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: July 20, 2007
(Appendices 1, 3, 6 & 7 updated June 26, 2008;
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 *P. ramorum* 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 *Phytophthora ramorum* 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 *P. ramorum*. 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 *Phytophthora ramorum*.

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 *Phytophthora ramorum*".

Infected plants:	Plants officially confirmed as being infected with *P. ramorum*, 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 *P. ramorum* 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 Nursery Delimitation Survey is completed 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 *P. ramorum.* Conducive conditions exist when climatic conditions match optimum disease etiology and are likely to express disease symptoms 50% or more of the time.
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 *P. ramorum* 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 *P. ramorum* is present.

Quarantine block: Area identified as a 10 meter radius around the destruction block (see Appendix 2) designed to determine if *P. ramorum* 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 *P. ramorum.* 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 *P. ramorum* 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 *P. ramorum* 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 HAP shipments within the 12 months prior to the first positive detection of *P. ramorum* at the nursery as per the protocol. [NOTE: For shipments to Canada provide a list of all HAP **genera** shipped within the 12 months prior to the first positive detection of *P. ramorum* at the nursery.] This information on shipments needs to be gathered, processed, and forwarded to Regional Office within 10 working days. 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 need to 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.

- 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)

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 100-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.

    - 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.

    <u>If found positive:</u>

    - 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*

(Revision dated 5 May 2008 (corrected 30 May))
This list is updated often.
The most current version is posted at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Proven Hosts Regulated for *Phytophthora ramorum*

Scientific Name (45)	Common Name(s)	Notes
Acer macrophyllum	Bigleaf maple	
*Acer pseudoplatanus**	Planetree maple	
Adiantum aleuticum	Western maidenhair fern	
Adiantum jordanii	California maidenhair fern	
Aesculus californica	California buckeye	
*Aesculus hippocastanum**	Horse chestnut	
Arbutus menziesii	Madrone	
Arctostaphylos manzanita	Manzanita	
Calluna vulgaris	Scotch heather	
Camellia spp.	Camellia - all species, hybrids and cultivars	
Castanea sativa	Sweet chestnut	
*Fagus sylvatica**	European beech	
Frangula californica (≡Rhamnus californica)	California coffeeberry	
Frangula purshiana (≡*Rhamnus purshiana*)	Cascara	
Fraxinus excelsior	European ash	
Griselinia littoralis	Griselinia	
Hamamelis virginiana	Witch hazel	
Heteromeles arbutifolia	Toyon	
Kalmia spp.	Mountain laurel - all species, hybrids and cultivars	
*Lithocarpus densiflorus**	Tanoak	
Lonicera hispidula	California honeysuckle	
Laurus nobilis	Bay laurel	
Magnolia doltsopa = Michelia doltsopa	Michelia	
Maianthemum racemosum (≡ *Smilacina racemosa*)	False Solomon's seal	
Parrotia persica	Persian ironwood	

Photinia fraseri	Red tip photinia	
Pieris spp.	Andromeda, Pieris - all species, hybrids and cultivars	
Pseudotsuga menziesii var. *menziesii*	Douglas fir	Also includes all other varieties and cultivars of nursery grown *P. menziesii*
*Quercus agrifolia**	Coast live oak	
*Quercus cerris**	European turkey oak	
*Quercus chrysolepis**	Canyon live oak	
*Quercus falcata**	Southern red oak	
Quercus ilex	Holm oak	
*Quercus kelloggii**	California black oak	
Quercus parvula var. *shrevei**	Shreve's oak	Also includes all other varieties and cultivars of nursery grown *Q. parvula*
Rhododendron spp.	Rhododendron (including azalea) – all species, hybrids and cultivars	
Rosa gymnocarpa	Wood rose	
Salix caprea	Goat willow	
Sequoia sempervirens	Coast redwood	
Syringa vulgaris	Lilac	
Taxus baccata	European yew	
Trientalis latifolia	Western starflower	
Umbellularia californica	California bay laurel, pepperwood, Oregon myrtle	
Vaccinium ovatum	Evergreen huckleberry	
Viburnum spp.	Viburnum – all species, hybrids and cultivars	

