
United States Department of Agriculture

# *Phytophthora ramorum* Domestic Regulatory Program Manual

First Edition Issued 2020

Phytophthora ramorum

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# Introduction

## Contents



## APHIS Mission

The Animal and Plant Health Inspection Service (APHIS) is an Agency within the United States Department of Agriculture (USDA). The mission of APHIS is to protect the health and value of American agriculture and natural resources.

## *Phytophthora ramorum* Program Objective

The goal of the APHIS *P. ramorum* program is to limit the spread of *P. ramorum* from regulated nurseries and quarantine areas to nonregulated nurseries and nonquarantine areas through regulatory strategies and adopting (voluntary and mandatory) best management practices.

## PPQ Mission

APHIS Plant Protection and Quarantine (PPQ), and operational program, safeguards agriculture and natural resources from the risks associated with the entry, establishment, or spread of animal and plant pests and noxious weeds.

## Intended Users

Users of this manual include State and Federal regulators who conduct or oversee surveys and inspections for *P. ramorum* in nurseries, on residential properties, and at managed landscapes and public gardens. It is publicly available for nursery owners, homeowners, managers of large landscapes and public gardens, and others interested in the Federal processes involved with the *P. ramorum* program.

## Manual Objective

The objective of the *Phytophthora ramorum* Manual is to help users detect the presence of *P. ramorum* in interstate shipping nurseries. This chapter describes methods for sampling plants, surface water, drainage water, water for irrigation, container mix, and any other articles designated by an inspector as possible sources of *P. ramorum* inoculum at the nursery.

## Scope

The chapters in this manual are:

## Revisions

The Manuals Unit of PPQ issues revisions using a USDA Stakeholder Registry announcement. Each announcement provides the following information:

- Transmittal number used to track revisions
- Purpose of the revision
- Page number(s) on which the revision(s) is located

## Authorities

This protocol incorporates requirements and procedures outlined in the regulations declared under the Plant Protection Act of June 20, 2000, as amended, Section 412(a), 7 U.S.C. 7712(a), which authorizes the Secretary of Agriculture to prohibit or restrict the movement in interstate commerce of any plant, plant part or article, or means of conveyance if the Secretary determines the prohibition or restriction is necessary to prevent the dissemination of a plant pest within the United States. These protocols are also issued in line with *P. ramorum* regulations found at 7 CFR 301.92 et seq.

Since the regulations were first published in 2022, *P. ramorum* has been detected in a significant number of nurseries. These detections prompted a standard protocol to be used by State and Federal regulators when responding to *P. ramorum* found in nurseries. To ensure there is consistency in responding to *P. ramorum* infestations, this manual describes the official activities performed within and around nurseries by USDA–APHIS staff in cooperation with State agriculture regulatory officials.

For States **with** regulations for quarantine pests, and/or specifically for *P. ramorum* equivalent to the Federal regulations or Federal Orders, State personnel may conduct specific actions required by the protocol, within and around the nursery, under State authority with Federal support. For States **without** regulations for quarantine pests and/or *P. ramorum* equivalent to the Federal regulations, specific actions required by this protocol within and around the nursery will be conducted under Federal authority, in cooperation with State and/or Federal personnel.

Consult the latest APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum* **prior** to beginning any survey, inspection, or delimitation.

# Reporting Issues With or Suggestions For the Manual

Refer to Table 1-1-1 to determine where to report problems or disagreements, or improvements that directly affect the contents of the manual.

**Table 1-1-1 Reporting Issues With or Suggestions for the Manual**

| If you: | Then: |
|---|---|
| ◆ Are unable to access the online manual<br>◆ Have a suggestion for improving the format (layout, spelling, etc.) | CONTACT PPQ Manuals Unit at PPQ.IRM.ISMU.Manuals.Feedback@usda.gov |
| Disagree with a policy or procedure, or have an urgent situation requiring an immediate response | CONTACT Phytophthora ramorum National Policy Manager at william.d.wesela@usda.gov or 301-851-2229.<br>4700 River Road<br>Riverdale, MD 20737 |

Chapter

2

# *Phytophthora ramorum* Inspection and Sampling Protocol for Nurseries

## Contents



## Introduction

The objective of the *Phytophthora ramorum* Inspection and Sampling Protocol is to detect the presence of *P. ramorum* in interstate-shipping nurseries. This protocol describes methods for sampling plants, surface water, drainage water, water for irrigation, container mix, and any other articles designated by an inspector as possible sources of *P. ramorum* inoculum at the nursery.

## Quick Guide for Conducting Nursery Inspection and Sampling

When sampling, remember to move from low-risk to high-risk areas to prevent potential spread of the pathogen.

1. Determine when to sample each nursery based on the time of year when climatic conditions will be most conducive for *P. ramorum* disease expression (refer to Timing Nursery Inspection and Sampling on page 2-1-3).
2. After determining when to sample, notify the laboratory **beforehand** to ensure supplies are available and the laboratory is prepared to receive the samples (refer to Notifying the Laboratory on page 2-1-4).

3. Ensure **all** supplies and equipment are available for the planned survey and review symptoms before arriving at the nursery (refer to Preparing for Nursery Inspection and Sampling on page 2-1-4).
4. Prior to inspection day, review:
   A. APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*
   B. Obtain and review the nursery inventory, if available
   C. Obtain and review any available maps of the nursery to determine areas to inspect and sample (refer to Inspecting and Sampling the Nursery on page 2-1-7)
   D. Nursery history, if available (e.g., has it been previously positive for *P. ramorum*? Is it new? Has it recently changed management or ownership? What are the best management practices and sanitation measures?)
   E. Fungicides can mask the presence of *P. ramorum*. Obtain the nursery's fungicide spray schedules to better understand how fungicide treatments may impact symptom expression. For inspector safety, plants should not be sprayed immediately prior to inspection.
5. Determine the approximate number of plant samples to take from each regulated plant genus (refer to Table 2-1-1).
6. On inspection day, begin by conducting a visual of the nursery as a whole. Note topography, water sources, drainage patterns, areas of high risk (cull piles, low-vigor plants, etc.) to compare your observations to any nursery map provided and verify or note any of the following items (refer to Inspecting and Sampling the Nursery on page 2-1-7).
7. Take samples of:
8. **Plants**: sample symptomatic plant tissue. Ensure that **at least** the minimum number of samples are collected (refer to Table 2-1-1) and keep plant genera samples separate from one another. Each sample must be bagged separately (refer to sample on page Glossary-1-6). Record the collection location of each sample on the outside of the sample bag. Each bag should have a unique identification number and the date. During implementation of this protocol, every plant sampled is on regulatory hold and should **not** be subject to scheduled nursery maintenance (refer to Plant Symptoms and Sampling for P. ramorum on page 2-1-4). While taking samples, **visibly and indelibly** flag or mark plants and areas sampled. Also mark sampled areas on a map of the nursery and take pictures, including areas of surface water (refer to Inspecting and Sampling the Nursery on page 2-1-7). Properly label and store collected samples for shipping to the laboratory (refer to Sampling and Submission Protocol on page 8-1-1).

A. **Water**: sample water in and around the nursery. Each area is its own discrete sample. Collect surface water in and around plant material blocks. Collect from holding ponds, drainage ditches, water around cull piles, etc. (refer to Water Sampling and Processing Protocol on page 10-1-1).

B. **Pots and containers**: if containers are recycled and stored at the nursery or if used pots are purchased, sample residual container mix from pots or other containers. Scrape container mix from pots filling a labeled, self-sealing plastic bag. Use the Soil and Container Mix Sampling and Processing Protocol on page 11-1-1.

C. **Cull piles**: examine any area where plants have recently been disposed. If regulated plants are present, sample symptomatic plant material and keep plant genera samples separate from one another. If there is any surface water, take **at least one** sample from each cull pile area.

D. **Other articles designated by an inspector as possible sources of *P. ramorum* inoculum**: at the inspector's discretion, sample any and all other possible sources of *P. ramorum* inoculum.

E. **Container mix**: **only** container mix from used container piles is sampled in this protocol (refer to Soil and Container Mix Sampling and Processing Protocol on page 11-1-1).

9. Sanitize tools and change or sanitize gloves between samples to prevent cross-contamination (refer to Inspecting and Sampling the Nursery on page 2-1-7).

10. Complete a PPQ Form 391 Specimens for Determination (or State equivalent) for **each** sample. Forward **all** samples to the appropriate laboratory (either NPPLAP-accredited or APHIS diagnostic laboratory; refer to Sampling and Submission Protocol on page 8-1-1 and U.S. State and Territory Plant Health Directors on page A-1-2 and APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*).

## Timing Nursery Inspection and Sampling

Nurseries should be inspected and sampled at a time of year when nursery conditions are optimal for *P. ramorum* disease expression.

- Disease expression typically begins between 30 and 90 days **after** bud break. Inspection and sampling should begin **after** the spring flush is underway when some of the leaves have fully expanded. Plants can express symptoms throughout the growing season, though isolating the pathogen may be more difficult during hot and dry periods.

- Nursery beds under shade cloth or overhead irrigation, greenhouses, and hot houses should be considered micro-climates where optimum conditions can occur **outside** of the typical fall/spring window.
- Plan inspections and samplings when nurseries receive shipments in the spring and fall when regulated plants will be present and nursery conditions will be optimal.
- Research suggests the most favorable climate for expressing symptoms (i.e., "conducive environmental conditions") is when ambient temperatures are between 3 °C (37.4 °F) and 28 °C (82.4 °F) (optimum 20 °C (68 °F)) and free moisture is present on regulated plant tissue for **at least** 12 hours over 10 **or more** days. In most areas, the most favorable timing is in the spring and fall.

## Notifying the Laboratory

Notify the laboratory of an upcoming sampling date. This will also ensure the laboratory is prepared to receive the samples and prepared to process them promptly.

## Preparing for Nursery Inspection and Sampling

Refer to Sampling Supplies and Equipment Checklist on page 8-1-4 and Water Sampling and Processing Protocol on page 10-1-1.

## Plant Symptoms and Sampling for *P. ramorum*

### Plant Symptom Resources

Inspectors **must** be trained to identify symptoms associated with *P. ramorum* on regulated plants. **At a minimum**, they should review photographs of the wide range of possible symptoms **before** starting the inspection and sampling. Photographs of typical and atypical symptoms are available in Biology and Symptoms of Phytophthora ramorum on page 7-1-1.

**NOTICE**

Symptoms of *P. ramorum* are variable and the greatest chance of detecting *P. ramorum* infections is through the collection of **any** unhealthy-looking plant tissue for laboratory analysis. Avoid desiccated or excessively decayed tissue.

Foliar symptoms of *P. ramorum* infection are highly variable and can range from pinpoint discolorations on the petiole and leaf surface to large "V"-shaped lesions along the leaf mid-vein. Inspect the lower, more shaded portions of plants and the interior of the canopy where moisture and high humidity may persist. Pay special attention to leaf areas in which water would

linger such as the midrib and leaf tips. Check for leaves inside the pot of asymptomatic, regulated plants because infection could cause premature leaf drop and symptomatic leaves could be found **only** in the pot or on the ground. Many *Phytophthora* spp., other pathogens, and environmental stressors can cause symptoms that **cannot** be distinguished from *P. ramorum* infection by visual inspection. Do **not** presume to know what **all** *P. ramorum* symptoms look like. Collect samples of leaves with symptoms that could be caused by abiotic stressors. If there is not enough symptomatic, regulated plant material to fulfill the required number of samples (refer to Table 2-1-1), surveyors shall also collect any symptomatic material on nonregulated plant material or any symptomatic material on adjacent landscape plants.

## Sampling by Symptom Type

### Leaf Spots and Lesions

1. Collect symptomatic leaves
   A. Some plants, i.e., *Camellia* or *Loropetalum*, may have very small pinpoint lesions.
   B. Some leaves have very subtle symptoms, such as flecking or chlorotic spots.
   C. For plants with very small leaves or needles, submit samples as twig sections with the leaves attached; in these cases, try to ensure the sample has a **minimum** of 2 square inches of symptomatic tissue.
   D. If there are not enough symptomatic leaves on the plant, collect symptomatic leaves that have dropped into the pot, provided they are **not** exhibiting dessication or extensive decay.
   E. If necessary to get the required amount of symptomatic tissue, collect composite leaves from **up to five** adjacent plants to make a composite sample.

### Twig Dieback

1. Cut the twigs below the cankered region (1 inch into healthy tissue). The sample must include canker margins and 1 inch of healthy tissue on either side.
2. Sterilize pruning equipment between samples using a diluted (10%) bleach solution, a quaternary ammonium solution at labeled rates, or disinfectant spray (with ETOH).

### Cankers on Boles and Branches of Regulated Plants

1. Some regulated plants do **not** have foliar symptoms but get cankers on boles or branches. Bole or branch cankers consistent with *P. ramorum* disease **must** be sampled.

2. In some States, nursery inspectors may sample trees, while in other States, forestry or other officials may be asked to sample trees.

## Inspecting and Sampling the Nursery

Two basic principles governing the inspection and sampling process are:

1. *P. ramorum* **cannot** be diagnosed by a visual inspection of symptoms alone and **only** laboratory testing can provide a definitive diagnosis; and
2. If there is any doubt as to whether the symptoms observed could be caused by *P. ramorum*, collect a sample.
3. Before the inspection season begins, review the APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*; if possible, time nursery inspection to periods when surface water is likely to be present. For example, this could be after a rain event, after irrigation, or early in the morning.
4. Prior to inspection day, if available, obtain and review a nursery plant inventory, a plant location map, an aerial map, and a topographic map of the nursery to determine areas to sample. Create a sampling plan based on the number of regulated plants present in the nursery and plan the areas to visit within the nursery (refer to Table 2-1-1). Initiate the survey and progress through the nursery beginning with the lower-risk areas, if possible. Within this protocol, the term “lower-risk” is meant to convey that these areas are less conducive to *P. ramorum*. More than one survey team can be deployed to the nursery and should be assigned specified areas (i.e., low risk versus high risk).
5. Begin the inspection by conducting a visual overview of the nursery as a whole to compare your observations to any nursery map provided and verify or note any of the following items. Identify cull piles, “plant hospitals” where low-vigor plants are kept for sale, and areas that may include plant returns. Determine irrigation water source (well, municipal, treated, or recycled). Note topography of the nursery and nursery drainage patterns and systems and irrigation method (i.e., overhead, drip, etc.). Confirm low-lying areas, surface water, nursery layout, the general condition of the plants, and the nursery environment.
6. Decontaminate inspection personnel, tools, and equipment between blocks in the nursery, between regulated plant genera within a block (refer to block on page Glossary-1-1), and between nursery sites. Wear rubber boots or other waterproof boots **without** sole crevices that can be treated with disinfectant. Sanitize or change gloves between samples. Use a spray bottle containing a diluted (10%) bleach solution, a quaternary ammonium solution at labeled rates, or disinfectant spray to treat all tools between samples. Brush loose dirt from boots, then spray boots to point of runoff with disinfection solution, or use foot bath, between nursery blocks.

Decontaminate **all** equipment **between** each sample and **before** leaving a nursery.

7. Indicate inspected and sampled areas on the nursery map. Note plants sampled, surface water areas sampled, cull piles sampled, etc. Photograph sampled areas, including areas of surface water.

## Sampling Instructions for Plants

Visually inspect **all** plants within a nursery paying careful attention to plants on the official APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*. Collect samples from **all** symptomatic regulated, plants. Additional samples can be collected at the inspector's discretion, all other nonregulated plant tissue with symptoms suggestive of *P. ramorum*.

Read Biology and Symptoms of Phytophthora ramorum on page 7-1-1 and view photos **prior** to entering the nursery. Each sample should consist of **at least** 2 square inches of symptomatic plant tissue; collect as many leaves as necessary to represent 2 square inches. It is strongly encouraged that each sample is from 1 plant; however, if there are not enough symptomatic leaves on the plant, collect symptomatic leaves that have dropped into the pot, provided they are not exhibiting desiccation or deterioration. The leaves still need to be mostly green. If the inspector is certain that leaves on the ground adjacent to the pot are from that plant, they can be used to complete the sample. Otherwise, symptomatic leaf debris (with distinct spots and margins) from the ground should be a separate sample and labeled as such.

The amount of leaf samples is necessitated by:

- Down-stream confirmatory testing
- Genetic analysis
- Initial testing

**NOTICE**

The more plant samples composited into one, the larger the resulting destruction and quarantine radii will be if the composite sample is confirmed to be positive for *P. ramorum*. This is why, if possible, it is important for the plants in a composite sample to be adjacent to one another.

Using Table 2-1-1 and the nursery inventory, determine the minimum number of samples to collect within the nursery.

If the number of regulated plants in a nursery is **greater than** one number (e.g., 405 is greater than 400), move to the next greater number listed (500). If there are more symptomatic plants, always take more samples than the calculated minimum. This allows pinpointing the location of *P. ramorum* in the nursery and lessening the potential regulatory impact on the nursery.

**Table 2-1-1 Minimum Number of Plant Samples to Take Based on the Number of Regulated Plants Within the Nursery**

| Regulated plants per nursery: | Minimum number of samples to collect (95% confidence of detecting a 1.0% disease incidence)[1]: |
|---|---|
| 25 | 25 |
| 50 | 50 |
| 100 | 100 |
| 200 | 173 |
| 300 | 211 |
| 400 | 234 |
| 500 | 250 |
| 600 | 262 |
| 700 | 270 |
| 800 | 277 |
| 900 | 283 |
| 1000 | 287 |
| 2000 | 308 |
| 3000 | 316 |
| 4000 | 320 |
| 5000 | 322 |
| 6000 | 324 |
| 7000 | 325 |
| 8000 | 326 |
| 9000 | 326 |
| 10000 | 327 |
| 20000+ | 332 |

1 Numbers are the **minimum** number of regulated plants that **must** be sampled in a confirmed-positive nursery to ensure detection at a 95% confidence level for a 1.0% incidence of disease.

After the survey results from the laboratory are reported to the inspector, the inspector may release all ELISA-, PCR, or culture-negative plants (**unless** within the quarantine radii or destruction radii of a confirmed-positive detection).

## Sampling Instructions for Water

Examine **all** areas within the nursery for surface water, particularly after a rain event or after irrigation occurs. With pin flags, demarcate each area in which water is collected. If demarcation is **not** possible, draw a sketch, take photos, and flag nearby areas. Label the water container and the flagging with corresponding numbers so any confirmed-positive samples can be located within the nursery. If helpful, take photos of each area in which water is collected. Areas of water sampling are **not** on hold awaiting diagnostic results, but must be visibly and indelibly marked in case of positive confirmation.

- Irrigation water—sample all types of irrigation water **except** from a municipal source or well water. Sample at end dispensers (sprinklers, nozzles, drip, etc.) instead of source pipe. Sample retention ponds regardless of the source, because they are likely to contain runoff from production areas.
- Standing water—as the first priority, sample standing water in and around blocks of regulated plant material and the drainage from regulated plant material blocks. Drains in greenhouses and hoop house systems containing regulated plant material can be accessed for sample collection after a rain event or after irrigation occurs. Collect a **minimum** of one 50- to 800-ml water sample per sample site from each general area in which surface water occurs, a **minimum** of one sample from each drainage ditch into which runoff from regulated plant blocks collects. Sample any water that is around or drains from cull piles as well.
- Nonrecycled retention ponds—collect a **minimum** of one 800-ml water sample from each nonrecycled holding pond.

Refer to the Water Sampling and Processing Protocol on page 10-1-1.

## Sampling Instructions for Soil

Standing water will be sampled in place of substrate soil sampling. **No substrate soil samples are required for this sampling protocol**.

## Sampling Instructions for Pots and Containers

If containers are recycled and stored at the nursery or if used pots are purchased, sample residual container mix from pots or other containers; scrape container mix from pots filling a labeled one-liter self-sealing plastic bag. During the inspection and sampling protocol, there are **no** holds on the nursery or container pile associated with sampling containers while waiting for diagnostic results. Refer to the Soil and Container Mix Sampling and Processing Protocol on page 11-1-1.

## Sampling Instructions for Cull Piles

Examine any area in which plants have recently been disposed. If regulated plants are present, sample symptomatic plant material and keep plant genera samples separate from one another. If there is any surface water, take **at least** one sample from each cull pile area. Demarcate for avoidance and do **not** disturb the cull/compost pile or that area of the cull/compost pile while waiting for diagnostic results because the material collected is symptomatic plant tissue.

## Sampling Instructions for Other Articles

At the inspector's discretion, sample **any and all** other possible sources of *P. ramorum* inoculum.

Chapter

3

# Interstate[1] Confirmed Nursery Protocol

## Protocol for Interstate Nurseries[1] Confirmed Positive for *Phytophthora ramorum*

## Contents



1 Interstate shipping retail nurseries, brokers, interstate and certain intrastate wholesale and production nurseries.

## Introduction

The intended use of this protocol is for nurseries that have been confirmed positive for *P. ramorum* in plants, water, or other regulated articles. The nursery types are:

- Interstate commerce brokers with a nursery site or holding lot
- Interstate-shipping propagation, wholesale, and re-wholesale nurseries
- Intrastate-shipping wholesale nurseries that distribute plants for interstate shipping (i.e., using an interstate shipper to broker plants to other States)
- Online interstate retail plant sellers

This protocol does **not** cover retail nursery dealers who **only** ship intrastate. Intrastate retail nurseries are covered in Chapter 4 Protocol for Intrastate Retail Nurseries and Retail Nursery Dealers When Phytophthora ramorum Is Present (rCNP) on page 4-1-1.

## Goal

The goal of this protocol is to limit the spread of *P. ramorum*, a quarantined plant pathogen, and to simplify the movement of *P. ramorum*-**free** nursery stock. When procedures described in this protocol are implemented, plant-to-plant spread and movement of the pathogen through nursery shipments should be minimized. Cooperation by nursery management personnel is essential. Early detection and reporting of potential *P. ramoram* plant infections are crucial to ensure the spread is contained.

## Trigger Events for Use of the Interstate Confirmed Nursery Protocol

This protocol shall be implemented by USDA–APHIS–PPQ, in cooperation with State Plant Regulatory Officials (SPROs), when the presence of *P. ramorum* has been confirmed in interstate-shipping nurseries from samples collected by regulatory officials. Samples may have been collected during surveys or inspections such as Cooperative Agricultural Pest Survey (CAPS), State Nursery Cleanliness Survey, national survey, State inspections, trace forward investigation, trace back investigation, or found by other means. APHIS regulatory authority can be used in all of these cases. The Interstate Confirmed Nursery Protocol (CNP) is triggered for **any** confirmed-positive sample, such as plants, water, soil, containers, container mix, or any other article.

Samples **must** be diagnosed using a method approved by USDA–APHIS–PPQ and consistent with the Potentially Actionable Suspect Samples (PASS) protocol (refer to the PPQ *Phytophthora ramorum* website for diagnostic information and the PASS protocol).

**NOTICE**

When planning to announce or make a public statement about the detection of a federally regulated pest, the State Plant Regulatory Official (SPRO) and/or the public information officer for the State department of agriculture **must** first contact the State Plant Health Director (SPHD) and/or USDA–APHIS–Office of Legal and Public Affairs (LPA).

## Disclaimers

**Any interpretation of this chapter or its procedures not consistent with the goal listed above, is a misinterpretation and misrepresentation.**

### Challenges

*P. ramorum* is a micro-organism and difficult to detect. It can infect plants; infest container mix, soil, and water; and persist in these substrates despite the best eradication efforts. Detection and management of this pathogen is informed by continually improving science. These protocols and regulations will be adjusted accordingly, based on the understanding of the pathogen biology.

### Field-Grown Stock

Field-grown stock can present different challenges and field personnel may need to adapt this protocol to those situations after discussion with the *P. ramorum* program National Operations Manager (NOM) until other appropriate modifications are incorporated.

## Interstate Confirmed Nursery Protocol Steps

In chronological order, the steps for the Interstate Confirmed Nursery Protocol (CNP) are as follows.

1. Communicate and notify
2. Conduct trace investigations (concurrently with securing the nursery)
3. Secure the nursery (concurrently with conducting trace investigations)
   A. Disinfest the nursery
   B. Delimit the nursery
   C. Delimiting survey results received

4. 90-day (minimum) quarantine activities
5. Release of plants in the nursery
6. Alternate quarantine-release strategy
7. Critical Control Point (CCP) assessment
8. Post-quarantine release monitoring

## Interstate Confirmed Nursery Protocol (CNP) Procedures

**NOTICE**

**Prior to** an APHIS-confirmed positive determination, the National Plant Protection Laboratory Accreditation Program (NPPLAP)-approved laboratory **must** communicate **all** suspect positive diagnostic samples to inspectors as soon as one of the following has occurred:

• Culture that matches the morphology for *P. ramorum* as determined and reported by an APHIS-approved laboratory; or
• Positive PCR using APHIS-approved work instructions by an APHIS-approved laboratory

For **all** Potentially Actionable Suspect Samples (PASS), laboratories **must** immediately forward the plant material and DNA to the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory (refer to Contact Information for the Phythopthora ramorum Program on page A-1-1) with a domestic ARM routing receipt (DARR) and notify the State's State Plant Health Director (SPHD; who generates DARR for laboratory), the State Plant Regulatory Official (SPRO) for the State of sample origin, and the National Operations Manager (NOM).

**Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 1 of 5)**

| If the step number: | Subnumber: | Then: |
|---|---|---|
| 1—Communicate and notify | → | When a sample is confirmed positive, laboratories need to notify the SPHD and the SPRO, the National Operations Manager (NOM), the National Program Manager (NPM), and the submitter. The SPRO (if State authority is used) or the SPHD (if Federal authority is used) may notify the owner of the nursery. These steps start immediately after SPHD and SPRO are notified of a confirmed-positive article in their State. SPHD and SPRO should designate an official to lead the activity. |
| | 1.1 | The designated official will notify the confirmed-positive nursery of the confirmed-positive and instruct the nursery **to place a hold on all** regulated plants at the nursery or as many plants as **deemed** necessary by the inspector. Regulated plants outside the D and Q radii will be released from hold once the block is cleared by inspection. |
| | 1.2 | SPHD and/or SPRO will notify the NOM of the nursery notification and the hold on the plants. |
| | 1.3 | SPHD and/or SPRO will provide a list of the identified facilities found through trace back and trace forward investigations to the NOM **within five business days** of a confirmed P. ramorum-positive sample in a nursery (refer to 2—Conduct investigations on page 4-1-5). The NOM will notify SPHDs and/or SPROs of States sending or receiving these shipments. |
| | 1.4 | SPHDs and/or SPROs will notify affected retail nurseries and retail nursery dealers within their States. |
| 2—Conduct trace investigations (concurrently with securing the nursery) | 2.1—Trace forward and trace back investigations | 1. Determine from provided information if the nursery has distributed regulated plants to another nursery. If so, implement Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed P. ramorum-Infested Nursery on page 5-2-1. Submit the trace forward list(s) to the NOM **within five business days**. If a nursery does not meet the five business day requirement, the SPHD, SPRO, and NOM should work together to decide potential consequences, such as holding the compliance agreement.<br>2. Determine from provided information if the confirmed-positive plants were received from another nursery. If so, implement Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed P. ramorum-Infested Nursery on page 5-3-1. Submit the trace back list(s) to the NOM **within five business days**. If a nursery does not meet the five business day requirement, the SPHD, SPRO, and NOM should work together to decide potential consequences, such as holding the compliance agreement. |
| | 2.2—Associated nursery sites | 1. Determine from provided information if additional locations (i.e., nursery sites) are owned and operated by the same nursery company.<br>2. Determine from provided information if nursery personnel are deployed to multiple locations.<br>3. Determine from provided information if regulated plants have moved to other sites or among nursery sites. If so, **all** nursery sites receiving regulated plants **must** be surveyed.<br>4. Determine from provided information if equipment used at the infested site is shared with additional locations (i.e., nursery sites, field areas, etc.). Document any shared equipment use in those additional locations. Equipment movement among nursery sites **must** use appropriate biosecurity measures (refer to Biosecurity Measures for Nurseries on page 9-1-1). |

**Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 2 of 5)**

| If the step number: | Subnumber: | Then: |
|---|---|---|
| 3—Secure the nursery (concurrent with conducting trace investigations) | 3.1 | 1. All regulated plants in the nursery should be held until they are cleared by inspection. To ensure material does not move prior to being cleared, use PPQ Form 523, Emergency Action Notification (EAN), or State equivalent.<br>2. Refer to the Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants on page 3-1-10 for guidance.<br>A. Arrive on site as soon as practicable. Identify locations of confirmed-positive sample sources. Obtain maps of the nursery (with bed layouts, if possible) and maps of the surrounding area for assessment.<br>B. Establish the quarantine (Q) and destruction (D) radii for each confirmed-positive source as described below.<br>C. For any type of confirmed positive, hold **all** plants within each established Q-radii for the entire quarantine period (**a minimum of 90 days of conducive environment for disease development**). Instruct nursery to **not** use fungicides registered for *Phytophthora* spp. in the Q radii.<br>D. During implementation of this protocol, every plant on regulatory hold should **not** be subject to scheduled nursery maintenance.<br>3. Restrict access to any D-radii until the inspector/nursery is prepared to begin disinfestation procedures; refer to Disinfest the Nursery on page 3-1-11.<br>4. Put nursery under initial compliance agreement for first-time detection. (Contact the PPQ NPM or NOM for a copy of the Compliance Agreement.)<br>5. Hold may include “any other product or article that an inspector determines to present a risk of spreading *P. ramorum*, if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations” (7 CFR part 301.92-2) within the infested nursery site. |
| | 3.2—Confirmed positive plant(s) | 1. Establish and demarcate D-radii by visibly and indelibly flagging 2 meters out from the confirmed-positive plant(s) (refer to Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants on page 3-1-10). Hold **all** plants within the 2-meter D-radius until **all** plants, pots, and potting medium are destroyed. A regulatory official must oversee plant destruction.<br>2. Establish an additional 2-meter radius around the D-radii and hold **all** plants within that 2-meter radius for the Q-radius. Restrict fungicide use on plants in the Q radii and restrict access to this area.<br>3. Each new confirmed-positive plant requires a new D-radius and Q-radius. |
| | 3.3—Confirmed-positive surface water or soil | 1. Establish and demarcate the confirmed-positive area by visibly and indelibly flagging 1 meter out from the margin of standing water. Make sure to include algal deposits, *Nostoc* spp., and aquatic plants in the margin of the demarcated area.<br>2. Restrict access. |
| | 3.4—Confirmed-positive cull pile | 1. Establish and demarcate the area by visibly and indelibly flagging 1 meter out from the perimeter of the cull pile.<br>2. Restrict access. |
| | 3.5—Confirmed-positive used containers | Flag for hold until sanitation is applied (refer to Treatments and Disinfectants on page 12-1-1). |

**Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 3 of 5)**

| If the step number: | Subnumber: | Then: |
|---|---|---|
| 3a—Disinfest the nursery | → | Depending on the complexity of the situation, disinfestation can occur before or after the delimiting survey.<br><br>Refer to Table 3-1-3 for disinfestation procedures. Refer to Treatments and Disinfectants on page 12-1-1. |
| 3b—Delimiting survey | 3b.1—All confirmed positives | For more information on the delimiting survey, refer to the Delimitation Sampling for Confirmed Plant Positives and Sample Handling and Submission Protocol on page 3-1-13.<br><br>1. Ensure conducive environmental conditions are present, as described in Timing Nursery Inspection and Sampling on page 2-1-3.<br>2. Ensure necessary sanitation measures are applied by regulatory officials while in the confirmed nursery.<br>3. Inspect **all** regulated and nonregulated plants within the nursery.<br>4. Sample **any** symptomatic tissue found and submit samples to the appropriate laboratory. Refer to Table 3-1-5 on page 3-1-15 to determine the number of samples to collect.<br>5. Refer to individual delimiting procedures for specific confirmed-positive material.<br>6. Place **all** sampled plants on hold.<br>7. Disinfest tools and equipment associated with any confirmed-positive materials.<br><br>For each type of confirmed-positive material, follow the specific delimiting instructions below. In addition, use the Phytophthora ramorum Inspection and Sampling Protocol for Nurseries on page 2-1-1 for guidance. |
| | 3b.2—Confirmed-positive plant(s) | 1. Immediately inspect **all** plants in the Q-radii and inspect other plants in that block. Take the number of samples required in Table 3-1-5 on page 3-1-15.<br>2. Sample **any** unhealthy tissue, provided it is **not** exhibiting desiccation or extensive decay.<br>3. Inspect **all** regulated and nonregulated plants within the nursery.<br>4. Sample surface water from underneath confirmed-positive plant(s), as well as throughout the D-radii and adjacent downslope areas (refer to Water Sampling and Processing Protocol on page 10-1-1 and Soil and Container Mix Sampling and Processing Protocol on page 11-1-1).<br>5. Mitigate **all** soil and gravel under plants within the D-radii and adjacent downslope areas to be able to allow host plants in the area. If there is no mitigation, then no host plants are allowed in the area.<br><br>For retail nurseries, once the delimiting survey and sampling are complete, any held plants may be consolidated and segregated. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery. Any movement of the segregated plants **must** be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction and supervision of a regulatory official. If the plants are **not** consolidated and segregated, the affected portion of the nursery **must** be closed to the public. Segregation **must** include storage on an impermeable surface (e.g., concrete, asphalt, or a 45-mil thick pond liner) and **not** within 2 meters of any other plant. The impermeable surface should be sloped to drain away from regulated plants. |

**Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 4 of 5)**

| If the step number: | Subnumber: | Then: |
|---|---|---|
| 3b—Delimiting survey (cont.) | 3b.3—Confirmed-positive surface water or soil | 1. Examine **all** plants in the Q radius of demarcated sample area as well as **all** plants within a 10-meter perimeter beyond the Q-radius demarcations.<br>2. Sample **any** unhealthy tissue, provided it is **not** exhibiting desiccation or extensive decay. |
| | 3b.4—Confirmed-positive cull pile | If **not** sampled in initial inspection, sample surface water or symptomatic plants adjacent to and/or downslope from cull pile. |
| | 3b.5—Confirmed-positive used containers | 1. Inspect **any** plants within 2 meters of container storage area.<br>2. Sample symptomatic plants.<br>3. Sample surface water or soil within the 2-meter radius. |
| | 3b.6—Perimeter survey | 1. Survey for symptoms on **all** plants located within 10 meters of the infested nursery.<br>2. Sample **all** symptomatic plants. |
| 3c—Delimiting survey results received | 3c.1—Confirmed-positive results from the delimiting survey | 1. Conduct a second delimiting survey of the entire nursery as described above in 2a and 2b. Wait until **all** diagnostic results are final because subsequent delimiting surveys may be necessary if further confirmed-positive results are reported.<br>2. With each new confirmed-positive diagnostic result, restart the 90-day quarantine period. Please note that confirmed-positive surface water or soil may require longer to process and receive diagnostic results. |
| | 3c.2—Confirmed-positive results from the second delimiting survey | At the inspector's discretion, after two positive delimitation surveys, the entire block of plants may be destroyed if the distribution of positive plants found outside the initial quarantine radius suggests an extensive and random pattern of infestation (refer to Trigger Sequence for Entire Block Destruction on page 3-1-14 for more details). |
| | 3c.3—Negative results from the delimiting survey | 1. Release sampled plants or other articles from hold if they test negative for *P. ramorum*. **However**, continue to hold **all** plants or other articles within the Q-radius around confirmed-positive plants or other articles for the 90-day period.<br>2. The 90-day quarantine starts when samples are submitted. |
| 4—90-day (minimum) quarantine activities | → | 1. The 90-day (minimum) quarantine period begins the day samples are collected if:<br>A. The delimiting survey is completed; and<br>B. **All** delimiting sample results are negative; and<br>C. PPQ Form 523, EAN or sufficient State equivalent is issued.<br>2. Update hold notice for specific plants on hold (e.g., official communication, PPQ Form 523, or State equivalent).<br>3. Within the Q-radius, do **not** allow applications of fungicides registered for *Phytophthora* spp. control during the quarantine period.<br>4. Visually inspect plants within Q-radii a **minimum** of two times. Sample **any** symptomatic plants, as above. Conduct the first inspection approximately halfway through the quarantine period. Near the end of the quarantine period, a second visual inspection in the Q-radius should be performed while a visual survey of the entire nursery is being completed.<br>5. During the quarantine period, **all** sample results **must** be negative for *P. ramorum* or the quarantine period shall be extended for an additional 90 days. |

**Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 5 of 5)**

| If the step number: | Subnumber: | Then: |
|---|---|---|
| 5—Release of plants in the nursery | | Plants placed under regulatory control may be released from that control by PPQ or its designated authority after the quarantine period, **if** the following three conditions are met:<br>◆ There are **no** additional detections of *P. ramorum* in regulated and non-regulated plants based on PPQ-approved plant inspection, sampling, and testing protocols during the preceding 90-day quarantine period; **and**<br>◆ If testing water, soil, and growing media is required, those sample results are negative for *P. ramorum* based on PPQ-approved sampling and testing protocols for the preceding quarantine period; **and**<br>◆ Any resulting samples from the second visual survey at the end of the 90-day quarantine period are negative for *P. ramorum*. |
| 6—Alternate quarantine-release strategy | | A nursery may avoid a quarantine period through the voluntary Alternate Quarantine Release Strategy if the following conditions are met. The Alternate Quarantine Release strategy is not available to nurseries that meet the criteria for Continuously Positive Nurseries. (Refer to Continuously Positive Nursery section on page 3-1-20).<br>1. The nursery **must** destroy everything (e.g., **all** plants, containers, growing media, etc.) in each D-radius by approved methods listed in the specific nursery compliance agreement; **and**<br>2. Inspect and sample **all** regulated plants within the Q-radius. Then destroy **all** regulated plants within the Q-radius along with their containers, media, debris, etc.; **and**<br>3. Mitigate soil of each D- and Q-radius, as per Disinfesting Soil and Container Mix on page 12-1-4. Sample and test drainage or recirculated irrigation water, as per Sampling Instructions for Water on page 2-1-10; **and**<br>4. Complete critical control point assessment (refer to 7—Conduct Critical Control Point (CCP) assessment on page 3-1-9); **and**<br>5. Revisit the nursery a minimum of 90 days after completing the alternate quarantine-release strategy and conduct a nursery-level survey inspection. The nursery is subject to “post-quarantine-release monitoring” (refer to 8—Post-quarantine release monitoring below). |
| 7—Conduct Critical Control Point (CCP) assessment | | After completing the delimiting survey and implementing or planning disinfestation procedures for each confirmed-positive article, use the CCP assessment and reference material (refer to Critical Control Point Assessment Procedures for P. ramorum-Confirmed- Positive Nursery Sites on page 3-1-19) to identify remediation and mitigation options, business/cultural practices, and best management practices (BMP) for the nursery’s site-specific plan to address *P. ramorum*. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions. |
| 8—Post-quarantine release monitoring | | Previously confirmed-positive nurseries shall be surveyed twice per year in successive years until there are 3 consecutive years of negative sample results. These nurseries are **not** under any other regulatory action unless there are additional *P. ramorum* detections. If there are further *P. ramorum* detections during the 3-year monitoring, the nursery **must** enter into a revised compliance agreement and restart 3 consecutive years of negative sample results. |

## Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants

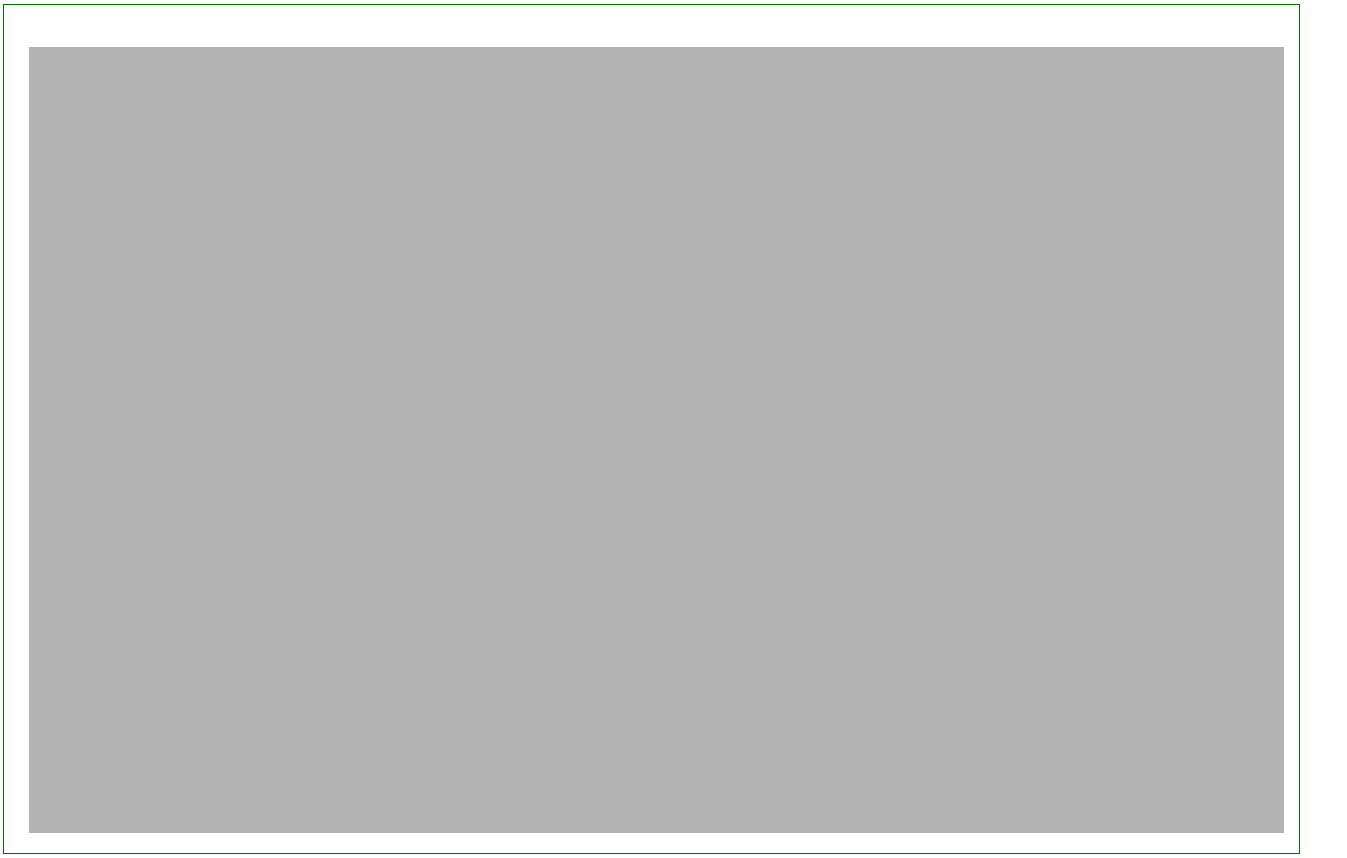

**Figure 3-1-1 Schematic of Destruction and Quarantine Radii of Positive Plants**

**Table 3-1-2 Legend of Destruction (D) and Quarantine (Q) Radii of Positive Plants Schematic**

| Area color: | Name: | Once D-radii and Q-radii are flagged, then: |
|---|---|---|
| Gray (*1) | Destruction (D) radii | Destroy **all** plants, pots, medium, and leaf debris |
| Light gray (*2) | Quarantine (Q) radii | Hold **all** plants from sale for 90 days or opt for the Alternative Quarantine-Release Strategy on page 3-1-17 |
| Gray (*3) | D-radii in block with nonregulated plants | Destroy **all** plants; nonregulated plant nursery stock could still move the pathogen |
| Light gray (*4) | Q-radii in block with nonregulated plants | Hold **all** plants from sale for 90 days; nonregulated plant nursery stock could still move the pathogen. |
| Dotted (*5) | Rest of the block with regulated plants | Release **all** plants for sale **only** when found to be **asymptomatic** during the delimiting survey |
| White (*6) | Rest of the block of nonregulated plants | Release **all** plant materials for sale if found to be **asymptomatic** during the delimiting survey |

## Notification Requirements for Interstate CNP

SPHDs and/or SPROs will notify nurseries and the NOM within 24 hours of final determination of results from samples collected in their State.

SPHD and/or SPROs will provide a list of the identified facilities found through trace back and trace forward investigations to the NOM **within five business days** of a confirmed *P. ramorum*-positive sample in a nursery (refer to Conduct Investigations on page 3-1-16). The NOM will notify SPHDs and SPROs of States sending or receiving these shipments, and SPHDs and/or SPROs will notify affected nurseries within their States.

## Disinfest the Nursery

Conduct the Critical Control Point (CCP) Assessment **only** after completing the delimiting survey and implementing or planning disinfestation procedures for each confirmed-positive article. Then, use the CCP assessment and reference material to identify remediation and mitigation options, business/cultural practices, and best management practices (BMP) for the nursery's site-specific plan to address *P. ramorum*. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions.

**Table 3-1-3 Disinfest the Nursery (page 1 of 2)**

| If material is: | Then: |
|---|---|
| Confirmed-positive plant(s) | 1. Destroy **all** plants, pots, medium, and leaf debris in the D-radii, per Treatments and Disinfectants on page 12-1-1; refer to Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants on page 3-1-10.<br>2. Remove and destroy **all** plant debris including container mix and any other plant parts found within the D-radii; refer to Treatments and Disinfectants on page 12-1-1 for proper removal and destruction.<br>3. A regulatory official must oversee plant destruction.<br>4. For field-grown stock, contact the NOM.<br>5. Sample surface water or soil underneath the D- and Q-radii; refer to Water Sampling and Processing Protocol on page 10-1-1 and Soil and Container Mix Sampling and Processing Protocol on page 11-1-1. |
| Confirmed-positive surface water | 1. Photograph area for the nursery owner and the CCP assessment team; site-specific conditions may apply depending on CCP assessment.<br>2. Maintain flagging for avoidance until remediation is chosen by the nursery owner with approval from the regulatory inspector **and** written into Exhibit D of the CA. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions. |
| Plants sitting in confirmed-positive surface water | Remove and destroy **all** plants, container mix, and pots sitting in confirmed-positive surface water as well as **all** plants within a 2-meter buffer, because these items have been exposed to water containing a quarantine organism. Site-specific recommendations may apply depending on CCP assessment. Confirmed-positive surface water samples initiate the 90-day quarantine period whether or not the plants standing in the water are confirmed positive. |
| Confirmed-positive irrigation water | 1. Cease using confirmed-positive irrigation source until treated; irrigation water sources **must be free** of *P. ramorum* as determined by water-testing protocols described in Soil and Container Mix Sampling and Processing Protocol on page 11-1-1.<br>2. Mitigate the irrigation water if it was sampled and tested positive for *P. ramorum* during the survey and delimitation of the infestation at the nursery; refer to Treatments and Disinfectants on page 12-1-1.<br>3. Confirmed-positive irrigation water samples initiate the minimum 90-day quarantine period for plants receiving positive irrigation water. |

**Table 3-1-3 Disinfest the Nursery (page 2 of 2)**

| If material is: | Then: |
|---|---|
| Confirmed-positive cull pile | 1. Immediately demarcate the cull pile to avoid pathogen dispersal (as a quarantine hold).<br>2. Dispose of **all** material (plants, plant material, water, growing media, or soil) from the cull pile if any material is confirmed positive for *P. ramorum*. For disposal, use one of the approved methods described in Treatments and Disinfectants on page 12-1-1.<br>3. Use the CCP assessment to address site-specific conditions and determine appropriate mitigation measures. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions. |
| Confirmed effluent water (e.g., culvert/ ditch, stream, nonrecycled retention pond) is positive | 1. Immediately demarcate the area around the confirmed-positive effluent water.<br>2. Identify remediation, mitigations, or business/cultural practices via the CCP assessment with the nursery owner.<br>3. Use the CCP assessment to address site-specific conditions and determine appropriate mitigation measures. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions. |
| Confirmed-positive soil (nursery substrate) | 1. Locate and reestablish boundary demarcation.<br>2. Place barrier mitigation and/or adopt appropriate avoidance practices while determining the disinfestation/remediation strategy.<br>3. Use the CCP assessment, Treatments and Disinfectants on page 12-1-1, and mitigation options available from nursery associations, county extensions, and State nursery practices manuals. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions. |
| Confirmed-positive tools or equipment | 1. Disinfest using options in Biosecurity Measures for Nurseries on page 9-1-1 and Treatments and Disinfectants on page 12-1-1.<br>2. Choose and institute cultural practices to ensure future sanitation (e.g., refer to the Best Management Practices Manual to Reduce the Risk of Introducing Soil-Borne Plant Pathogens into Horticultural Nurseries and Managed Wildland Landscapes).<br>3. Use the CCP assessment, Treatments and Disinfectants on page 12-1-1, and mitigation options available from nursery associations, county extension agents, and State nursery practices manuals. Refer to Compliance Agreement (obtained from PPQ NPM or NOM) for further instructions |

**Table 3-1-4 Quarantine Period for Plants in Q-Radii (Figure 3-1-1)**

| If step number: | For: | Then: |
|---|---|---|
| 1 —Quarantine period activities | → | The quarantine period, a **minimum** of 90 days of conducive weather conditions, begins when the nursery delimitation survey(s) are complete and **all** test results are negative. Plants, water, or other articles in Q-radii remain on hold for the full period.<br>During the quarantine period:<br>1. **Do not** use fungicides registered for *Phytophthora* spp. in the plant's Q-radii.<br>2. Regulatory officials will inspect plants in the Q-radii and regulated plants in the nursery a **minimum** of two times; once about halfway through the anticipated quarantine period and once near the end so test results coincide with the end of the period. **All** symptomatic regulated plants **must** be sampled and tested; the second inspection can serve as the quarantine release survey.<br>3. If confirmed-positive samples result from quarantine period surveys, return to steps 2 through 4 in Table 3-1-1 on page 3-1-5; quarantine period begins again. |

**Table 3-1-4 Quarantine Period for Plants in Q-Radii (Figure 3-1-1)**

| If step number: | For: | Then: |
|---|---|---|
| 2—Quarantine release survey | → | The quarantine release survey is the second of the two quarantine period inspections. It occurs near the end of the quarantine period. This survey includes Q-radii plant inspection and **all** regulated plants within the nursery. Sample and test any unhealthy plant tissue. |
| | Transition from EAN to new or revised CA | Q-radii: after 90 days of conducive weather conditions, if the quarantine release survey reveals **no** symptomatic plants or further confirmed-positive plants in Q-radii, and reveals **no** further positive surface water or soil in Q-radii, release Q-radii.[1] |
| | → | To retain interstate shipping status, or to otherwise distribute plants for interstate shipment (brokered), the nursery **must** enter into a compliance agreement. |

1 Surface water, effluent water (e.g., culvert, ditch, and nonrecycled retention pond water), soil, or cull piles may take longer than 90 days to disinfest/remediate; ensure avoidance/exclusion mitigations are in place for these confirmed-positive areas. This short-term mitigation and the longer-term remediation plan needs to be written into Exhibit D of the CA (refer to Compliance Agreement, obtained from PPQ NPM or NOM, for further instructions) **prior** to the end of the quarantine period. The end of the quarantine period infers the close of the Emergency Action Notification (EAN) and the beginning of the CA or the modification of the CA to contain measures to address *P. ramorum* in the nursery. Close the EAN **after** the following conditions are met:

- Tests of **all** symptomatic plants from the quarantine release survey are **negative**
- If surface water, effluent water (culvert, ditch, and nonrecycled retention pond water), soil, or cull pile is still confirmed positive awaiting remediation, ensure exclusionary/avoidance mitigation is in place and the specific temporary mitigation and the permanent remediation plan is written into Exhibit D of the CA **prior** to the end of the quarantine period. Contact the PPQ National Operations Manager (NOM) for a template and conformance instructions
- If the irrigation source water was confirmed positive, ensure it has been treated and found negative **prior** to the quarantine release survey, or, an alternate source is in place until the water tests negative. Ensure the alternate source and/or sanitation/mitigation plan is written into Exhibit D of the CA
- The new CA is signed

## Delimitation Sampling for Confirmed Plant Positives and Sample Handling and Submission Protocol

The delimitation survey begins once the confirmed-positive results are reported to the inspector. Conduct delimitation, inspection, and sampling after (on the same day(s)) the D-radii and Q-radii are established. The delimitation survey instructions for all other articles **other than** plants are provided in 3b—Delimiting survey on page 3-1-7.

**NOTICE**

**REMINDER**: disinfest personnel, tools, and equipment between blocks in the nursery, between regulated plant genera within a block, and between nurseries. Wear rubber boots or other waterproof boots **without** crevices. Sanitize or change gloves between samples. Use a spray bottle containing a diluted (10%) bleach solution, a quaternary ammonium solution at labeled rates, or disinfectant spray (with ETOH) to treat **all** tools between samples. Between nursery blocks, brush loose dirt from boots then spray boots with disinfectant solution in spray bottle, or use a foot bath. Disinfest **all** equipment used between **each** sample and before leaving the nursery.

When delimiting due to confirmed-positive detection in plants, focus on sampling the individual blocks in which the confirmed-positive plants were found. Follow the sampling method below for each individual block with a confirmed positive(s). Inspectors should sample **all** symptomatic material present. Each sample should consist of a **minimum** of 2 square inches of symptomatic plant tissue. The minimum number of plants to be sampled will depend on the total number of plants in the confirmed-positive nursery block.

## Collecting Samples

Collect samples from **all** symptomatic regulated plants and, at the inspector's discretion, all other nonregulated plant tissue with symptoms suggestive of *P. ramorum* (refer to Biology and Symptoms of Phytophthora ramorum on page 7-1-1). Foliar symptoms of *P. ramorum* infection are highly variable and can range from pinpoint discolorations on the leaf surface to large V-shaped lesions along the leaf mid-vein. Include inspection of the lower portions of plants where conditions favoring *P. ramorum* would be present. Moisture will tend to be present for longer periods of time on the plant surfaces on the lower portions of the plants, which can also result in higher humidity depending on plant spacing. Shading on the lower portions of the plants can promote cooler temperatures and offer protection from the effects of ultraviolet (UV) rays on spores. Pay attention to leaf areas at which water would run off or persist the longest such as the midrib and leaf tips. In some regulated plants (*Camellia* and *Rhododendron*) low levels of infection can cause premature leaf drop, resulting in infected plants that appear to be asymptomatic. As a result, leaves found in the pot should also be checked for possible symptoms and collected for laboratory analysis.

The purpose of the perimeter survey: 1) to ensure *P. ramorum* has **not** spread outside the infested nursery to the surrounding environment; and 2) to verify the infestation in the nursery did **not** originate in the surrounding environment.

## Trigger Sequence for Entire Block Destruction

At the inspector's discretion, the below sequence may trigger the destruction of an entire block of plants where the original *P. ramorum*-positive articles were detected. Two positive delimitations in the same block may trigger an entire block destruction if the distribution of positive plants found outside the initial quarantine radius suggests an extensive and random pattern of infestation. The trigger sequence is as follows:

1. An initial positive plant is detected in the block.
2. In the same block, positive plants are detected in the first delimitation survey, triggering a second delimitation.
3. In the same block, positive plants are detected during the second delimitation survey outside any positive plant quarantine radius, and suggest an extensive and random pattern of infestation in the block.

## Determining the Number of Samples to Take

Determine the **minimum** number of symptomatic plant samples of regulated plants to take within a confirmed-positive nursery. Samples should be targeted, **not** random. Inspectors should sample **all** symptomatic plant material present, including leaf tissue from the pots, or, if the inspector is absolutely certain the leaves are from a given plant, leaves may be collected from the ground immediately adjacent to the plant.

**Table 3-1-5 Minimum Number of Samples to Take Based on the Number of Regulated Plants**

| Regulated plants: | Minimum number of samples to collect (95% confidence of detecting a 1.0% disease incidence)[1]: |
|---|---|
| 25 | 25 |
| 50 | 50 |
| 100 | 100 |
| 200 | 173 |
| 300 | 211 |
| 400 | 234 |
| 500 | 250 |
| 600 | 262 |
| 700 | 270 |
| 800 | 277 |
| 900 | 283 |
| 1000 | 287 |
| 2000 | 308 |
| 3000 | 316 |
| 4000 | 320 |
| 5000 | 322 |
| 6000 | 324 |
| 7000 | 325 |
| 8000 | 326 |
| 9000 | 326 |
| 10000 | 327 |
| 20000+ | 332 |

1 Numbers are the **minimum** number of regulated plants that **must** be sampled in a confirmed-positive nursery to ensure detection at a 95% confidence level for a 1.0% incidence of disease.

Using a permanent marking method, label the sample bag with the following: date; genus species; cultivar; collector's identification number; location of sample site; and sample number. Visibly and indelibly mark the sampled plant (with flagging tape, stake, etc.), and label with the corresponding sample number, date, and other identifying information as required. This will facilitate

any additional work in the event of a confirmed-positive sample or the need for a second sample.

## Conduct Investigations

Concurrent to notifying and securing the nursery, require the nursery to research shipments of the past 6 months for the trace forward and trace back investigations and to provide this information within five business days.

### Associated Nursery Site Inspections

Determine whether additional locations (nursery sites) are maintained by the same nursery owner or if regulated plants are moved to or among other sites or between sites during the 6-month time period preceding the confirmed-positive detection.

- Equipment or other articles: determine if equipment or other articles used at the site is shared with other nursery sites or field areas; document any shared personnel, equipment, used containers, tools, etc., in different nursery sites or field areas. Equipment movement **without** appropriate biosecurity measures (refer to Biosecurity Measures for Nurseries on page 9-1-1) between nursery sites requires **all** nursery sites to be surveyed
- Plants: determine if regulated plants are moved among sites, and if so, **all** sites receiving regulated plants **must** be surveyed

### Trace Forward Investigations

At the time *P. ramorum* is confirmed in a nursery, it is necessary to determine if the nursery has shipped plants that could potentially be infected. The first step of the trace forward investigation is to determine if the nursery is required to notify receiving States, refer to Notification Requirements for Interstate CNP on page 3-1-10 for further instructions.

Initiate the trace forward investigation by identifying **all** plants shipped (domestic and international) within 6 months of the first (and any subsequent) confirmed-positive detection of *P. ramorum* at a nursery meeting the following criteria: 1) plants of the infected species/cultivar; 2) **all** regulated plants that originated in the D- and Q-radii; and 3) any plants of the high-risk genera: *Camellia* spp., *Kalmia* spp., *Pieris* spp., *Rhododendron* spp. (including azalea), and *Viburnum* spp. regardless of their location in the nursery. This combination of shipped plants is referred to as the “high-priority target plants.” These plants, including their shipment date(s); quantities; and respective destinations, make up the trace forward list. Identify these high-priority target plants using the best available information and to the lowest taxon possible (e.g., if the

plants can be identified to cultivar, trace forward activities may be conducted at the cultivar level).

**NOTICE**

**Forward the trace forward list(s) to PPQ's National Operations Manager (NOM) within five business days.**

Within 30 calendar days, the NOM will forward domestic trace forward lists to the States that have received plants. The NOM will forward international trace forward lists to the National policy Manager (NPM) who will work with Phytosanitary Issues Management (PIM) to inform internal trading partners of shipments to their countries. The plants sent to the receiving States **must** be inspected at the receiving nurseries (trace forward sites). Once the trace forward information is collected and communicated to the receiving States (or countries), the receiving regulatory officials will implement the trace forward protocol, refer to Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed P. ramorum-Infested Nursery on page 5-2-1.

## Trace Back Investigations

At the time *P. ramorum* is confirmed in a nursery, determine if confirmed-positive plants were shipped from another nursery. Trace back plants include **all** plants of the same genus of the infected plant regardless of size, location, or lot, back to the original propagation source (if it still exists).

The NOM will forward domestic trace back invoices to the States that have shipped plants. The NOM will forward trace back invoices from international sources to the NPM to inform PIM and the international trading partners of potentially positive shipments from their country. To view the trace back protocol refer to Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed P. ramorum-Infested Nursery on page 5-3-1.

## Alternative Quarantine-Release Strategy

A nursery may avoid a quarantine period through a voluntary management decision if the following conditions are met.

The alternate quarantine release strategy is not available to nurseries who meet the criteria of Continuously Positive Nurseries (refer to Continuously Positive Nurseries on page 3-1-19).

### If this nursery does not meet Continuously Positive Nursery Criteria, follow:

1. Steps 1 through 5 in section 6—Alternate quarantine-release strategy on page 3-1-9 of Table 3-1-1 **and all** steps in Table 3-1-3 are complete.

2. **All** plants, pots, media, debris, etc. in the D-radii and **all** regulated plants and their pots, media, debris, etc. in the Q-radii are destroyed.
3. The CCP assessment is complete.
4. Surface water, effluent water (e.g., culvert, ditch, and nonrecycled retention pond water), soil, or cull piles may take some time to disinfest/remediate; ensure avoidance/exclusion mitigations are in place for these confirmed-positive areas.
5. The compliance agreement shall be updated based on the CCP assessment and shall contain measures to address *P. ramorum* in the nursery and the agreement is signed by the nursery representatives and regulatory officials. (Refer to Compliance Agreement, obtained from PPQ NPM or NOM.) The nursery is under a compliance agreement for a **minimum** of 3 years from this date.
6. The short-term mitigations and the longer-term remediation plan are written into Exhibit D, updating PPQ Form 519, compliance agreement (CA). (Refer to Compliance Agreement, obtained from PPQ NPM or NOM.)
7. Inspect for compliance.

## Post-Confirmed Nursery Protocol Monitoring

Under the Compliance Program, if a nursery in the regulated or nonregulated area tests negative after 3 years (**all** samplings (6 or more) during the conducive time), it shall be released from compliance. For nurseries in the quarantine area, the sampling returns to the 7 CFR § 301.92 regimen.

Nurseries under compliance are required to notify state regulatory officials when host plants are shipped. They **must** notify state regulatory officials in both origin and receiving states at the time of shipment. The nursery **must** also notify state regulatory officials about shipments of all infected species/cultivars found in the nursery, as well as shipments of *Camellia* spp., *Kalmia* spp., *Pieris* spp., *Rhododendron* spp. (including azalea), and *Viburnum* spp. The USDA and state departments of agriculture will provide the information and requirements about the Notify system and its use.

# Critical Control Point Assessment Procedures for P. ramorum-Confirmed- Positive Nursery Sites

After positively confirming the presence of *P. ramorum* at a nursery site and completing the delimiting survey or completion of the alternative release strategy, a Critical Control Point (CCP) assessment of the nursery operation will be conducted by a team composed of State, Federal, and other subject matter experts (SMEs). CCPs are points in a nursery process or procedure that can result in the unintended spread or introduction of *P. ramorum*. Determining CCPs allows applicable best management practices (BMPs) and/or mitigations to be provided to the nursery in a systems approach. A systems approach consists of a defined set of phytosanitary procedures, at least two of which have an independent effect in mitigating pest risk associated with the movement of commodities. It is a proactive process to reduce the risk of infestation by correcting unsafe nursery practices leading to safe production practices that result in healthy plants. By addressing CCPs, nurseries can reduce the risk of a potential hazard and take corrective steps leading to *P. ramorum* mitigation and/or avoidance. Mitigation measures will be written in Exhibit D, updating PPQ Form 519, compliance agreement. **Please contact the PPQ S&T Representative for a copy of the fillable CCP template prior to the assessment**.

## Continuously Positive Nurseries

Nurseries that continue to find positive plants over a period of time will be assessed and additional mitigations will be required in Appendix D of the compliance agreement to ensure *P. ramorum* is **not** being moved. Best management practices (BMPs) can reduce or eliminate *P. ramorum* in a nursery setting and the required mitigations are part of the requirements for shipping plants in a nursery that has been found positive for *P. ramorum*.

A continuously positive nursery is defined as: a regulated nursery that has been under compliance for five consecutive years or more and has had a positive detection within the last six months.

Once a nursery falls under the definition of a continuously positive nursery, then the following actions will be required, as identified by the CCP team:

- Upon initial designation as a continuously positive nursery, State, federal, and other Subject Matter Experts (SMEs) will conduct a CCP inspection of the entire nursery (i.e., a systems approach audit). To bring in new perspectives, USDA recommends including SMEs who were not present at the first CCP. Inspectors will review the previous CCP and other historical information about the nursery. State and federal partners should agree in advance

who will lead the audit and what the process will be to streamline the CCP assessment.

- If possible, the nursery will designate a location where all incoming plants will arrive and be held for a minimum of three weeks.
- When inspectors find a positive plant, the continuously positive nursery will be required to, at minimum, destroy the D and Q radius. The nursery may be required to destroy the entire block if identified by the inspector.
- Nurseries will place only non-hosts on positive blocks that have mitigated soil.

**NOTICE**

Under 7 CFR 301.92, it is illegal to knowingly move the pathogen *P. ramorum* **without** a permit authorizing movement.

### Planning the CCP Assessment

The CCP assessment team should include **at least** two members who participated in the initiating inspection and sampling event. Schedule the assessment with the nursery owner and team well in advance.

**To initiate the assessment, the team must consider any available information prior to arrival**. Once at the site, the assessment team gathers additional information through discussions with nursery owners and managers, the inspection sampling team, and possibly county, State, and Federal personnel with knowledge of the nursery. Note that at the time of the site visit, **not all** nursery processes or situations may be present due to weather or change of seasons.

## CCP Assessment Day

Initially, the CCP assessment focuses on the areas of the nursery associated with confirmed-positive *P. ramorum* samples such as plants, water, soil, and pots. However, the assessment of the nursery operations may identify CCPs relating to larger nursery areas beyond the areas associated with confirmed-positive samples.

### Information Needed PRIOR to the CCP Assessment

This list is **not** meant to be inclusive.

1. Maps of the nursery (with bed layouts, if possible) and maps of the surrounding area for assessment planning purposes
2. Site assessment
   A. Perimeter—look for possible sources of inoculum (water or regulated plants)
   B. Site history

a. Prior ownership
b. Prior crops on site
c. Plants previously grown in ground
d. Failed crops or plants (specifically regulated plants)
e. History of prior confirmed-positive detections
f. History of pesticide use
g. History of on-site flooding
h. Weather patterns, rainfall, etc.
i. Location of high-risk areas (e.g., locations of regulated plant material and high-traffic areas)

3. Access to property
   A. Nursery rules for entering the site
   B. Public versus landscapers' access
   C. Any available logs or records of who has been on site
   D. Movement of plants and equipment between properties
4. Production practices/standard operating procedures (SOPs)
   A. Crop rotation practices
   B. Tracking movement of regulated plant material on site
   C. Are regulated plant materials treated or handled differently?
   D. Are holdovers mixed in with new stock or plants moved to fill in blocks?
5. Training
   A. Has there been any staff training for scouting *P. ramorum* symptoms?
   B. Are workers equipped with flagging as standard equipment to mark plants after scouting?

Please contact the PPQ–S&T representative for a fillable CCP template to use during the assessment. This template may also be used prior to the assessment team's arrival for information-gathering purposes and to focus the team's efforts and optimize time.

## After the CCP Assessment

After the information gathering and site assessment are completed, the team identifies the CCPs associated with the known, confirmed-positive sample sites and may include other components of the nursery operation. **Send a copy of the completed CCP template to the PPQ S&T Representative.** The State and PPQ regulatory officials/SMEs will provide as much information as possible about mitigation measures to assist the nursery owner. Nursery owners can request assistance from subject matter experts (county and university extension, nursery associations, etc.) to better understand required and recommended remediation measures, mitigations, and BMPs corresponding to the CCP assessment.

Note to CCP assessment team: surface water, effluent water (culvert, ditch, and nonrecycled retention pond water), soil, or cull piles may take longer than 90 days (the quarantine period) to disinfest/remediate. If the CCP assessment team makes specific mitigation recommendations, identify short-term mitigation versus longer-term remediation. Both short-term and longer-term measures agreed upon by the nursery owner/inspector will be written into Exhibit D, updating the compliance agreement with appropriate time periods.

**NOTICE**

Based on the CCP assessment, remediation, mitigations, and/or specific business/ cultural practices will be determined to address the presence of the pathogen within the nursery. Appendix D will contain the findings of the CCP assessment detailing the affected areas and will describe the remediation, mitigations, and/ or BMPs discussed with the nursery and agreed upon by the regulatory official.

## Critical Control Points

The following table provides examples of CCPs, remediations, mitigations, best management practices, and cultural changes that can be applied for *P. ramorum*. This list is not exclusive: other mitigations may be equally effective. For guidance on other potential mitigations, consult the BMP guides (refer to the References section on page 3-1-28), your state extension personnel, or the PPQ S&T representative.

**Table 3-1-6 Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices[1] (page 1 of 4)**

| Identified critical control point (CCP): | Mitigations that may have preceded CCP assessment: | Examples of remediations, mitigations, BMPs, and changes to business/cultural practices: |
|---|---|---|
| Regulated plant material | ◆ Double-bagged, identified material in (2 mil) plastic bags and deep burial (>2 m) burial in a site approved by regulatory authorities<br>◆ Incinerated at a site approved by regulatory authorities<br>◆ Heat sterilization; dry heat or steam (refer to the USDA *Treatment Manual* Schedule T415b) | ◆ Avoid accepting returned plant material; destroy or dispose of any returned regulated plant material<br>◆ Designate or assign specific personnel to work with regulated plant material for monitoring and management purposes<br>◆ Do **not** allow plant foliage to be in contact with the ground<br>◆ Do **not** mix incoming crops with existing regulated plant material<br>◆ Designate an area for unloading and holding regulated plant material for 30 days' monitoring<br>◆ Purchase from nurseries licensed or certified under **all** phytosanitary laws and applicable Federal and State regulations |
| General operation sanitation | ◆ Disinfestation of nonporous surfaces, concrete floors, benches, plastic sheeting, and tools | ◆ Adequately control weeds on the nursery site as they may potentially harbor the pathogen<br>◆ After every crop rotation, disinfest propagation mist beds, sorting area, cutting benches, machines, and tools to minimize the spread or introduction of pathogens<br>◆ Develop or review processes of cleaning carts and trailers used in moving plant materials, including tires<br>◆ Develop process for ensuring workers' clothing is clean and management tools are routinely cleaned and sanitized<br>◆ Do **not** allow trucks to sweep out debris on site<br>◆ Install foot baths in **all** high-risk areas, including for visitors to the production areas<br>◆ Prevent buildup of fallen leaves and plant debris from regulated plants in production areas and monitor with every crop rotation or quarterly, whichever is more frequent |
| General operation sanitation (cont.) | ◆ Disinfestation of nonporous surfaces, concrete floors, benches, plastic sheeting, and tools (cont.) | ◆ Properly dispose of any leaves or plant debris resulting from nursery operations or cleanup of areas or beds<br>◆ Routinely clean loading and shipping areas following shipment arrivals or after loading activities |

The following table provides examples of CCPs, remediations, mitigations, best management practices, and cultural changes that can be applied for *P. ramorum*. This list is not exclusive: other mitigations may be equally effective. For guidance on other potential mitigations, consult the BMP guides (refer to the References section on page 3-1-28), your state extension personnel, or the PPQ S&T representative.

