Phytophthora ramorum Domestic Regulatory Program Manual
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### Figures

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1-1</td>
<td>Schematic of Destruction and Quarantine Radii of Positive Plants 3-1-9</td>
</tr>
<tr>
<td>3-1-2</td>
<td>Assessment Checklist for Determining CCPS of <em>P. ramorum</em>-Confirmed-Positive Nursery Site 3-1-21</td>
</tr>
<tr>
<td>4-1-1</td>
<td>Schematic of Destruction and Quarantine Radii of Positive Plants 4-1-8</td>
</tr>
<tr>
<td>4-1-2</td>
<td>Nursery Questionnaire for a Confirmed-Positive Retail Nursery or Retail Nursery Dealer 4-1-14</td>
</tr>
<tr>
<td>5-4-1</td>
<td><em>Phytophthora ramorum</em> Trace Forward Questionnaire (Property Owner or Manager) 5-4-1</td>
</tr>
<tr>
<td>5-4-2</td>
<td><em>Phytophthora ramorum</em> Trace Back Questionnaire (Property Owner or Manager) 5-4-3</td>
</tr>
<tr>
<td>6-1-1</td>
<td>Diagram Showing Destruction Block, Quarantine Block, and Delimitation Survey 6-1-7</td>
</tr>
<tr>
<td>6-1-2</td>
<td><em>P. ramorum</em> Questionnaire for Property Owner or Manager 6-1-8</td>
</tr>
<tr>
<td>6-1-3</td>
<td>Follow-up Survey for Locations with Infected Plants 6-1-9</td>
</tr>
<tr>
<td>8-1-1</td>
<td>Instructions for Using PPQ Form 391, Specimens for Determination 8-1-3</td>
</tr>
<tr>
<td>10-1-1</td>
<td>Bottle of Bait (BoB) Materials and Leaf Pieces Plated into PARPH Media 10-1-5</td>
</tr>
<tr>
<td>10-1-2</td>
<td>Example of a Bait Bag Construction 10-1-7</td>
</tr>
<tr>
<td>10-1-3</td>
<td>Pattern of Mesh Screen to Use for Constructing a Bait Station 10-1-8</td>
</tr>
<tr>
<td>10-1-4</td>
<td>Completed Bait Station Construction 10-1-8</td>
</tr>
<tr>
<td>10-1-5</td>
<td>Characteristic Structure of <em>P. ramorum</em> 10-1-15</td>
</tr>
<tr>
<td>10-1-6</td>
<td>Vacuum Apparatus for Water Filtration and Filter Plated onto PARPH-V8 Plate 10-1-16</td>
</tr>
<tr>
<td>11-1-1</td>
<td>Baiting Soil Samples and Plating Baits 11-1-6</td>
</tr>
<tr>
<td>11-1-2</td>
<td>Characteristic Structure of <em>P. ramorum</em> 11-1-7</td>
</tr>
<tr>
<td>12-1-1</td>
<td>Steam Sock in Place on Soil Surface 12-1-5</td>
</tr>
<tr>
<td>12-1-2</td>
<td>Concrete Blocks Used to Support Tarp from Surface to Allow Steam Distribution from Steam Sock 12-1-6</td>
</tr>
<tr>
<td>12-1-3</td>
<td>Tarp with Sand Snakes in Place to Secure Edges 12-1-7</td>
</tr>
<tr>
<td>A-1-1</td>
<td>Example of PPQ Form 519, Compliance Agreement A-1-3</td>
</tr>
</tbody>
</table>
Figures
# Tables

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

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

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

**Table 3-1-3** Disinfest the Nursery

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

**Table 3-1-5** Minimum Number of Samples to Take Based on the Number of Regulated Plants Remaining With Confirmed-Positive Plants Following Destruction of Plants Within the D-Radii

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

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

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

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

**Table 5-2-1** Trace Forward Protocol Instruction

**Table 5-3-1** Trace Back Protocol Instructions

**Table 6-1-1** Confirmed Residential Protocol Instructions

**Table 6-1-2** Treatment and Disinfection Options for Residential or Commercial Landscaped Settings

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

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

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

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

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

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

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

**Table 12-1-1** Summary of Disinfectant Activities

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

Introduction

Contents

APHIS Mission  1-1-1
PPQ Mission  1-1-1
Manual Objective  1-1-1
Scope  1-1-2
Revisions  1-1-2
Questions Regarding this Manual  1-1-5

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.

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:

- **Introduction** on page 1-1-1
- **Phytophthora ramorum Inspection and Sampling Protocol for Nurseries** on page 2-1-1
- **Interstate Confirmed Nursery Protocol** on page 3-1-1
- **Confirmed Retail Nursery and Retail Nursery Dealer Protocol** on page 4-1-1
- **Trace Investigations** on page 5-1-1
- **Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed *P. ramorum*-Infested Nursery** on page 5-2-1
- **Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed *P. ramorum*-Infested Nursery** on page 5-3-1
- **Supporting Documents: Phytophthora ramorum Trace Investigation Questionnaires (Property Owner or Manager)** on page 5-4-1
- **Confirmed Residential Protocol** on page 6-1-1
- **Biology and Symptoms of Phytophthora ramorum** on page 7-1-1
- **Sampling and Submission Protocol** on page 8-1-1
- **Biosecurity Measures for Nurseries** on page 9-1-1
- **Water Sampling and Processing Protocol** on page 10-1-1
- **Soil and Container Mix Sampling and Processing Protocol** on page 11-1-1
- **Treatment and Disinfection Options** on page 12-1-1
- **Example of PPQ Form 519, Compliance Agreement** on page A-1-3
- **Glossary** on page Glossary-1-1
- **Index** on page Index-1-1

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
This protocol incorporates requirements and procedures outlined in the December 10, 2012 Federal Order (DA-2012-53) and in the January 10, 2014 (DA-2014-02) Federal Order (see Authorities on page 1-1-3). These Federal Orders are issued in line with the regulatory authority provided by 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. The Federal Orders are also issued in line with the regulations openly declared under the Plant Protection Act, and found at 7 CFR § 301.92 et. seq.

This chapter refers to wholesale and product nurseries throughout and includes interstate shipping retail nurseries and brokers whenever wholesale or production nurseries are mentioned.

In February 2005, USDA–Animal and Plant Health Inspection Service (APHIS)–Plant Protection and Quarantine (PPQ) published an interim rule revising Federal domestic regulations for \textit{P. ramorum} (7 CFR § 301.92). Since the regulations were first published in 2002, \textit{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 \textit{P. ramorum} found in nurseries. To ensure there is consistency in responding to \textit{P. ramorum} infestations, this chapter describes the official activities performed within and around nurseries by USDA–APHIS staff in cooperation with State agriculture regulatory officials.

**Authorities**

Consult the latest list of regulated plants prior to beginning any survey, inspection or delimitation. A current list can be found at APHIS List of \textit{Phytophthora ramorum}-Regulated Plants on page A-1-2.

To review the biology of \textit{P. ramorum}, see Biology and Symptoms of \textit{Phytophthora ramorum} on page 7-1-1.

1. For States with regulations for quarantine pests, and/or specifically for \textit{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.

2. For States without regulations for quarantine pests and/or for \textit{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.

3. Authority for this protocol is derived from the Section 414 of the Plant Protection Act, 7 U.S.C. 7714, 114 STAT. 445, PUBLIC LAW 106–224—June 20, 2000, as follows:

A. SEC 414, GENERAL REMEDIAL MEASURES FOR NEW PLANT PESTS AND NOXIOUS WEEDS. (a) AUTHORITY TO HOLD, TREAT, OR DESTROY ITEMS.—If the Secretary considers it necessary in order to prevent the dissemination of a plant pest or noxious weed that is new to or not known to be widely prevalent or distributed within and throughout the United States, the Secretary may hold, seize, quarantine, treat, apply other remedial measures to destroy, or otherwise dispose of any plant, plant pest, noxious weed, biological control organism, plant product, article, or means of conveyance that—

(1) is moving into or through the United States or interstate, or has moved into or through the United States or interstate;...

(b) is or has been otherwise in violation of this title; (2) has not been maintained in with a postentry quarantine requirements; (3) is the progeny of any plant, biological control organism, plant product, plant pest, or noxious weed that is moving into or through the United States or interstate, or has moved into the United States or interstate, in violation of this title.

B. (b) AUTHORITY TO ORDER AN OWNER TO TREAT OR DESTROY,—(1) IN GENERAL.—The Secretary may order the owner of any plant, biological control organism, plant product, plant pest, noxious weed, article, or means of conveyance subject to action under subsection (a), or the owner’s agent, to treat, apply other remedial measures to destroy, or otherwise dispose of the plant, biological control organism, plant product, plant pest, noxious weed, article, or means of conveyance, without cost to the Federal Government and in the manner the Secretary considers appropriate. (2) FAILURE TO COMPLY,—If the owner or agent of the owner fails to comply with the Secretary’s order under this subsection, the Secretary may take an action authorized by subsection (a) and recover from the owner or agent of the owner the costs of any care, handling, application of remedial measures, or disposal incurred by the Secretary in connection with action taken under subsection (a).

C. (c) CLASSIFICATION SYSTEM: (see Plant Protection Act, 7 U.S.C. 7714., 114 STAT. 445, PUBLIC LAW 106–224—JUNE 20, 2000)

Questions Regarding this Manual

Refer any questions concerning the use or content of this manual to the following office:

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Chapter 2

**Phytophthora ramorum**

Inspection and Sampling Protocol for Nurseries

## Contents

- Introduction 2-1-1
- Quick Guide for Conducting Nursery Inspection and Sampling 2-1-1
- Timing Nursery Inspection and Sampling 2-1-3
- Notifying the Laboratory 2-1-4
- Preparing for Nursery Inspection and Sampling 2-1-4
- Plant Symptoms and Sampling for *P. ramorum* 2-1-4
  - Plant Symptom Resources 2-1-4
  - Sampling by Symptom Type 2-1-5
- Inspecting and Sampling the Nursery 2-1-6
  - Sampling Instructions for Plants 2-1-7
  - Sampling Instructions for Water 2-1-9
  - Sampling Instructions for Soil 2-1-9
  - Sampling Instructions for Pots and Containers 2-1-10
  - Sampling Instructions for Cull Piles 2-1-10
  - Sampling Instructions for Other Articles 2-1-10

## 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 (see 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 (see 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 (see Preparing for Nursery Inspection and Sampling on page 2-1-4).

4. Prior to inspection day, review:
   A. APHIS List of *P. ramorum* Regulated Plants, see APHIS List of *Phytophthora ramorum*-Regulated Plants on page A-1-2
   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 (see Inspecting and Sampling the Nursery on page 2-1-6)
   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 spray schedules and sanitation measures?)

5. Determine the approximate number of plant samples to take from each regulated plant genus (see Table 2-1-1 on page 2-1-8).

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 (see Inspecting and Sampling the Nursery on page 2-1-6).

7. Take samples of:

8. Plants: sample symptomatic plant tissue. Ensure that at least the minimum number of samples are collected (see Table 2-1-1 on page 2-1-8) and keep plant genera samples separate from one another. Each sample must be bagged separately (see 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 (see 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 (see Inspecting and Sampling the Nursery on page 2-1-6). Properly label and store collected samples for shipping to the laboratory (see 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. (see 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 (see **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 (see **Inspecting and Sampling the Nursery** on page 2-1-6).

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; see **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 List of Phytophthora ramorum-Regulated Plants** on page A-1-2).

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**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


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 (see Table 2-1-1 on page 2-1-8), 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.
Inspection 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.

1. Before the inspection season begins, review the APHIS List of *Phytophthora ramorum*-Regulated Plants on page A-1-2; 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.

2. 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 (see Table 2-1-1 on page 2-1-8). 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).

3. 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.

4. Decontaminate inspection personnel, tools, and equipment between blocks in the nursery, between regulated plant genera within a block (see 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.

5. 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 List of *Phytophthora ramorum*-Regulated Plants on page A-1-2. 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.
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).

<table>
<thead>
<tr>
<th>Regulated plants per nursery:</th>
<th>Minimum number of samples to collect (95% confidence of detecting a 1.0% disease incidence)¹:</th>
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<td>283</td>
</tr>
<tr>
<td>1000</td>
<td>287</td>
</tr>
<tr>
<td>2000</td>
<td>308</td>
</tr>
<tr>
<td>3000</td>
<td>316</td>
</tr>
<tr>
<td>4000</td>
<td>320</td>
</tr>
<tr>
<td>5000</td>
<td>322</td>
</tr>
<tr>
<td>6000</td>
<td>324</td>
</tr>
<tr>
<td>7000</td>
<td>325</td>
</tr>
<tr>
<td>8000</td>
<td>326</td>
</tr>
<tr>
<td>9000</td>
<td>326</td>
</tr>
<tr>
<td>10000</td>
<td>327</td>
</tr>
<tr>
<td>20000+</td>
<td>332</td>
</tr>
</tbody>
</table>

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.
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.


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. Use the Soil and Container Mix Sampling and Processing Protocol on page 11-1-1 and also located at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/soil_protocol11-5-2010.pdf.

**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\(^1\) Containing

*Phytophthora ramorum*

Contents

Introduction 3-1-2
Goal 3-1-2
Trigger Events for Use of the Interstate Confirmed Nursery Protocol 3-1-2
Disclaimers 3-1-3
  Challenges 3-1-3
  Field-Grown Stock 3-1-3
Interstate Confirmed Nursery Protocol Steps 3-1-3
Interstate Confirmed Nursery Protocol (CNP) Procedures 3-1-4
  Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants 3-1-9
  Notification Requirements for Interstate CNP 3-1-10
Disinfect the Nursery 3-1-10
Delimitation Sampling for Confirmed Plant Positives and Sample Handling and Submission Protocol 3-1-13
  Collecting Samples 3-1-14
  Trigger Sequence for Entire Block Destruction 3-1-14
  Determining the Number of Samples to Take 3-1-15
Conduct Investigations 3-1-16
  Associated Nursery Site Inspections 3-1-16
  Trace Forward Investigations 3-1-16
  Trace Back Investigations 3-1-17
  Alternative Quarantine-Release Strategy 3-1-17
  Post-Confirmed Nursery Protocol Monitoring 3-1-18
Critical Control Point Assessment Procedures for *P. ramorum*-Confirmed-Positive Nursery Sites 3-1-18
  Planning the CCP Assessment 3-1-19
  CCP Assessment Day 3-1-19
  Critical Control Points 3-1-23
References 3-1-26

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\(^1\) Interstate shipping retail nurseries, brokers, interstate and certain intrastate wholesale and production nurseries.
Introduction

The intended use of this chapter 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)

Goal

The goal of this protocol is to prevent 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 survey, trace back survey, 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 (see the PPQ Phytophthora ramorum website for diagnostic information and the PASS protocol).
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. These protocols and regulations will be adjusted accordingly, based on the understanding of the pathogen biology. Detection and management of this pathogen is informed by continually improving science.

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. Secure the nursery
   A. Disinfest the nursery
   B. Delimit the nursery
   C. Delimiting survey results received
3. Conduct trace investigations
4. Ninety-day (minimum) quarantine activities
5. Release of plants in the nursery
6. Alternate quarantine-release strategy
7. Critical control point assessment

**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).
8. Post-quarantine release monitoring

After notifying the nursery, as concurrently as possible, conduct trace investigations while securing the nursery (see Conduct Investigations on page 3-1-16).

**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 regulatory officials as soon as one of the following has occurred:

- A positive PCR determination using APHIS-approved work instructions by an APHIS-approved laboratory; or
- A culture that matches the morphology for *P. ramorum* as determined and reported by an APHIS-approved laboratory.