Plants Associated with *Phytophthora ramorum*

(These are regulated only as nursery stock)

Scientific Name (72)	**Common Name, Date & Source of Report**	**Notes**
Abies concolor	White fir – Oct 05 (1)	
Abies grandis	Grand fir – June 03 (1)	
Abies magnifica	Red fir – Jan 06 (7)	
Acer circinatum	Vine maple – Feb 06 (5)	
Acer davidii	Striped bark maple – Jan 06 (9)	
Acer laevigatum	Evergreen Maple – Aug 05 (3)	
Arbutus unedo	Strawberry tree – Dec 02 (7)	
Arctostaphylos columbiana	Manzanita – Feb 06 (5)	
Arctostaphylos uva-ursi	Kinnikinnick, bearberry – Jan 07 (10)	
Ardisia japonica	Ardisia – Jan 06 (9)	
Berberis diversifolia =Mahonia aquifolium	Oregon grape – Aug 07 (9)	
Calycanthus occidentalis	Spicebush – May 05 (5)	
Castanopsis orthacantha	Castanopsis - Aug 06 (3)	
Ceanothus thyrsiflorus	Blueblossom – April 06 (5)	
#Cercis chinensis	Chinese redbud – April 08 (9)	New report from Canada
Cinnamomum camphora	Camphor tree – May 06 (3)	
Clintonia andrewsiana	Andrew's clintonia bead lily – May 04 (5)	
Cornus kousa x Cornus capitata	Cornus Norman Haddon – Aug 06 (3)	
Corylopsis spicata	Spike witch hazel – Nov 07 (9)	
Corylus cornuta	California hazelnut – Dec 02 (5)	
Distylium myricoides	Myrtle-leafed Distylium – Jul 06 (9)	
Drimys winteri	Winter's bark – July 04 (3)	
Dryopteris arguta	California wood fern – May 04 (5)	
Eucalyptus haemastoma	Scribbly gum – Aug 06 (3)	
Euonymus kiautschovicus	Spreading euonymus – Jan 06 (9)	

Fraxinus latifolia	Oregon ash – Aug 05 (5)	
Garrya elliptica	Silk tassel tree , coast silktassel – Aug 07 (3)	
Gaultheria shallon	Salal, Oregon wintergreen – Jan 06 (9)	
Hamamelis x intermedia (H. mollis & H. japonica)	Hybrid witchhazel – Jan 06 (9)	
Hamamelis mollis	Chinese witchhazel – Jan 05 (3)	
Ilex purpurea	Oriental holly – Jul 06 (9)	
Leucothoe axillaris	Fetterbush, dog hobble – Jan 06 (9)	
Leucothoe fontanesiana	Drooping leucothoe - Oct 03 (3)	
Loropetalum chinense	Loropetalum – Jul 06 (9)	
Magnolia denudata x salicifolia	Magnolia – Feb 08 (3)	
Magnolia ernestii = Michelia wilsonii	Michelia – Jan 06 (9)	
Magnolia figo	Banana shrub – April 08 (1)	New report from California. Trade name is *Michelia figo*
Magnolia grandiflora	Southern magnolia – Jan 06 (9)	
Magnolia kobus	Kobus magnolia – Feb 08 (9)	
Magnolia liliiflora =Magnolia quinquepeta	Purple magnolia – Feb 08 (3)	
Magnolia x loebneri	Loebner magnolia – Jan 05 (3)	
Magnolia maudiae =Michelia maudiae	Michelia – Jan 06 (9)	
Magnolia salicifolia =Magnolia proctoriana	Anise magnolia – Feb 08 (3)	
Magnolia x soulangeana	Saucer magnolia – Jan 05 (3)	
Magnolia stellata	Star magnolia – Jan 05 (3)	
Magnolia x thompsoniana (M. tripetala and M. virginiana)	Magnolia – Feb 08 (3)	
Manglietia insignis	Red lotus tree – Aug 06 (9)	
Nerium oleander	Oleander – June 06 (1)	
Nothofagus obliqua	Roble beech – Dec 04 (3)	

