth was slightly below 29°C (Jung *et al.*, 2002).

This species may be restricted to soils with permanently or seasonally high water tables, and was in two out or three stands isolated together with a *Saprolegnia* species, a freshwater mold. *P. uliginosa* was only recovered in the springtime.

Plant Hosts for *Phytophthora uliginosa*	Oaks (*Quercus robur*, *Q. patraea*) are reported hosts for *P. uliginosa*.

CONTENTS

SURVEY PROCEDURES

Introduction

Plant regulatory officials conduct detection, delimiting, and monitoring surveys. Detection surveys are performed to ascertain the presence or absence of a pest in an area where it is not known to occur. Delimiting surveys are performed to define the extent of an infestation. Monitoring surveys are performed to determine the success of control or mitigation activities conducted against a pest.

Use this chapter as a guide to conducting a survey for *Phytophthora* spp. in the natural environment and nurseries.

Precautions for Inspectors

Take the following precautions before starting a survey:

Pesticide Applications

Before starting inspections, always determine whether there have been recent pesticide applications that would make it unsafe to inspect the nurseries. Check with property owners or nursery managers for this information. Look for posted signs indicating recent pesticide applications, particularly in commercial operations.

Quarantines

Determine whether any quarantines for other forest or nursery pathogens, such as *Phytophthora ramorum*, are in effect for the area being surveyed. Comply with any and all quarantine requirements.

Private Property
Obtain permission from the landowner before entering a new property. See **Regulatory Procedures on page 5.1** for pertinent information.

Sanitation
When visiting commercial operations to conduct surveys or to take samples, everyone, including regulatory, officials must take strict measures to prevent contamination by plant pathogens and other pests between properties.

All activities that involve entry into a property or nursery where disease is suspected present a danger of inadvertent spread of the disease. Before entering a new property, make certain that footwear is clean and free of soil, or use disposable boots to avoid moving soilborne pests from one property to another. Disposable gloves must be worn or hands washed thoroughly with soap and water before exiting each field or garden. Hands must be washed on site in order to avoid contaminating other areas. Footwear must be disinfested with quaternary ammonium between nurseries and properties. In addition, all tools and equipment that come in contact with plants or soil must be disinfected between fields by washing with a quaternary ammonium compound. See Appendix A for details about disinfecting tools, supplies, and equipment.

National Survey

PPQ may conduct a national detection survey, similar to the National Survey conducted for *Phytophthora ramorum,* for exotic *Phytophthora* spp. in nurseries or forests, to find any infestations in the United States. A National Survey would most likely occur following a known positive finding and delimiting survey for a particular *Phytophthora* sp.

APHIS has established a protocol, *Phytophthora ramorum* Nursery Survey Manual, for systematic survey of *P. ramorum* in nursery settings. This manual is available online at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/surveyplan/surveymanual.pdf. States are encouraged to utilize the guidance in this document to best determine where to conduct survey activities for exotic *Phytophthora* spp. and as a source of general methodologies that may be employed to survey for *Phytophthora* spp. Select portions of this procedure are discussed below.

The USDA Forest Service Forest Health Monitoring Program

established a protocol for a systematic survey of *P. ramorum* in forest environments, which was employed to conduct a national *P. ramorum* survey using vegetation and water sampling. The survey strategy, based on risk-based polygons, reflected understanding of the biology and ecology of *P. ramorum*, known and potential hosts based on laboratory testing or taxonomic similarity, and the likely pathways for introduction. The following factors were used to assign risk and to develop the sampling polygons: 1) presence of known *P. ramorum* host species, host genera, and closely related genera; 2) locations of nurseries receiving *P. ramorum* host rhododendron stock; 3) length of yearly mesic/moist weather period; and 4) area outside limiting temperature extremes currently associated with *P. ramorum*. A similar risk-based sampling strategy may be employed in a national survey for the exotic *Phytophthora* spp. in a natural environment or forest setting after an initial U.S. detection. However, a single strategy may not be applicable to every exotic *Phytophthora* spp.; factors used to develop polygons and assign risk may need to change for each species, based on pathogen biology, epidemiology, and host range.

Note: At this time, some pertinent host range and biological information may be unknown for a particular exotic *Phytophthora* spp. covered within this NPRG. Therefore, extensive research may need to be conducted prior to employing a risk-based sampling strategy.

A risk-based stream sampling protocol is available that classifies streams from extreme to low risk, with corresponding methodology. This stream baiting protocol employed for *P. ramorum* can be found at http://www.fs.fed.us/foresthealth/fhm/sp/sod/methods/stream_baiting_protocol.doc

Sampling Procedures/ Survey Strategies

Visual inspection:
A visual inspection of host plants for characteristic symptoms or signs of a pathogen can be an effective survey methodology, providing that the symptoms or a combination of symptoms are characteristic of a particular pathogen. Characteristic symptoms (if any) associated with exotic *Phytophthora* species covered in this New Pest Response Guidelines are given in Table 3-1. Since symptoms of *Phytophthora* are not diagnostic, however, a laboratory confirmation will be necessary to confirm the presence of a *Phytophthora* spp. In general, during visual inspections, symptomatic plant tissue is collected and submitted for laboratory analysis (see Diagnostic Section 4).

There are many types of symptoms associated with *Phytophthora* spp. that may occur singly, or in combination. Common symptoms associated with *Phytophthora* infection include: root rot, stem canker, leaf blight, leaf spots, twig blight or dieback, lesions, internal and external discoloration, wilt, defoliation, production of abnormal exudates, chlorosis, abnormal leaf coloration, and plant death.

Signs associated with *Phytophthora* spp. can include: the presence of mycelium and/or microscopic spores on plant tissue or in soil.

Inspectors should receive training in identifying symptoms associated with *Phytophthora* spp. on host plants. At a minimum, inspectors should review photographs of the wide range of symptoms associated with *Phytophthora* infection before starting the survey**.**

Soil and Growing Media Sampling:
In natural areas, soil cores are collected using a standard soil probe to sample rhizosphere soil surrounding a symptomatic plant. In a nursery situation, growing media samples may also need to be collected. Soil and growing media samples should be collected as composite samples, but these two sample types should not be mixed. A composite sample consists of a mixture of sub-samples. Sub-samples are small amounts of soil (or media) removed from the ground (or pot) and added together to form a composite sample. The number of composite samples collected will depend upon the size of the natural area or nursery block being sampled, and is covered in the sections below.

Infested soil or growing media will look exactly the same as un-infested soil or growing media. Therefore, all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10 percent bleach solution or quaternary ammonium solution, or flame-sterilized with a propane torch between areas or blocks. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or growing media from one area or block to the next via shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new area or nursery block. Care must be taken to ensure that un-infested soil or growing media is not contaminated by infested material.

Soil samples are usually processed as shown below using a baiting technique. Soil baits are washed, rinsed, and drained well. The soil sample is covered and saturated with with about 2.5 cm (1") of sterile deionized water. The soil and water are not to be mixed. A minimum of two leaves of the preferred bait for the exotic *Phytophthora* spp. (Table 3-1) per soil sample should be used. Baits are immersed halfway in the soil/water solution for at least 48 hours at room temperature, then removed from the solution and washed. The baits are then incubated in a moist chamber at room temperature for 7 days to allow any potential disease symptoms develop. Baits are examined daily for developing symptoms; pieces are cut from the edge of a developing lesion or leaf spot and placed on a microbiological medium. Plates are sealed, incubated until growth is seen, and sent for identification if a *Phytophthora* spp. is suspected. Table 3-1 gives the baits that have been used in the literature for each *Phytophthora* spp. and the microbiological medium that has been used to culture each species.

Bait Selection

Healthy source plants are an absolute requirement. Source plants should be free of dieback and leaf symptoms. Use older leaves free of leaf symptoms (spots, blight, and chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full-leaf expansion and a period of hardening in summer. For *P. ramorum*, baits are recommended to be at least one year old. Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water

and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for washing or storing leaves, as these may harbor other *Phytophthora* spp. or other pathogens.

Water Sampling: The genus *Phytophthora* is in the class Oomycetes. Collectively these organisms are called "water molds" and are taxonomically closer to algae than to fungi. For this reason, it is necessary to test water collected from potentially infested nursery blocks or streams from potentially infested natural areas for the presence of *Phytophthora*.

There are two potential methods provided here to detect *Phytophthora* species in water. The first uses leaf baits in mesh bags followed by moist chamber incubation of the leaf baits. Known host material is floated in potentially *Phytophthora*-infested water. If *Phytophthora* motile zoospores are present, they are attracted to the host material. Infected leaves are plated onto microbiological media and the pathogen is cultured and then identified using morphological or molecular methods (see Diagnostic Section 4).

The second method uses water filtration. In general, the water is collected from a pond or stream and filtered with sterile filters. The filters are then placed on microbiological media to culture species present on the filter.

Survey Types

General Detection Surveys: Perform a detection survey to ascertain the presence or absence of a pest in an area where it is not known to occur.

The purpose of a general detection survey is to determine whether a pest is present in a defined area. This can be broad in scope, assessing the presence of the disease over large distances or it may be restricted to determining if a specific pest is present in a more narrowly focused area.

Statistically, a detection survey is **not** a valid tool to claim that a pest *does not* exist in an area, even if results are negative. Negative results can be used to provide clues as to mode of dispersal and temporal occurrence. Industry practices are an important consideration, particularly when combined with results from similar areas that are topographically, spatially, or geographically similar.

Delimiting Surveys: Delimiting surveys are performed to define the extent of an infestation for a detection of a confirmed pathogen. When the presence of disease caused by an exotic species of *Phytophthora* or viable propagules is confirmed in an area, a delimiting survey will be implemented to determine the size and level of the infestation.

Traceback/Traceforward Investigations: Traceback-traceforward investigations help determine priorities for delimiting survey activities after an initial U.S. detection. Traceback investigations attempt to determine the source of infection (*e.g.,* seed source). Traceforward investigations attempt to define further potential dissemination through means of natural and artificial spread (*e.g.,* distribution of infected plant material, areas that share common equipment, etc.). Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

For positive detections on homeowner properties, inquiries are conducted with the owner of the infected material to determine where it originated (seed source, nursery where purchased, etc.) and where it might have been further distributed (to neighbors, relatives, etc.).

Delimiting Survey after Initial U.S. Detection: Once a positive detection of an exotic *Phytophthora* species has occurred, additional surveys of natural areas or nurseries in the area will be necessary. Surveys after the first U.S. detection should be most intensive around the known positive detection(s) and at locations discovered through traceback / traceforward investigations.

Monitoring surveys: Monitoring surveys are performed to determine the success of control or mitigation activities conducted against a pest.

Data Collection: Data collection can be simplified by the use of pre-programmed hand-held units that allow ease of data recording with GPS capability. The data collected during surveys should include:

1) date of collection
2) sample number from predetermined numbering system
3) collector's name and agency

4) full address including county
5) type of property, *i.e.,* residential, natural area, nursery
6) property ID numbers if appropriate
7) GPS coordinates of the host plant and property
8) host species, and cultivar
9) observations of the number of infected plants
10) general conditions or any other relevant information
11) positive or negative results from testing (recorded later).

Recording negative results in surveys is just as important as positive detections, because it helps define an area of infestation. Data collection should include an efficient tracking system for suspect samples to document their status at various stages and laboratories in the confirmation process.

Survey Sites

Nursery Site

Visual:

The greatest chance of detecting *Phytophthora* infections is through the collection of **ANY** unhealthy-looking plant tissue for laboratory analysis. Prior to beginning the inspections, a visual assessment of the nursery as a whole needs to be conducted. During this survey, any low-lying areas or areas with standing water that may favor *Phytophthora* infection must be identified, and the nursery layout, general condition of the plants, and nursery environment assessed. This information is used to help guide inspection.

The following protocol for surveying nurseries draws from multiple sources for surveying nurseries for *Phytophthora ramorum*, and will serve as the baseline information for surveying for exotic *Phytophthora* spp. Slight deviations in methodology (*e.g.,* type of bait used) may be necessary to survey for a particular exotic *Phytophthora* species.

Determining the Number of Plants to Inspect:

A minimum number of host plants in each nursery should be visually inspected at random based on Table 3-2. For different exotic *Phytophthora* species, the host plants will vary considerably. At the discretion of the inspector, more plants may be visually inspected and sampled if conditions suggest this is necessary. Sampling and baiting downslope adjacent to the cull and/or compost pile can be an effective way of detecting the presence *Phytophthora* spp. in the nursery. Low-lying areas or areas with standing water may favor

Phytophthora infection, and should be examined.

Table 3-2. *Determining the number of hosts for visual inspection of disease symptoms associated with *Phytophthora* spp. within a nursery.

Hosts Plants Per Nursery	95 percent Confidence Limit of Detecting 0.5 percent Disease
n<500	All Plants
501<n<1,000	842
1001<n<5,000	1055
5,001<n<10,000	1087
n>10,001	1115

*Numbers are the minimum number of host plants that must be inspected in a nursery to ensure detection at a 95 percent confidence level when disease is present in 0.5 percent of the plants and when 75 percent of infected plants express symptoms.

Identifying Other Areas of the Nursery to Inspect:
Cull and/or compost piles: Cull piles of plant materials that have been discarded or taken off sale need to be located and inspected. Samples should be taken if symptomatic plant tissues are observed. These piles should be inspected after completed inspections of the rest of the nursery. Soil at the down slope edge of the cull pile should be sampled and tested for the presence of exotic *Phytophthora* spp.

Surveying the Nursery and Collecting Samples:
There are two basic principles that should govern the inspection and sampling processes.

1. *Phytophthora* species cannot be diagnosed by a visual inspection of symptoms alone; only laboratory testing can provide a definitive diagnosis.

2. If there is any doubt as to whether the symptoms observed could be caused by a particular *Phytophthora* species, collect a sample.

Plants chosen to be inspected should be carefully scrutinized. Foliar symptoms of *Phytophthora* infection are highly variable and can range from discoloration to lesion development to leaf

drop. Some *Phytophthora* spp. can cause premature leaf drop, yielding infected plants that appear to be asymptomatic. As a result, leaves found in the pot or on the ground below the plant should also be checked for symptoms and collected for laboratory analysis.

Any and all plant tissue that appears unhealthy needs to be collected and sampled. If there is a large amount of unhealthy tissue, collection of as many samples as needed to fully represent the symptoms seen on a genus/species/variety/block basis should be carried out. This does not mean sampling every symptomatic plant, but sampling enough of them in any given block so that the lab is sure to have the material it needs to make a correct diagnosis. Lack of certainty as to what *Phytophthora* symptoms might look like should not dictate whether to sample, as other pathogens, including *Phytophthora* spp. known to be present in the United States, as well as environmental stressors, can cause similar symptoms that cannot be identified based on visual inspection.

Samples should **not** be taken from healthy, asymptomatic plants. If no unhealthy plants are observed, note how many healthy host plants were inspected.

Each sample should consist of a minimum of five leaves; for small leaf hosts the terminal 3 inches of branch tips are collected. The testing laboratory may be consulted for detailed guidance on sample collection and documentation.

A Nursery Survey Data Collection Form **or equivalent** should be **c**ompleted for each location. Surveyors may wish to draw a map of the nursery and indicate areas inspected and sampled. This can be very useful if re-sampling is necessary.

These minimum decontamination procedures must be followed between nurseries and between hosts within a nursery:

- Decontaminate all tools or equipment used to take samples between blocks of nursery stock and before leaving a nursery. A dilute (10 percent) bleach solution or a quaternary ammonium solution must be sprayed over all tools between nursery blocks.

- Loose dirt must be brushed from boots and shoes, which

must be sprayed with disinfection solution between nursery blocks.

Collecting Sample Tissue by Tissue Type:

Leaves:

- Collect symptomatic or discolored leaves.
  - Symptomatic fallen leaves *within the pot* of a symptomatic plant can be included in the sample provided they are not exhibiting extensive decay.

  - For plants with very small leaves or needles, samples can be submitted as twig sections with the leaves attached. In these cases try to ensure that the sample has a total of approx. a 3" x 3" leaf surface area.

Twigs:

- Cut the twigs below the cankered regions (lesions, dieback) (well into healthy tissue).

- Sterilize pruning equipment between samples using a dilute (10 percent) bleach solution or a quaternary ammonium solution.

Branches of Trees:

- Follow procedures in your state for surveying and sampling trees.

- In some states, nursery inspectors may sample trees; in other states forestry or other officials may be asked to sample trees.

Roots:

- Collect fine roots while collecting soil from the rhizosphere.

Handling and Preparing Samples:

- Samples should be bagged in a moisture-retaining container, such as a polyethylene bag to prevent drying.

- **Do not** add extra moisture to the sample to keep it fresh. The extra moisture will actually speed deterioration of the sample.

• Keep the samples cool (around 3-6°C) – place them in foam cooler.

• Mail or deliver the sample as soon as possible to preserve freshness (if mailing use overnight mail).

• Remove gloves and place sample bag in a second protective bag.

• After double-bagging the sample, complete a lab sample form and attach it to the bag (you may also place the lab sample form inside the second bag; this reduces the risk that the form could deteriorate during shipping).

  o Use the sample submission form required by the receiving lab.

  o For samples going to an APHIS lab, use the PPQ Form 391.

• Always write identifying label remarks on the outside of the bag with a permanent marker.

  o Attach labels on the outside of bags; labels inside the bag may deteriorate due to moisture and become illegible.

  o Include on all labels with a permanent marker: time, date, collector's identification number, location of sample site, sample number.

• Keep the sample cool and out of the sun (have a foam cooler with cold packs available). **Do not** allow it to dry out or overheat.

Notifying the Lab:
Contact receiving lab and let staff know the samples are being sent.

Soil:
Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite

sample consists of a mixture of sub-samples. Sub-samples are small amounts of soil (or media) removed from the ground (or pot) and added together to form a composite sample. The use of sub-sampling increases the chances of finding *Phytophthora* spp. if they are present. Samples should contain a maximum of 500-ml (volume) of soil and/or growing media (1/2 of a quart-size Ziploc bag) for *P. ramorum*. The number of composite samples collected will depend upon the size of the nursery block being sampled (see Table 3-3). There should be at least two samples, one for growing media and one for soil, unless all plants and associated growing media were destroyed or the plants are not on soil (*e.g.,* on concrete or asphalt). If the surface of soil is covered with gravel, take sub-samples from the soil beneath the gravel. If water permeable weed block is present, either covered with gravel or under gravel, the weed block should be removed prior to soil sampling

Table 3-3: Number of composite soil or growing samples collected based on nursery block size for *Phytophthora* spp.

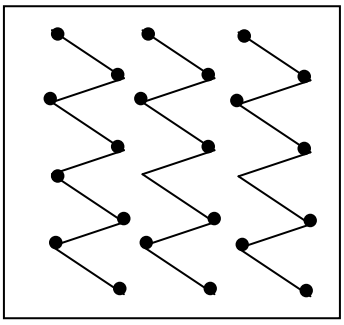
Size of site (acres)	Square feet	No. of soil and growing media samples collected (total)*
0.00< n < 0.25	n <10,890	5 (10)
0.25 < n < 0.5	10,890 <n < 21,780	10 (20)
0.50 < n < 1.0	21,780 <n < 43,560	20 (40)
n >1.0	n > 43,560	30 (60)

*The first number indicates the number of samples of growing media and the number of samples of soil. The number in parenthesis represents the total number of samples collected (*e.g.*, for a site between 0 and 0.25 acres, 5 soil samples plus 5 growing media samples will be collected for a total of 10 samples).

Each composite sample will consist of at least five sub-samples collected from soil or growing media within the targeted area. While five is a minimum, it is preferable to take 24 sub-samples of soil or growing media for each sample, provided the area is large enough (for soil samples) and enough plants are present (for growing media samples). Sub-

samples should be collected according the pattern in the diagram below (Figure 3-1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be maintained (preferably by GPS but at least by flagging) in case follow-up treatment of the soil or growing media for *P. ramorum* is required. Composite samples may also be collected from neighboring blocks of uninfested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/growing media samples from these blocks first to minimize the risk of contaminating un-infested soil/growing media. If all potentially infested growing media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the growing media of those plants that are downslope of the originally infected plants, based on watering patterns.

Figure 3-1: Recommended pattern for collection of sub-samples for composite soil and/or growing media samples.



Soil Baiting:
It is possible to follow the above procedure and not successfully bait and culture *Phytophthora* spp. This may be due to *Phytophthora* spp. not being present, but may also be due to dormancy of *Phytophthora* spp. To address this dormancy potential and to better enable the diagnostician to detect *Phytophthora* spp. when present, mix the soil well and split the soil samples when they arrive in the laboratory. Once the samples are well mixed and split, place one of the sample halves into cold storage at approximately 4°C for one month. Bring samples out from cold room after one month has passed,

leave samples at room temperature for two days and repeat soil baiting process. The samples should be processed as shown below. To prepare soil bait, briefly soak pears (select unripe green pears), *Rhododendron* leaves or leaves of the bait of choice for a particular *Phytophthora* spp. in a mild detergent solution to remove any pesticide residues. Rinse the baits well and drain. Leaving the soil in the Ziploc bag, add enough sterile deionized water to saturate and cover soil with about 2.5 cm (1") of water. Do not mix the soil and water. Use two pears or leaves per soil sample. With a black permanent marker, label one side of the pears or leaves with the soil sample number and date processed.

Carefully push each pear or leaf into the wet soil and water until the bait is immersed halfway. Leave the labeled side of the bait out of the water. Seal the Ziploc bag and leave bait in the soil/water mixture for at least 48 hours at room temperature. After 48 hours, remove the baits and wash off any clinging soil into Ziploc bag. Set the bait on a moistened paper towel in a sealed container at room temperature for 7 days to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal. Examine the bait daily for developing symptoms. Under a laminar flow hood, cut eight to 10 pieces of pear or leaf from the edge of a developing lesion or leaf spot and insert into the oomycete selective medium (PARP). Write the sample number and date processed on the underside of the Petri dish. Seal the dish with parafilm, incubate, and send for identification.

Water:
Water samples should be collected in a sterile wide-mouth bottle and kept at 5-10°C. Water samples should be taken from the surface to increase the likelihood of obtaining zoospores of *Phytophthora.* Sample size should be approximately 1000 ml. Samples should be processed within 48 hours of collection. Number of samples is determined by the size of the nursery pond or stream to be sampled. Information for the number of samples collected by pond size is given for *P. ramorum* in Table 3-4. A similar sample size is suggested for other *Phytophthora* species.

Table 3-4: Number of water samples collected based on pond size.

Size of pond/stream (acres)	No. of water samples collected (liters)
0.00 - 0.25	5
0.26 - 0.5	10
0.50 - 1.0	20
>1.00	30

Baiting: For *P. ramorum,* research supports using rhododendron leaves at least one year old. *Rhododendron* leaves are prepared as bait by trimming off the petiole end of each leaf. Three-to-four leaves are placed into each labeled mesh bag. The bags are labeled with a plastic tag listing the date, water source (location), and site name (*i.e.,* nursery license number). The mesh bags are placed into the water source for a minimum of 48 hours, preferably up to one week (baits will begin to decompose if left in the water longer than 1 week, so exceeding this period is not recommended). The bags are placed such that the leaves will remain submerged the entire time, even if water levels fluctuate within the water source. If possible, the baits are placed near the influent from the area closest to or containing the infested plants.

Suspect lesions that develop on the rhododendron leaves are plated on PARP at 18-20°C (64-68°F). Any *Phytophthora* species growing on the PARP need to be transferred to Corn Meal Agar (CMA) or V8 agar for identification to species. A control sample using a leaf bait in distilled water should be run simultaneously with the leaf bait sample. Specific host species used as baits for the exotic *Phytophthora* species covered in this New Pest Response Guideline and microbiological media used for culturing each species are given in Table 3-1.

Filtering: For *P. ramorum*, water is removed from the pond or stream and filtered with sterile filters, which are then placed on PARP. Once the filter is removed from PARP, any resultant *Phytophthora* colonies are transferred to Corn Meal Agar or V8 agar and identified to species.

Natural Area Site

General detection and monitoring protocols from the State of California and the State of Oregon are presented in this section

as examples of the various methods used to address different local conditions and objectives. Select the relevant parts and customize to fit your areas and the *Phytophthora* species for which you are surveying. Monitoring surveys are only needed when the pest is known to occur and information on distribution, treatment efficacy, and the like is needed. Protocol will vary for each type of survey.