**Table 3-1-6 Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices[1] (page 2 of 4)**

| Identified critical control point (CCP): | Mitigations that may have preceded CCP assessment: | Examples of remediations, mitigations, BMPs, and changes to business/cultural practices: |
|---|---|---|
| Potting media | ◆ Double-bagged, identified material in (2 mil) plastic bags and deep burial (>2 m) burial in a site approved by regulatory authorities<br>◆ Incinerated at a site approved by regulatory authorities<br>◆ Heat sterilization; dry heat or steam (refer to the USDA *Treatment Manual* Schedule T415b) | ◆ Do **not** reuse container mix from regulated plants<br>◆ Ensure area on which the growing media sits drains freely<br>◆ Ensure cull piles are clearly separated from container mix components<br>◆ Ensure growing container mix and/or components are from an area known to be **free** of *P. ramorum*<br>◆ Move container mix piles away from potential *P. ramorum* contamination sources<br>◆ Pasteurize potting media<br>◆ Place container mixes in containers, bins, or on hard surfaces that can be cleaned, and **not** in contact with site soil<br>◆ Purchase components from suppliers with the ability to supply media **free** of plant pathogens and pests and meets quality requirements<br>◆ Raise storage areas above the level of the surrounding land to a height of 10 to 12 cm to prevent all runoff water from entering the area or surrounding site with surface drains or diversion banks<br>◆ Sample and test media and media components at delivery or before use<br>◆ Steam-sterilize **any** container mix that is reused or composted according to strict national standards |
| Potting area | ◆ Disinfestation of nonporous surfaces, concrete floors, benches, plastic sheeting, and tools | ◆ Clean and disinfect **all** equipment used to transport media, e.g., front-end loader buckets, wheel barrows, mobile bins, trolleys, or plastic containers between uses<br>◆ Ensure staff members regularly wash their hands and maintain good hygiene<br>◆ Limit or divert traffic through soil-mixing area<br>◆ Regularly clean and disinfest potting facilities<br>◆ Regularly clean up and discard split media around potting facilities<br>◆ Schedule specific times to pot regulated plants and clean equipment and area **before or after** potting operations<br>◆ Use clean equipment to mix or load planting media |

The following table provides examples of CCPs, remediations, mitigations, best management practices, and cultural changes that can be applied for *P. ramorum*. This list is not exclusive: other mitigations may be equally effective. For guidance on other potential mitigations, consult the BMP guides (refer to the References section on page 3-1-28), your state extension personnel, or the PPQ S&T representative.

**Table 3-1-6 Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices[1] (page 3 of 4)**

| Identified critical control point (CCP): | Mitigations that may have preceded CCP assessment: | Examples of remediations, mitigations, BMPs, and changes to business/cultural practices: |
|---|---|---|
| Nursery beds | ◆ To avoid contact between infested soil/surface water and regulated plants, install permanent impermeable, nonporous barriers<br>◆ Steam soil<br>◆ Soil fumigation (e.g., dazomet, methyl bromide)<br>◆ Solarize soil | ◆ Maintain substrate, whether this is through additional gravel, repairing or replacing landscape cloth or covering, or leveling to improve or increase drainage<br>◆ Prevent buildup of fallen leaves and plant debris from regulated plants in production areas and monitor with every crop rotation or quarterly, whichever occurs most often |
| Irrigation water | ◆ Treat recycled water or water used for irrigation water with chlorine levels of 2 ppm or 2 mg/liter **or greater** | ◆ Avoid overhead irrigation of regulated plants or irrigate in a manner to avoid prolonged leaf wetness and splash<br>◆ Eliminate accumulations of surface water<br>◆ Irrigate regulated plant material around dawn, when possible, in order to prevent extended periods of leaf wetness<br>◆ Monitor and test (quarterly at a minimum) untreated irrigation water from any source **other than** a well or a municipal water supply to confirm it is **free** of the pathogen<br>◆ Monitor water treatment systems to verify the appropriate treatment measures are being applied<br>◆ Prevent surface water by **not** overwatering. Irrigate regulated plants based on water needs<br>◆ Treat water used for irrigation by using one or a combination of the following: bromine; chlorine; sodium hypochlorite; calcium hypochlorite; chlorine dioxide; ozone; activated peroxygen; ultraviolet radiation; copper ionization; heat treatment/pasteurization; or filtration |
| Water drainage | → | ◆ Divert soil and water movement from adjacent properties populated with regulated plants to prevent nursery contamination<br>◆ Ensure runoff from all cull piles is directed away from media components, media mixing areas, growing beds, nursery roadways or paths, and irrigation water to prevent contamination<br>◆ Insert a well-drained physical barrier (e.g., raised benches, effective gravel layer, asphalt, or concrete) between native soil and containers to prevent pathogen splash dispersal from potentially infested ground |

The following table provides examples of CCPs, remediations, mitigations, best management practices, and cultural changes that can be applied for *P. ramorum*. This list is not exclusive: other mitigations may be equally effective. For guidance on other potential mitigations, consult the BMP guides (refer to the References section on page 3-1-28), your state extension personnel, or the PPQ S&T representative.

**Table 3-1-6 Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices[1] (page 4 of 4)**

| Identified critical control point (CCP): | Mitigations that may have preceded CCP assessment: | Examples of remediations, mitigations, BMPs, and changes to business/cultural practices: |
|---|---|---|
| Pots/containers | ◆ Sterilization by steam or disinfestation by alcohols, chlorine, quaternary ammonium, or phenolics | ◆ Do **not** store used pots in areas in which water drainage could flow or splash into regulated plant beds<br>◆ Establish a procedure for cleaning and sanitizing pots with clear separation of clean pots from dirty pots<br>◆ Regularly control weeds in and around container storage facilities<br>◆ Store new and clean disinfested containers above ground level<br>◆ Store pots on a barrier that effectively separates them from underlying substrates<br>◆ Use pots that are: 1) new; 2) clean and properly disinfested; or 3) sanitized by steam sterilization or hot water dip |
| Roads/pathways | → | ◆ Cover pathways and roads adjacent to beds and benches with materials to reduce contamination with soil and water<br>◆ Maintain roads to avoid surface water; fill potholes and maintain drainage such that water will flow away<br>◆ Maintain substrate, leveling to improve or increase drainage<br>◆ Prevent buildup of fallen leaves and plant debris |
| Conveyance | → | ◆ Develop or review processes of cleaning tires and carts or trailers used in moving plant material<br>◆ Do **not** allow trucks to sweep out debris into nursery<br>◆ If a known *P. ramorum*-infested area has been visited, wash and sanitize shoes, tools, and vehicles that may have contacted contaminated soils **before** traveling to disease-**free** areas<br>◆ Regularly clean and disinfest transport equipment<br>◆ Require pick-up and delivery trucks to properly clean and sanitize truck bed, undercarriage, and tires **prior** to entering nursery operations<br>◆ Unload incoming deliveries onto a hard, impermeable surface area that is clean and **free** of **any** debris; collect **all** debris from plants, surface area, and delivery trucks |

1 Although this table tries to cover the most common potential hazards and the appropriate rectifications, it is unlikely to cover **all** of them. Hazards, CCPs, and potential responses will differ between nurseries.

## References

The following are examples of programs based on assessments to identify CCPs leading to BMPs and mitigations addressing associated risks.

- Best Management Practices Manual to reduce the risk of introducing Soil-borne Plant Pathogens into Horticultural Nurseries and Managed Wildland Landscapes
- The Systems Approach to Nursery Certification (SANC) Program
- *Phytophthora ramorum*: Best Management Practices
- Presidio Phytophthora Management Recommendations
- United States Nursery Certification Programs (USNCP)

Chapter

4

# Confirmed Retail Nursery and Retail Nursery Dealer Protocol

## Protocol for Intrastate Retail Nurseries and Retail Nursery Dealers When *Phytophthora ramorum* Is Present (rCNP)

## Contents



## Introduction

This protocol covers retail nurseries and retail nursery dealers who are intrastate shippers **only**. Any nursery that ships interstate is covered in Chapter 3 Interstate Confirmed Nursery Protocol on page 3-1-1.

Retail nurseries and retail nursery dealers represent a different type of risk from *Phytophthora ramorum* (*P. ramorum*) than nurseries that specialize in propagating and growing plants. The nature of the retail business tends to require that plants be moved more often in order to present them to the public for sale. Plants are **not** intended to remain on site for an extended period of time and plants do **not** tend to receive cultural controls like pruning or pesticides at the same frequency as they would during the plant production process.

As retail nurseries and retail nursery dealers are at the end of the production and distribution process, they normally represent a lower risk of distributing infected plants to other nurseries and facilities in the plant distribution system. Retail nurseries are the final location in the plant distribution system as well as the final location before infected plants are moved directly to the environment.

It is important that retail nurseries and retail nursery dealers do **not** become a location where noninfected plants could become infected en route to the point of final planting.

If the facility of concern is an intrastate commerce propagation, wholesale and rewholesale nursery, intrastate commerce grower, or an intrastate commerce broker with a nursery site or hold lot, please note the following:

If a State is interested in quarantining less than the entire State for *P. ramorum*, it **must** enforce restrictions on the intrastate movement of regulated, restricted, and associated articles that are substantially the same restrictions as those imposed by Federal regulation on the interstate movement of regulated, restricted, and associated articles.

Intrastate movement for nurseries that have been confirmed positive for *P. ramorum* in plants, water, or other regulated articles is **not** covered by Federal regulations. State inspectors have authority to limit movement intrastate. Creation and implementation of an intrastate protocol should closely resemble the Federal protocol for the movement of interstate material. Please refer to Interstate Confirmed Nursery Protocol on page 3-1-1 for guidance.

**NOTICE**

Online retail plant sellers would **not** be considered a retail nursery and should refer to Chapter 3 Interstate Confirmed Nursery Protocol on page 3-1-1 when positive plants are found.

## Goal

The goal of this protocol is to limit the spread of *Phytophthora ramorum*, a quarantine plant pathogen, and to simplify the movement of *P. ramorum*-**free** nursery stock. When procedures described in this protocol are implemented, plant-to-plant spread and movement of the pathogen through nursery shipments should be minimized. Cooperation by nursery management personnel is essential. Early detection and reporting of potential *P. ramorum* plant infections are crucial to ensure spread is contained.

**⚠ WARNING**

Any interpretation of this protocol or its procedures **NOT** consistent with the goal listed above, is a misinterpretation of the protocol.

## Trigger Events For Use of the Confirmed Retail Nursery and Retail Nursery Dealer Protocol

This protocol is to be implemented by USDA–APHIS–PPQ, in cooperation with State Plant Regulatory Officials (SPROs) when the presence of *P. ramorum* has been confirmed in a retail nursery or retail nursery dealer. Any detection of *P. ramorum*, a quarantine plant pathogen, requires a regulatory response. Samples may have been collected during surveys or inspections such as a Cooperative Agricultural Pest Survey (CAPS), State Nursery Cleanliness Survey, Plant Protection Act (PPA) Section 7721, or other surveys; State inspections, trace forward investigation, trace back investigation, or found by other means. The Retail Nursery and Retail Nursery Dealer protocol can be used in all of these cases.

Samples **must** be diagnosed using a method approved by USDA–APHIS–PPQ and consistent with the Potentially Actionable Suspect Sample (PASS) protocol (for diagnostic information and the PASS protocol, refer to the PPQ *Phytophthora ramorum* website).

**NOTICE**

When planning to announce or make a public statement about the detection of a federally regulated pest, the State Plant Regulatory Official (SPRO) and/or the public information officer for the State department of agriculture **must** first contact the State Plant Health Director (SPHD) and/or USDA–APHIS–Office of Legal and Public Affairs (LPA).

## Challenges

*P. ramorum* is a micro-organism and difficult to detect. It can infect plants; infest container mix, soil, and water; and persist in these substrates despite the best eradication efforts. Based on the understanding of the pathogen biology, these protocols and regulations will be adjusted accordingly. Detection and management of this pathogen is informed by continually improving science.

Additional challenges related to retail nurseries and/or retail nursery dealers include the following:

- Disposition of plants when **not** confirmed positive is a challenge when ownership is **not** clear
- If found positive, held plants, which are moved and commingled, lose their identity to surrounding plants that may be implicated for destruction and quarantine
- Lack of interest in ensuring plants are **free** of pathogens when plants are under consignment at a retail location

- Large retail chains create a unique pathway that is further complicated by the increasing use of consignment sales in ornamental plants
- Movement of nursery plants to plant brokers who bring material from larger nurseries and disseminate to multiple States increases the likelihood of accidental exposure to contamination of *P. ramorum* to other nursery products
- Plant traceability back to origin is difficult when multiple brokers/ nurseries are involved
- Plants under consignment complicate orders for plant destruction of confirmed positive plants because ownership is **not** local
- Potential reduction of effectiveness of the notification system is likely to occur when a nursery with a compliance agreement sells plants to a broker (receiving State is notified) and then the broker sells plants to a retail nursery dealer in multiple States and the receiving States are **not** notified
- Securing plant material at retail nursery dealers is a challenge, examples include space limitations and loss of communication between inspectors and managerial shifts at the facilities

## Confirmed Retail Nursery and Retail Nursery Dealer Steps

Any confirmed-positive article, such as a plant or water sample, triggers the confirmed nursery protocol. In chronological order, the steps for the Confirmed Retail Nursery and Retail Nursery Dealer protocol (rCNP) are as follows:

1. Communicate and notify.
2. Conduct trace investigations (concurrently with securing the nursery).
3. Secure the nursery (concurrently with conducting trace investigations).
4. Survey the nursery (delimitation inspection).
5. Disinfest the nursery.
6. Conduct 90-day (minimum) quarantine activities.
7. Release plants in the retail nursery or retail nursery dealer.
8. Conduct post-quarantine release monitoring.

**NOTICE**

**Prior to** an APHIS-confirmed positive determination, the National Plant Protection Laboratory Accreditation Program- (NPPLAP) approved laboratory **must** communicate **all** suspect positive diagnostic samples to inspectors as soon as one of the following has occurred:

• Culture that matches the morphology for *P. ramorum* as determined and reported by an APHIS-approved laboratory; or
• Positive PCR using APHIS-approved work instructions by an APHIS-approved laboratory

For **all** Potentially Actionable Suspect Samples (PASS), laboratories **must** immediately forward the plant material and DNA to the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory (refer to Contact Information for the Phythopthora ramorum Program on page A-1-1) with a domestic ARM routing receipt (DARR) and notify the State's State Plant Health Director (SPHD; generates DARR for laboratory) and State Plant Regulatory Official (SPRO) for the State of sample origin, and the National Operations Manager (NOM).

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 1—Communicate and notify | → | Laboratories need to notify the SPHD and the SPRO, the NOM, and the submitter. The SPRO (if State authority is used) or the SPHD (if Federal authority is used) will notify the owner of the nursery. |
| | 1.1 | The designated official will notify the confirmed-positive nursery of the confirmed-positive and instruct the nursery **to place a hold on all** regulated plants at the nursery and any other plants deemed necessary by the inspector. |
| | 1.2 | SPHD and SPRO will notify the NOM of the nursery notification and the hold on the plants. |
| | 1.3 | SPHD and/or SPRO will provide a list of the identified facilities found through trace back and trace forward investigations to the NOM within five business days of a confirmed *P. ramorum*-positive sample in a nursery (refer to 2—Conduct investigations on page 4-1-5). The NOM will notify SPHDs and/or SPROs of States sending or receiving these shipments. |
| | 1.4 | SPHDs and/or SPROs will notify affected retail nurseries and retail nursery dealers within their States. |
| 2—Conduct investigations | → | Conduct trace back and trace forward investigations concurrently with securing the nursery, as shipping lists **must** be provided to the NOM within five business days. If a nursery does not meet the five business day requirement, the SPHD, SPRO, and NOM should work together to decide potential consequences, such as holding the compliance agreement. |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 2—Conduct investigations<br>(cont.) | 2.1—Trace back investigation | At the time *P. ramorum* is confirmed in a retail nursery or retail nursery dealer facility, determine the source of the plant(s) to initiate the trace back investigation. Trace back plants include **all** plants from the source nursery of the same genus as the infected plant regardless of size, location, or lot, back to the original propagation source (if it still exists). Collect invoices for those purchases and provide to the NOM within five business days. For more detailed instructions, please refer to Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed P. ramorum-Infested Nursery on page 5-3-1.<br>**NOTICE**<br>Provide the trace back list(s) (including plant taxonomic details) to PPQ's NOM within 5 business days. |
| | 2.2—Trace forward investigation | Most retail establishments may **not** keep records of plant sales, however, it is necessary to inquire in order to perform a trace forward investigation. Any trace forward records **must** be sent to the NOM within five business days. If a nursery does not meet the five business day requirement, the SPHD, SPRO, and NOM should work together to decide potential consequences, such as holding the compliance agreement. Retail plant sales are likely to be intrastate and will be subject to the appropriate State regulations. If the retail nursery or retail nursery dealer has sales records, determine if the establishment has sold plants that could potentially be infected to another facility such as a landscaper. Initiate the trace forward investigation by identifying **all** plants (meeting the following criteria) sold within 6 months of the first confirmed-positive detection of *P. ramorum* at a nursery: 1) plants of the infected species/cultivar; 2) **all** regulated plants that originated in the destruction radius; and 3) any plants of the high-risk genera: *Camellia* spp., *Kalmia* spp., *Pieris* spp., *Rhododendron* spp. (including azalea), and *Viburnum* spp. regardless of their location in the nursery. This combination of shipped plants is referred to as the high-priority target plants. These plants, including their shipment date(s); quantities; and respective destinations, make up the trace forward list. Identify these high-priority plants using the best available information and to the lowest taxon possible (e.g., if the plants can be identified to cultivar, trace forward activities may be conducted at the cultivar level). |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 3—Secure the nursery | → | When the presence of *Phytophthora ramorum* has been confirmed in the nursery or retail nursery dealer, safeguard infected high-risk plants and high-risk plants nearby. The Schematic of Retail Nursery with Positive Plants (Figure 4-1-1 and Table 4-1-2) is included, but may **not** be appropriate for use in retail nursery dealer settings. |
| | 3.1—Establishing destruction and quarantine radii for confirmed-positive plant(s) | 3.1.1—**All** plants within 2 meters from the edge of any infected plants shall be held for destruction (destruction radius) |
| | | 3.1.2—**All** plants within a 2-meter perimeter (quarantine radius) beyond the 2 meters surrounding the infected plants (i.e., the retail destruction radius) shall be held for a **minimum** 90-day quarantine period **OR** plants can be voluntarily relinquished under official supervision at any time (refer to 7—Release the nursery on page 4-1-13). |
| | | 3.1.3—Inspect **all** regulated plants in the nursery that are **not** within the destruction and quarantine radii and hold any plants sampled. If an inspector determines any other product or article presented is at risk of spreading *P. ramorum* and the inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations, the product or article can be held (7 CFR Part 301.92-2). |
| | | 3.1.4—Refer to 5—Disinfest the nursery on page 4-1-11 for destruction and treatment protocols. |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 4—Survey the nursery | → | The goal of the survey is to locate *P. ramorum* in the nursery. A detailed and thorough inspection should be conducted in the nursery to determine the presence of *P. ramorum*. Samples should be collected from symptomatic plants (refer to Biology and Symptoms of Phytophthora ramorum on page 7-1-1). |
| | 4.1—Delimit the nursery | 4.1.1—Examine **all** plants within the retail nursery or retail nursery dealer and sample any symptomatic plant tissue found (refer to Plant Symptoms and Sampling for P. ramorum on page 2-1-4). Plants currently regulated in nurseries are included in the APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*. |
| | | 4.1.2—Hold **all** plants of that taxon (taxa) and **all** plants that are within 2 meters of the confirmed-positive plant. Or plants can be voluntarily relinquished for destruction under official supervision at any time (refer to 7—Release the nursery on page 4-1-13). |
| | | 4.1.3—Submit samples to the appropriate lab for analysis using a methodology approved by APHIS (refer to Sampling and Submission Protocol on page 8-1-1). |
| | | 4.1.4—Release **all** plants held if sample results are negative. |
| | | 4.1.5—Establish destruction and quarantine radius (radii) around plant(s) with positive diagnostic results (refer to 4.2—Establish destruction and quarantine radii for plants confirmed positive during delimiting survey on page 4-1-8) and delimit the nursery again. |
| | | 4.1.6—The 90-day plus quarantine period begins when the delimiting survey is complete. |
| | 4.2—Establish destruction and quarantine radii for plants confirmed positive during delimiting survey | 4.2.1—Plants at retail nursery dealers can be very transient, making it difficult to implement some of the following measures. Adapt as much of this protocol to the situation as practical. |
| | | 4.2.2—Establish destruction radius (radii) by flagging a 2-meter radius (a 4-meter diameter circle) around **all** infected plants (refer to Figure 4-1-1) **OR** inspector-witnessed relocation/segregation of plants within the nursery with safeguarding **OR** plants can be relinquished under official supervision at any time (refer to 7—Release the nursery on page 4-1-13); include plants on carts or other movable shelving situations. |
| | | 4.2.3—Establish quarantine radius (radii) by flagging a 4-meter radius (an 8-meter diameter circle) around **all** infected plants (refer to Figure 4-1-1) **OR** inspector-witnessed relocation/segregation of plants within the nursery with safeguarding **OR** voluntary destruction. |
| | | 4.2.4—Limit access to destruction and quarantine radius (radii) or other designated hold areas. Ensure proper sanitation measures are applied (refer to 5—Disinfest the nursery on page 4-1-11 and Biosecurity Measures for Nurseries on page 9-1-1). |
| | | 4.2.5—Inspectors should document **all** actions taken on EAN or State equivalent. |

## Schematic of Destruction (D) and Quarantine (Q) Radii

**Figure 4-1-1 Schematic of Destruction and Quarantine Radii of Positive Plants**

**Table 4-1-2 Legend of Destruction (D) and Quarantine (Q) Radii of Positive Plants Schematic**

| Area color: | Name: | Once D-radii and Q-radii are flagged, then: |
|---|---|---|
| Gray (*1) | Destruction (D) radii | Destroy **all** plants, pots, medium, and leaf debris |
| Light gray (*2) | Quarantine (Q) radii | Hold **all** plants from sale for 90 days |
| Gray (*3) | D-radii in block with nonregulated plants | Destroy **all** plants, containers, media, and leaf debris; nonregulated plant nursery stock could still move the pathogen |
| Light gray (*4) | Q-radii in block with nonregulated plants | Hold **all** plants for sale for 90 days; nonregulated plant nursery stock could still move the pathogen |
| Dotted (*5) | Rest of the block with regulated plants | Release **all** plants for sale **only** when found to be **asymptomatic** during the delimiting survey |
| White (*6) | Rest of the block of nonregulated plants | Release **all** plant materials for sale if found to be **asymptomatic** during the delimiting survey |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 4—Survey the nursery<br>(cont.) | 4.3—Sample other articles of concern at the nursery | 4.3.1—**Not all** of the following articles of concern will be found at the majority of retail nurseries and retail nursery dealers. Sample other articles of concern if they occur at the facility. |
| | | 4.3.2—Water sampling—<br>◆ Determine the source of water used at the nursery site and where drainage water flows. Note the type of irrigation system(s) in use, areas of standing water, any safeguards against water back flow in the irrigation system, as well as any water treatment practices if recirculated water is used.<br>◆ Water sampling is **not** required for irrigation water from municipal water facilities that treat their water **prior to** release.<br>◆ Sample any retention pond or surface water at the nursery (refer to Water Sampling and Processing Protocol on page 10-1-1). Bottle of bait is the preferred sampling method for surface water. |
| | | 4.3.3—Sampling instructions for soil—<br>◆ Standing water will be sampled in place of substrate soil sampling. **No substrate soil samples are required for this sampling protocol**. |
| | | 4.3.4—Cull pile and compost pile sampling—determine how the nursery disposes of culled and other waste plant material. Cull and compost piles will be uncommon in a retail nursery or retail nursery dealer, however, if cull or compost piles are present, record the location of any piles as these may be contaminated with infected plant material or associated soil and/or growing media.<br>◆ Check cull and compost piles for *P. ramorum* symptomatic plants and plant material and sample if observed.<br>◆ If a cull or compost pile is found to be positive, establish and demarcate the area by visibly and indelibly flagging 1 meter out from the perimeter of the cull pile.<br>◆ Sample and test soil for the presence of *P. ramorum* at the down slope edge of the cull pile.<br>◆ Determine how the nursery disposes of cull and compost piles.<br>◆ Restrict access.<br>◆ Determine the appropriate treatment or destruction method (refer to Treatments and Disinfectants on page 12-1-1). |
| | | 4.3.5—Segregation of plants on hold—<br>◆ Once inspection and sampling are complete, any held plants may be consolidated and segregated. If the plants are **not** consolidated and segregated, the affected portion of the nursery **must** be closed to the public. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery if properly safeguarded. Any movement of the segregated plants **must** be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction of an inspector. Segregation **must** include storage on an impermeable surface (e.g., a 45-mil thick pond liner or concrete or asphalt) and **not** within 2 meters of any other plants. The impermeable surface should ideally be situated to drain away from regulated plants. |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 5—Disinfest the nursery | → | Inspectors should use appropriate disinfection measures for conditions found at the establishment. For information on disinfesting and sanitizing any nursery, refer to Treatments and Disinfectants on page 12-1-1 and Biosecurity Measures for Nurseries on page 9-1-1. |
| | 5.1—Plant destruction | 5.1.1—When a *P. ramorum*-infected plant is found, **all** other plants, including pots and growing media within 2 meters (destruction radius) of the infected plant, will be removed and destroyed using one or more of the techniques detailed in Treatments and Disinfectants on page 12-1-1. This includes pots and potting media. A regulatory official must oversee plant destruction. |
| | | 5.1.2—Plants can be voluntarily relinquished under official supervision at any time (refer to 7—Release the nursery on page 4-1-13). |
| | 5.2—Debris removal | **All** plant debris, including growing media, leaves, stems, flowers, roots, and any other plant parts found within the destruction radius, will be removed and destroyed using one or more of the techniques detailed in Treatments and Disinfectants on page 12-1-1. |
| | 5.3—Nonporous surfaces | Nonporous surfaces will be disinfested beneath plants. For instance, wipe off shelves with Lysol or other approved disinfectant (refer to Treatments and Disinfectants on page 12-1-1 for recommended disinfestation options). |
| | 5.4—Porous surfaces | If the nursery is found to have an infested porous surface, remedial action **must** be developed and implemented with the written approval of an inspector. This is done in order to prevent contact of plants with soil or any other surface that **cannot** be immediately disinfested. A durable, impermeable ground barrier (e.g., a 45-mil pond liner) may be used as an inexpensive temporary measure. The condition of the barrier **must** be monitored and maintained and foot traffic minimized. Refer to Treatments and Disinfectants on page 12-1-1 for other recommended options. |
| | 5.5—Equipment and personnel | Use recommended disinfestation options and biosecurity measures (refer to Treatments and Disinfectants on page 12-1-1 and Biosecurity Measures for Nurseries on page 9-1-1). Use appropriate sanitation practices, such as disinfesting clippers used to cut plants or using disposable gloves to prevent cross-contamination. |
| | 5.6—Biosecurity measures | Biosecurity measures, such as tool sanitation, equipment disinfestation, and appropriate sanitation measures for employees, are designed to minimize the risk of introduction to or spread and survival of the pathogen in a nursery (refer to Biosecurity Measures for Nurseries on page 9-1-1for recommended biosecurity measures). |
| | 5.7—Treatments for cull piles, compost piles, water, soil, or growing media | It is unlikely cull piles, compost piles, water, soil, or growing media will need remediation at a retail nursery dealer, but they may need remediation at a retail nursery. If they are found to be positive, they will need to be treated (refer to Treatments and Disinfectants on page 12-1-1). |
| | 5.8—Considerations for inspectors | 5.8.1—Determine if plants will be placed on hold, destroyed on site, destroyed off site, or relinquished to an inspector. |
| | | 5.8.2—Determine sanitation protocols established for the facility including management oversight (e.g., how is information on holds communicated among employees and management staff?). |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 5—Disinfest the nursery (cont.) | 5.8—Considerations for inspectors (cont.) | 5.8.3—Determine management oversight and responsibility for plants for sale. How is information on holds communicated among employees and management staff? How will plants on hold be safeguarded? Who owns the plants and is responsible for destruction costs? |
| | | 5.8.4—Determine which disinfestation methods are appropriate for the site (refer to Treatments and Disinfectants on page 12-1-1). For instance, disinfesting concrete or asphalt may not be practical at a retail nursery dealer's based on the movement of the plants at the store. |
| | | 5.8.5—Determine the number of plants that **must** be destroyed and the number the nursery may voluntarily destroy or relinquish to an inspector. Determine the number of positive plants, the number of potentially positive plants, and the location of those plants in the nursery. The inspector can use the information collected to determine the State authority for holds, plant destruction, and other components of the nursery disinfestation. |
| 6—90-day (**minimum**) quarantine activities | → | Plants can be relinquished under official supervision at any time (refer to 7—Release the nursery on page 4-1-13). These activities follow the completion of the delimiting survey and may run concurrent with some of the disinfestation activities taking place at the nursery. If a retail nursery dealer that has been found to be infested has completed **all** of the following, it shall **not** undergo post-eradication monitoring **unless** additional plants or articles are found to be positive:<br>◆ Plants are **only** present seasonally; and<br>◆ Plants are placed **only** on nonporous surfaces that have been mitigated<br>If additional plants or articles are found to be positive, the nursery shall be placed in a compliance agreement and have post-eradication monitoring. |
| | 6.1—The quarantine period | The quarantine period begins when the delimiting survey is completed (i.e., the last sample is taken and an EAN or State equivalent is issued) and lasts a **minimum** of 90 days. During the quarantine period, inspection, sampling, and testing **must** reveal **no** further detection of *P. ramorum*, or the quarantine period will be extended. If a positive sample occurs, the 90-day (**minimum**) quarantine period restarts and the appropriate measures will be taken (refer to 3—Secure the nursery on page 4-1-7). |
| | 6.2—During the 90-day (**minimum**) quarantine period within the quarantine radius (radii) | 6.2.1—No fungicides registered for *Phytophthora* control shall be applied. |
| | | 6.2.2—Inspectors will visually inspect plants a **minimum** of two times—once about halfway through the 90-day (**minimum**) quarantine period, and once near the end of the 90-day (**minimum**) quarantine period in order to have test results coincide with the end of the quarantine period—according to the protocol (refer to Phytophthora ramorum Inspection and Sampling Protocol for Nurseries on page 2-1-1). Samples will **only** be taken if symptomatic tissue is observed. The second visual inspection in the quarantine radius (radii) can be done at the same time as the quarantine release survey as described below. |
| | | 6.2.3—If positive samples were collected during the delimiting survey, inspectors will collect soil and media samples and test them during the quarantine period according to the protocols (refer to Soil and Container Mix Sampling and Processing Protocol on page 11-1-1). |
| | | 6.2.4—If positive water samples were collected during the delimiting survey, inspectors will collect water samples and test them during the quarantine period according to the protocols (refer to Water Sampling and Processing Protocol on page 10-1-1). |

**Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (continued)**

| Step number: | Subnumber: | Then: |
|---|---|---|
| 6—90-day (**minimum**) quarantine activities (cont.) | 6.3—Quarantine release survey | A quarantine release survey of the entire nursery **must** be completed near the end of the **minimum** 90-day quarantine period. This survey includes visually inspecting **all** regulated plants within the nursery and sampling any symptomatic plant tissue, soil of destruction and quarantine radius (radii), and drainage or recirculated irrigation water. When the quarantine period is completed and **all** plant, soil, and water samples taken are negative for *P. ramorum*, the nursery can be released. |
| 7—Release the nursery | → | Nurseries and their plants that have been placed under regulatory control may be released from regulatory control by USDA–APHIS, or its designated authority, after the quarantine period **if** the following three conditions are met:<br>◆ There are **no** additional detection of *P. ramorum* in nursery stock based on USDA–APHIS-approved plant inspection sampling and testing protocols for the preceding quarantine period; **and**<br>◆ If water, soil, and growing media were tested, they must also have tested negative for *P. ramorum* based on USDA–APHIS-approved sampling and testing protocols for the preceding quarantine period (if testing of soil, water, and media is required); **and**<br>◆ The quarantine release survey is negative for *P. ramorum*.<br>**OR**<br>◆ The nursery operator may also choose to destroy plants that have been placed under quarantine at any time within the 90-day quarantine period, however, destruction **must** be under local authority supervision; **and**<br>◆ If **not** previously tested and determined to be negative, inspectors **must** sample and test drainage or recirculated irrigation water as per Water Sampling and Processing Protocol on page 10-1-1 or inspectors **must** disinfest porous and nonporous surfaces (refer to 5.3—Nonporous surfaces on page 4-1-11). If soil and water samples taken are negative for *P. ramorum*, the nursery can be released; **and**<br>◆ Inspectors **must** revisit the nursery after approximately 90 days and conduct **at least** a nursery-level survey inspection (refer to Phytophthora ramorum Inspection and Sampling Protocol for Nurseries on page 2-1-1). |

## APHIS List of Regulated Hosts and Plants Proven or Associated with *Phytophthora ramorum*

Refer to a current list of APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*.

Chapter

5-1

# Trace Investigations

## Introduction

## Contents



## Introduction

Trace investigations are required at nurseries confirmed positive for *Phytophthora ramorum*. The following sections include protocols for trace forward (Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed P. ramorum-Infested Nursery on page 5-2-1) and trace back (Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed P. ramorum-Infested Nursery on page 5-3-1) investigations. These two protocols are needed to complete trace investigations related to confirmed positive detections of *P. ramorum*. The *Phytophthora ramorum* Nursery Questionnaire (refer to Figure  on page A-1-2) is an available tool to collect required and other useful information on nurseries. Select the correct trace investigation in the Activity Action section. Contact the NOM for a fillable form.

## Intended Use

The intended use of these protocols is to identify where suspect *Phytophthora ramorum*-infected plants have been shipped. These protocols include plants shipped from the nursery confirmed positive for *P. ramorum* (Trace Forward) and plants shipped to that nursery (Trace Back). Any interpretation of these protocols that is contrary to this goal is a misinterpretation of the protocols. A detailed and thorough inspection shall take place at the field level to identify the presence of *P. ramorum*. Areas of consideration are to include, **but are not limited to**, plants, plant material and debris, soil, and water.

Chapter

5-2

# Trace Investigations

## Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed *P. ramorum*-Infested Nursery

**NOTICE**

If regulatory officials are conducting a trace forward investigation at a non-nursery locale (residence, commercial site, managed landscape), they should apply this protocol making appropriate adjustments to the instructions. Refer to Information Needed PRIOR to the CCP Assessment on page 3-1-20.

## Contents



## Intended Use

The intended use of this protocol is to identify where *Phytophthora ramorum*-infected plants have been shipped. These include:

- Interstate commerce brokers with a nursery site or holding lot
- Interstate commerce propagation, wholesale, and re-wholesale nurseries
- Interstate commerce retail nurseries or other retail outlets
- Intrastate commerce nurseries
- Residential, commercial sites, and managed landscapes/public gardens

## Goal

The goal of this protocol is to determine if *P. ramorum*-infected plants have been shipped from a confirmed-positive nursery. Any interpretation of this protocol that is contrary to this goal is a misinterpretation of the protocol. A detailed and thorough inspection shall take place at the field level to identify the presence of *P. ramorum*. Areas of consideration are to include, **but are not limited to**, plants, plant material and debris, soil, and water.

