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

Official Regulatory Protocol for Retail Nurseries Containing Plants Infected with *Phytophthora ramorum*

Retail

Confirmed Nursery Protocol (rCNP)
December 19, 2007
Version 1.0

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)

TABLE OF CONTENTS

APPENDICES

INTENDED USE

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

As retail nurseries are at the end of the production and distribution process, they represent a lower risk in distribution of infected plants to other nurseries and facilities in the plant distribution system. As retail nurseries are the final point in the plant distribution system, they are the last point before infected plants would be moved directly to the environment. It is also important that retail nurseries do not become a point where non-infected plants could become infected on the way to the place of final planting.

GOAL

The purpose and goal of this protocol is to ensure that any infestations of this pathogen are consistently and effectively addressed, mitigated, and eradicated when *Phytophthora ramorum* is detected in a retail setting. The actions described in the protocol are designed to ensure that any infestations of this pathogen are consistently and effectively addressed and the pathogen eradicated, while minimizing the expected downtime for the retail nursery. Cooperation by nursery management personnel is essential. Early detection and reporting of potential *P. ramorum* plant infections are crucial, to ensure that spread is contained. **The goal is to find and eradicate the pathogen if present in nurseries. Any interpretation of this protocol that is contrary to this goal is a misinterpretation of the protocol.**

PERSISTENT AND RE-INTRODUCTIONS OF P. RAMORUM

P. ramorum infestations in retail nurseries may be re-introduced, 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 (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).

DISCLAIMER

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).

Destruction radius: Block of plants to be destroyed. Within a nursery, for purposes of the **retail protocol**, the destruction radius is defined as all *P. ramorum* infected HAP and all other HAP within 2 meters of any infected HAP.

HAP: Host and associated host plants listed on the official APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum.*

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. Locations that grow trees for sale without roots (e.g., as Christmas trees) are considered to be nurseries. *Also see Retail nursery.*

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.

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

Quarantine radius: Block of plants to be quarantined. Within a nursery for purposes of the **retail protocol** this is an area identified as a 2 meter radius around the destruction radius (see Appendix 2) designed to determine if *P. ramorum* has spread beyond the destruction radius. (Use of Quarantine radius 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 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 within the nursery and sampling any unhealthy plant tissue, soil of the destruction radius (radii) and quarantine radius (radii) and drainage or recirculated irrigation water, as per Appendices. When the quarantine period is completed and all plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released.

Retail nursery: A nursery whose business is the sale of plants to the end user, typically a home owner.

Suspect infected plants: These are plants with visible symptoms likely of *P. ramorum* infection; and/or HAP that are a part of an infested radius or derived from an infested radius or Quarantine radius and/or plants that have tested positive using PCR or culturing, but have not been confirmed positive for *P. ramorum* by APHIS.

TRIGGER EVENTS FOR USE OF PROTOCOL

This protocol is to be implemented by APHIS-PPQ and/or its State Plant Regulatory cooperators when the presence of *P. ramorum* has been confirmed in a retail nursery. This may be from samples taken as part of a trace forward survey*, trace back survey*, a *P. ramorum* nursery survey*, annual compliance 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 equivalent 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 equivalent 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.

- Authority for this protocol is derived from the Section 414 of the Plant Protection Act, 7 USC 7714., 114 STAT. 445, PUBLIC LAW 106–224—JUNE 20, 2000, as follows: SEC. 414. GENERAL REMEDIAL MEASURES FOR NEW PLANT PESTS AND NOXIOUS WEEDS. (a) AUTHORITY TO HOLD, TREAT, OR DESTROY ITEMS.—If the Secretary considers it necessary in order to prevent the dissemination of a plant pest or noxious weed that is new to or not known to be widely prevalent or distributed within and throughout the United States, the Secretary may hold, seize, quarantine, treat, apply other remedial measures to, destroy, or otherwise dispose of any plant, plant pest, noxious weed, biological control organism, plant product, article, or means of conveyance that— (1) is moving into or through the United States or interstate, or has moved into or through the United States or interstate⋯, (B) is or has been otherwise in violation of this title; (2) has not been maintained in compliance with a post entry quarantine requirement; or (3) is the progeny of any plant, biological control organism, plant product, plant pest, or noxious weed that is moving into or through the United States or interstate, or has moved into the United States or interstate, in violation of this title.