Osmanthus decorus (≡Phillyrea decora; ≡P. vilmoriniana)	Osmanthus – Jan 06 (9)	
Osmanthus delavayi	Delavay Osmanthus, Delavay tea olive – Jan 07 (10)	
Osmanthus fragrans	Sweet olive – June 06 (1)	
Osmanthus heterophyllus	Holly olive – June 06 (1)	
Osmorhiza berteroi	Sweet Cicely – Aug 05 (5)	
Parakmeria lotungensis	Eastern joy lotus tree – Jul 06 (9)	
Physocarpus opulifolius	Ninebark – Oct 07 (9)	
Pittosporum undulatum	Victorian box – Dec 02 (6)	
Prunus lusitanica	Portuguese laurel cherry – Jan 06 (9)	
Prunus laurocerasus	English laurel, cherry laurel – Jan 07 (10)	
Pyracantha koidzumii	Formosa firethorn – Apr 04 (9)	
Quercus acuta	Japanese evergreen oak – May 06 (3)	
Quercus petraea	Sessile oak – Aug 05 (3)	
Quercus rubra	Northern red oak – Nov 03 (8)	
Rosa (specific cultivars) Royal Bonica (tagged: "MEImodac") Pink Meidiland (tagged: "MEIpoque") Pink Sevillana (tagged: "MEIgeroka")	Hybrid roses – Jan 06 (9)	
Rosa rugosa	Rugosa rose – Jan 06 (9)	
Rubus spectabilis	Salmonberry – Dec 02 (4)	
Schima wallichii	Chinese guger tree, needlewood – Nov 06 (3)	
Taxus brevifolia	Pacific yew – May 03 (5)	
Taxus x media	Yew – June 05 (8)	
Torreya californica	California nutmeg – Aug 05 (5)	
Toxicodendron diversilobum	Poison oak – Dec 02 (4)	
Vancouveria planipetala	Redwood ivy – Aug05 (5)	

30 May 2008 Cercis chinense - redbud changed to correctly read Cercis chinensis – Chinese redbud

(From parentheses numbers above) – Sources of reports of detections and identifications

[1] California Department of Food and Agriculture, Sacramento, CA
[2] Oregon Department of Agriculture. Salem, OR
[3] Department for Environment, Food and Rural Affairs, UK
[4] Everett Hanson, Oregon State University, Corvallis, OR
[5] David Rizzo, University of California, Davis, CA
[6] Matteo Garbelotto, University of California, Berkeley, CA
[7] Gary Chastagner, Washington State University, Puyallup, WA
[8] Plant Protection Service, Wageningen, Netherlands
[9] Canadian Food Inspection Agency, Ottawa, Ontario, Canada
[10] Washington State Department of Agriculture, Olympia, WA

***** Unmanufactured wood and wood products, including firewood, logs, and lumber of species listed above and marked with an asterisk (*) are regulated. See 7 CFR 301.92

Rationale for Lists:

Host Plants Regulated for *Phytophthora ramorum:*
Naturally infected associated plants are deemed host plants regulated for *P. ramorum* upon completion, documentation, review, and acceptance of traditional Koch's postulates. Details on regulated plants and articles can be found via links to "Phytophthora ramorum 7 CFR 301.92" and "Recent Modifications to Phytophthora ramorum Regulations" at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml

The plants listed in the original Interim Rule dated 14 February 2002 were adapted from a review and evaluation of lists of regulated plants from other regulatory agencies.

Plants Associated with *Phytophthora ramorum*:
Plants associated with *P. ramorum* are naturally infected plants from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). Traditional Koch's postulates have not yet been completed nor documented and reviewed for each of these associated plants. These reports must be documented and reviewed by PPQ before they will be listed.