# Trace Forward Protocol Instructions

**Table 5-2-1 Trace Forward Protocol Instruction (page 1 of 2)**

| Chronological or concurrent steps: | Actions: |
|---|---|
| 1) Communicate and notify the trace forward nursery | **Before inspection day**<br>1. The PPQ National Operations Manager (NOM) will send trace forward information generated from the Interstate Confirmed Nursery Protocol Steps on page 3-1-3 to the SPRO and SPHD who will determine who responds to the trace forward information.<br>2. The designated regulatory official will plan an inspection of the receiving facilities (hereinafter referred to as trace forward facilities) without delay. If favorable climatic conditions (refer to Timing Nursery Inspection and Sampling on page 2-1-3) are **not** present for disease development/expression when the initial inspection is conducted, an additional inspection **must** be conducted when conditions are conducive.<br>3. For Federal inspectors, notify the SPRO, or relevant State official, of your plans to inspect.<br>4. Coordinate the inspection day with the State inspector.<br>5. If required, Federal and State or county inspectors shall contact the property owners/managers **prior to** the visit to determine how many trace forward plants are still in stock and to arrange for the inspection.<br>6. If you are unable to visit the nursery within 24 hours of your contact with the nursery owner/manager, send a PPQ Form 523, Emergency Action Notification (EAN), or State equivalent, by email or FAX and request that they sign and immediately return it to you. The EAN will indicate what plants are on hold.<br>7. Review and bring with you the *P. ramorum* Biology and Symptoms of Phytophthora ramorum on page 7-1-1 and Biosecurity Measures for Nurseries on page 9-1-1.<br>8. Obtain sampling supplies, refer to Sampling and Submission Protocol on page 8-1-1 for checklist and review and bring with you. |
| 2) Investigation, inspection, and quarantine hold procedures | **Inspection day**<br>1. Identify yourself and agency to the nursery/facility owner/manager and explain the purpose of your visit.<br>2. Obtain copies of the shipping documents relating to the regulated plants shipped from the confirmed-positive nursery. Also, determine if the trace forward nursery shipped regulated plants to other wholesale or retail nurseries or facilities. If so, obtain those documents from the owner/manager.<br>3. Provide the owner/manager with a copy of the *Phytophthora ramorum* Nursery Questionnaire (refer to Figure A-1-1). Interview, review records, and observe the facility to fill out the questionnaire **with** the nursery owner/manager. Remember to ask the owner/manager the locations of the cull piles, compost piles, and waste bins or piles.<br>4. Verify the present or absence of any of the trace forward plants.<br>A. If trace forward plants are **not** in the nursery, verify whether additional locations (e.g., nursery sites) are maintained by the same nursery owner, of if any regulated plants moved to other sites or between sites during the 6-month period preceding the confirmed-positive detection.<br>B. Through interview or records, if possible, determine if other regulated plants may have come in contact with identified trace forward plants, duration of the contact, and where in the nursery the contact occurred.<br>5. Use the Sampling and Submission Protocol on page 8-1-1 and collect any symptomatic plant tissue found. While taking samples, visibly and indelibly flag or mark plants and areas sampled with sample ID and date. Also, mark areas on a map where plant samples were collected and/or take a GPS point or pictures. |

**Table 5-2-1 Trace Forward Protocol Instruction (page 2 of 2)**

| Chronological or concurrent steps: | Actions: |
|---|---|
| 2) Investigation, inspection, and quarantine hold procedures<br>(cont.) | 6. If trace forward regulated plants are in multiple locations within the nursery, the inspector **must** disinfect boots, tools, and hands between areas (refer to Treatments and Disinfectants on page 12-1-1).<br>7. Use PPQ Form 523, Emergency Action Notification (EAN) for the official Federal authorization of hold. In Section 16 of the EAN, state that those specific plants are prohibited in movement pending further notification by PPQ.<br>8. Visibly and indelibly flag 2-meter area around the sampled plant to hold for quarantine until all diagnostic results are final.<br>9. Hold the sampled plants and all other regulated plants within the 2-meter radius around the sampled plants.<br>10. Once the delimiting survey and sampling are complete, any held plants may be consolidated and segregated. If the plants are **not** consolidated and segregated, the affected portion of the nursery **must** be closed to the public. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery. Any movement of the segregated plants **must** be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction of a regulatory official. Segregation **must** include storage on an impermeable surface (e.g., a 45-mil thick pond liner, concrete, or asphalt) and **not** within 2 meters of any other plant. The impermeable surface should be sloped to drain away from regulated plants.<br>11. Per Federal and State authorities, inspectors may, at any time, place on hold other plants, plant products, or articles (e.g., pots) that present a risk of spreading *P. ramorum*.<br>12. Check any cull, waste, or debris piles for *P. ramorum*-symptomatic plants or plant material. Collect samples of symptomatic material. |
| 3) Sample collection and submission | Use Sampling and Submission Protocol on page 8-1-1. |
| 4a) If the trace forward nursery is confirmed positive, and is an **interstate** shipping nursery | Use the Interstate Confirmed Nursery Protocol on page 3-1-1 and contact the PPQ National Operations Manager (NOM). |
| 4b) If the trace forward nursery is confirmed positive and is an **intrastate** commerce only nursery | Use the Confirmed Retail Nursery and Retail Nursery Dealer Protocol on page 4-1-1 and contact the PPQ National Operations Manager (NOM). |
| 5) Questions | For PPQ *P. ramorum* program contacts, refer to Contact Information for the Phythopthora ramorum Program on page A-1-1. |

Chapter

5-3

# Trace Investigations

## Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed *P. ramorum*-Infested Nursery

**NOTICE**

If regulatory officials are conducting a trace forward investigation at a non-nursery locale (residence, commercial site, managed landscape), they should apply this protocol making appropriate adjustments to the instructions. Refer to Information Needed PRIOR to the CCP Assessment on page 3-1-20.

## Contents



## Intended Use

The intended use of this protocol is to identify the shipping/infection source(s) of confirmed-positive *Phytophthora ramorum* plants. These sources may include:

- Interstate commerce brokers with a nursery site or holding lot
- Interstate commerce propagation, wholesale, and re-wholesale nurseries
- Interstate commerce retail nurseries or other retail outlets

The intended use of this protocol is for nurseries that sell plants for **interstate** distribution. For nurseries that **only** sell plants for **intrastate** commerce, refer to Confirmed Retail Nursery and Retail Nursery Dealer Protocol on page 4-1-1.

## Goal

The goal of this protocol is to determine if suspect *Phytophthora ramorum*-infected plants have been shipped to a confirmed-positive nursery. The goal is to determine if *P. ramorum* is present at the originating nursery. Any interpretation of this protocol that is contrary to this goal is a misinterpretation of the protocol. A detailed and thorough inspection shall take place at the field level to identify the presence of *P. ramorum*. Areas of consideration are to include, **but are not limited to**, plants, plant material and debris, soil, and water used on plants.

# Trace Back Protocol Instructions

**Table 5-3-1 Trace Back Protocol Instructions (page 1 of 2)**

| Chronological or concurrent steps: | Actions: |
|---|---|
| 1) Communicate and notify the trace back nursery | **Before inspection day**<br>1. The PPQ National Operations Manager (NOM) will send trace back information generated from the Interstate Confirmed Nursery Protocol Steps on page 3-1-3 to the SPRO and SPHD who will determine who conducts the protocol.<br>2. The designated regulatory official/inspector will plan an inspection of the receiving facilities (hereinafter referred to as trace back facilities) without delay. If favorable climatic conditions are **not** present for disease development/expression when the initial inspection is conducted, an additional inspection **must** be conducted when conditions are conducive.<br>3. SPHD and SPRO should communicate on all trace back activities.<br>4. Coordinate the inspection day with the State inspector.<br>5. Federal and State or county inspectors should contact the property owners/managers **prior to** the visit to determine how many trace back plants are still in stock and to arrange for the inspection.<br>6. If you are unable to visit the nursery within 24 hours of your contact with the nursery owner/manager, send a PPQ Form 523, Emergency Action Notification (EAN), or State equivalent, by email or FAX and request that they sign and immediately return it to you. The EAN will indicate what plants are on hold.<br>7. Review and bring with you the *P. ramorum* Biology and Symptoms of Phytophthora ramorum on page 7-1-1 and Biosecurity Measures for Nurseries on page 9-1-1.<br>8. Obtain sampling supplies, refer to Sampling Supplies and Equipment Checklist on page 8-1-4 for checklist and review and bring with you Sampling and Submission Protocol on page 8-1-1 for checklist and review and bring with you. |
| 2) Investigation, inspection, and quarantine hold procedures | **Inspection day**<br>1. Identify yourself and agency to the nursery/facility owner/manager and explain the purpose of your visit.<br>2. Obtain copies of the shipping documents related to the regulated plants shipped from the confirmed-positive nursery. Also, determine if the nursery shipped regulated plants to other wholesale or retail nurseries or facilities. If so, obtain those documents from the owner/manager.<br>3. Provide the owner/manager with a copy of the *Phytophthora ramorum* Nursery Questionnaire (refer to Figure A-1-1). Interview, review records, and observe the facility to fill out the questionnaire **with** the nursery owner/manager. Remember to ask the owner/manager the locations of the cull piles, compost piles, and waste bins or piles.<br>4. Verify the presence or absence of any of the trace back plants.<br>A. If trace back plants are **not** in the nursery, verify whether additional locations (e.g., nursery sites) are maintained by the same nursery owner, of if any regulated plants were moved to other sites or between sites during the 6-month period preceding the confirmed-positive detection.<br>B. If trace back plants have had any contact, direct or indirect, with other plants or resources at the nursery, the entire nursery should/must be inspected.<br>5. Use the Sampling and Submission Protocol on page 8-1-1 and collect any symptomatic plant tissue found. While taking samples, visibly and indelibly flag or mark plants and areas sampled with sample ID and date. Also, mark areas on a map where plant samples were taken. |

**Table 5-3-1 Trace Back Protocol Instructions (page 2 of 2)**

| Chronological or concurrent steps: | Actions: |
|---|---|
| 2) Investigation, inspection, and quarantine hold procedures<br>(cont.) | 6. If trace back regulated plants are in multiple locations within the nursery, the inspector **must** disinfect boots, tools, and hands between areas (refer to Treatments and Disinfectants on page 12-1-1).<br>7. Use PPQ Form 523, Emergency Action Notification (EAN) for the official Federal authorization of hold. In Section 16 of the EAN, state that those specific plants are prohibited in movement pending further notification by PPQ.<br>8. Visibly and indelibly flag a 2-meter area around the sampled plant to hold for quarantine until all diagnostic results are final.<br>9. Hold the sampled plants and all other regulated plants within the 2-meter radius around the sampled plants.<br>10. Once the delimiting survey and sampling are complete, any held plants may be consolidated and segregated. If the plants are **not** consolidated and segregated, the affected portion of the nursery **must** be closed to the public. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery. Any movement of the segregated plants **must** be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction of a regulatory official. Segregation **must** include storage on an impermeable surface (e.g., a 45-mil thick pond liner, concrete, or asphalt) and **not** within 2 meters of any other plant. The impermeable surface should be sloped to drain away from regulated plants.<br>11. Per Federal and State authorities, inspectors may, at any time, place on hold other plants, plant products, or articles (e.g., pots) that present a risk of spreading *P. ramorum*.<br>12. Check any cull, waste, or debris piles for *P. ramorum*-symptomatic plants or plant material. Collect samples of symptomatic material. |
| 3) Sample collections and submission | Use Sampling and Submission Protocol on page 8-1-1. |
| 4a) If the trace back nursery is confirmed positive, and is an **interstate** shipping nursery | Use the Interstate Confirmed Nursery Protocol on page 3-1-1 and contact the PPQ National Operations Manager (NOM). |
| 4b) If the trace back nursery is confirmed positive and is an **intrastate** commerce only nursery | Use the Confirmed Retail Nursery and Retail Nursery Dealer Protocol on page 4-1-1 and contact the PPQ National Operations Manager (NOM). |
| 5) Questions | For PPQ *P. ramorum* program contacts, refer to Contact Information for the Phythopthora ramorum Program on page A-1-1. |

Chapter

6

# Confirmed Residential and Commercial Landscape Protocol

## Contents



## Intended Use

This protocol specifies actions that should be taken when a confirmed-positive *Phytophthora ramorum* infection is detected in plantings in residential or commercial landscape settings. If there are large areas of contiguous regulated plant material or large-caliper, infected plants encountered, consult with the PPQ National Operations Manager (NOM) for guidance. Guidance should include analysis of the environmental risks associated with treatments in residential and landscaped areas.

## Goal

The goal of this protocol is to ensure any infestations of this serious pathogen are consistently and effectively addressed, mitigated, and eradicated when possible. Cooperation by the homeowner is essential. Early detection and reporting of potential *P. ramorum* infestations is critical to ensure spread is contained. The strategies employed in the protocol are intended to ensure a rapid and appropriate response to prevent the spread of the pathogen.

# Trigger Events for Use of the Confirmed Residential and Commercial Landscape Protocol

This protocol outlines procedures that should be followed when the presence of *P. ramorum* has been confirmed positive in a residential or commercial landscape setting. Confirmed samples **must** have been diagnosed using a methodology approved by PPQ and consistent with the Potentially Actionable Suspect Sample (PASS) protocol (refer to the PPQ *Phytophthora ramorum* website for additional information regarding the PASS protocol).

# Disclaimers

**Any interpretation of this protocol or its procedures not consistent with the goal listed above is a misinterpretation and misrepresentation of the protocol.**

## Challenges

*Phytophthora ramorum* is a micro-organism and difficult to find and detect. It can infect plants; infest container mix, soil, and water; and persist in these substrates despite the best eradication efforts. These protocols and regulations will be adjusted accordingly, based on the understanding of the pathogen's biology. Detection and management of this pathogen is informed by continually improving science.

# Communication and Notification

**NOTICE**

**Prior to** an APHIS-confirmed positive determination, the National Plant Protection Laboratory Accreditation Program- (NPPLAP) approved laboratory **must** communicate **all** suspect positive diagnostic samples to inspectors as soon as one of the following has occurred:

• Culture that matches the morphology for *P. ramorum* as determined and reported by an APHIS-approved laboratory; or
• Positive PCR using APHIS-approved work instructions by an APHIS-approved laboratory

For **all** Potentially Actionable Suspect Samples (PASS), laboratories **must** immediately forward the plant material and DNA to the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory (refer to Contact Information for the Phythopthora ramorum Program on page A-1-1) with a domestic ARM routing receipt (DARR) and notify the State's State Plant Health Director (SPHD; generates DARR for laboratory) and State Plant Regulatory Official (SPRO) for the State of sample origin, and the National Operations Manager (NOM).

## Confirmed Residential Protocol Steps

In chronological order, the steps for the Confirmed Residential Protocol are as follows:

1. Communicate and notify
2. Secure the site
3. Survey the site and perimeter
4. Delimiting survey
5. Disinfest the site
6. 90-day quarantine activities
7. Release the site
8. Post-disinfestation monitoring

**Table 6-1-1 Confirmed Residential Protocol Instructions (page 1 of 4)**

| If step number: | For: | Then: |
|---|---|---|
| 1—Communicate and notify | → | 1. Immediately notify the SPHD and the SPRO of the State where the site is located. The SPHD will notify the PPQ National Operations Manager (NOM). (refer to Resources on page A-1-1). Laboratories need to notify the SPHD, the SPRO, the PPQ National Operations Manager, the National Policy Manager, and the submitter of the confirmed-positive samples.<br>2. In the event of a confirmed positive at a residential or commercial landscape setting, the appropriate regulatory official in the State (SPRO or SPHD) immediately informs the homeowner or commercial landscape owner of the confirmed positive.<br>3. Complete the questionnaire Phytophthora ramorum Nursery Questionnaire on page A-1-2. Complete the questionnaire as thoroughly as possible during the initial contact with the property owner or manager and when the site is secured and the confirmed-positive plant(s) and **all** associated plants are safeguarded. Complete the remainder of the questionnaire and Follow-up Survey for Residential Locations with Infected Plants (page 1 of 2) on page 6-1-13 at the time of the delimiting survey.<br>4. Document any proof of purchase the consumer may have, such as receipts, pot labels, etc. |
| 2—Secure the site | When the presence of *P. ramorum* has been confirmed in a residential or commercial setting | 1. Place on hold **all** regulated plant genera within a **minimum** of a 30-meter radius of the infected plant(s) under regulatory control as per the PPQ Form 523 Emergency Action Notification (EAN) or State equivalent. Safeguard these plants and keep them undisturbed until the delimitation survey and confirmation results are complete. Any regulatory control (hold) may also include "any other product or article that an inspector determines to present a risk of spreading *P. ramorum*..." (7 CFR part 30.92-2) within the infested site. |

**Table 6-1-1 Confirmed Residential Protocol Instructions (page 2 of 4)**

| If step number: | For: | Then: |
|---|---|---|
| 2—Secure the site<br>(cont.) | When the presence of *P. ramorum* has been confirmed in a residential or commercial setting<br>(cont.) | 2. Complete the questionnaire Follow-up Survey for Residential Locations with Infected Plants (page 1 of 2) on page 6-1-13 during the delimitation survey. Do **not** move **any** equipment used on the residential or landscaped commercial sites without proper treatment and disinfection (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15).<br>3. If necessary, detail any additional treatments and/or basic sanitary and precautionary measures on the EAN.<br>A. The EAN will be used as the official Federal authorization of hold. Detail the required treatments and/or basic sanitary and precautionary measures (e.g., biocontainment of suspected infected material, etc.) in the EAN. If the State initiated action, use the appropriate State notification.<br>4. If any other plants in the area are showing symptoms consistent with *P. ramorum*, immediately sample and test those plants for the presence of *P. ramorum*.<br>5. If necessary, when the infected plant is located on the boundary between properties, regulatory controls may be placed on multiple properties. In the event the infected plant is located in a public common area, such as a boulevard or roadside, the regulatory official determines the appropriate area to be placed under regulatory control. |
| 3—Survey the site and perimeter | → | The goal of the survey is to locate **all** *P. ramorum*-infected plants at the site, including the perimeter. A detailed and thorough inspection shall take place at the field level to identify the presence of *P. ramorum*. Collect samples from symptomatic plants, including any plants with minute symptoms such as tiny leaf spots, dropped leaves, or brown leaf tips. |
| | Establish the destruction and quarantine radii (refer to Diagram Showing Destruction Radius, Quarantine Radius, and Delimitation Survey on page 6-1-7) | Determine the destruction radius on a case-by-case basis, but it shall **not** have **less than** a 2-meter radius in total area (refer to rare exceptions in 2-meter D-radius below). If multiple plants are confirmed positive within 2 meters of each other, demarcate a destruction radius around all of them.<br>The destruction radius is established when diagnostic results from all delimiting samples have been reported. The 90-day quarantine period begins when the delimiting survey is complete.<br>1. Observe the slope of the ground on which the confirmed-positive plant(s) are located and note the moisture conditions and likely movement of water on the site. In sloping areas, the destruction radius may be an elliptical shape that angles downslope of the confirmed-positive plant. Determine if the natural and irrigation water movement and moisture conditions support increasing the destruction radius on the downslope side of the confirmed-positive plant.<br>2. Determine the plant debris area.<br>A. If slope **is** a factor, the destruction radius will be the combined area of the elliptical shape and the plant debris area.<br>B. If slope is **not** a factor, the plant debris area may increase the destruction radius **greater than** 2 meters.<br>3. The quarantine radius is a **minimum** of 10 meters beyond the destruction radius and follows the same general shape.<br>4. Limit access to destruction radius/radii. Ensure proper sanitation measures are applied (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15).<br>5. Destroy the *P. ramorum*-infected plants in an appropriate manner as soon as possible (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15). |

**Table 6-1-1 Confirmed Residential Protocol Instructions (page 3 of 4)**

| If step number: | For: | Then: |
|---|---|---|
| 4—Delimiting survey | → | 1. Inspect **all** regulated plants within a **minimum** of a 30-meter radius of the confirmed-positive *P. ramorum* plant(s) and sample any symptomatic plants. Subsequent detections of *P. ramorum* as a result of the delimitation survey will require **all** regulated plants within a **minimum** of a 30-meter radius of the newly detected, confirmed-positive plants to be surveyed and **all** symptomatic plants to be sampled.<br>2. If the infestation is widespread, consult with the PPQ National Operations Manager (NOM) to design and implement an appropriate delimiting survey.<br>3. Document the inspection and map **all** regulated plant locations.<br>4. **All** symptomatic plants shall be sampled, mapped, marked or tagged, and tested.<br>5. Samples **must** be analyzed using the APHIS-approved methodology. |
| | Soil sampling | 1. Take soil samples in the destruction radius at the time of plant removal.<br>2. When selecting sampling locations, take water drainage patterns into consideration and include soil downslope from the plant removal area. Soil within the destruction radius (radii) and the quarantine radius/radii **must** be sampled (refer to Soil and Container Mix Sampling and Processing Protocol on page 11-1-1). |
| | Water sampling | 1. If the source of the infected plant is **not** known, it may be caused by infested water.<br>2. Determine the source of water used at the residential or commercial site. Water sampling is **not** required for chlorinated irrigation water from municipal water facilities. If **not** chlorinated irrigation water from municipal water facilities, bait the water to determine if it is infested.<br>3. The infected plant(s) might spread disease via water runoff. Evaluate the drainage pattern in the area of the infected plant(s). From the point of infection, bait any runoff water as resources permit (refer to Water Sampling and Processing Protocol on page 10-1-1). |
| 5—Disinfest the site | Plant destruction | 1. Plants infected with *P. ramorum* **must** be removed and destroyed (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15). A regulatory official **must** oversee plant destruction.<br>2. Remove the infected plants and root systems as much as possible. Double-bag with appropriate-sized plastic bags to **at least** 4-mil thickness. Larger plants **must** be removed **at least** to the root collar, and the stumps **must** be treated in an APHIS-approved manner to prevent sprouting. Contact the PPQ National Operations Manager (NOM) for guidance.<br>3. Remove and destroy all parts of regulated plants (e.g., branches of larger shrubs or trees) within the D-radius (a 2-meter radius (radii) of a confirmed-positive plant). **Exception**: **bole hosts are less prone to disease; therefore, unless these plants show symptoms, they may be monitored for infection rather than being destroyed at the inspector's discretion.**<br>4. Approved methods of destruction include: incineration; deep burial; and steam sterilization (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15).<br>5. Using the survey follow-up questionnaire (Follow-up Survey for Residential Locations with Infected Plants (page 1 of 2) on page 6-1-13), maintain a record of the taxon and number of plants destroyed at each location. Record the owner's name, contact information, address, and the physical location of any infected plants. Draw a map, record landmarks, or enter the GPS coordinates for follow-up surveys. |

**Table 6-1-1 Confirmed Residential Protocol Instructions (page 4 of 4)**

| If step number: | For: | Then: |
|---|---|---|
| 5—Disinfest the site<br>(cont.) | Debris removal | Rake a sufficiently sized area to collect **all** plant debris associated with the destruction radius and **at least** 2 meters beyond. Double-bag debris, as described above. Rake from the outer edge of the area toward the infected plant(s). **All** debris **must** be destroyed by APHIS-approved methods (refer to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15). |
| 6—90-day quarantine activities | → | 1. Place **all** excepted regulated plants **not** destroyed, but located within the destruction radius under a 90-day quarantine.<br>2. The quarantined plants **must** be inspected and tested twice during the 90-day period. If the plants remain free of *P. ramorum* during this 90-day period, the site will be released from quarantine. The 90-day quarantine **must** occur during a time conducive to the expression of *P. ramorum* symptoms. |
| 7—Release the site | → | Plants from residential or commercial landscape sites placed under regulatory control may be released from that control by PPQ or its designated authority after the quarantine period, **if** the following conditions are met:<br>1. Regulated plants will **not** be replanted within a minimum of 2 meters of the destruction radius for a period of **at least** 2 years; **and**<br>2. There are **no** additional detections of *P. ramorum* on any plants at the site based on APHIS-approved plant inspection, sampling, and testing protocols during the preceding 90-day quarantine period; **and**<br>3. Water and soil have been tested, if necessary, and found free of *P. ramorum* based on APHIS-approved sampling and testing protocols for the preceding 90-day quarantine period. |
| | Alternate release strategy | A residential or commercial landscape site may avoid a quarantine period, through a voluntary management decision, **if** the following actions are taken:<br>1. Performed a delimiting survey and regulated plant genera found free of *P. ramorum*; **and**<br>2. Destroyed everything (**all** plants, pots, media, etc.) in the 2-meter destruction radius according to List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings on page 6-1-15; **and**<br>3. Destroyed the regulated plants in the 10-meter quarantine radii; **and**<br>4. Sampled soil and water from destruction and quarantine radii with negative results.<br>**Revisit the residential or commercial landscape site after approximately 90 days of conducive conditions and conduct a survey inspection of the residential or commercial landscape site to include sampling of the soil in the destruction radius (2-meter area).** |
| 8—Post-disinfestation monitoring | → | Sites that have been confirmed positive will continue to have the regulated plants monitored (inspected, sampled, and tested) when disease expression is anticipated for the 2 years after the site(s) has been released. These sites are **not** under any other regulatory action unless there are additional *P. ramorum* detections. |