For all Potentially Actionable Suspect Samples (PASS), laboratories must immediately forward them to an APHIS–PPQ laboratory (see Contact Information for the Phytophthora ramorum Program on page A-1-1) and notify their State’s State Plant Health Director (SPHD) and SPRO, and the NOM.

### Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 1 of 6)

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—Communicate and notify</td>
<td>In the event of a confirmed-positive sample at a nursery, the appropriate regulatory official in the State (SPRO or SPHD) immediately contacts the nursery owner to inform them of the confirmed-positive sample <strong>and to place a hold on all</strong> plants as described in <strong>Step 2—Secure the nursery</strong> on page 3-1-5. The SPRO and SPHD both inform the PPQ NOM about the confirmed-positive sample, nursery notification, and the hold on all plants. If there are unusual circumstances preventing timely arrival at the nursery, consult the PPQ cross-functional working group to determine next steps. <strong>Prior to</strong> arrival at the nursery, the inspectors shall review Interstate CNP in its entirety and acquire the necessary equipment and supplies specified in <strong>Biosecurity Measures for Nurseries</strong> on page 9-1-1. Upon arrival to the nursery, request shipping information for trace investigation <strong>concurrently</strong> with securing and delimiting the nursery as described below.</td>
<td></td>
</tr>
</tbody>
</table>
Interstate Confirmed Nursery Protocol  
Interstate Confirmed Nursery Protocol (CNP) Procedures

Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 2 of 6)

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| 2—Secure the nursery | 1. Concurrent with securing the nursery, conduct trace investigations.  
2. Refer to the Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants on page 3-1-9 for guidance.  
A. Arrive on site as soon as practicable. Identify locations of confirmed-positive sample sources.  
B. Establish the quarantine (Q) and destruction (D) radii for each confirmed-positive source as described below.  
C. For any type of confirmed positive, hold all regulated plants within each established Q-radii for the entire quarantine period (a minimum of 90 days of conducive environment for disease development).  
D. During implementation of this protocol, every plant on regulatory hold should not be subject to scheduled nursery maintenance.  
3. Restrict access to any D-radii until the inspector/nursery is prepared to begin disinfestation procedures; see Disinfest the Nursery on page 3-1-10.  
4. Put nursery under initial compliance agreement for first-time detection.  
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.  
6. To ensure material does not move prior to being cleared, use the PPQ Form 523, Emergency Action Notification (EAN), or State equivalent. | |
2b—Delimiting survey

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For: All confirmed positives</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ensure conducive environmental conditions are present, as described in Timing Nursery Inspection and Sampling on page 2-1-3.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Ensure necessary sanitation measures are applied by regulatory officials while in the confirmed nursery.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Examine all regulated and nonregulated plants within the nursery.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Sample any symptomatic tissue found and submit samples to the appropriate laboratory.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>See individual delimiting procedures for specific confirmed-positive material.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Place all sampled plants on hold.</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Disinfest tools and equipment associated with any confirmed-positive materials.</td>
<td></td>
</tr>
</tbody>
</table>

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.

Confirmed-positive plant(s)

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Immediately examine all plants in the Q-radii.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sample any unhealthy tissue, provided it is not exhibiting desiccation or extensive decay.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Inspect all regulated genera in the nursery.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Sample surface water from underneath confirmed-positive plant(s), as well as throughout the D-radii and adjacent downslope areas (see Water Sampling and Processing Protocol on page 10-1-1 and Soil and Container Mix Sampling and Processing Protocol on page 11-1-1).</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

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 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.

Confirmed-positive surface water or soil

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sample any unhealthy tissue, provided it is not exhibiting desiccation or extensive decay.</td>
<td></td>
</tr>
</tbody>
</table>

Confirmed-positive cull pile

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For: If not sampled in initial inspection, sample surface water, soil, or symptomatic plants adjacent to and/or downslope from cull pile.</th>
<th>Then:</th>
</tr>
</thead>
</table>

Confirmed-positive used containers

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Inspect any plants within 2 meters of container storage area.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sample symptomatic plants.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Sample surface water or soil within the 2-meter radius.</td>
<td></td>
</tr>
</tbody>
</table>

Perimeter survey

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Survey for symptoms on all plants located within 10 meters of the infested nursery.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Sample all symptomatic plants.</td>
<td></td>
</tr>
</tbody>
</table>
### Interstate Confirmed Nursery Protocol (CNP) Procedures

Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 4 of 6)

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| 2c—Delimiting survey results received | 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.  
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. |
|                   | 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 (see Trigger Sequence for Entire Block Destruction on page 3-1-14 for more details). |
|                   | 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.  
2. The 90-day quarantine starts when samples are submitted. |
| 3—Conduct trace investigations | Trace forward and 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 10 business days.  
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 10 business days. |
|                   | Associated nursery sites | 1. Determine from provided information if additional locations (i.e., nursery sites) are owned and operated by the same nursery company.  
2. Determine from provided information if nursery personnel are deployed to multiple locations.  
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.  
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 (see Biosecurity Measures for Nurseries on page 9-1-1). |
| 4—90-day (minimum) quarantine activities | | 1. The 90-day (minimum) quarantine period begins the day samples are submitted if:  
   A. The delimiting survey is completed; and  
   B. All delimiting sample results are negative; and  
   C. PPQ Form 523, EAN or sufficient State equivalent is issued.  
2. Update hold notice for specific plants on hold, e.g., official communication (PPQ Form 523, or State equivalent).  
3. Within the Q-radius, do not allow applications of fungicides registered for *Phytophthora* spp. control during the quarantine period. |
4—90-day (minimum) quarantine activities (cont.)

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.

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.

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:

- 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
- 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
- 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 elect to follow the alternate quarantine-release strategy (see Alternative Quarantine-Release Strategy on page 3-1-17 for more details) any time within any of the 90-day quarantine periods by fulfilling all the requirements in the order specified below:

1. The nursery must destroy everything (e.g., all regulated plants, containers, growing media, etc.) in each D-radius by approved methods listed in the specific nursery compliance agreement (see Example of PPQ Form 519, Compliance Agreement on page A-1-3); and
2. Inspect and sample all regulated plants within the Q-radius. Destroy all plants within the Q-radius only after receiving diagnostic results (please note new confirmed-positive diagnostic results will trigger new D- and Q-radii for additional destruction and sampling. Reference the Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants on page 3-1-9 for guidance); and
3. Mitigate soil of each D- and Q-radius, as per Disinfecting Soil and Container Mix on page 12-1-4. Sample and test drainage or recirculated irrigation water, if not previously tested and determined to be negative, as per Sampling Instructions for Water on page 2-1-9; and
4. 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” (see 8—Post-quarantine release monitoring below).
Table 3-1-1 Interstate Confirmed Nursery Protocol (CNP) Procedures (page 6 of 6)

<table>
<thead>
<tr>
<th>If the step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>7—Critical control point (CCP) assessment</td>
<td>After completing the delimiting survey and implementing or planning disinfection procedures for each confirmed-positive article, 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 <em>P. ramorum</em>. See Example of PPQ Form 519, Compliance Agreement on page A-1-3 for further instructions.</td>
<td></td>
</tr>
<tr>
<td>8—Post-quarantine release monitoring</td>
<td>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 <em>P. ramorum</em> detections. If there are further <em>P. ramorum</em> detections during the 3-year monitoring, the nursery must enter into a revised compliance agreement and restart 3 consecutive years of negative sample results.</td>
<td></td>
</tr>
</tbody>
</table>

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

Figure 3-1-1 Schematic of Destruction and Quarantine Radii of Positive Plants
Interstate Confirmed Nursery Protocol
Disinfest the Nursery

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

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

Notification Requirements for Interstate CNP

SPHDs and/or SPROs will notify nurseries and the NOM of final determinations 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 10 business days of a confirmed *P. ramorum*-positive sample in a nursery (see 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*. See Example of PPQ Form 519, Compliance Agreement on page A-1-3 for further instructions.
### Table 3-1-3  Disinfest the Nursery (page 1 of 2)

<table>
<thead>
<tr>
<th>If material is:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| Confirmed-positive plant(s)             | 1. Destroy **all** plants in the D-radii, including pots and container mix, per **Treatment and Disinfection Options** on page 12-1-1; see **Schematic of Destruction (D) and Quarantine (Q) Radii of Positive Plants** on page 3-1-9.  
2. Remove and destroy **all** plant debris including container mix and any other plant parts found within the D-radii; see **Treatment and Disinfection Options** on page 12-1-1 for proper removal and destruction.  
3. For field-grown stock, contact the NOM.  
4. Sample surface water or soil underneath the D- and Q-radii; see **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.  
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 (see **Example of PPQ Form 519, Compliance Agreement** on page A-1-3). |
| 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** from *P. ramorum* as determined by water-testing protocols described in **Soil and Container Mix Sampling and Processing Protocol** on page 11-1-1.  
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; see **Treatment and Disinfection Options** on page 12-1-1.  
3. Confirmed-positive irrigation water samples initiate the minimum 90-day quarantine period for plants receiving positive irrigation water. |
| Confirmed-positive cull pile             | 1. Immediately demarcate the cull pile to avoid pathogen dispersal (as a quarantine hold).  
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 **Treatment and Disinfection Options** on page 12-1-1.  
3. Use the CCP assessment to address site-specific conditions and determine appropriate mitigation measures, see Exhibit A (Compliance Agreement example) and Exhibit D (examples of CCP Assessment) in **Example of PPQ Form 519, Compliance Agreement** on page A-1-3 for further instructions. |
| Confirmed effluent water (e.g., culvert/ditch, stream, non-recycled retention pond) is positive | 1. Immediately demarcate the area around the confirmed-positive effluent water.  
2. Identify remediation, mitigations, or business/cultural practices via the CCP assessment with the nursery owner.  
3. Use the CCP assessment to address site-specific conditions and determine appropriate mitigation measures, see Exhibit A (Compliance Agreement example) and Exhibit D (examples of CCP Assessment) in **Example of PPQ Form 519, Compliance Agreement** on page A-1-3 for further instructions. |
Table 3-1-3 Disinfest the Nursery (page 2 of 2)

<table>
<thead>
<tr>
<th>If material is:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed-positive</td>
<td>1. Locate and reestablish boundary demarcation.</td>
</tr>
<tr>
<td>soil (nursery sub-strate)</td>
<td>2. Place barrier mitigation and/or adopt appropriate avoidance practices while determining the disinfestation/remediation strategy.</td>
</tr>
<tr>
<td></td>
<td>3. Use the CCP assessment, Treatment and Disinfection Options on page 12-1-1, and mitigation options available from nursery associations, county extensions, and State nursery practices manuals; also see Exhibit A (Compliance Agreement example) and Exhibit D (examples of CCP Assessment) in Example of PPQ Form 519, Compliance Agreement on page A-1-3 for further instructions.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>If step number:</th>
<th>For:</th>
<th>In nursery category¹:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 — Quarantine period activities</td>
<td>D and Q radius</td>
<td>The quarantine period, a minimum of 90 days, 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.  During the quarantine period:  1. Do not use fungicides registered for Phytophthora spp. in the plant’s Q-radii.  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.  3. If confirmed-positive samples result from quarantine period surveys, return to steps 2 through 4 in Table 3-1-1 on page 3-1-4; quarantine period begins again.</td>
<td></td>
</tr>
<tr>
<td>6—Quarantine release survey</td>
<td>D radius</td>
<td>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.  Q-radii: after 90 days, 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.²</td>
<td>Transition from EAN to new or revised CA  Q radius</td>
</tr>
</tbody>
</table>

¹ See Table 3-1-2 on page 3-1-10.
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 Table 3-1-1 on page 3-1-4.

**NOTICE**

**REMEMBER:** 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. Disinfect 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* (see *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 block. 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 Remaining With Confirmed-Positive Plants Following Destruction of Plants Within the D-Radii

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

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, inform the nursery to research shipments of the past 6 months for the trace forward and trace back investigations.

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 (see 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 *Phytophthora 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 under Federal Order DA-2012-53, see 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 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 destruction block, 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 trace forward/trace back 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).

**NOTICE**

**Forward the trace forward list(s) to PPQ's National Operations Manager (NOM) within 10 business days.**

The NOM will forward domestic trace forward lists to the States that have received plants. The NOM will inform international 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, see 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).

**NOTICE**

**Forward the trace back list(s) (including plant taxonomic details) to PPQ’s NOM within 10 business days.**

To view the trace back protocol see 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. This can occur if the following conditions are met:

1. **Steps 1 through 4 in Table 3-1-1 on page 3-1-4 and all steps in Table 3-1-3 on page 3-1-11** 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 disinfect/remediate; ensure avoidance/exclusion mitigations are in place for these confirmed-positive areas.
5. The compliance agreement shall contain measures to address *P. ramorum* in the nursery and the agreement is signed by the nursery representatives and regulatory officials (*see Example of PPQ Form 519, Compliance Agreement on page A-1-3*).

6. The short-term mitigations and the longer-term remediation plan are written into Exhibit D of the PPQ Form 519, Compliance Agreement (CA) (*see Example of PPQ Form 519, Compliance Agreement on page A-1-3*).

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 is no longer regulated. For nurseries in the quarantine area, the sampling returns to the 7 CFR § 301.92 regimen.

In addition to the 3 years of post-confirmed monitoring, the nursery shall notify the receiving States of shipments from the monitored nursery of *Camellia* spp., *Kalmia* spp., *Pieris* spp., *Rhododendron* spp. (including azalea), and *Viburnum* spp. nursery stock. Once samples test negative for 2 years, the nursery is released from this requirement. View the Notification Federal Order DA-2012-53 Instructions for Regulated and Quarantine Areas at the following address: [https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/DA-2012-53.pdf](https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/DA-2012-53.pdf).

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**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, 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 of the PPQ Form 519, Compliance Agreement (CA) (*see Example of PPQ Form 519, Compliance Agreement on page A-1-3*).
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. After deliberating on an approach, the assessment team walks the nursery site focusing on the known confirmed-positive sample areas. 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?

