



United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine



Official Regulatory Protocol for Wholesale and Production Nurseries Containing Plants Infected with *Phytophthora ramorum*

**Confirmed Nursery Protocol: Version 8.0
Revised: March 31, 2010**

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Appendices 3 & 7 updated June 26, 2008
Appendix 6 updated November 2010
Appendix 11 updated October 28, 2008**

**United States Department of Agriculture (USDA)
Animal Plant Health Inspection Service (APHIS)
Plant Protection and Quarantine (PPQ)**

**Center for Plant Health Science and Technology (CPHST)
Emergency and Domestic Programs (EDP)
Eastern Region (ER)
Western Region (WR)**

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INTENDED USE

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 *Phytophthora ramorum* (7 CFR 301.92). The complete text and other information may be found at the USDA APHIS PPQ web site:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Since the regulations were first published in 2002, *P. ramorum* has been detected in a significant number of nurseries. These detections prompted the need for a standard protocol for use by state and federal regulators to respond to finds of *P. ramorum* in nurseries. To ensure that there is consistency in responding to infestations of *P. ramorum*, this protocol describes the official activities performed within and around nurseries by USDA APHIS staff in cooperation with state agriculture regulatory officials.

The goal of this protocol is to ensure that any infestations of this serious pathogen are consistently and effectively addressed, mitigated, and eradicated. Cooperation by nursery management personnel is essential. Early detection and reporting of *P. ramorum* finds are critical to ensure that the infestation is contained and spread is minimized. The strategies employed in this protocol are consistent with those of the European Union, Canada, and other areas where eradications are being carried out with measures that ensure rapid suppression of infection, and which prevent the spread of the pathogen.

P. ramorum infestations in nurseries may be introduced via three critical pathways.

- The movement of infected plant material from one nursery to another;
- The natural environmental movement of spores from a nursery or infected wild plants to infect plants in a nursery;
- The transmission of the pathogen from non-plant pathways to plant material (e.g. the introduction of infested soil, water, growing media, equipment, etc.)

Other pathways are possible, but are not yet known.

Nurseries found with *P. ramorum* infestations more than once

P. ramorum infestations in nurseries may also be re-introduced by the above means, or the effort to eradicate the disease may fail. In the event that a nursery has *P. ramorum* detected on site after the initial release from the Emergency Action Notification (EAN) or state equivalent, it is necessary to implement additional measures to ensure that the risks associated with *P. ramorum* are properly mitigated. See **Appendix 11** for details of these additional measures.

GOAL

The goal of this protocol is to find and eradicate the pathogen in nurseries. Any interpretation of this protocol or its procedures that are not consistent with this goal is a misinterpretation of this protocol

DISCLAIMERS

FIELD GROWN STOCK: We have received comments that this protocol fails to adequately address situations found in nurseries with field grown stock. We recognize this limitation and leave it to field personnel to properly adapt this protocol to those situations when they occur until appropriate modifications can be incorporated.

RETAIL SITES: We recognize that we need a protocol for retail nurseries. Until that can be issued, regulatory officials must use this protocol and apply it to each situation.

CHALLENGES: *P. ramorum* is a microorganism. Thus it can be elusive and difficult to detect and difficult to eradicate. It can infect plants, infest media, soil and water and persist despite best intentions and best efforts. It can wash into nearby waterways and can be expected to do so and be present during eradication and monitoring procedures. Scientists continue to learn and report on basic biology and enhanced detection and eradication techniques. We continue to learn from science and our successes and failures and those will be reflected in updated protocols and regulations.

DEFINITIONS

Associated plants:	Associated plants are those reported found naturally infected and from which <i>P. ramorum</i> has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed. See Appendix 1.
Biosecurity measures:	Actions taken to reduce or mitigate the potential introduction or spread of <i>Phytophthora ramorum</i> from one area or site to another area or site of a nursery. See Appendix 9.
Compost pile:	A heap of mixture of decaying organic matter, as from leaves and manure, used to improve soil structure and provide nutrients.
Cull pile:	An area where discarded plant material is deposited. Also known as a waste or trash pile.
Delimitation survey:	A survey done to determine the extent of the infestation within a nursery site. The quarantine period begins when all delimitation sampling is completed.
Destruction block:	Block of plants to be destroyed. Within a nursery, this is a contiguous block of HAP containing one or more plants known to be infected with <i>P. ramorum</i> . The block will be considered contiguous until there is a 2 meter break of either no plants or no HAP.
Emergency Action Notification (EAN):	PPQ Form 523 or equivalent State document, is used to specify the regulatory actions to be taken within a nursery.
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. 10, 1999)
HAP:	Host and associated host plants listed on the official APHIS List of Regulated Hosts and Plants Associated with <i>Phytophthora ramorum</i> .
Hold block:	This term no longer in use; See Quarantine Block.
Host plants:	Naturally infected plants verified with completion, documentation, review and acceptance of traditional Koch's postulates and listed in the "APHIS List of Regulated Hosts and Plants Associated with <i>Phytophthora ramorum</i> ".

Infected plants:	Plants officially confirmed as being infected with <i>P. ramorum</i> , based on the use of APHIS approved diagnostics, and following the PASS system.
Nursery/Facility:	Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed, including locations that grow trees to be sold without roots, such as Christmas trees.
Nursery block:	A contiguous grouping of plants separated by at least two meters from other contiguous groupings of plants.
Nursery site:	A geographically separate location of a Nursery/Facility that has a distinct physical address and appropriate biosecurity measures (See Appendix 9) to prevent the movement of <i>P. ramorum</i> between locations.
Nursery site quarantine:	This is a period of time during which host plants and associated plants shall not be moved within or out of the quarantine block (see Appendix 2). This quarantine period begins when the <u>Nursery Delimitation Survey is completed</u> and lasts for 90 days during which proscribed activities must occur. During the quarantine period, inspection, sampling, and testing must reveal no further detection of <i>P. ramorum</i> . Conducive conditions exist when climatic conditions match <u>optimum disease etiology and are likely to express disease symptoms 50% or more of the time</u> .
Nursery stock:	Any plants for planting, including houseplants, propagative material that are grown in a nursery and tree seedlings for reforestation.
Parallel quarantine:	A quarantine or regulation imposed by a State or local plant regulatory authority that is essentially the same as a federally promulgated quarantine. These regulations can be more restrictive for intrastate movement and internal controls.
PASS (Potentially Actionable Suspect Sample):	A presumptive positive <i>P. ramorum</i> sample diagnosed or identified by a provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation. (For more information see: “PASS System Policy” at

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml

Presumptive positive:	A preliminary diagnostic test result from a laboratory indicating <i>P. ramorum</i> is present.
Quarantine block:	Area identified as a 10 meter radius around the destruction block (see Appendix 2) designed to determine if <i>P. ramorum</i> has spread beyond the destruction block. (Use of Quarantine block 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. 10, 1999]).
Quarantine period:	A minimum of 90 days that begins when the Nursery Delimitation Survey is completed and lasts until such time as both plant parts and climatic conditions conducive to disease expression have occurred. During the quarantine period , inspection, sampling, and testing must reveal no further detection of <i>P. ramorum</i> . Conducive conditions exist when climatic conditions match optimum disease etiology and are likely to express disease symptoms 50% or more of the time.
Quarantine release survey:	This is the second quarantine period inspection that occurs near the end of the quarantine period. This survey includes visually inspecting all HAP genera within the nursery and sampling any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water, as per Appendices 4, 6 and 7, respectively. When the quarantine period is completed and all plant, soil and water samples taken are negative for <i>P. ramorum</i> the nursery can be released.
Regulated area:	Any state, or portion of a state, in which only nurseries that ship HAP interstate are regulated to prevent the spread of <i>P. ramorum</i> and the only regulated article is nursery stock. These areas are detailed in the regulations posted at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/
SPHD:	The State Plant Health Director of a particular state. Lead APHIS contact in each state responsible for overseeing all Plant Protection and Quarantine activities in that state.
SPRO:	The State Plant Regulatory Official in any given state’s department of agriculture. This is the person primarily responsible for plant

health programs in that state. SPROs can be found listed at:
www.nationalplantboard.org/member/index.html

TRIGGER EVENTS FOR USE OF PROTOCOL

This protocol shall be implemented by APHIS-PPQ and/or its State Plant Regulatory cooperators when the presence of *P. ramorum* has been confirmed in a nursery from samples collected as part of a trace forward survey*, trace back survey*, *P. ramorum* nursery survey*, or found by other means. Confirmed samples must have been diagnosed using a methodology approved by USDA, APHIS, PPQ and consistent with the Potentially Actionable Suspect Sample (PASS) protocol*.

*See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for links with details on trace forward survey, trace back survey, *P. ramorum* nursery survey, and the PASS protocol.

AUTHORITIES

- For states with quarantines equivalent to the Federal regulation, State personnel will conduct specific actions required by the protocol, within and around the nursery, under State authority with Federal support.
- For States without quarantines for *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 personnel.

COMMUNICATE AND NOTIFY

Communicate suspect finds using the bullets below as soon as one of the following has occurred:

1. A positive PCR determination
 2. A culture that matches the morphology for *P. ramorum* (i.e. isolation of *P. ramorum*)
- Immediately notify the State Plant Health Director (SPHD) and the State Plant Regulatory Official (SPRO) of the State in which the nursery is located. The SPHD will notify the Regional Office and National Headquarters Office. See Appendix 3, Resource and Contact List.
 - SPHD's and SPRO's, shall notify facilities within their states that are impacted by the trace backs and trace forwards and provide a list of these facilities to their PPQ Regional offices. See "Conduct Investigations" Section.
 - Laboratories need to notify, the SPHD, and the SPRO, the Regional Office, National Program Manager, and the submitter. Ideally the SPRO should notify the owner of the nursery, but either the SPRO (if State authority is used) or the SPHD (if Federal authority is used) may notify the owner of the nursery.
 - The SPRO and SPHD will use state channels, including public affairs offices to make any public announcements, as necessary. The SPHD will ensure that the USDA APHIS Office of Legislative and Public Affairs is aware of any pending release, via the Regional Office and National Headquarters Office.