State of California
Prepared by Donald R. Owen, California Department of Forestry and Fire Protection. March 2003.
Don.Owen@fire.ca.gov

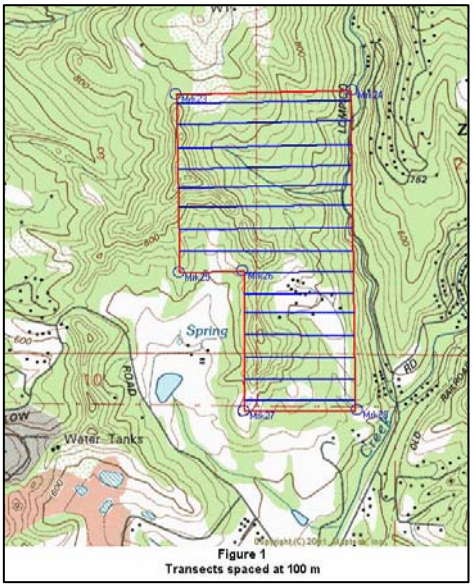
Detection Survey:
Visual: The best method for conducting a detection survey is to traverse the area to be surveyed following a series of parallel, evenly spaced transect lines, continually looking for disease symptoms as you walk. In effect, the area being surveyed is a strip that extends outward a certain distance on either side of transect lines. All known hosts are visually scanned for symptoms, both to the right and left of the transect line, within the boundaries of the strip. All transects should be walked in the course of the survey, but the surveyor should be willing to make deviations from survey strips in order to further investigate areas of specific interest, *e.g.,* areas with a concentration of symptoms, or areas suspected to have an abundance of hosts but are not within the boundaries of the survey strip.

The intensity of the survey will determine the likelihood of finding *Phytophthora* spp. if present. Intensity can be measured as a percentage of the area visually inspected, and will vary based on the width and spacing of survey strips. From a practical standpoint, the width of the strip is roughly estimated based on the distances to the right and left that a surveyor can effectively scan for symptoms. This is obviously not a precise measurement, but it does provide a means for determining the approximate area of land that has been visually surveyed. For small areas (under 10 acres), it may be possible to survey close to 100 percent of the property; this would be impractical for larger properties. As a minimum standard for a detection survey, a 20 percent strip survey is recommended.

Figure 3-2 illustrates how this could be achieved. Parallel

transect lines are plotted on the map at 100 m intervals. The surveyor uses a strip width of 20 m (10 m on either side of the transect line) and visually scans all hosts within the boundaries of the strip as the transects are walked. This example could be used with any size or configuration of land to achieve a 20 percent survey. Strips of the same width spaced at 50 m intervals would achieve a 40 percent survey. Strip width can be varied to meet the preferences of the surveyor(s) or to better conform to site conditions (*e.g.*, heavy vegetative cover may warrant narrower strips). If strip width is decreased, spacing between strips will also need to be decreased to maintain survey intensity. From a practical standpoint, about 20 m is the maximum strip width that a single surveyor should consider using.

Figure 3-2: Transects spaced at 100 m



Topography can have significant influence on host and disease distribution. For this reason, it is generally best to plot transect lines roughly perpendicular to contour lines (up and down the slope). If lines are plotted parallel to contour lines, certain topographic features (*e.g.*, ridgelines, stream bottoms) may be missed or poorly represented in the survey. It may not be

necessary or practical to use a single method for surveying an entire property. For larger properties in particular, the best approach may be to partition the property into more uniform subunits. Topographic maps, aerial photos, and other sources of information can aid in this process. Changes in topography may warrant changes in the orientation of transect lines. Changes in vegetative cover may warrant different survey intensities. Some areas may be devoid of hosts and be excluded from the survey, while others may be of lower or higher risk for disease depending on the kinds and numbers of hosts present.

During the survey, closely inspect all symptomatic hosts to decide whether a diagnostic sample is warranted. Determining the presence of *Phytophthora* spp. requires lab confirmation. This is essential when conducting a detection survey in an area where the pathogen's presence is uncertain. Keep a record of *Phytophthora* hosts and symptoms encountered. Record the locations of symptomatic hosts and where diagnostic samples are taken, so that these areas can easily be returned to. Each sampling location should have a unique identifier.

Even with the most thorough survey, there is always the possibility the disease will not be detected even though it is present. It is also possible that symptoms will be found, but the pathogen cannot be detected in samples submitted for lab analysis. The best time for symptom recognition and sample collection will vary with climate type and *Phytophthora* spp. For example, for *P. ramorum*, the best time in California is during winter and spring. In Washington State, fall sampling is recommended. Also, the pathogen may be more readily isolated from foliar samples than from bark and wood or from certain host plants. All samples should be kept cool and processed as quickly as possible. Survey and sample collection can occur at any time of year, but the aforementioned factors may influence the outcome of the survey. Follow-up surveys may be warranted.

Keep different host plant species collected on the same transect double bagged and separate from each other. Do not transport plant parts or bark samples unless double bagged, labeled inside and out and sealed, along with an accompanying "Chain of Custody" form. A clean dry paper towel should be inserted in bag with leaf samples to absorb excess moisture. Bark samples should be covered in plastic wrap, double

bagged, and labeled inside and out. All samples should be protected from direct sunlight and kept in a cooler, sealed coolant bag, or refrigerator until shipped. Mail samples to the appropriate diagnostic lab via overnight mail.

Delimitation or Delineation Survey

A delimitation survey has a different purpose than a detection survey, but the basic survey methodology is the same, *i.e.,* traverse the area following a series of parallel, evenly spaced transect lines while visually scanning all known hosts within a certain distance, both to the right and left, of transect lines (refer to the discussion under detection survey). The purpose may simply be to map the area-wide distribution (presence or absence) of disease, but more than likely additional information will be desired: On which hosts is the disease present? What are the relative levels of infestation (high, medium, low, or absent)? Diagnostic samples may not be necessary if there is good evidence that disease already exists in the general area, in which case disease presence or level of infestation is inferred from the symptoms observed. Diagnostic verification is needed for regulatory action to occur.

The intensity of the survey will depend upon the desired accuracy and the relative abundance of disease. If a high level of resolution is desired and/or the disease is believed to be relatively rare, greater survey intensity is warranted, *i.e.,* consider a strip survey of $\geq$20 percent. Also consider doing the survey in stages. For example, a 5-10 percent strip survey might be sufficient to establish the general disease distribution across the property. This could be followed by more intense surveys that are limited to particular areas of special interest. Meandering searches, *i.e.,* those that do not follow transect lines, can be used to better define distribution patterns and their boundaries. As was described for the detection survey, the property can be partitioned into more uniform subunits that are surveyed according to their particular attributes. A delineation survey need not follow a strict protocol; be flexible in designing a survey that best meets your needs.

Distribution data is best expressed as a continuum. A major advantage of a systematic strip survey is that it allows you to effectively sample a large area of land and to view conditions of interest as a continuum as you walk the property. If you are interested in specific information, *e.g.,* the distribution of

symptomatic oak trees, it may be relatively easy to map the occurrence of each symptomatic and non-symptomatic oak within the boundaries of the strip survey. Collecting greater amounts of information, however, can be overwhelming and time consuming, *e.g.,* attempting to map the occurrence of every symptomatic species of host plant. Keep in mind the purpose of the survey. Because you are doing a delineation survey, it is important that you continually observe conditions as you walk – this will enable you to better discern distribution patterns. Especially look for changes or unusual conditions and record them. Other data, which provides details to your overall observations, need not be recorded on a continual basis. You may decide to collect detailed data only at given points along the transect. To avoid bias, it is best to predetermine how this will be done. For example, every 40 m along the transect, stop and record data on conditions within a given distance of your position.
If symptoms of a particular *Phytophthora* spp. are known to exist in the survey area, diagnostic samples should only be taken to confirm potentially new or unusual occurrences of disease, *e.g.,* symptoms on an unusual portion of a host plant, or suspicious symptoms on a non-host. If diagnostic samples are taken, record each sampling location and provide it with a unique identifier. You may also consider taking samples to determine if other pathogens are in the area.

State of Oregon:
Oregon Post-treatment *P. ramorum* monitoring protocol
Nancy Osterbauer, nosterba@oda.state.or.us

Monitoring Survey: Pathogen is known to be established.
Phytophthora ramorum has been detected in a small part of one county in Oregon. The Oregon Department of Agriculture (ODA) issued a quarantine against *Phytophthora ramorum* to prevent further spread and to protect Oregon's agricultural and timber industries and natural resources. The following survey for *P. ramorum* has been implemented to assist in the detection and eradication of the pathogen. This post-treatment *P. ramorum* monitoring standard operating procedure is followed when surveying and processing samples from known positive sites in Curry County, Oregon for the pathogen *Phytophthora ramorum*.

Sample Collection and Sample Handling
The *P. ramorum* survey will be conducted every winter,

spring, and summer for 24 months after the initial eradication treatment. All oak and tanoak samples collected must be processed in the field. All other samples should be processed in the field whenever possible. Samples will be delivered to the laboratory in sealed plastic bags (*e.g.,* Ziploc bags) in a cooler and then processed within 48 hours upon arrival. Plates should arrive sealed and in a cooler. Samples without a sample submission form will not be accepted.

Aseptic technique will be used with all sample plating. Plated samples will be stored in the absence of light at room temperature. Footwear must be sprayed thoroughly with a 10 percent bleach solution after each site inspection to avoid spread of the pathogen. Vehicle tires must be washed clean of soil before leaving the area. All field and laboratory tools must be sanitized/sterilized after each use. All samples and plated samples must be sterilized in the autoclave at 121°C and 15 psi for 30 minutes at the conclusion of the survey.

Procedure

Visually survey each treated site for host plants symptomatic for *P. ramorum.* Use Table 3-5 to determine the acreage to be visually inspected and then run a transect survey through each treated site. The number of plant and soil samples to be collected from each treated site is also listed in Table 3-5. Determine the transect to be followed. Survey as much of the treated site as possible within the guidelines given in Table 3-5. Examine all host plants within the transect for *P. ramorum*-like symptoms. Collect host and soil samples while walking the transect(s). To determine how often to collect a sample, divide the length of the transect by the number of samples (host and soil) to be collected. Using Example 1 (8.6 acre treated site) and the information given in Table 3-5, a host sample would be collected every 24 yards along the transect and a soil sample every 48 yards. Soil samples need only be collected during the winter and spring survey periods.

Collect plant samples from hosts with suspicious symptoms. If no symptoms are present, collect samples from asymptomatic hosts. Mark the location of the host with GPS. Record this information and a description of the sample on the sample submission form. Assign each plant and soil sample a unique number. Label the host with yellow flagging and an aluminum marking tag. Write the sample number and date on the flagging and tag.

During the winter and spring survey periods only: Collect soil samples at the base of host plants. Preferentially collect soil samples at the base of symptomatic hosts. If no symptoms are present, collect samples at the base of asymptomatic hosts. Mark the location of the nearest host with GPS. Record this information and a description of the sample on the sample submission form. Label the nearest host with an aluminum marking tag. Write the sample number and date on the tag. Assign each soil sample a unique number. Wash the soles of your shoes and your tools with a 10 percent bleach solution using a hand-held sprayer before leaving the area.

Table 3-5. Sampling table for examination of treated sites for *Phytophthora ramorum*.

Size of Treated Site (acres)	Area visually inspected (percent)	No. of Plant Samples Collected	No. of Soil Samples Collected
0.00 – 1.00	80.0	8	4
1.01 – 1.25	72.0	10	5
1.26 – 1.50	67.0	12	6
1.51 – 2.00	55.0	16	8
2.01 – 2.50	48.0	20	10
2.51 – 3.00	43.3	24	12
3.01 – 4.00	35.0	32	16
4.01 – 5.00	30.0	40	20
5.01 – 6.00	26.2	48	24
6.01 – 7.00	23.6	56	28
7.01 – 8.00	21.9	64	32
8.01 – 9.00	20.6	72	36
9.01 – 10.00	19.5	80	40
10.01 – 50.00	4.0	80	40
50.01 – 100.00	2.5	80	40
100.01 or more	2.0	80	40

Table 3-1. Host range, symptoms, and survey information for exotic *Phytophthora* spp.

Phytophthora species	Hosts	Symptoms	Survey Specifics	Baits used	Media used for culturing	Key Reference(s)
Phytophthora alni (subsp. *alni, multiformis,* and *uniformis*)	*Alnus glutinosa*, *A. incana*, *A. cordata*, and *A. viridis* (alder).	**Leaves:** Small, sparse, yellow, premature drop. **Crown/collar:** Overall decline, rot. **Stem:** Lower portion marked by black/rusty colored exudate ('tarry spots'), adventitious roots. **Fruit:** Early and excessive fructification with unusually small cones. **Roots:** Rot.	Visual survey based on symptoms. Isolated primarily from bark lesions or from rhizosphere soil around symptomatic trees. Also isolated from water but efficiency was low.	Rhododendron, alder twigs, apple, eucalyptus, oak leaflets	PVPH (Tsao and Guy, 1977), PARPNH (Jung *et al.*, 1996a), Corn Meal Agar, and PARBHy (Streito *et al.*, 2002).	Baiting Methodology-Alaska Brasier *et al.* (1995) Jung and Blashke (2004)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora alticola	*Eucalyptus* spp. (*E. badjensis*, *E. dunnii*, and *E. macarthurii*).	**Leaves:** Progressive wilting. **Crown/collar:** Rot, girdling. **Stem:** Bleeding lesions and formation of epicormic shoots. **Roots:** Rot.	Four soil samples were taken from the top 10 cm at the base of dying trees and pooled. Plant tissue was collected from infected root collars.	Citrus leaf disks (5mm, diam.) or *Eucalyptus sieberi* cotyledons.	NARPH (Huberli *et al.*, 2000)	Maseko *et al.* (2007)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora austrocedrae	*Austrocedrus chilensis* (Cordilleran cypress).	**Overall:** Progressive withering and defoliation of the tree, which dies while standing. **Leaves**: Chlorotic foliage, may change from yellow to red in color. **Stem:** Basal resinous exudates and red-brown necrotic lesion in the inner bark are visible extending up the stem from killed roots. **Roots:** Rot, dead tissue.	Direct plating of necrotic tissues onto selective medium; immediately after collection and after washing necrotic tissue with running tap water for 24-48 hours	None Listed	PARNBP, PAR, NAR, and BARP (Greselebin *et al.*, 2007).	Greselebin *et al.* (2007)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora boehmeriae	*Acacia mearnsii* (black wattle), *Ailanthus altissima* (tree-of-heaven), *Araucaria hetrophila* (Norfolk Island Pine), *Avicennia* spp. (mangrove), *Boehmeria* spp. (nakai, ramie), *Broussonetia papyrifera* (paper mulberry), *Cedrus deodara* (Deodar cedar), *Chamelaucium uncinatum* (Geraldton waxplant), *Citrus* spp. (citrus), *Eucalyptus* spp. (eucalyptus), *Ficus* spp. (ficus), *Gossypium* spp. (cotton), *Malus* spp. (apple), *Persea americana* (avocado), *Persoonia longifolia* (long-leaf Personnia), *Pinus* spp. (pine), *Pterocarya stenoptera* (Chinese wingnut), and *Solanum melogena* (eggplant).	Symptoms vary with host (see Section 2). **Overall:** Wilting, and plant death (cotton). **Leaves**: Leafspots (paper mulberry, Chinese wingnut), round water-soaked lesions, and leaf fall (cotton). **Trunk:** Gummosis, lesions at the trunk base with gum exudation (black wattle). **Roots:** Root rot (citrus, pine, cotton), brown streaks (cotton), and root stock gummosis (citrus).	Direct plating, soil sampling. Soil and root samples were taken from four trees per site. One soil sample was sampled in the rhizosphere of each tree. The top 5 cm of soil was removed and ~1 kg of soil sampled at a depth of 5-25 cm. Root samples consisted of diseased sections of adventitious roots with feeder roots.	*Eucalyptus sieberi* cotyledons, lupin, citrus leaf disks	PDA, CMA, CMA +A, 3P, PVPH, PARP, PARPH, NARPH	Gerettson-Cornell (1976) Linde *et al.* (1994)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora captiosa	*Eucalyptus* spp.(eucalyptus) (*E. botryoides*, and *saligna*).	**Overall:** Dieback. **Leaves**: Leaf spots, lesions. **Crown/collar:** Dieback, defoliation. **Stem/petioles/twig/ branches:** Lesions. **Seeds:** Lesions. Symptoms can be observed as high as 20 meters up in the canopy.	Direct plating of diseased leaf lamina, petiole, or twig material; soil near the base of diseased trees.	*Eucalyptus* petiole material with a small portion of leaf lamina still attached.	Modified PARPH – malt extract base, CA	Dick *et al.* (2006)

Phytophthora species	Hosts	Symptoms	Survey Specifics	Baits used	Media used for culturing	Key Reference(s)
Phytophthora colocasiae	*Alocasia* spp. (taro), *Amorphophallus campanulatus* (elephant-foot yam), *Bougainvillea spectabilis* (bouganvilla), *Cantharanthus roseus* (periwinkle), *Colocasia* spp. (elephant's ear, taro), *Dracontium polyphyllum* (guapa), *Hevea brasilensis* (rubber), *Panax quinquefolius* (American ginseng), *Piper* spp. (betel, black pepper), *Ricinis communis* (castorbean), *Vinca rosea* (periwinkle), and *Xanthosoma* spp. (yautia, blue taro).	**Leaves**: Small dark brown to olive green spots, lesions, enlarge to turn purplish brown with yellow margins. Lesions are zonate and exude drops of yellowish liquid. Petiole infection leads to long, brown rot. **Stem/stalk:** Tissue softens, breaks **Corms:** Gray-brown to dark blue lesions, decay or rot.	Direct plating of diseased leaves and petioles. Isolated from soils using leaf baits or by sieving a soil-water solution.	Taro whole leaf baits, taro leaf disks	NAP, V8, PARP, 3P-LBA (Gollifer *et al.*, 1980).	Gollifer *et al.* (1980) Narula and Mehrotra (1984) Ann *et al.* (1986) Quintugua and Trujillo (1998)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora fallax	*Eucalyptus* spp. (eucalyptus) (*E. delegatensis*, *E. fastigata*, *E. nitens*, and *E. regnans*).	**Overall:** Dieback. **Leaves/Petioles**: Leaf spots, lesions. **Crown/collar:** Dieback, defoliation. **Stems/ Twigs/Branches:** Lesions. **Seeds:** Lesions. Symptoms can be observed as high as 20 meters up in the canopy.	Direct plating of diseased leaf lamina, petiole, or twig material; soil near the base of diseased trees.	*Eucalyptus* petiole material with a small portion of leaf lamina still attached.	Modified PARPH – malt extract base, CA	Dick *et al.* (2006)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora frigida	*Acacia* spp. (black wattle) (*A. decurrens* and *A. mernsii*), *Eucalyptus* spp.(eucalyptus) (*E. dunnii* and *E. smithii*)	**Leaves:** Progressive wilting. **Crown/collar:** Rot, girdling. **Stem:** Bleeding lesions and formation of epicormic shoots. **Roots:** Rot	Four soil samples were taken from the top 10 cm at the base of dying trees and pooled. Plant tissue was collected from infected root collars.	Citrus leaf disks (5mm, diam.) or *Eucalyptus sieberi* cotyledons	NARPH (Huberli *et al.*, 2000)	Maseko *et al.* (2007)
Phytophthora gallica	*Querus robur* (English oak), *Salix alba* (white willow), *Alnus glutinosa* (alder), and *Fagus sylvatica* (European beech).	**Overall:** Decline, lesions on cuttings (experimental inoculation)	Isolated from rhizosphere soil of declining oak and common reed (*Phragmites australis*)	*Quercus robur* leaflets	PARPNH	Jung and Nechwatal (2008)
Phytophthora idaei	*Rubus ideaus* (red raspberry).	**Roots:** Rot. Reduced cane height for cultivars grown in the ground not under greenhouse conditions.	Direct plating of necrotic root and cane bases	None listed	Modified French Bean Agar (FBA) (Kennedy and Duncan,1995)	Kennedy and Duncan (1995)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora iranica	*Solanum melongena* (eggplant) (isolated from), *Solanum tuberosum* (potato), *Lycopersicon esculentum* (tomato), and *Beta vulgaris* (sugar beet).	Isolated from eggplant, but was not pathogenic to eggplant by experimental inoculation. Caused a pink rot in potato tubers when exposed to air.	Direct plating of eggplant root tissue.	None listed	Not known	Ershad (1971) Erwin and Ribeiro (1996)
Phytophthora italica	*Myrtle communis* (myrtle). Weakly pathogenic to *Lycopersicon esculentum* (tomato) *Malus* spp. (apple).	**Roots:** Rot. Wilted seedlings. Tomato and apple – brown discoloration.	Direct plating of rotted roots of wilted seedlings.	None listed	BNPRAH (Masago *et al.*, 1977).	Belisario *et al.* (1993)

Phytophthora species	Hosts	Symptoms	Survey Specifics	Baits used	Media used for culturing	Key Reference(s)
Phytophthora kernoviae	*Annona cherimola* (cherimoya), *Castanea sativa* (European chestnut), *Drimys winteri* (winter's bark), *Fagus sylvatica* (beech), *Gevuina avellana* (Chilean hazelnut), *Hedera helix* (ivy), *Ilex aquifolium* (variegated holly), *Liriodendron tulipfera* (tulip tree), *Lomatia myricoides* (river lomatia), *Magnolia* spp. (magnolia) *Michelia doltsopa* (sweet michelia), *Pieris* spp. (pieris), *Podocarpus salignus* (willow podocarp), *Prunus laurocerasus* (cherry laurel), *Quercus* spp.(oak), *Rhododendron* spp.,(rhododendron), *Sesquiadendron giganteum* (giant sequoia), and *Vaccinium myrtillus* (bilberry).	**Leaves:** Leaf blights (blackening of leaf petiole, leaf tip, leaf base), necrotic lesions, dieback, wilting. Rhododendron leaves often fall within a few weeks of infection. **Stem:** Bleeding bark cankers, can girdle/kill trees, dieback. Bleeding cankers may be sunken or demarcated by black lines.	Direct plating of symptomatic tissue (necrotic inner bark or leaf lesions). Brown and Brasier (2007) recommend attempting to isolate from the xylem if discoloration is present.	Rhododendron 'Cunningham's White leaf disks (Benson *et al.*, 2008)	SMA + MRP (Elliot *et al.*, 1966), PARPH	Brasier *et al.* (2005) Beales *et al.* (2006)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora melonis	*Cucumis sativus* (cucumber), *Cucumis melo* (cantaloupe), *Citrullus lanatus* (watermelon), *Lens culinaris* (lentil), *Pistacia vera* (pistachio), and *Trichosanthes dioica* (pointed gourd).	**Overall:** Rapid wilting, plant death. **Leaves:** Lesions, late blight (cucumber). **Crown/collar:** Gummosis (pistachio), rot (cucurbits). **Stem/Vine:** Soft, shrinks, falls over, rot (cucumber, pointed gourd). **Fruit:** Water-soaked lesions, soft rot (cucumber, pointed gourd). **Roots:** Rot, turn brown. (pistachio).	Direct plating of symptomatic tissue.	Diseased cantaloupes were divided and mixed with sterile soil; cantaloupe seeds planted in infested soil; germinated seedlings had symptoms.	PCNB-Grated carrot agar (Maden and Karahan, 1980). CMA, PARP (with carbendazim)	Alavi and Strange (1979) Guharoy *et al.* (2006)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora multivesiculata	*Cymbidium* spp. (orchids)	**Leaves:** Under wet conditions, lesions that expand rapidly to form large irregular patches of water-soaked tissue; Under dry conditions, a change of color to brown with typical zebra-like stripes, about 0.5 cm wide with lighter discoloration in the middle and dark brown to black margin. Shoots turn gray-green color and lose turgor **Pseudobulbs (modified stems):** Internal rot; distinctive internal, blue-black or purplish-brown discoloration and a sour odor.	Direct plating of symptomatic tissue.	None listed	Cherry decoction agar, WA	Ilieva *et al.* (1998)