*Figure 3-1-2 on page 3-21* is a checklist for a preassessment or walk-through of a confirmed-positive nursery site. This checklist may be used prior to the assessment team’s arrival for information-gathering purposes and to focus the team’s efforts and optimize time.
### Interstate Confirmed Nursery Protocol

**CCP Assessment Day**

**01/2022-04**

**Phytophthora ramorum**

Manual/Interstate Confirmed Nursery Protocol 3-1-21

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#### Figure 3-1-2  Assessment Checklist for Determining CCPS of *P. ramorum*-Confirmed-Positive Nursery Site (page 1 of 2)

<table>
<thead>
<tr>
<th>Nursery Name:</th>
<th>Nursery License Number:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Location:</th>
<th>Phytophthora ramorum regulated plants present:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Rhododendron □ Camellia □ Kalmia □ Viburnum □ Pieris □ Other</td>
</tr>
</tbody>
</table>

I. REGULATED PLANT BUY-INS AND SALES

<table>
<thead>
<tr>
<th>Records kept for sales:</th>
<th>BMP for buy-ins:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Plants shipped/received</td>
<td>□ Inspected on arrival</td>
</tr>
<tr>
<td>□ Received contact info</td>
<td>□ Isolated for 30-days</td>
</tr>
<tr>
<td>□ Invoice number</td>
<td>□ Trucks cleaned on arrival</td>
</tr>
<tr>
<td>□ Fungicide records</td>
<td>□ Loading dock cleaned after delivery</td>
</tr>
<tr>
<td>□ Kept 24-months</td>
<td>□ Returns treated like buy-ins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

II. GENERAL SANITATION

<table>
<thead>
<tr>
<th>Loading dock:</th>
<th>Plant debris present, pathways, beds:</th>
<th>Cull pile:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Clean</td>
<td>□ Heavy □ Medium □ Light □ Other</td>
<td>□ Isolated □ No drainage □ Off-site □ Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

III. POTTING MEDIA

<table>
<thead>
<tr>
<th>Sourced from</th>
<th>Storage:</th>
<th>Components:</th>
<th>Treated:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Commercial</td>
<td>□ Concrete</td>
<td>□ Bark □ Used media □ Pumice □ Peat □ Other</td>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

IV. POTTING AREA

<table>
<thead>
<tr>
<th>How potted:</th>
<th>Sanitation:</th>
<th>Pots:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Machine □ Hand</td>
<td>□ Clean tools □ Limit traffic □ Dedicated tools □ Other</td>
<td>□ New □ Cleaned □ Sanitized □ Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

V. PLANTS GROWN IN CONTAINERS

<table>
<thead>
<tr>
<th>Pots placed on:</th>
<th>Gravel depth:</th>
<th>Sanitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Gravel □ Native soil</td>
<td>□ ≥ 4” □ ≥ 6” □ Compacted □ Other</td>
<td>□ Plant debris □ Weeds □ Re-used pots □ Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

VI. IRRIGATION WATER*

<table>
<thead>
<tr>
<th>Source:</th>
<th>Type</th>
<th>Treatments received:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Well □ Municipal □ River</td>
<td>□ Overhead □ Hose □ Microsprinkler □ Drop</td>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>

VII. SOIL DRAINAGE

<table>
<thead>
<tr>
<th>Surface water present:</th>
<th>(check all that apply)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Plant blocks □ Greenhouses</td>
<td>□ Roadways □ Media piles</td>
</tr>
<tr>
<td>□ None □ Other</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments:</th>
<th></th>
</tr>
</thead>
</table>
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. Remediation measures, mitigations, and BMP options corresponding to the CCPs will be researched by the nursery owner with assistance from the county or university extension, State or nursery associations, and the inspector. The State and PPQ regulatory officials/SMEs will provide as much information as possible about mitigation measures to assist the nursery owner.

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 chosen by the nursery owner/inspector will be written into Exhibit D of the Compliance Agreement with appropriate time periods (see Example of PPQ Form 519, Compliance Agreement on page A-1-3).

NOTICE

After considering the recommended measures, the nursery and inspector will determine which measures to implement to specifically address the known, confirmed-positive sample sites. The nursery may also suggest its own measures for consideration to address specific CCPs. The agreed-upon measures will become part of a Compliance Agreement in Exhibit D with specific actions, timelines, and outcomes. Over time, additional BMPs and mitigations may be implemented by the nursery as feasible.
## Critical Control Points

Table 3-1-6  Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices¹ (page 1 of 4)

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

¹ For more detailed information on each CCP, please refer to the_Phyllostrophora ramorum_Manual/Interstate Confirmed Nursery Protocol.
<table>
<thead>
<tr>
<th>Identified critical control point (CCP):</th>
<th>Mitigations that may have preceded CCP assessment:</th>
<th>Examples of remediations, mitigations, BMPs, and changes to business/cultural practices:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potting media</td>
<td>◆ Double-bagged, identified material in (2 mm) self-sealing plastic bags and deep burial (&gt;2 m) burial in a site approved by regulatory authorities ◆ Incinerated at a site approved by regulatory authorities ◆ Heat sterilization; dry heat or steam (see USDA Treatment Manual Schedule T415b)</td>
<td>◆ Do not reuse container mix from regulated plants ◆ Ensure area on which the growing media sits drains freely ◆ Ensure cull piles are clearly separated from container mix components ◆ Ensure growing container mix and/or components are from an area known to be free from \textit{P. ramorum} ◆ Move container mix piles away from potential \textit{P. ramorum} contamination sources ◆ Pasteurize potting media ◆ Place container mixes in containers, bins, or on hard surfaces that can be cleaned, and \textbf{not} in contact with site soil ◆ Purchase components from suppliers with the ability to supply media free from plant pathogens and pests and meets quality requirements ◆ 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 ◆ Sample and test media and media components at delivery or before use ◆ Steam-sterilize \textbf{any} container mix that is reused or composted according to strict national standards</td>
</tr>
<tr>
<td>Potting area</td>
<td>◆ Disinfestation of nonporous surfaces, concrete floors, benches, plastic sheeting, and tools</td>
<td>◆ Clean and disinfect \textbf{all} equipment used to transport media, e.g., front-end loader buckets, wheel barrows, mobile bins, trolleys, or plastic containers between uses ◆ Ensure staff members regularly wash their hands and maintain good hygiene ◆ Limit or divert traffic through soil-mixing area ◆ Regularly clean and disinfest potting facilities ◆ Regularly clean up and discard split media around potting facilities ◆ Schedule specific times to pot regulated plants and clean equipment and area before and after potting operations ◆ Use clean equipment to mix or load planting media</td>
</tr>
<tr>
<td>Nursery beds</td>
<td>◆ To avoid contact between infested soil/surface water and regulated plants, install permanent impermeable, nonporous barriers ◆ Steam soil ◆ Soil fumigation (e.g., dazomet, methyl bromide) ◆ Solarize soil</td>
<td>◆ Maintain substrate, whether this is through additional gravel, repairing or replacing landscape cloth or covering, or leveling to improve or increase drainage ◆ 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</td>
</tr>
<tr>
<td>Identified critical control point (CCP):</td>
<td>Mitigations that may have preceded CCP assessment:</td>
<td>Examples of remediations, mitigations, BMPs, and changes to business/cultural practices:</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Irrigation water</td>
<td>Treat recycled water or water used for irrigation water with chlorine levels of 2 ppm or 2 mg/liter or greater</td>
<td>Avoid overhead irrigation of regulated plants or irrigate in a manner to avoid prolonged leaf wetness and splash</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eliminate accumulations of surface water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Irrigate regulated plant material around dawn, when possible, in order to prevent extended periods of leaf wetness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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 from the pathogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monitor water treatment systems to verify the appropriate treatment measures are being applied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevent surface water by not overwatering. Irrigate regulated plants based on water needs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td>Water drainage</td>
<td></td>
<td>Divert soil and water movement from adjacent properties populated with regulated plants to prevent nursery contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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</td>
</tr>
<tr>
<td>Pots/containers</td>
<td>Sterilization by steam or disinfection by alcohols, chlorine, quaternary ammonium, or phenolics</td>
<td>Do not store used pots in areas in which water drainage could flow or splash into regulated plant beds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Establish a procedure for cleaning and sanitizing pots with clear separation of clean pots from dirty pots</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regularly control weeds in and around container storage facilities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Store new and clean disinfested containers above ground level</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Store pots on a barrier that effectively separates them from underlying substrates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use pots that are: 1) new; 2) clean and properly disinfested; or 3) sanitized by steam sterilization or hot water dip</td>
</tr>
</tbody>
</table>
Table 3-1-6 Examples of CCPs, Remediations, Mitigations, Best Management Practices (BMPs), and Changes to Business/Cultural Practices\(^1\) (page 4 of 4)

<table>
<thead>
<tr>
<th>Identified critical control point (CCP):</th>
<th>Mitigations that may have preceded CCP assessment:</th>
<th>Examples of remediations, mitigations, BMPs, and changes to business/cultural practices:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roads/pathways</td>
<td>◆ Cover pathways and roads adjacent to beds and benches with materials to reduce contamination with soil and water &lt;br&gt; ◆ Maintain roads to avoid surface water; fill potholes and maintain drainage such that water will flow away &lt;br&gt; ◆ Maintain substrate, leveling to improve or increase drainage &lt;br&gt; ◆ Prevent buildup of fallen leaves and plant debris</td>
<td></td>
</tr>
<tr>
<td>Conveyance</td>
<td>◆ Develop or review processes of cleaning tires and carts or trailers used in moving plant material &lt;br&gt; ◆ Do not allow trucks to sweep out debris into nursery &lt;br&gt; ◆ 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 &lt;br&gt; ◆ Regularly clean and disinfect transport equipment &lt;br&gt; ◆ Require pick-up and delivery trucks to properly clean and sanitize truck bed, undercarriage, and tires prior to entering nursery operations &lt;br&gt; ◆ 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</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) 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.

◆ **Australia BioSecure HACCP Guidelines, Nursery Industry Accreditation Scheme, and United States Nursery Certification Program (USNCP)**

◆ **National Plant Board A Systems Approach to Nursery Certification (SANC)—Nursery Practices**

◆ **Nursery Industry Best Management Practices for \(P.\ ramorum\) to Prevent the Introduction or Establishment in California Nursery Operations**

◆ **Oregon Grower Assisted Inspection Program (GAIP)**

◆ **Presidio Phytophthora Management Recommendations**

◆ **United States Nursery Certification Program (USNCP)**
Introduction

This chapter covers retail nurseries and retail nursery dealers who are intrastate shippers only. Any nursery that ships interstate is covered in Chapter 3 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 re-wholesale nursery, intrastate commerce grower, or an intrastate commerce broker with a nursery site of hold lot, please note the following:

If a State is interested in quarantine less than the entire State for \textit{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 \textit{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 \textit{Interstate Confirmed Nursery Protocol} on page 3-1-1 for guidance.

\textbf{NOTICE}

Online retail plant sellers would not be considered a retail nursery and should use Chapter 3 on page 3-1-1 when positive plants are found.

\textbf{Goal}

The goal of this protocol is to prevent the spread of \textit{Phytophthora ramorum}, a quarantine plant pathogen, and to simplify the movement of \textit{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 \textit{P. ramorum} plant infections are crucial to ensure spread is contained.

\textbf{Disclaimer}

Any interpretation of this chapter 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 survey, trace back survey, 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 (see the PPQ *Phytophthora ramorum* website for diagnostic information and the PASS protocol).

### 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 comingled, 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
Confirmed Retail Nursery and Retail Nursery Dealer Protocol

Confirmed Retail Nursery and Retail Nursery Dealer Steps

◆ Large retail chains create a unique pathway that is further complicated by the increasing use of consignment sales in ornamental plants
◆ Movement of [by?] nursery industry 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.
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 APHIS–PPQ–S&T Beltsville Laboratory (see Contact Information for the Phytophthora ramorum Program on page A-1-1) and notify the State’s State Plant Health Director (SPHD) and State Plant Regulatory Official (SPRO) 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    | When a sample is confirmed positive, laboratories need to notify the SPHD and the SPRO, the National Operations Manager (NOM), the National Program Manager, and the submitter. The SPRO (if State authority is used) or the SPHD (if Federal authority is used) may notify the owners 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. |
| 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 10 business days of a confirmed *P. ramorum*-positive sample in a nursery (SEE 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. |
| 1.5                        | Laboratories need to notify the SPHD and the SPRO, the NOM, the National Program Manager, 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. |
| 2—Conduct investigations   | Conduct trace back and trace forward investigations within a similar time frame to securing the nursery as is practical because invoices and shipping lists are to be provided to the NOM within 10 business days. Trace back investigations will be more common than trace forward investigations, especially at retail nursery dealers. Most retail establishments may not keep records of plant sales, however, it is necessary to inquire. |
Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps

<table>
<thead>
<tr>
<th>Step number: Subnumber: Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2—Conduct investigations (cont.)</strong></td>
</tr>
<tr>
<td>2.1—Trace back investigation</td>
</tr>
<tr>
<td>At the time <em>P. ramorum</em> 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 10 days. For more detailed instructions, please see Trace Back Protocol on page 5-3-1.</td>
</tr>
<tr>
<td><strong>2.2—Trace forward investigation</strong></td>
</tr>
<tr>
<td>Most retail establishments may not keep records of plant sales, however, it is necessary to inquire in order to perform a trace forward investigation. 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 to another facility, such as a landscaper, plants that could potentially be infected. Initiate the trace forward investigation by identifying all plants (meeting the following criteria) sold within 6 months of the first confirmed-positive detection of <em>P. ramorum</em> at a nursery: 1) plants of the infected species/cultivar; 2) all regulated plants that originated in the destruction block; and 3) any plants of the high-risk genera: <em>Camellia</em> spp., <em>Kalmia</em> spp., <em>Pieris</em> spp., <em>Rhododendron</em> spp. (including azalea), and <em>Viburnum</em> 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).</td>
</tr>
<tr>
<td><strong>3—Secure the nursery</strong></td>
</tr>
<tr>
<td>When the presence of <em>Phytophthora ramorum</em> 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 on page 4-1-8 and Table 4-1-2 on page 4-1-9) is included, but may not be appropriate for use in retail nursery dealer settings.</td>
</tr>
<tr>
<td>3.1—Establishing destruction and quarantine radius (radii) for confirmed-positive plant(s)</td>
</tr>
<tr>
<td>3.1.1—All plants within 2 meters of any infected plants shall be held for destruction (destruction radius)</td>
</tr>
<tr>
<td>3.1.2—All regulated 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 (SEE 7—Release the nursery on page 4-1-13).</td>
</tr>
<tr>
<td>3.1.3—Inspect all regulated plants in the nursery that are not within the destruction and quarantine radii and hold any plants sampled. Any other product or article an inspector determines presents a risk of spreading <em>P. ramorum</em>, if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations, can be held (7 CFR Part 301.92-2).</td>
</tr>
<tr>
<td>3.1.4—Refer to 5—Disinfest the nursery on page 4-1-10 for destruction and treatment protocols.</td>
</tr>
</tbody>
</table>
### Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps

<table>
<thead>
<tr>
<th>Step number:</th>
<th>Subnumber:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>4—Survey the nursery</td>
<td></td>
<td>The goal of the survey is to locate <em>P. ramorum</em> in the nursery. A detailed and thorough inspection should be conducted in the nursery to determine the presence of <em>P. ramorum</em>. Samples should be collected from symptomatic plants (SEE Biology and Symptoms of <em>P. ramorum</em> on page 7-1-1).</td>
</tr>
<tr>
<td>4.1—Delimit the nursery</td>
<td>4.1.1—Examine all plants within the retail nursery or retail nursery dealer and sample any symptomatic plant tissue found (SEE Plant Symptoms and Sampling for <em>P. ramorum</em> on page 2-1-4). Plants currently regulated in nurseries are included in the APHIS List of Regulated Hosts and Plants Associated with <em>Phytophthora ramorum</em> located on the USDA–APHIS–PPQ website (<a href="http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/">http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/</a>).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>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 (SEE 7—Release the nursery on page 4-1-12).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1.3—Submit samples to the appropriate lab for analysis using a methodology approved by APHIS (SEE Sampling and Submission Protocol on page 8-1-1).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1.4—Release all plants held if sample results are negative.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1.5—Establish destruction and quarantine radii around plant(s) with positive diagnostic results (SEE 4.2—Establish destruction and quarantine radii for plants confirmed positive during delimiting survey on page 4-1-7) and delimit the nursery again.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1.6—The 90-day plus quarantine period begins when the delimiting survey is complete.</td>
<td></td>
</tr>
<tr>
<td>4.2—Establish destruction and quarantine radii for plants confirmed positive during delimiting survey</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2.2—Establish destruction radius (radii) by flagging a 2-meter radius (a 4-meter diameter circle) around all infected plants (SEE Figure 4-1-1 on page 4-1-8) OR inspector-witnessed relocation/segregation of plants within the nursery with safeguarding OR plants can be relinquished under official supervision at any time (SEE 7—Release the nursery on page 4-1-13); include plants on carts or other movable shelving situations.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2.3—Establish quarantine radius (radii) by flagging a 4-meter radius (an 8-meter diameter circle) around all infected plants (SEE Figure 4-1-1 on page 4-1-8) OR inspector-witnessed relocation/segregation of plants within the nursery with safeguarding OR voluntary destruction.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2.4—Limit access to destruction and quarantine radius (radii) or other designated hold areas. Ensure proper sanitation measures are applied (SEE 5—Disinfest the nursery on page 4-1-10 and Biosecurity Measures for Nurseries on page 9-1-1).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2.5—Inspectors should document all actions taken on EAN or State equivalent.</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4-1-1  Schematic of Destruction and Quarantine Radii of Positive Plants

Gray = destruction zone
Light gray = quarantine zone
Dotted = block of regulated plants
White = block of nonregulated plants
Table 4-1-2  Legend of Destruction (D) and Quarantine (Q) Radii of Positive Plants Schematic

<table>
<thead>
<tr>
<th>Color</th>
<th>Description</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray (*1)</td>
<td>Destruction block</td>
<td>Destroy all plants, containers, and leaf debris</td>
</tr>
<tr>
<td>Light gray(*2)</td>
<td>Quarantine block</td>
<td>Hold all plants from sale for a <strong>minimum</strong> of 90 days</td>
</tr>
<tr>
<td>Gray (*3)</td>
<td>D-radii in block with nonregulated plants</td>
<td>Destroy all plants; nonregulated plant nursery stock could still move the pathogen</td>
</tr>
<tr>
<td>Light gray(*4)</td>
<td>Q-radii in block with nonregulated plant</td>
<td>Hold all plants from sale during the delimiting survey until <strong>all</strong> diagnostic results are final</td>
</tr>
<tr>
<td>Dotted (*5)</td>
<td>Rest of the block with regulated plants</td>
<td>Positive block needs to be intensely sampled and held until positive block delimitation samples are returned</td>
</tr>
<tr>
<td>White (*6)</td>
<td>Rest of the block of nonregulated plants</td>
<td>Release all plant materials for sale if found to be asymptomatic during the delimiting survey</td>
</tr>
</tbody>
</table>

Table 4-1-1  Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (cont.)