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

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

- (d) APPLICATION OF LEAST DRASTIC ACTION: (see Plant Protection Act, 7 USC 7714., 114 STAT. 445, PUBLIC LAW 106–224—JUNE 20, 2000)

COMMUNICATE AND NOTIFY

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

1. A positive PCR determination as determined and reported by an APHIS approved laboratory.

2. A culture that matches the morphology for *P. ramorum* (i.e. isolation of *P. ramorum*) as determined and reported by an APHIS approved laboratory.

- The Regional Program manager will 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.

- SPHD's and SPRO's, shall notify facilities within their states that are impacted by the trace backs 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.

- This Protocol is the official document to be followed. At the end of this document is a "job aid" provided for you to use for reference and as a device to communicate the protocol.

CONDUCT INVESTIGATIONS

Trace Forward Investigation:

If the nursery has distributed HAP to another nursery, the Trace Forward Protocol shall be applied.

Implement the current Trace Forward Protocol present on the *Phytophthora ramorum* website located 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.

- **Plants:** Determine if HAP has moved to other sites or between nursery sites. If so, than all nursery sites receiving HAPs must be surveyed as per the "Survey the Nursery" section of this protocol.

- **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 in terms of the survey of the nursery section below.

Destruction Options:

USDA, APHIS or State regulatory official will identify options for destruction*

- Deep burial or open burning at nursery site
- Burial at a locally approved landfill
- Availability of incinerator for burning

**List and provide to nursery owner upon request*

SECURE THE NURSERY

When the presence of *Phytophthora ramorum* has been confirmed in the nursery, you must safeguard infected HAP and HAP nearby as described below:

- All infected HAP and all other HAP within 2 meters* of any infected HAP shall be held for destruction.

- All HAP within a 2 meter perimeter beyond the 2 meters surrounding the infected HAP (i.e. the retail destruction radius) shall be held for a 90 day quarantine period (see Appendix 2).

- All HAP in the nursery that is not infected and not within 4 meters from the infected plant(s) shall be held under this protocol only until the next step is completed under "Survey the Nursery. However, 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.

*Rational: Retail nurseries represent a lower risk in the establishment and distribution of infected plants to other nurseries and facilities. As such, a two meter destruction radius and a two meter quarantine radius are used in this protocol, rather than the block concept found in the Production/Wholesale CNP. In a retail setting the plants are frequently moved thus there is less risk of plant to plant transfer of the disease. Another factor that reduces risk of plant infection developing within a retail nursery is that the product moves quickly and so does not remain on site for an extended period of time.

SURVEY THE NURSERY

The goal of the survey is to locate *P. ramorum* in the nursery. A detailed and thorough inspection should be conducted in the nursery to determine the presence of *P. ramorum*. Samples should be collected from 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 Radius (Radii):

- Examine all plants (nursery stock and decorative) within the nursery and sample any unhealthy plant tissue found.
    - Hold all plants of that taxon (taxa) that are within 2 meters of the sampled plant, including all HAP that is within those 2 meters.
- Samples must be analyzed using a methodology approved by APHIS (see Appendix 5).
- Release held radius (radii) with negative results as reported.
- The final destruction and quarantine radius (radii) 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 radius (radii) by flagging a 2 meter radius (a 4 meter diameter circle) around all infected plants. (see Appendix 2).
- Establish quarantine radius (radii) by flagging a 4 meter radius (an 8 meter diameter circle) around all infected plants. (see Appendix 2).
- Limit access to destruction and quarantine radius (radii). Ensure that proper sanitation measures are applied (see Appendix 8).

Soil and Growing Media Sampling:

- See Appendix 6 for detailed soil and media sampling protocol. Keep soil samples separate from growing media samples.
- Soil within the destruction and quarantine radius (radii) must be sampled.
- Growing media from HAP within the destruction and quarantine radius (radii) must be sampled.
- Soil and growing media from nursery areas immediately down slope from the destruction and quarantine radius (radii) must also be sampled.
- Growing media from the plant potting area shall be sampled.

- If reported positive, determine the content, origin, storage and handling of growing media used at the nursery site.

Note - **Soil** is the substrate underneath the pots and **growing media** is located within the pots of the plants in the destruction and quarantine radius (radii).

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 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. If water is to be sampled; See Appendix 7 for detailed water sampling protocol.

Cull Pile Sampling:

In a retail nursery this is likely not present, however if a cull pile is present, 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:

In a retail nursery this is likely not present, however if a compost pile is present, 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*.

