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 7.0
Revised September 1, 2006
(Host List Updated September 11, 2006)

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 2002, USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) issued a federal domestic regulation for *Phytophthora ramorum* (7 CFR 301.92). The complete text and other information may be found at the USDA APHIS PPQ web site: www.aphis.usda.gov/ppq/ispm/pramorum/

Since the regulations were first published, *P. ramorum* has been detected in a 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.

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: These are actions taken to reduce or mitigate the potential spread of *Phytophthora ramorum* from one area or site to another area or site of a nursery. These include but are not limited to best management practices for nursery cleanliness and pathogen reduction.

Buffer zone: This term no longer in use; See Quarantine Block.

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 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 Hosts and Plants Associated with *Phytophthora ramorum*".

Infected plants: Plants confirmed as being infected with *P. ramorum* based on the use of APHIS approved diagnostics.

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 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 until such time as host plants and climatic conditions conducive to disease expression have occurred for at least 90 days, and inspection, sampling, and testing reveals no further detection of *P. ramorum.* Conducive conditions occur (exist) when climatic conditions match optimum disease etiology and plants 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 the same as a federally promulgated quarantine.

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 www.aphis.usda.gov/ppq/ispm/pramorum/protocols.html

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/ppq/ispm/pramorum

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*, national *P. ramorum* survey*, regulatory survey as per Federal Order*, or found by other means. Presumptive positive samples must have been confirmed at an APHIS approved laboratory.

*See www.aphis.usda.gov/ppq/ispm/pramorum for links with details.

AUTHORITIES

- For States with parallel quarantines for *P. ramorum,* specific actions required by this protocol within and around the nursery are expected to be conducted by the State personnel under State authority with Federal support.

- For States without parallel quarantines for *P. ramorum,* 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 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.

- State plant regulatory officials (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 submitter, the SPHD, and the SPRO, the Regional Office and National Program Manager. 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 the 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 high priority HAP genera* and other HAP species within the 12 months prior to the first positive detection of *P. ramorum* at the nursery. 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 interstate 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 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.

> * "High priority HAP genera" refers to the genera of HAP located in the Destruction Block. This designation recognizes that the exposure of non-listed species (members of the same genus) reflect a greater risk, hence the designation "high priority".

See the "Trace Forward Protocol" at http://www.aphis.usda.gov/ppq/ispm/pramorum/

Trace Back Investigation:

Implement the current traceback protocol present on the *Phytophthora ramorum* website located at http://www.aphis.usda.gov/ppq/ispm/pramorum/

Nursery Sites:

Determine whether additional locations are maintained by the same nursery personnel.

- **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 between nursery sites requires that all nursery sites utilizing the equipment 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. 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 (e.g. 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 or www.aphis.usda.gov/ppq/ispm/pramorum

- 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 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*.

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.

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.

Best Management Practices:

These Best Management Practices (BMP's) are designed to minimize the risk of introduction or survival of the pathogen in a nursery. See Appendix 9 for recommended best management practices.

NINETY (90) DAY QUARANTINE ACTIVITIES

These concurrent activities follow completion of the delimiting survey:

- Any non-HAP remaining in a destruction block will be held in place 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 "Work Aid" 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.

RELEASING 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 national 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 national 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 11 September 2006)
This list is continually being updated.
The most current version is posted at: http://www.aphis.usda.gov/ppq/ispm/pramorum

Proven Hosts Regulated for *Phytophthora ramorum*

Scientific Name (47)	**Common Name(s)**	**Notes**
Acer macrophyllum	Bigleaf maple	
Acer pseudoplatanus	Planetree maple	Koch's postulates completed
Aesculus hippocastanum	Horse chestnut	Koch's postulates completed
Adiantum aleuticum	Western maidenhair fern	
Adiantum jordanii	California maidenhair fern	
Aesculus californica	California buckeye	
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 latifolia	Mountain laurel	
Lithocarpus densiflorus	Tanoak	
Lonicera hispidula	California honeysuckle	
Laurus nobilis	Bay laurel	Koch's postulates completed
Maianthemum racemosum (≡ *Smilacina racemosa*)	False Solomon's seal	
Michelia doltsopa	Michelia	Koch's postulates completed