<table>
<thead>
<tr>
<th>Step number:</th>
<th>Subnumber:</th>
<th>Then:</th>
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</table>
| 4—Survey the nursery (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—  
  ◆ 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.  
  ◆ Water sampling is **not** required for irrigation water from municipal water facilities that treat their water prior to released.  
  ◆ Sample any retention pond or surface water at the nursery (SEE 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—  
  ◆ 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.  
  ◆ Check cull and compost piles for *P. ramorum* symptomatic plants and plant material and sample if observed.  
  ◆ 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.  
  ◆ Sample and test soil for the presence of *P. ramorum* at the down slope edge of the cull pile.  
  ◆ Determine how the nursery disposes of cull and compost piles.  
  ◆ Restrict access.  
  ◆ Determine the appropriate treatment or destruction method (SEE Treatment and Disinfection Options on page 12-1-1).
Table 4-1-1  Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (cont.)

<table>
<thead>
<tr>
<th>Step number:</th>
<th>Subnumber:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| 4—Survey the nursery (cont.) | 4.3—Sample other articles of concern at the nursery (cont.) | 4.3.5—Segregation of plants on hold—
| | | ◆ 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. |
| | | 4—Survey the nursery |
| | | Inspectors should use appropriate disinfection measures for conditions found at the establishment. For information on disinfecting and sanitizing any nursery, SEE Treatment and Disinfection Options on page 12-1-1 and Biosecurity Measures for Nurseries on page 9-1-1. |
| 5—Disinfest the nursery | 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 Treatment and Disinfection Options on page 12-1-1. This includes pots and potting media. |
| | | 5.1.2—Plants can be voluntarily relinquished under official supervision at any time (SEE 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 Treatment and Disinfection Options 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 (SEE Treatment and Disinfection Options on page 12-1-1 for recommended disinfection 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. SEE Treatment and Disinfection Options on page 12-1-1 for other recommended options. |
| | 5.5—Equipment and personnel | Use recommended disinfection options and biosecurity measures (SEE Treatment and Disinfection Options on page 12-1-1 and Biosecurity Measures for Nurseries on page 9-1-1). Use appropriate sanitation practices, such as disinfecting clippers used to cut plants or using disposable gloves to prevent cross-contamination. |
| | 5.6—Biosecurity measures | Biosecurity measures, such as tool sanitation, equipment disinfection, 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 (SEE Biosecurity Measures for Nurseries on page 9-1-1 for recommended biosecurity measures). |
Table 4-1-1 Confirmed Retail Nursery and Retail Nursery Dealer Protocol Steps (cont.)

<table>
<thead>
<tr>
<th>Step number:</th>
<th>Subnumber:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>5—Disinfest the nursery (cont.)</td>
<td>5.7—Treatment for cull piles, compost piles, water, soil, or growing media</td>
<td>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 (SEE Treatment and Disinfection Options on page 12-1).</td>
</tr>
<tr>
<td>5.8—Considerations for inspectors</td>
<td>5.8.1—Determine if plants will be placed on hold, destroyed on site, destroyed off site, or relinquished to an inspector.</td>
<td>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?).</td>
</tr>
<tr>
<td></td>
<td>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?</td>
<td>5.8.4—Determine which disinfestation methods are appropriate for the site (SEE Treatment and Disinfection Options on page 12-1). 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.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td>Plants can be relinquished under official supervision at any time (SEE 7—Release the nursery on page 4-13). These activities follow the completion of the delimiting survey and may run concurrent with some of the disinfestation activities taking place at the nursery. [...] “facilities with temporary supply of plants”—Amy note: it is unclear to me where this phrase belongs.</td>
</tr>
<tr>
<td>6—Ninety-day (minimum) quarantine activities</td>
<td>5.8.1—Determine if plants will be placed on hold, destroyed on site, destroyed off site, or relinquished to an inspector.</td>
<td>6.1—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 (SEE 3—Secure the nursery on page 4-1).</td>
</tr>
<tr>
<td></td>
<td>6.1.1—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 (SEE 3—Secure the nursery on page 4-1).</td>
<td></td>
</tr>
<tr>
<td>6.2—During the 90-day (minimum) quarantine period within the quarantine radius (radii)</td>
<td>6.2.1—No fungicides registered for Phytophthora control shall be applied.</td>
<td></td>
</tr>
<tr>
<td>Step number:</td>
<td>Subnumber:</td>
<td>Then:</td>
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<td>-------------</td>
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</tr>
<tr>
<td>6—Ninety-day (minimum) quarantine activities (cont.)</td>
<td>6.2—During the 90-day (minimum) quarantine period within the quarantine radius (radii) (cont.)</td>
<td>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 (SEE <em>Phytophthora ramorum</em> 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.</td>
</tr>
<tr>
<td></td>
<td>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 (SEE Soil and Container Mix Samples and processing Protocol on page 11-1-1).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>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 (SEE Water Sampling and Processing protocol on page 10-1-1).</td>
<td></td>
</tr>
<tr>
<td>6.3—Quarantine release survey</td>
<td>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 <em>P. ramorum</em>, the nursery can be released.</td>
<td></td>
</tr>
<tr>
<td>7—Release the nursery</td>
<td>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:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>◆ There are no additional detection of <em>P. ramorum</em> in nursery stock based on USDA–APHIS-approved plant inspection sampling and testing protocols for the preceding quarantine period; and</td>
<td></td>
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<tr>
<td></td>
<td>◆ If water, soil, and growing media were tested, at the inspector’s discretion, then they must also have tested negative for <em>P. ramorum</em> based on USDA–APHIS-approved sampling and testing protocols for the preceding quarantine period (if testing of soil, water, and media is required); and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>◆ The quarantine release survey is negative for <em>P. ramorum</em>. <strong>OR</strong></td>
<td></td>
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<td></td>
<td>◆ 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</td>
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<td>◆ 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 inspector must disinfest porous and nonporous surfaces (SEE 5.3—Nonporous surfaces on page 4-1-10). If soil and water samples taken are negative for <em>P. ramorum</em>, the nursery can be released; and</td>
<td></td>
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</table>
7—Release the nursery (cont.)

Inspectors must revisit the nursery after approximately 90 days and conduct at least a nursery-level survey inspection (SEE Inspection and Sampling Protocol for Nurseries on page 2-1-1). Also, the nursery is subject to “Post-Eradication Monitoring” (SEE 8—Post-eradication monitoring on page 4-1-13).

8—Post-eradication monitoring

Upon successful completion of the 90-day (minimum) quarantine period (SEE 6—Ninety-day (minimum) quarantine activities on page 4-1-11), a retail nursery dealer will not undergo post-eradication monitoring unless:

◆ Additional plants or articles are found to be positive. If additional plants or articles are found to be positive, the nursery shall be placed in a compliance agreement and have post-eradication monitoring.

◆ Nurseries that possess regulated plants year-round and/or have the plants on porous surfaces, such as soil, and have had a confirmed positive for P. ramorum will be visually monitored for 2 years. This monitoring shall occur twice a year during conditions conducive for detecting P. ramorum.

◆ If symptomatic tissue is observed, follow the Inspection and Sampling Protocol for Nurseries on page 2-1-1. If there are no additional detections, these nurseries are not under any other quarantine or regulatory action.
Nursery Questionnaire for a Confirmed-Positive Retail Nursery or Retail Nursery Dealer

Revised, TBD, 2020

*Location Name: ____________________________ *(see naming conventions on instructions page)

*Address 1 & 2: ________________________________

*City/State/ZIP: ____________________________ / ____________________________ / ____________________________

*County: ____________________________ *Coordinates: ____________________________ / ____________________________

*Contact Name: ____________________________ *(Type: ☐ Owner ☐ Operator ☐ Employee ☐ Resident)

Phone/Email: ________________________________

Secondary Contact: ____________________________ *(Type: ☐ Owner ☐ Operator ☐ Employee ☐ Resident)

Phone/Email: ________________________________

Location Comments:

*Nursery Category [select all that apply]: ☐ Wholesale ☐ Propagator ☐ Retail Nursery ☐ Retail Outlet

*Host Plants Sold [select all that apply]: ☐ Camellia spp. ☐ Kalmia spp. ☐ Rhododendron spp.

☐ Viburnum spp. ☐ Other Hosts

Who owns the plants? ☐ Nursery ☐ Plant on consignment (Y/N) if yes, who owns the plants? ________

Does Nursery Propagate Host Plants? ____ (Y/N)

Does Nursery Receive Host Plants? ____ (Y/N) if yes, from which nursery(ies)?____________________

*Does Nursery Ship Interstate? ____ (Y/N) Was Nursery Previously Positive? ____ (Y/N)

Month and Year: ____________________________

Nursery Part of Trace Out Investigations? [select all that apply] ☐ None ☐ Trace Back ☐ Trace Forward

☐ Unknown

Host Plant Placement [select all that apply]: ☐ Directly on soil ☐ In soil ☐ Benches ☐ Gravel

☐ Standing water ☐ Other, please specify ______________________________

Planting Containers Sanitation [select all that apply]: ☐ New ☐ Recycled with sanitation

☐ Recycled without sanitation

---

Figure 4-1-2 Nursery Questionnaire for a Confirmed-Positive Retail Nursery or Retail Nursery Dealer (page 1 of 2)
Figure 4-1-2 Nursery Questionnaire for a Confirmed-Positive Retail Nursery or Retail Nursery Dealer (page 2 of 2)
**Inspection Information**

Sites inspected, e.g., hoop house, block of plants, standing water, cull pile, planting media, etc.

Site Name: ___________________________ Latitude: ___________________ Longitude: ___________________

# Inspected: Camellia_____ Kalmia_______ Pieris_______ Rhod_______ Vibumum____ Other Hosts

Site Name: ___________________________ Latitude: ___________________ Longitude: ___________________

# Inspected: Camellia_____ Kalmia_______ Pieris_______ Rhod_______ Vibumum____ Other Hosts

Site Name: ___________________________ Latitude: ___________________ Longitude: ___________________

# Inspected: Camellia_____ Kalmia_______ Pieris_______ Rhod_______ Vibumum____ Other Hosts

Site Name: ___________________________ Latitude: ___________________ Longitude: ___________________

# Inspected: Camellia_____ Kalmia_______ Pieris_______ Rhod_______ Vibumum____ Other Hosts

Site Name: ___________________________ Latitude: ___________________ Longitude: ___________________

# Inspected: Camellia_____ Kalmia_______ Pieris_______ Rhod_______ Vibumum____ Other Hosts

**Sampling Information**

Inspector: __________________________________________________________

Date: ______________________________________________________________________________________

Use most specific taxonomic level possible (e.g., *Camellia japonica* “Silver Flame”). Sample type may be water, soil, or plant material.

<table>
<thead>
<tr>
<th>Site Name</th>
<th># Plants Inspected</th>
<th>Sample #</th>
<th>Sample Type</th>
<th>Genus/Species</th>
<th>Variety/Cultivar/ssp.</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>
Nursery Naming Conventions

Use the full proper business name as it is used for the mailing address of the company.

1. For large businesses with multiple locations and headquarters offices (i.e., “Really Big Nursery Company”) where possible, use the company’s method of distinguishing each location (i.e., Store #212) at the end of the name.

2. For smaller businesses with multiple locations, but no central headquarters, where possible, use the company’s method of distinguishing each location (i.e., Westside, Eastside) at the end of the name.

   A. If the business has no such designation method:

      a. Businesses with stores in multiple States/cities, use the State/city at the end of the name.

      b. Businesses with stores within the same city, use the street name at the end of the name.
3. Do not include program name, year, or a person’s name or title as part of the location name.

4. If more than one business shares the same street address either use the suite number in the address or use the nine-digit postal code in the ZIP code field (i.e., 12345-6789). Do not include information beyond the business name in the location name.
Chapter 5-1

Trace Investigations

Introduction

Contents

Trace Forward Protocol for Nurseries that Received Plant Material Shipped from a Confirmed *P. ramorum*-Infested Nursery  5-2-1
Trace Back Protocol for Nurseries that Shipped Plant Material to a Confirmed *P. ramorum*-Infested Nursery  5-3-1
Supporting Documents: *Phytophthora ramorum* Trace Investigation Questionnaires (Property Owner or Manager)  5-4-1

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 following section also includes the *P. ramorum* Trace Investigation Questionnaire (Property Owner or Manager) (Supporting Documents: *Phytophthora ramorum* Trace Investigation Questionnaires (Property Owner or Manager) on page 5-4-1).

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.
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. See *Information Needed PRIOR to the CCP Assessment* on page 3-1-19.