Segregation of Plants on hold:

Once inspection and sampling are complete (see Survey the Nursery section), any held plants may be consolidated and segregated. If the plants are not consolidated and segregated, then the affected portion of the nursery must be closed to the public. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery. Any movement of the segregated plants must be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction of a regulatory official. Segregation must include storage on an impermeable surface (e.g. a 45 mil thick pond liner or concrete or asphalt) and not within 2 meters of any other plant. The impermeable surface should ideally be situated to drain away from HAP.

Alternative Release Strategy:

See Release the Nursery Section.

DISINFEST THE NURSERY

Plant Destruction:

Where a *P. ramorum* infected plant(s) is found, all infected HAP, all other HAP within 2 meters (destruction radius) of the infected plant(s) and plant parts will be removed and destroyed using one or more of the techniques detailed in Appendix 8. This includes pots and related media.

Debris Removal:

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

Porous Surfaces:

If the nursery is found to have an infested porous surface, remedial action must be developed and implemented with the written approval of a regulatory official. This is done in order to prevent contact of plants with soil or any other surface which cannot be immediately disinfested. A durable, impermeable ground barrier (such as a 45 mil pond liner) may be used as an inexpensive temporary measure. The condition of the barrier must be monitored and maintained, and foot traffic minimized.

Water Treatment:

If water was sampled and tested positive for *P. ramorum* during the survey and delimitation of the infestation at the nursery, treatment is required (see Appendix 8 for recommended disinfestation options).

Soil and Growing Media Treatment:

If soil, growing media or plant debris in a destruction or quarantine block tested 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. A durable, impermeable ground barrier (such as a 45 mil pond liner) may be used as an inexpensive temporary measure to prevent contact of plants with soil. The condition of the barrier must be monitored and maintained, and foot traffic minimized.
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 and may run concurrent with some of the disinfestation activities taking place at the nursery:

- The quarantine period begins when the delimiting survey is completed (i.e. the last sample is taken and an EAN or state equivalent is issued in a timely manner) and lasts for 90 days. During the quarantine period, inspection, sampling, and testing must reveal no further detection of *P. ramorum*, or the quarantine period will be extended (see "If found positive", below).

- During the 90 day quarantine period within the quarantine radius (radii):

    - 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 radius (radii) can be done at the same time as the quarantine release survey as described below.

    - Regulatory officials will collect soil and media samples and test during the quarantine period according to the protocols detailed in Appendix 6.

    - Regulatory officials will collect water samples and test during the quarantine period according to the protocols detailed in Appendix 7, if water samples were collected and tested during the delimiting survey (see "Water Sampling" within the Survey the Nursery section).

    <u>If found positive:</u>

    - If a plant sample tests positive for *P. ramorum*, the destruction radius (radii) and quarantine radius (radii) shall be redefined via sampling and the quarantine period reset.

    - If a soil sample is found to be positive, the soil shall be treated, and any plants in the radius (radii) with the infested soil will be placed on hold and the area re-delimited.

    - The growing media in the potting shed must be tested. If there are any positives for *P. ramorum* from the media in the shed confer with the Regional Program Manager

    - If water, soil, and/or media samples tested positive for *P. ramorum* during the delimiting survey and the water, soil, and/or media was subsequently 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.

1. If samples of the treated water test positive, re-treatment per Appendix 8 will be required. 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.

2. If a treated soil sample is found to be positive, the soil shall be re-treated, sampled and tested. 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.

3. For nurseries with established quarantine radius (radii) undergoing a 90 day quarantine period, the 90 day quarantine period re-starts after the second delimiting survey is completed.

4. Once re-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.

- **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 within the nursery and sampling any unhealthy plant tissue, soil of destruction and quarantine radius (radii) and drainage or recirculated irrigation water. When the quarantine period is completed and all plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released.

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 detection 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 if testing of soil, water and media is required: and

- The quarantine release survey is negative for *P. ramorum*.