Regulation at the genus level:
Plants included in either of the above lists may be regulated at the genus level. This will ensure appropriate and effective inspection in quarantine areas, regulated nurseries, and regulated articles to mitigate the spread of *P. ramorum*. Examples of this include when the number of individual species, hybrids, or cultivars listed or to be listed are determined to hinder appropriate and effective inspection or regulation; or when sufficient numbers of member species of a genus are known susceptible to the disease causing organism, all members of that genus have a demonstrable risk of spreading that disease. Thus, to prevent the spread of disease, all members of that genus will be treated the same in our regulation.

Nomenclature:
We intend to have this list consistent with the listing in the Agricultural Research Service (ARS), Germplasm Resources Information Network (GRIN) database.
http://www.ars-grin.gov/npgs/aboutgrin.html

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Rationale for Lists:

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Regulation at the genus level:
Plants included in either of the above lists may be regulated at the genus level. This will ensure appropriate and effective inspection in quarantine areas, regulated nurseries, and regulated articles to mitigate the spread of *P. ramorum*. Examples are when the number of individual species, hybrids, or cultivars listed or to be listed is determined to hinder appropriate and effective inspection or regulation; or when sufficient numbers of member species of a genus are known susceptible to the disease causing organism, all members of that genus have a demonstrable risk of spreading that disease. Thus, to prevent the spread of disease, all members of that genus will be treated the same in our regulation.

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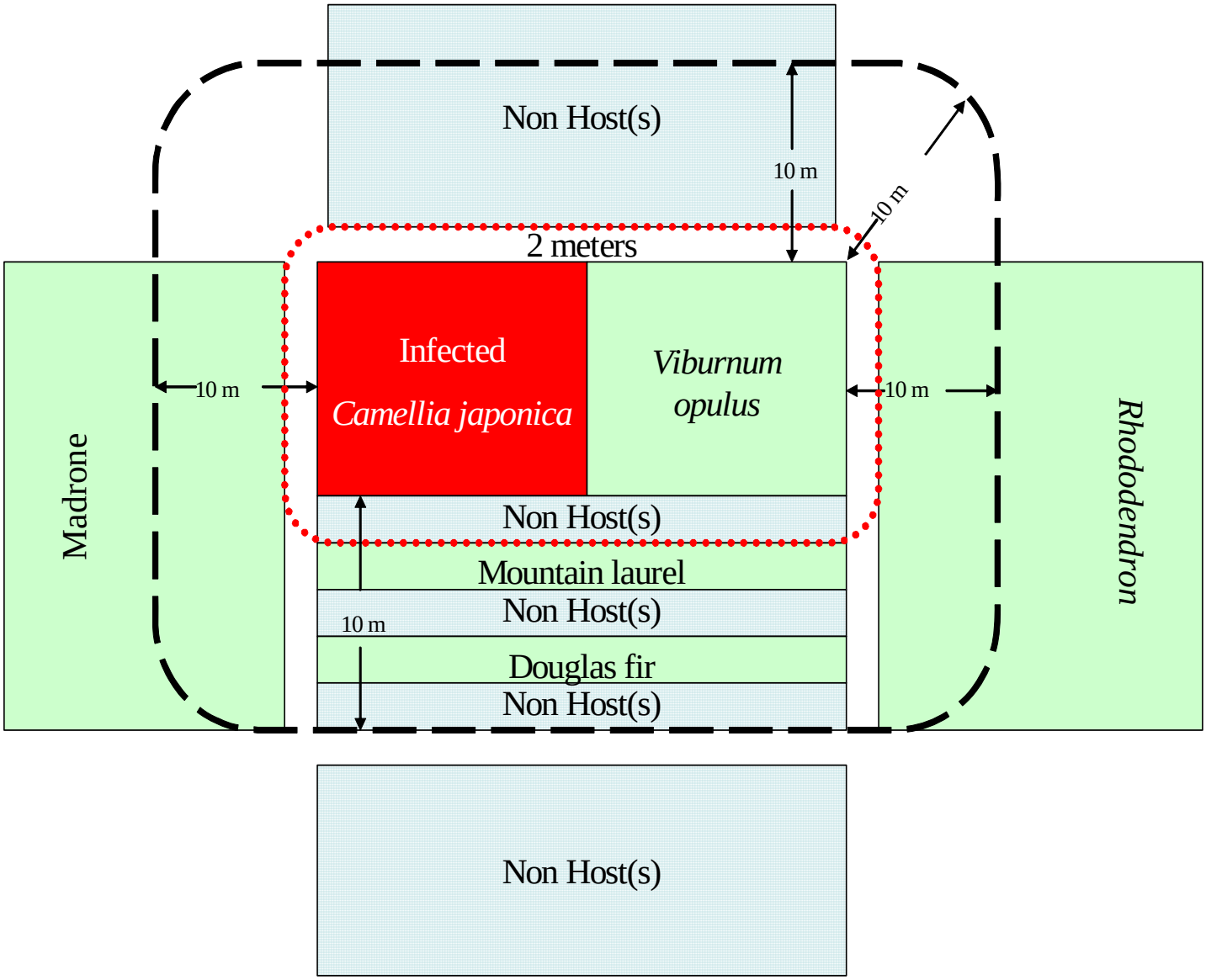
APPENDIX 2