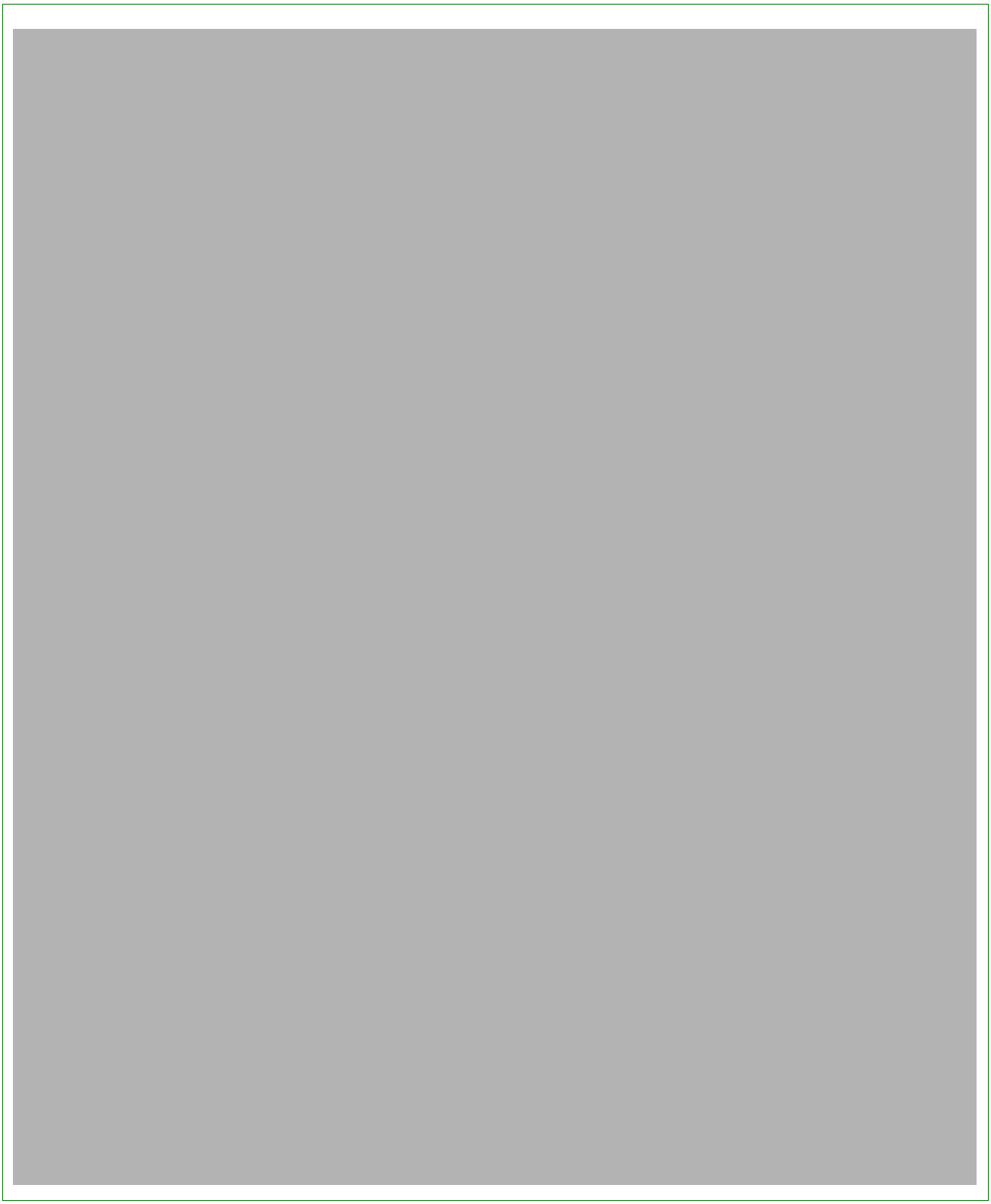

**Figure 6-1-1 Diagram Showing Destruction Radius, Quarantine Radius, and Delimitation Survey**

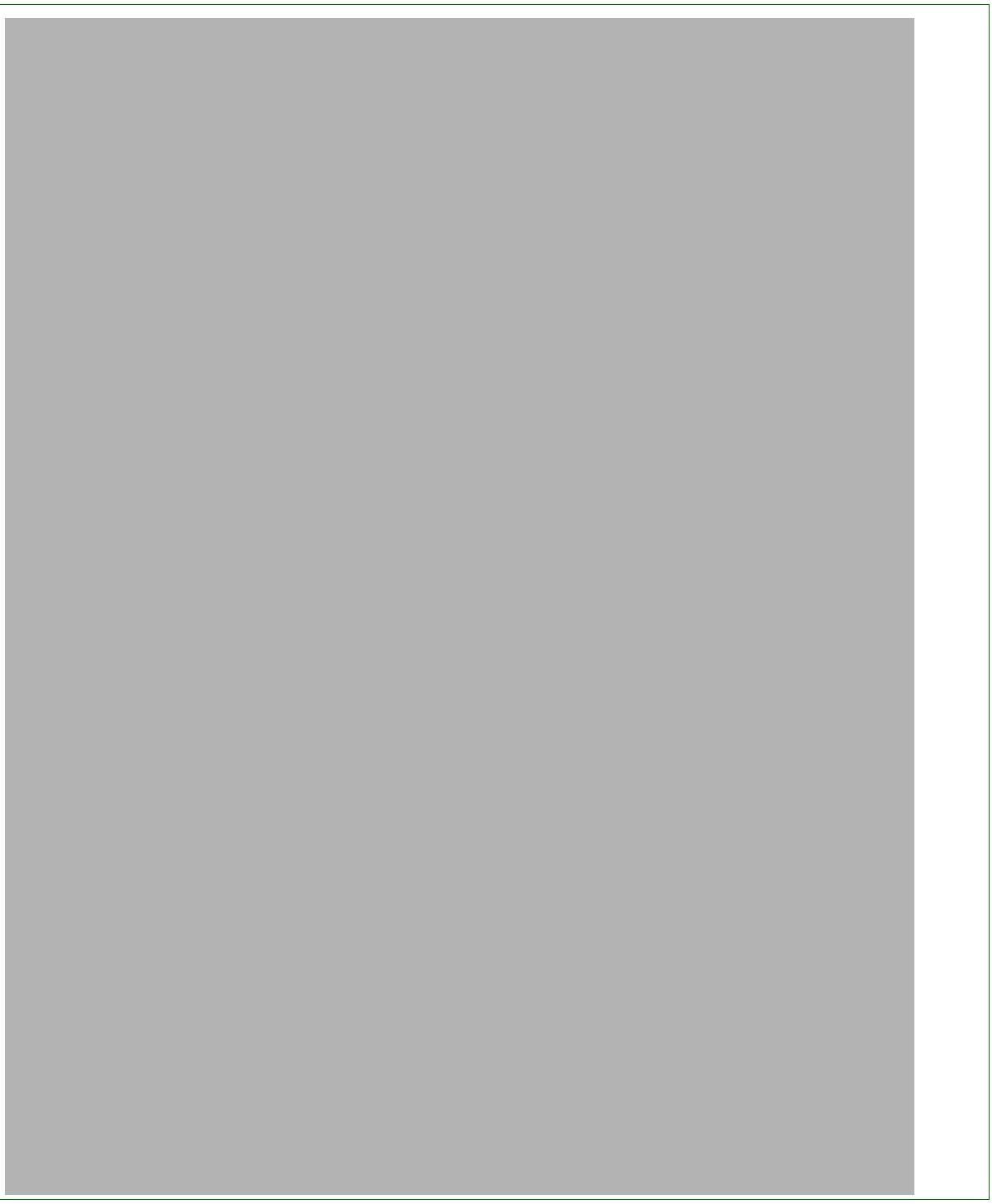

**Figure 6-1-2 *P. ramorum* Nursery Questionnaire (page 1 of 5)**

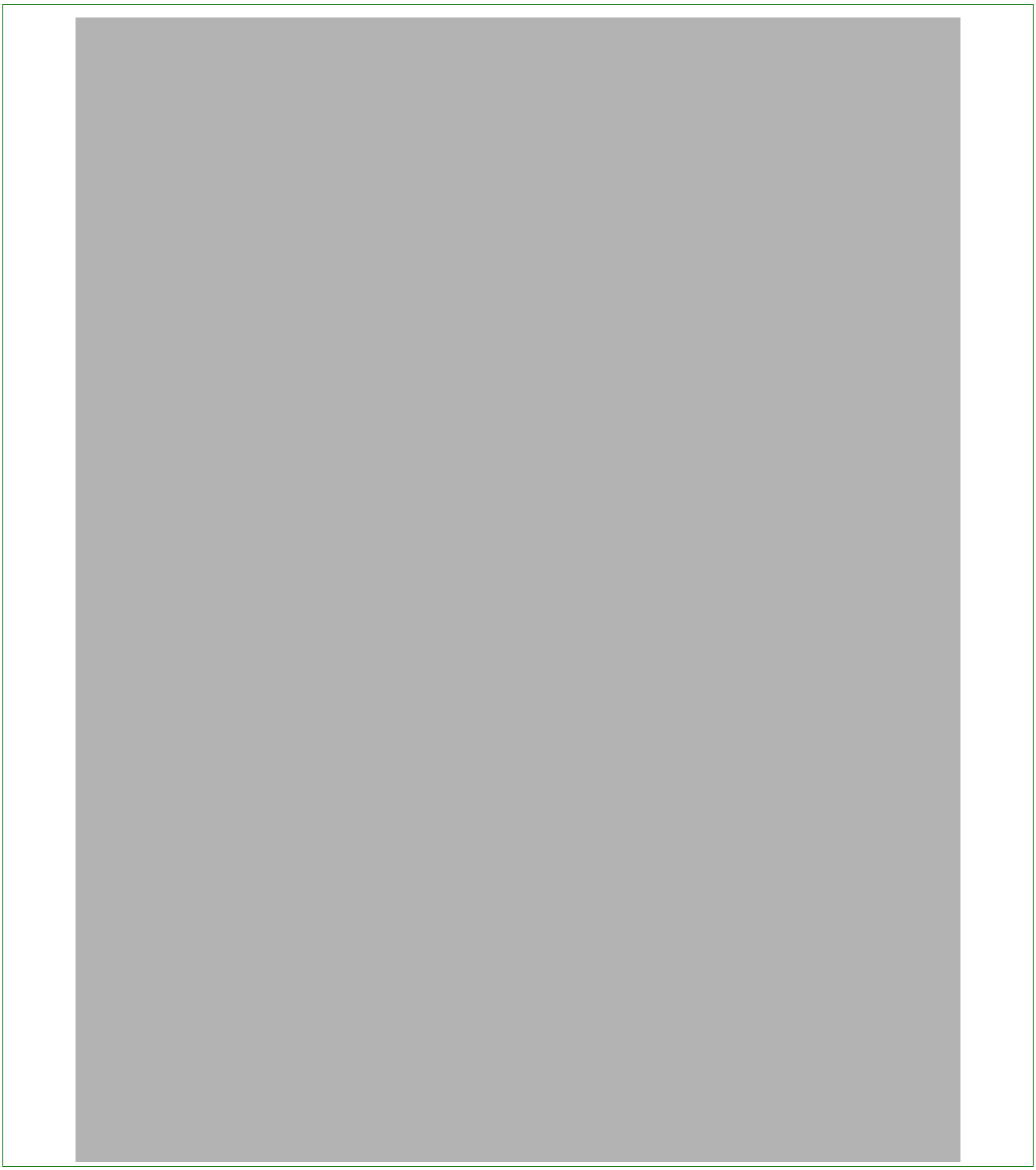

**Figure 6-1-2 *P. ramorum* Nursery Questionnaire (page 2 of 5)**

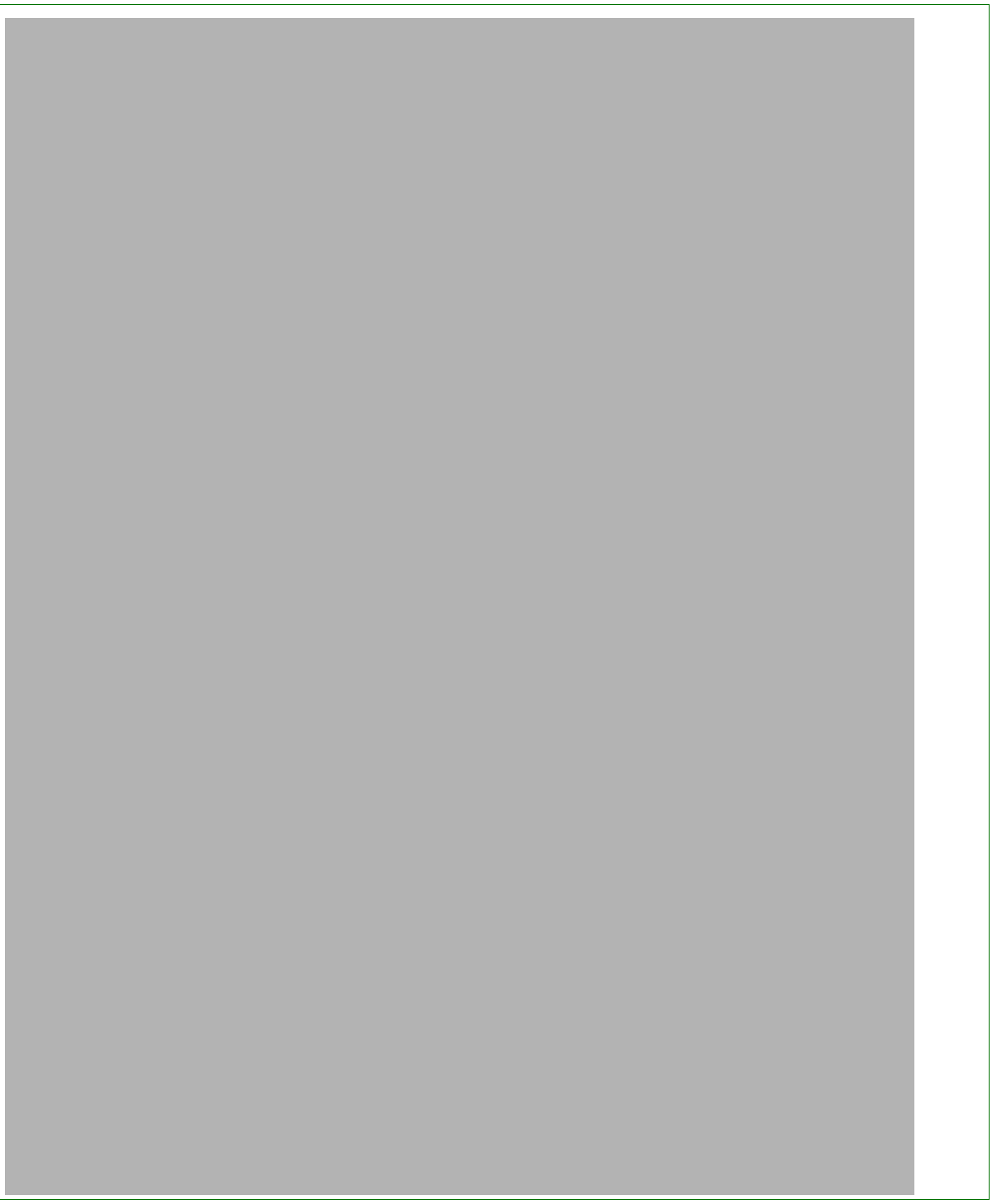

**Figure 6-1-2 *P. ramorum* Nursery Questionnaire (page 3 of 5)**

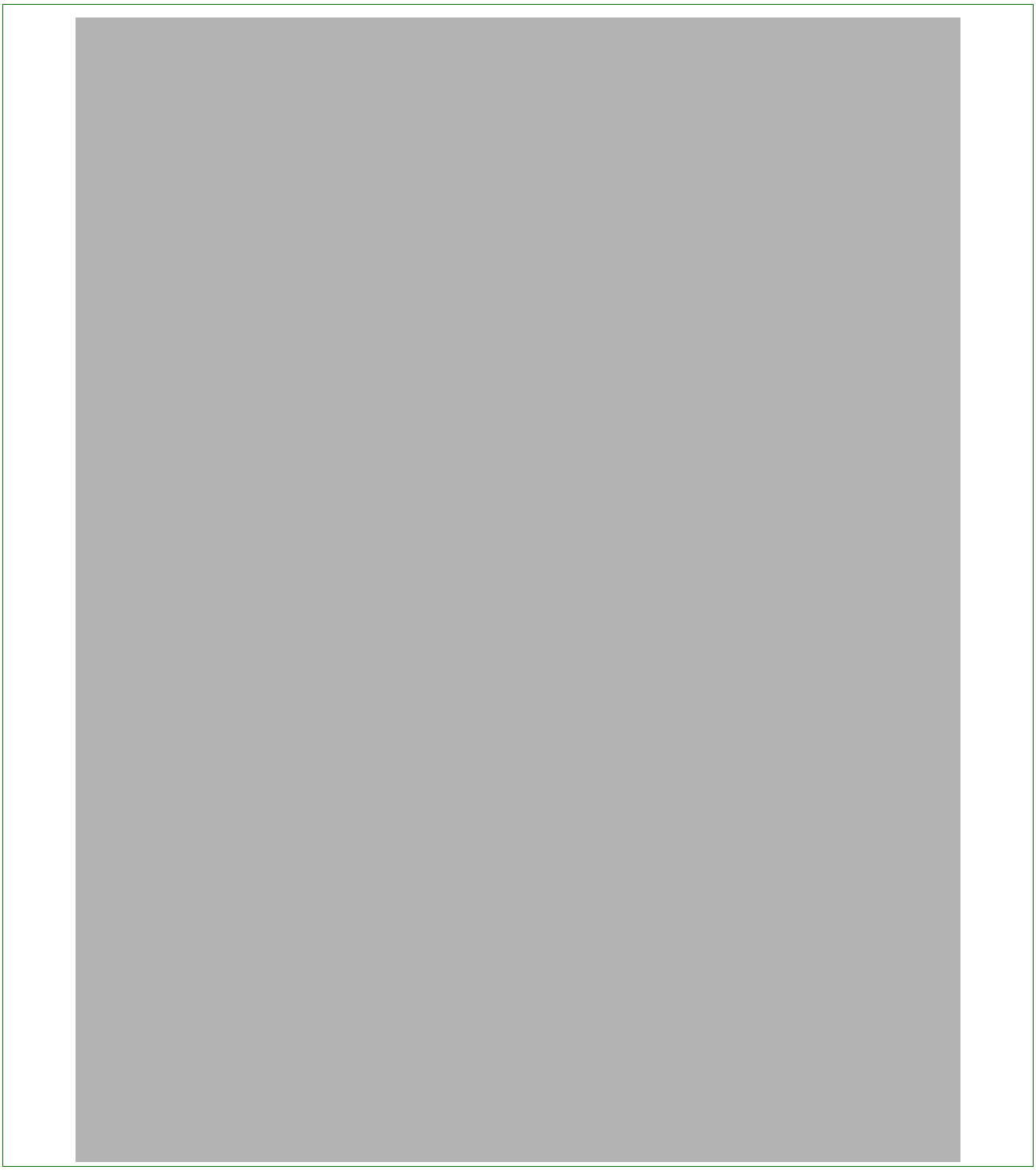

**Figure 6-1-2 *P. ramorum* Nursery Questionnaire (page 4 of 5)**

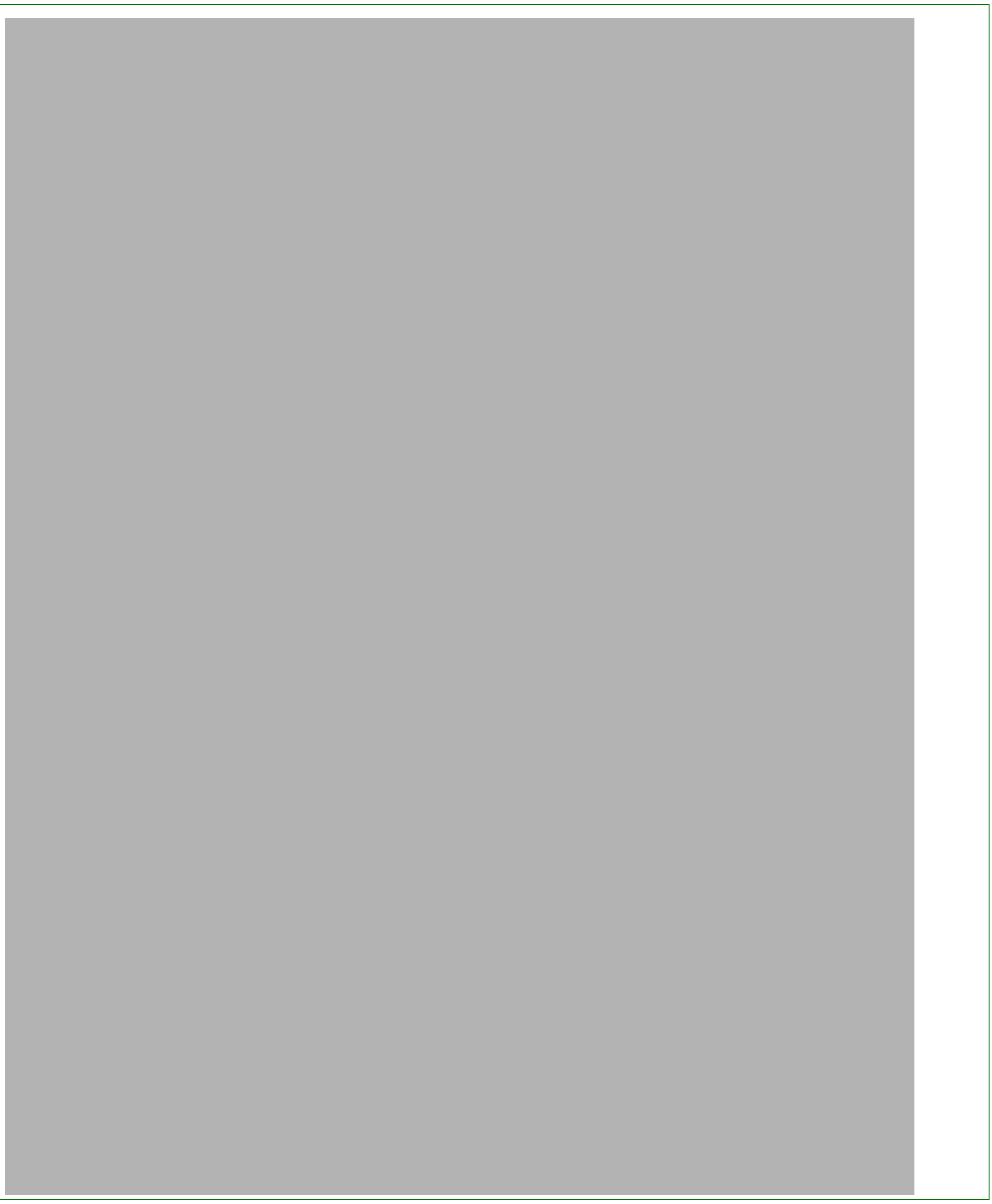

**Figure 6-1-2 *P. ramorum* Nursery Questionnaire (page 5 of 5)**

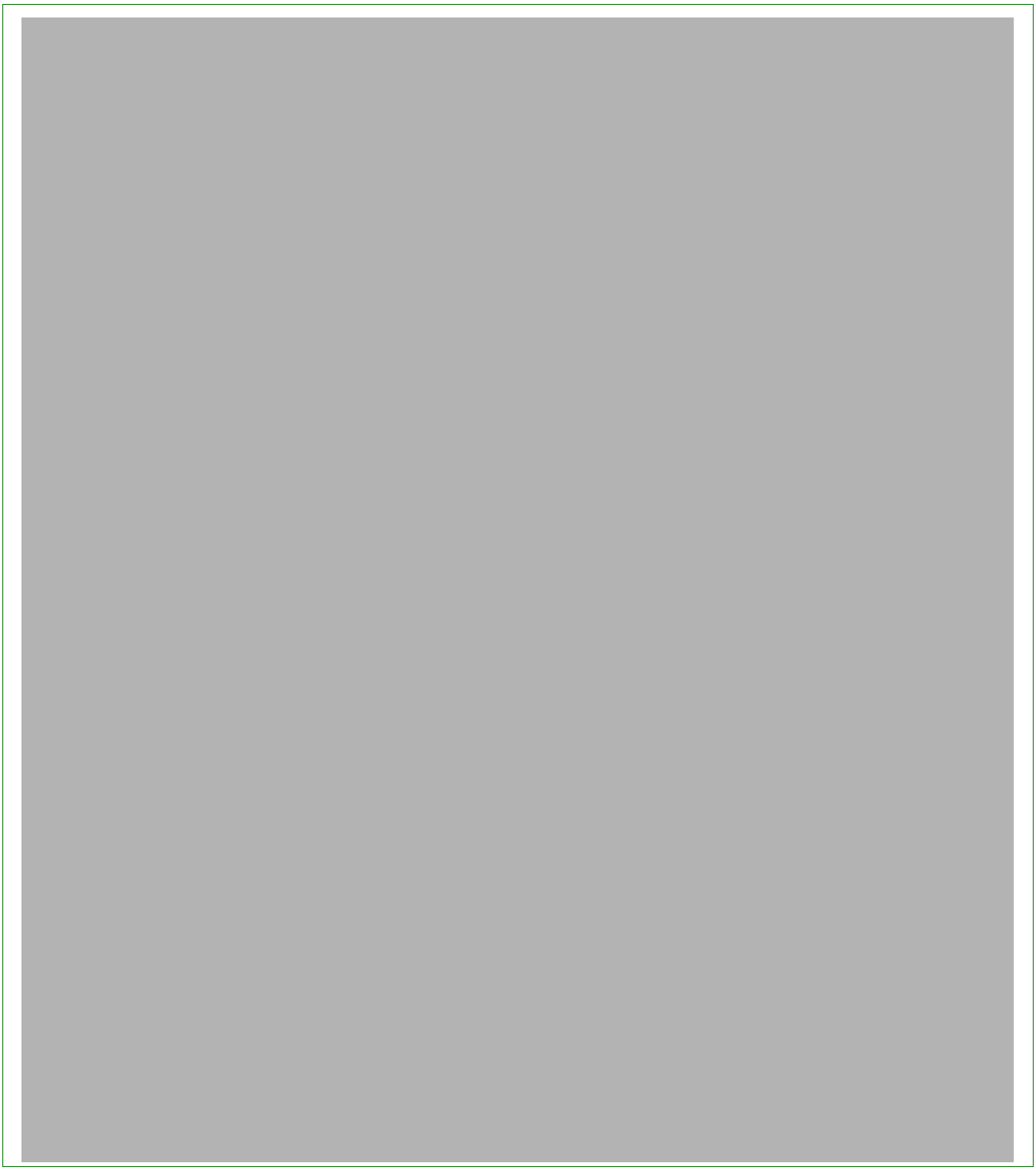

**Figure 6-1-3 Follow-up Survey for Residential Locations with Infected Plants (page 1 of 2)**

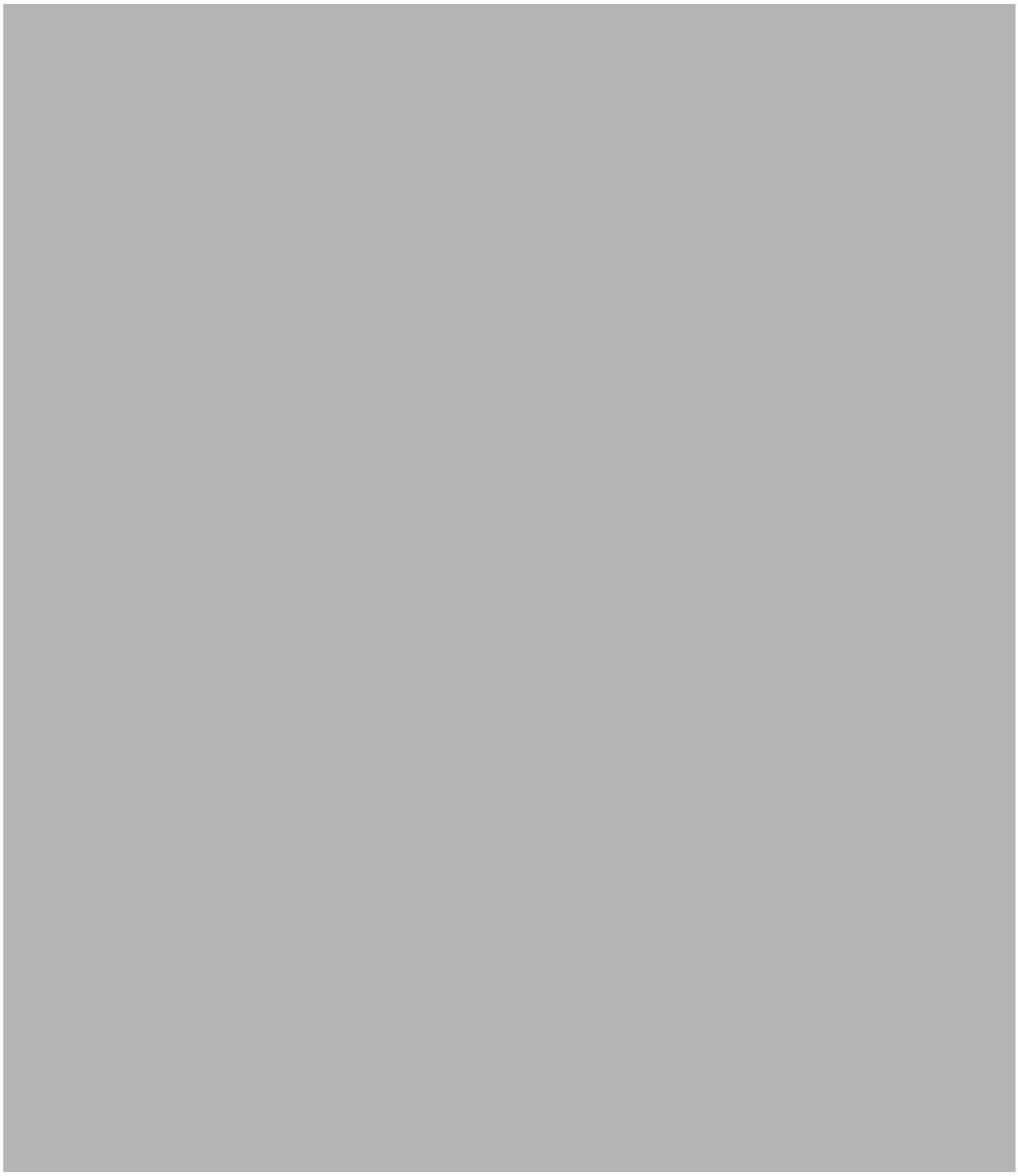

**Figure 6-1-3 Follow-up Survey for Residential Locations with Infected Plants (page 2 of 2)**

# List of Treatments and Disinfectants for Residential or Commercial Landscaped Settings

The following techniques may be used to control *P. ramorum* in residential and commercial landscape sites found to contain infected plants. Prior to use, please confirm the chosen method is approved for your State. Always follow label directions.

**Table 6-1-2 Treatments and Disinfectants for Residential or Commercial Landscaped Settings (page 1 of 2)**

| For: | Then: |
|---|---|
| 1—Confirmed-positive plants | 1. **Incineration (burning to ash)**: infected plants, associated container mix, associated containers (i.e., pots and trays), and **all** leaf debris in and around the area where plants were stored may be incinerated at a facility or other location (e.g., on site) approved by APHIS and permitted within State and municipal statutes or regulations. Properly safeguard off-site movement and take every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration. Burning may be through open burning or in an incinerator.<br>2. **Deep burial**: infected plants, associated container mix, associated containers (i.e., pots and trays), and **all** leaf debris in and around the area where plants were stored **must** be double-bagged using plastic bags to **at least** a 4-mil thickness or greater and buried to a depth of **no less than** 2 meters to the top of the debris. Bury the material at an APHIS-approved site, on site, or in a municipal landfill, which will be undisturbed. Take every effort to prevent plant debris or soil from being dislodged from the plants.<br>3. **Steam sterilization**: dry heat or steam commonly heated to internal temperatures of 212 °F (100 °C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA *Treatment Manual* for "insect pests and pathogens in garbage, Schedule T415-b.<br>4. **Nonporous surfaces**: most disinfectants are **not** labeled for use in soil and are **only** useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants registered for use on nonporous surfaces may effectively reduce populations of *Phytophthora* species. If it is practical, tools such as knives, pruners, water breakers, water wands, and other implements used in the quarantine area should **only** be used in the quarantine area. The Summary of Disinfect Activities (Table 6-1-3 on page 6-1-17 examines the effects of different classes of disinfectants on pathogenic micro-organisms. This list is for explanation and information **only**. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **bold**. All labels for the disinfectants listed below **must** be strictly adhered to for maximum efficacy and environmental and worker safety. |
| 2—Confirmed-positive water | 1. **For dust abatement, fire suppression, and equipment cleaning**: Clorox® (sodium hypochlorite) is labeled (EPA Reg. No. 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora* spp. via water used for dust abatement, fire suppression, and equipment cleaning. The active ingredient level **must** be measured from water collected at the sprinkler head.<br>2. **For irrigation**: chlorine levels of 2 ppm or 2 mg/liter **or greater** has been correlated with the control of *Phytophthora* spp. in recirculated irrigation systems. Recirculated, nonmunicipal water **must** be chlorinated at an active chlorine concentration **equal to or greater than** 2 mg/liter of water and monitored to maintain the proper chlorine levels. |

**Table 6-1-2 Treatments and Disinfectants for Residential or Commercial Landscaped Settings (page 2 of 2)**

| For: | Then: |
|---|---|
| 3—Confirmed-positive soil and container mix | 1. **Container mix**: heat container mix such that the internal temperature in the center of the load reaches **at least** 180 °F for 30 minutes or treat with an approved fumigant. Treatment **must** be conducted in the presence of an inspector.<br>2. **Soil treatment**:<br>A. Heat a load of soil being treated such that the temperature in the center of the load reaches **at least** 180 °F for 30 minutes.<br>B. Conduct field soil treatments in the presence of an inspector and treat with an approved fumigant as per the label.<br><br>If considering the use of solarization for soil treatments, contact the PPQ NOM and USDA–PPQ Science and Technology (S&T) representative (refer to Contact Information for the Phythopthora ramorum Program on page A-1-1). |
| 4—Confirmed-positive containment soil | Mitigation of infested soil in the destruction radius can also be achieved by installing permanent, impermeable, and impervious barriers that consists of cement, concrete, or asphalt 3 inches in depth and extending 6 feet beyond the infested area. Construct these barriers such that **no** native soil is exposed. Grade the barriers such that **no** standing water collects. |
| 5—Equipment and personnel | 1. Limit or minimize access to infested areas and quarantine areas. Everyone entering and leaving the residential or commercial landscape site **must** scrape off loose pieces of soil from their person into the destruction radius. Those working with or in contact with suspected infested material (including plants), **must** wash hands using soap or approved disinfectant immediately after completing the task. There are no products currently labeled for use on porous materials for *Phytophthora* control.<br>2. Conduct activity in the destruction zone wearing disposable shoe covers and dispose of the covers immediately upon exiting the area. Properly dispose of the shoe covers. If shoe covers are **not** used, clean and disinfect shoes with a disinfectant listed in Table 6-1-3 upon exiting the area.<br>3. Tires (or other parts in contact with the soil or plants, such as truck beds) of vehicles **must** be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. If at all possible, do **not** allow vehicles in the destruction radii. Any efficacious product labeled for use on nonporous surfaces may be used on tires or vehicle undercarriages.<br>4. Do **not** visit other sites or areas in potentially contaminated work clothing. |

**Table 6-1-3 Summary of Disinfectant Activities**[1]

| Disinfectant: | Trade names: | Comments: | Contact time: |
|---|---|---|---|
| Alcohols (ethyl and isopropyl) (60% – 85%) | Lysol spray® | Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable | 10 – 15 minutes |
| Phenolics (0.4% – 5%) | Phenocen® | Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue | 10 – 15 minutes |
| Quaternary ammonium (0.5% – 1.5%) | ◆ Consan triple action 20<br>◆ Physan 20®<br>◆ Green-Shield®<br>◆ Formula 409® | Effective for nonporous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels | 10 – 15 minutes |
| Chlorine (100ppm – 1,000ppm) | ◆ 10% Clorox®<br>◆ 10% bleach | Inactivated by organic matter; fresh solutions of hypochlorite (Clorox®) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. Exposure to sunlight further reduces hypocholorite efficacy. Keep solution in opaque container. | 10 – 15 minutes |

1 Modified table from Columbia Research Environmental Health and Safety (EH&S).

Chapter

7

# Biology and Symptoms of *Phytophthora ramorum*

## Contents



## Biology and Symptoms of *Phytophthora ramorum*

### Biology

Hosts of *P. ramorum* usually fall into one of three disease categories based on visual symptoms: canker hosts; leaf hosts; and twig hosts. Infections in leaf and twig hosts are rarely fatal, but act as a reservoir for the pathogen. *P. ramorum* has also been shown to infect roots of host plants without resulting in above-ground visual symptoms. Host plants with latent root infections can remain symptomless for months.

Infected plant debris and *P. ramorum* spores from infected plants, on or under the soil surface, can result in areas remaining persistently confirmed positive for months or years following regulated plant material removal and above-ground litter removal. Recent research in Europe has shown this pathogen can persist in substrate and be detected in water runoff from *P. ramorum*-confirmed-positive sites for up to 5 years.

Pathways for long-distance dispersal of the pathogen include movement of infected plant material (wood, green material products, and nursery stock), soil, water (rain, runoff, streams, rivers, and irrigation water), animals, and aerial dissemination during major weather events.

For more detailed information regarding *P. ramorum* biology, refer to the USDA National Invasive Species Information Center website and the American Phytopathological Society (APS) website.

## Symptoms

Three different syndromes are attributed to *P. ramorum*: stem or bole canker; leaf blight; and twig blight or dieback. Regulated plant material prominent in the nursery trade include *Rhododendron*, *Camellia*, *Viburnum*, *Pieris*, *Kalmia*, and *Syringa*. Symptoms on *Rhododendron* closely resemble those caused by other *Phytophthora* species or those caused by environmental stress (drought, etc.), making inspection for the disease more complicated and detection challenging. With *Lithocarpus* species, drooping or wilting of new growth occurs before other symptoms appear. Cankers typically occur in the lower 3 meters and are restricted to above the soil line. Occasionally cankers have been found 20 meters above ground. Cankers can eventually kill the tree by attacking the phloem and girdling the tree. Bleeding symptoms of the canker are easier to detect during dry weather.

For more detailed information regarding *P. ramorum* symptoms, refer to the APHIS–PPQ *P. ramorum* website.

Chapter

8

# Sampling and Submission Protocol

## Contents



## Sample Handling and Submission Protocol

### Sample Handling Information

Perform the following tasks in order to correctly and accurately handle and submit samples.

1. **Always** write out the identifying label remarks on the **outside** of the bag with a permanent marking method.
   A. Attach labels to the **outside** of bags because labels inside the bag may deteriorate due to moisture and may become illegible.
   B. Include on **all** labels (with a permanent marking method) the following: date; collector's identification number; location of sample site; sample number; and other required information.
2. **Do not** add extra moisture to the sample to keep it fresh. **Do not** wrap leaves in paper towels when shipping. Extra moisture and paper towel use can speed deterioration of the sample.
3. **Sanitize** or remove gloves and place sample bag in a second protective bag. To provide extra insurance against accidental release during shipping, double bag the labeled specimen bag(s), i.e., first place the specimen in a self-locking labeled plastic bag, then place that labeled specimen bag inside a second self-sealing plastic bag. Place PPQ Form 391 Specimens for Determination (or State equivalent) inside the outer bag.
4. **Place samples in a cooler out of the sun as soon as possible**. When sampling large areas, bring coolers out to the sampling areas. In sunlight, samples can heat up quickly when placed in self-sealing plastic bags, even for short periods of time. If it is **not** possible to have coolers in the

sampling area, place the samples in a shaded area until they can be collected and placed in a cooler.

5. **Refrigerate** samples while awaiting shipment. Place double-bagged samples in a sturdy cardboard box or heavy styrofoam container so the samples are not damaged during shipping. Ship with an ice pack with buffer space between the ice and leaves. Thoroughly seal **all** seams on the container with shipping tape. To preserve freshness, mail or deliver the sample(s) to the laboratory **as soon as possible** (if mailing, use overnight delivery). **Do not ship on Fridays**. It is better to hold the sample(s) in the refrigerator over a weekend than to have them sit in unknown environmental conditions.

## Sample Submission Information

Follow the laboratory's standard operating procedure (SOP). Typically, have ready the following required information: 1) tracking number; 2) number of samples being shipped; and 3) the disease for which the sample is being tested. **All** samples **must** have **either** a completed PPQ Form 391Specimens for Determination **or** an equivalent State documentation. The laboratory may be a NPPLAP-accredited State laboratory, a cooperating National Plant Diagnostic Network (NPDN) laboratory, or a USDA–APHIS–PPQ laboratory. If submitters are **not** sure to which laboratory they should send samples, contact the State Plant Health Director (SPHD).

If PPQ Form 391 is electronic, it can be emailed when notifying the laboratory about the pending shipment. **Remember to also attach a hard copy to the sample.** On PPQ Form 391 complete blocks **1 through 5, 7, 10, 11, 16, 22**, and **23** (refer to Figure 8-1-1).

## Sample Forwarding and Reporting

Nursery plant samples that are ELISA or ImmunoStrip positive for the genus *Phytophthora* **must** be forwarded to the cooperating NPPLAP-accredited laboratory and/or to an APHIS diagnostic laboratory to determine if the species is *P. ramorum*. **Every** initial sample from nurseries in nonregulated areas **must** be forwarded to APHIS for confirmatory testing. If APHIS confirms a sample is positive, **all** subsequent samples may be diagnosed by any NPPLAP-accredited laboratory.

For laboratories with Federal confirmation authority, the laboratory **must** report confirmed positives to the SPRO/SPHD, then to the *P. ramorum* Field Operations Manager **within 24 hours of the diagnostic result**. **All** subsequent confirmed-positive samples taken at a confirmed-positive nursery **must** also be reported in the same way.

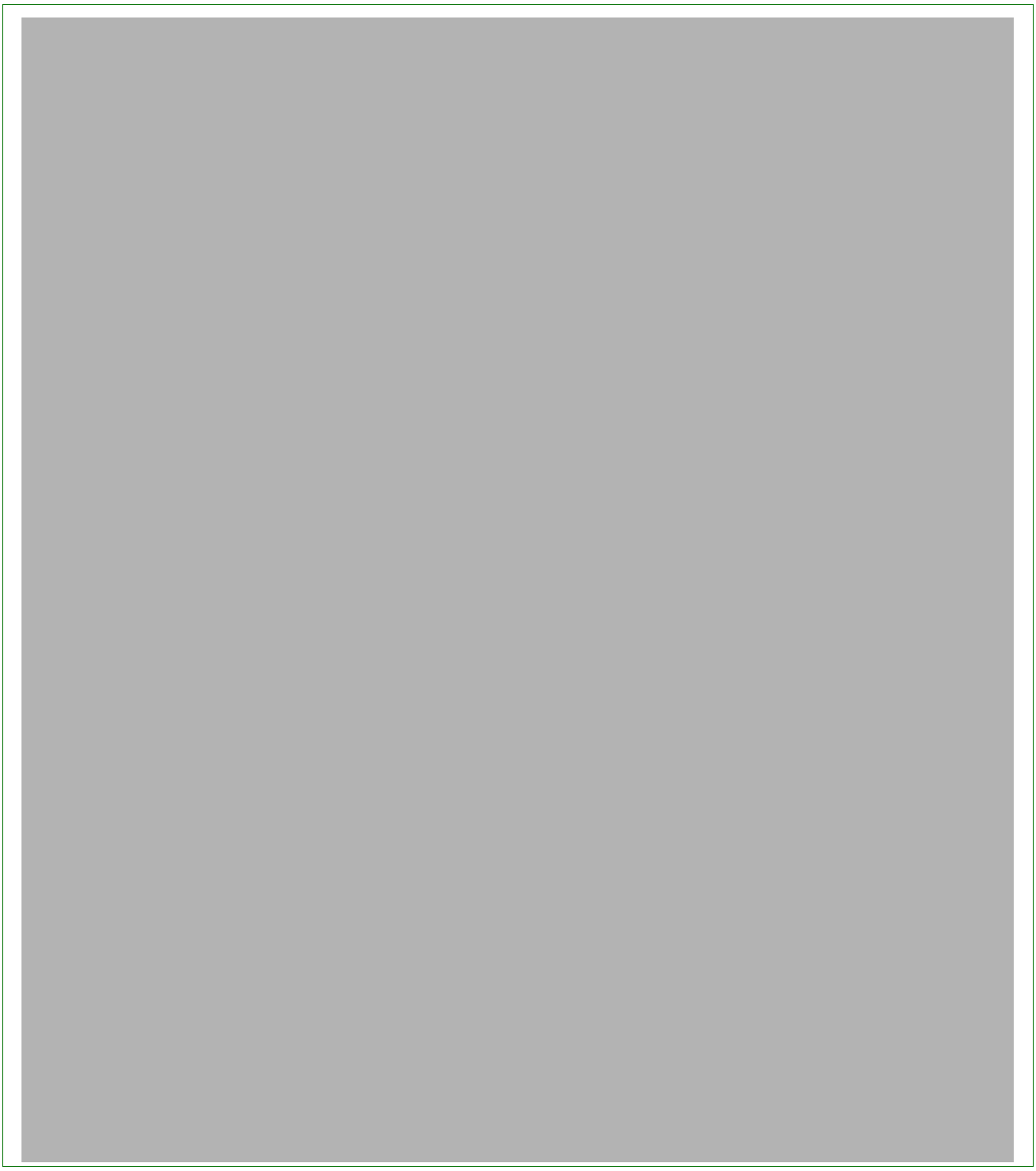

**Figure 8-1-1 Instructions for Using PPQ Form 391, Specimens for Determination**

## Sampling Supplies and Equipment Checklist

- ❒ APHIS Lists of Proven Hosts of and Plants Associated with *P. ramorum*
- ❒ Bigger collection bags in which to carry samples while inspecting and sampling
- ❒ Box for mailing samples
- ❒ Camera
- ❒ Clipboard or PDA, PPQ Form 391 Specimens for Determination (or State equivalent) nursery inspection and sampling forms, paper, etc.
- ❒ Cooler, coolant, and newspapers to keep samples cool until mailed
- ❒ Disposable gloves
- ❒ Flagging, pin flag, or label sticks to mark sampled plants/blocks
- ❒ Foot bath bin
- ❒ GPS (optional)
- ❒ Hand sanitizer to sanitize gloves between samples
- ❒ Larger bags for mailing samples (**must** arrive in the laboratory double bagged)
- ❒ Nursery maps and nursery inventory
- ❒ Permanent marking method
- ❒ Pruners to sample twigs and branches
- ❒ Quaternary ammonium solution or other approved disinfectant, at labeled rates 1” deep in bath
- ❒ Rubber boots
- ❒ Self-sealing plastic sample bags
- ❒ Spray bottle of an approved disinfectant for *P. ramorum*
- ❒ Toilet brush or other stiff brush for scrubbing dirt off shoes
- ❒ Writing pen

Chapter

9

# Biosecurity Measures for Nurseries

## Contents



## Introduction

Decontaminate personnel, tools, and equipment between blocks in the nursery, between regulated genera within a nursery, and between nurseries. Sanitize gloves with antiseptic rubs/gels/rinses (containing a **minimum** of 60% ethyl alcohol and rub hands vigorously to decrease drying time). Wear rubber boots or other waterproof boots without crevices. Sanitize or change gloves between samples. Use spray bottle containing a dilute (10%) bleach solution, a quaternary ammonium solution at labeled rates, or spray disinfectant (with ETOH) to treat all tools between samples. Brush loose dirt from boots then spray boots with disinfection solution in spray bottle, or use foot bath, between nursery blocks/areas. Decontaminate **all** equipment used between each sample and before leaving a nursery.