Alternate Release Strategy:

A nursery may avoid a quarantine period. Nursery management may through a voluntary management decision elect to follow **all** the requirements specified below:

- **The nursery must destroy everything (all plants, pots, media, etc.) in the destruction radius (radii) by approved methods, (see Appendix 8).** The nursery operator may also choose to destroy plants that have been placed under quarantine at any time within the 90 day quarantine period, however inspection and sampling must take place prior to destruction; **and**

- **Inspectors must** sample and test soil of destruction and quarantine radius (radii) and drainage or recirculated irrigation water if not previously tested and determined to be negative, as per Appendices 5, 6, and 7. If soil and water samples taken are negative for *P. ramorum* the nursery can be released; **and**

- **Inspectors must** revisit the nursery after approximately 90 days and conduct at least a nursery level survey inspection (per the current Nursery Survey Manual) to include sampling of the soil in the destruction radius (radii). Also, the nursery is subject to "Post Eradication Monitoring" (see below).

POST ERADICATION MONITORING

Nurseries that have been infested will be monitored the following two years by inspection and survey as per the nursery survey protocol. These nurseries are not under any other quarantine or regulatory action, unless there are additional detections.

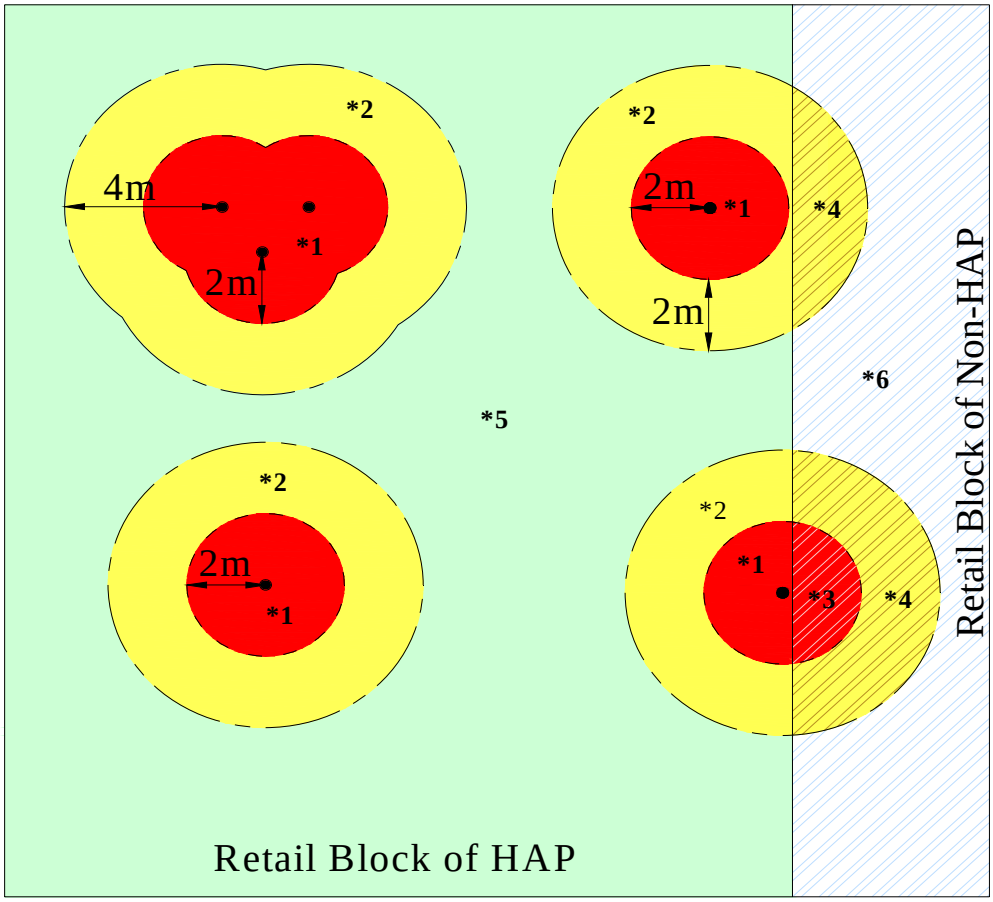
APPENDIX 1

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

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

APPENDIX 2

Schematic of Retail Nursery with Infected Host Plant(s)
July 9, 2007



Red (* 1)	Destruction block	Destroy infected plant and all HAP
Yellow (*2)	Quarantine block	Hold HAP from sale for 90 days
Hatch Over Red (*3)		Release non-HAP from sale
Hatch Over Yellow (*4)		Release non-HAP for sale
Green (*5)		Release HAP for sale
Blue Hatch (*6)		Release non-HAP for sale

APPENDIX 3

Reserved

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 plants unless unhealthy tissue is present.