Phytophthora species	Hosts	Symptoms	Survey Specifics	Baits used	Media used for culturing	Key Reference(s)
Phytophthora multivora	*Eucalyptus* spp. (jarrah) (*E. gomphocephala* and *marginata*), *Agonis flexuosa* (peppermint tree), *Banksia* spp. (banksias), *Bossiaea* spp., *Conospermum* spp., *Gastrolobium spinosum* (prickly poisonbush), *Leucopogon verticillatus* (tassle flower), *Patersonia* spp., *Pinus radiata* (Monterey pine - plantation), *Podocarpus drouyniana* (Drouyn's podocarpus), and *Xanthorrhoea gracilis* (graceful grass tree).	**Overall:** Dieback, wilt, decline, mortality. **Leaves:** Clustering of leaves. **Crown/collar:** Dieback, thinning, girdling of collar. **Stem/Branches:** Dieback. Tongue-shaped, orange-brown necrosis of the inner bark (*Banksia*).	Rhizosphere soil sampling, baiting, and isolation. Soil sampled below the upper 5 cm organic layer to a depth of 30 cm (sampling along main lateral roots). Four subsamples from each tree were bulked and baited.	Juvenile leaves of *Quercus ilex*, *Q. suber*, and *Pittosporum undulatum*.	PARPNH	Scott *et al.* (2009)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora pinifolia	*Pinus radiate* (Monterey Pine)	**Overall:** Decline, wilting, mortality. **Leaves:** Needle blight (reddening to gray color), exudation of resin at the bases of needle brachyblasts. Dead needles are initially retained on the branches. When needles fall, can lead to near complete defoliation. **Stem/Branches:** Necrotic lesions in the phloem and cambium, which eventually girdle the branches	Direct plating of symptomatic tissue (bases of newly infected needles, resinous bands on needles, phloem tissue below infected needles on the branches and stems)	None listed	NARP – Corn Meal Agar based	Duran *et al.* (2008)
Phytophthora polonica	*Alnus glutinosa* (alder)	**Twigs:** discoloration – slightly pathogenic after inoculation.	Rhizosphere soil samples.	Rhododendron leaves	PARP, PARPNH	Belbahri *et al.* (2006)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora porri	*Allium* spp. (onion, leek, garlic), *Campanula persicifolia* (peach-bells), *Chrysanthemum* spp., *Daucus carota* subsp. *sativus* (carrot), *Dianthus caryophyllus* (carnation), *Gladiolus* spp., *Hyacinthus orientalis* (hyacinth), *Lactuca sativa* (lettuce), *Parthenium argentatum* (guayule), *Pastinaca sativa* (parsnip), *Rosa* spp. (rose), and *Tulipa* spp. (tulips).	**Overall:** Wilt, complete collapse of plants, rot. **Leaves:** Yellowing, dying of leaf tips, followed by the area turning white, leaf twisting, water-logged areas (*Allium* spp.); wet rot (*Gladiolus* spp.) **Crown:** Gray-brown to chocolate discoloration, spreading upwards and downwards (*Campanula* spp.); Rot (carnation) **Stem/Stalk:** Dark, wet lesions that later become gray and dry (carnation); firm rot of stems (lettuce). **Roots:** Pale pink to brown and later turn dark brown. (*Campanula* spp.).	Direct plating of symptomatic tissue.	None listed	PVP, CMA amended with vancomycin, oat extract agar	Griffen and Jones (1977) Sitepu and Bumbieris (1981) Smilde *et al.* (1996b)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora primulae	*Primula* spp. (polyanthus, primrose) and *Petroselinum crispum* (parsley)	**Overall:** Wilt, collapse dwarfed/stunted plants; plant death. **Leaves:** Wilt, collapse, in succession from outside to the center; chlorosis, wilting of leaves (parsley). **Crown:** Rot (parsley). **Roots:** Poorly developed root system; with a conspicuous absence of fine lateral roots; some main roots are decayed backwards from the tip, complete brown discoloration of vascular tissue (*Primula* spp. and parsley); the steles of old infected roots contain large numbers of oospores (*Primula* spp.); rot (parsley).	Direct plating of symptomatic tissue (roots, plants)	None listed	Maize extract agar, Corn Meal Agar	Tomlinson (1952)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora psychrophila	*Quercus* spp. (oak) (*Q. ilex, Q. petraea, Q. robur*),	Associated with oak decline in Europe but role unknown. **Roots:** Necrotic bark lesions on suberized tap roots (*Q. robur* seedlings)	Rhizosphere soil samples around base of *Quercus robur* and *Q. petraea.* One liter of soil samples were collected, mixed, and 200 ml subsamples flooded to a depth of 3 cm and baited.	Oak leaflets (2-5 day old) *(Quercus robur*), Cypress (*Chamaecyparis lawsoniana)* twigs	PARPNH, CARPHBHy	Jung *et al.* (2002)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora quercina	Oak (*Quercus cerris*, *Q. hartwissiana*, *Q. frainetto*, Q. *ilex*, *Q. robur*, *Q. petraea, Q. pubescens*, *Q. suber*, and *Q. vulcanica*).	Associated with oak decline throughout Europe. **Overall:** Yellowing and wilting; dieback, eventual tree death. **Leaves:** Yellowing, wilting, necrosis. **Crown:** Thinning. **Roots:** Dieback (necrosis) of nonsuberized and suberized roots, abnormal root branching.	Rhizosphere soil samples, including necrotic fine roots around the stem and base of declining oak trees; direct plating of necrotic roots	Young oak leaves; Apple and pear baits were unsuccessful.	PARPNH	Jung *et al.* (1999) Balcy and Halmschlager (2002b) Jönsson-Belyazio and Rosengren (2006)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora tentaculata	*Aucklandia lappa* (mu xiang), *Chrysanthemum* spp. (*frutescens*, *leucanthemum*), *Delphinium ajacis* (larkspur), *Gerbera jamesonii* (African daisy), *Origanum* spp. (oregano), *Verbena* spp., and *Santolina chamaecyparissus* (lavender cotton).	**Overall:** Wilting; plant death **Leaves:** Blighted leaves (African Daisy), Leaf russeting, wilt, and chlorosis (oregano). **Collar/Crown:** Rot (African Daisy, *Chrysanthemum*, *Verbena*, *Delphinium*) **Stem/Stalk:** Rot (African Daisy, *Chrysanthemum*, *Verbena*, *Delphinium*), defoliation, and dieback of twigs, browning and rot of the basal stem (oregano). **Roots:** Rot (*Chrysanthemum*, *Verbena*, *Delphinium*, oregano, *Santolina chamaecyparissus*).	Soil, direct plating of symptomatic tissue.	Apple	PARBH, PARP BNPRAH	Moralejo *et al.* (2004) Alvarez *et al.* (2006) Martini *et al.* (2007)

Phytophthora species	**Hosts**	**Symptoms**	**Survey Specifics**	**Baits used**	**Media used for culturing**	**Key Reference(s)**
Phytophthora uliginosa	*Quercus* spp. (oak) (*Q. petraea, Q. robur*),	Associated with oak decline in Europe but role unknown. **Roots:** Reduction of fine root length and number of fine root tips, dieback of tap roots (*Q. robur* seedlings).	Rhizosphere soil samples around base of *Quercus robur* and *Q. petraea.* May be restricted to soils with permanently or seasonally high water tables. One liter of soil samples were taken, mixed, and 200 ml subsamples flooded to a depth of 3 cm and baited.	Oak leaflets (2-5 day old) *(Quercus robur*), Cypress (*Chamaecyparis lawsoniana)* twigs	PARPNH, CARPHBHy	Jung *et al.* (2002)

CONTENTS

DIAGNOSTICS AND IDENTIFICATION

Importance

Accurate identification *Phytophthora* spp. is pivotal to assessing its potential risk, developing a survey strategy, and deciding the level and manner of control.

Authorities

Plant Protection Act

The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status. A permit is required for any laboratory receiving such material shipped interstate. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services at (301) 734-8758.

Identification

Enzyme-linked immunosorbent assay (ELISA) is a biochemical technique used to detect the presence of an antibody or an antigen in a sample. ELISA can be used as a screening test for the presence of *Phytophthora* species in a sample. ELISA is not species-specific and cannot be used to diagnosis which *Phytophthora* spp is causing the infection.

Characteristic symptoms or combination of symptoms can be useful for identification for each exotic *Phytophthora* spp. Characteristic symptoms (if available) are provided below for each species covered in this NPRG. To confirm a particular *Phytophthora* species, however, additional laboratory testing will be required.

Identification of *Phytophthora* spp. can be made through isolation of the organism by culturing on various microbiological media, and subsequent identification via morphological similarities or differences with other species, and through molecular methodologies, including PCR. Morphological identification will be necessary for a number of *Phytophthora* spp. in this New Pest Response Guidelines, based on similarity and differences between fungal structures, including gametangia, sporangia, colony type and more. Specific information is provided for each *Phytophthora* spp. below.

Culture methods and PCR can detect the organism in plant tissue,

plant debris, soil, potting media, and water. Recovery rates during culturing may vary with season and host, and are facilitated by the use of the selective medium, PARP. PCR methods are very sensitive and seem less influenced by these factors.

***Phytophthora alni*:**

Characteristic symptoms: Crown decline and the tarry spot symptoms occurring together reliably indicate the presence of a basal stem necrosis produced by *Phytophthora*. After removing the outer bark layers around the tarry spots a red-brown to black discolored necrotic area is exposed. It is mostly tongue-shaped, growing upwards as well as in a periclinal direction (Cech, 1998).

Morphological: Phloem tissue from the outer edges of necrotic lesions may be washed in running water and plated directly onto a selective medium and incubated at 20°C (Tsao and Guy, 1977). The pathogen has been cultured using the apple method (Campbell, 1949; Brasier and Strouts, 1976).

Morphologically, the gametangia of *P. alni* are similar to *P. cambivora*, but mycologists can distinguish these pathogens in a laboratory through colony type, their ability to self-fertilize (homothallic), oogonia shape, lower optimal growth temperature, and other characteristics (Brasier *et al.*, 1999; Brasier *et al.*, 2004; Jung and Blaschke, 2004). Main morphological characteristics are given in Table 4-1. Brasier *et al.* (2004) gives the complete species descriptions for each subspecies of *P. alni*.

Molecular: According to Olsson (1999), an improved DAS-ELISA technique developed for *Phytophthora* spp. from strawberry and raspberry are suitable for mass testing of plant material and for diagnosing the alder *Phytophthora* in Sweden. The sensitivity of this method was found to be comparable to that of DNA-based methods using PCR, although specific data are not given. The pathogen can be isolated by direct plating of necrotic bark tissue and from water using alder twigs as bait, but the efficiency of this method is low (Streito *et al.*, 2002).

De Merlier *et al.* (2005) developed a PCR test to identify *P. alni* subsp. *alni* and *P. alni* subsp. *uniformis*. However, this test did not detect *P. alni* subsp. *multiformis* (Ioos *et al.*, 2005). Ioos *et al.* (2005) developed a PCR-based test that detects and differentiates the various subspecies of *P. alni*. Where the De Merlier *et al.* (2005) method used DNA extracted from

laboratory-grown cultures, the Ioos *et al.* (2005) method extracted DNA directly from soil, water, or wood. Additionally, Bakonyl *et al.* (2006) used SAP and SWAP PCR primers to identify all three subspecies of *P. alni* from pure culture and from plant tissue. This test can detect a minimum of 20 pg of DNA from pure cultures or DNA extracted from as few as 10 zoospores.

Note: Single-strand conformation polymorphism (SSCP) analysis of PCR-amplified ribosomal DNA internal transcribed region I has now been used to identify and provide molecular fingerprints for 59 *Phytophthora* species, including *P. alni* subsp. *alni* (Kong *et al.*, 2003; Kong *et al.*, 2004; Gallegly and Hong, 2008).

***Phytophthora alticola*:**

Morphological: Symptoms of *P. alticola* are not characteristic as they are common for all *Phytophthora* species causing root and crown/collar rot. Identification requires the use of morphological characteristics (Table 4-1). According to Maseko *et al.* (2007), *P. alticola* is similar morphologically to *P. arecae*. Note: Erwin and Ribeiro (1996) note that *P. arecae* should be merged with *P. palmivora*. *P. arecae* differs from *P. alticola* in culture morphology, sporangial shape and size, and heterothallism. Maseko *et al.* (2007) give the complete species description for *P. alticola*.

***Phytophthora austrocedrae*:**

Morphological: Characteristic symptoms on *Austrocedrus chilensis* coupled with morphology are key to identifying this pathogen. Symptoms include: progressive withering and subsequent defoliation of the tree, which dies while standing, foliage chlorotic to red, basal resinous exudates and a red-brown necrotic lesion in the inner bark that is visible extending up the stem from killed roots, and root rot. This new species is characterized by a combination of a very slow growth rate, semipapillate, non-caducous and non-proliferating sporangia, oogonia with amphigynous antheridia formed in a single culture, and low (17°C) optimal temperature for growth (Table 4-1). In addition, the morphology of the mycelium is often characteristic and may be useful for identification of the species in combination with other characters. Greslebin *et al.* (2007) gives the complete species description for *P. austrocedrae*.

P. austrocedrae can be distinguished from its closest relative, *P. syringae*, by colony pattern and antheridia. *P. syringae* has a petaloid colony pattern on V8, TA, and PDA, and oogonia with

paragynous antheridia that, in isolates from Argentina, are usually formed only in media amended with oil and at temperatures below 12°C.

***Phytophthora boehmeriae*:**

Morphological: Symptomatology can be combined with morphology to identify *P. boehmeriae*. Sawada (1927) published the original species description, but the description is not in English. Erwin and Ribeiro (1996) summarize the Sawada paper and others. This information is provided in Table 4-1. *P. boehmeriae* can be recognized by the production of abundant oogonia and oospores in single culture, amphigynous antheridia, and broadly ovoid to subspherical conspicuously papillate sporangia. *P. boehmeriae* differs from *P. parasitica* and *P. citrophthora* by production of oospores in single culture and from *P. megasperma* by production of papillate sporangia.

Molecular: Shen *et al.* (1995) developed a PCR assay for the molecular detection of *P. boehmeriae* in infected cotton. PCR primers PB1 and PB2 resulted in the amplification of a product of approximately 750 base pairs only from isolates of *P. boehmeriae*. Using primers PB1 and PB2, detection sensitivity was approximately 10 fg DNA/µl. In inoculated plant material, *P. boehmeriae* could be detected in tissue one day after inoculation prior to the appearance of symptoms.

Note: SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. boehmeriae* (Gallegly and Hong, 2008).

Phytophthora captiosa:

Morphological: Foliar symptomatology on *Eucalyptus botryoides* and *E. saligna* can be combined with morphology to identify *P. captiosa*. Dick *et al.* (2006) gives the complete species description for *P. captiosa*. *P. captiosa* can be distinguished culturally from similar group VI species (Waterhouse, 1963) by its combination of homothallism, sometimes rather conical amphigynous antheridia, sporangial dimensions, the absence of hyphal swellings and chlamydospores, and its growth-temperature relationships. It has some similarities with *P. cajani* and *P. melonis*. The first formed sporangia of *P. cajani*, however, are larger than those of *P. captiosa* at 60 x 32 µm, and later formed sporangia of *P. cajani* are more irregular in shape. *P. cajani* is also reportedly host-specific to pigeon pea (*Cajanus cajan*). *P. melonis* is most

probably heterothallic. A factor that can clearly distinguish *P. melonis* from *P. captiosa* is its minimum, optimum, and maximum temperatures for growth of 9°C, 28-32°C, and 37°C respectively, compared with 0-5°C, 25°C, and 32°C, for *P. captiosa* (Dick *et al.*, 2006).

Among the *Phytophthora* species in ITS clades 9 and 10, *P. captiosa* is most similar to *P. richardiae*. *P. captiosa*, however, does not produce paragynous antheridia and has a lower minimum growth temperature than *P. richardiae* (2°C), which does not grow below 10°C. Morphologically, *P. captiosa* can be distinguished from *P. insolita* by its formation of antheridia, as *P. insolita* has oogonia devoid of antheridia. It can be distinguished from *P. macrochlamydospora* by the absence of chlamydospores (Dick *et al.*, 2006).

Phytophthora colocasiae:

Characteristic symptoms: On taro, symptoms are quite characteristic for this disease. Symptoms include: small dark brown to olive-green spots (often water-soaked) on leaves, which enlarge rapidly and turn purplish brown with yellowish margins. The lesions frequently form concentric zones and exude drops of yellowish liquid. As the disease progresses, the lesions (mostly along the leaf margin) continue to expand and frequently coalesce. Exudates associated with disease tissue become yellow to brown and form small crusts. Affected corms are almost completely decayed at 8 days after harvest in wet conditions. In the early stages of the disease, it may be confused with *Phyllosticta colocasiae*. This pathogen causes brown spots with yellow borders similar to those caused by *Phytophthora colcasiae,* but the spots do not enlarge or coalesce, and the centers usually fall out, causing a 'shot hole' type of symptom ('shot holes' are only seen in *Phytophthora colocasiae* in tolerant or resistant cultivars). A postharvest rot of the corms can also occur. This diseased tissue is firm and leathery in contrast to the soft rot caused by *Pythium* or *Botryodiploidia* species (Erwin and Riberio, 1996).

Morphological: On other hosts, symptoms coupled with pathogen morphology are key to identifying this pathogen. The causal organism of leaf spot on taro (*Colocasia esculenta*) was first described as *Phytophthora colocasiae* in Java (Indonesia) (Raciborsky, 1900). Morphological characteristics are summarized in Erwin and Ribeiro (1996) and given in Table 4-1. Ho (1992) and Ho *et al.* (1995) give dichotomous keys for *Phytophthora* species occurring in Taiwan, including *P.*

colocasiae.

Molecular: Mishra *et al.* (2010) developed a species-specific PCR assay for *P. colocasiae*. SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above, has now been used to identify and provide molecular fingerprints for *P. colocasiae* (Gallegly and Hong, 2008).

Phytophthora fallax:

Morphological: Foliar symptomatology on *Eucalyptus delegatensis*, *E. fastigata, E. nitens,* and *E. regnans* can be combined with morphology to identify *P. fallax*. Dick *et al.* (2006) gives the complete species description for *P. fallax*. *P. fallax* can be distinguished from other *Phytophthora* species in groups V and VI of Waterhouse's classification system by its combination of both amphigynous and paragynous antheridia, and by its often distorted sporangia. Among the species grouped together on the phylogenetic tree, *P. fallax* most closely resembles *P. richardiae* and *P. captiosa*. It is distinguished from these species by the occurrence of paragynous antheridia and the presence of distorted sporangia. Also, it can be distinguished from *P. richardiae* by a lower minimum growth temperature, and from *P. captiosa* by its rather different growth-temperature responses (Dick *et al.*, 2006).

Phytophthora frigida:

Morphological: Symptoms of *P. frigida* are not characteristic, as they are common for all *Phytophthora* species causing root and crown/collar rot. Identification requires the use of morphological characteristics (Table 4-1). *P. frigida* is similar morphologically to *P. multivesiculata* (also exotic to the United States and covered in this NPRG). In terms of mating behavior, *P. frigida* is heterothallic, whereas *P. multivesiculata* is homothallic. *P. frigida* has papillate sporangia rather than semi-papillate sporangia. *P. frigida* also has corraloid hyphae rather than coiled hyphae, and large spherical hyphal swellings rather than the catenulate hyphal swellings found in *P. multivesiculata*. Maseko *et al.* (2007) give the complete species description for *P. frigida*.

Phytophthora gallica:

Morphological: Symptoms of *P. gallica* are not characteristic, as they are common for all *Phytophthora* species causing overall decline. Identification requires the use of morphological characteristics (Table 4-1). Jung and Nechwatal (2008) give the complete species description for *P. gallica*. *Phytophthora gallica* is an apparently sterile species with nonpapillate sporangia, and

belongs to group VI of the Waterhouse (1963) morphological classification system. DNA sequence analyses places *P. gallica* in ITS clade 10 of the Cook *et al.* (2000) and clade 8 of Kroon *et al.* (2004), and therefore, it is related to *P. kervoviae* and *P. boehmeriae* (both exotic species to the United States covered in this NPRG). *P. kernoviae* can easily be distinguished from *P. gallica* by its production of oogonia in single culture, absence of chlamydospores and hyphal swellings, production of papillate caducous sporangia, different colony growth patterns on CA, and different cardinal temperatures for growth. Moreover, *P. gallica* has, as yet, only been recovered from soil at wet sites, whereas *P. kernoviae* spreads aerially in ornamental gardens and beech woodlands with dense rhododendron understory. *P. boehmeriae* is distinguished from *P. gallica* by its homothallism, the production of papillate caducous sporangia, the absence of elongated pyriform, club-shaped, and irregular chlamydospores, different colony growth patterns and higher cardinal temperatures for growth, different host ranges, and different ITS and mtDNA sequences (Jung and Nechwatal, 2008).

Phytophthora idaei:

Morphological: Symptoms of *P. idaei* are not characteristic, as they are common for all *Phytophthora* species causing root rot. Identification requires the use of morphological characteristics (Table 4-1). Kennedy and Duncan (1995) give the complete species description for *P. idaei. P. idaei* isolates are clearly homothallic, and with their predominately paragynous antheridia and distinctly papillate sporangia, they fit into Group I of the key to species of *Phytophthora* de Bary (Waterhouse, 1963). *P. idaei* differ from other species in Group I: from *P. iranica* by not producing brown, intercalary chlamydospores; from *P. pseudotsugae* in increased sporangial numbers in liquid culture, more branched sporangiophores, and the pigmentation produced on CHT medium; and from *P. clandestina* by the absence of hyphal swellings, persistent sporangia, subterminal or digitate antheridia, and much smaller levels of amphigyny. They also grow faster and have no prominent basal plug in the sporangium.

P. idaei isolates are distinguished easily from the two species belonging to Group III that have been isolated from raspberry, namely *P. citricola* and *P. syringae*, by the depth of the apical thickening of the sporangium, as well as other characters. Other papillate species on raspberry belonging to Group II can be distinguished from *P. idaei* in that they are primarily heterothallic, have amphigynous antheridia, and have higher cardinal temperatures for growth (Kennedy and Duncan, 1995).

Kennedy and Duncan (1995) note that *P. idaei* and *P. cactorum* can be distinguished by a range of cultural and morphological characteristics including but not limited to: a uniform colony growth on MEA compared to the rosette appearance of *P. cactorum*, wider hyphae than *P. cactorum*, persistent sporangia and slightly larger oogonia and oospores, reduced numbers of sporangia, slower growth rate on solid medium, and sympodial branching of sporangiophores in liquid culture.

Molecular: The Scottish Crop Research Institute mentions that, "A sensitive, species-specific polymerase chain reaction (PCR)-based detection assay capable of detecting *P. idaei* was developed at SCRI (http://www.fruitdisease.co.uk/RootRotResearchPage1.asp), but a scientific paper with the details of the PCR reaction could not be located. A poster presented by Cooke *et al.* (n.d.) illustrates a primer that is specific to *P. cactorum/P. idaei* in strawberry and raspberry, but a species-specific primer is not mentioned.

Note: SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above, has now been used to identify and provide molecular fingerprints for *P. idaei* (Gallegly and Hong, 2008).

Phytophthora iranica:

Morphological: Symptoms of *P. iranica* are not characteristic, as they are common for all *Phytophthora* species causing root rot and are similar to *P. erthyroseptica* causing pink rot of potatoes. Identification requires the use of morphological characteristics (Table 4-1). Ershad (1971) gives the complete species description for *P. iranica*, but the description is not in English. Erwin and Ribeiro (1996) summarize the Ershad species description. *P. iranica* differs from *P. cactorum* by its production of noncaducous sporangia, sporangia with more than one papilla, and thicker oospore walls.

Molecular: SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. iranica* (Gallegly and Hong, 2008).

Phytophthora italica:

Morphological: Symptoms of *P. italica* are not characteristic, as they are common for all *Phytophthora* species causing root rot. Identification requires the use of morphological characteristics (Table 4-1). *P. italica*, isolated from myrtle in Italy, was initially

misidentified as *P. iranica* (Belisario *et al.*, 1993). Cacciola *et al.* (1996) compared the myrtle isolate with an authentic *P. iranica* isolate. The myrtle isolate could be distinguished from *P. iranica* and *P. citricola* and *P. pseudotsugae* by morphological characteristics as well as by polyacrylamide gel electrophoresis of mycelial proteins and esterase. The complete species description is given by Cacciola *et al.* (1996).

The oogonia of *P. italica* are similar in shape and size to those of *P. iranica*. The antheridia of *P. italica* are always paragynous, whereas antheridia of *P. iranica* are either amphigynous or paragynous (most frequent) and on cylindrical amphigynous antheridia, fingerlike appendages often form. The average diameter of antheridia is consistently smaller for *P. italica* than for *P. cactorum*, *P. citricola*, *P. iranica*, and *P. pseudotsugae*. Hyphae produced by *P. italica* are straight to irregularly branched, but hyphae of *P. iranica* are sinuate with ends typically coiled. *P. italica* produce distinctly different protein and esterase patterns when compared to *P. cactorum*, *P. citricola*, *P. iranica*, and *P. pseudotsugae*.

Phytophthora kernoviae:

Serological: In addition to the ELISA procedure described above, a lateral flow device developed by Pocket Diagnostics (Central Science Lab, York, U.K.) will also detect *Phytophthora* at the genus level (Lane *et al.*, 2007). This device was tested for *P. ramorum* and *P. kernoviae* but did not differentiate the two species.