CONDUCT INVESTIGATIONS

Trace Forward Investigation:

Initiate trace forward investigations. Identify all domestic and international shipments of the High Risk HAP Genera: *Camellia*, *Rhododendron*, *Pieris*, *Viburnum* and *Kalmia* and HAP shipments of the infected plant species within the 6 months prior to the first positive detection of *P. ramorum* at the nursery as per the protocol. This information on shipments needs to be gathered, processed, and forwarded to Regional Office **within 10 working days**. Reporting trace forwards within 10 days is essential. If requested or necessary, Smuggling Interdiction and Trade Compliance (SITC) or Investigative and Enforcement Services (IES) may be asked to assist in the information gathering, as appropriate. The Regional Offices will forward these domestic lists to the States that have received plants. Headquarters will inform international trading partners of shipments to their countries. The plants sent to the receiving States must be inspected at the receiving nurseries.

Use the Trace Forward Protocol posted at

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Trace Back Investigation:

Implement the current Trace Back Protocol present on the *Phytophthora ramorum* website located at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Nursery Sites:

Determine whether additional locations (nursery sites) are maintained by the same nursery personnel, or if HAP move to other sites or between sites.

- **Equipment:** Determine if equipment used at the site is shared with other nursery sites or field areas. Document any shared equipment utilization in different nursery sites or field areas. Equipment movement without appropriate biosecurity measures (See Appendix 9) between nursery sites requires that all nursery sites utilizing the equipment be included under this protocol.
- **Plants:** Determine if HAP move between sites. If so, than all sites receiving HAPs must be included under this protocol.

SECURE THE NURSERY

When the presence of *Phytophthora ramorum* has been confirmed in a nursery:

- All plants (including non-host plants) in the destruction block shall remain under regulatory control as per the Emergency Action Notification (EAN) or State equivalent document. All plants within the destruction block shall be cordoned off with no unauthorized access until delimitation survey is complete and all destruction block(s) is(are) defined.
- All HAP genera in the nursery are to be placed under regulatory control as per EAN. This action may also include any item that an inspector determines to present a risk of spreading *P. ramorum* within or from the nursery; and,
- A delimitation survey will take place on the nursery site as per this protocol; and,
- All HAP genera must be held until delimitation within the nursery is complete, that is, until the samples taken have diagnoses reported that allow release of blocks of HAP. This hold may also include “any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum*, if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations” (7CFR part 301.92-2) within the infested nursery site; and,
- Secure the cull pile until all testing is complete.
- Ensure that equipment used on nursery site is not moved from the site without proper disinfestation.
- Any additional treatments and/or basic sanitary and precautionary measures shall be detailed on the EAN.
 - PPQ form 523, Emergency Action Notification will be used as the official Federal authorization of hold. The required treatments and/or basic sanitary and precautionary measures (e.g. bio-containment of suspected infected material, etc.) should be included in the PPQ form 523. If the State initiated action, then the appropriate State notification would be used. Stop Sales notices should be placed on the nursery by the appropriate State Regulatory Official.
- If any plants not on hold are showing symptoms consistent with diseases caused by *P. ramorum*:
 - These plants must be sampled and tested for the presence of *P. ramorum*.

SURVEY THE NURSERY AND PERIMETER

The goal of the survey is to locate *P. ramorum* in the nursery and perimeter. A detailed and thorough inspection should be conducted at the field level to determine the presence of *P. ramorum*. Samples should be collected from unhealthy looking plants, including any plants with any minute symptoms such as tiny leaf spots or brown leaf tips.

Delimiting Survey and Establishing Destruction and Quarantine Block(s):

- Inspect all plants held, for sale or propagation, of HAP genera in the nursery and decorative plants (permanent landscape plants within the nursery that are not for sale).
- Examine all HAP genera within 10 meters of the positive block(s) in the nursery as per Appendix 4. Sample any unhealthy tissue.
- All HAP genera within 10 meters of the positive block(s) shall be considered exposed to *Phytophthora ramorum* and shall be held for the quarantine period.
- Examine all plants within the nursery and sample any unhealthy plant tissue found. A minimum of 40 samples shall be taken in a small* nursery, 80 samples in a medium* nursery and 120 samples in a large* nursery. These are absolute minimums. To assure proper delimiting it is expected that the actual numbers will commonly be in the hundreds.
- Samples must be analyzed using a methodology approved by APHIS (see Appendix 5).
- The destruction and quarantine block(s) is (are) established when diagnostic results from all delimiting samples have been reported. The 90 day quarantine period begins when the delimiting survey is complete.
- Establish destruction block(s) by flagging the perimeter of the block(s) of HAP containing one or more plants known to be infected with *P. ramorum*. The block is considered contiguous until there is a 2 meter break of either no plants or no HAP.
- Limit access to destruction block. Ensure that proper sanitation measures are applied (See Appendix 8).
- The HAP (note: not all plants nor all HAP genera) in the destruction block shall be destroyed in an appropriate manner (see Appendix 8)

*A small nursery can be considered one containing less than 200,000 HAP plants. A medium sized nursery can be considered as one containing more than 200,000 and less than 999,999 HAP plants. A large sized nursery can be considered as one containing more than 1,000,000 HAP plants.

Soil and Growing Media Sampling:

- Soil from within the destruction and quarantine block(s) must be sampled, and

- Growing media from non-HAP within the destruction block(s) and from all types of plants in the quarantine block(s) must be sampled, and
- Soil and growing medium from nursery blocks down slope from destruction and quarantine block(s) must also be sampled.
- Growing media from the plant potting area shall be sampled.
- Soil is the substrate underneath pots and growing medium is located within pots with the plants in the blocks.
- If reported positive, determine the content, origin, storage and handling of growing media used at the nursery site. See Appendix 6 for detailed soil and media sampling protocol. Keep soil samples separate from growing media samples.

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 and any safeguards against water back flow in the irrigation system, as well as any water treatment practices if recirculated water is used. Water is to be sampled; See Appendix 7 for detailed water sampling protocol. Water sampling is not required for irrigation water from municipal water facilities that treat their water prior to release, but any retention pond or area where water collects at the nursery site must be sampled.

Cull Pile Sampling:

Record the location of any cull piles as these may be contaminated with infected plant material or associated soil and/or growing media. Check any cull piles for *P. ramorum* symptomatic plants and plant material and sample if observed. Determine how the nursery disposes of culled plant material. Sample and test soil at the down slope edge of the cull pile for the presence of *P. ramorum*.

Compost Pile Sampling:

Record the location of any compost piles as these may be contaminated with infected plant material or associated soil and/or growing media. Check any compost piles for *P. ramorum* symptomatic plants and plant material and sample if observed. Determine how the nursery disposes of composted plant material. Sample and test soil at the down slope edge of the compost pile for the presence of *P. ramorum*.

Perimeter Survey:

The purpose of the perimeter survey is twofold: (1) to ensure that *P. ramorum* has not spread from the infested nursery to the surrounding environment and (2) to verify that the infection in the nursery did not originate in the surrounding environment. Conduct a survey concentrating on plants of all HAP genera located within 10-meters of the infested nursery for symptoms of disease caused by *P. ramorum*. Sample all plants with suspicious symptoms. Samples must be labeled and sent to a laboratory for testing using a method approved by APHIS (see Appendix 5). Detection of *P. ramorum* in the perimeter may be indicative of a more widespread infestation. In this case, notify your PPQ Regional Office immediately as further regulatory actions may be required depending on the quarantine status of the area.

DISINFEST THE NURSERY

Plant Destruction:

Where a *P. ramorum* infected plant(s) is found, all HAP and plant parts within a destruction block will be removed and destroyed using one or more of the techniques detailed in Appendix 8.

Debris Removal:

All plant debris including growth medium, leaves, stems, flowers, roots, and any other plant parts found within the destruction block will be removed and destroyed using one or more of the techniques detailed in Appendix 8.

Cull Pile Treatment:

If any plants, plant material, growing media or soil from a cull pile is positive for *P. ramorum*, all material in the cull pile shall be properly disposed. See Appendix 8 for recommended destruction/disinfestation options.

Compost Pile Treatment:

If any plants, plant material, growing media or soil from a compost pile is positive for *P. ramorum*, all material in the compost pile shall be properly disposed. See Appendix 8 for recommended destruction/disinfestation options.

Non-porous Surfaces:

Non-porous surfaces will be disinfested. See Appendix 8 for recommended disinfestation options.

Water Treatment:

If water tests positive for *P. ramorum*, treatment is required (see Appendix 8 for recommended disinfestation options) and an additional delimitation of the nursery must be completed. For nurseries with established quarantine block(s) undergoing a 90 day quarantine period, the 90 day quarantine period re-starts after the second delimiting survey is completed. Also, plants and growing media that may have been irrigated with infested water must also be resampled and retested within the new 90 day quarantine period.

Soil and Growing Media Treatment:

If soil, growing media or plant debris in a destruction or quarantine block test positive, soil treatment is required. The destruction block is the most likely area of soil or growing media infestation (underneath and around the diseased plants, and in containerized stock) and the most likely area where reinfestation of new host material would occur. See Appendix 8 for recommended destruction/ disinfestation options.

Equipment and Personnel:

See Appendix 8 for recommended disinfestation options.

Biosecurity Measures:

Biosecurity measures are designed to minimize the risk of introduction or, spread and survival of the pathogen in a nursery. See Appendix 9 for recommended biosecurity measures.

NINETY (90) DAY QUARANTINE ACTIVITIES

These concurrent activities follow completion of the delimiting survey:

- Any non-HAP that were present in a destruction block will be held in place, or moved under official supervision to a safeguarded area with a non-porous surface, during the quarantine period and be subject to the same conditions as the HAP in the quarantine block(s).
- For nurseries with HAP genera in the quarantine block(s) (see Appendix 2), these HAP genera shall not be moved within or out of the quarantine block(s) during the quarantine period. This quarantine period begins when the delimiting survey is completed (i.e. the last sample is taken and an EAN is issued) and lasts until such time as both plant parts and climatic conditions conducive to disease expression have occurred for at least 90 days. If the quarantine period (90 days) does not include climatic conditions conducive for disease development then the quarantine period shall be extended to an appropriate length to include conducive climatic conditions for a total of 90 days. During the quarantine period, inspection, sampling, and testing must reveal no further detection of *P. ramorum*.
- During the 90 day quarantine period within the 10 meter quarantine block(s):
 - No fungicides registered for *Phytophthora* control shall be applied.
 - Regulatory officials will visually inspect plants a minimum of two times, once about half-way through the anticipated quarantine period and once near enough to the end to have test results coincide with the end of the quarantine period, according to the protocol detailed in Appendix 4. This second visual inspection in the quarantine block(s) can be done at the same time as the quarantine release survey as described below. A minimum of 40 samples shall be taken in a small* nursery, 80 samples in a medium nursery and 120 samples in a large nursery. These are absolute minimums.
 - Regulatory officials will collect water, soil, and media samples and test during the quarantine period according to the protocols detailed in Appendices 6 and 7.