- 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 and Testing Protocol

Revised: November 15 2007

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:

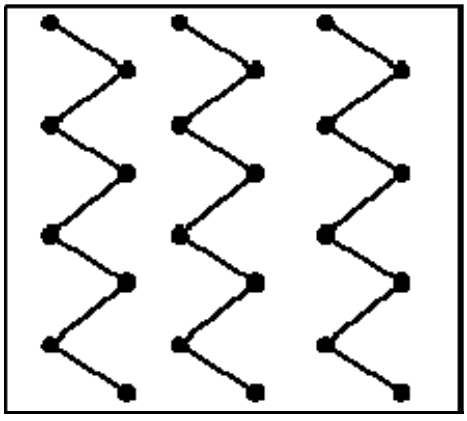
- Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or 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 and covered with gravel, the weed block should be removed prior to soil sampling.

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

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 < 1,780	10 (20)
0.50 < n < 1.0	21,780 <n < 3,560	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 at least 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 meters for retail nurseries and 2 meters to 10 meters for production nurseries of the originally infected plants that have been placed on hold. Preferentially target the growing media of those plants that are downslope (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 in the Laboratory

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 also 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**. 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 the month has passed, add water as below and leave samples at room temperature for two days and repeat soil baiting process. This baiting can be done in conjunction with the final soil baiting required for the quarantine release survey. The samples should be processed as shown below.

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 and leave at room temperature for two days.

To prepare soil bait, briefly soak the Rhododendron leaves in a mild detergent solution to remove any pesticide residues. Rinse the bait well and drain.

Use two leaves per soil sample. With a black sharpie pen, label one side of the 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 *P. ramorum* 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 *P. ramorum* 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 place of one.

**This applies only to initial soil samples at a location (quarantine block, destruction block, cull pile, etc.) at the infested nursery site.

- 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 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 days to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal.

Examine the bait daily for developing symptoms.

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

Under the laminar flow hood, cut eight to 10 pieces of 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: November 15, 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 body of water, 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 leaf bait in distilled water should be run simultaneously with the leaf bait 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 *P. ramorum* 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 *P. ramorum* 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 month 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 each of two mesh bags. Label the bags with a plastic tag listing the date, water source (location), and nursery (i.e., nursery license number). Place the mesh bags into the water source for one to two weeks (see U. S. Forest Protocol. Do not leave the bait in the water source for longer than 2-weeks 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 confer with Dr. Steve Jeffers at Clemson University.

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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.

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

Fumigant	Trade names	Comments
	Metam 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. If entry is unavoidable, follow disinfection procedures in this section.

- 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". There is no effective way to test or treat wood surfaces for contamination.

APPENDIX 9

Biosecurity Measures for Nurseries

April 2007 (Revised November 15, 2007)

Apply these measures for use in the specific retail nursery that is undergoing eradication of *Phytophthora ramorum* from the nursery.

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 disease-causing 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.

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, to be utilized by Nursery personnel and Regulatory authorities, are intended to reduce the likelihood of this pathway.

- **Avoidance:**
    - Vehicles should only be driven and parked on paved, concrete or gravel areas to avoid contact with soil and organic matter. Loading of nursery stock onto other than the nursery's vehicles should, if at all possible, 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.
    - If the retail nursery is large enough for vehicles to be used in unpaved

areas, vehicle use in these areas should be minimized whenever possible. When unavoidable, it is important to have an area designated for soil removal from wheel wells and undercarriages. A suitable disinfectant needs to be used after this cleaning. It may be appropriate to clean off clumps of soil, and then visit a nearby car-wash. In no event should another site be visited by the same vehicle without cleaning and disinfection.

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. Work should always be completed working from the areas of lowest to highest risk.

Access:

Access to infested areas and quarantine areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving the infested and quarantine areas must scrape off loose pieces of soil into the destruction radius. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after 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 personnel entering and exiting the quarantine area and entering and exiting the destruction area 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 area. 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 infested and quarantine areas.

- 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.

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.) used in the infested and quarantine areas 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 in a bag marked for disinfection.

Visitors:

- Access to infested areas and quarantine areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving these areas of a 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.