Contents

Intended Use  5-2-1
Goal  5-2-1
Trace Forward Protocol Instructions  5-2-2

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)

<table>
<thead>
<tr>
<th>Chronological or concurrent steps:</th>
<th>Actions:</th>
</tr>
</thead>
</table>
| 1) Communicate and notify the trace forward nursery | **Before inspection day**  
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.  
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 (see *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.  
3. For Federal inspectors, notify the SPRO, or relevant State official, of your plans to inspect.  
4. Coordinate the inspection day with the State inspector.  
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.  
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.  
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.  
8. Obtain sampling supplies, see *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**  
1. Identify yourself and agency to the nursery/facility owner/manager and explain the purpose of your visit.  
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.  
3. Provide the owner/manager with a copy of the trace investigation questionnaire (see Supporting Documents: Phytophthora ramorum Trace Investigation Questionnaires (Property Owner or Manager) on page 5-4-1). 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.  
4. Verify the present or absence of any of the trace forward plants.  
   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.  
   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.  
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)

<table>
<thead>
<tr>
<th>Chronological or concurrent steps:</th>
<th>Actions:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2) Investigation, inspection, and quarantine hold procedures (cont.)</td>
<td>6. If trace forward regulated plants are in multiple locations within the nursery, the inspector <strong>must</strong> disinfect boots, tools, and hands between areas (see Treatment and Disinfection Options on page 12-1-1).&lt;br&gt;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.&lt;br&gt;8. Visibly and indelibly flag 2-meter area around the sampled plant to hold for quarantine until all diagnostic results are final.&lt;br&gt;9. Hold the sampled plants and all other regulated plants within the 2-meter radius around the sampled plants.&lt;br&gt;10. Once the delimiting survey and sampling are complete, any held plants may be consolidated and segregated. If the plants are <strong>not</strong> consolidated and segregated, the affected portion of the nursery <strong>must</strong> 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 <strong>must</strong> 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 <strong>must</strong> include storage on an impermeable surface (e.g., a 45-mil thick pond liner, concrete, or asphalt) and <strong>not</strong> within 2 meters of any other plant. The impermeable surface should be sloped to drain away from regulated plants.&lt;br&gt;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 <em>P. ramorum</em>.&lt;br&gt;12. Check any cull, waste, or debris piles for <em>P. ramorum</em>-symptomatic plants or plant material. Collect samples of symptomatic material.</td>
</tr>
<tr>
<td>3) Sample collection and submission</td>
<td>Use <strong>Sampling and Submission Protocol</strong> on page 8-1-1.</td>
</tr>
<tr>
<td>4a) If the trace forward nursery is confirmed positive, and is an <strong>interstate</strong> shipping nursery</td>
<td>Use the <strong>Interstate Confirmed Nursery Protocol</strong> on page 3-1-1 and contact the PPQ National Operations Manager (NOM).</td>
</tr>
<tr>
<td>4b) If the trace forward nursery is confirmed positive and is an <strong>intrastate</strong> commerce only nursery</td>
<td>Use the <strong>Confirmed Retail Nursery and Retail Nursery Dealer Protocol</strong> on page 4-1-1 and contact the PPQ National Operations Manager (NOM).</td>
</tr>
<tr>
<td>5) Questions</td>
<td>For PPQ <em>P. ramorum</em> program contacts, see <strong>Contact Information for the Phytophthora ramorum Program</strong> on page A-1-1.</td>
</tr>
</tbody>
</table>
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. See Information Needed PRIOR to the CCP Assessment on page 3-1-19.

Contents

| Intended Use | 5-3-1 |
| Goal | 5-3-1 |
| Trace Back Protocol Instructions | 5-3-2 |

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, see 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)**

<table>
<thead>
<tr>
<th>Chronological or concurrent steps:</th>
<th>Actions:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) Communicate and notify the trace back nursery</strong></td>
<td><strong>Before inspection day</strong></td>
</tr>
<tr>
<td>1) Communicate and notify the trace back nursery</td>
<td>1. The PPQ National Operations Manager (NOM) will send trace back information generated from the <a href="#">Interstate Confirmed Nursery Protocol Steps</a> on page 3-1-3 to the SPRO and SPHD who will determine who conducts the protocol.</td>
</tr>
<tr>
<td></td>
<td>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/expressions when the initial inspection is conducted, an additional inspection must be conducted when conditions are conducive.</td>
</tr>
<tr>
<td></td>
<td>3. SPHD and SPRO should communicate on all trace back activities.</td>
</tr>
<tr>
<td></td>
<td>4. Coordinate the inspection day with the State inspector.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>7. Review and bring with you the <em>P. ramorum</em> Biology and Symptoms of Phytophthora <em>ramorum</em> on page 7-1-1 and Biosecurity Measures for Nurseries on page 8-1-1.</td>
</tr>
<tr>
<td></td>
<td>8. Obtain sampling supplies, see <a href="#">Sampling Supplies and Equipment Checklist</a> 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.</td>
</tr>
<tr>
<td>2) Investigation, inspection, and quarantine hold procedures</td>
<td><strong>Inspection day</strong></td>
</tr>
<tr>
<td>2) Investigation, inspection, and quarantine hold procedures</td>
<td>1. Identify yourself and agency to the nursery/facility owner/manager and explain the purpose of your visit.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>3. Provide the owner/manager with a copy of the trace investigation questionnaire (see XXX). 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.</td>
</tr>
<tr>
<td></td>
<td>4. Verify the presence or absence of any of the trace back plants.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>5. Use the <a href="#">Sampling and Submission Protocol</a> 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.</td>
</tr>
</tbody>
</table>
### Table 5-3-1 Trace Back Protocol Instructions (page 2 of 2)

<table>
<thead>
<tr>
<th>Chronological or concurrent steps:</th>
<th>Actions:</th>
</tr>
</thead>
</table>
| 2) Investigation, inspection, and quarantine hold procedures (cont.) | 6. If trace back regulated plants are in multiple locations within the nursery, the inspector **must** disinfect boots, tools, and hands between areas (see Treatment and Disinfection Options on page 12-1-1).  
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.  
8. Visibly and indelibly flag a 2-meter area around the sampled plant to hold for quarantine until all diagnostic results are final.  
9. Hold the sampled plants and all other regulated plants within the 2-meter radius around the sampled plants.  
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.  
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*.  
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, see Contact Information for the Phytophthora ramorum Program on page A-1-1. |
Trace Forward Questionnaire

*Phytophthora ramorum* Trace Forward Investigation Questionnaire

Name of Nursery or Garden Store: ________________________________
Name of Owner or Manager: ________________________________
Email address of Owner or Manager: ________________________________
Name and title of person interviewed: ________________________________
Address of site: ________________________________
City: ________________, State: ________________, ZIP code: ________________
Contact Name: ________________________________, Title: ________________________________
Office Number: ________________________________, Cell Number: ________________________________
GPS Coordinates: ________________________________
Type of Site (circle): Nursery, Greenhouse, Wholesale, Retailer, Other: ________________________________

Does this facility sell and/or ship plants? (circle) Intra-state, Interstate, Both, Other: ________________________________

Have trace forward/back plants been shipped to other wholesale or retail nurseries or facilities? ________________________________

1. What types and varieties were they? ________________________________
2. When did that shipping occur? ________________________________
3. What is the address of that location? ________________________________

Did you purchase the plant(s) in question? (If “no,” seek information on individual who purchased material in question) ________________________________

4. How long ago did you purchase the plant(s)? ________________________________
5. Did you purchase any other plants from this same nursery? ________________________________
   a. Were these plants purchased from a broker? ________. If so, please provide broker’s information in trace documentation. ________________________________
6. Have you noticed any other problems with plants on your property? ________________________________
7. Do you have multiple locations of your business? ________. If not, skip to question #8. ________________________________

Figure 5-4-1 *Phytophthora ramorum* Trace Forward Questionnaire (Property Owner or Manager) (page 1 of 2)
Have any plants, received from the infested nursery, been moved from your primary retail location to a different location? ________________________________

NOTE: Provide invoices or shipping documents that include types and varieties, dates of shipping and address of the shipping/receiving entities (brokers, nurseries, landscapers, residential locations, etc.).

Have any plants been shipped to this facility from a different location? ________________________________

NOTE: Provide invoices or shipping documents that include types and varieties, dates of shipping and address of the shipping/receiving entities (brokers, nurseries, landscapers, residential locations, etc.).

8. What is your source of water? ________________________________

9. What are the locations of cull piles, compost piles, and waste/refuse bins or piles on this site? ________________________________

10. Have the plants been trimmed or pruned? ________________________________

11. How are the trimmings disposed of? ________________________________

12. What are the sanitation practices at the facility? ________________________________

13. Where does the growing media come from? ________________________________

14. Do they propagate their own growing material? ________________________________

15. Did the plant material come in pots? ________________________________

16. If the pots were reused or stored, describe how the pots were handled: ________________________________
Trace Back Questionnaire

 Phytophthora ramorum Trace Back Investigation Questionnaire

Name of Nursery or Garden Store: ________________________________
Name of Owner or Manager: ____________________________________
Email address of Owner or Manager: ____________________________
Name and title of person interviewed: ___________________________
Address of site: ______________________________________________
City: __________________, State: ______, ZIP code: _______________
Contact Name: __________________, Title: _______________________
Office Number: __________________, Cell Number: ________________
GPS Coordinates: ____________________________________________
Type of Site (circle): Nursery Greenhouse Wholesale Retailer
Other: _______________________________________________________
Does this facility sell and/or ship plants? (circle) Intrastate Interstate Both

Have trace forward/back plants been shipped to other wholesale or retail nurseries or facilities?

1. What types and varieties were they? __________________________
2. When did that shipping occur? _______________________________
3. What is the address of that location? __________________________

Did you purchase the plant(s) in question? (If “no,” seek information on individual who purchased material in question)

4. How long ago did you purchase the plant(s)? __________________
5. Did you purchase any other plants from this same nursery?
   a. Were these plants purchased from a broker? ______ If so, please provide broker’s information in trace documentation.
6. Have you noticed any other problems with plants on your property? ______________
7. Do you have multiple locations of your business? ______ If not, skip to question #8.

Figure 5-4-2 Phytophthora ramorum Trace Back Questionnaire (Property Owner or Manager) (page 1 of 2)
Have any plants, received from the infested nursery, been moved from your primary retail location to a different location?

NOTE: Provide invoices or shipping documents that include types and varieties, dates of shipping and address of the shipping/receiving entities (brokers, nurseries, landscapers, residential locations, etc.).

Have any plants been shipped to this facility from a different location?

NOTE: Provide invoices or shipping documents that include types and varieties, dates of shipping and address of the shipping/receiving entities (brokers, nurseries, landscapers, residential locations, etc.).

8. What is your source of water?

9. What are the locations of cull piles, compost piles, and waste/refuge bins or piles on this site?

10. Have the plants been trimmed or pruned?

11. How are the trimmings disposed of?

12. What are the sanitation practices at the facility?

13. Where does the growing media come from?

14. Do they propagate their own growing material?

15. Did the plant material come in pots?

16. If the pots were reused or stored, describe how the pots were handled:
Chapter 6

Confirmed Residential Protocol

Contents

- Intended Use 6-1-1
- Goal 6-1-1
- Trigger Events for Use of the Confirmed Residential Protocol 6-1-2
- Disclaimers 6-1-2
  - Challenges 6-1-2
- Communication and Notification 6-1-2
- List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings 6-1-11

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, infested 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 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 (see 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 regulatory officials as soon as one of the following has occurred:

1. A confirmed-positive PCR determination using APHIS-approved work instructions by an APHIS-approved laboratory; or
2. A culture that matches the morphology for *P. ramorum* (i.e., isolation of *P. ramorum*) as determined and reported by an APHIS-approved laboratory.

For all Potentially Actionable Suspect Samples (PASS), laboratories must immediately forward to an APHIS–PPQ laboratory (see Contact Information for the *Phytophthora ramorum Program* on page A-1-1) and notify their State’s State Plant Health Direction (SPHD) and State Plant Regulatory Official (SPRO), and the PPQ 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. Ninety-day quarantine activities
7. Release the site
8. Post-disinfestation monitoring

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

<table>
<thead>
<tr>
<th>If step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| 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). See 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. | 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.  
3. Complete the questionnaire *P. ramorum Questionnaire for Property Owner or Manager* on page 6-1-8. 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 Locations with Infected Plants* (page 1 of 2) on page 6-1-9 at the time of the delimiting survey.  
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 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. |
2—Secure the site (cont.)

When the presence of *P. ramorum* has been confirmed in a residential or commercial setting (cont.)

2. Complete the questionnaire Follow-up Survey for Locations with Infected Plants (page 1 of 2) on page 6-1-9 during the delimitation survey. Do not move any equipment used on the residential or landscaped commercial sites without proper treatment and disinfection (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).

3. If necessary, detail any additional treatments and/or basic sanitary and precautionary measures on the EAN.
   
   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.

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*.

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 blocks (see Diagram Showing Destruction Block, Quarantine Block, and Delimitation Survey on page 6-1-7)

Determine the destruction block on a case-by-case basis, but it shall not have less than a 2-meter radius in total area (see rare exceptions in 2-meter D-radius below). If multiple plants are confirmed positive within 2 meters of each other, demarcate a destruction block around all of them.

The destruction block is established when diagnostic results from all delimiting samples have been reported. The 90-day quarantine period begins when the delimiting survey is complete.

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 block 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 block on the downslope side of the confirmed-positive plant.

2. Determine the plant debris area.
   
   A. If slope is a factor, the destruction block will be the combined area of the elliptical shape and the plant debris area.

   B. If slope is not a factor, the plant debris area may increase the destruction block greater than 2 meters.

3. The quarantine zone is a minimum of 30 meters beyond the destruction block and follows the same general shape.

4. Limit access to destruction block(s). Ensure proper sanitation measures are applied (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).

5. Destroy the *P. ramorum*-infected plants in an appropriate manner as soon as possible (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).
## Table 6-1-1 Confirmed Residential Protocol Instructions (page 3 of 4)

<table>
<thead>
<tr>
<th>If step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| **4—Delimiting survey** | | 1. Inspect **all** regulated plants within 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 30-meter radius of the newly detected, confirmed-positive plants to be surveyed and **all** symptomatic plants to be sampled.  
2. If the infestation is widespread, consult with the PPQ National Operations Manager (NOM) to design and implement an appropriate delimiting survey.  
3. Document the inspection and map **all** regulated plant locations.  
4. **All** symptomatic plants shall be sampled, mapped, marked or tagged, and tested.  
5. Samples **must** be analyzed using the APHIS-approved methodology. |
| **Soil sampling** | | 1. Take soil samples in the destruction block at the time of plant removal.  
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 area(s) **must** be sampled (see 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.  
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.  
| **5—Disinfest the site** | **Plant destruction** | 1. Plants infected with *P. ramorum** must** be removed and destroyed (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).  
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.  
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.**  
4. Approved methods of destruction include: incineration; deep burial; and steam sterilization (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).  
5. Using the survey follow-up questionnaire (Follow-up Survey for Locations with Infected Plants (page 1 of 2) on page 6-1-9), 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)

<table>
<thead>
<tr>
<th>If step number:</th>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>5—Disinfest the site (cont.)</td>
<td>Debris removal</td>
<td>Rake a sufficiently sized area to collect <strong>all</strong> plant debris associated with the destruction block and <strong>at least</strong> 2 meters beyond. Double-bag debris, as described above. Rake from the outer edge of the area toward the infected plant(s). <strong>All</strong> debris <strong>must</strong> be destroyed by APHIS-approved methods (see List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11).</td>
</tr>
</tbody>
</table>
| 6—90-day quarantine activities |                        | 1. Place **all** excepted regulated plants **not** destroyed, but located within the destruction block under a 90-day quarantine.  
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: |  1. Regulated plants will **not** be replanted within a minimum of 2 meters of the destruction block for a period of **at least** 2 years; **and**  
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**  
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: |  1. Performed a delimiting survey and regulated plant genera found free of *P. ramorum*; **and**  
2. Destroyed everything (**all** plants, pots, media, etc.) in the 2-meter destruction block according to List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings on page 6-1-11; **and**  
3. Destroyed the regulated plants in the 30-meter quarantine blocks; **and**  
4. Sampled soil and water from destruction and quarantine blocks with negative results.  
**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 block (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 Block, Quarantine Block, and Delimitation Survey

DB:
1) Inspect all regulated plant genera and sample any unhealthy plants.
2) Destroy regulated plants ASAP.
3) Sample soil and water where it drains.
4) Hold any regulated plants that are not destroyed for 90 days.
5) Limit access.

QB:
1) Inspect all regulated plant genera and sample any unhealthy plants.
2) Sample soil and water where it drains.
3) All regulated plants, 90-day hold.
4) Limit access.