Morphological: Symptoms of *P. kernoviae* are not characteristic, as they are common for other forest and nursery *Phytophthora* species, including *P. ramorum*. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Brasier *et al.* (1995) give the complete species description for *P. kernoviae*. *P. kernoviae* is homothallic and produces amphigynous antheridia and caducous and conspicuously papillate sporangia. *P. boehmeriae* is the only known species that has the same characters. *P. kernoviae*, however, can be easily separated from *P. boehmeriae* by the shape of oogonial stalks (tapered vs. not tapered) and sporangium (often asymmetric vs. spherical/ovoid), and pedicel length (medium vs. short). Other species with similar morphology include *P. botryosa*, *P. heveae*, *P. hibernalis*, *P. ilicis*, *P. katsurae*, *P. meadii*, *P. megakarya*, and *P. nemorosa*, but they all have distinct morphological characteristics from *P. kernoviae* (Gallegly and Hong, 2008). Specifically, *P. heveae*

and *P. katsurae* are homothallic with tapered oogonial stalks and amphigynous antheridia; their sporangia are papillate but they are non-caducous. Similarly, *P. ilicis*, *P. hibernalis* and *P. nemorosa* are homothallic, produce amphigynous antheridia and caducous sporangia with medium-length pedicels, but they are semi-papillate. In addition, *P. botryosa*, *P. meadii*, and *P. megakarya* produce amphigynous antheridia and caducous papillate sporangia, but they are heterothallic (Brasier *et al.*, 1995; Benson *et al.*, 2008).

Molecular: Presently, there are two real-time TaqMan diagnostic procedures for *P. kernoviae* that have been reported in the literature, one relying on the ITS region developed at the Central Science Laboratory, York, U.K. (Defra, 2005) and the other using the spacer region in the *ras*-related protein *Ypt*1 gene (Schena *et al.*, 2006; Schena *et al.*, 2008). In addition to *P. kernoviae*, the multiplex real-time PCR developed by Schena *et al.* (2006) can also detect *P. ramorum*, *P. citricola*, and *P. quercina* with a detection limit of 100 fg of target DNA. Schena *et al.* (2008) developed a PCR-based molecular tool box to identify 15 *Phytophthora* species that damage forests and trees: *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. europaea*, *P. inundata*, *P. lateralis*, *P. megasperma*, *P. nemorosa*, *P. kernoviae*, *P. pseudosyringae*, *P. psychrophila*, *P. quercina*, *P. ramorum*, and *P. ilicis*. Detection limits ranged between 100 and 10 pg target DNA/µ1. Amplification with *Phytophthora*-genus-specific primers prior to amplification with the various species-specific primers (nested PCR) increased the sensitivity of detection over amplification with species-specific primers only (100 fg/µ1). Nested PCR was required for soil and water samples. Note: Primers designed for *P. cactorum* and *P. ilicis* cross-reacted with *P. idaei* and *P. nemorosa*.

Phytophthora melonis:

Morphological: Symptoms of *P. melonis* are not characteristic, as they are common for other *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. *Phytophthora melonis* was first described by Katsura (1968, 1976) from diseased cucumber in Japan. Ho *et al.* (2007) published a redescription of *Phytophthora melonis* to correct errors in the original description.

Phytophthora melonis is morphologically quite similar (often indistinguishable) to *P. drechsleri*, but quite different at the molecular level. Banihashemi and Mirtalebi (2008) used safflower seedlings as a selective host to discriminate between

the morphologically similar *P. melonis* and *P. drechsleri*. *P. melonis* could not infect safflower seedlings; while *P. drechsleri* from various hosts attacked safflower within a short period. At least six other *Phytophthora* species (*asparagi*, *cactorum*, *cryptogea*, *erythroseptica*, *palmivora*, and *quercina*) are capable of infecting safflower. This method should only be used once a morphological identity has been established. Additionally *P. melonis* does not induce pink rot symptoms in potato tubers unlike *P. drechsleri* (Mostowfizadeh-Ghalamfarsa *et al.*, 2005; Esmaili-Shirazifard and Banihashemi, 2008).

Molecular: Wang *et al.* (2007) developed a PCR assay for the detection of *Phytophthora melonis* in plant tissue, water, and soil. Traditional, nested, and real-time assays were developed using PCR primers specific to *P. melonis* (Pm1 and Pm2), which were designed using sequences from the ITS region of nuclear ribosomal DNA.

SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for P. *melonis* (Gallegly and Hong, 2008).

Phytophthora multivesiculata:

Characteristic symptoms: The combination of symptoms on *Cymbidium* spp. is quite characteristic of *P. multivesiculata*. The leafy parts of the plants and the pseuduobulbs (modified stems) are attacked. According to Hill (2004), the disease first appears as dark green lesions that expand rapidly under humid conditions to form large irregular patches of water-soaked tissue on mature leaves. The appearance of leaf lesions often signals the presence of a more damaging form of the disease (severe internal rot). Initially the rot is not visible and, often, the first indication that infection is present is a change in the color of the leaves. Infected shoots become gray-green and rapidly lose turgor. Often by this stage, the infection has already spread through the attachment point of the shoot into the adjacent pseudobulb. If the plants are young and have only one or two pseuduobulbs, the infection may prove fatal. Infected young pseudobulbs have a distinctive internal, blue-black or purplish-brown discoloration and a sour odor. The roots do not become infected and remain grayish-white, apparently unaffected even after the rest of the plant has turned brown (Hill, 2004).

According to Ilieva *et al.* (1998), dry rot of leaves (with a somewhat waxy-looking surface) that change color to brown

with typical horizontal zebra-like stripes (about 0.5 cm wide with lighter discoloration in the middle and a dark brown to black margin) are observed on *Cymbidium* plants infected with *P. multivesiculata*.

Morphological: Final species identification requires the use of morphological characteristics (Table 4-1). Ilieva *et al.* (1998) give the complete species description for *P. multivesiculata. P. multivesiculata* resembles *P. porri* and *P. megasperma*. Several morphological characteristics can be used to separate these three species. *P. porri* isolates: 1) sparsely form sporangia on solid medium, 2) have sporangia more often with a tapered base than *P. multivesiculata*, 3) never have internal proliferation of sporangia, 4) have differences in hyphal swellings from *P. multivesiculata*, 5) have paragynous antheridia, 6) exhibit slower growth on V8 agar, 7) have differences in culture characteristics from *P. multivesiculata*, and 8) have differences in isozyme analysis from *P. multivesiculata*. *P. megasperma* isolates: 1) produce sporangia only in water culture, 2) more commonly show internal proliferation of sporangia than *P. multivesiculata*, 3) rarely produce of hyphal swellings, 4) have paragynous antheridia 5) exhibit faster growth on V8 agar than *P. multivesiculata*, 6) show differences in culture characteristics from *P. multivesiculata*, and 7) have differences in isozyme analysis from *P. multivesiculata*.

Phytophthora multivora:

Morphological: Symptoms of *P. multivora* are not characteristic, as they are common for other *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular phylogenetic techniques. Scott *et al.* (2009a) give the complete species description for *P. multivesiculata. P. multivesiculata* is very similar to *P. citricola* and has been misidentified as *P. citricola* in the past. Phylogenetic analyses of the ITS and *cox*1 gene regions show that *P. multivora* is unique and comprises a discrete cluster within the major ITS clade 2 of Cook *et al.* (2000); its closest relative is *P. citricola*. *P. multivora* and *P. citricola* (ex-type isolate) produce different colony growth patterns on V8, MEA, and CMA with the most distinct variation on V8 agar. *P. multivora* has a clear optimum growth temperature of 25 °C while the optimum growth rate of *P. citricola* is at 22.5 °C. Overall for the whole temperature range (except the optimal temperature of 25 °C), *P. multivora* isolates are slower growing than *P. citricola*. Sporangial shapes of *P. multivora* are generally more uniform while in *P. citricola* sporangia are more variable

and the frequency of distorted shapes is significantly higher. *P. multivora* produces on average significantly smaller oogonia and oospores, and significantly thicker oospore walls than *P. citricola* (Scott *et al.*, 2009a).

Phytophthora pinifolia:

Characteristic symptoms: *P. pinifolia* is the only species of *Phytophthora* known to infect green shoots and needles of a *Pinus* spp. Other *Phytophthora* species infecting pine are soilborne and do not infect pine shoots and needles. The pathogen causes serious needle blight. Overall decline, wilting, and mortality are common. Exudation of resin at the bases of needle brachyblasts is also observed. Dead needles are initially retained on the branches. When needles fall, it can lead to near-complete defoliation. Necrotic lesions in the phloem and cambium will eventually girdle the branches.

Morphological: Final identification requires the use of morphological characteristics (Table 4-1). Duran *et al.* (2008) gives the complete species description for *P. pinifolia.* The closest relatives of *P. pinifolia* based on DNA sequence data are *P. megasperma*, *P. gonapodyides*, *P. humicola*, and *P. inundata*. These species all reside in Clade 6 in the Cook *et al.* (2000) phylogenetic classification of *Phytophthora* spp. Members of this clade mostly occur in forests or riparian ecosystems, and cultures are mostly sexually sterile or inbreeding. These species are unlikely to be confused with *P. pinifolia*, because they are all soilborne pathogens and have nested or extended sporangium proliferation unlike *P. pinifolia*. It is, therefore, easy to discriminate between *P. pinifolia* and these species of *Phytophthora* even in the absence of oogoonia, simply on the basis of habit (aerial vs. soilborne) and morphology of sporangia (Duran *et al.*, 2008).

Phytophthora polonica:

Morphological: Symptoms of *P. polonica* are not characteristic, as they are common for other forest *Phytophthora* species, including *P. alni*. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Belbahri *et al.* (2006) give the complete species description for *P. polonica. P. polonica* belongs to Group V of the morphological classification scheme, as it is homothallic with paragynous antheridia, and bears nonpapillate sporangia with internal proliferation (Waterhouse, 1963). Other similar *Phytophthora* species with Group V include: *P. fragariae* var. *typ*., *P. fragariae* var. *rubi, P. humicola*, *P. insolita*, *P.*

megasperma, *P. quininea*. Unlike *P. polonica*, both varieties of *P. fragariae*, as well as species within the '*P. megasperma* complex', can be readily distinguished by having lower cardinal growth temperatures. *P. humicola* has unusually high optimal temperatures like *P. polonica* but does not form chlamydospores. *P. insolita* superficially resembles *P. polonica* in its cardinal temperatures and colony pattern, and in the formation of hyphal swellings and small chlamydospores, but it is easily distinguished by its parthenogenetic oospores (*i.e.,* without attached antheridia). *P. quininea* differs by producing larger oogonia (Belbahri *et al.*, 2006).

Phytophthora porri:

Characteristic symptoms: On *Allium* spp., symptoms are quite characteristic for this disease. Symptoms include: yellowing and dying of the tips of leaves, followed by this area turning white (hence the name for the disease 'white tip'); leaves becoming twisted; and water-logged area developing half-way down or at the base of the leaf (Foister, 1931). Roots are not affected. A similar disease of onions and shallots known as 'shanking' has been reported in England. In this case, infection is spread through roots and bulbs. The roots are completely destroyed, the scales of the bulbs become soft and water soaked, and the leaves turn yellow and shrivel (Erwin and Ribeiro, 1996).

Morphological: On other hosts, symptoms (rot and wet rot) are not specific. Symptoms coupled with pathogen morphology are key to identifying this pathogen. Morphological characteristics are given in Table 4-1. Foister (1931) gives the complete species description for *P. porri.* A key morphological characteristic that separates *P. porri* from other *Phytophthora* species is the presence of coiling hyphae on solid media (Smilde *et al.*, 1996b). Isolates of *P. brassicae* (a new species that infects *Brassica* spp. and was once included in *P. porri*) are morphologically similar to *P. porri* but they show rather different mitochondrial DNA restriction-fragment patterns, abundantly produce sporangia on agar, lack oogonial production after subculturing, and have mainly amphigynous antheridia (De Cock *et al.*, 1992).

Molecular: Restriction fragment patterns of mitochondrial DNA was used to separate *P. porri* from *P. brassicae* (De Cock *et al.*, 1992). SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. porri* (Gallegly and Hong, 2008).

Phytophthora primulae:

Morphological: Symptoms of *P. primulae* are not characteristic, as they are common for other *Phytophthora* species, including *P. fragariae* (Red Core disease). Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Tomlinson (1952) gives the complete species description for *P. primulae*. *P. primulae* forms coiled hyphae on solid media similar to those of *P. porri*. Sporangia of *P. primulae* do not form new sporangia by external or internal proliferation. *P. fragariae* gives rise to new sporangia by proliferation; *P. porri* has both paragynous and amphigynous antheridia, and produces new sporangia by sympodial branching (Tomlinson, 1952; Erwin and Ribiero, 1996).

Molecular: SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. primulae* (Gallegly and Hong, 2008).

Phytophthora psychrophila:

Morphological: Symptoms of *P. psychrophila* are not characteristic, as they are common for other forest *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Jung *et al.*, (2002) gives the complete species description for *P. psychrophila*. *P. psychrophila* belongs to morphological Group IV (Waterhouse, 1963) characterized by homothallic species with amphigynous antheridia and semipapillate sporangia. ITS-DNA sequences indicate a distinct species belonging to Clade 3 of Cook *et al.* (2000), allied with *P. ilicis*. *P. psychrophila* can be distinguished from *P. ilicis* by the former's significantly larger sporangia with more variable shapes and with shorter pedicels (<5 μm) when caducous, its larger oogonia and oospores, the occurrence of sympodially branched hyphae, its lower optimum temperature for growth, different colony morphologies on all agars tested, and different pigmentation on CHT agar. Furthermore, *P. ilicis* occurs only on the above-ground parts of *Ilex aquifolium*. *P. psychrophila* and *P. quercina* exhibit similar growth patterns, but *P. quercina* has paragynous antheridia, distinctly papillate sporangia, and higher optimum and maximum temperatures for growth.

Molecular: As previously described for other species that damage forests and trees, the PCR-based molecular tool box developed by Schena *et al.* (2008), can be used to identify *P. psychrophila*.

SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. psychrophila* (Gallegly and Hong, 2008).

Phytophthora quercina:

Morphological: Symptoms of *P. quercina* are not characteristic, as they are common for other forest *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Jung *et al.*, (1999) gives the complete species description for *P. quercina.* Jung *et al.* (1999) summarizes the major morphological differences among *P. quercina*, *P. cactorum*, *P. clandestina*, *P. idaei*, *P. iranica*, *P. psuedotsugae*, *P. tentaculata*, and *P. citricola* (Group III).

Molecular: Schubert *et al.* (1999) developed primers for PCR-based detection of *Phytophthora citricola*, *P. cambivora*, and *P. quercina.* Nechwatal *et al.* (2001) developed a PCR technique utilizing these species-specific primers and could detect *P. quercina* and *P. citricola* from baited soil samples taken from oak stands. The pathogen could be detected from the oak baits, and from the water used in the baiting tests after removal of floating organic matter. Nested PCR with the primers allowed the detection of as few as five zoospores of *P. citricola* and 300 zoospores of *P. quercina* in a volume of 100 µl. This method allowed detection and identification of species of Phytophthora in soil without the need for direct extraction of the soil samples and without the specific knowledge required to make a morphological identification of each species.

Presently, there is a real-time TaqMan diagnostic procedure for *P. quercina* that has been reported in the literature using the spacer region in the ras-related protein *Ypt*1 gene (Schena et al, 2006; Schena *et al.*, 2008). In addition to *P. quercina*, the multiplex real-time PCR developed by Schena *et al.* (2006) can also detect *P. ramorum*, *P. citricola*, and *P. kernoviae* with a detection limit of 100 fg of target DNA. As previously described for other species that damage forests and trees, the PCR-based molecular tool box developed by Schena *et al.* (2008), can be used to identify *P. quercina*.

SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. quercina* (Gallegly and Hong, 2008).

Phytophthora tentaculata:

Morphological: Symptoms of *P. tentaculata* are not characteristic, as they are common for other *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Kroeber and Marwitz, (1993) give the complete species description for *P. tentaculata.* Erwin and Ribeiro (1996) summarize the main morphological characteristics of *P. tentaculata*. In Group I, *P. tentaculata* differs from *P. cactorum* by production of hyphal swellings, the different shape of sporangia, and by its low number of caducous sporangia. *P. cactorum* sporangia are all nearly caducuous. *P. tentaculata* has larger oogonia and oospores than *P. cactorum* does, and also differs from both *P. clandestina* and *P. pseudotsugae*, in that it produces chlamydospores, whereas *P. clandestina* and *P. tentaculata* do not (Erwin and Ribeiro, 1996).

Molecular: Camele *et al.* (2005) conducted RFLP and sequence analysis of PCR amplified nuclear ribosomal DNA to confirm the presence of *P. tentaculata* in Italy. SSCP analysis of PCR-amplified ribosomal DNA internal transcribed region I, as described above for *P. alni*, has now been used to identify and provide molecular fingerprints for *P. tentaculata* (Gallegly and Hong, 2008).

Phytophthora uliginosa:

Morphological: Symptoms of *P. uliginosa* are not characteristic, as they are common for other forest *Phytophthora* species. Identification requires the use of morphological characteristics (Table 4-1) or molecular techniques. Jung *et al.*, (2002) gives the complete species description for *P. uliginosa. P. uliginosa* belongs to morphological Group V (Waterhouse, 1963) with homothallic species with paragynous antheridia and nonpapillate sporangia with a shallow apical thickening. ITS-DNA sequences indicate a distinct species belonging to Clade 7a of Cook *et al.* (2000), allied with *P. cambivora, P. fragariae, P. alni*, and *P. europaea*. *P. uliginosa* shares many morphological and growth features with members of the *P. cambivora* clade. *P. uliginosa* differs from other species in this clade in its combination of smooth-walled, homothallic oogonia with one-celled paragynous antheridia. *P. uliginosa* is distinguished from *P. europaea* by having larger oogonia without tapering base, larger oospores, wider exit pores, slower growth rates, lower optimum and maximum temperature for growth, different colony morphology on CMA, V8, MEA, and PDA, and different ITS-DNA

sequences. Furthermore, it seems to be more aggressive to *Q. robur* than *P. europaea*.

Sample Packaging and Documentation

All samples must be packaged in a large zip-lock bag made leak proof, then placed in a sturdy cardboard outer box with insulation to prevent movement within the box during shipping. Include the completed PPQ form 391, and any relevant tags or barcodes that came with the sample.

Sample Labeling, Numbering, and Record Keeping

An electronic data collection system for survey and sample collection is currently being developed. The Incident Commander or Program officials should provide the appropriate equipment for recording the sample collection information. Until the protocols for that system are finalized, complete a PPQ form 391 (*Specimens for Determination*) for each sample.

Place a hard copy of the PPQ form 391 inside the outside bag of double-bagged samples. Assign and record for each sample a unique ID sample number with a predetermined format. Assure that the sample is linked to any survey data collected for that sample by including the Survey ID number on the form. This will enable the linkage of the sample to all the field collection information.

In Block 1 of the PPQ Form 391, enter and label the assigned sample ID number first: the first two letters designated the state code. Also enter the survey ID in parenthesis. A state-specific lab sample accession number can also be added for record keeping. Use the following format:

Sample ID # XX-00000 (Survey ID # _____)

In the remarks section (Block 22), provide the name of the office or diagnostic laboratory forwarding the sample, plus a contact name, e-mail address, and phone number. Include the date forwarded to state diagnostic lab, USDA-APHIS NIS, or the USDA Beltsville laboratory.

In Block 23, enter the preliminary diagnosis (e.g., "High suspect *Phytophthora* spp.").

Inspectors must provide all relevant collection information with samples. This information should be communicated within a State and with the regional office program contact. If a sample tracking database is available at the time of the detection, enter collection information in the system as soon as possible.

State or Other Diagnostic Screening Laboratories Results

When plant samples are collected they should be completed and submitted to the state or university diagnostic facility within the state of collection. Samples are not to be directly submitted to the USDA Beltsville laboratory. Each state land grant university diagnostic laboratory, as part of the National Plant Diagnostic Network (NPDN), has a protocol for preliminary identification of *Phytophthora spp.* from plant material, and instructions for sending suspicious samples to the USDA lab for confirmation and species identification. Diagnostic screening laboratories receiving samples are to communicate the date of receipt to their State Plant Regulatory Official and/or State Plant Health Director. All relevant sample information, along with the diagnostic lab's determinations, must be communicated as soon as possible within a State and with the PPQ regional office program contact.

Approved Laboratory for Confirmatory Testing

Morphological identification of *Phytophthora spp.* will be performed by the National Mycologist at the USDA-APHIS-PPQ Plant Safeguarding and Pest Identification National Identification Services in Beltsville, MD. Confirmatory molecular testing and validation of primers for PCR or serological tests for the pathogens is performed at the USDA, APHIS, PPQ CPHST Lab in Beltsville, Maryland

Potentially Actionable Suspect Sample (PASS) Policy for Exotic *Phytophthora* species

Potentially Actionable Suspect Sample and Federal Confirmation in PPQ

A Potentially Actionable Suspect Sample (PASS), in its simplest form, is a sample of a pest or pathogen of regulatory concern (including bacteria, viruses, nematodes, weeds, insects, mites, mollusks, etc.) that has been presumptively identified by a laboratory without federal confirmatory authority. Under these circumstances, confirmatory testing by an appropriately approved Federal laboratory is required. These pests are defined by the Plant Protection Act (2000) as "new to or not widely distributed" and are regulated by APHIS in order to protect foreign and interstate commerce. Confirmation by an APHIS-approved lab means that Federal action will result from the identification, and/or that Federal funds will be utilized in response to the identification of the pest or pathogen.

Presumptive identification of a PASS by a non-approved laboratory results in the sample(s)/specimen(s) or its biological extract (DNA, RNA, proteins, etc.) being forwarded to a

designated Federal laboratory for confirmatory testing. Subsequent 'suspect' positive samples from within an APHIS-defined regulated area of the first PASS do not require federal confirmatory testing, but new finds outside of the defined area are considered a new PASS. Typically, a sample considered a PASS would also encompass any sample that involves unusual or unexpected circumstances, (*e.g.*, a new host, new location, an atypical biology, or potential bio-terrorism act). The PASS policy for a specific pest or pathogen is based on what is known about the biology of the organism and epidemiology of the disease.

PASS Policy for Exotic *Phytophthora* spp.

A PASS policy similar to that of *P. ramorum* is used for other exotic *Phytophthora* spp. The PASS policy for *P. ramorum* is available at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/passpolicy_mar08.pdf

Diagnostic and Identification Protocols

Soil sample extraction and morphological identification protocols are being developed. Molecular protocols are currently being validated at the CPHST Beltsville Laboratory and protocols are expected in the future. When available, all protocols will be posted on the PPQ website:
http://www.aphis.usda.gov/plant_health/plant_pest_info/

Completing the PPQ form 391 Determination Section

Diagnostic screening laboratories must report their determinations for each sample using the PPQ form 391 that came with the sample. Include the name and phone number of the responsible diagnostician, keep a copy, and follow the sample packaging instructions described above.

Saturday Delivery

Normally, it is not recommended that samples be sent on Thursdays or Fridays to NIS or the CPHST Beltsville Lab because of the possibility of deterioration over the weekend. Depending on need and when approved by APHIS officials, samples may be sent on Thursdays or Fridays by FedEx® because it is possible to have Saturday delivery by overnight carriers to the Beltsville facility. **However this must be determined by consultation and arrangement with APHIS and state officials prior to assuming that the laboratory will be operating on Saturday**. If you verify with APHIS officials that samples will be accepted on Saturday, the FedEx® tracking number to the CPHST Beltsville Lab must be provided, via email, no later than 2 PM EST Friday so the Fedex® local office

can be notified in time to authorize Saturday delivery**.**

Notification of State Officials of Sample Submissions and Results

Notify the State Plant Health Director and State Plant Regulatory Officials in the sample state of origin and fax the PPQ regional office of any sample forwarding information and completed documentation, including overnight freight tracking information. Once results are known, States will be notified through the official communication chain.

All communication of sample results will be carried out in accordance with the official communication policies of the USDA and APHIS PPQ. **Do not contact the CPHST Beltsville Lab or MDL for sample results. This information will be reported through the appropriate and approved reporting lines to the regions and States from PPQ headquarters as soon as they are available**. The NPGBL and MDL will direct any inquiries to PPQ headquarters.