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 $3^{o} – 6^{o}$ 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 Growing Medium Sampling Protocol

Revised April 22, 2008

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

Soil and Growing Media Sampling:

- Infested soil or growing media will look exactly the same as un-infested soil or growing media. Therefore all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10% bleach solution, quaternary ammonium solution or flame-sterilized with a propane torch between blocks. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or growing media from one block to the next on shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new nursery block. Care must be taken to ensure that un-infested soil or growing media is not contaminated by infested soil or growing media. If the areas of soil/media infestation are known or suspected sample these quarantine block and work toward the destruction block(s).

Preparing for sampling:

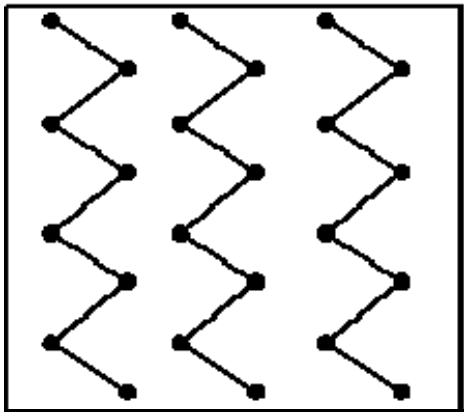
- Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or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

Table 1: Number of composite samples collected based on nursery block size.

Size of Treated Site (acres)	Sq Ft	No. of Soil and Growing Media Samples Collected (total)
0.00< n < 0.25	n <10,890	5 (10)
0.25 < n < 0.5	10,890 <n < 21,780	10 (20)
0.50 < n < 1.0	21,780 <n < 43,560	20 (40)
n >1.0	n > 43,560	30 (60)

- Each composite sample will consist of at least five sub-samples collected from soil or growing media within the targeted area. While five is a minimum, it is preferable to take 24 sub-samples of soil or growing media for each sample, provided the area is large enough (for soil samples) and enough plants are present (for growing media samples). Sub-samples should be collected according the pattern in the diagram below (Figure 1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be maintained (preferably by GPS but at least by flagging) in case follow-up treatment of the soil or growing media for *P. ramorum* is required. Composite samples may also be collected from neighboring blocks of un-infested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/growing media samples from these blocks first to minimize the risk of contaminating un-infested soil/growing media. If all potentially-infested growing media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the growing media of those plants that are down slope (e.g., based on watering patterns) of the originally infected plants.