Parrotia persica	Persian ironwood	
Photinia fraseri	Red tip photinia	
Pieris floribunda and *Pieris floribunda x japonica* & all hybrids of *P. floribunda*	Mountain Andromeda	
Pieris formosa and *P. formosa x japonica* & all hybrids of *P. formosa*	Himalaya Andromeda	
Pieris japonica & all hybrids of *P. japonica*	Japanese Pieris	
Pseudotsuga menziesii var. *menziesii* & all nursery grown *P. menziesii*	Douglas fir	
Quercus agrifolia	Coast live oak	
Quercus chrysolepis	Canyon live oak	
Quercus cerris	European turkey oak	
Quercus falcata	Southern red oak	
Quercus ilex	Holm oak	
Quercus kelloggii	California black oak	
Quercus parvula var. *shrevei* & all nursery grown *Q. parvula*	Shreve's oak	
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 (58)	**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)	
Ardisia japonica	Ardisia – Jan 06 (9)	
Calycanthus occidentalis	Spicebush – May 05 (5)	
Castanopsis orthacantha	Castanopsis - Aug 06 (3)	New listing - Reported found in the UK
Ceanothus thyrsiflorus	Blueblossom – April 06 (5)	
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)	New listing - Reported found in the UK
Corylus cornuta	California hazelnut – Dec 02 (5)	
Distylium myricoides	Myrtle-leafed Distylium – Jul 06 (9)	New listing - Reported found in Canada
Drimys winteri	Winter's bark – July 04 (3)	
Dryopteris arguta	California wood fern – May 04 (5)	
Eucalyptus haemastoma	Scribbly gum – Aug 06 (3)	New listing - Reported found in the UK
Euonymus kiautschovicus	Spreading euonymus – Jan 06 (9)	
Fraxinus latifolia	Oregon ash – Aug 05 (5)	
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)	New listing - Reported found in Canada
Kalmia angustifolia	Sheep laurel – May 06 (3)	
Leucothoe axillaris	Fetterbush, dog hobble – Jan 06 (9)	
Leucothoe fontanesiana	Drooping leucothoe - Oct 03 (3)	
Loropetalum chinense	Loropetalum – Jul 06 (9)	New listing - Reported found in Canada
Manglietia insignis	Red lotus tree – Aug 06 (9)	New listing - Reported found in Canada
Magnolia grandiflora	Southern magnolia – Jan 06 (9)	
Magnolia stellata	Star magnolia – Jan 05 (3)	
Magnolia x loebneri	Loebner magnolia – Jan 05 (3)	
Magnolia x soulangeana	Saucer magnolia – Jan 05 (3)	
Michelia maudiae	Michelia – Jan 06 (9)	
Michelia wilsonii	Michelia – Jan 06 (9)	
Nerium oleander	Oleander – June 06 (1)	
Nothofagus obliqua	Roble beech – Dec 04 (3)	
Osmorhiza berteroi	Sweet Cicely – Aug 05 (5)	
Osmanthus decorus (≡Phillyrea decora; ≡P. vilmoriniana)	Osmanthus – Jan 06 (9)	
Osmanthus fragrans	Sweet olive – June 06 (1)	
Osmanthus heterophyllus	Holly olive – June 06 (1)	
Parakmeria lotungensis	Eastern joy lotus tree – Jul 06 (9)	New listing - Reported found in Canada
Pittosporum undulatum	Victorian box – Dec 02 (6)	
Prunus lusitanica	Portuguese laurel cherry – Jan 06 (9)	
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:	Hybrid roses – Jan 06 (9)	Revised listing - Note that these are specific registered cultivars which can be identified by the listed tags

"MEIpoque") Pink Sevillana (tagged: "MEIgeroka")		
Rosa rugosa	Rugosa rose – Jan 06 (9)	
Rubus spectabilis	Salmonberry – Dec 02 (4)	
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)	

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[2] Oregon Department of Agriculture. Salem, OR
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[10] (Reserved)
[11] (Reserved)

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/ppq/ispm/pramorum

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 and 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*. An example is when the number of individual species, hybrids, or cultivars listed or to be listed is determined to hinder appropriate and effective inspection or regulation.