If found positive:

- If a plant sample tests positive for *P. ramorum*, the destruction block(s) and 10 meter quarantine block(s) shall be redefined via sampling and the quarantine period reset.
- If water, soil, and/or media samples tested positive for *P. ramorum* during the delimiting survey, it must be treated per Appendix 8. Once successfully treated, samples of the infested water, soil, and/or media material will be taken and tested during each of the two quarantine period nursery inspections per the protocols detailed in Appendices 6 and 7.

- If irrigation water is found to be positive, then any portion of the nursery that has been irrigated with the *P. ramorum* infested water shall be placed on hold and the irrigated area re-delimited.
- If a soil sample is found to be positive, the soil shall be treated, then any plants in the block with the infested soil are placed on hold and the area re-delimited.
- The growing media in the potting shed must be tested. Any positives for *P. ramorum* from the media in the shed confer with the Regional Program Manager.
- **A quarantine release survey of the entire nursery must be completed near the end of the 90 day quarantine period.** This survey includes visually inspecting all HAP genera within the nursery and sampling any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water. When the quarantine period is completed and all plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released.

RELEASE THE NURSERY

Nurseries and their plants that have been placed under regulatory control may be released from regulatory control by USDA-APHIS or its designated authority after the quarantine period if the following three conditions are met:

- There are no additional detections of *P. ramorum* in nursery stock based on USDA APHIS approved plant inspection, sampling and testing protocols for the preceding quarantine period; and
- Water, soil and growing media have also tested negative for *P. ramorum* based on USDA APHIS approved sampling and testing protocols for the preceding quarantine period; and
- The quarantine release survey is negative for *P. ramorum*.

Alternative Release Strategy:

A nursery may avoid a quarantine period, through a voluntary management decision, by:

- Destroying everything (all plants, pots, media, etc.) in the destruction block(s); and
- Destroying the HAP genera and plant parts in the quarantine block(s); and
- Visually inspecting all HAP genera within the nursery and sampling and testing any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water, as per Appendices 4, 6 and 7, respectively. If plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released., and
- Revisit the nursery after approximately 90 days of conducive conditions and conduct at least a nation-wide survey level inspection to include sampling of the soil in the destruction block.

POST ERADICATION MONITORING

Nurseries that have been infested will continue to be monitored when disease expression is anticipated for the following two years at the nursery survey protocol levels. These nurseries are not under any quarantine or regulatory action, unless there are additional detections.

CONFIRMED NURSERY PROTOCOL FLOWCHART

A flow chart of these protocols is shown in Appendix 10.

APPENDIX 1

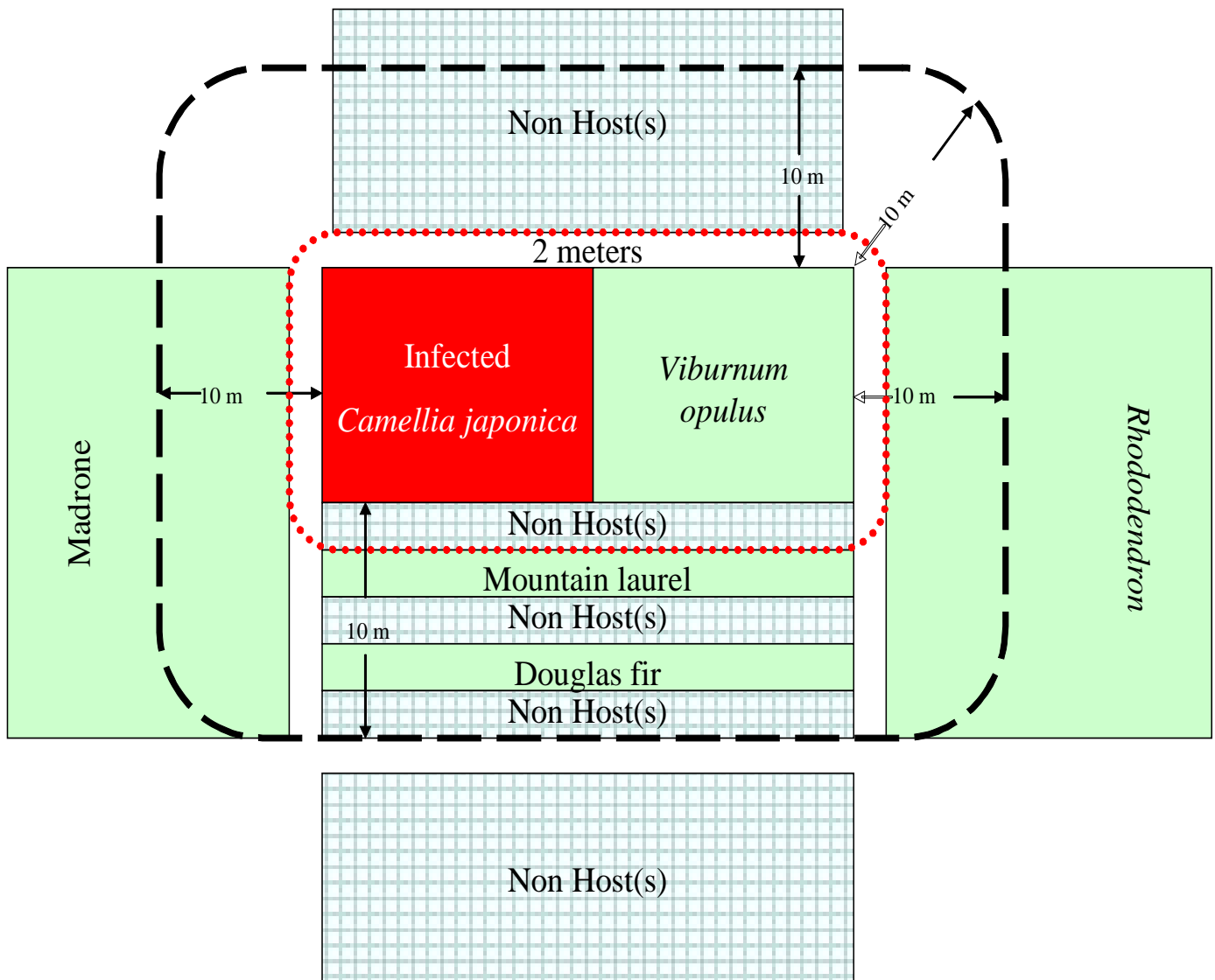
APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*

A current list may be found at the USDA APHIS PPQ website at
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

APPENDIX 2

Schematic of Wholesale/Production Nursery with Infected Host Plant(s)

Revised: August 31, 2006



**Destruction Block**

Action: Destroy *Camellia japonica* and *Viburnum opulus*. Hold and monitor all non-hosts.

**Quarantine Block**

Action: Hold and monitor all Mountain laurel and Douglas fir, as well as some Madrone and Rhododendron.

APPENDIX 3

Resource and Contact List

Revised: May 2007

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APPENDIX 4

Delimiting Survey Protocol

Delimiting Survey Protocol to Detect *Phytophthora ramorum*
In Plants at Confirmed Nurseries
Revised: July 19, 2007

Objective:

The objective of this document is to provide guidelines for the delimiting survey in nurseries where the regulated pathogen, *Phytophthora ramorum* has been confirmed. This survey method is designed using the best available scientific principles to determine apparent freedom from *P. ramorum* in nursery plants. In order to achieve this freedom from *P. ramorum*, accurate and successful inspection of HAP (genera for wholesale/production) must be accomplished at an appropriate confidence level to ensure detection of disease.

Sampling method:

The goal is targeted sampling of plant tissue to determine the presence of *P. ramorum* with a 95% confidence of finding the disease at a very low level (0.5% of plants are infected with *P. ramorum*) by inspecting a minimum of 850 HAP plants in each block (or all the plants if there are less than 850). A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. Do not sample asymptomatic plants.

- Inspector should contact the nursery manager to set up the inspection and find out approximately how many HAP are present in each nursery block (i.e. a nursery map).
- These visually inspected plants should be chosen at random, but if certain areas of the block contain plants exhibiting unhealthy tissue or are more prone to disease development (such as low areas where water might puddle or places where mist or fog persists) these areas should be included in the sampling process.
- Disposable rubber gloves and tyvek booties should be worn and should be changed or disinfested using 10% bleach solution **or** a quaternary ammonium solution (at the labeled rate) between each block. Additionally, waterproof raingear and rubber boots may be used and disinfested between each block. Washtubs with ~ 1/2 inch of disinfectant to step in for booties and 3 inches in buckets to dip gloved hands should be sufficient.
- To visually inspect a plant, carefully lift the plant from surrounding plants, if possible, and carefully examine all plant leaves and stems for unhealthy tissue particularly for the presence of water-soaked or necrotic lesions consistent with *P. ramorum* infection, however all unhealthy tissue should be considered suspect. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms. Be sure to properly disinfest booties and gloves

between all nursery blocks. Because this is a confirmed nursery, proper use of sanitation is imperative to reduce the potential for pathogen transport from an infested part of the nursery to an un-infested nursery block.

- Sample plant tissue from any and all visually inspected plants that appear unhealthy. Each sample should consist of a minimum of five leaves; for *Vaccinium* and other small leaf hosts collect the terminal last 3 inches of branch tips, if present, from each unhealthy plant. If, however, only one leaf is unhealthy include only the one leaf with lesions. Examine any other leaves on the plant for the presence of lesions, because chances are much smaller lesions may be present on other leaves of the same plant.
- Samples should be placed in a re-sealable leak proof plastic bag labeled with the appropriate nursery designation and sample number. Samples should be double-bagged in an additional re-sealable leak proof plastic bag with a completed PPQ391 form for each sample submitted.
- Keep the samples cool by placing them in a cooler (around 3° – 6° C or 37 – 43 F).
- Overnight mail or deliver the sample to the laboratory as soon as possible to preserve freshness.
- All samples must be analyzed following the APHIS diagnostic protocols.
- Continue inspecting 850 plants in each block that contains HAP (genera for wholesale/production).
- Examine all HAP (genera for wholesale/production) in cull piles for the presence of tissue symptomatic for *P. ramorum* and take symptomatic tissue from any and all plants with symptoms.