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



Soil Baiting

It is possible to follow the below procedure and not successfully bait and culture *P. ramorum.* This may be due to *P. ramorum* not being present, but may be due to dormancy of *P. ramorum*. To address this dormancy potential and to better enable the diagnostician to detect *P. ramorum* when present, mix the soil well and split the soil samples when they arrive in the laboratory. Once the samples are well mixed and split, place one of the split sample halves into cold storage at approximately 4 degrees C for one month. Bring samples out from cold room after one month has passed, leave samples at room temperature for two days and repeat soil baiting process This baiting can be done in conjunction with the final baiting required fore the quarantine release survey. The samples should be processed as shown below.

To prepare soil bait, briefly soak the pears (select unripe green pears) or Rhododendron leaves in a mild detergent solution to remove any pesticide residues. Rinse the baits well and drain.

Leaving the soil in the Ziploc bag, add enough sterile deionized water to saturate and cover soil with about 2.5 cm (1") of water. Do not mix the soil and water.

Use two pears or leaves per soil sample. With a black sharpie pen, label one side of the pears or leaves with the soil sample number and date processed. The USDA Forest Service recommends the following bait selection criteria in *Stream Baiting Protocol: 2007 National Phytophthora ramorum Early Detection Survey of Forests*, issued March 20, 2007. See http://fhm.fs.fed.us/sp/sod/sod.shtm for latest approved protocol.

Bait Selection

- Use leaves from a population of native or naturalized rhododendrons, if possible. The population should be sufficiently large to supply needed leaves for the survey duration.

- Variation in Pr susceptibility among rhododendron species/cultivars in laboratory inoculation has been published, but field and lab studies have shown that leaves of common native and naturalized species perform acceptably as Pr bait.

- Leaf size can vary considerably among species and cultivars. If bait leaves are quite small (8 cm x 3 cm at the widest point or smaller), use 2 leaves in each pocket of the bait bag.

- If the source of leaves is nursery-grown or naturalized landscape plants, ensure that they have been free of fungicides and other pesticides for a minimum of 6 weeks before using as bait.

- Source plants should be mostly free of dieback and leaf symptoms. Use 1 year-old leaves as free as possible from leaf symptoms (spots, blight, chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full leaf expansion and a period of hardening in summer.
- Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for stored leaves.

Carefully push each pear or leaf into the wet soil and water until the bait is immersed halfway. Leave the labeled side of the bait out of the water. Seal the Ziploc bag and leave bait in the soil/water mixture for at least 48- hr at room temperature.

After 48-hr, remove the baits and wash off any clinging soil into Ziploc bag. Set the bait on a moistened paper towel in a sealed container at room temperature for 7-d to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal.

Examine the bait daily for developing symptoms. Pears infected with *P. ramorum* will display lesions that are round, brown, somewhat leathery in texture, with undefined edges. Colorless, watery, and/or soft lesions are generally caused by other pathogens (especially *Pythium* spp.).

Rhododendron leaves that have become infected with *P. ramorum* will exhibit 'diffuse' leaf spots usually with the midvein most affected.

Under the laminar flow hood, cut eight to 10 pieces of pear or leaf from the edge of the developing lesion or leaf spot and insert into the PARP medium. Write the sample number and date processed on the underside of the Petri dish. Seal the dish with parafilm and incubate and treat as described in the USDA approved *Guidelines for Isolation by Culture and Morphological Identification of Phytophthora ramorum* at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml

APPENDIX 7

Water Sampling Protocol
Revised April 2007

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

Phytophthora ramorum is an oomycete, belonging to the group that includes *Pythium* species. Collectively these organisms are called "water molds" and are taxonomically related closer to algae than to fungi. For this reason, water collected from potentially infested nursery blocks must be tested for the presence of *P. ramorum*.

There are two potential methods provided here to detect *Phytophthora* species in water. The first uses rhododendron leaf baits in mesh bags followed by moist chamber incubation of the leaf baits. As of April 2007, research supports using leaves at least one year old, so that is recommended. Any suspect lesions that develop on the rhododendron leaves would be plated on PARP at 18-20°C (64-68°F). Any *Phytophthora* species growing on the PARP would need to be transferred to Corn meal agar or V8 agar for identification to species.