DS:
1) Inspect all regulated plant genera and sample any unhealthy plants.
2) Sample water if necessary.
Figure 6-1-2  *P. ramorum* Questionnaire for Property Owner or Manager

1. Who owns the property? (name, address, phone number(s))

2. Who purchased the plant(s)?

3. Where were the plant(s) purchased? (name and address)

   A. When were the plant(s) purchased and planted?

   B. Did you purchase any other plants from this same nursery?

4. Have you noticed any other problems with plants on your property?

5. Have you moved any of these or other nearby plants to a different location?

6. Did you move any plants here from a different location?

   A. What types and varieties were they? (if regulated plant material proceed to questions 11 & 12)

   B. When were they moved?

   C. What is the address of the location?

7. Do you have a landscape company that did any planting for you?

   A. What is the contact information for the landscape company?

8. Have you added any mulch, potting soil, or top soil to the yard recently?

   A. Where did you get this material?

9. Information on plant material for inspector visiting property:

   A. What is the variety and number of plants?

   B. What is the condition of the plant material?

   C. Have the suspect plants been trimmed or pruned?

   D. How were the trimming disposed of?

   E. Did the plant material come in pots/containers?

   F. Did you dispose of the pots/containers or reuse them?

   G. If the pots/containers were reused or stored, describe how they were handled.

   H. Where are the pots/containers now?
Follow-up Survey for Locations with Infected Plants

Locations found to have infected plants will be monitored for 2 years. The affected area will not be under any quarantine or regulatory control unless additional outbreaks are detected.

Property Owner or Manager Survey:

1. Date of plant collection: __________________________
2. Property owner or manager name: __________________________
3. Location: __________________________
4. Name(s) and numbers of plants collected for destruction:
   a. ____________________________  # __________________
   b. ____________________________  # __________________
   c. ____________________________  # __________________
   d. ____________________________  # __________________
   e. ____________________________  # __________________
   f. ____________________________  # __________________
   g. ____________________________  # __________________
   h. ____________________________  # __________________
   i. ____________________________  # __________________
5. Where were the plants purchased?
   a. ____________________________  # __________________
   b. ____________________________  # __________________
   c. ____________________________  # __________________
   d. ____________________________  # __________________
   e. ____________________________  # __________________
   f. ____________________________  # __________________
   g. ____________________________  # __________________
   h. ____________________________  # __________________
   i. ____________________________  # __________________
6. When were the plants purchased?
   a. ____________________________________________  # __________________
   b. ____________________________________________  # __________________
   c. ____________________________________________  # __________________
   d. ____________________________________________  # __________________
   e. ____________________________________________  # __________________
   f. ____________________________________________  # __________________
   g. ____________________________________________  # __________________
   h. ____________________________________________  # __________________
   i. ____________________________________________  # __________________

7. Any proof of purchase or documentation such as receipts, pot labels, etc.?
   List here: ______________________________________________________________________

8. Survey types of regulated plants in the landscape and list named and numbers:
   a. ____________________________________________  # __________________
   b. ____________________________________________  # __________________
   c. ____________________________________________  # __________________
   d. ____________________________________________  # __________________
   e. ____________________________________________  # __________________
   f. ____________________________________________  # __________________
   g. ____________________________________________  # __________________
   h. ____________________________________________  # __________________
   i. ____________________________________________  # __________________

9. Samples of symptomatic regulated landscape plants taken?  □ No  □ Yes
10. Soil samples taken? □ Yes  □ No (plants still in pots)
11. Comments:

---

Figure 6-1-3  Follow-up Survey for Locations with Infected Plants (page 2 of 2)
List of Treatment and Disinfection Options 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>
<thead>
<tr>
<th>For: 1—Confirmed-positive plants</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incineration (burning to ash):</strong> 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.</td>
<td>1.</td>
</tr>
<tr>
<td><strong>Deep burial:</strong> 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.</td>
<td>2.</td>
</tr>
<tr>
<td><strong>Steam sterilization:</strong> 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.”</td>
<td>3.</td>
</tr>
<tr>
<td><strong>Nonporous surfaces:</strong> 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 <em>Phytophthora</em> 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. This list is for explanation and information only. Few disinfectants are specifically labeled for <em>Phytophthora</em> 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.</td>
<td>4.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For: 2—Confirmed-positive water</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For dust abatement, fire suppression, and equipment cleaning:</strong> Clorox® (sodium hypochlorite) is labeled (EPA Reg. No. 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of <em>Phytophthora</em> 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.</td>
<td>1.</td>
</tr>
<tr>
<td><strong>For irrigation:</strong> chlorine levels of 2 ppm or 2 mg/liter or greater has been correlated with the control of <em>Phytophthora</em> 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.</td>
<td>2.</td>
</tr>
</tbody>
</table>
Table 6-1-2 Treatment and Disinfection Options for Residential or Commercial Landscaped Settings (page 2 of 2)

<table>
<thead>
<tr>
<th>For:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| 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.  
2. **Soil treatment:**  
   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.  
   B. Conduct field soil treatments in the presence of an inspector and treat with an approved fumigant as per the label.  
   If considering the use of solarization for soil treatments, contact the PPQ NOM and USDA–PPQ Center for Plant Health Science and Technology (CPHST).  |
| 4—Confirmed-positive containment soil | Mitigation of infested soil in the destruction block 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 block. 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.  
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** on page 6-1-13 upon exiting the area.  
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 blocks. Any efficacious product labeled for use on nonporous surfaces may be used on tires or vehicle undercarriages.  
4. Do **not** visit other sites or areas in potentially contaminated work clothing. |
Table 6-1-3  Summary of Disinfectant Activities

<table>
<thead>
<tr>
<th>Disinfectant:</th>
<th>Trade names:</th>
<th>Comments:</th>
<th>Contact time:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols (ethyl and isopropyl) (60% – 85%)</td>
<td>Lysol spray®</td>
<td>Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable</td>
<td>10 – 15 minutes</td>
</tr>
<tr>
<td>Phenolics (0.4% – 5%)</td>
<td>Phenocen®</td>
<td>Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue</td>
<td>10 – 15 minutes</td>
</tr>
<tr>
<td>Quaternary ammonium (0.5% – 1.5%)</td>
<td>Consan triple action 20</td>
<td>Effective for nonporous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels</td>
<td>10 – 15 minutes</td>
</tr>
<tr>
<td></td>
<td>Physan 20®</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green-Shield®</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formula 409®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine (100ppm – 1,000ppm)</td>
<td>10% Clorox®</td>
<td>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 hypochlorite efficacy. Keep solution in opaque container.</td>
<td>10 – 15 minutes</td>
</tr>
<tr>
<td></td>
<td>10% bleach</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Confirmed Residential Protocol
List of Treatment and Disinfection Options for Residential or Commercial Landscaped Settings
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.

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.

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 391 Specimens 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 (see Figure 8-1-1 on page 8-3).

**Sample Forwarding and Reporting Under DA-2014-02**

DA-2014-02, APHIS Revises *Phytophthora ramorum* Domestic Quarantine Regulatory Requirements for Certain Host Nurseries, instructs that 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.
**INSTRUCTIONS:** Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

<table>
<thead>
<tr>
<th>BLOCK</th>
<th>INSTRUCTIONS</th>
</tr>
</thead>
</table>
| 1     | 1. Assign a number for each collection beginning with the year, followed by the collector’s initials and collector’s number.  
   **EXAMPLE:** In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001.  
   2. Enter the collection number. |
| 2     | Enter date   |
| 3     | Enter name of sender |
| 4     | Enter type of property specimen obtained from (farm, nursery, feed mill, etc.) |
| 5     | Enter address |
| 6     | Enter name and address of property owner |
| 8A–8L | Check all appropriate blocks |
| 9     | Leave blank |
| 10    | Enter scientific name of host, if possible |
| 11    | Enter quantity of host and plants affected |
| 12    | Check block to indicate distribution of plant |
| 13    | Check appropriate blocks to indicate plant parts affected |
| 14    | Check block to indicate pest distribution |
| 15    | * Check appropriate block to indicate type of specimen  
   * Enter number of specimens submitted under appropriate column |
| 16    | Enter sampling method |
| 17    | Enter type of trap and lure |
| 18    | Enter trap number |
| 19    | Enter X in block to indicate isolated or general plant symptoms |
| 20    | Enter X in appropriate block for weed density |
| 21    | Enter X in appropriate block for weed growth stage |
| 22    | Provide a brief explanation if prompt or URGENT identification is requested |
| 23    | Enter a tentative determination if you made one |
| 24    | Leave blank |

**DISTRIBUTION of PPQ Form 391**
1. Send original along with the sample to your area identifier.  
2. Retain and file a copy for your records.

---

**Figure 8-1-1 Instructions for Using PPQ Form 391, Specimens for Determination**
Sampling Supplies and Equipment Checklist

- APHIS List of *P. ramorum*-regulated Plants (see APHIS List of *Phytophthora ramorum*-Regulated Plants on page A-1-2)
- 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 9-1-1
Communications 9-1-2
Vehicles 9-1-2
  Avoidance 9-1-2
  Cleaning 9-1-3
Nursery Personnel 9-1-3
  Access 9-1-3
  Boots 9-1-4
  Hands 9-1-4
  Equipment 9-1-4
Sampling Supplies and Equipment Checklist 9-1-5

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.
Biosecurity Measures for Nurseries

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 block 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 block 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 block 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 List of P. ramorum-Regulated Plants (see APHIS List of Phytophthora ramorum-Regulated Plants on page A-1-2)
- 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
**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 sampling and processing protocol

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

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

Contact APHIS–PPQ–CPHST Beltsville Diagnostics at: PPQ.CPHST.Beltsville.Diagnostics@aphis.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
Water Sampling and Processing Protocol
In Situ Water Sampling with Regulated Plant Material Leaf Baits

- 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 Media¹

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 (**see Figure 10-1-2** on page 10-1-7). 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.
In Situ Water Sampling with Regulated Plant Material Leaf Baits

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.

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 (see Figure 10-1-3 on page 10-1-8 and Figure 10-1-4 on page 10-1-8 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.

<table>
<thead>
<tr>
<th>If the temperature is:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 °C</td>
<td>Leave in water for 7 days</td>
</tr>
<tr>
<td>≥ 8 °C, but &lt; 22 °C</td>
<td>Leave in water for 7 to 14 days</td>
</tr>
</tbody>
</table>
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 (see Table 10-1-1 on page 10-1-7).

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 \textit{minimum} of two bait stations per pond.

\textit{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.

\textit{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 (see Figure 10-1-3 on page 10-1-8).
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 (see Figure 10-1-4 on page 10-1-8).

\textbf{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 \textbf{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 \textbf{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 \textbf{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.

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**Water Sampling for Filtration**

**Sample Collection**

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.

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1 Water sampling for filtration is an optional water sample protocol.
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. 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) (see Figure 10-1-6 on page 10-1-16).

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 (see Figure 10-1-6 on page 10-1-16).

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 disinfect 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 (see Figure 10-1-6 on page 10-1-16); mark these with a permanent, waterproof marking method.


12. 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.

13. 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.
14. 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).

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

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 Treatment and Disinfection Options 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 (see 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 PASS, the DNA can be forwarded to the PPQ–S&T Beltsville 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 Beltsville 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 Treatment and Disinfection Options 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 Treatment and Disinfection Options 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.
## 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 at [http://www.sterlitech.com](http://www.sterlitech.com))
- 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 (see 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

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

<table>
<thead>
<tr>
<th>Ingredient:</th>
<th>Amount per:</th>
<th>1.0 liter</th>
<th>0.5 liter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal medium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarified V8 Concentrate¹</td>
<td>50 ml</td>
<td>25 ml</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>950 ml</td>
<td>475 ml</td>
<td></td>
</tr>
<tr>
<td>Difco Bacto agar</td>
<td>15 g</td>
<td>7.5 g</td>
<td></td>
</tr>
<tr>
<td><strong>Amendments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delvocid [50% pimaricin]</td>
<td>10 mg</td>
<td>5 mg</td>
<td></td>
</tr>
<tr>
<td>Ampicillin sodium</td>
<td>250 mg</td>
<td>125 mg</td>
<td></td>
</tr>
<tr>
<td>Rifamycin-SV [sodium salt]</td>
<td>10 mg</td>
<td>5 mg</td>
<td></td>
</tr>
<tr>
<td>Terraclor [75% PCNB]</td>
<td>66.7 mg</td>
<td>33.4 mg</td>
<td></td>
</tr>
<tr>
<td>Hymexazol</td>
<td>50 mg</td>
<td>25 mg</td>
<td></td>
</tr>
</tbody>
</table>

1 Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaC₃/100 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.
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**

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount per:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 liter:</td>
</tr>
<tr>
<td><strong>Basal medium</strong></td>
<td></td>
</tr>
<tr>
<td>Clarified V8 concentrate¹</td>
<td>50 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>950 ml</td>
</tr>
<tr>
<td>Difco Bacto agar</td>
<td>15 g</td>
</tr>
<tr>
<td><strong>Amendments</strong></td>
<td></td>
</tr>
<tr>
<td>Delvocid [50% pimaricin]</td>
<td>10 mg</td>
</tr>
<tr>
<td>Ampicillin sodium</td>
<td>250 mg</td>
</tr>
<tr>
<td>Rifamycin-SV [sodium salt]</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

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.


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.

References


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 on page 11-1-2 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

<table>
<thead>
<tr>
<th>Survey area size:</th>
<th>Composite samples of soil:</th>
<th>Composite samples of container mix:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(m²)</td>
<td>(ac)</td>
<td></td>
</tr>
<tr>
<td>&lt;1,000</td>
<td>&lt;0.25</td>
<td>5</td>
</tr>
<tr>
<td>1,000 to 2,000</td>
<td>0.25 to 0.5</td>
<td>10</td>
</tr>
<tr>
<td>2,000 to 4,000</td>
<td>0.5 to 1</td>
<td>20</td>
</tr>
<tr>
<td>&gt;4,000</td>
<td>&gt;1</td>
<td>30</td>
</tr>
</tbody>
</table>

**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. Betzen  
   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 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.

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.

---

1 These procedures are provided by S.N. Jeffers, Clemson University, 2010.
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 (see Figure 11-1-1 on page 11-1-6). 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 (see Figure 11-1-1).

---

2. *See Growing Media Formulae* on page 11-1-10.
Soil and Container Mix Sampling and Processing Protocol
Procedure for Baiting Soil and Container Mix Samples

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 (see Figure 11-1-2 on page 11-1-7); mark these with a permanent, waterproof marking method.


**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](image)

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.
15. *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.

16. Subculture isolates to fresh PARPH-V8 and then to PAR-V8³.

**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](image)

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

³ See Growing Media Formulae on page 11-1-10.
17. 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 Treatment and Disinfection Options 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 (see 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 PASS, the DNA can be forwarded to the PPQ–S&T Beltsville 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 Beltsville 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
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 Treatment and Disinfection Options 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 mm 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) (see Figure 11-1-1 on page 11-1-6)
- 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

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

<table>
<thead>
<tr>
<th>Ingredient:</th>
<th>Amount per:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 liter</td>
</tr>
<tr>
<td><strong>Basal medium</strong></td>
<td></td>
</tr>
<tr>
<td>Clarified V8 concentrate(^1)</td>
<td>50 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>950 ml</td>
</tr>
<tr>
<td>Difco Bacto agar</td>
<td>15 g</td>
</tr>
<tr>
<td><strong>Amendments</strong></td>
<td></td>
</tr>
<tr>
<td>Delvocid [50% pimaricin]</td>
<td>10 mg</td>
</tr>
<tr>
<td>Ampicillin sodium</td>
<td>250 mg</td>
</tr>
<tr>
<td>Rifamycin-SV [sodium salt]</td>
<td>10 mg</td>
</tr>
<tr>
<td>Terraclor [75% PCNB]</td>
<td>66.7 mg</td>
</tr>
<tr>
<td>Hymexazol</td>
<td>50 mg</td>
</tr>
</tbody>
</table>

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.