APPENDIX 5

Diagnostics

Revised: April 2007

Samples must be analyzed using a methodology approved by APHIS. See techniques posted at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

APPENDIX 6

Soil and Container Mix Protocol

Protocol for Detecting *Phytophthora ramorum* in Soil and Container mix Revised November 2010

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Soil or container mix infested with *Phytophthora ramorum* appears to the unaided eye exactly the same as non-infested soil or container mix. Therefore, all soil and container mix samples must be handled carefully. All tools used to collect soil or container mix samples must be disinfested with a solution of 10% bleach, quaternary ammonium at the labeled rate, or full-strength Lysol™ spray, then rinsed thoroughly with distilled water and allowed to dry. Alternatively, tools may be flame-sterilized with a propane torch between sample collections. All soil and organic material should be removed from the tools prior to being disinfested. Care should be taken to not transfer soil or container mix from one sample location to another on shoes, gloves, or clothing. All sampling equipment should be cleaned and disinfested prior to entering a new nursery sampling location or block. Care must be taken to ensure that soil or container mix, within a sampling location, are not cross-contaminated in any manner. To reduce the risk of contamination, samples should be collected starting with the least infested area and moving toward the most infested area.

Definitions

Aliquot – a volume of substrate from a composite sample that is placed into a container and assayed; usually three aliquots (approx. 50-150 ml or 2-5 oz) from each composite sample are baited.

Block of plants – A contiguous group of host and associated plants with less than a 2 m (6.5 ft) break of non-host plants or empty space.

Composite sample – A mixture of subsamples that are physically combined to form a single representative sample from a designated area.

Container mix – Soilless substrates (also referred to as growing media) placed in pots and used to grow plants; usually consists of bark and peat but also may contain slow-release fertilizer, sand, vermiculite, perlite, etc.

Lot – Set of plants that can be identified or grouped by shipment, cultivar, or production unit.

Soil – Substrates in the field on which potted plants are located; often this consists of peat and bark fines washed from pots, plant debris, gravel, or any combination of these.

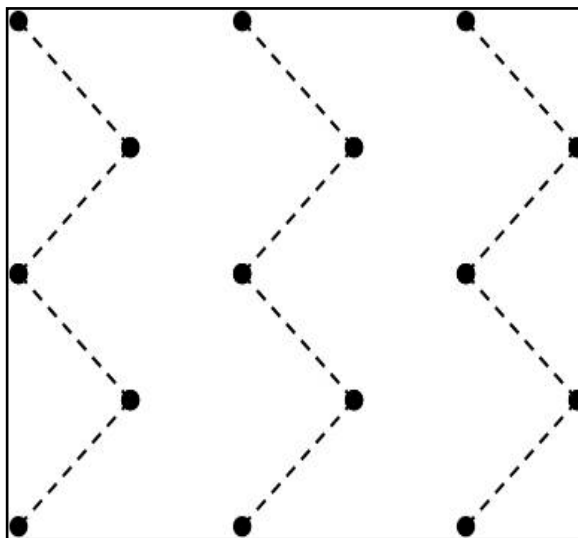
Subsamples – Small amounts of soil or container mix that are combined to form a single, composite sample; collecting subsamples increases the chances of finding *P. ramorum* if it is present.

Sampling Soils and Container Mixes

Sample Collection

1. Record the physical location (address) of the nursery site along with GPS reference coordinates. (*Tip: 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. Subsamples should be collected in zigzag transects according to the pattern in the diagram below (Figure 1).

Figure 1. Recommended pattern for collecting subsamples of soil or container mix to produce a representative composite sample



4. Referencing Table 1, collect composite samples from both soil and container mix for each block of plants. An exception to this would be if all plants (including container mix and pots) were destroyed or the plants are not on a soil substrate (e.g., concrete or asphalt). Each sample should contain approximately 1 L/1 qt (volume) of soil or container mix and be placed in a 4 L (1 gallon) 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 1).

Table 1. Number of composite samples to collect per block

Survey Area Size		Composite Samples of Soil	Composite Samples of Container Mix
(m ²)	(ac)		
<1,000	<0.25	5	5
1,000-2,000	0.25-0.5	10	10
2,000-4,000	0.5-1	20	20
>4,000	>1	30	30

Note: If the soil surface is covered with gravel having 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, small slits should be made in the cloth to allow for sample collection.

Soil

- a. 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.) with a trowel to a depth of approximately 5-10 cm (2-4 in). (**Tip:** If soil is loosely packed, a plastic spoon can be used to collect 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. Samples should be collected 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.

Container mix

- d. 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. Moisten the sample with distilled water if it appears dry, 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 Lysol™ spray) between samples to prevent

potential dissemination of the pathogen. Next, thoroughly rinse tools with distilled water to remove all disinfection product residues or allow tool to dry. (*Tip: Rinsing off disinfection 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 (4-10°C/39-50°F) for a maximum of two days.
2. Before shipping, samples should be double-bagged using gallon-size zip-to-close, self-sealing plastic bags, making sure to clearly label each bag using a permanent marker. To further protect samples, each sample can then be placed inside a 2 L (2 qt) disposable storage container which is also clearly labeled using a permanent marker. PPQ 391 forms (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/PPQ_Form_391.pdf) should be filled out completely and placed inside a separate zip-to-close bag and placed 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. Samples should be sent to a qualified state laboratory or to a USDA-APHIS-PPQ regional laboratory (see below).

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Western Region: Craig A. Webb, Ph.D.
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Procedure for Baiting Soil and Container Mix Samples (*S. N. Jeffers, Clemson University, 2010*)

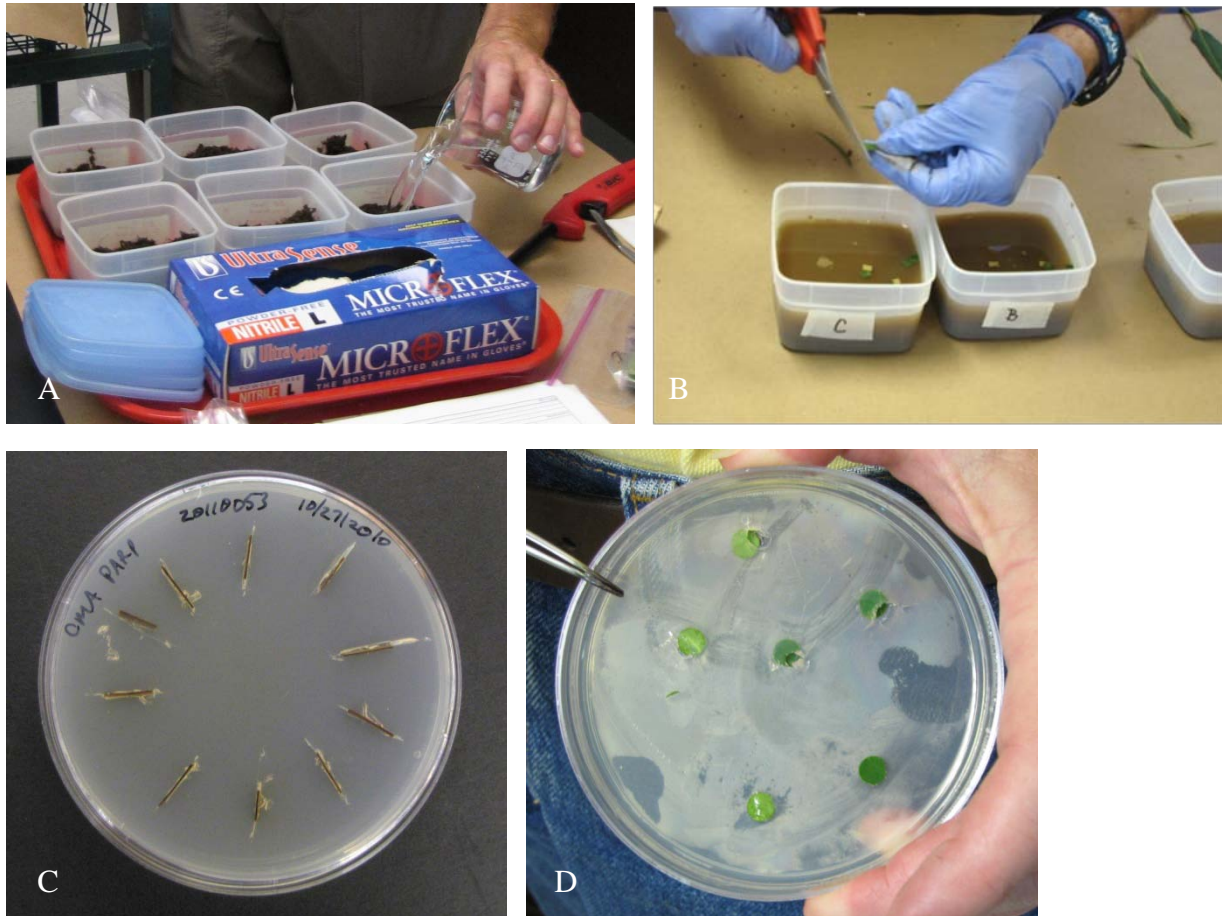
1. Once samples arrive in the lab, they should be protected from exposure to direct sunlight near windows. If samples cannot be processed immediately, they should be stored in a refrigerator or cold room (4-10°C/39-50°F). Do not permit the samples to freeze or dry out.