APPENDIX 10

Reserved

APPENDIX 11

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

May 2007 (Revised December 17, 2007)

These mitigations apply for nurseries detected as positive for *P. ramorum* within one year of release from an Emergency Action Notification (EAN) 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 retail nursery has *P. ramorum* detected on site after the initial release from the Emergency Action Notification (or state equivalent), it is necessary to implement additional measures to ensure that the risks associated with *P. ramorum* are properly mitigated. The biosecurity measures described here are the minimum measures to be taken by the nursery and must be maintained for 2 years from the date of release of the last EAN. These additional measures are:

1. Regulatory inspections of all listed plants in spring, summer, and fall for a period of two years.

   Official inspections are to be made of all plants within this nursery that are found on the "APHIS List of Regulated Proven Hosts and Plants Associated with *Phytophthora ramorum*" in the spring, summer and fall.

2. Implementation of appropriate site-specific best management practices. These are to be determined by discussions among the nursery and federal and state regulatory officials. These are to be incorporated into a Compliance Agreement and remain in place as long as the Compliance Agreement is required.

   See http://nature.berkeley.edu/comtf/html/nursery_best_mgmt_practices.html for a listing of best management practices. These have been developed among a group of nurserymen and scientists with input from state and federal program managers. They contain management practices which, if properly applied, can be expected to mitigate risks associated with *P. ramorum* and, incidentally, other plant pests, in a nursery. This site lists the management practice and the rationale for including that practice. State and Federal program officials should team with the nursery to determine which practices are appropriate for implementation at the infested site. The appropriate practices are to be included as part of a Compliance Agreement with specific timelines for implementation. These need to verified and evaluated during the inspections discussed in bullet number one above.

3. 45 days after implementation of this protocol, evaluate the situation in the retail nursery and identify the best locations to run a series of soil and water samples in the nursery. Then take and analyze these samples for the presence of *P. ramorum*.

   The presence of *P. ramorum* in soil or water likely contributes to the occurrence of disease in the nursery. It is necessary to conduct this sampling

and testing, and if found eradicate to prevent re-occurrence of the disease in the nursery. See Appendices 6 and 7 for how to conduct sampling.

4. If the *Rhododendron* and/or *Camellia* have been confirmed positive in the nursery, leaf and debris will be removed from *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. This should occur immediately prior to the required inspections and be confirmed during the regulatory visit.

   Camellia and other hosts are well known to shed infected leaves. This will result in further infection and likely infestation of soil with resultant spread of infection. To address this, it is important for these dropped leaves and related debris to be removed and destroyed or buried. Blowing these leaves away to somewhere else is not an appropriate mitigation.

5. Nurseries are to inspect all *Rhododendron* and *Camellia* brought into the nursery, whether buy-ins or returns and report to inspectors any plants with suspicious symptoms.

   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. If you should accept nursery stock returns, based on the nursery's policy, then destroy those using approved methods.

   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. Other taxa found positive in a nursery present the same risk and should be handled the same way.

6. For retailers that ship interstate, a one year pre-shipment notification to the office of the State Plant Regulatory Official (SPRO), of the receiving State, 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(s) 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

APPENDIX 12

Nursery Questionnaire for a Nursery Confirmed Positive
Revised November 15, 2007

***Phytophthora ramorum* Questionnaire (Property Owner or Manager): Part 1**

Name of Nursery or Garden Store: _____.

Name of Owner or Manager: _____

Address of Site: _____

City: _____, State: _____, Zip Code: _____

Contact Name: _____, Title: _____

Phone Number: _____, Fax Number: _____

GIS Coordinates (if available): _____

Type of Facility (circle): Nursery Greenhouse Wholesale Retailer
Other _____

1. Are you the owner or manager of the property or facility? If not please provide owner contact information._____

2. Did you purchase the plant(s) in question? (If "no", seek information on individual who purchased the material in question)_____

3. When were the plant(s) purchased? _____

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

5. Have you noticed any similar problems with other plants on your property?
_____

6. Have you moved any plants from this location to any different locations in the past year?

_____

a) What types and varieties were they?

_____

_____

b) When was that? _____

c) What is the address of that location?

_____

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

a) What types and varieties were they?

_____

b) How long ago was that? _____

c) What is the address of that location?