Sample Processing Time

Growers, cooperators, and laboratories need to be aware that, once received by the CPHST Beltsville Lab or MDL, sample processing and testing time of at least 48 hours is required. This is in addition to the time it takes to process and forward samples from the intermediate state or cooperating university diagnostic laboratories.

Generic life cycle of Phytophthora spp.

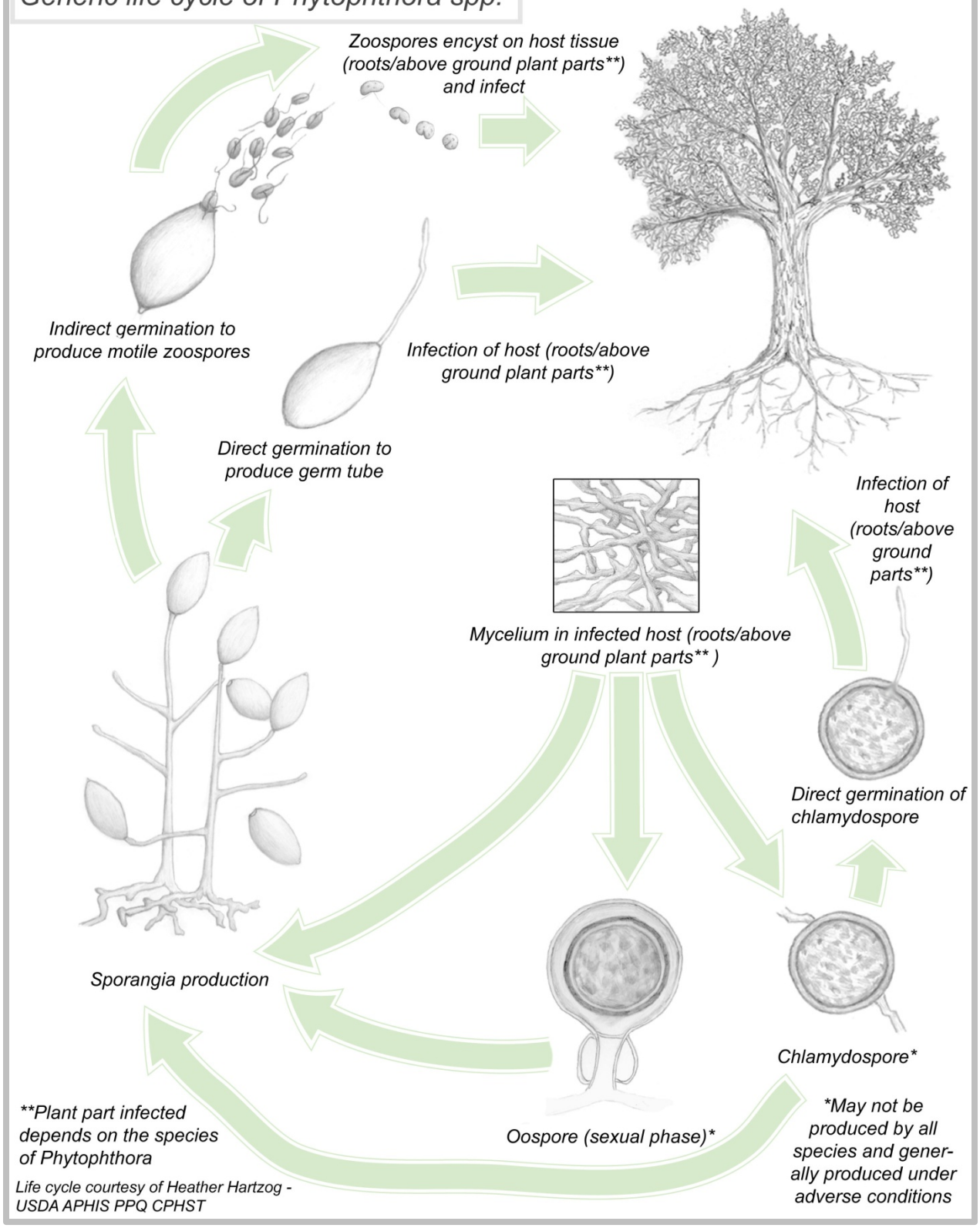


Table 4-1: Morphological characteristics of exotic *Phytophthora* spp.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora alni subsp. *alni*	Hyphal swellings are not reported.	Not seen on CA, sparse on pea broth. Born singly on long sporangiophores, ellipsoid, non-papillate, non-caducous, with a broad exit pore. Length range 35-70 µm. Breadth range 27.5-50 µm. Length-breadth ratio: 1.32-1.62. After zoospore release showing nested and internal proliferation.	None observed.	Homothallic	Abundant. With tapered stalks, variably warty with bullate proturbances. Diameter range 37-55 µm. Some with small diameters of 25-35 µm. High proportion either fully aborted or with thin-walled oospores.	Plerotic. Diameter range – 27.5-50 µm.	Predominately two-celled and amphigynous. Length overall range from 20-30 µm. Width overall range of 15-20 µm.	CA- uniform, appresed-felty with no or very sparse aerial mycelium; colonies irregular in outline. Maximum temperature for growth is 29 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora alni subsp. *multiformis*	Hyphal swellings are not reported.	Similar to *P. alni* subsp. *alni.*	None observed.	Homothallic	Range of different forms from near smooth to extremely ornamented. Diameters range 45-65 µm.	Information not provided.	Wide variety of types from single celled to two celled amphigynous and occasionally predominately paragynous.	CA- irregular, often with moderate to dense aerial mycelium. Comprises a range of forms with different phenotypes.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora alni subsp. *uniformis*	Hyphal swellings are not reported.	Similar to *P. alni* subsp. *alni.*	None observed.	Homothallic	Gametangia generally frequent. Oogonia mostly smooth-walled, but some slightly wavy edged-verrucose. Occasionally, oogonia have large, distorted beak-like proturbances. Diameter range 37.5-55 µm.	Diameter range – 30-47.5 µm.	Consistently two-celled and amphigynous. Length overall range from 17.5-27.5 µm.	CA- irregular, appressed colony, often with a little wooly aerial mycelium in the colony center but submerged growth at the edge. Some cultures areas highly unstable and chimeric, with gametangia produced only beneath patches of aerial mycelium.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora alticola	Smooth up to 5 µm. Hyphal swellings not present.	Sporangia papillate, occasionally bipapillate, variable in size and shape (ovoid, globose, obturbinate, limoniform, and various distorted shapes). Terminal sporangia caducous with short pedicel and conspicuous basal plugs. Exit pore diameter 4-8 µm (mean 6 µm). Length-breadth 30-45 µm x 20-35 µm (mean 36 x 28 µm). Length-breadth ratio mean 1.4.	Rarely produced, terminal and spherical in shape between 20 and 35 µm (mean 28 µm).	Homothallic	Smooth walled, terminal with tapered stalks with diameters ranging from 24-31 µm (mean 26 µm).	Thick inner walls. Markedly aplerotic 24-36 µm.	Mainly amphigynous but paragynous antheridia also present. Antheridia had a tendency to detach from the oogonia.	V8, CA, MEA, CMA, and PDA-No distinctive growth pattern. Mycelium dome-shaped and fluffy with scant to moderate aerial mycelium on V8, CMA, and MEA. Colonies tend to be appressed with thinly spread aerial mycelium on CMA. Sensitive to hymexazol.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora austrocedrae	Quite variable based on medium used, 4-8 µm in diameter. Hyphal swellings usually formed in solid and liquid media, but more abundant in solid medium. Globose to subglobose and catenulated, sometimes with distorted shapes. Growth very slow and favored by cool temperatures (17.5 °C)	Sporangiophores simple, 3-11 µm diam., frequently with hyphal swellings. Sporangia borne terminally. Ovoid, obpyriform, limoniform or ellipsoidal, infrequently with distorted shapes; semi-papillate, papilla 1-3 µm thick, non-papillate sporangia were infrequently observed; 22-83 x 15-58 µm. Length-breadth ratio on average 1.4 $\pm$0.2. Sporangia with hyphal projections, lateral attachment of the sporangiophore were frequently observed. Non-caducous, non-proliferating sporangia.	None reported.	Homothallic	Oogonia formed more quickly on media with antibiotics than that without antibiotics (20 days). Globose or nearly so, range 22-56 µm with hyaline to light brown, smooth walls.	Plerotic, globose, 17-48 µm, hyaline, with smooth walls 1-3 µm thick.	Amphigynous, hyaline, one-celled 10-30 x 8-20 µm (average 18$\pm$3.5 x 14$\pm$2 µm)	V8, TA, and TAβ -colony uniform, without pattern, cottony dome-shaped in the center, appressed or mostly submerged at the margins. CMA - colony was appressed, with little or no aerial mycelium; the submerged mycelium showed an arachnoid pattern. PDA-colony was uniform, without pattern, densely felty to wooly, with abundant and dense aerial mycelium and defined margins.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora boehmeriae	Gnarled hyphae, grows in a tortuous manner. No hyphal swellings observed.	Papillate, spherical, ovoid, ellipsoidal, or obturbinate. The spores measure 20-72 x 20-51 µm. Length-breadth ratios reported are 1.25 to 1.4. Sporangia are caducous with a pedicel length of 5.0 µm or less. Sporangiophores are sympodial.	Produced infrequently. Diameters range from 17 to 51 µm. Walls are 2 µm thick.	Homothallic. Zhou *et al.* (1997) showed that growing *P. boehmeriae* on medium containing the fungicide ethazol caused self-sterile sectors (change in mating type).	Smooth-walled and hyaline to yellow. Diameters are 21.7-41 µm.	Abundant in host tissues. Oospores nearly fill the oogonium (plerotic). The wall is 2 µm or less in thickness. Diameter from 16-34 µm.	Amphigynous and almost spherical in shape, often with a residual oil globule. Dimensions reported vary from 8-21 x 12.5-21 µm. Gao *et al.* (1998) showed that high concentration of nutrients in culture media favored amphigynous antheridia production; low concentration of nutrients favored paragynous antheridia.	Colonies are uniform and compact with well-defined margins and dense aerial mycelia. Oospores, and sporangia form abundantly in an agar medium in 7 to 10 days.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora captiosa	No hyphal swellings observed.	Non-papillate, non-caducous, ovoid sporangia are sometimes produced on host petiole material floated in soil water. Sporangia average 24±4.5 x 25.5 ±4.5 µm with isolate means of 30.5-40.5 x 20.5-29.5 µm. Length-breadth ratio is 1.2-1.8. Sporangial proliferation is both internal and external from the base of the existing sporangium.	No chlamydospores observed.	Homothallic	Spherical, 30-41.5 µm, often with a tapered base, generally turning pale brown with age. Oogonial stalk often two celled. Oogonia produced in host tissue are brown and slightly smaller than those produced on CA 27.5 ± 0.5 µm in diameter.	Initially plerotic, occasionally becoming aplertoic with age. Averaging 29.5 ± 4 µm in diameter. Oospore wall averaging 1.5 ± 0.5 µm thick; occasionally up to 3 µm.	Amphigynous, ranging from cylindrical to conical, often with coiled hyphae at the base, occasionally two-celled, the bottom cell about one third the size of the top cell, averaging 19.5 ± 3 x 14 ± 2.5 µm.	Optimal temperature: 25 °C. Moderate growth on CA at 20 °C in the dark. Forms a stellate to rosaceous, appressed to fluffy colonies after 10 days on CA in darkness.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora colocasiae	No hyphal swellings reported.	Sporangiophores in culture are irregularly branched, but on leaf surfaces are short and unbranched. Semipapillate, caducous sporangia (pedicel length 3.5-10 µm); ovoid, ellipsoid, or sometimes fusiform; and 40-70 µm long x 17-28 µm wide. Length-breadth ratio of 1.6:1 and a tapered base with an occasional lateral attachment, sometimes intercalary. A conspicuous basal plug is present at the point of attachment of the sporangium to the sporangiophore.	Abundant in some isolates and rare in others. Often hyaline in color. They are 17-38 µm in diameter (average 27 µm), and the wall is 2-3 µm thick. Formation is either terminal or intercalary in the mycelium).	Heterothallic.	Oogonia are 20-35 µm in diameter, average 29.0 µm, spherical and yellowish.	Oospores are 18-30 µm in diameter, average 23.0 µm and aplerotic.	Antheridia are amphigynous and subterminal.	The minimum growth temperature is >10 °C, optimum 27-30 °C, and maximum >35 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora fallax	No hyphal swellings.	Non-papillate, non-caducous, obpyriform to distorted sporangia, often with a distinctive elongated neck and conspicuous basal plug. Sporangia average 55.5±5 x 32 ±3 µm with isolate means of 50.5-61.5 x 28-34 µm. Length-breadth ratio is about 1.7. Hyphal projections are often seen at the apex of mature sporangia. Sporangia proliferate both internally and externally from the base of an existing sporangia.	Spherical, terminal chlamydospores measuring 12-26 µm in diameter were produced in soil water in two isolates.	Homothallic	Spherical, 30-39 µm, often becoming pale brown with age.	Initially plerotic, becoming aplerotic with age. Averaging 31.5 ± 2.5 µm in diameter. Oospore wall averaging 2 ± 0.5 µm thick.	Antheridia both amphigynous and paragynous. Structure difficult to determine due to coiled and distorted hyphae around the stalk of the oogonia. Antheridia are occasionally absent (older culture). Amphigynous antheridia are cylindrical, single celled and average 18.5±4 x 14±1 µm. Paragynous antheridia are globose, and average 9±2 µm, most often attached near the stalk.	Optimal temperature: 20 °C. Slow growth on CA at 20 °C in the dark. Forms a stellate to rosaceous, often with densely fluffy mycelium after 10 days on CA in darkness.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora frigida	Corraloid, irregular, and sympodially branched, fairly uniform, up to 5 µm. Hyphal swellings globose and intercalary.	Sporangiophores thin branches, arising near or directly from hyphal swellings. Sporangia terminal or sometimes intercalary, readily produced in solid or liquid media, conspicuously papillate. Caducous with short pedicels. Exit pore 3-7 µm (mean 5 µm), ovoid, obpyriform, or irregular shaped. Length x breadth 24-40 µm x 20-33 µm (mean 33 x 27 µm).	Terminal, globose, mean diameter 20-35 µm (mean 25 µm), thin or thick-walled, and brown.	Heterothallic	Terminal, spherical with smooth walls, often thicker and golden brown with age and mostly 25-42 (mean 33 µm) in diameter.	Aplerotic 19-38 µm (mean 28 µm) diameter, wall 1.5-3 µm thick, often light yellow or colorless.	Amphigynous (95 percent), elongated, cylindrical or spherical to ellipsoidal.	V8, CA, MEA, CMA, and PDA-stellate to rosaceous colony type. Cottony colonies with irregular growth patterns are produced on V8, CA, MEA, and PDA. Submerged colonies with only sparse aerial mycelium on CMA. Tolerant to hymexazol.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora gallica	Primary hyphae on CA 2.5-7.5 µm (average 4.5±1.5 µm) wide. Spherical and irregular hyphal swellings produced in water culture.	Sporangia produced abundantly in water culture. Borne terminally, on unbranched sporangiophores or more rarely in lax sympodia. Non-papillate, proliferating internally in an extended or more rarely in a nested way. Shapes vary from obpyriform, ovoid, peanut-shaped, or limoniform. Average 52.5±11 x 27±5 µm. Length-breadth ratio 2±0.5. Zoospores discharged through an exit pore 7.5-19 µm wide.	Globose and elongated, pyriform, club-shaped, and irregular. Attached terminally or laterally. Globose chlamydospores averaging 47.5±7 µm. Elongated pyriform, club-shaped, and irregular chlamydospores averaging 72±19 x 34±7 µm. Most chlamydospores starting to abort a few days after formation.	Sterile	Not produced in single culture or in paired cultures.	-	-	Colonies generally appressed with submerged margins and dendroid sectors of aerial mycelium on CA, largely submerged with a rosaceous pattern on MEA, and faintly stellate with narrow-lobed to chrysanthemum margins on CMA.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora idaei	Hyphae hyaline, non-septate, 4.8-6 µm wide. Hyphal swellings not reported.	Sporangiophores simple or sparingly branched with irregular lax sympodium; width 2.4-3.6 µm, length variable averaging 3.1-3.3 µm. Sporangia persistent, terminal or occasionally intercalary, proliferating externally; spherical to ovoid, 41.4-55.2 x 29.9-43.7 µm, papilla conspicuous protruding beyond contour of sporangium; apical thickening hyaline, depth 2.4-6 µm. Zoospores 12-16.8 µm x 7.2-9.1 µm discharged through a pore 6-7.4 µm wide.	Chlamydospores not reported.	Homothallic	Oogonia terminal on stalks 5.5-10.5 µm wide and 6.1-20.9 µm long, spherical, smooth-walled, 27-38.4 µm in diameter, sometimes pigmented when mature.	Aplerotic 22.8-31.2 µm diameter with a wall 1.9-2.7 µm.	Hyaline, simple, club-shaped, occasionally spherical, 9.1-15.7 µm wide and 12.6-26.2 µm long, with stalks 4.1-6.6 µm wide and 6.9-23.1µm long. Predominately (97 percent) paragynous and attachment commonly near oogonial stalk.	Colonies were uniform with no obvious pattern on CMA, MEA, V8, and SAMA. They formed no aerial mycelium on CMA but produced limited aerial mycelium on V8, MEA, and SAMA. Minimum growth at or below 5 °C, optimum 20 - 22.5 °C, and maximum 27 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora iranica	Hyphae were sinuate, with typically coiling ends. Hyphal swellings are not formed.	Sporangiophores are short and sympodially branched. Sporangia papillate (sometimes bipapillate), ovoid, obpyriform, ellipsoid to subspherical, 30-72 µm long x 22-51 µm wide. Length-breadth ratio: 1.3:1; and persistent on the stalk.	Rare, 17-41 µm in diameter, average 28.7 µm; mostly intercalary; and rarely terminal.	Homothallic	Oogonia subspherical and 21-45 µm in diameter (average 34 µm).	Aplerotic, 15-37 µm in diameter (average 29.3 µm). The wall is 1-5 µm thick (average 3 µm).	Mostly paragynous, but occasionally an amphigynous antheridium is found. Amphigynous antheridia were cylindrical and often showed finger-like appendices. More than one antheridium per oogonium was observed in this species.	The minimum growth temperature is 10 °C, optimum 27.5 °C, and maximum 35 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora italica	Hyphae hyaline, 3-6 µm in diameter, poorly branched, becoming septate with age. Hyphal swellings are not formed.	Sporangiophores simple, sympodial. Sporangia terminal, occasionally intercalary, distinctly papillate, frequently bipapillate, sometimes with three papillae, persistent, varying in shape and dimensions. On V8 agar-sporangia ovoid, obpyriform, subspherical or broadly ellipsoid, 14-56 µm long x 11-38 µm wide; length-breadth ratio: 1.1-1.5.	Rarely produced.	Homothallic	Oogonia subspherical, often slightly flattened or pyriform, with a short stalk and smooth walls, varying in size; diameter on V8 agar 15-29 µm at the poles (mean diameter 22±3.4 µm).	Single, markedly aplerotic, 11-23 µm in diameter (mean 16.8±2.9 µm).	Single, unicellular, paragynous, mostly subspherical (mean diameter 7.1±1.6 µm), sometimes ovoid, 6-12 µm long x 4-10 µm wide.	On PDA-slight stellate patterns; On V8-uniform, white to yellowish-brown with aerial mycelium wooly and dense like felt. On CMA-white, appressed and slightly radiate, with sparse aerial mycelium when mature. Growth occurs between 10 and 35 °C, with optimum at 26 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora kernoviae	Hyphae sometimes denticulate or tuberculate.	Sporangia occasional on CA, common on plugs immersed in soil water or soil leachate; sympodial sporangiophores. Sporangia papillate, caduceus, from regular ovoid or limoniform to distinctly asymmetrical or 'mouse-shaped' with one rounded and one flatter side. Most have a conspicuous vacuole. Sporangia length x width range of means 38.5-45.5 x 22.5-27 µm. Length-breath ration: average 1.5 µm. Sporangial pedicels range of means 8.6-14.1 µm.	No chlamydospores observed.	Homothallic	Oogonia, diameter range of means 23.5-25.5 µm; often with tapered stalks.	Plerotic, diameter range of means 21.1-22.5 µm; wall thickness averages about 3.5 µm.	Amphigynous. Length/width range of means 11.5-12.5 x 10-10.8 µm.	On CA-largely submerged in darkness. On exposure to light, small central patchy aerial mycelium. Diurnal light, alternating rings of aerial mycelium. Maximum growth temperature 26 °C, optimum at 18 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora melonis	Main hyphae tubular, nonseptate, smooth, 4-6 µm wide, branching freely but becoming irregular with age. Hyphal swellings occasionally produced in water, spherical to oval (under 25 µm), terminal, intercalary, or in small networks.	Sporangia sparse on agar; abundant in water. Sporangia single, terminal, nonpapillate, noncaducous, on long mostly unbranched or lax, sympodially branched undifferentiated sporangiophores, ovoid to ellipsoid, average 35-84 x 14-44 µm; length-breadth ration 1.4-3.1; internal proliferation dominant. Empty sporangia partially collapse after zoospore release with exit pores 10-15 µm wide. Encysted zoospores 8.3-9.4 µm diameter.	Not observed.	Heterothallic	Yellow to golden brown, globose, smooth, 26-49 µm diameter.	Single, globose, hyaline, plerotic to slightly aplerotic, 19-42 µm in diameter. Wall 2-4 µm thick.	Amphigynous, cylindrical to oval, unicellular but sometime bicellular, 16-18 x 16-17 µm.	On V8-colonies slightly radiate to rosette with scant to abundant aerial mycelium. Cardinal temperatures 10-13 °C (minimum), 27-28 °C (optimum), and 36-37 °C (maximum).