2. If the soil or container mix sample is desiccated when it arrives in the lab, moisten with distilled water, re-seal the bag, and allow the moistened sample to sit for 48-72 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 (4-10°C/39-50°F) for a minimum of 30 days.
4. Prepare and label three containers (e.g., small 0.5 L (1 pt) plastic containers, resealable 1 L (1 qt) bags, etc.) for each composite sample to be baited.
5. Thoroughly mix the remaining sample, then place an aliquot approx. 1 to 2 cm (0.5 to 1 in) deep into each of the three 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 in) above the soil surface; stir the mixture and allow it to settle. *Note: 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/container) to bait all containers (Figure 2). Leaf pieces cut with scissors should be approximately 5 mm (< 0.25 in) across. (*Tip: Use different-shaped leaf pieces to differentiate between bait types if two types of bait are being used.*)
8. Using sterile forceps, add 8-10 leaf pieces of each bait type (or 15-20 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 18-22°C/64-72°F for three days (an incubator maintained at 20°C/68°F or a closed cabinet works best).
10. For each container, remove six baits of each host type (or 12 baits of one host type) with sterile forceps and blot dry on a clean paper towel. Dispose of paper towels after each sample.
11. Place the six bait pieces of each host type from one container on a separate plate of PARPH-V8¹ medium (e.g., one plate with six rhododendron leaf pieces and one plate with six camellia leaf pieces, or alternatively, two plates of same host 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 six plates and 36 baits from each composite sample: 3 containers × 2 plates/container × 6 baits/plate (Figure 2). (*Tip: Baits can be inserted into the medium vertically, which prevents shadowing during microscopic examination. To*

¹ See Appendix A for recipe

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 plates no longer have to be read on both sides.)

Figure 2. Baiting soil samples and plating baits (images courtesy of Grace O’Keefe, PPQ and Jennifer Falacy, Washington State Dept. of Ag)

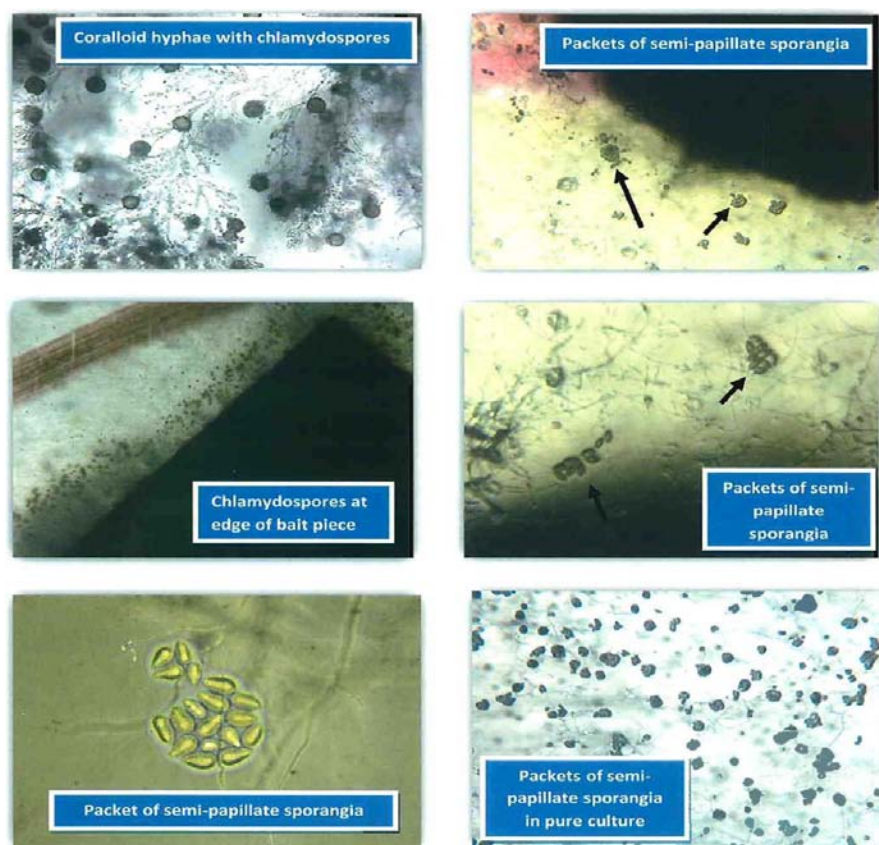


A) Preparing soil samples for baiting. B) Baiting soil samples using *Rhododendron* and *Camellia* leaves. C) Plate with baits inserted vertically. D) Plate with baits inserted horizontally.

12. Place plates upside-down in a plastic box or zip-to-close bag to prevent desiccation; incubate plates at 15-20°C/59-68°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.
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 (Figure 3); mark these with a permanent marker or grease pencil.

14. Once colonies form on plates, follow the morphological identification protocol found at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/culture_protocol6-07.pdf
15. *P. ramorum* hyphae often are visible 2-5 days after baits have been plated. However, patience, persistence, and good observational skills often are the keys to finding *P. ramorum* on the isolation plates; *P. ramorum* may be recovered from only one 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-V8². **Note:** *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 over grown by faster-growing organisms.

Figure 3. Characteristic structures of *Phytophthora ramorum* (images courtesy of S. N. Jeffers, Clemson University)



Second Baiting of Soil and/or Container Mix Samples

² See Appendix B for recipe

17. Remove composite samples from cold storage and hold at room temperature (22-24°C/72-75°F for 3 days to acclimate before baiting begins.
18. Bait samples again as described above in Steps 1-14.
19. 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

- Disposable gloves
- 4 L (1gallon) zip-to-close bags (at least 4 mm in thickness), avoid the bags with a “zipper” mechanism
- Trowel or other soil-sampling tool
- Wide bore soil tube (2.5 cm/1 in or larger)
- Permanent marker
- Insulated ice chest (with ice in bags or blue ice if external temperatures are above 21°C/70°F)
- Disinfesting solution (10% bleach, quarternary ammonium at the labeled rate or full-strength Lysol™ spray)
- Plastic or glass containers with lids; square, wide bottom containers work best (e.g., 0.5 L (1 pt) freezer boxes)(see Figure 2)
- Distilled water
- Single hole punchers, scissors or razor blades
- Forceps and scalpel
- 70% alcohol and flame for sterilizing laboratory utensils
- Paper towels
- Baits –Use rhododendron and/or camellia leaves that have been on the plant for at least one year; leaf pieces should be 5×5 mm squares (< 0.25 in) or 5 mm (< 0.25 in) dia. disks
- PARPH-V8 selective medium; 2 plates per baited container
- PAR-V8 selective medium (as needed for subcultures)

Growing Media Formulae

PARPH-V8 Selective Medium: For *Phytophthora* species (Adapted from Jeffers and Martin, 1986; Ferguson and Jeffers, 1999)

Ingredient	Amount Per:	
	1.0 Liter	0.5 Liter
<u>Basal Medium</u>		
Clarified V8 Concentrate*	50 ml	25 ml
Distilled Water	950 ml	475 ml
Difco Bacto Agar	15 g	7.5g
<u>Amendments</u>		
Delvocid [50% pimaricin]	10 mg	5 mg
Sodium Ampicillin	250 mg	125 mg
Rifamycin-SV [sodium salt]	10 mg	5 mg
Terracolor [75% PCNB]	66.7 mg	33.4 mg
Hymexazol	50 mg	25 mg

* Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaCO₃/100 ml V8 Juice) clarified in one of three ways:

- centrifugation at 4000 RPM for 20 min followed by filtration using two layers of Whatman No. 1 filter paper under vacuum
- centrifugation at 7000 rpm for 10 min then filtration is not necessary
- vacuum filtration alone through a 1-2 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, & PARH]

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

Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
2. Autoclave for 20 min at 121°C and 15 psi; turn waterbath on to ~50°C
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix
4. Cool medium in waterbath
5. Slowly stir medium with a magnetic stirrer in laminar flow hood
6. Vortex each amendment thoroughly and add to mixing basal medium
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 plastic bags in the dark in a refrigerator
11. Plates should be used within 30 days

PAR-V8 Selective Medium: For *Phytophthora* species (Adapted from Ferguson and Jeffers, 1999)

Ingredient	Amount Per:	
	1.0 Liter	0.5 Liter
<u>Basal Medium</u>		
Clarified V8 Concentrate*	50 ml	25 ml
Distilled Water	950 ml	475 ml
Difco Bacto Agar	15 g	7.5g
<u>Amendments</u>		
Delvocid [50% pimaricin]	10 mg	5 mg
Sodium Ampicillin	250 mg	125 mg
Rifamycin-SV [sodium salt]	10 mg	5 mg

* Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaCO₃/100 ml V8 Juice) clarified in one of three ways:

- centrifugation at 4000 RPM for 20 min followed by filtration using two layers of Whatman No. 1 filter paper under vacuum
- centrifugation at 7000 rpm for 10 min then filtration is not necessary
- vacuum filtration alone through a 1-2 cm deep layer of Celite

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

Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
2. Autoclave for 20 min at 121°C and 15 psi; turn waterbath on to ~50°C
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix
4. Cool medium in waterbath
5. Slowly stir medium with a magnetic stirrer in laminar flow hood
6. Vortex each amendment thoroughly and add to mixing basal medium
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 plastic bags in the dark in a refrigerator
11. Plates should be used within 30 days

References

- Jeffers, S.N., and Martin, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70: 1038-1043.
- Ferguson, A.J., and Jeffers, S.N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83: 1129-1136.

APPENDIX 7

Water Sampling Protocol Revised March 2010

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Phytophthora ramorum is a soil-borne plant pathogen well adapted to water-logged soil environments. 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 blocks can be tested for the presence of *P. ramorum* spores.

The following methods should be utilized to detect *P. ramorum* on a landscape or watershed level. A detection of *P. ramorum* does not mean that nursery stock is infected with the pathogen, but that an infestation within the watershed exists. Detection of *P. ramorum* by this method indicates a problem exists, thereby triggering a more intensive survey.

There are two methods summarized here for detecting *Phytophthora* species spores from water. Additional updates to this protocol are expected soon. The first and preferred sample collection method, for use in waterways or ponds located within and around a nursery, relies on host material (*Rhododendron*, *Camellia*, etc.) leaves contained in mesh bags or containers that function to “bait” or attract *Phytophthora* spores. This method is most effective when water temperatures are warmer than 39°F but cooler than summer-heated levels. After exposure, leaf baits are held briefly in a moist chamber to promote disease development and symptom expression.

The second method, water filtration, may be used where drainage outflows, diversion boxes, standing water and streams are found within the nursery perimeter. More time-sensitive than the first method, water is collected in one-liter plastic bottles and filtered through sterile particulate filters. Following exposure, filters are placed on specialized growth media to support pathogen establishment.

***In situ* Water Sampling with Host Material Leaf Baits**

Bait Selection

- Collect leaves that are known to be free of disease from a population of native or naturalized *Rhododendron*, *Camellia*, *Syringa* (lilac), or *Viburnum* spp. that have susceptible responses to *P. ramorum*. Source host material must not have been sprayed with fungicide within the last three months. Avoid using newly acquired host 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.
- Use healthy leaves that have been on the plant for at least one 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 summer.