# Biosecurity Measure for Nurseries

In the course of daily work, nursery personnel are frequently required to visit a number of different nursery sites, greenhouses, fields, and facilities. These actions could potentially provide a pathway for transferring quarantine organisms from one work site to another. It should be recognized that even if a single work site is visited per day, precautions **must** be taken to avoid using contaminated clothing and equipment at a new site the following day. Further, visitors to these same facilities present the same risks and could vector disease-causing-organisms from other sites.

Biosecurity measures must be taken by nurseries and be required of nursery personnel and visitors to avoid and mitigate the spread of *P. ramorum*. The biosecurity measures described here are the **minimum** measures to be taken by the nursery.

# Communications

**All** nursery personnel **must** be trained and visitors informed of the biosecurity requirements enacted by the facility. As new scientific data and technology becomes available, the facility **must** update its biosecurity requirements and retrain its personnel.

# Vehicles

Vehicles can become contaminated with soil; a primary vector for quarantine pests. The following guidelines seek to reduce the likelihood of this pathway.

## Avoidance

Once at the inspection site, if possible, the vehicle should **only** be driven and parked on paved, concrete, or gravel areas to avoid contact with soil and organic matter. Visitors should consider requesting a facility employee to drive them to their designated location in one of the nursery's vehicles. Load nursery stock onto any vehicle, **other than** the nursery's vehicles, in an area with a concrete or asphalt pad located near the gate and **not** inside the nursery.

### Cleaning

To ensure there is no buildup of soil, debris, or other items, clean nursery vehicle interiors. When the vehicle **must** go into a field, the vehicle **must** be driven to the edge of the facility where the tires, wheel wells, and accessible areas of the vehicle's undercarriage **must** be cleaned with a brush or a water hose, followed by a spray down with suitable disinfectant. When the undercarriage has been coated with soil, it is recommended that after cleaning and disinfecting a vehicle at the work site, a vehicle should go through a car wash in order to clean the vehicle's undercarriage **before** proceeding to another work site. If a car wash is **not** available, avoid driving the vehicle to the next work site. To ensure the entire surface of the tires is cleaned it will also be necessary to move the vehicle forward by approximately a foot to clean the portion of the tire in contact with the ground.

Vehicle tires (or other vehicle parts in contact with soil or plants, e.g., truck beds) **must** be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products **before** leaving the infested site. Any product labeled for use on nonporous surfaces may be used on tires or vehicle undercarriages.

A portion of the vehicle **must** be designated as a "clean area" in which clean work supplies and equipment can be kept. A designated "dirty area" of the vehicle, such as the trunk of the car or a specified enclosed area of a truck bed, **must also** be identified for use to hold double-bagged clothes or dirty equipment requiring cleaning. For situations in which pool vehicles are used, adopt a set procedure for **all** personnel.

## Nursery Personnel

Nursery personnel routinely come into contact with potentially contaminated soil, plants, and organic matter requiring the personnel to address a number of biosecurity measures. If the inspection site has distinct levels of biosecurity for different areas in the nursery, it is necessary to work from the areas of lowest to highest risk.

### Access

Access to infested areas and hold areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction radius and use a disinfection spray on foot wear or use a foot bath. Those working with, or in contact with suspected infested material (including plants), must wear gloves and rub them with an approved disinfectant between samples and between blocks. There are **no** products currently labeled for use on porous materials for *Phytophthora* control.

1. Personnel should **not** have access to production areas of the nursery after entering the destruction radius on the same day
2. Place a disinfectant foot bath for use by personnel entering and exiting the quarantine area and entering and exiting the destruction radius at the infested nursery site, where the movement of soil or plant debris on footwear is likely. The foot bath **must** be filled with fresh disinfectant **at least** on a daily basis, or more frequently if contaminated with dirt or debris, in accordance with label directions.
3. Do **not** visit other nursery sites in potentially contaminated work clothing and footwear.

## Boots

Wearing rubber boots is strongly encouraged when working in an infested area(s). Wear waterproof, smooth boots that can be disinfected. As a last resort, disposable waterproof boot covers can be worn over work boots. The rubber boots **must** be disinfected upon arrival, even if the boots were disinfected when leaving the last work site. At the conclusion of any inspection, clean the boots of soil and disinfect them between the infested block and other blocks, as well as **prior** to placing them in the vehicle area designated as a "clean area." Dispose used boot covers by double bagging and placing them in the designated "dirty area" of the vehicle for proper disposal. After removing the boot covers, the soles of the work boots **must** be inspected for soil and, if soil is present, **must** be cleaned and treated with disinfectant.

## Hands

Thoroughly wash hands with soap and water before entering and after leaving the work site. Wet hands with warm running water then lather with soap for **at least** 20 seconds before rinsing and drying hands. When sampling, wear gloves and use antiseptic rubs, gels, or rinses (containing a **minimum** of 70% ethyl alcohol) between samples. To avoid cross-contaminations, disinfect hands or change gloves after handling **any** plants or other contaminated matter in the infested area.

## Equipment

Any equipment used (pruners, measuring tapes, clipboards, pens, etc.) at a work site **must** be disinfected **prior** to leaving the work site. Where practical, equipment should be disinfected as frequently as possible at each work site. Use a spray bottle containing a dilute (10%) bleach solution, a quaternary ammonium solution at labeled rates, or disinfectant spray (with ETOH) to treat all tools between samples. When equipment **must** leave the work site for disinfection, it **must** be double bagged and placed in the vehicle's designated "dirty area."

## Sampling Supplies and Equipment Checklist

- ❒ APHIS Lists of Proven Hosts of and Plants Associated with *P. ramorum*
- ❒ Bigger collection bags in which to carry samples while inspecting and sampling
- ❒ Box for mailing samples
- ❒ Camera
- ❒ Clipboard or PDA, PPQ Form 391 Specimens for Determination (or State equivalent) nursery inspection and sampling forms, paper, etc.
- ❒ Cooler, coolant, and newspapers to keep samples cool until mailed
- ❒ Disposable gloves
- ❒ Flagging, pin flag, or label sticks to mark sampled plants/blocks
- ❒ Foot bath bin
- ❒ GPS (optional)
- ❒ Hand sanitizer to sanitize gloves between samples
- ❒ Larger bags for mailing samples (**must** arrive in the laboratory double bagged)
- ❒ Nursery maps and nursery inventory
- ❒ Permanent marking method
- ❒ Pruners to sample twigs and branches
- ❒ Quaternary ammonium solution or other approved disinfectant, at labeled rates 1” deep in bath
- ❒ Rubber boots
- ❒ Self-sealing plastic sample bags
- ❒ Spray bottle of an approved disinfectant for *P. ramorum*
- ❒ Toilet brush or other stiff brush for scrubbing dirt off shoes
- ❒ Writing pen

Chapter

10

# Water Sampling and Processing Protocol

## Contents



## Introduction

*Phytophthora ramorum* is a soilborne plant pathogen well adapted to dispersal and movement via water. Described as a "water mold," *P. ramorum* is more closely related to algae than fungi. For this reason, water samples collected from potentially infested nursery areas can be tested for the presence of *P. ramorum* spores.

- Contact the National Plant Protection Laboratory Accreditation Program (NPPLAP)-approved laboratory if *P. ramorum* is suspected from **any** samples

- Water filtration is best used in cases where you expect low inoculum levels

1. Bottle of bait or "BoB"—BoB relies on using regulated plant material for "baiting" collected water samples. After a water sample is collected in a 1-liter bottle, an intact leaf and/or leaf pieces from a regulated plant leaf are placed in the bottle as soon as possible. Bottles are incubated at 18 °C to 22 °C in the dark for 3 days, with the leaf pieces tested directly and the intact leaf incubated for symptom development, as described in Sample Processing in Receiving Laboratory on page 10-1-4. This is the preferred method of testing for *Phytophthora* spp. in most nursery settings.
2. Bait bags or bait stations—bait bags containing leaves of *P. ramorum* host plants (e.g., *Rhododendron* or *Camellia*) are used to attract *Phytophthora* spp. This method is used for testing large holding ponds, lakes, streams, and rivers. This method is most effective when water temperatures are between 46 °F (8 °C) and 71 °F (22 °C). After exposure, leaf baits are held for a minimum of 7 to 10 days at 18 °C to 22 °C in a dark, moist chamber to promote disease development and symptom expression.

## In Vitro Water Sampling with Regulated Plant Material Leaf Baits—Bottle of Bait (BoB) Technique

### Bait Selection

1. Collect healthy leaves from a population of *P. ramorum* host plants (e.g., *Rhododendron* or *Camellia*). Bait-source host plant material must **not** have been sprayed with fungicide within the last 3 months. Avoid using newly acquired plants for this reason. Bait-source plants should be sufficiently large, robust, and numerous enough to supply leaves during the entire duration of the survey.
2. Use healthy leaves that have been on the plant for **at least** 1 year and are as free as possible from insect and mechanical damage. Do **not** use succulent, newly formed leaves. Present-year leaf growth may be used after full leaf expansion and a period of hardening in the summer.
3. Place bait leaves in self-sealing plastic bags for refrigerated storage for **no more than** 14 days before use.

### Sample Collection

1. The number of water samples collected is based on the number of water bodies present, availability of run-off water, and the overall size of the nursery. If this sampling method is selected, collect a minimum of one 800-ml sample per sampling site. More samples will be needed for larger nurseries with more water and irrigation and drainage sources.

2. Collect 800 ml of water from each sample point and record water temperature. If possible, sample should be collected from upper surface of the water; collect the cleanest sample possible by minimizing sediment disturbance, while avoiding plant and other floating debris. Use a 100-ml measuring cup or disposable paper cup to fill a 1-liter screw cap plastic bottle in increments rather than filling the container all at once. Collect 100 ml of water from each sample point; use 8 different sample points, which would total 800 ml.
3. Record and mark the location of the sample site. If possible, record GPS coordinates or write a description of the sample collection location for each sample.
4. Affix identification labels (e.g., laboratory tape or masking tape) **both** to the screw cap **and** the outside of each water collection bottle using a waterproof marking method. Sufficiently code labels to correspond with datasheet entries for each nursery and water body and include date collected, water source (location), a unique sample number, and nursery (e.g., nursery name or license number).
5. When sampling drainage ditches or areas of moving water within nurseries, rinse bottles downstream with the water about to be sampled **before** water is collected.
6. Immediately bait each sample with 20 freshly punched leaf pieces, and 1 healthy, intact host plant leaf. This must be done on site, using a hand-held hole punch to get uniform leaf pieces. Sometimes, the punched pieces can become too degraded or have too many other organisms growing on them to successfully isolate *P. ramorum*, if present. Because of this, the whole leaf is also used.
7. Maintain collected water samples on their side in a cooler (without ice if external temperatures are cool or with enough ice to gradually cool water samples) for transport to the sample processing laboratory. Pack and ship water samples with the host plant leaf baits via overnight mail with a cold pack or drive the samples directly to the laboratory.
8. Maintain a record of the water sample information. Assign a unique sample number to each bottle.
9. Wash each 100-ml measuring cup with warm, soapy water or use a new disposable cup between sample collections. Thoroughly and completely rinse each item. For best results, use an automatic dishwasher with a heated drying cycle or an autoclave to wash collection bottles. Contact the receiving laboratory to determine if they will provide samplers with clean 1-liter water collection bottles.
10. Prepare enough cup/bottle sets for water collection at a number of sample sites per nursery. Use only clean, sanitized collection materials at each site and water source.

## Sample Processing in Receiving Laboratory

Email APHIS–PPQ–Plant Pathogen Confirmatory Diagnostics Laboratory at: PPQ.CPHST.Beltsville.Diagnostics@usda.gov for the latest version of the *P. ramorum* diagnostic protocols.

1. Place bottles containing water samples and leaf baits on their sides and incubate for 3 days at 18 °C to 22 °C in the dark.
2. Following incubation, remove baits (leaf pieces and whole leaf), rinse them in distilled water, and blot dry. Immediately process leaf pieces for detection, but place whole leaf in a moist chamber in the dark for a **minimum** of 7 to 10 days at 18 °C to 22 °C to promote disease development and symptom expression. In the event that no lesions develop, the sample can be considered negative and discarded.
3. If symptoms appear on the incubated whole leaf, process the leaf using the same methods as for nursery leaf samples. The approved molecular diagnostic work instructions **must** be followed for these regulatory samples.
4. Leaf pieces can be placed into selective PARPH-V8 medium to isolate *P. ramorum* and then tested using approved molecular diagnostics, or they can be tested directly using the USDA-approved PCR protocols. Leaf pieces that are to be tested directly can also be frozen and held for testing at a later time. The pieces from one sample can also be split with a portion of them being tested through PCR and the remainder either going to plating and/or freezing.
5. If symptoms appear on the incubated whole leaf, process the leaf using the same methods as for nursery leaf samples.
6. Transfer the isolation plates immediately to a State or Federally approved processing laboratory via overnight courier **OR** maintain the agar plates at 20 °C in the dark for **at least** 3 days.
7. At regular time intervals, using an inverted or dissecting microscope under low magnification, check the plates for colonies with typical morphological characters of *P. ramorum* (e.g., coralloid hyphae, semi-papillate sporangia, and large chlamydospores).
8. Contact laboratory personnel if *P. ramorum* is suspected from **any** samples.

## Bottle of Bait (BoB) Materials List

- Bait leaves in plastic bag kept in a cooler until needed
- Hand-held paper hole punch (heart-shaped preferred)
- Ice chest cooler (with a small amount of ice or other refrigerant if temperatures are warm outside)—isolate ice from sample with newspaper

- Multiple 100-ml plastic measuring cups or beakers and large-capacity (100 ml) syringe per water sample site—disposable paper cups are also suitable for collecting water
- One-liter bottles per sample site

**Figure 10-1-1 Bottle of Bait (BoB) Materials and Leaf Pieces Plated into PARPH Media[1]**

1 Photos courtesy of Steve Oak, USDA–Forest Service, Southern Region FHP and Dr. Craig Webb, USDA–APHIS–PPQ.

# In Situ Water Sampling with Regulated Plant Material Leaf Baits

## Bait Selection

1. Collect healthy leaves from a population of native or naturalized *Rhododendron* spp*., Camellia* spp*., Viburnum* spp., or other hosts that have susceptible responses to *P. ramorum*. Source-regulated plant material must **not** have been sprayed with fungicide within the last 3 months. Avoid using newly acquired regulated plants for this reason. Bait-source plants should be sufficiently large, robust, and numerous enough to supply leaves during the entire duration of the survey.
2. Use healthy leaves that have been on the plant for **at least** 1 year and are as free as possible from insect and mechanical damage. Do **not** use newly formed, succulent leaves. Present-year leaf growth may be used after full leaf expansion and a period of hardening in the summer.
3. If bait leaves are **smaller** in size than 8 cm x 3 cm (3.2” x 1.2”) at the widest point, use 8 leaves at each sampling location (1 in each mesh bait bag). If leaves are **larger** than this dimension, 4 leaves per site can be used.
4. Bait leaves may be stored wrapped in **dry** paper towels and sealed in self-sealing plastic bags for refrigerated storage for **no more than** 14 days before use. Dry towels help prevent leaves from breaking down during storage.
5. Place four to eight leaves with the petioles (the stalk-like tissue that attaches the leaf to a stem) attached into each container (depending on leaf size as discussed above) at each sampling site. **Insert a uniquely numbered plastic tag into each bait bag for identification**. On a datasheet, record the dates (when bait was established and when bait was collected), water source (location), nursery information (i.e., nursery license number), tag number, water temperatures (initial temperature when bait was established and final temperature when bait was collected), and GPS coordinates. If forwarding these samples to a confirmatory laboratory, this information will be necessary to complete a PPQ Form 391 for specimens for determination.

## Baiting Techniques

### Bait Bags

Bait bags (approximately 12” x 12”) should be constructed of a durable, coarse nylon mesh material (e.g., non-wire window screening) and fastened together on 3 sides to allow sufficient overlapping material to seal bag edges (refer to Figure 10-1-2). Single-use bait bags can be fashioned from muslin. Bait bags **must** have a separate pocket for each leaf, even if several small leaves are being used. This maximizes the surface area in the water. Exact configuration is **not** crucial and any bag type that can be closed and securely fastened (drawstring, flapped, rolled, etc.) is sufficient. Once leaves and numbered plastic tag are placed into the bag, secure the bag so it cannot float out and away.

**Figure 10-1-2 Example of a Bait Bag Construction**

1. Firmly secure each bag by tethering it to a stake driven into the ground or by suspending it from a rope that spans the width of the watercourse or pond. Bags should float near or just below the water’s surface for 7 to 14 days depending on water temperature (8 °C to 22 °C). Suspend baiting when water temperatures exceed 22 °C. If necessary, water can be tested using the BoB method. Place bait bags in an area where water flows more slowly and pools. Locate the bags such that the leaves remain submerged even if water levels fluctuate. Do **not** leave the bait in the water for **more than** 7 days if the water temperatures are at the higher range (22 °C) as the leaf tissue will degrade and baiting efficacy will be sharply reduced.

**Table 10-1-1 Temperature Versus Days for Floating Baits in Water**

| If the temperature is: | Then: |
|---|---|
| 22 °C | Leave in water for 7 days |
| ≥ 8 °C, but < 22 °C | Leave in water for 7 to 14 days |

2. When possible, choose shaded locations.
3. Record the water temperature.
4. When placing bait bags in retention ponds, give priority to inflow and outflow points, preferably in shaded areas. Deploy a **minimum** of two bait bags per pond.

### Bait Stations

An alternative to bait bags is a bait station, which consists of an enclosure constructed from a PVC frame with plastic cable ties attaching half-inch plastic fencing material (refer to Figure 10-1-3 and Figure 10-1-4 for photo and construction details). Leaves are attached with binder clips secured to the bottom of the enclosure. The numbered plastic tag is placed inside the enclosure **before** sealing.

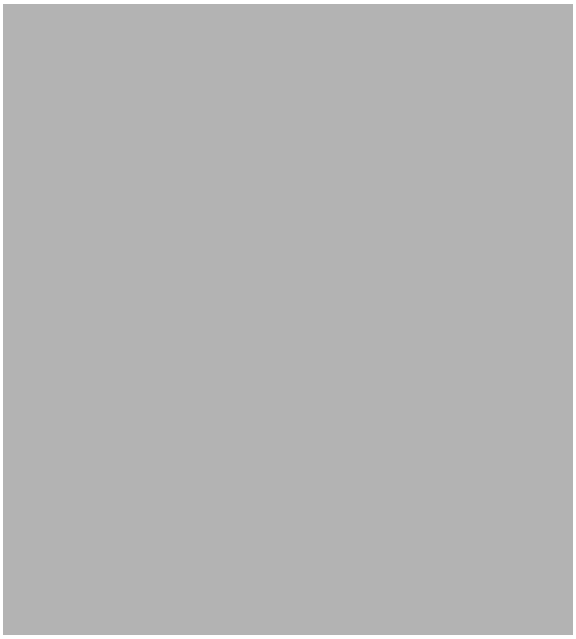

**Figure 10-1-3 Pattern of Mesh Screen to Use for Constructing a Bait Station**[1]

1 Photo courtesy of Dr. Steve Jeffers, Clemson University.

**Figure 10-1-4 Completed Bait Station Construction**[1]

1 Photo courtesy of Dr. Steve Jeffers, Clemson University.

1. Attach the station to a stake driven into the ground or by suspending it from a rope that spans the width of the watercourse or pond. Deploy the station for **at least** 7 days (refer to Table 10-1-1).
2. When possible, choose shaded locations.
3. When placing bait stations in retention ponds, give priority to inflow and outflow points located in shaded areas. Deploy a **minimum** of two bait stations per pond.

*Bait Station Construction Details—Frame.*

1. Frame is made using 1/2-inch diameter PVC pipe and four elbow joints.
2. Cut four lengths of PVC—two 11 1/2-inch and two 10 1/2-inch lengths.
3. Glue pipes and joints into a rectangular shape using PVC primer and cement.

*Bait Station Construction Details—Mesh.* The mesh used is a plastic, 1/2-inch fence material (with 3/8-inch openings) available at any national home improvement chain store.

1. Dimensions are given both in inches and are also based on number of squares (refer to Figure 10-1-3).
2. Secure mesh to the frame using plastic cable ties.
3. Leave one side of mesh half secured until leaves are inserted.
4. Tie four small binder clips into mesh using plastic-covered twist ties; these clips hold leaves in place by the petioles. This maintains separation between bait leaves, allowing for maximum water flow exposure for each bait tissue (refer to Figure 10-1-4).

### Bait Retrieval

1. After 7 to 14 days, depending on water temperature (8 °C to 22 °C), remove bait leaves (and the numbered tag) from each bag or station and rinse using water from the stream or pond, thereby reducing the foreign matter (organic and soil particles) on the bait leaves.
2. Wrap leaves in a 1-gallon, self-sealing plastic bag. Be certain to place the numbered plastic tag from **each** bait bag into the plastic bag of the corresponding leaf bait tissues. Double bag the samples to prevent contamination or desiccation in the event a bag ruptures.
3. Place **all** sample bags in an insulated cooler with cold packs for transport to the laboratory. Do **not** place bait samples directly on the ice or cold pack; cardboard can be used to separate the ice from the bait samples.
4. Do **not** leave bags exposed to direct sunlight or in hot conditions for an extended length of time before placing into a cooler. Clear plastic bags can solarize and ruin leaf bait samples if left in direct sunlight. Leaf bait samples **not** placed in a cooler first can also be negatively affected if left in the hot interior of a vehicle.
5. Record the date of bait retrieval as well as the water temperature at time of retrieval.
6. Following each use, clean bait bags with either 95% ethanol or a 10% household bleach solution. To decontaminate, bait bags should be either sprayed until runoff occurs or soaked. To ensure adequate time to decontaminate and dry, wait **at least** 4 hours **prior** to reuse. Upon completing the cleaning process, thoroughly rinse bags with chlorinated tap or sterile water. Check for signs of deterioration and bag failure and replace bags accordingly.

## Sample Transport, Storage, Shipping, and Processing

1. Keep samples in a cooler with a cold pack or in a refrigerator until shipped. Do **not** permit the samples to freeze or dry out. Before shipping, contact laboratory personnel to coordinate sample reception and processing. Pack leaf bait samples **just prior** to shipping via overnight courier. Do **not** prepare and store packed samples if they will not be shipped immediately.
2. Laboratory personnel should process bait samples using the same methods as for nursery leaf samples.

# Water Sampling for Filtration

## Sample Collection[1]

**NOTICE**

Samples should be processed **within** 8 hours of collection to optimize detection of *Phytophthora* spores. Samples will begin to degrade or decline as a detection tool after storing for 12 hours **or more**.

1. Record and mark the location of the sample site. If possible, record GPS coordinates for each sample. When feasible, record the water temperature.
2. Affix identification labels (time tape or masking tape) **both** to the screw cap **and** the outside of each water collection bottle using a waterproof marking method. Sufficiently code labels to correspond with datasheet entries for each nursery and water body and include date collected, water source (location), a unique sample number, and nursery (e.g., nursery name or license number).
3. When sampling drainage ditches or areas of moving water within nurseries, rinse bottles downstream with the water about to be sampled **before** water is collected.
4. Maintain collected water samples in a cooler.
5. Maintain a record of the water sample information, assign a unique sample number to each bottle.
6. Between sample collection trips, wash each 100-ml measuring cup and 1-liter bottle with warm, soapy water. Thoroughly and completely rinse each item. For best results, use an automatic dishwasher with a heated drying cycle. Care should be taken when using soap and/or bleach as the residue could potentially affect the retrieval process.
7. Prepare enough cup/bottle sets for water collection for a number of sample sites per nursery. Use **only** clean collection materials at each site and water source.

## Sample Processing

Most water samples can be vacuum filtered through polycarbonate membrane filters with 3-µm pores. Turbid or muddy water samples will need to be filtered through membrane filters with 5-µm pores. Typically, a **minimum** of 10 plates and 10 filters will be required if 100 ml of water per filter are used. Surveyors should always take extra plates and filters to ensure they have enough in the field.

1 Water sampling for filtration is an optional water sample protocol.

1. Place a filter funnel into a filter flask with a capacity of **at least** 1 liter and connect the flask to a vacuum source using plastic tubing; use a second filter flask as a trap between the flask with the funnel and the vacuum source (i.e., electric vacuum pump or a hand vacuum pump) (refer to Figure 10-1-6).
2. Wet the filter holder with distilled water and place a polycarbonate membrane with the shiny side up or a polyvinylidene fluoride membrane with smoothest side up. Ensure the paper between the filters has been removed, the filter is aligned over the perforated area of the funnel, and the filter is **not** wrinkled. Assemble the filter funnel and clamp it in place.
3. Thoroughly mix the water sample by inverting the plastic bottle and/or swirling. Pour 100 ml of sample into the funnel. If the water is highly turbid, 100 ml may not be completely filtered by a single filter and smaller volumes should be used per filter to complete the sample. Conversely, if the water is extremely clear, additional water (up to 200 ml) may be processed by a single filter. More than 1 filter will be necessary to completely filter the 1-liter sample.
4. Initially, turn on the vacuum source at a low setting to filter water subsamples and adjust as necessary. Turn off the air **just prior** to complete filtration of each subsample to avoid building up excessive vacuum pressure, which could damage *Phytophthora* spores.
5. Rinse the inner surface of the funnel with distilled water to wash down any spores onto the filter that may be on the funnel wall. Briefly apply the vacuum to remove excess water.
6. Gently remove sizable organic debris or soil particles trapped on the surface of the filter if it will prevent complete contact of the filter with the surface of PARPH-clarified V8 agar contained in petri plates. Using forceps, gently lift the filter from the filter funnel and invert it so the collection side contacts the media surface. Smooth the filter with the forceps to remove air bubbles that may have formed between the filter and the agar media surface (refer to Figure 10-1-6).
7. Repeat the above steps until the entire 1-liter water sample has been filtered. A **minimum** of 10 agar plates per collection bottle should be produced resulting from filtration if 100 ml of sample is used per filter. Always have additional filters and plates prepared as the number required per sample can vary.
8. Rinse the filter funnel assembly and forceps under hot, running tap water after each sample to avoid cross-contamination between samples. Do **not** disinfest the funnel with a bleach solution or alcohol as any chemical residue may affect spore viability.
9. Place plates upside down in a plastic box or zip-to-close bag to prevent desiccation; incubate plates at 16 to 22 °C (64 to 71 °F) in the dark for 10 to

14 days. Because *P. ramorum* can grow slowly in culture, additional incubation up to 28 days is optional. A designated incubator works best, but a closed cabinet in an air-conditioned room can also be used.

10. Using a dissecting or inverted microscope, examine plates frequently (starting 2 days after baits have been plated) for colonies resembling *P. ramorum*—i.e., those with typical coralloid hyphae, large golden chlamydospores, and packets of semi-papillate sporangia on the surface (refer to Figure 10-1-6); mark these with a permanent, waterproof marking method.
11. *P. ramorum* hyphae are often visible 2 to 5 days after baits have been plated. However, because *P. ramorum* can grow slowly in culture, it may not be recognizable until several weeks after baits are plated. Patience, persistence, and good observational skills are often the keys to finding *P. ramorum* on the isolation plates. *P. ramorum* grows slower than most other species of *Phytophthora* commonly found in water, leaving the filters on the agar plates for a 3-day incubation period is critical for recovery of this species.
12. After the incubation period, remove the filters with sterile forceps and gently rinse the surface of the agar medium with running tap water to wash off small particles and bacterial colonies that may interfere with microscopic observation. Filters and rinse water should be treated and properly discarded to eliminate any risk associated with *P. ramorum*.
13. Under low magnification and using an inverted or dissecting microscope, check the plates at regular intervals for colonies with typical morphological characteristics of *P. ramorum* (e.g., coralloid hyphae, semi-papillate sporangia, and large chlamydospores).
14. Subculture isolates to fresh PARPH-V8 and then to PAR-V8.
15. If morphology or PCR testing does **not** indicate a positive *P. ramorum* by 14 days of culture, the sample is considered negative for regulatory purposes. The culture can be disposed of in the manner described in Treatments and Disinfectants on page 12-1-1.

## Additional Guidance on Culture and Identification

1. Be sure to culture mostly green tissue at the margin of the lesion, and **not** too much brown, dead tissue.
2. If the initial culture media is PARP-H, take care to adjust the concentration of Hymexazole. Adding too much Hymexazole to the media will inhibit *P. ramorum* growth.
3. If a surface sterilization step is added, do **not** oversterilize leaf pieces, 5% bleach solution for 30 seconds is generally sufficient for this purpose.
4. The culture conditions should be at a constant temperature between 16 and 22 °C (64 to 71 °F) with inverted plates placed in the dark.

5. Cultures should be checked up to 10 to 14 days for hyphal growth and morphological identification. Real-time PCR can be attempted on hyphal growth suspected to be *P. ramorum*.
6. Additional steps and procedures can be carried out in attempts to isolate *P. ramorum*, but this is **not** required for regulatory purposes. However, if *P. ramorum* is morphologically identified using these extra procedures, the sample is a presumptive positive (refer to below).

## Diagnostic Determination for Cultures

1. If the bait or mycelia growing from the bait is tested positive by PCR in a NPPLAP-accredited laboratory and it qualifies as a Potentially Actionable Suspect Sample (PASS), the DNA can be forwarded to the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory. If it is a **non**-PASS, the sample is considered positive for regulatory purposes.
2. If the culture is morphologically identified as *P. ramorum*, regardless of PCR results, the sample is determined as presumptive positive. If the original sample is a PASS, the culture **must** be confirmed at the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory according to the instructions in this program manual.
3. If the key morphological features of *P. ramorum* are clearly observed, attempt to transfer to a fresh plate for singular isolation. If **no** morphology is observed, PCR can be attempted.

**NOTICE**

If the initial *P. ramorum* identification is **not** made at the laboratory, transfer the plates immediately to a State or Federally approved processing laboratory via overnight courier. Do **not** permit the samples to freeze or dry out at any time. Before shipping, contact laboratory personnel to coordinate sample reception and processing. If you do **not** have contact with an appropriate laboratory, notify the S&T representative (refer to Contact Information for the Phythopthora ramorum Program on page A-1-1) to arrange for culture forwarding and identification.

## Disposing of Cultures After Diagnosis

1. Please contact the *P. ramorum* program via email at P.ramorum@usda.gov for instructions on the final disposition of non-PASS cultures. These cultures may be found useful for regulatory research or methods development needs. If the required PPQ permit is in place, arrangements can be made to forward cultures to the research and method development scientific community. If **not** needed for this purpose, the cultures can be disposed of as instructed in Treatments and Disinfectants on page 12-1-1.
2. If morphology or PCR testing does **not** indicate a positive *P. ramorum* by 14 days of culture, the sample is considered negative for regulatory purposes. The culture can be disposed of as instructed in Treatments and Disinfectants on page 12-1-1.

3. Place plates upside down in a plastic box or zip-to-close bag to prevent desiccation. Incubate plates at 15 to 20 °C (59 to 68 °F) in the dark for **up to** 28 days. A designated incubator works best, but a closed cabinet in an air-conditioned room can also be used.

**Figure 10-1-5 Characteristic Structure of *P. ramorum*[1]**

1 Photos courtesy of Dr. Steve Jeffers, Clemson University.

**Figure 10-1-6 Vacuum Apparatus for Water Filtration and Filter Plated onto PARPH-V8 Plate[1]**

1 Photos courtesy of Dr. Steve Jeffers, Clemson University.

## Water Filtration Methods Materials List

- 1-liter bottles for sample collection (Nalgene® preferred)
- 47-mm diameter polycarbonate membrane filters with 3-µm pores (e.g., Sterlitech SKU No. PCT3047100)
- 47-mm diameter polyvinylidene fluoride (Durapore®) membrane filters with 5-µm pores (e.g., Fisher Scientific #SVLP04700)
- 100-ml plastic measuring cup or beaker per sample site; or 5-ounce paper cups
- Bent-tip forceps
- Clamp-type filter funnel (Nalgene® preferred)
- Disposable pipette or syringe for shallow water collection
- Electric vacuum pump or hand-operated vacuum pump
- Ice chest cooler (with a small amount of ice or other refrigerant if temperatures are warm outside)
- Inverted or dissecting microscope
- Plastic tubing
- Sterile PARPH-clarified V8 selective medium (refer to PARPH-V8 Selective Medium: for Phytophthora Species on page 10-1-17) in disposable petri plates; 10 plates per collection bottle; media plates can be stored in sealed plastic sleeves or bags in a refrigerator for 2 months before use
- Squeeze bottle containing distilled water

- Thermometer (water-resistant type preferred)
- Two 1- or 2-liter filtering flasks (plastic or glass)

# Growing Media Formulae

## PARPH-V8 Selective Medium: for *Phytophthora* Species[2]

**Table 10-1-2 PARPH-V8 Selective Medium: for *Phytophthora* Species**

| Ingredient: | Amount per: | |
|---|---|---|
| | **1.0 liter** | **0.5 liter** |
| **Basal medium** | | |
| Clarified V8 Concentrate[1] | 50 ml | 25 ml |
| Distilled water | 950 ml | 475 ml |
| Difco Bacto agar | 15 g | 7.5 g |
| **Amendments** | | |
| Delvocid [50% pimaricin] | 10 mg | 5 mg |
| Ampicillin sodium | 250 mg | 125 mg |
| Rifamycin-SV [sodium salt] | 10 mg | 5 mg |
| Terraclor [75% PCNB] | 66.7 mg | 33.4 mg |
| Hymexazol | 50 mg | 25 mg |

1 Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaC)3/100 ml V8 Juice) clarified in one of three ways:

Centrifugation at 4000 RPM for 20 minutes followed by filtration using 2 layers of Whatman No. 1 filter paper under vacuum
Centrifugation at 7000 RPM for 10 minutes; then filtration is **not** necessary
Vacuum filtration alone through a 1- to 2-cm deep layer of Celite
Clarified V8 should be frozen at -20 °C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes). Pentachloronitrobenzene (PCNB) and hymexazol are optional and can be omitted (e.g., to make PAR, PARP, and PARH):

PCNB is useful to inhibit soilborne fungi on soil dilution plates
Hymexazol inhibits **most** species of *Pythium* while allowing **most** species of *Phytophthora* to grow, although they may grow more slowly

### Directions

1. Add ingredients for basal medium to a 2-liter flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask.
2. Autoclave for 20 minutes at 121 °C and 15 psi; turn waterbath on to ~50 °C.
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix.
4. Cool medium in waterbath.
5. Slowly stir medium with a magnetic stirrer in laminar flow hood.
6. Vortex each amendment thoroughly and add to mixing basal medium.