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora multivesiculata	Mycelium branched, main hyphae 6.0 µm in CMA. Hyphal swellings numerous; rounded, ellipsoid, catenulate, and clustered.	Sporangiophores long, slender (2-3 µm), often twisted, proliferating percurrently through the empty sporangium, sometimes sympodially from near the base. Sporangia 30-60 x 20-41 µm, ovoid, obpyriform, non-papillae or semi-papillate; most with a rounded base, occasionally on a tapered base; exit pore 8-14 µm wide.	None reported.	Homothallic	Smooth-walled, spherical, readily produced in host tissue and culture. On V8 – 28-50 µm.	Mostly aplerotic 24-42 µm.	Antheridia mostly amphigynous (95 percent), some diclinous.	Irregular and slightly fluffy.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora multivora	Primary hyphae 3.8-4.6 µm in diameter.	Sporangia semipapillate and either ovoid; limoniform, ellipsoid, or obpyriform, sometimes with shallow apical thickening, non-caducous, occasionally forming a conspicuous basal plug. Sporangia with 2 or 3 papillae or distorted shapes were formed at times. Typically borne terminally. External proliferation was regularly observed, either irregular or in lax or dense sympodia. Mean sporangial dimensions were overall 25-97 x 13-63 µm with length-breadth ratio of 1.3-3.3 µm overall.	None reported.	Homothallic	Oogonia borne terminally with smooth walls, globose to slightly subglobose. Mean diameter of 26.5±1.9 µm.	Globose, nearly plerotic, 23.9±2 µm. Thick oospore walls 2.6+0.5 µm (overall range 1.4-4.6 µm). Oospore wall index (0.52 +0.07).	Ovoid, club-shaped, or irregular, almost exclusively paragynous, diclinous and typically attached close to oogonial stalk. Intercalary and amphigynous antheridia were rarely observed.	V8- produce stellate growth patterns with clearly delimited, submerged margin. MEA- faintly stellate CMA- Appressed to submerged colonies with a faintly stellate to petaloid pattern. PDA- petaloid felty to fluffy colonies.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora pinifolia	Hyphae coralloid with unusual single spherical swellings, sometimes with radiating hyphae, swellings 4-8 µm diameter.	Sporangia borne predominately on unbranched, simple sporangiophores (22-44 µm long). Sporangia non-papillate, 39-61 x 27-45 µm, semiglobose to ovoid, caducous, no internal proliferation, exit pore 15 µm wide, occasional free with pedicels 14-32 µm long.	None reported	Heterothallic?	Not Known – *P. pinifolia* from Chile may consist of a single mating type unable to undergo sexual recombination.	Not Known	Not Known	Cultures were NARP-Submerged with coralloid morphology. CA-V8- white with fluffy aerial mycelium, regular margin or a rosaceous or petallate pattern. Optimal temperature for growth 25 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora polonica	Main hyphae 8 µm wide. Hyphal swellings abundantly, usually large (up to 50 µm long), single or more frequently catenulate, intercalary or lateral, or aggregated, inflated, toruloid, irregularly shaped to globose.	None on culture media or in water soil extract, few in gelatine solution. Borne on long nonbranching sporangiophores, mostly ovoid to ellipsoid, 52-67 x 32-44 µm, noncaducous, nonpapillate, proliferating internally, often nested or catenulate. Zoospores discharged through an exit pore 10-18 µm wide.	Abundant, spherical to subglobose or pyriform, average diameter 48.4 µm (ranging from 16 to 69 µm), moderately thin-walled (1-2 µm), intercalary, lateral or terminal on short branches. In water, forming extensive networks of geniculate hyphae with lateral chlamydospores at the joints.	Homothallic	Abundant. Mostly borne on stalked branches, spherical to globose, smooth- and thin-walled, 41.8 $\pm$2.8 SD µm diameter.	From aplerotic to nearly filling the oogonia, 38.1$\pm$2.5 µm diameter. A high proportion of aborted oogonia were seen.	Mostly clavate to irregularly shaped, less frequently spherical to barrel shaped, 16.2$\pm$2.8 µm x 13$\pm$2.1 µm wide; borne on long stalks, mostly attached near the oogonial base, occasionally with hyphal extensions. Predominately paragynous but sometimes amphigynous.	CA- aerial mycelium appressed to limited, slightly stellate to rosaceous; concentric growth rings somewhat noticeable on underside of Petri dish CMA- submerged to somewhat radiate MEA-aerial mycelium appressed, fairly felty, markedly rosaceous PDA-felty, broadly lobed, rosaceous. Optimum temperature 30 °C, minimum temperature 5 °C, and maximum temperature 38 °C.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora porri	Mycelium branched, non-septate when young, septate and empty when old, coiled in spirals in region of sexual activity. Hyphal swellings are round, ellipsoid, or angular and occur singly or in chains. Form abundantly in culture.	Sporangia obpyriform, oval, or ellipsoid, 51 x 35 µm (31-82 x 23-52 µm), produced at end of sporangiophores or intercalary, semipapillate, persistent on the stalk, nonproliferating, and borne successively. Length-breadth ratio is 1.29-1.46. On *Allium* spp. occasionally with 5-12 x 10-12 µm; apical thickening 2.5-5.0 µm and hyaline.	Form in culture medium after prolonged incubation in water and measure 20.8-35.3 µm in diameter, average 30 µm.	Homothallic	Spherical, with an unevenly thickened wall, 38-39 µm (29-46 µm), fertilized mostly by paragynous antheridia, sometimes by amphigynous antheridia, and sometimes by both types at once.	Spherical, honey-yellow when old, 32-33 µm (22-39 µm), with a thick wall 3-4.5 µm, aplerotic.	Oval or flattened spheres, terminal or intercalary. Not on the same hyphae as oogonium; amphigynous 7.3-19.4 µm (avg. 12.5 µm) and paragynous 7.3-10 µm.	Although Foister (1931) originally reported a maximum temperature for growth that was <35 °C, it is now reported as a maximum of 27 °C or less.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora primulae	Mycelium hyaline, non-septate when young, septate and devoid of protoplasm when old; occasionally coiled in spirals. Hyphal swellings not reported by Tomlinson (1952), but are common in other isolates (Gallegly and Hong, 2008).	Sporangia terminal, developed on undifferentiated sporangiophores, variable in shape and size, some, single structures ovoid, bluntly ellipsoid, limoniform, or obpyriform. 33-165 x 23-52 µm; others compound forms, elongated consisting of 2 to 11 spherical or more irregularly shaped sporangial segments delimited at one or both ends by septa or hyaline plugs and connected in chain fashion, 100-351 x 6-36 µm; semipapillate, noncaducous. Zoospores 10-15 µm.	None reported	Homothallic	Oogonia terminal or lateral, globose, 23-43 µm (avg. 37 µm) in diameter.	Spherical, hyaline when young, sometimes light yellow when old, 17-33 µm (avg. 30 µm) in diameter, with smooth wall 3 µm in thickness, aplerotic.	Terminal, rarely intercalary, diclinous and paragynous, mostly 14-18 µm in diameter.	Temperatures for growth: minimum (1°C), optimum (15-20 °C), and maximum (<27 °C).

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora psychrophila	Primary hyphae sometimes show sympodial branching with a mother hyphae ending in a short projection. Hyphal swellings have not been reported.	Sporangia semipapillate, sometimes bipapillate, some caducous with pedicel lengths less than 5 µm. Sporangia are variable in shape and size, usually ovoid to obpyriform with a rounded base, but sporangia with unusual feathers such as curved apices, lateral displacement of papilla, lateral attachment of sporangiophore, short hyphal projections, or a conspicuous basal plug are common. Sporangia average 58±10.3 x 39±5.9 µm. Length-breadth ration: 1.5. Proliferate only externally.	None reported.	Homothallic	34-39 µm. Oogonial walls are often markedly thicker than oospore walls.	Plerotic usually, but can be aplerotic in oogonia with distorted shapes. Oogonia with two oospores are occasionally found. Diameter average 29±3.0 µm. Oospore walls average 2.4±0.5 µm in diameter, sometimes turning golden yellow when aging. Oospore abortion can be found in all isolates.	Amphigynous, one-celled, and average 15±2.2 x 13±1.5 µm.	CMA- faint petaloid pattern sparse aerial hyphae, VA and MEA- dome-shaped and fluffy, petaloid to broad-lobed growth patter, PDA-irregular to stoloniferous pattern with appressed to submerged growth, felty and dome-shaped. Optimal temperature (15-17°C), maximum (25°C).

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora quercina	Primary hyphae were 3.8-9.2 µm wide. Terminal, sympodial branching with mother hyphae ending in a short proturbance. Hyphae were sometimes undulate.	Sporangiophores simple or forming irregular lax sympodium, 1.5-5.8 µm in diameter, sometimes wider near the point of attachment to the sporangium. Terminal, occasionally intercalary. Noncaducous, papillate or rarely bipapillate with a papillium depth of 2.3-6 µm. Sometimes with a conspicuous basal plug. Variable in size and shape, 18.8-112.5 x 13.8-47.5 µm. Avg. length-breath ratio: 1.45. Range from subglobose, ovoid and obpyriform to ampulliform, banana- or peanut-like	Occasionally produced by some isolates, spherical, terminal, or intercalary 17-35 µm diameter.	Homothallic	Borne terminally , range in shape from spherical to ovoid and ellipsoid, 45 percent being markedly elongated. On MEA, 19-45 µm in diameter. Oogonial walls were smooth and ranged in thickness from 0.5-2.1 µm.	Spherical to ovoid, 17.9-38 µm in diameter, markedly aplerotic and thick-walled (0.8-5 µm). Older oospore walls often turned golden yellow.	Hyaline, single, terminal, spherical or club-shaped to irregular, paragynous, 8.3-26 x 6.3-15 µm. Usually inserted near the oogonial stalk.	V8, MEA-domeshaped and fluffy; becoming appressed with age PDA- appressed dense-felty and dome-shaped CMA-sparse aerial mycelium.

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
		distorted shapes.						
Phytophthora tentaculata	On CA, the mycelium is arachnoid. Hyphal swellings are relatively small and occur where mycelium branches.	Papillate, some are bipapillate. Spherical or ovoid to obpyriform; some are distorted. A few are caducous with a short pedicel. They are 10-81 x 13-52 µm (average 35.7 x 27.4 µm). Length-breadth ration is 1.3:1.	Intercalary or terminal, spherical thin-walled, and 10-45 µm in diameter (average 26.6 µm)	Homothallic	Spherical, some lobed at the base, mostly thick-walled, hyaline, 20-49 µm in diameter (average 34 µm).	Spherical, hyaline, aplerotic, and 14-38 µm in diameter (average 28.1 µm)	Spherical to elliptical 13-23 x 9-16 µm. One to two, rarely three paragynous antheridia are attached to oogonium; a few are amphigynous. Antheridia are diclinous. Antheridia stalks are long and sometimes branched. A few stalks completely encircle the oogonium. Some stalks have tooth-shaped projections.	Temperatures for growth: minimum (7°C), optimum (15-25 °C), and maximum (<32 °C).

***Phytophthora* spp.**	**Main hyphae**	**Sporangia**	**Chlamydospores**	**Heterothallic vs. Homothallic**	**Oogonia**	**Oospores**	**Antheridia**	**Growth Characteristics ***
Phytophthora uliginosa	Main and lateral hyphae are irregular in diameter and short thick lateral hyphae without restricted bases, as well as irregular hyphal swellings are common. In water, clusters of hyphae with chains of deltoid to irregular swellings are formed. Hyphae often taper towards a point where they suddenly become wider again; hyphal tips appear arrow-like.	Nonpapillate sporangia with a flattened broad apex, a very thin apical thickening, and an extremely wide exit pore. Empty sporangia are sometimes funnel-shaped with slightly curled walls toward the exit pore. Broadly ellipsoid or obpyriform, sometimes with an undulating wall or conspicuous basal plug, averaging 49±6 x 30±4.2 µm. Length-breadth ratio is about 1.6. Sporangial proliferation is external or internal sometimes nested.	None reported	Homothallic	Oogonia single, globose, terminal, average 43+4.4 µm.	Plerotic and aplerotic oospores with thick walls (average 4±0.7 µm), globose, and average 39±4.4 in diameter.	Paragynous, single, terminal, unicellular, hyaline, about 17±3.3 x 13 ±2.6 µm, and attached near the stalk.	CMA, V8, and MEA – colony is uniform without any growth patter, with some aerial mycelium but not dome-shaped. PDA- almost no growth. Optimal temperature (17-18°C), maximum (29°C).

* CA- Carrot Agar, CMA- Corn Meal Agar, MEA- Malt Extract Agar, PDA- Potato Dextrose Agar, SAMA – Defined sucrose, asparagine, and mineral salts media containing β-sitosterol, TA- Tomato Juice Agar, V8- V8 Juice Agar, β after any medium indicated addition of β-sitosterol

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REGULATORY PROCEDURES

Instructions To Officers

Agricultural officers must follow instructions for regulatory control measures, treatments, or other procedures when authorizing the movement of regulated articles. A full understanding of the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatments may be used in accordance with labeling restrictions. During all field visits, please ensure that proper sanitation procedures are followed as outlined in the Survey section.

Quarantine Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification (PPQ form 523, Appendix D) may be issued to hold articles or facilities, pending positive identification by a USDA, APHIS, PPQ recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register*.

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides for authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under state authority. State departments of agriculture normally work in conjunction with federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the measures taken by the state are inadequate, USDA can take intrastate regulatory action, provided that the governor of the state has been consulted and a notice has been published in the *Federal Register.* If intrastate action cannot or will not be taken by a state, PPQ may find it necessary to quarantine an entire state.

PPQ works in conjunction with state departments of agriculture and forestry to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property in the absence of owner permission. PPQ prefers to work with the state to facilitate access when permission is denied. Each state government, however, has varying authorities regarding entering private property. A General Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas where PPQ and the state department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected state.

Tribal Governments

PPQ also works with Federally Recognized Indian tribes to conduct surveys, enforce regulations and take control actions. Each tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to state governments. Permission is required to enter and access tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Indian tribal governments about actions that may have substantial direct effects on tribes. Whether an action is substantial and direct is determined by the tribes. Effects are not limited to current tribal land boundaries (reservations) and may include effects on off-reservation land or resources which tribes customarily use, or even effects on historic or sacred sites in

states where tribes no longer exist.

Consultation is a specialized form of communication and coordination between the federal government and tribal government. Consultation must be conducted early in the development of a regulatory action to ensure that tribes have opportunity to identify resources which may be affected by the action and to recommend the best ways to take actions on tribal lands or affecting tribal resources. Communication with tribal leadership follows special communication protocols.

For additional information, contact PPQ's Tribal Liaison. To determine whether there are Federally Recognized Tribes in a state, contact the state plant health director. To determine whether there are sacred or historic sites in an area, contact the State Historic Preservation Officer (SHPO).

For clarification, check with your state plant health director (SPHD) or State Plant Regulatory Official (SPRO) in the affected state.

Overview of Regulatory Program for Exotic *Phytophthora* Species After a U.S. Detection

Once an initial U.S. detection is confirmed, holds may be placed on the property by the issuance of an Emergency Action Notification (EAN). Immediately place a hold on the property to prevent the removal of any potential *Phytophthora* host plants and/or soil. All potential host plants for *Phytophthora* spp. should be included until delimiting surveys on the property can be performed. However, particular emphasis should be placed on the known positive plant host(s).

Trace-back/trace-forward investigations from the property will determine the need for subsequent holds for testing and/or taking further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures prescribed.

Record Keeping

Record keeping and documentation is important for any holds and subsequent actions taken. Rely on receipts, shipping records, and information provided by homeowners and nurseries to determine where plant material was shipped, how plant material may have moved within the facility, and any cultural or sanitation practices employed.

Keep a detailed accounting of the numbers and types of plant material held, destroyed, or requiring treatments or control actions. Consult a master list of properties distributed with the

lists of suspect properties based on trace-back/trace-forward investigations. Draw maps of the facility layout to locate suspect plants and other potentially infected areas. When appropriate, take photographs of the plant symptoms and property layout, and document plant propagation methods, labeling, and any other situation that may be useful for further investigations and analysis.

Keep all written records filed with EAN (PPQ form 523) copies, including copies of sample submission forms, documentation of control activities, and related State-issued documents if available.

Issuing an Emergency Action Notification

An EAN is issued to hold all host plant material at facilities that have the suspect plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not suspect or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in natural areas or nurseries pending positive identification of suspect samples. When a decision to destroy plants is made, or in the case of submitted samples, once positive confirmation is received, the same EAN for which the plants are on hold is also used to document any actions taken such as destruction and disinfection. Additional quarantine action may be warranted in the case of additional areas or nurseries with positive finds of exotic *Phytophthora* spp.

If plant lots or shipments are to be held as separate units, it is advisable to issue separate EAN's for each held unit of suspect plant material associated with that unit. EAN's are issued under the authority of the Plant Protection Act of 2000 (statute 7 USC 7701-7758). It is advised that States issue their own hold orders parallel to the EAN to ensure that plant material cannot move intrastate.

When using EAN's to hold articles, it is most important that the EAN language clearly specify the actions to be taken. An EAN issued for positive testing and positive associated plant material must clearly state that the material must be disposed of, or destroyed, and that areas must be disinfected. Include language that these actions will take place at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, be sure to document on the same EAN any

disposal, destruction, and disinfection orders resulting from investigations or testing.

For Block 1, enter the name of location of the nearest PPQ office. Under "Name of Article" in Block 3, enter the host scientific name and cultivar. In Block 4, enter the property address, or field number, or other information indicating the location of the plant material held. Under "Shipper" Block 6, enter the plant material source if known. Blocks 7 and 8 can be left blank unless that information is known.

To place plant material on a property on "Hold," in Block 12 of the EAN, enter for the Pest: "Exotic *Phytophthora* species". The authority under which actions are taken is The Plant Protection Act of 2000, Statute 7 USC 7701-7758. In Block 15, under "Action Required" suggested text is as follows:

"All host plants of the Exotic *Phytophthora* species are prohibited from movement from the property pending further notification by USDA APHIS PPQ and/or the State department of agriculture. No other host material may leave the property until further evaluations can be made. After further investigations are conducted on the listed plants and other host material, if a positive detection is confirmed on the property, material will be treated/destroyed under supervision, with approved methods in accordance with USDA and state policies."

Regulated Articles

The following articles may be regulated if an Exotic *Phytophthora* species is detected in a nursery or forest setting:

- Nursery hosts and forest host plants and host plant parts
- Soil
- Equipment (harvesting and cultivation as well as conveyance)
- Nurseries
- Forests or regions

Quarantine Actions

Regulatory action may be required if:

- An Exotic *Phytophthora* species is found during a nursery or forest survey.

Regulated Area Establishment

Field personnel will attempt to detect the pest within the regulated area at all establishments where regulated articles are sold, grown, handled, moved or processed. Establishments that might be included are airports, landfill sites, processing plants, produce and flea markets, nurseries, horticultural shops, flower shops, farms,

home gardens, and any other establishment that handles regulated articles. Surveys may be set up at establishments deemed to be at risk by project personnel.

Primary Plant Host List

The primary host plants for Exotic *Phytophthora* species differ with each species. Consult the biology and life cycle section for more definitive information.

Regulatory Treatments for Exotic *Phytophthora* spp.

Approved regulatory treatments appropriate for this pest are determined by program management and/or a Technical Advisory Committee in conjunction with the Center for Plant Health, Science, and Technology. Check the PPQ Treatment Manual for current recommendations.

Treatment options include:
Sanitation, and/or
Destruction of wild and cultivated hosts, and/or
Application of fumigants and/or
Application of other cultural controls, and/or
Application of biological controls

Four treatments are currently available after an exotic *Phytophthora* spp. has been found:

1. **Destruction of wild and cultivated hosts:** This can be accomplished by double bagging and proper disposal in an approved landfill, incineration to ash (T415-a), or steam sterilization (T415b).

2. **Fumigation:** After removal of plant material, nurseries are fumigated with methyl bromide, basamid or similar biocidal fumigant. If post-treatment sampling reveals active Exotic *Phytophthora* species, fumigations should be repeated.

3. **Disinfection** of all equipment and implements that have come in contact with host plants or soil.

4. **Prohibition** of host crops in nurseries in the regulated area should remain in effect until the pathogen is considered eradicated.

Other treatments may be available in the near future but will require validation (see research recommendations). This screening should be initiated immediately to identify alternatives rapidly, but longer-term studies may be necessary

to address all regulatory issues.

Quarantine Area

Quarantine Areas will be based on an assessment of the number of areas or nurseries found positive for Exotic *Phytophthora* species.

Grower Requirements Under Quarantine

Depending upon decisions made by Federal and State regulatory officials in consultation with the Exotic *Phytophthora* Technical Working Group, quarantine areas may have certain other requirements for commercial nurseries in that area.

Any chemicals used to control Exotic *Phytophthora* species will require labeling for their specific use, or emergency exemptions must be in place to allow the use of other materials.

Establishing a Federal Quarantine

Regulatory actions undertaken using EAN's continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfection orders or longer-term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers, and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing, and risk assessment will determine the area to be designated for a Federal and parallel State quarantine. Risk factors will take into account positive testing, and positive associated or potentially infested exposed fields. Boundaries drawn may include a buffer area based on risk factors and epidemiology.

PPQ may issue a Federal Order, followed by an interim rule establishing a quarantine to be published in the Federal Register, which is normally drafted by PPQ headquarters staff in consultation with the region, SPHD, and SPRO. The conditions that growers must abide by within a quarantine area are included in the rule. Regulated articles and conditions allowing movement of articles out of the regulated area are determined and included in the regulation, along with other administrative requirements.

Removing Areas from Quarantine

Project managers identify and remove areas from quarantine requirements after the exotic *Phytophthora* spp. is declared eradicated. Eradication is assumed when sufficient time has passed since the last specimen recovery. APHIS will publish a Notice of Quarantine Revocation in the *Federal Register* when

areas are removed from quarantine requirements.

Regulatory Records	Maintain standardized regulatory records and database(s) in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

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CONTROL PROCEDURES

Overview

Plant Protection and Quarantine develops control measures and makes them available to involved states. Environmental Protection Agency (EPA)-approved and labeled treatments will be recommended when available. If additional treatments selected are not labeled for use against the organism or in a particular environment, an emergency exemption can be requested and obtained under Section 18, or 24(c), special local need (SLN), of FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act), as amended.

Control Decisions and Oversight

All quarantine actions related to destruction are to be witnessed, supervised, and documented by a federal and/or state plant regulatory official whenever possible.

Once it becomes clear that an Exotic *Phytophthora* sp. is present on a property, an assessment and appropriate delimiting survey should be accomplished. After assessment and an effective delimiting survey, control or containment of the Exotic *Phytophthora* sp. may include a number of control options. Recommendations from the Exotic *Phytophthora* Technical Working Group may include host plant removal and destruction, as well as fumigation within an area.

Control of Exotic *Phytophthora* Species

Exclusion. The best control strategy for any *Phytophthora* sp. is exclusion. Avoiding the introduction of a pest into the natural or nursery environment is the only method that guarantees a given disease will not develop. Exclusion can occur by regulating (not allowing) plants or seed

from countries or areas where a disease is known to occur.

Exclusion can also occur by:

- Authorized and trained personnel visually inspecting all incoming nursery stock (buy-ins, transfers, and returns), regardless of origin, for symptoms of *Phytophthora* spp. prior to introduction into the nursery facility.

- Offloading incoming high-risk plant shipments to an area that can be cleaned of leafy debris; sweeping incoming plant debris from the receiving area and the delivery truck; and collecting debris and disposing of it appropriately are ways to remove potential sources of disease inoculum.

- Monitor sanitation practices of delivery trucks that ship high-risk plants. Assure that trucks are properly cleaned of plant debris, and that mud or soil is washed off tires and truck body between shipments, because basic sanitization can remove sources of disease, and trucks are a potential source of inoculum if not cleaned properly.

Eradication. Once an exotic *Phytophthora* spp. is detected, the overall APHIS strategy for nurseries infested with exotic *Phytophthora* species is eradication after delimiting surveys to determine the extent of the infestation. Fumigation, destruction of host plants, and other methods will likely be employed in the regulated area. Quarantines will be issued to limit the spread of the exotic *Phytophthora* species. Quarantines can restrict the movement of infested commodities out of the regulated area(s) and reduce the potential for infested nursery stock to move outside of an infested nursery. Furthermore, proper identification of the organism is required to identify the location and regulate the movement of the pathogen.

Integrated Pest Management Methods. If eradication is not possible, adequate control/management of exotic *Phytophthora* species, as with common *Phytophthora* diseases, will require the integration of chemical, cultural, biological control, and host resistance methods to reduce disease losses. In most cases, an integrated approach for pathogen management gives the best results and is less toxic to the environment. For best results, cultural practices and chemical controls should be applied preventatively.

Chemical control. Fungicides are chemical compounds that either destroy a fungus or inhibit or suppress its growth. Most conventional fungicides are not effective against *Phytophthora* species, because they

are not true fungi. There are several classes of compounds that have activity against *Phytophthora* species by protecting the plants from infection. These compounds, however, rarely kill *Phytophthora* if the plant is already infected, and may mask symptom development and lead to inadvertent transport of infected nursery stock and spread the disease. Infested soil or infected roots may also provide hidden pathways for pathogen dispersal, evading detection, and thwarting quarantine efforts. Fungicides that do not completely eliminate the pathogen need to be avoided.

Certain systemic compounds, called the phenylamide fungicides, which includes metalaxyl (now mefenoxam), are capable of moving upward (acropetally) from soil or foliage. Because phenylamide fungicides have a specific mode of action, metalaxyl-resistant strains of *Phytophthora* spp. can displace the sensitive wild-type isolates in field populations. Management of fungicide resistance has become a problem for control of *Phytophthora* spp.; resistance development must be monitored if using fungicides (Erwin and Ribeiro, 1996).

Fosetyl-Aluminum (Fosetyl-Al) (an ethyl phosphonate) is another type of systemic fungicide used for *Phytophthora* species. It is taken up into the plants and translocated both upward and downward (basipetally). Fosetyl-Al has been effective for control of many *Phytophthora*-caused diseases but not all *Phytophthora*-caused diseases, including *P. infestan*s.

Phosphonates are currently being evaluated for control of *P. ramorum* and other *Phytophthora* spp. and may prove useful for managing diseases caused by exotic species in agronomic, nursery, and forest settings (Garbeloto *et al.*, 2002, 2003, 2007, 2009). Comparison of metalaxyl-M, phosphonic acid (a phosphonate), and copper hydroxide was conducted *in vitro* and *in planta* for activity against *P. ramorum*. *P. ramorum* was only moderately sensitive to phosphonic acid *in vitro*, but was highly sensitive to copper hydroxide. *In planta* experiments indicated the broad efficacy of phosphonic acid injections and of copper hydroxide sprays in preventing growth of *P. ramorum* in oaks and bay laurels, respectively. Although metalaxyl-M was effective *in vitro*, drenches of potted oak trees using this active ingredient were largely ineffective in reducing the growth rate of the pathogen *in planta* (Garbelotto *et al.*, 2009). In contrast, Linderman and Davis (2008) showed that metalaxyl (as Subdue MAXX) was the most effective chemical for suppressing infections on a range of *Phytophthora* spp. (*P. ramorum*, *P. citricola*, *P. citrophthora*, and *P. nicotianae*) that occur commonly in nursery crops. Agri-Fos (potassium phosphite), Alliete (fosetyl-Al), Banaol (propamocarb hydrochloride), Biophos (potassium phosphate), Fenamidone, Fosphite (potassium phoshite), Heritage

(azoxystrobin), Insignia (pyraclostrobin), Ranman (cyazofamid), StatureDM (dimethomorph), Subdue MAXX (mefenoxam), Tanos 50WG (famoxadone, cymoxanil), Truban 30 WP (terrazol), and an untreated control were also tested against the five *Phytophthora* species.