- If bait leaves are smaller in size than 8 cm x 3 cm at the widest point, use 8 leaves at each sampling location (one in each mesh bait bag). If leaves are larger than this dimension, four leaves per site can be used.
- Bait leaves may be wrapped in moistened paper towels and sealed in self-sealing plastic bags for refrigerated storage for no more than 14 days before use. Paper towels should be moistened with chlorinated tap or sterile water. **Do not use underground well water or water from a stream to moisten towels.**
- Place 4-8 leaves with the petioles (the stalk-like tissue that attaches the leaf to a stem) attached into each container depending on leaf size (discussed above) at each sampling site. Insert a uniquely numbered plastic tag into each bait bag. The dates (date 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 baiting was established and final temperature when bait was collected) and GPS coordinate should be recorded on a data sheet.

Baiting Techniques

Bait Bags

- Multiple-use bait bags (approximately 12 inches by 12 inches) should be constructed of a durable, coarse nylon mesh material (e.g., window screening) and fastened together on three sides to allow sufficient overlapping material to seal bag edges (Figure I-1). Single-use bait bags can be fashioned from muslin. Bait bags must have a separate pocket for each leaf to maximize 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 are placed into bag, it should be secured so leaves cannot float out and away.



Figure I-1. Example of a bait bag construction.

- Firmly secure each bag by tethering to a stake driven into the ground or by suspending from a rope that spans the width of a stream. Bags should float near or just below the water's surface for 7 days. Bait bags should be placed in an area of the stream where water flows more slowly and pools. Do not place the bait in the fast moving current of the stream. 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 seven days as the leaf tissue will degrade and baiting efficacy will be sharply reduced.
- If possible, place the bait near the site where water flows into the body of water from the nursery. When locating bait bags in waterways, priority should be given to sampling areas downstream from host material, and at the upstream and downstream edges of the nursery perimeter. When possible, shaded locations should be chosen.
- When placing bait bags in retention ponds, priority should be given to inflow and outflow points, preferably in shaded areas. A minimum of two bait bags per pond should be deployed.

Bait Stations

- An alternative to bait bags is a bait station, constructed of a PVC frame to which is attached ½ inch plastic fencing material (see below for photo and construction details). Leaves are attached with binder clips secured to the bottom of the enclosure.
- Attach the station to a stake driven into the ground or by suspending from a rope that spans the width of the stream or pond. The station should be deployed for 7 days. This period of time allows sufficient exposure time for *Phytophthora* spores to locate leaf tissues while limiting the effects of degradation and interference from the colonization of other water-mold organisms.
- If possible, place the bait near the water inflow point from the nursery. When locating bait stations in waterways, priority should be given to sampling areas downstream from host material, and at the upstream and downstream edges of the nursery perimeter. When possible, shaded locations should be chosen.
- When placing bait stations in retention ponds, priority should be given to inflow and outflow points, located in shaded areas. A minimum of two bait stations per pond should be deployed.

Bait Retrieval

- After 7 days, remove bait leaves and the 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.
- Wrap leaves in a moistened paper towel and place in a 1 gallon self-sealing plastic bag. Be certain to place the numbered 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.

- Place all sample bags in an insulated cooler with blue ice or another sealed cooling media for transport to the laboratory. Do not place bait samples directly on the ice; cardboard can be used to separate the ice from the bait samples.
- Record the date of bait retrieval as well as the water temperature at time of retrieval. This information should be entered into an appropriate database (see Appendix G of Nursery Survey Manual).
- Multi-use bait bags should be cleaned and sterilized with either 95% ethanol or a 10% household bleach solution for at least 4 hours prior to reuse. Rinse bags thoroughly with chlorinated tap or sterile water after sterilization. Check for signs of deterioration and bag failure, replacing bags accordingly.

Sample Transport, Storage, Shipping and Processing

- Samples should be kept in a cooler on a sealed coolant bag or in a refrigerator until shipped. ***Do not permit the samples to freeze or dry out at any time.*** Contact laboratory personnel before shipping to advise them that a sample will be arriving. Ship samples via overnight courier; do not store samples prior to shipping.
- Laboratory personnel should process bait samples using the same methods as for foliage samples, according to the diagnostic protocol found here http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/cultureprotocol6-07.pdf

Water Sampling for Filtration

Sample Collection

- The number of water samples collected is based upon the number of water bodies present; availability of run-off water; and the overall size of the nursery. If this sampling method is selected, a minimum of two 1-liter samples should be collected per site. More samples will be needed for larger nurseries with more water and irrigation drainage sources.
- Collect one liter of water from each sample point. Collect the cleanest sample possible by minimizing disturbance to the sediment, while avoiding plant and other floating debris. Use a 100-ml measuring cup or beaker to fill a 1-liter screw cap plastic bottle in increments rather than filling the container all at once. If possible, collect the 1-liter sample from several collection points at each sample site.
- Measure and record water temperature at each sample location. When possible, GPS coordinates should be recorded for each sample.
- Identification labels (time tape or masking tape) should be affixed both to the screw cap and the outside of each water collection bottle using a waterproof pen. Labels should be

sufficiently coded to correspond with datasheet entries for each nursery and water body and should include date collected, water source (location), and nursery (i.e., nursery license number).

- Before water is collected, rinse bottles downstream with the water about to be sampled. Maintain collected water samples in a cooler (without ice if external temperatures are cool or with enough ice to gradually cool water samples). Samples should be processed within 8 hours of collection to maximize detection of *Phytophthora* spores. Samples cannot be stored for more than 12 hours without compromising the quality of survey results.
- Log the water samples into the appropriate database. Assign a unique sample number to each bottle.
- Wash each 100-ml measuring cup and 1-liter bottles with warm, soapy water between sample collections. Thoroughly and completely rinse each item. For best results, use an automatic dishwasher with a heated drying cycle. Have several cup/bottle sets on hand to support water collection at a number of bodies of water each day. Use only clean, sanitized collection materials at each site and water source.

Sample Processing

- Most samples can be filtered through polycarbonate membrane filters with 3- μ m pores however, turbid or dirty water samples (from ponds or standing water) will need to be filtered through Durapore membrane filters with 5- μ m pores
- Place a filter funnel into a filter flask with a capacity of at least one liter and connect the flask to a vacuum source using plastic tubing; put 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).
- Wet filter holder with distilled water and place a polycarbonate membrane with the shiny side up or a polyvinylidene fluoride membrane with smoothest side up. Be sure the paper between the filters has been removed, the filter is aligned over the perforated area of the funnel, and that the filter is not wrinkled. Assemble the filter funnel and clamp it in place.
- Thoroughly mix the water sample by inverting the plastic bottle and/or swirling and 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; conversely, if the water is extremely clear, additional water (up to 200 ml) may be processed by a single filter.
- Turn on the vacuum source to filter water subsamples. The air should be turned off immediately after each subsample is completed (approx. 10 seconds) to avoid building up excessive vacuum pressure, which could damage *Phytophthora* spores. Rinse the inner surface of the funnel with distilled water to dislodge spores that may be adhered to funnel wall.

- Gently remove sizable organic debris or soil particles trapped on the surface of the filter if it prevents complete contact of the filter with the surface of PARPH-clarified V8 agar contained in Petri plates. Using forceps, gently lift the filter 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.
- Repeat steps 1 to 6 until the entire 1 liter water sample has been filtered. Unless water is very clear, this should result in a minimum of 10 agar plates per collection bottle and 20 agar plates per collection site.
- 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 residues may affect spore viability.
- Transfer the plates immediately to a state or federally-approved processing laboratory via overnight courier, or
- Maintain the agar plates with filters at 20°C in the dark for at least three days. Because *P. ramorum* grows slower than most other species of *Phytophthora* commonly found in water, leaving the filters on the agar plates for a three day incubation period is critical for recovery of this species.
- 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 bacteria that may interfere with microscopic observation. Filters and rinse water should be treated and properly discarded.
- Check the plates at regular intervals under low magnification—using an inverted or dissecting microscope—for colonies with typical morphological characters of *P. ramorum* (e.g., coralloid hyphae, semi-papillate sporangia, and large chlamydospores).
- Contact laboratory personnel if *P. ramorum* is suspected from any samples. See Appendix 3 for shipping details in the culture and morphology protocol at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/cultureprotocol6-07.pdf

Bait Station Construction Details

Frame

- Frame is made using ½-inch-diameter PVC pipe and four elbow joints.
- Cut four lengths of PVC—two 11 ½ inch and two 10 ½ inch lengths.
- Glue pipes and joints into a rectangular shape using PVC primer and cement.

Mesh

- The mesh used is a plastic, ½-inch fence material (with 3/8-inch openings) available at any national home improvement chain store
- Dimensions are given both in inches and also based on number of squares (Figure I-2)
- Mesh is secured to frame using plastic zip-ties.
- Leave one side of mesh half secured until leaves are inserted.
- Tie four small binder clips into mesh using plastic covered twist-ties; these clips hold leaves in place by petioles. This maintains separation between bait leaves, allowing for maximum water flow exposure for each bait tissue (Figure I-3).