---

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

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount per:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 liter:</td>
</tr>
<tr>
<td><strong>Basal medium</strong></td>
<td></td>
</tr>
<tr>
<td>Clarified V8 concentrate¹</td>
<td>50 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>950 ml</td>
</tr>
<tr>
<td>Difco Bacto agar</td>
<td>15 g</td>
</tr>
<tr>
<td><strong>Amendments</strong></td>
<td></td>
</tr>
<tr>
<td>Delvocid [50% pimaricin]</td>
<td>10 mg</td>
</tr>
<tr>
<td>Ampicillin sodium</td>
<td>250 mg</td>
</tr>
<tr>
<td>Rifamycin-SV [sodium salt]</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

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).

---

5 Adapted from Ferguson and Jeffers, 1999.
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. 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


Treatment and Disinfection Options

Contents

Treatment Options  12-1-1
Infected Plants and Associated Potting Mix and Containers  12-1-1
   Incineration (Burning to Ash)  12-1-2
   Deep Burial  12-1-2
   Steam Sterilization  12-1-2
Disinfection Options  12-1-3
   Disinfecting Nonporous Surfaces  12-1-3
   Disinfesting Water  12-1-4
   Disinfecting Soil and Container Mix  12-1-4

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 disinfect 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), 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), all leaf debris in and around the area where plants were stored may be double bagged using self-sealing plastic bags of 2-mm 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), 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. See http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf.

1 Associated containers (e.g., pots and trays) may be treated for re-use. Guidelines for safeguarding should be determined and approved to prevent movement or potential contamination of P. ramorum to noninfected 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 disinfectant methods for the correct contact time.
Disinfection Options

Disinfecting 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 Disinfectant Activities

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

Disinfecting 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.

Disinfecting 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 disinfect 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.

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\(^2\) 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
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) 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


Figure 12-1-2  Concrete Blocks Used to Support Tarp from Surface to Allow Steam Distribution from Steam Sock
Fumigation may be the most efficacious and economical option to disinfect 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.

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.
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

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

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 disinfect 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 (see 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

Resources

Contents

Contact Information for the *Phytophthora ramorum* Program  A-I-1
U.S. State and Territory Plant Health Directors  A-I-2
APHIS List of *Phytophthora ramorum*-Regulated Plants  A-I-2
Example of PPQ Form 519, Compliance Agreement  A-I-3

Contact Information for the *Phytophthora 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. Betzen, 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 Nicole O’Donahue:

USDA–APHIS–PPQ–CPHST Laboratory in Beltsville, MD.
BARC-East Building 580 Powder Mill Road
Beltsville, MD 20705-2350
(301) 313-9204
nicole.l.odonahue@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
# Example of PPQ Form 519, Compliance Agreement

According to the Framework Regulation Act of 1996, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control numbers for this information collection are 9731-0004, 0605-0136, 0201, 0818-0017, 9202, 9207, 9208, 9209, and 9210. The time required to complete this information collection is estimated to average 1.39 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

<table>
<thead>
<tr>
<th>UNITED STATES DEPARTMENT OF AGRICULTURE</th>
<th>COMPLIANCE AGREEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLANT PROTECTION AND Quarantine</td>
<td></td>
</tr>
<tr>
<td>1. NAME AND MAILING ADDRESS OF PERSON OR FIRM</td>
<td>2. LOCATION</td>
</tr>
<tr>
<td>Name</td>
<td>Full Address</td>
</tr>
<tr>
<td>Business Name</td>
<td>(or enter &quot;Same&quot; if mailing address is the same)</td>
</tr>
<tr>
<td>Phone#</td>
<td>e-mail</td>
</tr>
<tr>
<td>Fax#</td>
<td></td>
</tr>
</tbody>
</table>

3. **Regulated Articles**: Hosts and Associated Hosts of *Phytophthora ramorum* and other articles

4. **Applicable Federal Quarantine or Regulations**:
   7 CFR 301.92, and, Section 414 of the Plant Protection Act, 7 USC 7714, 114 STAT 445, PUBLIC LAW 106-224—JUNE 20, 2000, SEC. 414. (a) and (b).

5. **I WE AGREE TO THE FOLLOWING**: USDA,APHIS,PPQ, will permit your establishment to execute the regulatory requirements outlined in the 7 Code of Federal Regulations (CFR) 301.92 and Federal Order DA-2012-53. Procedures, protocols and limitations are outlined and attached as exhibits, inclusive and incorporated into this agreement by reference as if fully set out. The exhibits (attached) are binding: Exhibit A - Compliance Terms, Conditions, and Procedures. Exhibit B - Authorization for Certification. Exhibit C - *Phytophthora ramorum* host and associated host plants. Exhibit D – CCP Assessment Findings, Requirement, Remediation, Mitigation Measure, and/or Business or Cultural Practice Records Table, Compliance Table, and Correction Action Log, if applicable.

This agreement becomes effective on signing and shall remain in effect until canceled by either party on 30 days’ notice to the other at the address appearing above. However, the Department may accelerate the notice to immediate for cause, including but not limited to the Establishment’s abandonment of the procedures outlined in the attached Exhibits. This compliance agreement is non-transferable.

The Establishment assumes liability, if any, arising from the manner in which Establishment sells, handles or distributes any regulated host material. NOTICE: Any signatory or employee of any signatory who violates the terms of this compliance agreement may be subject to Civil Penalties pursuant to 7 CFR 301.92, and the Plant Protection Act of 2000. Specifically, any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. 7701 et seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. 8001 et seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, a one-year prison term or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater. “Establishment” here fore referred to as “nursery” as defined in 7 CFR 301.92.1.

<table>
<thead>
<tr>
<th>6. SIGNATURE</th>
<th>7. TITLE</th>
<th>8. DATE SIGNED</th>
</tr>
</thead>
<tbody>
<tr>
<td>The affixing of the signatures below will validate this agreement which shall remain in effect until canceled, but may be revised as necessary or revoked for non-compliance.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>11. PPQ OFFICIAL (NAME AND TITLE)</th>
<th>12. ADDRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPQ (See designee)</td>
<td>1200 Cherry Brook Drive, Little Rock, Arkansas 72211</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13. SIGNATURE</th>
<th>14. STATE AGENCY OFFICIAL (NAME AND TITLE)</th>
<th>15. ADDRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPQ (See designee)</td>
<td>SPRO (See designee)</td>
<td>SPRO Office</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>16. SIGNATURE</th>
<th>PPQ FORM 519 SEP 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRO (See designee)</td>
<td>Previous editions before 2010 are obsolete.</td>
</tr>
</tbody>
</table>
EXHIBITS

PLANT PROTECTION AND QUARANTINE PROGRAMS

COMPLIANCE AGREEMENT TERMS, CONDITIONS AND PROCEDURES FOR
REGULATED NURSERIES (DA-2014-02; Positive March 31, 2011 and thereafter)

EXHIBIT A

7 CFR 301.92-1 Definitions: Nursery. Any location where nursery stock is grown, propagated, stored, or sold, or any location from which nursery stock is distributed. Locations that grow trees for sale without roots (e.g., as Christmas trees) are considered to be nurseries for the purposes of this subpart.

In order to prevent the dissemination of *Phytophthora ramorum*, the Administrator of Animal and Plant Health Inspection Service (APHIS) considers it necessary to establish restrictions on the interstate movement of nursery stock from nurseries described in 7 CFR 301.92 and Federal Order DA-2012-53.

The goal of this regulation is to effectively remove *P. ramorum* from nursery cultural and conveyance systems, 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, and is likewise issued pursuant to the regulations, promulgated under the Plant Protection Act, and found at 7 CFR 301.92 et seq. For nurseries (positive March 31, 2011 or thereafter) that wish to ship or distribute nursery stock listed in Exhibit C of this document interstate must be inspected, sampled and tested, certified and enter into this compliance agreement. Shipments must be accompanied by a certificate issued under this compliance agreement.

The nursery agrees to the following:

1. Inspection, Sampling, and Testing of Regulated Articles which includes but is not limited to nursery stock, soil, container mix, cull piles, pots, water, and other associated articles that the inspector determines may pose a risk of spreading *P. ramorum*.

   a. The nursery shall allow inspection and sampling of nursery stock and other regulated articles as defined in 7 CFR 301.92.

   b. In order to remain under compliance, the nursery must undergo a minimum of two inspections per year, and be tested for *P. ramorum* following procedures as authorized by 7 CFR 301.92. As specified in 7 CFR 301.92 - 11 (c)(3), if annual certification expires prior to re-inspection, all plants in the nursery are prohibited interstate movement until the nursery is inspected, tested and re-certified in accordance with this section and CFR 301.92-12 unless authorized by a regulatory official.

   c. The State Department of Agriculture shall use sampling procedures that meet USDA standards for nurseries as detailed in protocols pursuant 7 CFR 301.92. The department or other USDA approved laboratory, using federally approved laboratory protocols, will test the samples.

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7731 et. seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8331 et. seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties and one-year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012) Nursery Owner Initial/Date ________________________

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Figure A-1-1 Example of PPQ Form 519, Compliance Agreement [Exhibit A] (page 2 of 9)
2. If *P. ramorum* is detected, all host nursery stock is on hold until an inspector conducts the delimiting survey. Areas of the nursery will remain on hold and some areas released depending on positive findings and further determinations by the inspector and the laboratory. The nursery must allow the implementation of the Confirmed Nursery Protocol (CNP) and a follow up Critical Control Point (CCP) assessment to retain interstate shipping status. Based on the assessment, the nursery shall choose remediation and/or mitigations and specific business or cultural practices to address the presence of the pathogen. These measures will be recorded in the compliance agreement.

3. After initial detection, if *P. ramorum* is not detected during any sampling of articles for three years under this compliance agreement, the nursery is no longer required to be under a compliance agreement and may ship without certification and a federal shield.

4. The nursery will comply with Federal Order DA-2012-53 by notifying receiving state regulatory officials at the time of shipment. The notification time period is upon detection of *P. ramorum* in the nursery until 2 years after the CNP is complete. When the nursery tests negative for two years (4 or more samplings), the nursery is released from the notification requirement. The nursery is not required to notify during the third year under compliance if sampling results remain negative.

5. Shipping Period

Listed plants (Exhibit C) may be moved interstate from the date the above inspection(s) is/are completed, which includes laboratory analysis results of tested articles, or directed otherwise by an authorized Regulatory Official.

6. Restriction on Sources of Regulated Articles

The nursery may receive plants from sources within the quarantine areas or from regulated nurseries only under the following conditions:

- If Plants listed in Exhibit C originate from nurseries located in the quarantine areas, those nurseries must have a PPQ approved compliance agreement for *P. ramorum* and are accompanied by an appropriate certificate.*
  - Interstate movement of plants listed in Exhibit C from a nursery located in the quarantine area without a compliance agreement is a violation of 7 CFR 301.92.

- If Plants listed in Exhibit C originate from *P. ramorum* regulated nurseries, those nurseries must have a PPQ approved compliance agreement for *P. ramorum* and are accompanied by an appropriate certificate.*
  - Interstate movement of plants listed in Exhibit C from a regulated nursery without a compliance agreement is a violation of 7 CFR 301.92.

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7731 et. seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8301 et. seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012) Nursery Owner Initial/Date

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**Figure A-1-1 Example of PPQ Form 519, Compliance Agreement [Exhibit A]** (page 3 of 9)
EXHIBITS

7. Maintain Identity

The nursery shall maintain the identity of all listed plants in Exhibit C. The nursery shall maintain the identity of all listed plants that originated from other sources.

8. Requirements for Interstate Movement

The nursery shall ensure that a USDA certificate or stamp (Exhibit B) stating compliance with 7 CFR 301.92, DA-2012-53, and DA-2014-02 accompany each shipment of plants listed in Exhibit C that is moved interstate.

The USDA certificate or stamp (PPQ form 570, Exhibit B) may also be used to indicate nursery compliance with 7 CFR 301.92 for the movement of listed (Exhibit C) nursery stock interstate.

The nursery agrees to comply with all other applicable federal and state regulations related to the importation or distribution of plant material.

8. Records

The nursery agrees to maintain records of all incoming and outgoing shipments of host and associated plants. This information is to be used to track and record shipments and for the development of any Trace Forward or Trace Back Shipments. The following data must be maintained: Plants shipped or received (Genus, species, variety if known): Origin of plants; Receiver name; Street address, City, State, and Zip code; Contact Phone Number; Date of Shipment; Invoice Number; Number of Plants Shipped; and if applicable Store Number. The nursery shall also maintain records of fungicide applications. All records are to be kept for a minimum of 36 months. These records shall be made available for periodic inspection by an approved Regulatory Official upon request to verify compliance with this provision.

If *Phytophthora ramorum* is detected in the nursery, the nursery agrees to keep records for auditing purposes, as appropriate, that involve a given remediation, mitigations, and/or specific business or cultural practices chosen by the nursery. These records will be kept for at least 36 months or as long as the nursery is in this compliance program.

9. Detection of *P. ramorum* and implementation of the CNP, CCP assessment, and mitigations

   a. In the event *P. ramorum* is detected in the nursery, the USDA/State Department of Agriculture will delimit and mitigate the presence of the pathogen using the CNP. To remain under compliance, the nursery must allow the implementation of a follow up CCP assessment.

   b. Based on the CCP assessment, the nursery shall choose remediation, mitigations, and/or specific business/cultural practices to address the presence of the pathogen within the nursery.

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7701 et. seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 6901 et. seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term; or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012)  Nursery Owner Initial/Date ____________________________

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Figure A-1-1  Example of PPQ Form 519, Compliance Agreement [Exhibit A] (page 4 of 9)
EXHIBITS

nursery. The regulatory official will review and accept measures efficacious for mitigation. The compliance agreement will contain the findings of the critical control point assessment detailing the affected areas and will describe the remediation, mitigations, and/or BMPs chosen by nursery and accepted by the regulatory official as efficacious.

c. The compliance agreement will contain implementation timelines for the mitigation measures. The nursery agrees to report and correct any deviations from the chosen mitigation and/or specific business/cultural practices. If the nursery is found to be non-compliant with a specific measure within the specified time period, a notice and a correction action request (CAR) will be issued. The notice will include the corrective action(s) that detail the needed recourse for the non-conformity. The notice will provide the expected timeline. The protocol for conforming to compliance agreement requirements will be discussed with and provided to the nursery.

10. Monitoring

Federal or State Regulatory Officials shall be granted access to the nursery during normal business hours to evaluate whether the nursery and its operations are in compliance with the applicable provisions of this agreement. Violation of any of these compliance agreement provisions may be cause for termination of the agreement.

11. Notice

The nursery shall provide advance notice to the State Department of Agriculture Regulatory Official when requesting an inspection.

12. Continuance of Compliance Agreement

This agreement is valid through __________________________

*A USDA form 527, or 540 (certificate or stamp) is an appropriate certificate.

This agreement may be immediately canceled or revoked for noncompliance. Violation of these Federal regulations can result in a criminal penalty of up to a $5,000 fine, one year in jail, or both, or a civil penalty of up to $1,000 per violation.

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7701 et. Seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8301 et. Seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012) Nursery Owner Initial/Date __________________________
Authorization for Certification

Nursery Name

Nursery agrees to the following:

1. Reproduce the Federal certificate following dimensions in example below and using exact language in the example.

2. Use Federal certificate to certify interstate shipments of regulated plant material shipped from the nursery named in this compliance agreement.

3. Use Federal certificate to certify regulated plant material shipped from the nursery that has been inspected and found free of Phytophthora ramorum by agricultural officials during the annual inspection.

4. Maintain records of all interstate shipments certified with Federal certificate and make such records available to agricultural officials upon request.

5. Delegate to one person only the authority to reproduce Federal certificate and use to certify interstate shipments of regulated articles.