Cultural control. Disease ensues when 1) a susceptible host is exposed to a 2) virulent pathogen under 3) optimal environmental conditions (sometimes referred to as the disease triangle). Manipulation of any of the three factors via cultural controls can help control disease.

1. Moisture: The environmental factor to which plants are most vulnerable and to which *Phytophthora* spp. have a critical dependency is moisture.

Avoid overhead irrigation of high risk plants and irrigate in a manner to avoid prolonged leaf wetness. Properly timed irrigation events reduce conditions favorable for disease development. Extended leaf wetness (such as overnight) is conducive to infection by the pathogen.

Monitor, and annually test, untreated irrigation water from any source other than a well or a municipal water supply to confirm that it is free from the pathogen. For growing operations that utilize open irrigation water sources (ponds, lakes, streams), or blend both well and surface water sources for irrigation purposes, proper water treatment *(i.e.*, ozonation, chlorination or other water disinfection program) is recommended.

Avoid or minimize accumulation of standing surface water in high risk plant beds, as *Phytophthora* spp. are transmitted via water. Repeat finds occur more often in high-risk plant beds where standing water accumulates. The pathogen may potentially enter through the roots or by splashing onto leaf surfaces.

Good soil drainage and air movement will discourage the development of disease. Raising depressions to eliminate poor drainage has been employed to manage *Phytophthora* diseases. Avoid planting in poorly drained sites, on heavy clay soils, and in low lying areas.

2. Sanitation: Sanitation is defined as the prevention of deposition of inoculum and/or removal of diseased plant material from the field. Proper sanitation and removal of plants and foliage with suspicious symptoms is important (see exclusion above). Avoiding contact of containers with soil, and cleaning and disinfection of equipment, shoes, and hands before moving into another planting area are important.

3. Plant spacing: Use the widest in-row spacing economically feasible,

because the most severe and rapidly spreading outbreaks of *Phytophthora* have been observed in very dense plantings. Additionally, having a physical barrier or placing/planting non-host plants near high risk plants is useful to reduce spread from highly susceptible plants to other possible host plants.

4. Crop rotation or leaving fields fallow has been used for agricultural crops. Crop rotation and fallowing fields have been used to reduce the initial pathogen inoculum. *Phytophthora* spp. in general, form resistant spores that can persist for years in the absence of a host. The inoculum of *Phytophthora*, however, is subject to microbial antagonism, and *Phytophthora* spp. are not competitive saprophytically (*i.e.*, they would not be expected to persist in high populations in the absence of a susceptible host).

5. Reducing injury to hosts: Injury provides an entry point for pathogenic organisms including *Phytophthora* species.

6. Proper weed control: Weedy hosts may harbor the pathogen.

7. Optimal fertilization practices: Over-fertilization can lead to increased host susceptibility.

8. pH: Increase in soil pH has been associated with *Phytophthora* outbreaks.

9. Eliminate stress: Drought stress has been shown to be an important factor in disease caused by *Phytophthora* spp.

Biological control.
Biological control agents are useful for suppressing pest populations, but do not eradicate them. Biological control can be useful if rigorous screening on non-target organisms is tested. Proper permitting must be obtained prior to testing in the United States. They can be effective when used in combination with other techniques. They are characterized as predators, parasites, parasitoids, or pathogens.

Little information is currently available on possible biological options for exotic *Phytophthora* spp. None have been widely adopted in field use, and efficacy information is limited.

Antagonistic effects have been shown for a range of fungi against *Phytophthora* species in general (Erwin and Ribeiro, 1996). Antagonistic fungi exert lytic effects on mycelium, chlamydospores, sporangia, and oospores. Although most of these effects are demonstrable, very few have been utilized successfully in field

situations (Erwin and Ribeiro, 1996).

Soils that favor the expression of disease are termed conducive; soils inhospitable to some plant pathogens (*i.e*., pathogens cannot be established or, if established, cannot initiate disease are termed) suppressive soils. Conversely, in some cases with continued culture of the crop, the intensity of disease lessens. There are numerous examples of suppression of *P. cinnamomi* in Australian soils (Broadbent *et al.*, 1971; Broadbent and Baker, 1974 a, b; Baker, 1978; Weste and Vithange, 1977, 1978 a, b; Malajczuk and McComb, 1977; Malajckuk 1979 a, b; Halsall, 1982 a, b). Certain soils were shown to be suppressive to *P. cactorum*, the cause of root rot of ginseng in Korea (Chung *et al.*, 1984). The seedling disease caused by *P. palmivora* was controlled simply by filling the transplanting holes with noninfested soil (Ko 1971, 1982).

Myrothecium verucarria and *Streptomyces* spp. inhibited the growth of *P. cinnamomi* on potato-dextrose agar. Munnecke (1984) used the strategy of infesting field soils with the microorganisms after methyl bromide fumigation of land naturally infested with *P. cinnamomi* in an avocado orchard. It was found that although methyl bromide had suppressed *P. cinnamomi* in microbe amended and non-amended plots, the fungus became reestablished after 9 months.

Trichoderma significantly reduced mortality caused by *P. cactorum* after natural and artificial inoculations of young apple trees (Valdebenito-Sanhueza, 1987). Root rot of soybean, caused by *P. sojae*, was partially controlled in a series of field experiments by row application of spore formulations of *Bacillus cereus* strain UW85, previously reported as an antagonist of *P. medicaginis* by Handelsman *et al.* (1990) (Osburn *et al.*, 1995).

Host resistance. Resistance is defined as the ability of the host to hinder development of the pathogen. Host resistance (partial, general, horizontal, field, rate-limiting) and/or single-gene (vertical, race-specific)) is possible against many *Phytophthora* species depending on the plant host. Deployment of single-gene (also known as vertical resistance) can lead to the development of pathogenic races, which can quickly overcome the resistance.

Control Procedures for Positive Commercial Nursery Detections

The following guidelines may be used in commercial fields in the event of an initial detection where there is no evidence of a general infection in surrounding areas.

Several actions need to occur immediately upon confirmation that a field is positive for an exotic *Phytophthora* species: 1) If not done at the time

of the suspect forwarding, an immediate quarantine hold must be placed on the property to prevent the movement of plant host material and soil from the premises. 2) Check all records to obtain information about seed source, sale of host plants from nurseries, etc. 3) Commence delimitation survey operations as soon as possible.

In consultation with the Exotic *Phytophthora* Technical Working Group, consideration should be given by state and federal regulatory officials to removing all host material.

Following completion of host removal operations, fumigation should be completed. Trace-backs should begin as soon as possible, with initial survey directed at adjacent and contact fields based on risk and proximity to the index or infested field.

Destruction of Wild and Cultivated Host Plants

Use the following methods to destroy all host material completely:

- **Spray herbicide on cultivated host plants and weeds.** Before any other activities, be sure to spray any underbrush and weeds. All are suspect until proven otherwise. Always follow label directions and wear protective equipment. Check areas to avoid drift and damage/chemical exposure to adjacent areas.

- **Burning, incinerating, and removal**. The dead plant material is subsequently destroyed by burning or incineration, or removed and sent to an approved landfill. Collect, pile, and burn host material, including debris, if local ordinances permit.

Cultural Control of Exotic *Phytophthora* spp.

Cultural controls are available that can be effective for all *Phytophthora* spp. in general (see section above). For the exotic *Phytophthora* spp. covered in this NPRG specifically:

P. alni: From the outset, it was realized that a 'sanitation approach' based on destroying diseased alder trees was not feasible in the natural or semi-natural environment because diseased trees do not show crown symptoms until most of the bark at the base of the tree has been killed (Gibbs *et al.*, 2003). By the time crown symptoms are observed, the affected trees have produced inoculum for months, if not years. The process of tree removal would also be extremely difficult and highly destructive in the riparian habitat.

Coppicing is a traditional method of managing riparian alder by encouraging new growth. Generally, trees are cut for coppicing 20-30 cm above ground level, leaving a tall stump to develop new shoots under favorable space and light conditions. Coppicing is especially important if the tree has a diseased root system that can no longer

support the entire crown; it prevents diseased trees from becoming unstable and causing damage to the anchoring riverbank. A number of studies have been established, but most are still in an early stage of development. Preliminary results have shown that regrowth is abundant and vigorous where trees were healthy at the time of cutting, but some severely diseased stumps can also produce healthy shoots (Gibbs *et al.*, 2003). However, fewer shoots regenerate from diseased stumps than from healthy ones (Webber *et al.*, 2004). Jung and Blaschke (2004) found a low sprouting rate from diseased stumps, wilting of shoots during hot periods, and re-infections of sprouts during the first two years of their study, which further reduced the number of surviving stumps.

In a nursery/forest nursery situation, a 3-year fallow period between bare-rooted alder crops was recommended for control in infested areas, as the pathogen appears to have a relatively poor long-term survival rate in soil (Jung and Blaschke, 2004). Because infections occur predominately via free water, it is recommended that alders not be planted in areas prone to flooding (Cech, 1998). Alders and surface water in contact with alders should not be exposed to contaminated soil or diseased plants. Water from streams or rivers is not recommended for irrigation of plants, as a diseased alder stand may exist upstream from the point of extraction.

P. austrocedrae: Although specific cultural controls have not been investigated at this time, disease occurrence and the spatial pattern of disease is associated with soil properties related to poor internal drainage, such as proximity to water streams, non-allophanized soils of fine textures, and redoximorphic features (Mann and Rajchenberg, 2004).

P. boehmeriae: Elena and Paplomatas (1998) noted that the cotton field in Greece where bollrot was observed was irrigated prior to a heavy rainfall. Another field was sprinkler irrigated. The authors recommend using drip irrigation instead of sprinkler irrigation to prevent soil with spores from splashing onto cotton fruits.

After harvest, residues of diseased plants should be cleared promptly, and crop rotations should be practiced (if possible). Before sowing, seeds should be sunned and then treated in hot water after selection in order to increase seed vigor. These practices will reduce carry-over and/or spread of disease. Reasonable plant densities and avoiding excessive levels of nitrogen fertilizer will help decrease moisture stress and disease severity (Shen, 1992; Zhou, 1997).

P. colocasiae: Cultural practices towards disease control include minimizing the source of inoculum, use of disease-free plant material,

roguing (removing) infected leaves, and avoiding excessive levels of moisture. Where rainfall is high, taro leaf blight is difficult to control; cultural controls can be tried, but are often ineffective. New gardens should be started as far away as possible from old ones. Young taro should be inspected twice a week and infected leaves removed as soon as they are noticed. Removal of leaves, however, will only delay the disease where rainfall is high. Suckers without leaves attached should be planted. Taro stalks for planting should be cut only when the 'tops' are dry. Apart from the above, the best cultural practice is to make gardens in areas with cooler temperatures (Tsatsia and Jackson, n. d.).

P. kernoviae: So far, control measures are based on the destruction of infected plants, and in particular of rhododendrons in infected woodlands. Leaf litter should also be removed and burned. The remaining stumps should be treated with herbicide to prevent regrowth. Strict hygiene precautions for tools and machinery should also be followed. More studies are needed on possible control measures. All steps in the cultural control section above for *Phytophthora* spp. are applicable to *P. kernoviae*.

P. melonis: Due to the confusion of *P. melonis* with *P. drechsleri*, little specific control information is available for *P. melonis*. *P. melonis* is most active after heavy rains. Rainwater accumulating around the stem of the plant results in invasion of the collar region. Cucumbers should be planted in well-drained areas (Erwin and Ribeiro, 1996).

P. multivora: Nursery hygiene and the quarantine of infected areas is required to control pathogen spread (Scott *et al.*, 2009b).

P. pinifolia: It seems that only *P. radiata* is affected by *P. pinifolia*. Thus, other *Pinus* species may be chosen for plantation development.

P. porri: Prevention of infection by *P. porri* can be obtained by mulching to avoid rain splash (Poll, 1996; Alofs and Pijnenburg, 1998; CABI, 2006). *P. porri* survives in soil on plant debris and may persist for 3 years; thus, long rotations can be used to avoid crop reinfection. Soil solarization has not been shown to reduce initial inoculum level in the Netherlands (Smilde *et al.*, 1996b).

P. primulae: Poorly drained sites subject to waterlogging should be avoided. In such situations, the crop may be grown on small ridges or raised beds (EPPO, 2000). Because *P. primulae* can remain viable in the soil as oospores for several years, *Polyanthus* and other hosts should not be planted in soil where the disease has occurred previously (Erwin and Ribeiro, 1996).

P. quercina: Rotation of tree species between nursery beds may reduce *P. quercina* populations in the soil. Due to the long survival time of *P. quercina* oospores, this measure alone is not appropriate to eradicate the pathogen (Jung, 2003b). Heating and steaming of soil is effective against other soilborne *Phytophthora* species, and this may be an option for *P. quercina* in small nursery beds (Jung, 2003b). Lowering soil pH may also reduce *P. quercina* populations in nursery beds (Jung, 2000; Jung, 2003b).

Cultural controls have not been investigated for *P. alticola*, *P. captiosa*, *P. fallax, P. frigida*, *P. gallica*, *P. idaei*, *P. iranica*, *P. italica*, *P. multivesiculata*, *P. polonica*, *P. psychrophila*, *P. tentaculata*, and *P. uliginosa*.

Chemical Control of Exotic *Phytophthora* spp.

P. alni: Chemical treatments remain untried but offer little scope for use. The most promising candidate material is phosphate (phosphonate).

P. boehmeriae: This is a seed-borne disease, so treating seeds with systemic fungicides produces good protection for cotton and ramie seedlings. At the growing stage, fungicides effective against oomycetes, including systemic treatments such as metalaxyl, fosetyl-aluminum, cymoxanil, and protective treatments such as mancozeb and chlorothalonil, can be applied as foliar sprays or as root pours. For the control of cotton blight, the chemicals can be sprayed at the seedling stage for the control of seedling blight, and in the boll-bearing period for the control of boll blight (Shen, 1992; Wang *et al.*, 1997; CABI, 2006). Systemic and protective fungicides should be used alternately to prevent or delay development of pathogen resistance to the fungicides (Gao *et al.*, 1997, 1999).

P. colocasiae: Fungicidal control is largely practiced against *P. colocasiae* in taro cultivation. Heavy rains often make repeat applications necessary for chemical control. In many countries and island nations, taro is a subsistence crop and routine chemical use is neither economically practical nor environmentally suitable (Brooks, 2005). Currently, widely used products are systemic (metalaxyl) and non-systemic fungicides (copper oxychloride, mancozeb, captafol, zineb), and are applied as foliar sprays. Copper-based fungicides (especially copper oxychloride) are available, but they have to be used often and are only recommended where taro is grown for sale. The use of metalaxyl (a systemic product) in combination with copper improves control, but costs are high. Phosphoric acid alternating with mancozeb has used successfully in Samoa (Tsatsia and Jackson, n. d.; Semis *et al.*, 1998).

In India, spraying metalaxyl at intervals of 15 days was effective in

controlling the disease under field conditions and maximized net financial return (Ghosh and Pan, 1991). Aggarwal and Mehrotra (1987) showed the best control of the disease with chloroneb and captafol, good control with metalaxyl, fair control with copper fungicides, and poor control with thiophanate and zineb. In another study, excellent control was obtained with captafol, good control with metalaxyl, and fair control with copper oxychloride (Aggarwal *et al.*, 1987). Sahu *et al.* (1989) report that four sprays of zineb at 15-day intervals reduced the incidence of *P. colocasiae* and increased the yield. In Papua New Guinea five applications of metalaxyl at 3-week intervals resulted in an almost 50 percent increase of corm yields (Cox and Kasimani, 1990). Applications of mancozeb at 7-day intervals gave substantial disease control and increased yields in Hawaii (Bergquist, 1974). With mist blower application in the Solomon Islands, copper oxychloride at 2.25 kg/ha was effective in controlling *Phytophthora colocasiae* and increasing corn yield, but mancozeb at 3.6 kg/ha was not. Phytotoxicity from captafol at 1.8 and 3.6 kg/ha nullified any potential gain in yield from control of blight (Jackson *et al.*, 1980).

P. melonis: Due to the confusion of *P. melonis* with *P. drechsleri*, little specific control information is available for *P. melonis*. Lin and Wu (1985) showed that Ridomil MZ (metalaxyl) at 1450 ppm inhibited disease development, but disease levels of greater than 50 percent were still observed.

P. multivora: Initial results indicated that infected *E. gomphocephala* respond well to phosphate application (Scott *et al.*, 2009b).

P. porri: Chemical control, especially with protectant fungicides, is difficult due to the unpredictable onset of epidemics. Dobson and Clarkson (1989) tested a range of fungicides for control of white top on leeks, caused by *P. porri*, including metalaxyl plus mancozeb (Fubol), metalaxyl plus chlorothalonil (Folio), chlorothalonil, and captafol. Metalaxyl-containing fungicides significantly reduced the severity of white tip when compared to the other fungicide treatments. At harvest, only the metalaxyl-containing fungicides significantly increased marketable and total yield. Metalaxyl resistance, however, has been identified in *P. porri* populations in the United Kingdom (Locke *et al.*, 1997). Protective (preventative) programs of captafol and dithiocarbamates were shown to give adequate control of *P. porri* in leeks and salad onions, respectively (Griffin and Jones, 1977).

P. primulae: High volume fungicide sprays of metalaxyl and thiram should be applied when symptoms are first observed (EPPO, 2000). Clarkson and Phillips (1987) compared fungicides, etridiazole, fosetyl-aluminum, metalaxyl plus mancozeb, and propamocarb hydrochloride

for control of *P. primulae* in parsley. Metalaxyl plus mancozeb increased the percentage plant cover significantly on both beds and ridges, and reduced percentage plants affect significantly on the ridges.

P. quercina: It has been shown that soil drenching with metalaxyl does not eliminate the pathogen from infested soil (Jung, 2003b). Potassium phosphonate may be effective against *P. quercina*, although test results are not available (Jung, 2003b).

Chemical controls have not been investigated fully, and fungicide sensitivity data is not available, for *P. alticola*, *P. austrocedrae, P. captiosa*, *P. fallax, P. frigida*, *P. gallica*, *P. idaei*, *P. iranica*, *P. italica, P. kernoviae, P. multivesiculata*, *P. pinifolia*, *P. polonica*, *P. psychrophila, P. tentaculata,* and *P. uliginosa.*

Biological Control of Exotic *Phytophthora* spp.

P. boehmeriae: Inhibitory effects of antagonists such as *Trichoderma* spp., *Pythium oligandrum*, *Bacillus* sp. (Ma and Shen, 1993; Ma *et al.*, 1998) and *Xenorhabdus* spp. (Yang *et al.*, 1998) have been reported, suggesting that these antagonists have potential for biocontrol of the diseases caused by *P. boehmeriae.*

Biological control has not been investigated for *P. alni*, *P. alticola*, *P. austrocedrae, P. captiosa*, *P. colocasiae*, *P. fallax*, *P. frigida*, *P. gallica*, *P. idaei*, *P. iranica*, *P. italica, P. kernoviae, P. melonis, P. multivesiculata*, *P. multivora*, *P. pinifolia*, *P. polonica*, *P. porri*, *P. primulae, P. psychrophila, P. quercina, P. tentaculata*, and *P. uliginosa.*

Host Resistance for control of Exotic *Phytophthora* spp.

P. alni: As yet, studies on host resistance have focused on *Alnus glutinosa* and have given disappointing results, suggesting that resistance genes may be rare, and that a long-term resistance screening program will be required (Gibbs *et al.*, 2003).

P. boehmeriae: Host cultivars resistant to cotton and ramie blight caused by *P. boehmeriae* have been reported (Zhou, 1984; Huang and Yao, 1995), and are widely used for control of these diseases (Chen, 1986; Ji and Fan, 1988). Growing resistant cultivars can significantly decrease losses caused by these blights.

P. colocasiae: Host cultivars resistant to leaf blight have been the most important method of disease control. However, desirable cultural characteristics and eating qualities are often lost during breeding. Current breeding efforts are focused on improving yield, suckering (desirable for vegetative propagation), time to maturity, taste, and

texture (Brooks, 2005).

Breeding programs in Papua New Guinea and Samoa have produced plants resistant to taro leaf blight. In Solomon Islands, a hybrid, LA16, has been found to be resistant. In Bangladesh, among 50 lines tested by artificial inoculation in the field, two were highly resistant to *P. colocasiae*, five resistant, 12 moderately resistant and the rest moderately to highly susceptible (Goswami, 1993). Of 270 *Colocasia esculenta* lines screened for natural resistance to leaf blight in the field at Trivandrum, India, 119 lines were resistant (Santha-Pillai *et al.*, 1993). In tests carried out in Arunachal Pradesh, India, 23 varieties of taro were screened for resistance to *P. colocasiae*: five varieties were immune and one was moderately resistant (Chaudhary and Rai, 1988). Of 11 cultivars screened under natural epiphytotics, Burdwar local was the best for commercial cultivation in west Bengal, India (Ghosh and Pan, 1991). In the British Solomon Islands, none of the 181 local cultivars tested were highly resistant to the fungus (Gollifer and Brown, 1974). More than 200 local varieties have been screened for resistance to the fungus; only Abrueme has shown promise (Jackson and Gollifer, 1975).

Resistance to *P. colocasiae* was found in a wild taro (*Colocasia esculenta*) accession introduced from Thailand and designated Bangkok. Data from crosses between Bangkok and local cultivars indicated that resistance is controlled by a single dominant gene (Patel *et al.*, 1984).

P. kernoviae: No host resistance has been detected at this time, but little research has been accomplished to date.

P. melonis: Due to the confusion of *P. melonis* with *P. drechsleri*, little specific control information is available for *P. melonis*. Grafting of cucumber plants onto Kurodane or Shintosa squash cultivars (Erwin and Ribeiro, 1996) controlled the disease. The method may not be practical, however, outside the greenhouse environment.

P. pinifolia: There is evidence that genotypes of *P. radiata* differ in susceptibility to P. pinifolia, indicating that host resistance may be available (Duran *et al.*, 2008).

P. porri: Different levels of partial resistance to *P. porri* were identified in winter leek and in wild and cultivated relatives (Smilde *et al.*, 1995, 1997).

Adequate levels of host resistance against many exotic *Phytophthora* spp., including *P. alticola*, *P. austrocedrae*, *P. captiosa*, *P. fallax, P. frigida*, *P. gallica*, *P. idaei*, *P. iranica*, *P. italica, P. multivesiculata*, *P.*

multivora, P. polonica, *P. primulae, P. psychrophila, P. quercina, P. tentaculata,* and *P. uliginosa*, have not been found at this time.

Control Records Attach any forms, receipts, etc. that document these actions. Program personnel must maintain records and maps noting the locations of all detections, the number and type of plants subjected to control actions, and the materials and formulations used in each treated area. Attach all documentation to the office EAN copy.

Environmental Monitoring Contact PPQ headquarters for guidance on environmental documentation and monitoring.

CONTENTS

PATHWAYS FOR INTRODUCTION OF EXOTIC *PHYTOPTHORA* SPP.

A Quarantine Pest and its Pathway

Introduction Potential

Early detection will require that potential pathways for pest entry be regularly monitored, and that quality assurance be built in to assure tracking (monitoring) in each link in the pathway. This set of guideline addresses what needs to occur between the ports and those points at risk within the continental United States.

Pathway Components – for Exotic *Phytophthora* spp.

Exotic *Phytophthora* species covered in this NPRG are quarantine pests of economic and environmental importance, and do not occur in the United States. The most likely method of entry into the United States is intentional (smuggling) or unintentional introduction of host material infected with the pathogen through the international trade of nursery stock or wood products. Different risks are associated with each of these potential pathways relating to their accessibility, transmissibility of the disease organism, and likelihood of interdiction.

Local spread may occur through the movement of fungal spores in soil, water, or in contaminated soil adhering to plants, equipment, implements, footwear or other carriers.

Phytophthora infestations in nurseries may be introduced via three critical pathways.

• The movement of infected plant material from one nursery to another;

• The natural environmental movement of spores from a nursery or infected wild plants to infect plants in a nursery;

• The transmission of the pathogen from non-plant pathways to plant material (*e.g.*, the introduction of infested soil, water, growing media, equipment, etc.)

Other pathways are possible, but are not yet known.