Water Filtration Methods Materials List

- 2, 1-liter bottles per collection site (Nalgene preferred)
- 100-ml plastic measuring cup or beaker per collection site
- thermometer (water-resistant type preferred)
- ice chest cooler (with a small amount of ice or other refrigerant if temperatures are warm outside)
- electric vacuum pump or hand-operated vacuum pump
- Sterile PARPH-clarified V8 selective medium (see protocol recipe) in disposable Petri plates; 10 plates per collection bottle or 20 plates per collection site; media plates can be stored in sealed plastic sleeves or bags in a refrigerator for two months before use
- 47-mm-diameter polycarbonate membrane filters with 3-µm pores (e.g., Sterlitech SKU No. PCT3047100 at <http://www.sterlitech.com>)
- 47-mm-diameter polyvinylidene fluoride (Durapore) membrane filters with 5-µm pores (e.g., Fisher Scientific #SVLP04700)
- clamp-type filter funnel (Nalgene preferred)
- two 1- or 2-liter filtering flasks (plastic or glass)
- plastic tubing
- bent-tip forceps
- Squeeze bottle containing distilled water
- inverted or dissecting microscope

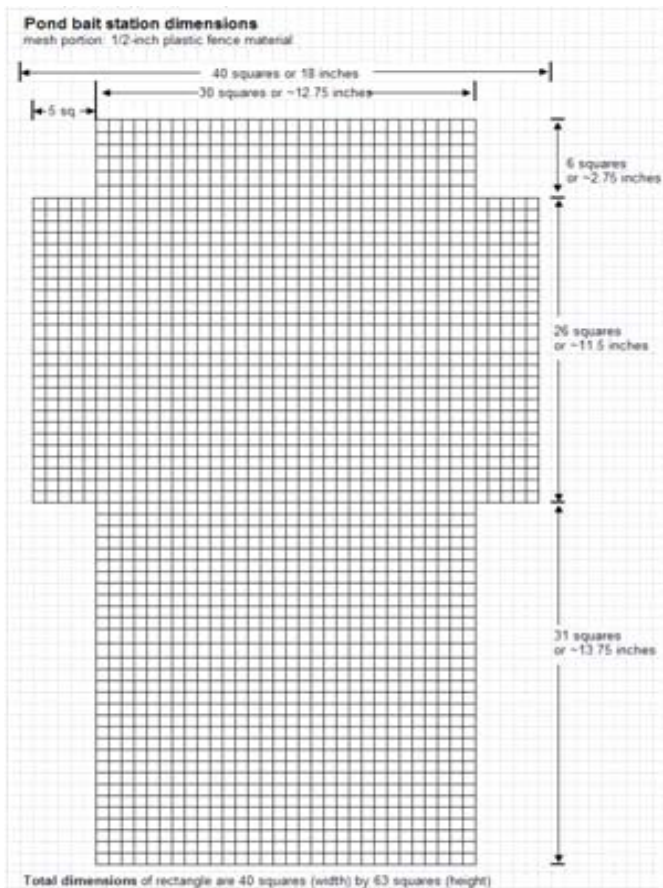


Figure I-2. Pattern of mesh screen to use for constructing a bait station.



Figure I-3. Completed bait station construction. *Photo courtesy of Dr. Steve Jeffers, Clemson University*

PAR(PH)-V8 Selective Medium: For *Phytophthora* species. Adapted from *Jeffers & Martin, 1986; Ferguson & Jeffers, 1999.*

Ingredient	Amount Per:		Concentration (PPM)
	1.0 Liter	0.5 Liter	
<u>Basal Medium</u>			
Clarified V8 Concentrate	50 ml	25 ml	
Distilled Water	950 ml	475 ml	
Difco Bacto Agar	15 g	7.5g	
<u>Amendments</u>			
Delvocid (50% pimaricin)	10 mg = 0.01 g	5 mg	5
Sodium Ampicillin	250 mg = 0.25 g	125 mg	~250
Rifamycin-SV (sodium salt)	10 mg = 0.01 g	5 mg	~10
Terraclor [75% PCNB]*	66.7 mg = 0.0667	33.4 mg	50
Hymexazol*	50 mg = 0.05	25 mg	50

Clarified V8 concentrate is made from V8 Juice, buffered with the addition of CaCO₃ (1.0 g CaCO₃/100 ml V8 Juice) and clarified in one of three ways:

- centrifugation @4000 RPM for 20 min followed by filtration using 2 layers of Whatman No. 1 under vacuum, or;
- centrifugation @ 7000 rpm for 10 min then filtration is not necessary, or;
- vacuum filtration alone with Celite.

Following clarification, 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, & PARH). PCNB is useful to inhibit soilborne fungi on soil dilution plates and hymexazol inhibits **most** *Pythium* spp. while allowing **most** *Phytophthora* spp. to grow

Directions

12. Add ingredients for **basal medium only** to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
13. Autoclave for 20 min at 121 C and 15 psi; turn waterbath on to ~50°C
14. Add each amendment to separate sterile water blanks [5 ml distilled water in a 16-mm test tube]; vortex each to mix or suspend
15. Cool medium in waterbath
16. Slowly stir medium with a magnetic stirrer in laminar flow hood
17. Vortex each amendment thoroughly and add to mixing basal medium
18. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium
19. 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
20. Cool plates at room temperature
21. Store plates inverted in plastic bags in the dark in a refrigerator
22. Best if plates are used within several weeks—but they will keep for months

References

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APPENDIX 8

Treatment and Disinfection

Revised April 2007

The following techniques are approved by USDA APHIS PPQ for control of *P. ramorum* in nurseries found to contain plants infected with *P. ramorum*.

Infected Plants:

Note: HAP material, including leaf litter, must not be placed in compost piles or be removed from the nursery site as trash or in debris removal. HAP material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.

- **Incineration (burning to ash):** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.
- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.
- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for “insect pests and pathogens in garbage”, Schedule T415b.
http://www.aphis.usda.gov/ppq/manuals/port/Treatment_Chapters.htm

Non-Porous 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, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the quarantine block. The following table modified from <http://cpmcnet.columbia.edu/dept/ehs/decon.html> examines the effects of different classes of

disinfectants on microbial populations. This list is for explanation and information only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

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

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 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, recirculated, non-municipal 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.

Soil and Potting Media:

- **Potting media:** Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- **Soil:** Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. 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 *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is not a consideration as a viable option for soil treatment.

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

Summary of Labeled Soil Fumigants

Fumigant	Trade names	Comments
Chloropicrin	Chlor-O-Pic Metapicrin Timberfume Tri-Clor	Often used in combination with methyl bromide due to its ability to be detected in small quantities.
Dazomet	Basamid	Methyl isothiocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated. Requires careful soil preparation and incorporation into soil. All application must be made in accordance with labeling.
Metam-sodium	Busan 1020 Busan 1180 Busan 1236 Metam Vapam	Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling.

Fumigant	Trade names	Comments
Methyl Bromide	Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom	Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential.

Physical Treatment of Soil:

- Mitigation of infested soil can also be achieved by installing permanent impermeable, non-porous barriers that consist of cement, concrete or asphalt. These barriers must be constructed so that no native soil within the destruction block is visible. The barriers should be graded such that no standing water can be observed.

Equipment and Personnel (Inspectors and employees):

- Access to infested areas and hold areas should be limited, as much as possible, to officials and necessary employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil 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 completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- Personnel should not have access to other production areas of the nursery after entering the destruction block on the same day.
- A disinfectant foot bath should be placed near the exit to the destruction blocks and quarantine blocks and used by all personnel entering and exiting the quarantine block and entering and exiting the destruction block at the infested nursery site, where the contact with potentially infested soil or plant debris by footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with soil or organic debris, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine block or destruction block. The disposable shoe covers must be placed in bags and incinerated, deep-buried or properly disposed in a sanitary landfill.
- The tires (or other parts in contact with the soil or plants, such as the bed of trucks) 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, vehicles should not be allowed in the destruction blocks at all. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.

- Do not visit other nursery sites in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the nursery, the nursery should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris by the visitor.
- Wood surfaces suspected of contamination with *P. ramorum* should be disposed of as stated above under “Infected Plants.”

APPENDIX 9

Biosecurity Measures for Nurseries

April 2007

In the course of daily work, nursery personnel are frequently required to visit a number of different nurseries sites, greenhouses, fields, and facilities. These actions could potentially provide a pathway for transferring quarantine organisms from one work site to another during the work day. It should also be recognized that even if a single work site is visited per day, precautions must be taken to avoid contaminated clothing and equipment from being used at a new site the following day. Further, visitors to these same facilities present the same risks and additionally 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 should be trained and visitors informed of these biosecurity requirements that have been put in place by the facility. As new scientific data and technology is learned, the facility needs to update their biosecurity requirements and retrain their 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 with one of the nursery's vehicles. Loading of nursery stock onto other than the nursery's vehicles should be done in an area with concrete or asphalt pad located near the gate and not in the interior of the nursery.

Cleaning:

Interior of nursery vehicles should be cleaned to ensure no build-up of soil, debris or other items.

Where it is not possible to avoid the vehicle going onto the fields, the vehicle must be driven to the edge of the facility where the tires, wheel wells and accessible areas of the undercarriage of the vehicle must be cleaned of soil and organic matter with a brush or a water hose followed by a spray down with a suitable disinfectant. In situations where the undercarriage has been coated with soil it is recommended that after cleaning and disinfecting at the work site an effort be made

to go through a car wash that has the ability to clean the undercarriage before proceeding to another work site. If a car wash is not available, avoid driving onto the next work site. To ensure the entire surface of the tires are cleaned it will also be necessary to move the vehicle forward a foot or so to permit cleaning of the portion of the tire in contact with the ground.

The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.

A portion of the vehicle must be designated as a “clean area” where 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 that require cleaning. In situations where pool vehicles are used, the work site should adopt a set procedure for all personnel.

Nursery Personnel

Nursery personnel routinely come in contact with potentially contaminated soil, plants and organic matter and this requires 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 be aware of this situation. Work should always be completed working 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. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.

- Personnel should not have access to production areas of the nursery after entering the destruction block on the same day.
- A disinfectant foot bath should be placed and used 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. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine area or destruction block. The disposable shoe covers must be placed in bags and incinerated or deep-buried.
- Do not visit other nursery sites in potentially contaminated work clothing and footwear.

Boots:

Rubber boots which can be disinfected should be worn and if they are not available disposable boot covers must be worn over work boots in any infested or possibly infested area. The rubber boots must be disinfected on arrival, even if this has been done at the time of departure from the last work site. At the conclusion of the inspection, the boots must be cleaned of soil and disinfected prior to placing into the vehicle area designated as a “clean area”. Dispose of used boot covers by double bagging and place into the designated “dirty area” of the vehicle for proper disposal. After removing boot covers, the soles of the work boots must be inspected for soil and if present, must be cleaned of soil and treated with disinfectant.

Hands:

Thoroughly wash hands with soap and water before entering and after leaving the work site. Follow these four simple steps to keeping hands clean.

- Wet hands with warm running water.
- Add soap, and then rub hands together, making a soapy lather. Do this away from the running water for at least 20 seconds, being careful not to wash the lather away. Wash the front and back of hands, as well as between fingers and under nails.
- Rinse hands under warm running water. Let the water run back into the sink, not down the elbows. Turn off the water with a paper towel and dispose in a proper receptacle.
- Dry hands thoroughly with a clean towel

If a hand washing station is not available, antiseptic rubs/gels/rinses (containing a minimum of 70% ethyl alcohol and left on for 10 - 15 minutes) must be used. Follow these basic steps for using antiseptic rubs/gels/rinses.