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7701 et. seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8301 et. seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012)  Nursery Owner Initial/Date ____________________________

Figure A-1-1  Example of PPQ Form 519, Compliance Agreement [Exhibit B] (page 6 of 9)
EXHIBITS

Stamp Description:
Minimum Size: 2"x4"
Font: Arial Black
Font Size: 11

Nursery or Compliance Agreement Numbering System:
Each state shall use the Nursery number or Compliance Agreement numbering system that is appropriate for their state. If a nursery has multiple growing locations, the state shall ensure that the individual growing locations can be distinguished, one from the other, via a Compliance Agreement numbering system that is appropriate for their state.

Authorization for Certification

By signing below, Nursery agrees to follow all instructions contained in Exhibit B, Authorization for Certification.

Printed Name of Nursery Representative

Signature of Nursery Representative Date

Printed Name of Federal, State or County Representative

Signature of Federal, State or County Representative Date

Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7701 et. seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8301 et. seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012) Nursery Owner Initial/Date

Figure A-1-1 Example of PPQ Form 519, Compliance Agreement [Exhibit B] (page 7 of 9)
EXHIBIT C

APHIS List of Regulated Hosts and Plants Associated with Phytophthora ramorum

Please see the most current host list at:


Figure A-1-1 Example of PPQ Form 519, Compliance Agreement [Exhibit C] (page 8 of 9)
## Exhibit D

### CCP Assessment Findings, Requirements including Remediation, Mitigation Measure, And/or Business or Cultural Practice Records Table

**Required Action Table**

<table>
<thead>
<tr>
<th>CCP Assessment Date/Issue Identification</th>
<th>CCPs to Address or Issue to be Addressed</th>
<th>Requirement, Remediation, Mitigation, Bus. or Cultural Practice Measure to Address CCP</th>
<th>Time Frame to Implement</th>
<th>Standard or Performance Measure Description for compliance inspection*</th>
<th>Mitigation/Action Agreed to by Nursery owner</th>
<th>Measure ID #</th>
</tr>
</thead>
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* The standard or performance measure is what the nursery and the inspector notes as successful or requires correction. So it must be as objective, tangible and measurable.

### Compliance Tables

These two tables can be one long table

<table>
<thead>
<tr>
<th>Measure Identification Number</th>
<th>Compliance Incident? (Y/N)</th>
<th>Root Cause</th>
<th><em>CARS # (1st or 2nd)</em></th>
<th>CAR issue date</th>
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</table>

*C CORS Action Requests (CARS) outline 1) what was required and what the failure was, 2) Root cause (why it happened), 3) specific required mitigation (what is required to correct it), and 4) the timeframe allotted.

### Detailed Corrective Action Log

<table>
<thead>
<tr>
<th>Measure Identification #</th>
<th>CARS # (1st or 2nd)</th>
<th>CAR Issue Date</th>
<th>Letter of Finding or Warning Letter?</th>
<th>Findings of CAR visit with Nursery: Problem/Follow Up Action Description (root cause of failure, corrective action, and new timeframe allotted, and, regulatory action if appropriate)</th>
<th>Problem Rectified Date or Moved to Critical Non-Compliance?</th>
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</thead>
<tbody>
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Any person who knowingly violates the Plant Protection Act (PPA) (7 U.S.C. §§ 7731 et. Seq.) and/or the Animal Health Protection Act (AHPA) (7 U.S.C. §§ 8301 et. Seq.) may be criminally prosecuted and found guilty of a misdemeanor which can result in penalties, and one year prison term, or both. Additionally, any person violating the PPA and/or the AHPA may be assessed civil penalties of up to $250,000 per violation or twice the gross gain or gross loss for any violation that results in the person deriving pecuniary gain or causing pecuniary loss to another, whichever is greater.

Attachment to PPQ FORM 519 (Sept 2012)  Nursery Owner Initial/Date ___________________________

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Figure A-1-1  Example of PPQ Form 519, Compliance Agreement [Exhibit D] (page 9 of 9)
Resources
Example of PPQ Form 519, Compliance Agreement
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 can be found at the USDA–APHIS–PPQ website at: https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/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 break of **either** no plants **or** no 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; see Biosecurity Measure for Nurseries on page 9-1-2

**block of plants.** contiguous group of regulated plants with **less than** a 2 m (6.5 foot) break of nonregulated plants or empty space

**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 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

**EAN.** (see below)

**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
**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 (see confirmed positive on page Glossary-1-2)

**free from.** 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 block 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 (see Potentially Actionable Suspect Sample (PASS) on page Glossary-1-5)

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

**NDPN.** National Plant Diagnostic Network

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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
2. 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
3. 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
**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 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 block.** Contiguous grouping of plants separated by some distance by a path; preferably at least two meters from other contiguous groupings of plants.

**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 (see 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 (see Schematic of Destruction and Quarantine Radii of Positive Plants on page 3-1-9); 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.

**PASS.** see Potentially Actionable Suspect Sample (PASS) on page Glossary-1-5.
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, see “PASS System Policy” at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram

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 (see Schematic of Destruction and Quarantine Radii of Positive Plants on page 3-1-9) designed to determine if *P. ramorum* has spread beyond the destruction block; 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, see 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 (see 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.


**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, see 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.
SPHD. *see* below

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

SPRO. *see* below

**State Plant Regulatory Official (SPRO).** Primary person responsible for plant health programs in each State; a list of SPROs can be found at: [http://nationalplantboard.org/membership/](http://nationalplantboard.org/membership/)

**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
A
aliquot
definition of Glossary-1-1
ampicillin sodium 10-1-17, 10-1-18, 11-1-10, 11-1-11
Animal and Plant Health Inspection Service (APHIS) Glossary-1-1
APHIS list of regulated hosts and associated plants with P. ramorum 1-1-3, 2-1-2, 8-1-4, 9-1-5
associated mitigations
examples of 3-1-23
associated plants
definition of Glossary-1-1
B
bait bags 10-1-6
construction 10-1-7
bait selection 10-1-2, 10-1-5
bait stations 10-1-7
construction of 10-1-8
baiting soil
soil and container mix sampling 11-1-4
baiting techniques 10-1-6
Basamid
trade name for dazomet 12-1-8
biosecurity measures for nurseries
communications 9-1-2
definition of Glossary-1-1
nursery personnel 9-1-3
equipment 9-1-4
hands 9-1-4
nursery personnel access 9-1-3
nursery personnel boots 9-1-4
sampling supplies and equipment checklist 9-1-5
vehicles 9-1-2
avoidance 9-1-2
cleaning 9-1-3
block
definition of Glossary-1-1
of plants Glossary-1-1
BMPs
see business management practices 3-1-23
BoB
see bottle of bait
boots 9-1-4
bottle of bait (BoB)
materials list 10-1-4
Busan 1020
trade name for metam-sodium 12-1-8
Busan 1180
trade name for metam-sodium 12-1-8
Busan 1236 Metam
trade name for metam-sodium 12-1-8
business management practices (BMPs)
examples of 3-1-23
C
Camellia 2-1-5, 3-1-14, 10-1-2, 10-1-5, 11-1-5, 11-1-9, Glossary-1-3
Camellia japonica 11-1-5
camera 8-1-4, 9-1-5
cankers on boles and branches 2-1-5
CCP
see critical control point 3-1-23
CFR
see Code of Federal Regulations
checklists
critical control point (CCP) assessment procedure 3-1-21
sampling supplies and equipment 8-1-4
chlorine 12-1-3
Chlor-O-Pic
trade name for chloropicrin 12-1-8
chloropicrin 12-1-8
clarified V8 concentrate 10-1-17, 10-1-18, 11-1-10, 11-1-11
Chlorox
trade name for chlorine 12-1-3
communications
for biosecurity measures 9-1-2
composite sample
definition of Glossary-1-2
compost pile
definition of Glossary-1-2
confirmed nursery protocol (CNP) procedures
disinfest the nursery 3-1-10
quarantine period 3-1-12
confirmed positive
definition of Glossary-1-2
Consan Triple Action 20
trade name for a quarternary ammonium 12-1-3
container mix
definition of Glossary-1-2
disinfesting 12-1-4
containers
sampling 2-1-10
treating 12-1-1
critical control point (CCP) 3-1-10
assessment procedures
assessment day 3-1-19
information needed 3-1-19
checklist 3-1-21
planning of 3-1-19
post-assessment 3-1-22
examples of 3-1-23
cull piles
definition of Glossary-1-2
sampling 2-1-10

D
dazomet 12-1-8
deep burial 12-1-2
delimitation sampling for plant positives and sample handling and submission protocol
sample forwarding and reporting under DA-2014-02 8-1-2
sample handling and submission protocol 8-1-1
delimitation survey
definition of Glossary-1-2
Delvocid 10-1-17, 10-1-18, 11-1-10, 11-1-11
destruction radii
definition of Glossary-1-2
destruction radius
definition of Glossary-1-2
Difco Bacto agar 10-1-17, 10-1-18, 11-1-10, 11-1-11
distilled water 10-1-17, 10-1-18, 11-1-10, 11-1-11
dust abatement 12-1-4

E
Emergency Action Notification (EAN)
definition of Glossary-1-2
equipment
of nursery personnel 9-1-4
equipment cleaning 12-1-4

F
Federal confirmatory authority
definition of Glossary-1-2
filtration
water sampling 10-1-10
final determination
definition of Glossary-1-3
fire suppression 12-1-4
footwear 9-1-4
free from
definition of Glossary-1-3
fumigants
soil 12-1-8

G
glossary Glossary-1-1
Green-Shield 20
trade name for a quarternary ammonium 12-1-3
growing media formulae 10-1-17, 11-1-10

H
hands 9-1-4
high-risk genera
definition of Glossary-1-3
Hymexazol 10-1-17, 11-1-10

I
in situ water sampling with host material leaf baits 10-1-5
bait retrieval 10-1-9
bait selection 10-1-5
baiting techniques 10-1-6
in vitro water sampling and processing
bait selection 10-1-2
BoB technique 10-1-2
sample collection 10-1-2
sample processing in receiving laboratory 10-1-4
incineration 12-1-2
infected plants
definition of Glossary-1-3
interstate shipping propagation wholesale nurseries 3-1-2
intrastate shipping wholesale nurseries 3-1-2
irrigation 12-1-4
irrigation water 2-1-9

K
Kalmia Glossary-1-3

L
labeled soil fumigants 12-1-8
laboratory notification 2-1-4
leaf lesions 2-1-5
leaf spots 2-1-5
Loropetalum 2-1-5
lot
definition of Glossary-1-3

M
materials list
for bottle of bait (BoB) 10-1-4
for water filtration 10-1-16
metam-sodium 12-1-8
Metapricrin
trade name for chloropicrin 12-1-8
methyl bromide 12-1-8

N
National Operations Manager 3-1-17
National Plant Diagnostic Network (NPDN) 8-1-2
National Plant Protection Laboratory Accreditation Program (NPPLAP) 2-1-3
non-PASS
definition of Glossary-1-4
nonporous surfaces
disinfecting 12-1-3
nonrecycled retention ponds 2-1-9
NPDN see National Plant Diagnostic Network
NPPLAP see National Plant Protection Laboratory Accreditation Program
nurseries
regulatory protocol for 3-1-1
wholesale
intrastate shipping 3-1-2
nursery
block Glossary-1-4
dealer Glossary-1-4
definition of Glossary-1-4
personnel
biosecurity measures 9-1-3
quarantine radii/radius definition of Glossary-1-5
quarantine release survey definition of Glossary-1-5
quaternary ammonium 12-1-3
R
regulatory protocol for nurseries 3-1-1
retail nursery definition of Glossary-1-6
Rhododendron 10-1-5, 11-1-5, 11-1-9, Glossary-1-3
Rhododendron catawbiense 11-1-5
Rifamycin-SV 10-1-17, 10-1-18, 11-1-10, 11-1-11
S
sample definition of Glossary-1-6
sample collection for BoB 10-1-2
soil and container mix 11-1-2
sample forwarding and reporting under DA-2011-02 8-1-2
under DA-2014-02 8-1-2
sample handling information 8-1-1
sample numbers
minimum 2-1-8, 3-1-15
sample processing in receiving laboratory 10-1-4
sample submission information 8-1-2
sampling by symptom type 2-1-9
sampling supplies and equipment checklist 8-1-4, 9-1-5
SME see subject matter expert
soil definition of Glossary-1-6
disinfecting 12-1-4
in nursery beds 12-1-5
in situ 12-1-5
physical treatment 12-1-8
sampling 2-1-9
soil and container mix sampling
baiting soil 11-1-4
growing media formulae 10-1-17, 11-1-10
sample collection 11-1-2
transport, storage, shipping, and processing 11-1-3
SPHD see State Plant Health Director
SPRO see State Plant Regulatory Official
State Plant Health Director (SPHD) definition of Glossary-1-7
State Plant Regulatory Official (SPRO) definition of Glossary-1-7
steam sterilization 12-1-2
subject matter expert (SME) 3-1-18
subsamples definition of Glossary-1-7
surface water 2-1-9
suspect plant material definition of Glossary-1-7

P
P. ramorum
plant symptoms 2-1-4
parallel quarantine definition of Glossary-1-4
PARPH-V8 selective medium 10-1-17, 11-1-10
PAR-V8 selective medium 10-1-18, 11-1-11
PCR see polymerase chain reaction
Pheno-cen tradename for a phenolic 12-1-3
phenolics 12-1-3
Phytophthora ramorum plant symptoms 2-1-4
cankers on boles and branches 2-1-5
leaf spots 2-1-5
lesions 2-1-5
twig dieback 2-1-5
plant symptoms of P. ramorum 2-1-4
plants treating 12-1-1
polymerase chain reaction (PCR) 12-1-1
ponds nonrecycled retention 2-1-9
pots sampling 2-1-10
potting mix treating 12-1-1
PPQ see Plant Protection and Quarantine
Preplant Soil Fumigant race name for methyl bromide 12-1-8
presumptive positive definition of Glossary-1-5
Q
quarantine period definition of Glossary-1-5
symptom type
   sampling of 2-1-5
symptoms of *P. ramorum*
   plant 2-1-4

T
Terraclor 10-1-17, 11-1-10
   trade name for methyl bromide 12-1-8
Terr-O-Gas
   trade name for chloropicrin 12-1-8
Timberfume
   trade name for chloropicrin 12-1-8
trace back (TB)
   plants Glossary-1-7
   protocol 5-3-1
   site Glossary-1-7
trace forward (TF)
   plants Glossary-1-7
   protocol 5-2-1
   site Glossary-1-7
trace protocols
   trace back (TB) protocol 5-3-1
   trace forward (TF) protocol 5-2-1
transport, storage, shipping, and processing
   soil and container mix sampling 11-1-3
treatment and disinfection options 12-1-1
   deep burial 12-1-2
   disinfecting nonporous surfaces 12-1-3
   disinfecting soil and container mix 12-1-4
   disinfecting water 12-1-4
   disinfection options 12-1-3
   equipment and personnel 12-1-8
   incineration 12-1-2
   infected plants and associated potting mix and containers 12-1-1
   physical soil treatment 12-1-8
   soil in nursery beds 12-1-5
   soil in situ 12-1-5
   steam sterilization 12-1-2
   treatment options 12-1-1
Tri-Clor
   trade name for chloropicrin 12-1-8
Tri-Con
   trade name for methyl bromide 12-1-8
twig dieback 2-1-5

U
United States Department of Agriculture (USDA)
   Glossary-1-7
   see United States Department of Agriculture
V
Vapam
   trade name for metam-sodium 12-1-8
vehicles 9-1-2
Viburnum 10-1-5, Glossary-1-3
W
water
   disinfecting 12-1-4
   irrigation 2-1-9
   surface 2-1-9
   water filtration materials list 10-1-16