The second method uses water filtration. Water is removed from the pond, filtered with sterile filters and the filters placed on PARP. Once the filter is removed from PARP, any resultant *Phytophthora* colonies are transferred to Corn Meal Agar or V8 agar and identified to species.

In situ Water Sampling with Rhododendron Leaf Baits:

A control sample using a leaf bait in distilled water should be run simultaneously with the leaf bait sample in the nursery site water. The USDA Forest Service recommends the following bait selection criteria in *Stream Baiting Protocol: 2007 National Phytophthora ramorum Early Detection Survey of Forests*, issued March 20, 2007. See http://fhm.fs.fed.us/sp/sod/sod.shtm for latest approved protocol.

Bait Selection

- Use leaves from a population of native or naturalized rhododendrons, if possible. The population should be sufficiently large to supply needed leaves for the survey duration.

- Variation in Pr susceptibility among rhododendron species/cultivars in laboratory inoculation has been published, but field and lab studies have shown that leaves of common native and naturalized species perform acceptably as Pr bait.

- Leaf size can vary considerably among species and cultivars. If bait leaves are quite small (8 cm x 3 cm at the widest point or smaller), use 2 leaves in each pocket of the bait bag.

- If the source of leaves is nursery-grown or naturalized landscape plants, ensure that they have been free of fungicides and other pesticides for a minimum of 6 weeks before using as bait.
- Source plants should be mostly free of dieback and leaf symptoms. Use 1 year-old leaves as free as possible from leaf symptoms (spots, blight, and chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full leaf expansion and a period of hardening in summer.

- Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for stored leaves.

Prepare the rhododendron leaves as bait by trimming off the petiole end of each leaf. Place 3-4 cut leaves into a mesh bag. Label the bag with a plastic tag listing the date, water source (location), and nursery (i.e., nursery license number). Place the mesh bag into the water source for a minimum of 48-hours to 1-week (preferable). Do not leave the bait in the water source for longer than 1-week as the bait will begin to decompose. Place the bags such that the leaves will remain submerged the entire time (i.e., even if water levels fluctuate within the water source). If possible, place the bait near the influent coming from the area closest to or containing the infested plants.

Remove the bait from the water source and transfer to a sealable bag for transport to the laboratory. Label the bag with the information on the plastic tag, including the date collected. Log the leaf samples into the appropriate database. Assign a unique sample number to the bait(s) from each nursery.

Water Sampling for Filtration:

Water samples should be collected in a sterile wide-mouth bottle and kept at 5 – 10 C. Water samples should be taken from the surface to increase the likelihood of obtaining zoospores of *Phytophthora.*

Sample size should be approximately 1000 ml. Samples should be processed within 48 hours of collection or the samples should be discarded and new samples obtained and processed within 48 hours. Number of samples is determined by the size of the nursery pond to be sampled (Table 1)

Table 1: Number of composite samples collected based on pond size.

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

Note, if you have not used water filtration before and choose to do so, it is recommended you contact Dr. Steve Jeffers at Clemson University for further details on this technique.

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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^{o} F (100^{o} 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.

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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 isothyocyanate (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	Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling.

Fumigant	Trade names	Comments
	Vapam	
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.