2 Adapted from Jeffers and Martin, 1986; Ferguson and Jeffers, 1999.

7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium.
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does **not** quite cover the entire plate; thus, plates will need to be swirled gently to evenly distribute medium before it hardens.
9. Cool plates at room temperature.
10. Store plates inverted in self-sealing plastic bags in the dark in a refrigerator.
11. Use plates **within** 30 days.

## PAR-V8 Selective Medium: for *Phytophthora* Species[3]

**Table 10-1-3 PAR-V8 Selective Medium: for *Phytophthora* Species**

| Ingredient: | Amount per: | |
|---|---|---|
| | **1.0 liter:** | **0.5 liter:** |
| **Basal medium** | | |
| Clarified V8 concentrate[1] | 50 ml | 25 ml |
| Distilled water | 950 ml | 475 ml |
| Difco Bacto agar | 15 g | 7.5 g |
| **Amendments** | | |
| Delvocid [50% pimaricin] | 10 mg | 5 mg |
| Ampicillin sodium | 250 mg | 125 mg |
| Rifamycin-SV [sodium salt] | 10 mg | 5 mg |

1 Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaC)3/100 ml V8 Juice) clarified in one of three ways:

Centrifugation at 4000 RPM for 20 minutes followed by filtration using 2 layers of Whatman No. 1 filter paper under vacuum
Centrifugation at 7000 RPM for 10 minutes; then filtration is **not** necessary
Vacuum filtration alone through a 1- to 2-cm deep layer of Celite
Clarified V8 should be frozen at -20 °C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes).

### Directions

1. Add ingredients for basal medium to a 2-liter flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask.
2. Autoclave for 20 minutes at 121 °C and 15 psi; turn waterbath on to ~50 °C.
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix.
4. Cool medium in waterbath.
5. Slowly stir medium with a magnetic stirrer in laminar flow hood.
6. Vortex each amendment thoroughly and add to mixing basal medium.

3 Adapted from Ferguson and Jeffers, 1999.

7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium.
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does **not** quite cover the entire plate; thus, plates will need to be swirled gently to evenly distribute medium before it hardens.
9. Cool plates at room temperature.
10. Store plates inverted in self-sealing plastic bags in the dark in a refrigerator.
11. Use plates **within** 30 days.

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Chapter

11

# Soil and Container Mix Sampling and Processing Protocol

## Contents



## Introduction

Soil or container mix sampling is a preferred method of collecting samples from symptomatic plants grown in pots or grown on, or adjacent to, soil. Soil sampling is **not** required during a detection survey. Soil or container mix infested with *Phytophthora ramorum* appears to the unaided eye exactly the same as noninfested soil or container mix. Therefore, **all** soil and container mix samples **must** be handled carefully.

# Sampling Soils and Container Mixes

## Sample Collection

1. Record the physical location (address) of the nursery site along with GPS reference coordinates.

**NOTICE**

If a GPS unit is **not** available, many cellular phones have GPS capabilities. Also Google Earth can be used to obtain coordinates using an address.

2. Prepare or secure from the facility manager/owner a diagram of the nursery or sampling area, which includes row or block numbers and plant species/cultivars. If possible, collect reference GPS coordinates for each block of plants.
3. Each 1-L (1-qt) composite sample should consist of a **minimum** of 15 subsamples collected from soil or container mix within the targeted area. Collect subsamples in zigzag transects according to the pattern in the diagram below. Collect subsamples from underneath positive plants. If plants are on benches or gravel, take a composite from each pot comprising one liter of soil thoroughly mixed.
4. Referencing Table 11-1-1 collect composite samples from **both** soil and container mix for each block of plants. An exception to this would be if all plants (including container mix and pots) were destroyed or the plants are **not** on a soil substrate (e.g., concrete or asphalt). Each sample should contain approximately 1 L/1 qt (volume) of soil or container mix and be placed in a 4-L (1-gal) size zip-to-close plastic bag. The number of composite samples collected will depend on the size of the block of plants being sampled.

**Table 11-1-1 Number of Composite Samples to Collect Per Block**

| Survey area size: | | Composite samples of soil: | Composite samples of container mix: |
|---|---|---|---|
| ($m^2$) | (ac) | | |
| <1,000 | <0.25 | 5 | 5 |
| 1,000 to 2,000 | 0.25 to 0.5 | 10 | 10 |
| 2,000 to 4,000 | 0.5 to 1 | 20 | 20 |
| >4,000 | >1 | 30 | 30 |

**NOTICE**

If the soil surface is covered with gravel with a large amount of plant debris on top, collect as much debris as practical. If the gravel has little plant debris on top, collect subsamples from the soil beneath the gravel. If water-permeable weed block (landscape cloth) is present, either covered with gravel or under gravel, make small slits in the cloth to allow for sample collection.

A. Soil

a. With a trowel, collect a representative composite sample (approximately 1 L (1 qt)) from the surveyed area (e.g., a block of plants, a nursery bed, a shade house, etc.) to a depth of approximately 5 to 10 cm (2” to 4”).

**NOTICE**

If soil is loosely packed, a plastic spoon can be used to collect the sample. The spoon can then be sealed in the corresponding sample’s plastic bag for easy disposal in the laboratory. This method is **not** recommended for sampling container mix because substrate at the bottom of pots cannot be sampled.

b. Collect samples from around and under pots containing plants suspected of being infested or infected with *P. ramorum* or from areas where diseased plants were previously located. This may require scraping soil from on or under nursery cloth or anything else on which pots are or were located.

c. Place each composite sample into an individual plastic bag; if the soil is wet or saturated from rain or excessive irrigation, double bag the slurry to avoid leaks.

B. Container mix

a. Collect a representative composite sample from each block of plants using a wide-bore soil tube (highly recommended); one core from each or every other pot in the block of plants is sufficient depending on the number of pots present.

5. For each composite sample, break up clods and root masses, then thoroughly mix the sample in the bag; this can be done in the field or laboratory.
6. If it appears dry, moisten the sample with distilled water, as desiccation will severely affect the ability to recover *P. ramorum* from a soil sample.
7. Disinfest sampling tools and soles of shoes (e.g., 10% bleach, quaternary ammonium at the labeled rate, or full-strength disinfectant spray (with ETOH)) between samples to prevent potential dissemination of the pathogen. Next, thoroughly rinse tools with distilled water to remove all disinfestation product residues or allow tools to dry.

**NOTICE**

Rinsing off disinfestation residues and allowing the tool to dry prevents possible sterilization of your next sample. Distilled water can be purchased at most grocery and big-box stores.

### Sample Transport, Storage, Shipping, and Processing

1. Place all samples in a cooled, insulated ice chest for transport to the laboratory or until samples are shipped. If samples **cannot** be shipped immediately, hold them in a refrigerator or cold room (4 to 10 °C/39 to 50 °F) for a **maximum** of 2 days.
2. Before shipping, double bag samples using gallon-size zip-to-close, self-sealing plastic bags, ensuring each bag is clearly labeled using a permanent, waterproof pen. To further protect samples, each sample can then be placed inside a 2-L (2-qt) disposable storage container that is also clearly labeled using a permanent, waterproof marking method. Completely fill out PPQ Form 391 and place inside a separate zip-to-close bag and place in the same box as the samples.
3. Contact laboratory personnel before shipping to advise them that a sample will be arriving. Ship samples via overnight courier. Avoid shipping on Fridays and prior to holidays to avoid shipping delays that may compromise the quality of the sample.
4. Send samples to a qualified State laboratory or a USDA–APHIS–PPQ regional laboratory at:

   Bliss M. Coffin
   USDA–APHIS–PPQ
   Dep't of Plant Pathology
   Kansas State University
   4024 Throckmorton Plant Sciences
   Manhattan, KS 66506-5502
   (785) 532-1349
   bliss.betzen@usda.gov

## Procedure for Baiting Soil and Container Mix Samples

1. Once[1] samples arrive in the laboratory, protect them from exposure to direct sunlight near windows. If samples **cannot** be processed immediately, store them in a refrigerator or cold room (4 to 10 °C/39 to 50 °F). Do **not** permit the samples to freeze or dry out.
2. If the soil or container mix sample is desiccated when it arrives in the laboratory, moisten with distilled water, reseal the bag, and allow the moistened sample to sit for 40 to 72 hours before processing.
3. Thoroughly mix the 1-L (1-qt) sample within the bag, breaking up any clods. Divide the sample equally into two 500-ml (17-oz.) aliquots, placing one sample into a new sealed zip-to-close bag or container then placing it in cold storage (4 to 10 °C/39 to 50 °F) for a **minimum** of 30 days.

1 These procedures are provided by S.N. Jeffers, Clemson University, 2010.

4. Prepare and label 3 containers (e.g., small 0.5-L (1-pt.) plastic containers, self-sealing plastic 1-liter (1-qt) bags, etc.) for each composite sample to be baited.
5. Thoroughly mix the remaining sample, then place an aliquot approximately 1 to 2 cm (0.5” to 1”) deep into each of the 3 containers; soil deeper than this may inhibit zoospores from swimming to the surface.
6. Add distilled water to a depth of 2.5 cm (1”) above the soil surface; stir the mixture and allow it to settle.

**NOTICE**

Organic debris may continue floating.

7. Bait leaves should be free of blemishes, damage, disease, and pesticides. *Rhododendron catawbiense* and/or *Camellia japonica* are recommended bait types (if both bait types are available it is recommended to use both). Using a standard hole-punch or scissors, prepare enough leaf pieces (~10 per container) to bait all containers (refer to Figure 11-1-1). Leaf pieces cut with scissors should be approximately 5 mm (<0.25”) across.

**NOTICE**

Use different-shaped leaf pieces to differentiate between bait types if two types of bait are being used.

8. Using sterile forceps, add 8 to 10 leaf pieces of each bait type (or 15 to 20 leaf pieces if only using one bait type) to each container. Baits should float on the water surface. If some of the baits sink, do **not** remove them, instead, add additional baits. Cover containers to avoid evaporation and desiccation.
9. Store containers at 18 to 22 °C/64 to 72 °F for 3 days (an incubator maintained at 20 °C/68 °F or a closed cabinet works best).
10. For each container, remove 6 baits of each regulated plant type (or 12 baits of 1 regulated plant type) with sterile forceps and blot dry on a clean paper towel. Dispose of paper towels after each sample.
11. Place the 6 bait pieces of each regulated plant type from 1 container on a separate plate of PARPH-V8[2] medium (e.g., 1 plate with 6 *Rhododendron* leaf pieces and 1 plate with 6 *Camellia* leaf pieces, or alternatively, 2 plates of same regulated plant tissue baits) so they are embedded completely in the agar. Leaf pieces placed on the agar surface will dry out and curl up. There should be 6 plates and 36 baits from each composite sample: 3 containers x 2 plates/container x 6 baits/plate (refer to Figure 11-1-1).

2 Refer to Growing Media Formulae on page 11-1-10.

**NOTICE**

Baits can be vertically inserted into the medium, which prevents shadowing during microscopic examination. To prevent media tearing, a scalpel can be used to make small incisions where baits are going to be placed. Vertical placement may require slightly thicker agar or smaller bait pieces, however, the places no longer have to be read on both sides.

**Figure 11-1-1 Baiting Soil Samples and Plating Baits[1]**

1 Photos courtesy of Grace O'Keefe, PPQ and Jennifer Falacy, Washington State Department of Agriculture. A) Preparing soil samples for baiting; B) baiting soil samples using *Rhododendron* and *Camellia* leaves; C) plate with baits inserted vertically; and D) plate with baits inserted horizontally.

12. Place plates upside down in a plastic box or zip-to-close bag to prevent desiccation; incubate plates at 18 to 22 °C (64 to 71°F) in the dark for 10 to 14 days. Because *P. ramorum* can grow slowly in cultures, additional incubation up to 28 days is optional. A designated incubator works best, but a closed cabinet in an air-conditioned room can also be used.
13. Using a dissecting or inverted microscope, examine plates frequently (starting 2 days after baits have been plated) for colonies that resemble *P. ramorum*—i.e., those with typical coralloid hyphae, large golden chlamydospores, and packets of semi-papillate sporangia on the surface (refer to Figure 11-1-2); mark these with a permanent, waterproof marking method.
14. *P. ramorum* hyphae are often visible 2 to 5 days after baits have been plated. However, patience, persistence, and good observational skills are often the keys to finding *P. ramorum* on the isolation plates; *P. ramorum*

may be recovered from only 1 of the 36 bait pieces and may not be recognizable until several weeks after baits are plated.

15. Subculture isolates to fresh PARPH-V8 and then to PAR-V8[3].

**NOTICE**

*P. ramorum* grows and sporulates better in the absence of hymexazol (i.e., on PAR-V8). It is best to subculture from suspect colonies early, **before** these colonies become overgrown by fast-growing organisms.

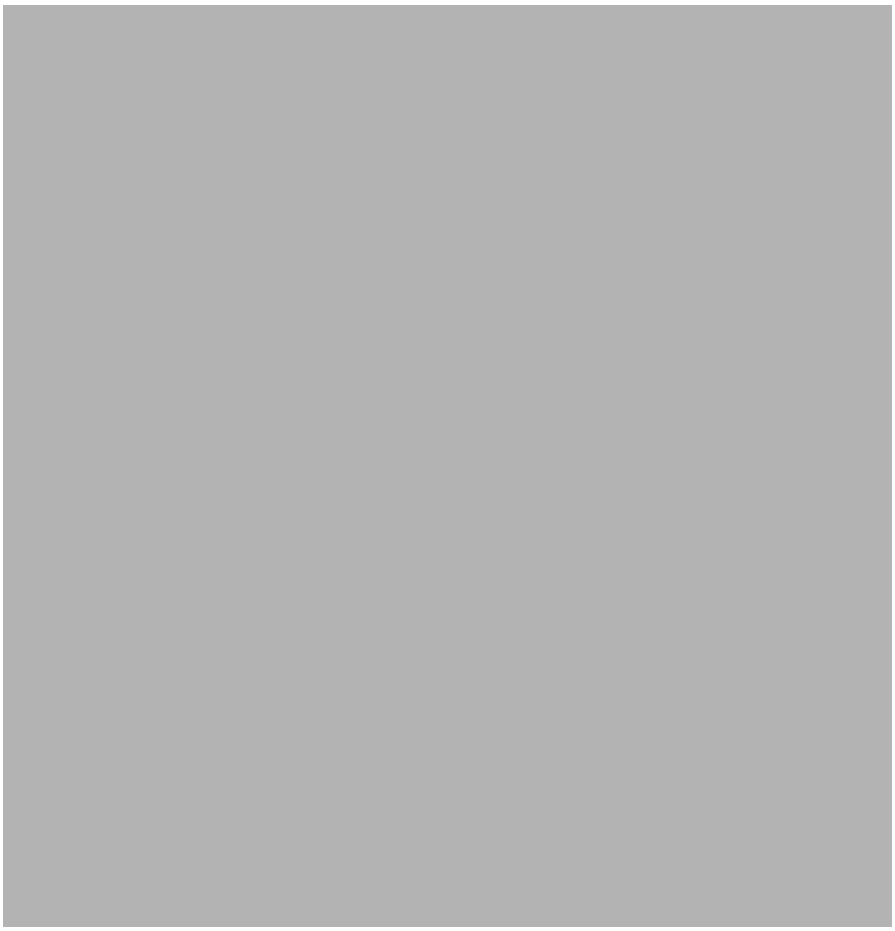

**Figure 11-1-2 Characteristic Structure of *P. ramorum*[1]**

1 Photos courtesy of Dr. Steve Jeffers, Clemson University.

16. If morphology or PCR testing does **not** indicate a positive *P. ramorum* by 14 days of culture, the sample is considered negative for regulatory purposes. The culture can be disposed of in the manner described in Treatments and Disinfectants on page 12-1-1.

3 Refer to Growing Media Formulae on page 11-1-10.

## Additional Guidance on Culture and Identification

1. Be sure to culture mostly green tissue at the margin of the lesion, and **not** too much brown, dead tissue.
2. If the initial culture media is PARP-H, take care to adjust the concentration of Hymexazole. Adding too much Hymexazole to the media will inhibit *P. ramorum* growth.
3. If a surface sterilization step is added, do **not** oversterilize leaf pieces, 5% bleach solution for 30 seconds is generally sufficient for this purpose.
4. The culture conditions should be at a constant temperature between 16 and 22 °C (64 to 71 °F) with inverted plates placed in the dark.
5. Cultures should be checked up to 10 to 14 days for hyphal growth and morphological identification. Real-time PCR can be attempted on hyphal growth suspected to be *P. ramorum*.
6. Additional steps and procedures can be carried out in attempts to isolate *P. ramorum*, but this is **not** required for regulatory purposes. However, if *P. ramorum* is morphologically identified using these extra procedures, the sample is a presumptive positive (refer to below).

## Diagnostic Determination for Cultures

1. If the bait or mycelia growing from the bait is tested positive by PCR in a NPPLAP-accredited laboratory and it qualifies as a Potentially Actionable Suspect Sample (PASS), the DNA can be forwarded to the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory. If it is a **non**-PASS, the sample is considered positive for regulatory purposes.
2. If the culture is morphologically identified as *P. ramorum*, regardless of PCR results, the sample is determined as presumptive positive. If the original sample is a PASS, the culture **must** be confirmed at the PPQ–S&T Plant Pathogen Confirmatory Diagnostics Laboratory according to the instructions in this program manual.
3. If the key morphological features of *P. ramorum* are clearly observed, attempt to transfer to a fresh plate for singular isolation. If **no** morphology is observed, PCR can be attempted.

## Disposing of Cultures After Diagnosis

1. Please contact the *P. ramorum* program via email at P.ramorum@usda.gov for instructions on the final disposition of non-PASS cultures. These cultures may be found useful for regulatory research or methods development needs. If the required PPQ permit is in place, arrangements can be made to forward cultures to the research and method development scientific community. If **not** needed for this purpose, the cultures can be disposed of as instructed in Treatments and Disinfectants on page 12-1-1.

2. If morphology or PCR testing does **not** indicate a positive *P. ramorum* by 14 days of culture, the sample is considered negative for regulatory purposes. The culture can be disposed of as instructed in Treatments and Disinfectants on page 12-1-1.
3. Remove composite samples from cold storage and hold at room temperature (22 to 24 °C/72 to 75 °F) for 3 days to acclimate before baiting begins.

### Second Baiting of Soil and/or Container Mix Samples

1. Remove composite samples from cold storage and hold at room temperature (22 to 24 °C/72 to 75 °F) for 3 days to acclimate before baiting begins.
2. Bait samples again as described above in Procedure for Baiting Soil and Container Mix Samples on page 11-1-4.
3. After samples have been baited a second time, destroy or sterilize any remaining soil and/or container mix using an appropriate method (e.g., autoclaving).

## Materials and Supplies for the Soil and Container Mix Protocol

- ❒ 4-L (1-gal) self-sealing plastic bags (**at least** 4 mil in thickness); avoid the bags with the "zipper" mechanism
- ❒ 70% alcohol and flame for sterilizing laboratory utensils
- ❒ Baits—use *Rhododendron* and/or *Camellia* leaves that have been on the plant for **at least** 1 year; leaf pieces should be 5 x 5 mm squares (<0.25") or 5 mm (<0.25") in diameter disks
- ❒ Disinfesting solution (10% bleach, quarternary ammonium at the labeled rate, or full-strength disinfectant spray (with ETOH))
- ❒ Disposable gloves
- ❒ Distilled water
- ❒ Forceps and scalpel
- ❒ Insulated ice chest (with ice in bags or blue ice if external temperatures are **above** 21 °C/70 °F)
- ❒ PAR-V8 selective medium (as needed for subcultures)
- ❒ PARPH-V8 selective medium; two plates per baited container
- ❒ Paper towels
- ❒ Permanent, waterproof marking method
- ❒ Plastic or glass containers with lids; square, wide-bottom containers work best (e.g., 0.5 L (1 pt) freezer boxes) (refer to Figure 11-1-1)

- ❒ Single-hole punches, scissors, or razor blades
- ❒ Trowel or other soil-sampling tool
- ❒ Wide-bore soil tube (2.5 cm/1" or larger)

# Growing Media Formulae

## PARPH-V8 Selective Medium: for *Phytophthora* Species[4]

**Table 11-1-2 PARPH-V8 Selective Medium: for *Phytophthora* Species**

| Ingredient: | Amount per: | |
|---|---|---|
| | **1.0 liter** | **0.5 liter** |
| **Basal medium** | | |
| Clarified V8 concentrate[1] | 50 ml | 25 ml |
| Distilled water | 950 ml | 475 ml |
| Difco Bacto agar | 15 g | 7.5 g |
| **Amendments** | | |
| Delvocid [50% pimaricin] | 10 mg | 5 mg |
| Ampicillin sodium | 250 mg | 125 mg |
| Rifamycin-SV [sodium salt] | 10 mg | 5 mg |
| Terraclor [75% PCNB] | 66.7 mg | 33.4 mg |
| Hymexazol | 50 mg | 25 mg |

1 Clarified V8 concentrate is made from buffered V8 Juice (1.0 g CaC)3/100 ml V8 Juice) clarified in one of three ways:

- ◆ Centrifugation at 4000 RPM for 20 minutes followed by filtration using 2 layers of Whatman No. 1 filter paper under vacuum
- ◆ Centrifugation at 7000 RPM for 10 minutes; then filtration is **not** necessary
- ◆ Vacuum filtration alone through a 1- to 2-cm deep layer of Celite

Clarified V8 should be frozen at -20 °C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes). PCNB and hymexazol are optional and can be omitted (e.g., to make PAR, PARP, and PARH):

- ◆ PCNB is useful to inhibit soilborne fungi on soil dilution plates
- ◆ Hymexazol inhibits **most** species of *Pythium* while allowing **most** species of *Phytophthora* to grow, although they may grow more slowly

### Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask.
2. Autoclave for 20 minutes at 121 °C and 15 psi; turn waterbath on to ~50 °C.
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix.
4. Cool medium in waterbath.

4 Adapted from Jeffers and Martin, 1986; Ferguson and Jeffers, 1999.

5. Slowly stir medium with a magnetic stirrer in laminar flow hood.
6. Vortex each amendment thoroughly and add to mixing basal medium.
7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium.
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does **not** quite cover the entire plate; therefore, plates will need to be swirled gently to evenly distribute medium before it hardens.
9. Cool plates at room temperature.
10. Store plates inverted in self-sealing plastic bags in the dark in a refrigerator.
11. Use plates **within** 30 days.

## PAR-V8 Selective Medium: for *Phytophthora* Species[5]

**Table 11-1-3 PAR-V8 Selective Medium: for *Phytophthora* Species**

| Ingredient: | Amount per: | |
|---|---|---|
| | **1.0 liter:** | **0.5 liter:** |
| **Basal medium** | | |
| Clarified V8 concentrate[1] | 50 ml | 25 ml |
| Distilled water | 950 ml | 475 ml |
| Difco Bacto agar | 15 g | 7.5 g |
| **Amendments** | | |
| Delvocid [50% pimaricin] | 10 mg | 5 mg |
| Ampicillin sodium | 250 mg | 125 mg |
| Rifamycin-SV [sodium salt] | 10 mg | 5 mg |

1 Clarified V8 concentrate is made from buffered V8 Juice (1.0 g CaC)3/100 ml V8 Juice) clarified in one of three ways:

- Centrifugation at 4000 RPM for 20 minutes followed by filtration using 2 layers of Whatman No. 1 filter paper under vacuum
- Centrifugation at 7000 RPM for 10 minutes; then filtration is **not** necessary
- Vacuum filtration alone through a 1- to 2-cm deep layer of Celite

Clarified V8 should be frozen at -20 °C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes).

### Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask.
2. Autoclave for 20 minutes at 121 °C and 15 psi; turn waterbath on to ~50 °C.
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix.

5 Adapted from Ferguson and Jeffers, 1999.

4. Cool medium in waterbath.
5. Slowly stir medium with a magnetic stirrer in laminar flow hood.
6. Vortex each amendment thoroughly and add to mixing basal medium.
7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium.
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does **not** quite cover the entire plate; therefore, plates will need to be swirled gently to evenly distribute medium before it hardens.
9. Cool plates at room temperature.
10. Store plates inverted in self-sealing plastic bags in the dark in a refrigerator.
11. Use plates **within** 30 days.

## References

Ferguson, A.J. and Jeffers, S.N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83:1129-1136.

Jeffers, S.N. and Martin, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.

Chapter

12

# Treatments and Disinfectants

## Contents



## Treatment Options

The following techniques have been determined to effectively control *P. ramorum* in nurseries with sample site confirmed positives. Any of these options may be required to mitigate *P. ramorum* infestations, providing the options are appropriately labeled for use in the State. A systems approach to treatment should be considered rather than relying on just one method. **Always** follow label directions when applying any chemical treatment.

## Infected Plants and Associated Potting Mix and Containers

**SAFETY**

Do **not** place regulated plant material, including leaf litter, in compost piles or remove regulated plant material from the nursery site as trash or with debris removal. Regulated plant material should be collected and incinerated, double bagged and deep buried in a site approved by USDA–APHIS or delegated regulatory authority, or steam sterilized. Properly disinfest **all** tools or materials used for cleanup or material movement. To prevent unintentional inoculum movement, **all** personnel involved with cleanup should follow appropriate procedures. To prevent contamination of other areas and methods of conveyance, follow appropriate procedures concerning **all** material movement on the nursery site.

## Incineration (Burning to Ash)

Infected plants, associated growth media, associated containers (e.g., pots and trays[1]), **all** leaf debris in and around the area where plants were stored may be incinerated at a facility or other location (e.g., on site). The facility or other location **must** be approved by USDA **and** permitted within State and municipal statutes or regulations. Off-nursery (off-site) movement **must** be properly safeguarded and every effort taken to prevent plant debris or soil from being dislodged from the plants **prior to** incineration. Incineration may be through open burning or in an incinerator.

## Deep Burial

Infected plants, associated growth media, associated containers (e.g., pots and trays[1]), **all** leaf debris in and around the area where plants were stored may be double bagged using plastic bags of **2-mil thickness or greater** and buried to a depth of **no less than 2 m**. The material **must** be buried at a USDA-approved site, on site, or a municipal landfill, where it is expected to remain undisturbed. Take every effort to prevent plant debris or soil from being dislodged from the plants.

## Steam Sterilization

Infected plants, associated growth media, associated containers (e.g., pots and trays[1]), **all** leaf debris in and around the area where plants were stored may be treated with steam sterilization or dry heat commonly heated to internal temperatures of 176 °F (80 °C) for 60 minutes (steam) or 120 minutes (dry heat), or as otherwise detailed in the USDA *Treatment Manual* Schedule T521 for plant pathogenic fungi and bacteria.

1 Associated containers (e.g., pots and trays) may be treated for reuse. Guidelines for safeguarding should be determined and approved to prevent movement or potential contamination of *P. ramorum* to noninfested areas of the nursery. **All** potting mix should be removed to the extent possible **prior to** treating. Dispose of the potting mix using one of the above methods. Containers may be sterilized by treatment with steam **at or above** 122 °F (50 °C) for 30 minutes **or more** (most containers will withstand 140 °F to 160 °F) or other approved disinfestant methods for the correct contact time.

# Disinfestation Options

## Disinfesting Nonporous Surfaces

Most disinfectants are **not** labeled for use in soil and are **only** useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is practical, tools such as knives, pruners, water breakers, water wands, and other implements used in the quarantine area should **only** be used in the quarantine area. If tools and other implements **must** be moved from the quarantine area, regular disinfection using an appropriate disinfectant for controlling *P. ramorum* is recommended **prior to** removing from the quarantine area. Table 12-1-1 examines the effects of different classes of disinfectants on microbial populations. This table is for explanation and information **only**. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in the table in **bold** type.

Strictly adhere to **all** labels for the disinfectants listed in Table 12-1-1 for maximum efficacy and environmental and worker safety. The contact time for the products **must** be followed to ensure efficacy. If the surface dries before the contact time is reached, re-wet the surface until the contact time is achieved.

**Table 12-1-1 Summary of Disinfestant Activities**[1]

| Disinfectant: | Trade name(s): | Comments: | Contact time: |
|---|---|---|---|
| Alcohols (ethyl and isopropyl) (60% to 85%) | ◆ Clorox Disinfecting Spray | ◆ Evaporates quickly meaning adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable | 10 to 15 minutes |
| Phenolics (0.4% to 5%) | ◆ **Pheno-cen** | ◆ Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue | 10 to 15 minutes |
| Quaternary ammonium (0.1% to 1.5%) | ◆ **Consan Triple Action 20**<br>◆ **Physan 20**<br>◆ Green-Shield 20 | ◆ Effective for nonporous surfaces sanitation (floors, walls, benches, pots); low odor and low irritation; use according to labels | 10 to 15 minutes |
| Chlorine (100 to 1,000 ppm) | ◆ **10% Clorox**<br>◆ **10% bleach** | ◆ Inactivated by organic matter; prepare fresh solutions of hypochorite (Clorox) every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin; **exposure to sunlight further reduces hypocholrite efficacy**; keep solution in opaque container | 10 to 15 minutes |

1 Modified table from Columbia Research Environmental Health and Safety (EH&S).

## Disinfesting Water

### For Dust Abatement, Fire Suppression, and Equipment Cleaning

Clorox (sodium hypochlorite) is labeled (EPA Reg. No. 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression, and equipment cleaning. The active ingredient level **must** be measured from water collected at the sprinkler head.

### For Irrigation

Chlorine levels of 2 ppm or 2 mg/liter **or greater** has been correlated with the control of *Phytophthora* spp. in recirculated irrigation systems. For irrigation purposes, recirculated, nonmunicipal water **must** be chlorinated at an active chlorine concentration **equal to or greater than** 2 mg/liter of water; for facilities that recycle water, this chlorine level **must** be monitored.

Other systems that can be approved for treating water can include one or a combination of the following: bromine; chlorine; sodium hypochlorite; calcium hypochlorite; chlorine dioxide; ozone; activated peroxygen; ultraviolet radiation; copper ionization; heat treatment/pasteurization; and filtration.

## Disinfesting Soil[2] and Container Mix

### Container Mix

Container mix **must** be heated so the temperature in the center of the load reaches **at least** 60 °C (140 °F) for 30 minutes. Heat treatment **must** be conducted in the presence of an inspector.

Fumigation may be the most efficacious and economical option to disinfest container mix.

### Soil/Container Mix in Pots

Soil **must** be heated so the temperature in the center of the load reaches **at least** 60 °C (140 °F) for 30 minutes. Heat treatment **must** be conducted in the presence of an inspector.

2 Soil treatment through the use of solarization is being developed. If you are considering solarization treatment for infested nursery beds, consult the regulatory agencies in your State for further information and guidance.

### Soil in Situ or in Nursery Beds[3]

Nursery beds can be treated with steam following specific protocols to ensure soil heating to 50 °C (122 °F) for 30 minutes. Check soil water content **prior to** steaming to avoid uneven heating. Monitor treatment with thermocouples placed in locations throughout the bed (15 cm deep[4]) with sensors placed in locations most likely to reach the correct temperature the slowest. Place a steam sock on the nursery bed and cover with a tarp sealed on the edges with sand snakes. Concrete blocks can be used to support the tarp off of the bed surface. Treatment timing begins when the last sensor reaches the target temperature of 50 °C (122 °F). Steam treatment **must** be conducted in the presence of an inspector.

**Figure 12-1-1 Steam Sock in Place on Soil Surface**

3 Schweigkofler, W., Kosta, K., Huffman, V., Sharma, S., Suslow, K., and Ghosh, S. 2014. Steaming inactivates *Phytophthora ramorum* causal agent of sudden oak death and ramorum blight, from infested nursery soils in California. Plant Health Progress doi: 10.1094/PHP-RS-13-0111.

4 Dart, N., Chastagner, G., Rugarber, E., and Riley, K. 2007. Recovery frequency of *Phytophthora ramorum* and other *Phytophthora* spp. in soil profiled of ornamental retail nurseries. Plant Dis. 91:1419-1422.

**Figure 12-1-2 Concrete Blocks Used to Support Tarp from Surface to Allow Steam Distribution from Steam Sock**

**Figure 12-1-3 Tarp with Sand Snakes in Place to Secure Edges**

**NOTICE**

Soil treatment through solarization is being developed. If you are considering solarization treatment of infested nursery beds, consult the regulatory agencies in your State for further information and guidance.

Fumigation may be the most efficacious and economical option to disinfest soil. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *Phytophthora cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is currently being evaluated as an option for soil treatment.

### Summary of Labeled Soil Fumigants

**All** fumigants are restricted use and **must** be applied according to labels by a licensed applicator. Any pesticide used in any manner **not** listed on the label is unlawful.

**Table 12-1-2 Labeled Soil Fumigants**

| Fumigant: | Trade names: | Comments: |
|---|---|---|
| Chloropicrin | ◆ Chlor-O-Pic<br>◆ Metapicrin<br>◆ Timberfume<br>◆ Tri-Clor | ◆ Often used in combination with methyl bromide due to its ability to be detected in small quantities |
| Dazomet | ◆ Basamid | ◆ Methyl isothyocyanate (MITC) breaks down into cyanide gas<br>◆ Requires careful soil preparation and incorporation into soil<br>◆ Water-activated granular formulation<br>◆ **All** applications **must** be made in accordance with labeling |
| Metam-sodium | ◆ Busan 1020<br>◆ Busan 1180<br>◆ Busan 1236 Metam<br>◆ Vapam | ◆ Metam-sodium can be applied through irrigation<br>◆ Tarping can increase efficacy<br>◆ **All** applications **must** be made in accordance with labeling |
| Methyl bromide | ◆ Tri-Con<br>◆ Terr-O-Gas<br>◆ Preplant Soil Fumigant<br>◆ Pic-Brom | ◆ Colorless and odorless<br>◆ Use is restricted due to ozone depletion potential<br>◆ Usually combined in various concentrations with Chloropicrin (tear gas) |

### Physical Soil Treatment

Mitigating infested soil can be achieved by installing permanent impermeable, nonporous barriers consisting of cement, concrete, or asphalt. These barriers **must** be constructed such that **no** native soil within the block is visible. Grade the barriers such that **no** surface water can be observed. When soil treatment is **absolutely impossible** due to human health concerns, certain soil hydrologic conditions, or due to city, county, or State regulations, nurseries under an agreement may use avoidance and exclusionary methods on a case-by-case basis.