Questions Regarding Detection

i. **Is the inspection protocol in place for regulated commodity and plant material at the designated ports of entry?**

ii. **Are signs and/or symptoms visible on commodity if this pest/pathogen is present:** (Yes/No), Characteristic symptoms are given in Chapter 2 and Table 3-1 of this document.

iii. **Is the inspection staff trained to detect the pathogen in question?**

iv. **Can the pest be detected by visual inspection?**

v. **Can samples be processed in NPPO identification lab for non-visible agents?**

vi. **Is the sample size adequate to detect the pest/pathogen? (**Yes/No) Yes only if inspectors are trained to inspect plant material correctly. Confiscation of any contraband is to be expected of inspectors.

Examination of Pathway Risk

i. **Does environment at destination have exotic *Phytophthora* hosts?** (Yes/No) A number of alternate hosts have been identified and may potentially serve as reservoirs for exotic phytophthoras (although whether these alternative hosts are epidemiologically important is debatable). It is important to note 'susceptible hosts' sites, potential time of exposure, and relative climate, if known.

ii. **Is there a delivery system for infected/infested plant material, to host areas associated with Consumers, Distributors?** (Yes/No) Consider trucking, rail and foot traffic. Consider end use and disposal of dead or dying infected plant materials and associated infested vector.

iii. **Are climatic and biotic conditions suitable if exotic Phytophthoras follows the pathway to destination?** (Yes/No) Consider host material around ports if entry is along pathways to the final disposition locations. Refer to exotic *Phytophthora* host list to determine if pathways are susceptible. If so, set up detection plans to evaluate and inspect pathways. This is especially important in areas where the primary host affected by this exotic *Phytophthora* grows.

iv. **Is disposal of discarded plant material at landfills, in gardens, as compost or fertilizer likely?** (Yes/No) Determine the longevity and disposal of imported products. Locate disposal sites and waste areas that represent potential reservoirs for establishing new infestations.

CONTENTS

Overview

A key element in designing a program or an emergency response is consultation with Environmental Services (ES), a unit of APHIS' Policy and Program Development Staff (PPD). ES prepares environmental documentation such as environmental impact statements (EIS) and environmental assessments (EA) to aid in program operational decisions, as well as Endangered Species consultation. ES also coordinates pesticide registration and approvals for APHIS pest control and eradication programs, ensuring that registrations and approvals meet program use needs and conform to pesticide use requirements. Refer to the Resources Section of this document for additional information.

Disclaimer

All uses of fumigants must be registered or approved by appropriate Federal, State, and/or Tribal agencies before they can be applied. The information provided on pesticide labels may not reflect all of the actual information, including precautions and instructions for use, which you are required to follow in your specific State or locality. It is the responsibility of persons intending to use a pesticide to read and abide by the label, including labeling that has been approved for the particular State or locality in which the chemical is to be used, and to comply with all Federal, State, Tribal, and local laws and regulations relating to the use of the pesticide. APHIS program staffs are responsible for their compliance with applicable environmental regulations.

National Environmental Policy Act

Agencies should prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) concurrently and integrated with environmental impact analyses, surveys, and studies required by the Fish and Wildlife Coordination Act, National Historic Preservation Act of 1966, Endangered Species Act, and other laws and executive orders. Environmental documents prepared to comply with other acts

also may be incorporated into National Environmental Policy Act (NEPA) documents as part of the NEPA process.

Categorical Exclusions

Categorical exclusions (CE) are categories of actions that do not have a significant effect on the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is generally required.

APHIS managers are encouraged to use categorical exclusions where appropriate to reduce paperwork and speed the decision-making process. Proposed actions are subject to sufficient environmental review to determine whether they fall within the broadly defined categories. Each time a specific categorical exclusion is used, the required review must be done. An EA may be prepared for proposed actions otherwise excluded when the manager determines that the action may have potential to significantly affect the environment or an EA would be helpful in planning or decision making.

Environmental Assessments

An environmental assessment (EA) is a concise public document that briefly provides sufficient evidence and analysis for determining whether to prepare an environmental impact statement (EIS) or finding of no significant impact (FONSI). An EA aids an agency's compliance with the National Environmental Policy Act (NEPA) when no EIS is necessary and facilitates the preparation of an EIS when necessary. Generally, an EA leads to a FONSI or an EIS, but it could also lead to abandonment of a proposed action.

The content of an EA must include brief discussions of the need, alternatives, and potential environmental impacts of the proposal and a list of agencies and persons consulted.

Environmental Impact Statements

An environmental impact statement (EIS) is a detailed statement that must be included in every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment. The primary purpose of an EIS is to serve as an action-forcing device to insure that the policies and goals defined in the National Environmental Policy Act (NEPA) are infused into the ongoing programs and actions of the Federal government. Generally, EIS's are prepared when Federal agencies recognize that their actions have the potential for significant environmental effects (adverse or beneficial), or when an environmental assessment leads to a finding of potential significant impact.

APHIS prepares EIS's for administrative proceedings that establish broad scale significant impact-generating strategies, methods, or techniques such as large-scale aerial pesticide applications. This can

include contingency or emergency strategies that are comprehensive in scope or long-range plans with potential for significant environmental impact. APHIS also prepares programmatic EIS's to examine strategies and options for dealing with issues with important implications for the maintenance and enhancement of environmental quality.

Environmental Monitoring

PPQ requests assistance from ES before PPQ personnel or funding are used for control operations. Additionally, program staff should consult with the PPQ EDP Environmental Monitoring staff to determine if an environmental monitoring plan is required for the operation. State, regional, and national program managers determine counties where treatments may be needed.

Program personnel should evaluate the success of biological control agents and herbicide treatments used in eradication or suppression of the target FNW or host weeds and avoid damage to non-target plants.

Biological Assessment

A biological assessment (BA) is an analysis of the effects that a Federal agency action may have on listed or proposed endangered or threatened species and designated critical habitat. The Endangered Species Act (ESA) requires this analysis if the proposed action may affect a listed species. In such a case consultation with the U.S. Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS) is required. Federal agencies are required to insure that any action authorized, funded, or carried out is not likely to jeopardize listed species or result in adverse modification of designated critical habitat.

CONTENTS

Overview

Another very important part of the New Pest Response Guidelines is determining what is not known about the pathogen. Once these research areas are identified, additional funding may be secured to evaluate the potential effectiveness of these different areas on control of exotic *Phytophthora* species.

Research Needs

For most of the *Phytophthora* species covered in this NPRG, little information is available on the lifecycle and biology, especially under field conditions. Information is also lacking on the economic and ecological importance of each species. This information would be very useful for designing a targeted survey for each exotic *Phytophthora* sp.

- Determination of the susceptibility of North American alder species to *Phytophthora alni* (all subspecies).
- Determination of the ecology and ecological importance of *Phytophthora alni* in North America.
- Elucidation of the biology and life cycle of *Phytophthora alni* under field conditions, including the role of the oospore in the biology and survival of *P. alni*.
- Determination of the importance of *Phytophthora alticola* and *P. frigida* as tree pathogens in the *Phytophthora* complex associated with collar rot of cold-tolerant *Eucalyptus* spp.
- Elucidation of the biology and life cycle of *Phytophthora alticola* and *P. frigida*.
- Elucidation of the biology and life cycle of *Phytophthora austrocedrae*.
- Clarification of the economic importance and ecological importance of *Phytophthora boehmeriae* on hosts other than cotton.
- Elucidation of the biology and life cycle of *Phytophthora boehmeriae* under field conditions, including the role of the

chlamydospore and oospore in the survival of *P. boehmeriae*.

- Determination of the mode of infection and dispersal of *Phytophthora captiosa* and *P. fallax* (*e.g.,* how does inoculum reach Eucalyptus foliage as high as 20-60 meters?).
- Clarification of the economic and ecological importance of *Phytophthora captiosa* and *P. fallax*.
- Determination of the center of origin for *Phytophthora colocasiae*.
- Clarification of the role of the chlamydospore and oospore in the survival of *P. colocasiae* where taro is grown seasonally (there is no evidence either structure is produced under field conditions).
- Determination of whether *Phytophthora gallica* is a pathogen or a saprotroph.
- Clarification of the economic and ecological importance of *Phytophthora gallica.*
- Elucidation of the biology and life cycle of *Phytophthora gallica* under field conditions, including whether *P. gallica* is sexually sterile.
- Clarification of the economic and ecological importance of *Phytophthora idaei.*
- Elucidation of the biology and life cycle of *Phytophthora idaei*.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora iranica*.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora italica*.
- Determination of the full host range of *Phytophthora kernoviae*.
- Elucidation of the biology and life cycle of *Phytophthora kernoviae*, particularly survival strategies as the oospore has not been observed in nature.
- Clarification of the economic and ecological importance of *Phytophthora melonis.*
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora multivesiculata*.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora multivora*.
- Elucidation of the biology and life cycle of *Phytophthora pinifolia*.
- Clarification of pathogenic status, lifecycle, biology, and

economic and ecological importance of *Phytophthora polonica*.

- Elucidation of the biology and life cycle of *Phytophthora primulae*.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora psychrophila.*
- Determination of the extent of economic damage and how much *Phytophthora quercina* is involved in oak decline in Europe.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora quercina.*
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora tentaculata.*
- Full knowledge of the potential host range of *Phytophthora tentaculata*, as the host range is expanding.
- Clarification of lifecycle, biology, and economic and ecological importance of *Phytophthora uliginosa.*

DEFINITIONS

Abiotic	Pertaining to the absence of life; diseases not caused by living organisms.
Actinomycete	A group of microorganisms similar to bacteria that produce long filaments.
Adventitious Roots	Arising from other than the usual place. Roots from a stem rather than as branches of a root.
AFLP	Amplified Fragment Length Polymorphism. A technique that uses PCR to amplify genomic DNA, cleaved by restriction enzymes, in order to generate DNA fingerprints; it is a combination of RFLP and arbitrary primer PCR. It does not require prior sequence knowledge.
Allopolyploid	A polyploid has more than two sets of chromosomes (e.g., triploid, tetraploid). In an allopolyploid, the chromosomes are derived from two or more different species. In an autopolyploid, all the chromosomes are derived from the same species.
Amphigynous	Having an antheridium through which the oogonium grows, as in many *Phytophthora* species (see paragynous).
Antheridium (-ia (plural))	Male sexual organ (male gametangium) found in some fungi.
Aplerotic	An oospore, not filling the oogonium (see plerotic).
Appressed	Pressed close to or lying flat.
Appressoria	Swollen, flattened portion of a fungal filament that adheres to the surface of a higher plant, providing anchorage for invasion by a fungus.
Arachnoid	Spider-web like.
Basal	Located at the base.

Biflagellate	Having two flagella
Biotic	Relating to life; diseases caused by living organisms.
Bole	The trunk of a tree.
Brachyblasts	A short lateral branch.
Bullate	Appearing puckered as if blistered.
Caducous	Sporangia that detach easily from the sporangiophore by wind and water.
Calcareous	Composed of, containing, or characteristic of calcium carbonate, calcium, or limestone; chalky.
Cambium	Formative one-cell layer of tissue between xylem and phloem in most vascular plants that is responsible for secondary growth.
Cankers	A plant disease characterized (in woody plants) by the death of cambium tissue and loss and/or malformation of bark, or (in non-woody plants) by the formation of sharply delineated, dry, necrotic, localized lesions on the stem; "canker" may also be used to refer to the lesion itself, particularly in woody plants.
Catenulate	Arranged in a series of rings or chains.
Chimeric	Composed of parts of different origin.
Chlamydospore	Thick-walled or double-walled asexual resting spore formed from hyphal cells (terminal or intercalary) or by transformation of conidial cells that can function as an overwintering stage.
Chlorosis	Failure of chlorophyll development, caused by disease or a nutritional disturbance; fading of green plant color to light green, yellow, or white.

Clavate	Resembling a club, becoming increasingly wide from the base to the distal end.
Coenocytic	Having multiple nuclei embedded in cytoplasm without cross walls; nonseptate.
Corm	Solid swollen underground bulb-shaped stem or stem base and serving as a reproductive structure.
Cotyledons	Embryonic leaf in seed-bearing plants.
Crown	The part of a plant, usually at ground level, where the stem and roots merge.
Decontamination	The application of an approved chemical or other treatment to contaminated implements, material, or buildings for killing or deactivating a pathogen. Also called disinfection or disinfestation.
Delimiting Survey	After the initial first detection in an area, this type of survey is conducted to define the geographic range of the infection/infestation.
Dendroid	Resembling a tree in form and branching structure.
Denticulate	Having a very finely toothed margin.
Dicotyledonous	Flowering plant with two cotyledons; the stem grows by deposit on its outside.
Dieback	Progressive death of shoots, leaves, or roots, beginning at the tips.
Eradication	Control of plant disease by eliminating the pathogen after it is established or by eliminating the plants that carry the pathogen.
Elicitins	Small cysteine-rich lipid-binding proteins.
Elipsoid	Surface whose plane sections are all ellipses or circles.

Encysted	To form a cyst or protective covering, to lose motility.
Endophytes	An endosymbiont, often a bacterium or fungus, which lives within a plant for at least part of its life without causing apparent disease.
Epicormic shoots	New shoots arising near the base of the plant.
Exudate	Liquid excreted or discharged from diseased tissues, from roots and leaves, or by fungi.
Fascicles	Dense cluster or bundle.
Flagellum	Hair-like, whip-like, or tinsel-like appendage of a motile cell, bacterium or zoospore that provides locomotion.
Fructification	The bearing of fruit.
Fungus	A eukaryotic organism that is usually filamentous (forming a mycelium) and heterotrophic, has cell walls composed of chitin, and reproduces by sexual and/or asexual spores.
Fusiform	Spindle-shaped; tapering at each end.
Gametangia	Cell containing gametes or nuclei that act as gametes.
General Detection Survey	A survey conducted over a large area to discover new potential infestations/infections in areas where the pest/disease is not known to occur.
Geniculate	Bent at a sharp angle.
Girdle	To circle and cut through a stem or the bark and outer few rings of wood, disrupting the phloem and xylem.
Globose	Ball-shaped.

Gummosis	A plant disease in which the lesions exude a sticky liquid.
Haustoria	Specialized branch of a parasite formed inside host cells to absorb nutrients.
Heterothallism	Condition in which sexual reproduction can occur only in the presence of genetically different mycelia (see homothallism).
Homothallism	Condition in which sexual reproduction occurs with a single thallus; self-fertile (see heterothallism).
Host	A plant which is invaded by a parasite or pathogen and from which it obtains its nutrients.
Hyaline	Transparent or nearly so; translucent; often used in the sense of colorless.
Hybrid	Offspring of two individuals of different genotypes.
Hyphae	Single, tubular filament of a fungal thallus or mycelium; the basic structural unit of a fungus.
Identification Authority	Authority to confirm the presence of a particular pest organism issued by the APHIS National Identification Services to diagnosticians that have demonstrated proficiency in identifying.
Incident Command System	An expandable and contractible system to manage emergencies, based on the Forest Service's Forest Fire Management System.
Infection	The establishment of a parasite on or within a host plant.
Intercalary	Inserted between.
Isozyme	Enzymes that differ in amino acid sequence but catalyze the same chemical reaction.

Leaf Spot	A plant disease lesion typically restricted in development in the leaf after reaching a characteristic size.
Lesion	Localized diseased area or wound.
Limoniform	Shaped like a lemon.
Monitoring or Evaluation Survey	A survey conducted at a site where a disease was found and where an eradication program is being performed.
Mottle	Disease symptom comprising light and dark areas in an irregular pattern, usually caused by a virus; often used interchangeably with mosaic.
Mycelium	Mass of hyphae constituting the body (thallus) of a fungus.
Necrosis	Death of cells or tissue, usually accompanied by black or brown darkening.
Obligate Parasite	Organism that can grow only as a parasite in association with its host plant and cannot be grown in artificial culture media.
Obpyriform	Resembling a pear which is attached at the narrower end.
Oogonia	Female gametangium of oomycetes, containing one or more gametes.
Oospore	Thick-walled, sexually-derived resting spore of oomycetes.
Ovoid	Egg-shaped.
Papilla	Nipple-like projection; used to describe the tip of some sporangia and the localized wall thickenings on the inner surface of plant cell walls at sites penetrated by fungi.
Papillate	Having papilla.
Paragynous	Having the antheridium contact the

	oogonium on the side, as in many *Pythium* sp. (see amphigynous).
Pathogen	Any organism that can incite a disease.
PCR	An acronym for Polymerase Chain Reaction, a laboratory technique that amplifies DNA sequences using pieces of DNA called primers. These primers attach to different ends of specific segments of the target DNA and assemble the DNA from opposite directions. This technique is used in order to determine if a host is infected with a known pathogen.
Pedicle	Small stalk.
Peduncle	The stalk of an inflorescence or a stalk bearing a solitary flower in a one-flowered inflorescence.
Phenotype	A phenotype is any observable characteristic or trait of an organism: such as its morphology, development, biochemical or physiological properties, or behavior.
Phloem	Tissue that conducts synthesized food substances (*e.g.*, from leaves) to parts where needed; consists primarily of sieve tubes.
Plerotic	An oospore filling the oogonium.
Potentially Actionable Suspect Sample	Also known as PASS, a presumptive positive sample diagnosed or identified by provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation.
Presumptive Positive	Such a result may require confirmatory testing if the sample is a PASS sample.

Protuberance	Something, such as a bulge, knob, or swelling, that protrudes.
Pyriform	Pear-shaped.
Reniform	Kidney-shaped.
Rhizosphere	Microenvironment in the soil, immediately around plant root.
Riparian	Relating to or located on the banks of a river or stream.
Rosaceous	Like a rose.
Sanitation	Destruction or removal of infected and infested plants or plant parts; decontamination of tools, equipment, containers, work space, hands, etc.
Saprophyte	An organism that obtains nourishment from non-living organic matter.
Saprotroph	An organism that lives off of dead or decaying organic material.
Septate	With cross walls; having septa.
Sinuate	Curved or curving in and out.
Soilborne	Carried on or beneath the soil surface.
Sporangia	Saclike fungal structure in which the entire contents are converted into an indefinite number of asexual spores.
Spores	Reproductive structure of fungi and some other organisms, containing one or more cells; a bacterial cell modified to survive an adverse environment.
Stellate	Arranged like rays or radii.
Subglobose	Nearly globose.
Submerged	Beneath the surface.

Subspherical	Nearly spherical.
Symbiotic	Mutually beneficial association of two different kinds of organisms.
Sympodial	Pertaining to proliferation of axes, in which each successive spore or branch develops behind and to one side of the previous apex where growth has ceased.
Symptom	The external and internal reactions or alterations of a plant as the result of a disease.
Terminal	Situated at the ends.
Trace-back	To investigate the origin of infested plants from initial detection location back through intermediate steps in commercial distribution channels to the origin.
Trace-forward	To investigate where infected plants may have been distributed from a known infestation through steps in commercial distribution channels or wholesale or retail procurement.
Tuberculate	Covered with tubercles (warty-like projections).
Undulate	To cause to move in a smooth wavelike motion.
Uninucleate	A cell having one nucleus.
Vacuole	Generally spherical organelle within a plant cell bound by a membrane and containing dissolved materials such as metabolic precursors, storage materials, or waste products.
Verrucose	Covered with warts or projections that resemble warts.
Viable	The state of being alive; able to germinate, as seeds, fungus spores, sclerotia, etc.;

	capable of growth.
Water-soaked	Describing disease symptom of plants or lesions that appear wet, dark, and usually sunken and translucent.
Wilt	Drooping of leaves and stems from lack of water (inadequate water supply or excessive transpiration); vascular disease that interrupts normal water uptake.
Xylem	The woody part of plants: the supporting and water-conducting tissue, consisting primarily of tracheids and vessels.
Zonate	Marked with zones or bands; belted; striped.
Zoospores	Fungal spore with flagella, capable of locomotion in water.
Zygotic	Relating to a zygote.

APPENDIX A: Disinfection Protocol

Introduction	Plant pathogens can persist on pruning shears, knives, harvesters, tractors and other implements used for cutting, digging, or taking soil samples. Any piece of equipment that comes in contact with plant material or soil could potentially harbor *Phytophthora* spp. Disinfection of all equipment and footwear is required prior to leaving a site.
Instructions	**Quaternary Ammonium Compounds** For safety and comfort, applicators are required to wear rainwear such as hats, coats, rubber boots and face shields. Soil should not be removed from equipment before treatment if there is a possibility the soil will contaminate the site. Once soil is saturated with quaternary ammonium or 10 percent sodium hypochlorite solution (bleach), it is no longer considered contaminated. Please follow all label directions. Use of any product inconsistent with label directions is illegal and may be dangerous, and is not recommended. a. To disinfest storage areas, drench area thoroughly with a solution of quaternary ammonium solution at labeled rates or 10 percent sodium hypochlorite solution. Do not rinse. b. To disinfest vehicles, portions of vehicles where soil is likely to adhere, such as tires, wheel wells, and under the chassis, should be washed thoroughly with a 0.15 percent *a.i.* solution of quaternary ammonium. Do not rinse for at least 1 hour (hr). After 1 hr, equipment should be rinsed only if specifically required by owner or operators. Because quaternary ammonium can kill vegetation on contact, it should be used to wash equipment in non-planted area. Equipment should be dry at the time of treatment to facilitate efficacy of the solution. For large pieces of equipment, a high pressure delivery system, such as a Hotsy steam pressure wash system, is recommended to penetrate the soil and debris which may still adhere. Equipment must be wet to saturation with the quaternary ammonium solution. c. To disinfest tools and boots, remove adhering soil and thoroughly wet them with a 0.15 percent *a.i.* quaternary ammonium solution. Do not rinse. An emergency exemption may be required for the above rates and uses of quaternary ammonium compounds.

APPENDIX B: Symptoms of Exotic *Phytophthora* spp.

PPQ Form 391

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions. *See reverse for additional OMB information.* **FORM APPROVED OMB NO. 0579-0010**

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER
2. DATE (MO / DA / YR)
3. SUBMITTING AGENCY ☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN
4. NAME OF SENDER
6. ADDRESS OF SENDER
ZIP

INTERCEPTION SITE
5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*
7. NAME AND ADDRESS OF PROPERTY OR OWNER
COUNTRY/ COUNTY

PURPOSE
8. REASON FOR IDENTIFICATION *("X" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)	E. ☐ Livestock, Domestic Animal Pest
B. ☐ Damaging Crops/Plants	F. ☐ Possible Immigrant *(Explain in* REMARKS)
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS)	G. ☐ Survey *(Explain in* REMARKS)
D. ☐ Stored Product Pest	H. ☐ Other *(Explain in* REMARKS)

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

HOST DATA
10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*
11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure and Indicate* ☐ Number ☐ Percent*):*

12. PLANT DISTRIBUTION
☐ LIMITED ☐ SCATTERED ☐ WIDESPREAD

13. PLANT PARTS AFFECTED

☐ Leaves, Upper Surface	☐ Trunk/Bark	☐ Bulbs, Tubers, Corms	☐ Seeds
☐ Leaves, Lower Surface	☐ Branches	☐ Buds	
☐ Petiole	☐ Growing Tips	☐ Flowers	
☐ Stem	☐ Roots	☐ Fruits or Nuts	

PEST DATA
14. PEST DISTRIBUTION
☐ FEW ☐ COMMON ☐ ABUNDANT ☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

SIGNATURE DATE

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

PPQ FORM 391 *Previous editions are obsolete.*
(AUG 02)

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

PPQ Form 523

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED - OMB NO. 0579-0102

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION | 2. DATE ISSUED

3. NAME AND QUANTITY OF ARTICLE(S)

4. LOCATION OF ARTICLES

5. DESTINATION OF ARTICLES

6. SHIPPER

7. NAME OF CARRIER

8. SHIPMENT ID NO.(S)

9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. FAX NO.

SS NO. TAX ID NO.

10. PORT OF LADING | 11. DATE OF ARRIVAL

12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)

12a. PEST ID NO. | 12b. DATE INTERCEPTED

13. COUNTRY OF ORIGIN | 14. GROWER NO.

15. FOREIGN CERTIFICATE NO.

15a. PLACE ISSUED | 15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT: _____

☐ RE-EXPORTATION: _____

☐ DESTRUCTION: _____

☐ OTHER: _____

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):

18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE: | DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER: | DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

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APPENDIX E: Diagnostic Protocols

Purpose	
Materials and Equipment	
Safety	
INSTRUCTIONS	
Sub-sampling	
Sieving	
Chloroform Extraction	
Cleaning and Drying Sieves	
Microscopic Examination of Samples	

Technical Working Group Members:
None at this time

P. ramorum Science Panel, 2004

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