_____

8. Do you have a landscape company that purchases plants from you?_____

9. What is the contact information for the landscape company? _____

_____

***Phytophthora ramorum* Questionnaire (Property Owner or Manager): Part 2**

Information on plant material for inspector visiting property:

1. What is the variety and number of plants? _____
2. What is the condition of the plant material? _____
3. Have the suspect plants been trimmed or pruned? _____
4. How are the trimmings disposed of? _____
5. Did the plant material come in pots? _____.
    a) Did you dispose of the pots or Re-use them? _____
6. If the pots were reused or stored, describe how the pots were handled.
    _____
    _____
7. What type of watering techniques are used on site (drip, overhead irrigation, other)?
    _____
8. What is the source(s) of water (municipal, retention pond, other)?
    _____

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Work Flow Check List for rCNP

November 15, 2007

Conduct Investigations

1. Determine if nursery has distributed HAP to another nursery. If so, implement Trace Forward protocol
2. Implement Trace Back protocol
3. Determine if there are additional nursery sites used by the nursery
4. Determine if there are additional sites used by this nursery and if so, if HAP is moved between these sites
5. Determine if equipment is moved from one site to another, and if so, what biosecurity measures are utilized and if this presents a risk to be addressed
6. Identify options for destruction and provide to nursery owner, upon request

Secure the Nursery

1. Hold all infected HAP and all other HAP within 2 meters of infected HAP for destruction.
2. Hold all HAP within a 2 meter radius beyond the 2 meter radius surrounding the infected HAP to determine if they show infection over time.
3. Determine if there is any other product or article that may present a risk of spreading *P. ramorum* that needs to be held.
4. (Do not hold any HAP that is not within 4 meters of the infected plants.)

Survey the Nursery

Delimiting Survey and Establishing Destruction and Quarantine Radius (Radii)

1. Examine all plants (nursery stock and decorative) within the nursery and sample any unhealthy plant tissue found
2. Hold all plants of taxon (taxa) sampled that are within 2 meters plus all HAP within those two meters.
3. Release held radius (radii) that are negative for *P. ramorum* as the results come in.
4. EAN or State equivalent is issued for the 90 day Quarantine Period (commences once delimiting survey is complete)
5. Establish destruction radius (radii)
6. Establish quarantine radius (radii)

Soil and Growing Media Sampling

1. Sample soil in the destruction and quarantine radius (radii)
2. Sample growing media from HAP in destruction and quarantine radius (radii).

3. Sample soil and growing media from down slope areas of the destruction and quarantine radius (radii).
4. Sample growing media from the plant potting area
5. Determine the content, origin, storage and handling of the growing media if the growing media is reported positive for *P. ramorum*.

Water Sampling

1. Determine source of irrigation water
2. Determine drainage water flow/pattern
3. Determine irrigation system used, presence of standing water
4. Sample the water if appropriate.

Cull Pile Sampling (if present)

1. Record cull pile location
2. Inspect cull pile(s)
3. Sample *P. ramorum* symptomatic plants/plant material on cull pile
4. Determine how nursery disposes of culled plant material
5. Sample soil from the down slope edge of the cull pile

Compost Pile Sampling (if present)

1. Record compost pile location
2. Inspect compost pile
3. Sample *P. ramorum* symptomatic plants/plant material on compost pile
4. Determine how nursery disposes of composted plant material
5. Sample soil from the down slope edge of the compost pile

Disinfest the Nursery

1. Destroy HAP in the destruction radius (radii) & pots and related media
2. Remove and destroy debris within destruction radius.
3. Dispose of cull pile if plants, plant material, growing media or soil from cull pile is positive for *P. ramorum*.
4. Dispose of compost pile if plants, plant material, growing media or soil from cull pile is positive for *P. ramorum*.
5. If applicable disinfest any non-porous surface.
6. Take remedial action if there is an infested porous surface.
7. Treat the water, if the water tested positive for *P. ramorum*
8. Treat any soil in the nursery that tested positive for *P. ramorum*
9. Dispose of any media that tested positive for P. *ramorum*.

Ninety (90) Day Quarantine Activities

1. Insure that no fungicides registered for *Phytophthora* control is applied
2. Inspect the quarantine radius (radii) twice during the 90 day quarantine period.
    a. Take samples of unhealthy plant tissue
3. Take samples of water, soil and growing media

4. Conduct a Quarantine Release Survey of the nursery (Can coincide with the second required 90 day quarantine inspection).

Release the Nursery

Rescind all EAN's at the end of the hold period and if all required treatments and mitigations are done and if there are no further detections of *P. ramorum* in plants, soil water, and media.

For the next two springs conduct protocol level inspections in the nursery.

If a repeat nursery, put required measures in place (see Appendix 11).