### Equipment and Personnel (Inspectors and Employees)

Rubber boots or other very smooth and crevice-free waterproof boots are strongly encouraged as other footwear is too porous. When feasible, limit access to infested areas and hold areas to officials and necessary employees. Everyone entering and leaving the nursery site **must** scrape off loose pieces of soil into the infested site and disinfest footwear. Those working with or in contact with suspected infested material (including plants) **must** wear gloves and remove them or rub/wash them with an approved disinfectant between samples and between blocks. Currently, there are **no** products labeled for use on porous materials for *Phytophthora* control.

Place a disinfectant foot bath near the exit to the destruction-radii and quarantine-radii. Because of the higher potential of footwear coming in contact with infested soil or plant debris, **all** personnel entering and exiting the infested site **must** use the foot bath. The foot bath **must** be filled with fresh disinfectant **at least** daily (or more frequently if contaminated with soil or organic debris) and **must** be in accordance with label directions.

Vehicle tires (or other vehicle parts in contact with soil or plants (e.g., truck beds) **must** be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products **before** leaving the infested site. If at all possible, **do not** allow vehicles in the infested site **at all**. Any product labeled for use on nonporous surfaces may be used on tires or vehicle undercarriages.

**Do not** visit other nursery sites in potentially contaminated work clothing and footwear. If it is necessary for a visitor to enter the nursery, the nursery should ensure every precaution is taken to prevent movement, by the visitor, of infected and/or contaminated plants, soil, or debris.

Dispose of wood surfaces suspected of *P. ramorum* contamination (refer to Infected Plants and Associated Potting Mix and Containers on page 12-1-1). There is **no** effective way to test or treat wood surfaces for contamination.

Appendix

A

# Resources

## Contents



## Contact Information for the *Phythopthora ramorum* Program

1. William D. Wesela, National Policy Manager
   USDA–APHIS–PPQ
   4700 River Rd.
   Riverdale, MD 20737
   (301) 851-2229
   FAX (301) 734-8584
   william.d.wesela@usda.gov
2. Betsy Randall-Schadel, National Operations Manager
   USDA–APHIS–PPQ Field Operations
   920 Main Campus Drive, Suite #500
   Raleigh, NC 27606
   (919) 855-7544
   betsy.randall-schadel@usda.gov
3. Patrick J. Shiel, S&T Representative
   920 Main Campus Drive, Suite #500
   Raleigh, NC 27606
   (919) 855-7416
   FAX (919) 855-7480
   patrick.j.shiel@usda.gov
4. Bliss M. Coffin, Plant Pathologist
   USDA–APHIS–PPQ
   Department of Plant Pathology
   Kansas State University
   4024 Throckmorton Plant Sciences Center
   Manhattan, KS 66506-5502
   (785) 532-1349
   bliss.betzen@usda.gov

For questions regarding diagnostic work instructions, contact:

USDA–APHIS–Plant Pathogen Confirmatory Diagnostics Laboratory
BARC-East Building 580
9901 Powder Mill Road
Laurel, MD 20708
(301) 313-9204
APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov

For the National Plant Pathogen Laboratory Accreditation Program (NPPLAP), contact Dr. Patrick Shiel at (919) 855-7416 or patrick.j.shiel@usda.gov.

Please send incoming samples to the following email address: PPQ.OpsKS.Manhattan.Lab@usda.gov.

## U.S. State and Territory Plant Health Directors

For an up-to-date list of **all** U.S. State and Territory Plant Health Directors, please visit the U.S. State Plant Health Directors website.

## APHIS List of *Phytophthora ramorum*-Regulated Plants

Consult the latest list of regulated plants **prior** to beginning any survey, inspection, or delimitation. A current list can be found at the APHIS–PPQ *P. ramorum* website.

## *Phytophthora ramorum* Nursery Questionnaire

The *P. ramorum* Nursery Questionnaire (refer to Figure A-1-1) is a tool available to gather data about a positive nursery. The Questionnaire is most useful on the first visit to a nursery, but is also useful for verifying if nursery information has changed on subsequent visits. Information required for the *Phytophthora ramorum* program is marked with an asterisk. Additional information is helpful to understanding the nursery operation. Other data required by the *P. ramorum* program is listed in the core data document. To obtain the core data document and a fillable PDF of the *P. ramorum* Nursery Questionnaire, please contact the NOM.

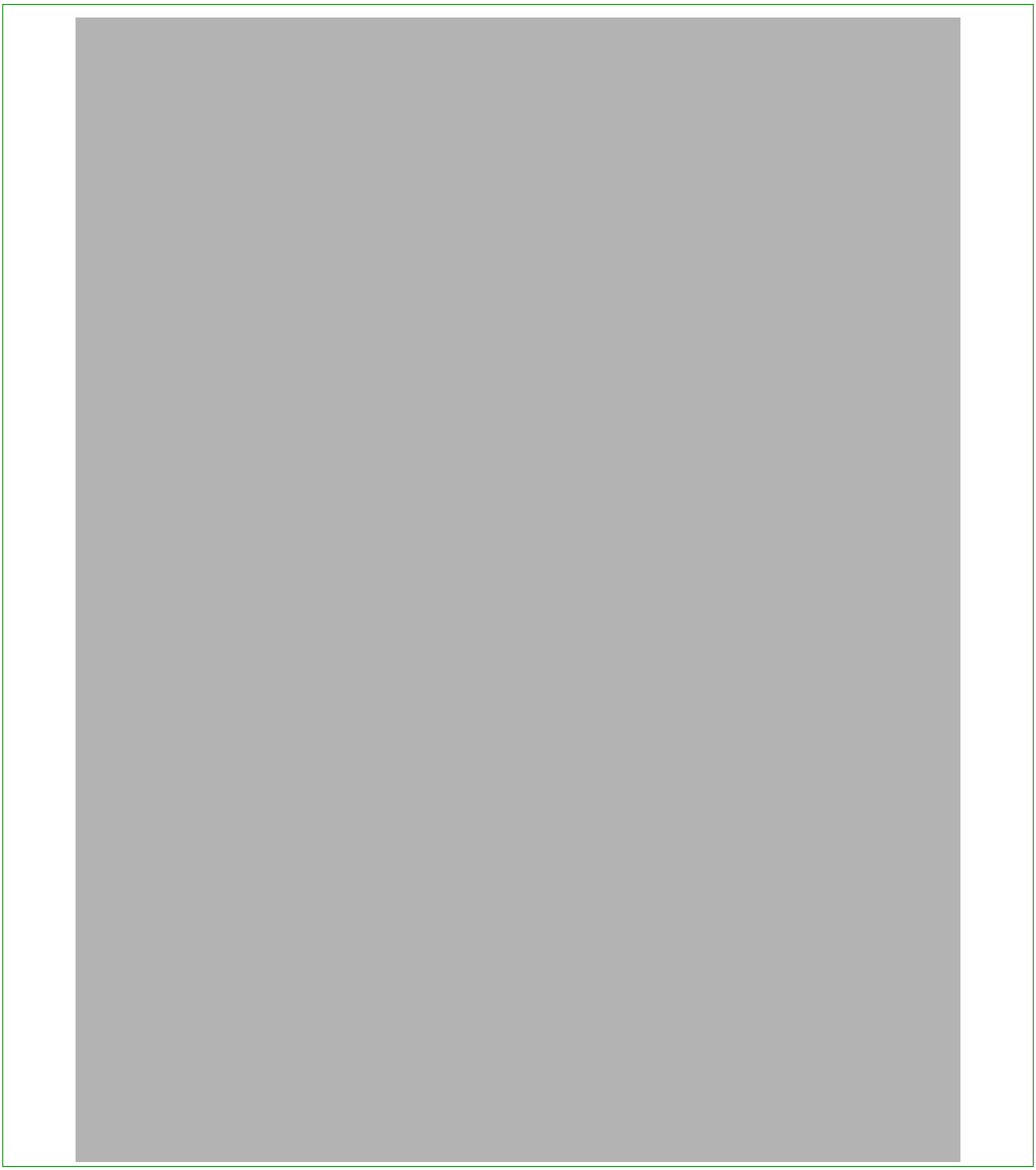

**Figure A-1-1 *P. ramorum* Nursery Questionnaire (page 1 of 5)**

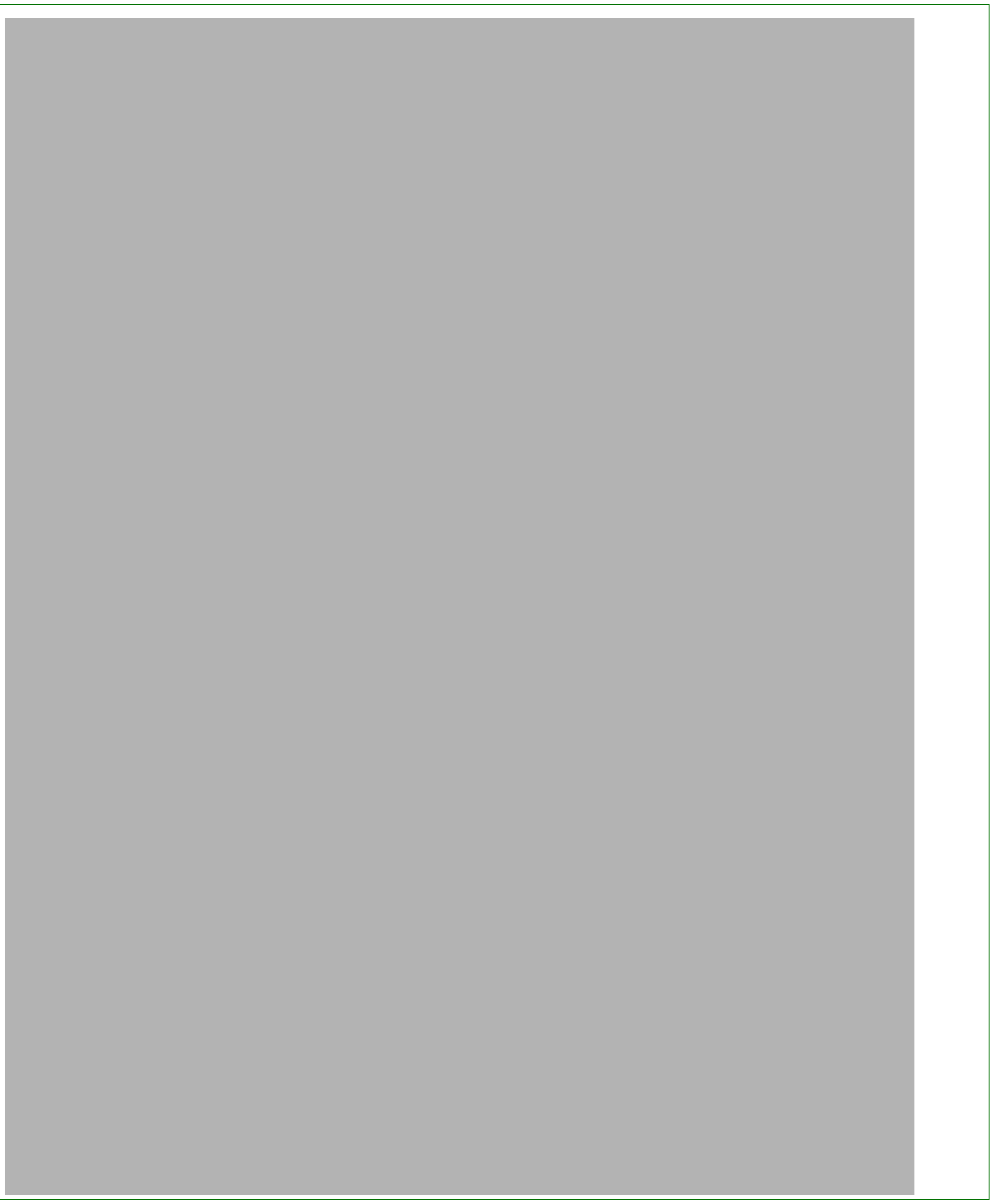

**Figure A-1-2 *P. ramorum* Nursery Questionnaire (page 2 of 5)**

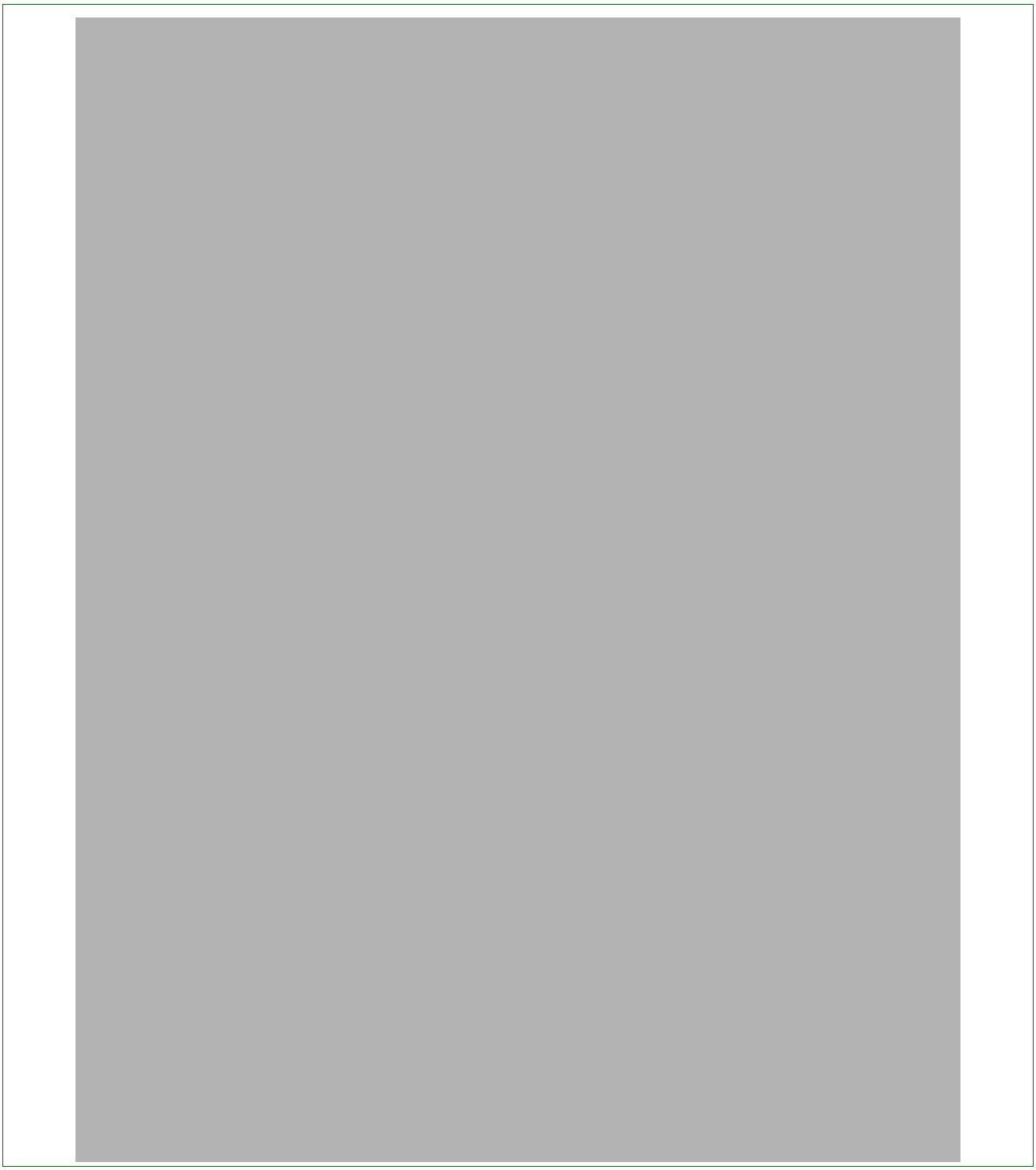

**Figure A-1-2 *P. ramorum* Nursery Questionnaire (page 3 of 5)**

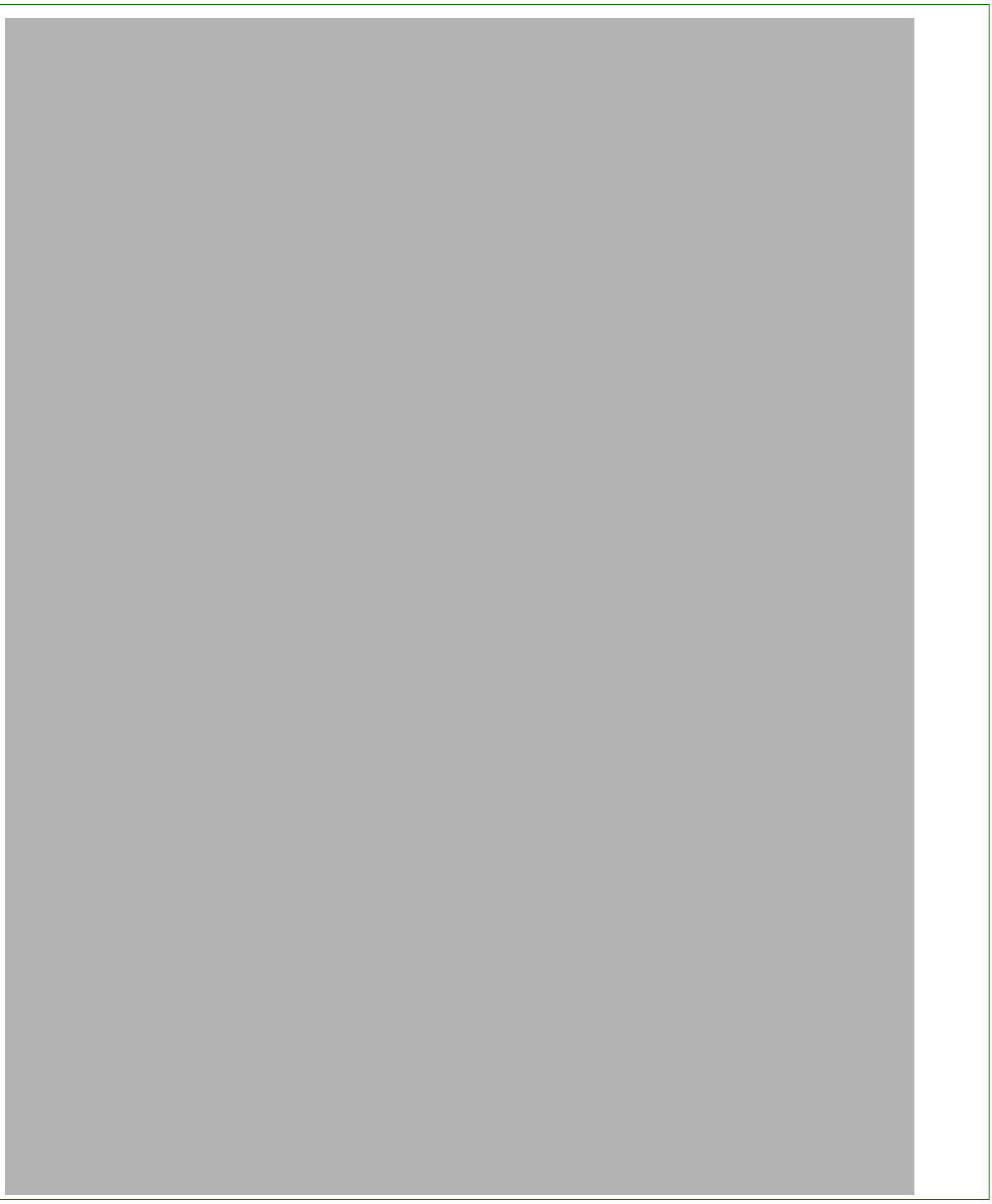

**Figure A-1-2 *P. ramorum* Nursery Questionnaire (page 4 of 5)**

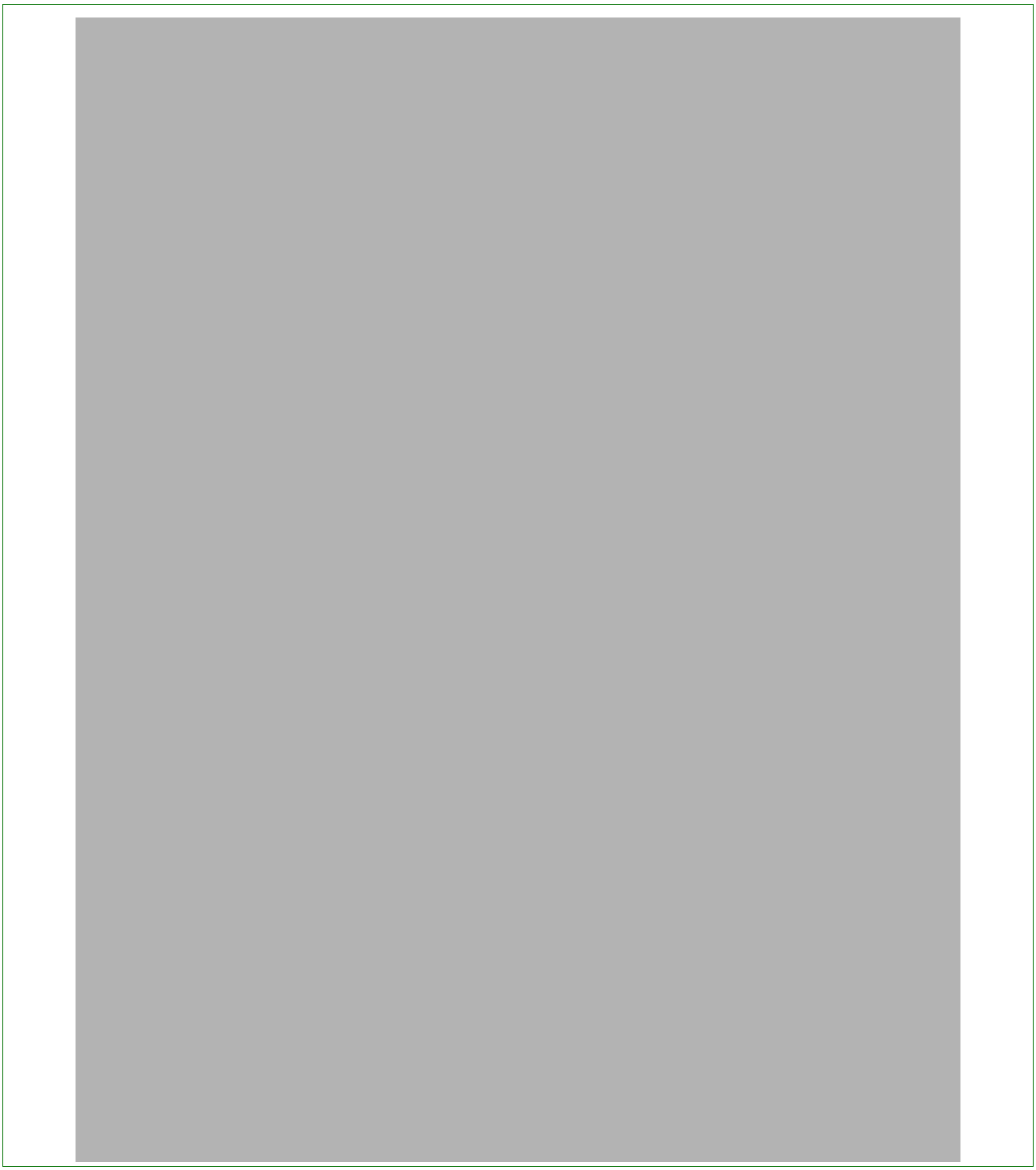

**Figure A-1-2 *P. ramorum* Nursery Questionnaire (page 5 of 5)**

*Phytophthora ramorum*

# Glossary

## Introduction

Use this glossary to find the meaning of specialized words, abbreviations, acronyms, and terms used in this manual. To locate where in the manual a given definition, term, or abbreviation is mentioned, refer to the index.

## Definitions, Terms, and Abbreviations

**aliquot.** volume of substrate from a composite sample that is placed into a container and assayed; usually 3 aliquots (approximately 50 to 150 ml or 2 to 5 oz.) from each composite sample are baited

**APHIS.** Animal and Plant Health Inspection Service

**associated plants.** naturally infected plants from which *P. ramorum* has been cultured and/or detected using Polymerase Chain Reaction (PCR); for each of these plants, traditional Koch's postulates have **not** yet been completed or documented and reviewed; a current list of associated plants is available at APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*.

**block.** within a nursery, this is a contiguous block of regulated plant material; the block will be considered contiguous until there is a two-meter (6.5 foot) break of **either** no plants **or** non-regulated plant material

**BMP.** best management practices

**biosecurity measures.** actions taken to reduce or mitigate the potential introduction or spread of *P. ramorum* from one area or site to another area or site of a nursery; refer to Biosecurity Measure for Nurseries on page 9-1-2

**CCP.** critical control point

**CNP.** confirmed nursery protocol

**CFR.** Code of Federal Regulations

**composite sample.** mixture of subsamples that are physically combined to form a single representative sample from a designated area

**compost pile.** mixture of decomposed organic matter of different origins, including plant debris, soil residues, as well as other substances found in the nursery; the finished product is used as a potting media component

**confirmed positive.** the presence of *Phytophthora ramorum* is confirmed by an APHIS-accredited diagnostic laboratory, only using current APHIS *P. ramorum* diagnostic work instructions. This may include plant, soil, and/or water samples. A final determination of a positive sample allows for Federal regulatory action.

**container mix.** substrates without soil (also referred to as growing media) and/or other materials placed in containers and used to grow plants, usually consisting of bark and peat but also may contain soil, slow-release fertilizer, sand, vermiculite, perlite, etc.

**cull pile.** an area in which discarded plant material is deposited; this area may also be known as a waste pile; may contain any combination of plants, plant material, water, container mix, compost, or soil.

**delimitation survey.** survey to determine the extent of the infestation(s) within a nursery site; the quarantine period begins when **all** delimitation sampling and testing is completed

**destruction radii.** two meters from the edge (plant drip line or canopy) of the known positive plant(s); one meter around the periphery of positive water; destruction radii is surrounded by the quarantine radii/radius on page Glossary-1-5

**destruction radius.** area of plants to be destroyed; within a nursery, for purposes of the **retail protocol**, the destruction radius is defined as all *P. ramorum*-infected regulated plant material and all other regulated plant material within 2 meters of any infected regulated plant material

**Emergency Action Notification (EAN).** PPQ Form 523 or equivalent State document used to specify regulatory requirements and actions within a nursery

**Federal confirmatory authority.** authority to make a final determination on a regulatory sample[1]

**final determination.** last diagnostic result(s) necessary for a regulatory sample indicating whether *P. ramorum* is present or not; the final determination test(s) are conducted by analysts/laboratories with Federal

confirmatory authority—if the final determination is positive, regulatory action may commence (refer to confirmed positive on page Glossary-1-2)

**free of.** without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures (ISPM Pub. No. 5, 2007)

**high-priority target plants.** any regulated plant material that originated in the destruction radius at the infested (source) nursery; these plants are to be identified using the best-available information and to the lowest-available taxonomy (e.g., if high-priority target plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level); **all** domestic and international shipments of the high-risk regulated plant genera: *Camellia*; *Rhododendron*; *Pieris*; *Viburnum*; and *Kalmia* and regulated plant shipments of the infected plant species within the six months **prior to** the first positive detection of *P. ramorum* at the nursery as per the protocol

**high-risk genera.** *Camellia, Rhododendron, Pieris, Viburnum*, and *Kalmia*

**HR.** high risk

**IES.** Investigative and Enforcement Services

**infected plants.** officially confirmed plants verified as being infected with *P. ramorum* based on APHIS-approved diagnostics and following the PASS system (refer to Potentially Actionable Suspect Sample (PASS) on page Glossary-1-4)

**lot.** set of plants that can be identified or grouped by shipment, cultivar, or production unit

**NDPN.** National Plant Diagnostic Network

**non-PASS.** in nonregulated areas, once APHIS confirms a nursery is positive by during a given calendar year, **all** subsequent samples are considered "non-PASS," meaning an NPPLAP-accredited laboratory outside of APHIS can

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1
- National Plant Protection Laboratory Accreditation Program (NPPLAP) accredited APHIS laboratories have authority to make a final determination on **any** *P. ramorum* regulatory sample
- State NPPLAP accredited laboratories in the three regulated States (CA, OR, and WA) have authority to make the final determination on **any** regulatory sample in a previously positive nursery
- State or National Plant Diagnostic Network (NPDN) NPPLAP accredited laboratories outside the regulated States have authority for subsequent samples to the initial APHIS confirmed positive for a given nursery during the EAN period; once the EAN period has lapsed and a new positive is detected, it **must** be forwarded to APHIS for final determination

make the final determination using APHIS NPPLAP diagnostic work instructions; in the regulated areas, for a nursery that has been historically positive, **all** samples are "non-PASS" if tested by the NPPLAP-accredited State laboratory; if the diagnostic result is positive, the sample is confirmed positive and reported to the APHIS–PPQ operations manager **within 24 hours**; regulatory action can commence based on that positive sample

**NPPLAP.** National Plant Pathogen Laboratory Accreditation Program

**nursery/facility.** any location in which nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed; locations that grow trees to be sold without roots (i.e., Christmas trees) and locations at which such trees are stored or distributed are also considered nurseries

**nursery dealer.** nurseries that are resellers—wholesale or retail—of nursery plants

**nursery site.** geographically separate location of a nursery/facility on page Glossary-1-4 that has a distinct physical address and appropriate biosecurity measures (refer to Biosecurity Measures for Nurseries on page 9-1-1) to prevent the movement of *P. ramorum* between locations

**nursery site quarantine period.** period of time during which regulated plants will **not** be moved within or out of the quarantine radii (refer to Schematic of Destruction and Quarantine Radii of Positive Plants on page 3-1-10); **this period begins when the nursery delimitation survey is completed and last for 90 days** during which proscribed activities **must** occur

**nursery stock.** any plants, including houseplants, propagative material grown in a nursery and tree seedlings for reforestation

**parallel quarantine.** quarantine or regulation imposed by a State or local plant regulatory authority essentially the same as a federally promulgated quarantine; these regulations can be more restrictive for intrastate movement and internal controls

**PCR.** polymerase chain reaction

**Potentially Actionable Suspect Sample (PASS).** presumptive positive *P. ramorum* sample that requires confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation (for more information, refer to PASS System Policy

**PPQ.** Plant Protection and Quarantine

**presumptive positive.** preliminary diagnostic test result from a laboratory indicating *P. ramorum* is present; a final determination (the confirmatory test) is the next and final step

**quarantine period. minimum** of 90 days beginning when the nursery delimitation survey is completed and lasting until both plant parts and climatic conditions conducive to disease expression have occurred; plants, water, or other articles in quarantine hold radii remain on hold during this period; regulatory officials will inspect plants in the quarantine radii/radius on page Glossary-1-5 and all regulated plant on page Glossary-1-6 in the nursery a **minimum** of two additional times, once about halfway through the anticipated quarantine period and once near enough to the end to have test results coincide with the end of the quarantine period—**all** symptomatic plants during these surveys **must** be sampled and tested (the second inspection can be considered the quarantine release survey on page Glossary-1-5 at the discretion of the inspector)

**quarantine radii/radius.** for plant positives, quarantine radii is a two-meter radius around the destruction radii (refer to Schematic of Destruction and Quarantine Radii of Positive Plants on page 3-1-10) designed to determine if *P. ramorum* has spread beyond the destruction radius; use of this term is an adaptation from the definition: "An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production, or a pest-free production site and in which phytosanitary measures are taken to prevent spread of the pest" (ISPM Pub. No. 5, 2007); also known as "Q-radii"

**quarantine release survey.** second of the two quarantine period inspections occurring near the end of the quarantine period; this survey includes inspection of plants in the quarantine radii/radius on page Glossary-1-5 and **all** regulated plant on page Glossary-1-6 within the nursery; sample and test any unhealthy plant tissue—for quarantine hold areas involving positive plants **only**, those areas can be released from quarantine if plant inspection, sampling, and testing reveal **no** further *P. ramorum* detection; for water and other regulated article positives, refer to Table 3-1-4 on page 3-1-12 for quarantine release instructions. Soil and surface or nonrecycled pond water may take longer than 90 days to remediate; an avoidance/exclusion mitigation plan for these positive areas is written into Appendix D (refer to Example of PPQ Form 519, Compliance Agreement on page A-1-3) of the agreement prior to the end of the quarantine period

**regulated establishment.** nursery confirmed positive for *Phytophthora ramorum*, **not** located in a quarantined area, that ships regulated, restricted, or associated articles interstate

**regulated plant.** listed on the official APHIS Lists of Proven Hosts of and Plants Associated with *Phytophthora ramorum*—naturally infected plants verified with completion, documentation, review, and acceptance of traditional Koch's postulates

**retail nursery dealer.** nursery (e.g., big-box stored) that is a reseller of nursery plants to the end user

**retail nursery/facility.** nursery (e.g., big-box store) whose business is the sale of plants to the end user, typically a home owner

**sample.** referring to a **single bag** of *P. ramorum* leaves; the goal is to have **at least** two square inches of symptomatic (unhealthy) plant tissue per sample for the diagnostician to test, therefore, each sample will contain approximately five symptomatic leaves from medium leaf species and five to twelve leaves for small leaf species; for species with twig dieback as a symptom, include the terminal three inches of a symptomatic branch including one inch of live stem; regulatory action may commence on one sample confirmed positive. In *P. ramorum* diagnostics, sample may refer to a 25-microliter aliquot of DNA or a culture derived from one plant sample; for container mix and water baiting samples, refer to Soil and Container Mix Sampling and Processing Protocol on page 11-1-1

**SITC.** Smuggling, Interdiction, and Trade

**soil.** loose surface material of the earth usually consisting of disintegrated rock with an admixture of organic material; the reference to soil in this manual is the surface or substrate under plant containers, the bare ground, and/or gravel; often with plant debris, peat, and bark fines are washed from plant containers

**SOP.** standard operating procedure

**State Plant Health Director (SPHD).** lead APHIS contact in each State responsible for overseeing all PPQ activities in that State

**State Plant Regulatory Official (SPRO).** primary person responsible for plant health programs in each State; a list of SPROs is available at the National Plant Board (NPB) website

**subsamples.** small amounts of soil or container mix that are combined to form a single, composite sample; collection subsamples increased the changes of finding *P. ramorum* if it is present

**surface water.** water that collects on the surface of the ground.

**suspect plant material.** plants with visible symptoms of *P. ramorum* infection; and/or regulated plant material that are a part of destruction or quarantine radii; and/or plants that have tested positive using PCR or culturing, **but** have **not** been confirmed positive for *P. ramorum* by APHIS

**trace back (TB) plants.** all plants of the same taxon (i.e., genus, species, hybrid, variety, or cultivar) of the infected plant regardless of size, location, or lot, back to the original propagation source (if it still exists)

**trace back (TB) site.** any location that shipped high-priority target plants on page Glossary-1-3 to a confirmed positive nursery, residence, or commercial landscapes

**trace forward (TF) plants.** list of high-priority target plants on page Glossary-1-3 that were shipped within six months **prior** to detection at the nursery; list includes the shipment date(s), quantities, and destination nursery

**trace forward (TF) site.** any location that received high-priority target plants on page Glossary-1-3 from a confirmed infested source nursery; including residential or commercial landscapes

**USDA.** United States Department of Agriculture

**wholesale nursery.** any place of production at which nursery stock is grown, propagated, stored, sold, or distributed to other wholesale or retail nurseries or landscapers

*Phytophthora ramorum*

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