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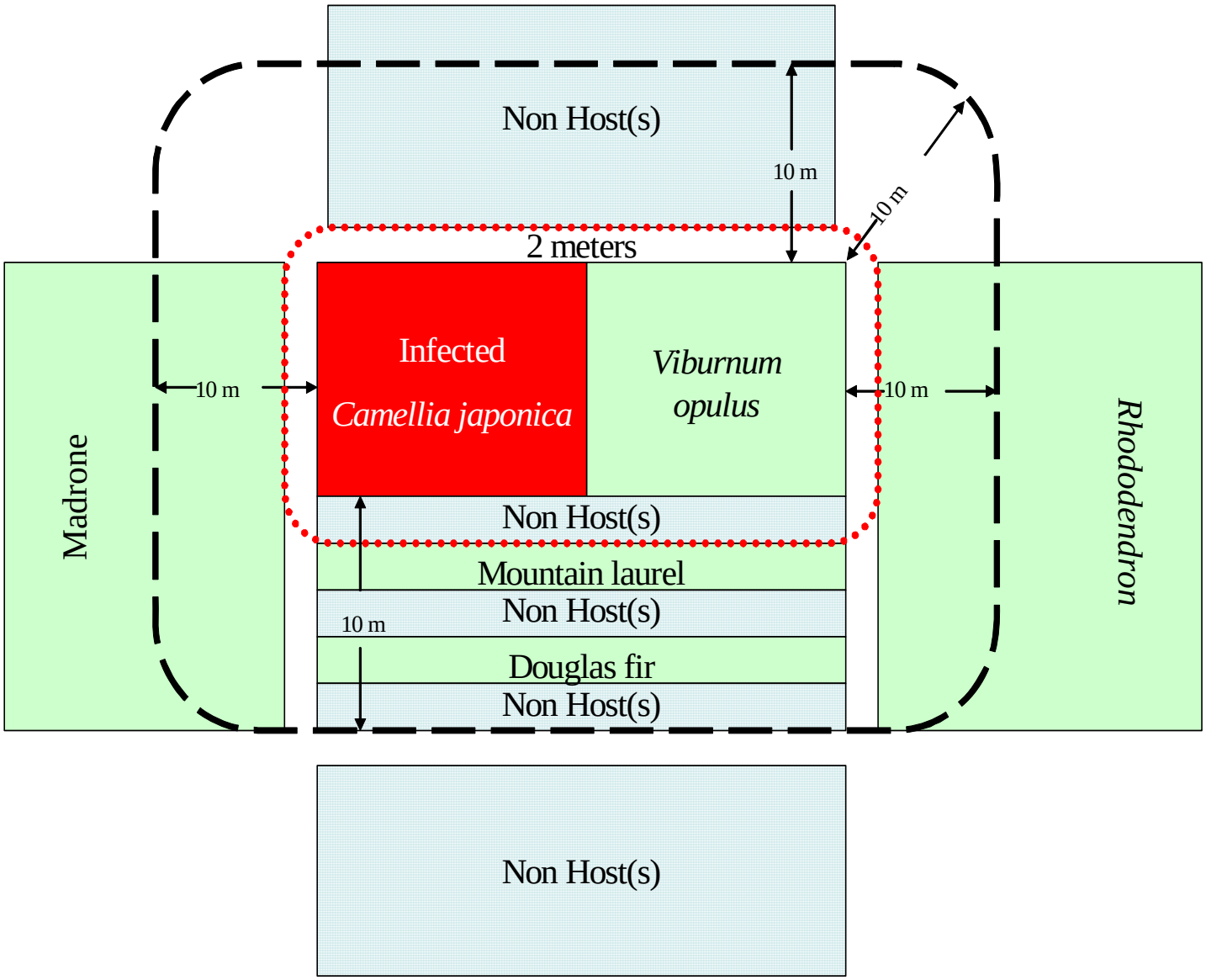
APPENDIX 2

Work Aid – Schematic of 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

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APPENDIX 4

Delimiting Survey Protocol

Delimiting Survey Protocol to Detect
Phytophthora ramorum
In Plants at Confirmed Nurseries

May 24, 2005

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 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.

- Examine all HAP genera 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

Samples must be analyzed using a methodology approved by APHIS. See techniques posted at: http://www.aphis.usda.gov/ppq/ispm/pramorum/

APPENDIX 6

Soil and Growing Medium Sampling Protocol

(See http://www.aphis.usda.gov/ppq/ispm/pramorum/soilsamplingprotocol.html 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 areas last. In other words, begin soil and potting medium sampling at outer edges of buffer zone and work toward the destruction block(s).

Preparing for sampling:

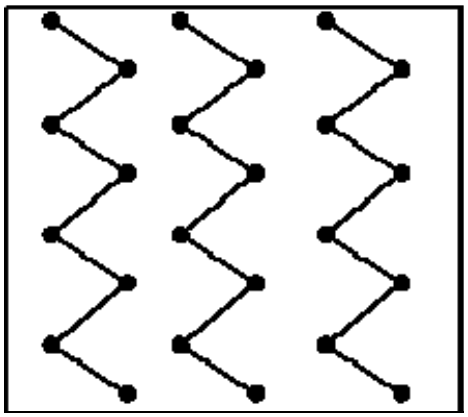
- Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or 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 week 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 "downstream" (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.



APPENDIX 7

Water Sampling Protocol for Retention Ponds

See http://www.aphis.usda.gov/ppq/ispm/pramorum/watersamplingprotocol.html 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. 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.

- Prepare the rhododendron leaves as bait by cutting the leaves in a herringbone pattern into (but not through) the mid-vein or 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

APPENDIX 8

Treatment and Disinfection

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/pdf_files/Treatment%20Chapters/05-05-T400-5.pdf).

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 buffer area should only be used in the buffer area. If tools and other implements must be moved from the buffer area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the buffer zone. 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.

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.
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.
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 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 parts 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 buffer zone 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 filth, 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 buffer zone or destruction block. The disposable shoe covers must be placed in bags and incinerated or deep-buried.

- 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.

- 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

Best Management Practices for Nurseries

Best Management Practices (BMP) are inherently site specific and will need to be determined as a cooperative effort. For the latest BMPs, visit www.suddenoakdeath.org

APPENDIX 10

Confirmed Nursery Protocol Flowchart