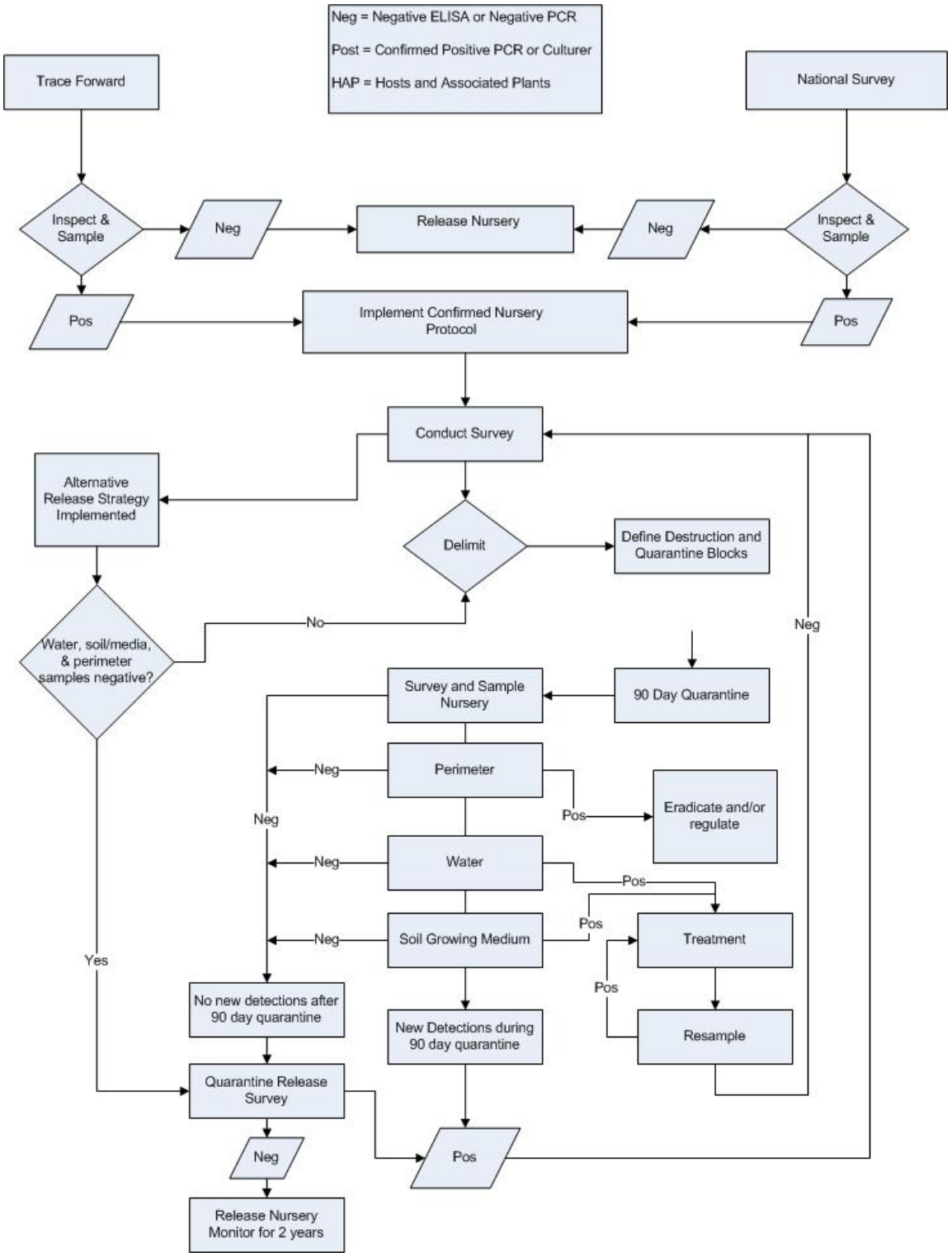
Visitors:

- 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)

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. A regulatory inspection of all listed plants was conducted in the nursery. 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*; or, if there is significant water runoff, and the water has not yet tested positive, conduct seasonal baiting of that water.

   Either official baiting of water draining from the nursery (and nursery inspections if any water is found positive) or a nursery inspection of all plants within the nursery that are found on the "APHIS List of Regulated Proven Hosts and Plants Associated Plants with *Phytophthora ramorum*" are to be conducted in the two best remaining seasons, that are conducive to the development of symptoms for *P. ramorum*. Any plants observed with symptoms will be sampled sufficiently to represent the plants with 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