- Remove soil from hands.
- If hands are wet, dry as much as possible.
- Apply enough disinfectant (amount about the size of a quarter) onto hands to cover all areas, including under the nails. Use a rubbing motion to evenly distribute the disinfectant product for about 15 seconds.

If antiseptic rubs/gels/rinses are used, avoid formulations with moisturizers as they leave a gummy residue. Disposable gloves may be used, however they have the tendency to rip and become uncomfortably wet after a short period. Rubber gloves which withstand more abuse than disposable gloves have the same drawbacks as disposable gloves, however will be more practical when handling materials that are sharp or jagged. If rubber gloves are used in cold weather it is recommended to purchase rubber gloves with cotton or acrylic liners. Both disposable and/or rubber gloves must be double bagged after use if working in an infested area and placed into the

“dirty area” of the vehicle for disposal or cleaning. If on-site disposal of the gloves are available this option should be chosen. After disposal of gloves, hands must be washed or sanitized. To avoid cross contamination, disinfection of hands must take place 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. Where equipment must leave the work site for disinfection it must be double bagged and place in the designated “dirty area” of the vehicle.

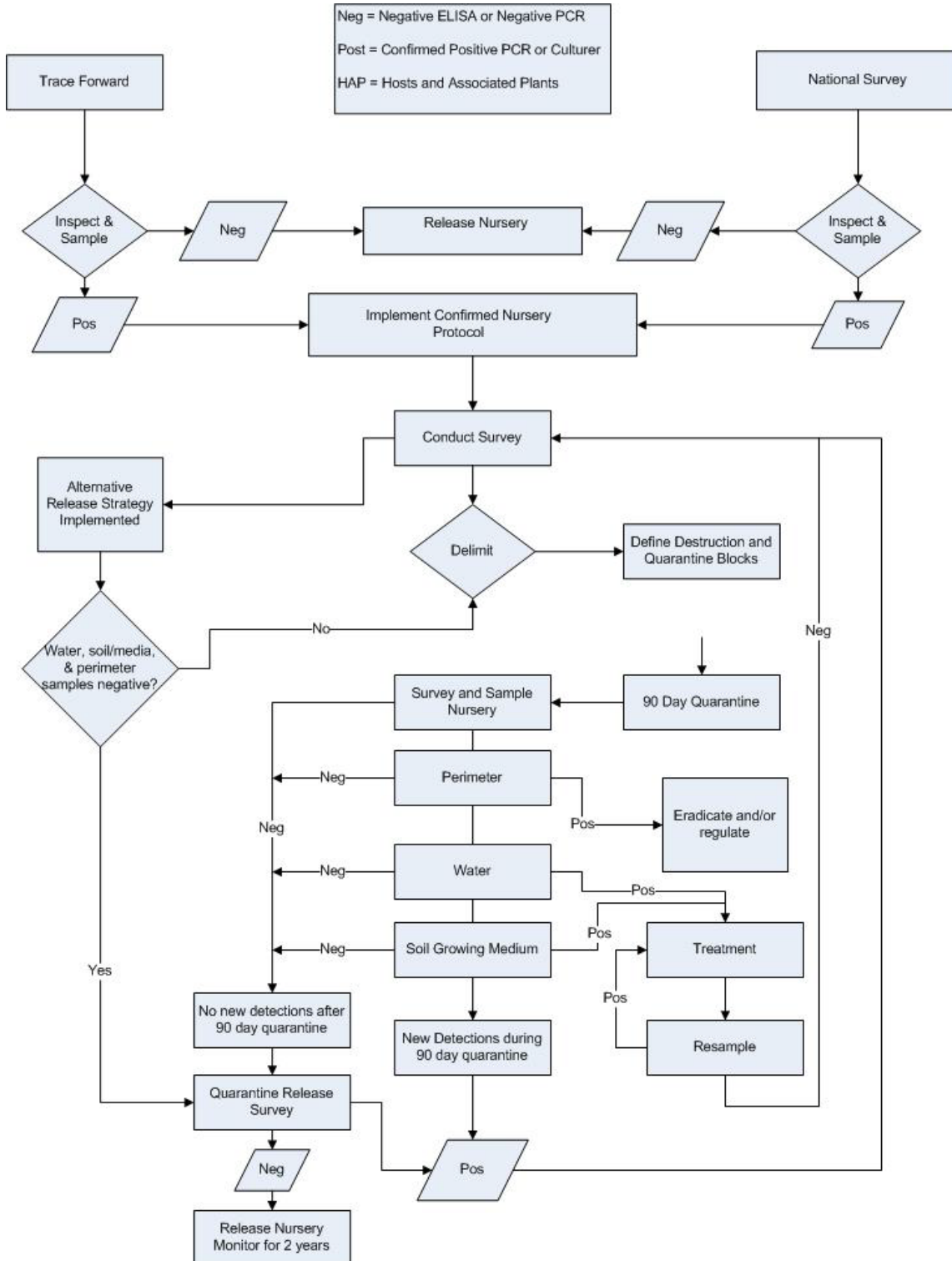
Visitors:

- 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. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- A disinfectant foot bath should be placed and used by all entering and exiting the nursery site. These should be placed at all entrances and exits. 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.

APPENDIX 10

Confirmed Nursery Protocol Flowchart for First Time Positive Nurseries

Revised: April 2007



APPENDIX 11

Mitigations for Nurseries Found with *P. ramorum* More Than Once

May 2007

(Modified October 28, 2008, Updated May 14, 2010)

These mitigations apply for nurseries detected as positive for *P. ramorum* within one year of release from an EAN (Emergency Action Notification) or state equivalent. *P. ramorum* infestations in nurseries may be re-introduced or the effort to eradicate the disease may fail. In the event that a production or wholesale nursery has *P. ramorum* detected on site after the initial release from the EAN or state equivalent, it is necessary to implement additional measures to ensure that the risks associated with *P. ramorum* are properly mitigated. These seven additional measures are to be implemented:

1. Additional monitoring of the nursery through water-baiting and regulatory inspections.

a) Water Baiting

- If there is significant water run-off and the water has not yet tested positive, conduct seasonal baiting of that water.
- If any water sample, either draining **from** or within the nursery premises is determined to be positive, **then the option defaults to option b**, and a nursery inspection is required of all plants within the nursery that are listed on **the** APHIS list of Regulated Proven Hosts and Plants Associated with *P. ramorum*.

b) Regulatory Inspections of host and associated plants:

- Conduct two additional inspections, during the two out of the three best remaining seasons that are conducive to the development of symptoms for *P. ramorum*.
- Inspect all plants within the nursery that are listed on **the** APHIS list of Regulated Proven Hosts and Plants Associated with *P. ramorum*.
- Any plants observed with the symptoms will be sampled sufficiently to represent the plants with the symptoms being expressed and those samples are to be analyzed for *P. ramorum*.

2. Appropriate biosecurity measures are to be incorporated into the EAN or Compliance Agreement and remain in place until two years of negative survey are completed.

See Appendix 9 for biosecurity measures. These contain practices which, if properly applied, can be expected to effectively mitigate risks associated with *P. ramorum* in a nursery. In areas of the country not regulated these need to remain in place for two years via the EAN. In regulated and quarantine areas these practices are to be included as part of a Compliance Agreement. In all cases, appropriate and specific timelines for implementation will be established. Additionally, these will be periodically verified, perhaps best done at the seasonal re-inspections.

3. 45 days after implementation of the CNP, a series of soil samples will be taken in the destruction and quarantine blocks as well as any water drainage areas will be baited or sampled and analyzed for the presence of *P. ramorum*.

The presence of *P. ramorum* in soil or water may contribute to the occurrence of disease in the nursery and puts the local area at risk. Thus it is necessary to conduct these sampling and testing procedures and if found, eradication is to take place. See Appendices 6 and 7 for how to conduct sampling and Appendix 8 for details on treatment and eradication procedures.

4. Fallen leaves and plant debris will be removed from pots, soil and within the immediate area of *Rhododendron* and *Camellia* on a quarterly basis to the best ability of the nursery to prevent possibly infested dropped leaves from infesting the soil or other plants. Verify this at the seasonal inspections.

Camellia and other hosts are known to shed infected and infested leaves. This may result in further infection and soil infestation with a potential for resultant spread of infection. To address this potential, it is important for these leaves and related debris be removed and destroyed or buried. The use of a blower to move these leaves away to a different location is not an appropriate mitigation.

5. Nurseries that ship interstate must undergo approved training in the risks, recognition and mitigation of *P. ramorum*. The nursery shall develop and maintain a database/list showing names of staff and date of training and make it available to regulatory officials upon request.

Appropriate nursery personnel must complete training approved by APHIS (contact the Regional Program Manager for currently approved training) and provide appropriate guidance to other nursery personnel as demonstrated by the training.

6. Nurseries are to inspect all *Rhododendron* and *Camellia* brought into the nursery. *P. ramorum* has been re-introduced to nurseries through buy-ins and customer returns. Therefore, neither of these two genera, nor any other taxa of plants found positive in the nursery, is to be returned to stock upon a customer's return or when purchased as seconds. If the nursery has a policy to accept nursery stock returns, then destroy those using appropriate methods. If seconds of these two taxa are purchased, these plants must be safeguarded, segregated, and withheld from interstate movement until the plants are officially inspected, sampled, tested and found free of evidence of *Phytophthora ramorum*.

P. ramorum is occurring in these two genera at greater levels, as compared to other genera. It is essential that *Rhododendron* and *Camellia* be carefully examined for any signs of this disease and samples provided for analysis should any be detected. If customer returns, do not return members of these genera to stock but rather destroy them appropriately. Other taxa found positive in a nursery present the same risk. Seconds of these two genera present a similar risk.

7. A one year pre-shipment notification to the office of the SPRO of all shipments containing any plants of the genera, *Rhododendron*, *Camellia*, *Viburnum*, *Pieris*, and *Kalmia*.

Upon being confirmed positive for *P. ramorum*, the nursery is required to notify the SPRO of any interstate shipment made containing these five hosts. This notification is expected to be a fax (or agreed upon equivalent) containing all the information needed to identify the shipper, receiver, contents of the shipment, expected arrival date and appropriate contact information. It is to be sent to the office of the SPRO and identified as “Pre-shipment notification of *P. ramorum* hosts as required by USDA-APHIS”. SPRO contact information can be found at: www.nationalplantboard.org/member/index.html