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

*Interstate shipping retail nurseries, brokers, inter/intrastate wholesale and production nurseries

Confirmed Nursery Protocol: Version 8.2
Revised: August 6, 2014

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)
Plant Health Programs (PHP)
Field Operations (FO)
TABLE OF CONTENTS

INTENDED USE ........................................................................................................ 3
GOAL ......................................................................................................................... 3
TRIGGER EVENTS FOR USE OF THIS PROTOCOL .................................................. 4
DISCLAIMERS ........................................................................................................... 4
AUTHORITIES .......................................................................................................... 5
DEFINITIONS ............................................................................................................. 6
COMMUNICATE AND NOTIFY .................................................................................. 11
TABLE 1 Confirmed Nursery Protocol (CNP) Procedures by Nursery Category ........... 12
TABLE 2 CNP STEPS AND PROCEDURES .............................................................. 12
TABLE 3 CNP STEPS AND PROCEDURES - Disinfest the Nursery ......................... 15
TABLE 4 CNP STEPS AND PROCEDURES – Quarantine Period .......................... 16
CONDUCT INVESTIGATIONS ................................................................................... 18
Appendix 1- APHIS List of Regulated Hosts and Associated Plants ......................... 20
Appendix 2 - Schematic of Destruction and Quarantine Radii ................................. 21
Appendix 3 - Contact List for APHIS-PPQ .............................................................. 22
Appendix 4 – Delimitation Sampling for Plant Positives ........................................... 23

| Table A4. Minimum number of samples to take based on the number of HAP within a positive nursery block. | 24 |

SAMPLE HANDLING AND SUBMISSION PROTOCOL ........................................... 25

Sample Submission Information ............................................................................. 25
Sample Forwarding and Reporting Under DA-2014-02 ........................................ 26

Appendix 5 - Diagnostics ......................................................................................... 28
Appendix 6 - Soil and Container Mix Protocol ......................................................... 29
Appendix 7 - Water Sampling Protocol .................................................................. 30
Appendix 8 - List of Treatment and Disinfection Options ....................................... 31
Appendix 9 - Biosecurity Measures for Nurseries ................................................... 37

Sampling Supplies and Equipment Check List ..................................................... 40

Appendix 10 – Reserved ......................................................................................... 41
Appendix 11 – Notification Requirements .............................................................. 42
Appendix 12 – Critical Control Point (CCP) Assessment Procedures ...................... 43
INTENDED USE

The intended use of this Regulatory Protocol is for nurseries containing *Phytophthora ramorum* in plants, water, or other regulated articles; the nursery types are:

1) **Interstate shipping retail nurseries**  
2) **Brokers**  
3) **Intrastate shipping wholesale** who distribute plants for interstate shipping (use an interstate shipper to broker plants interstate)  
4) **Interstate shipping propagation, wholesale, and re-wholesale nurseries**

This protocol incorporates requirements and procedures outlined in the December 10, 2012 Federal Order (DA-2012-53) and in the January 10, 2014 (DA-2014-02) Federal Order. These Federal Orders are issued pursuant to the regulatory authority provided by the Plant Protection Act of June 20, 2000, as amended, Section 412(a), 7 U.S.C. 7712(a), which authorizes the Secretary of Agriculture to prohibit or restrict the movement in interstate commerce of any plant, plant part, or article, or means of conveyance, if the Secretary determines the prohibition or restriction is necessary to prevent the dissemination of a plant pest within the United States, and is likewise issued pursuant to the regulations, promulgated under the Plant Protection Act, and found at 7 CFR 301.92 *et. seq.* This protocol refers to wholesale and production nurseries throughout the document and includes interstate shipping retail nurseries and brokers whenever wholesale or production nurseries are mentioned.

In February 2005, USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) published an interim rule revising federal domestic regulations for *Phytophthora ramorum* (7 CFR 301.92). Since the regulations were first published in 2002, *P. ramorum* has been detected in a significant number of nurseries. These detections prompted the need for a standard protocol for use by state and federal regulators to respond to finds of *P. ramorum* in nurseries. To ensure that there is consistency in responding to infestations of *P. ramorum*, this protocol describes the official activities performed within and around nurseries by USDA APHIS staff in cooperation with state agriculture regulatory officials.

GOAL

The goal of this protocol is to prevent the spread of this quarantined plant pathogen and to facilitate movement of *P. ramorum*-free nursery stock. If procedures described in this document are implemented, the expected result is that infested nursery stock will be removed and plant to plant spread of the pathogen and the conveyance of the pathogen through plant shipments will be prevented or minimized.
TRIGGER EVENTS FOR USE OF THIS PROTOCOL

This protocol shall be implemented by APHIS-PPQ in cooperation with its State Plant Regulatory Cooperators when the presence of *P. ramorum* has been confirmed in interstate shipping nurseries from samples collected by regulatory officials during any survey or inspection. In addition, use this protocol if the nursery is a wholesaler, re-wholesaler or propagator if they received positive nursery stock interstate; APHIS regulatory authority will be used in these cases. Samples may have been collected during surveys or inspections such as Cooperative Agricultural Pest Survey (CAPS), State Nursery Cleanliness Survey, National Survey, State Compliance Inspections, Trace Forward Survey, and Trace Back Survey or found by other means by regulatory officials. This CNP is triggered for any positive sample, such as plants, water, soil, containers, container mix, or any other article. Samples must be diagnosed using a methodology approved by USDA APHIS PPQ and consistent with the Potentially Actionable Suspect Sample (PASS) protocol. See the PPQ [Phytophthora ramorum Website](#) for links with details on diagnostics, trace forward survey, trace back survey, *P. ramorum* nursery survey, and the PASS protocol.

DISCLAIMERS

Any interpretation of this protocol or its procedures that is not consistent with this goal is a misinterpretation and misrepresentation of this protocol.

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

INTRASTATE SALES WHOLESALE OR RETAIL SITES: Utilize the Intrastate Confirmed Nursery Protocol. This protocol is for nurseries that are strictly retail or wholesale nursery who produce host plants not for interstate shipment.

CHALLENGES: *P. ramorum* is a microorganism, thus, it can be elusive and difficult to detect. It can infect plants, infest container mix, soil and water and persist in these substrates despite the best efforts at eradication. These protocols and regulations will be adjusted accordingly based on our understanding of the biology, detection and management of this pathogen as informed by continually improving science.
AUTHORITIES

• For states with regulations for quarantine pests, and/or specifically for *P. ramorum* equivalent to the Federal regulation or Federal Orders, State personnel may conduct specific actions required by the protocol, within and around the nursery, under State authority with Federal support.

• For States without regulations for quarantine pests, and/or for *P. ramorum* equivalent to the Federal regulations, specific actions required by this protocol within and around the nursery will be conducted under Federal authority, in cooperation with State and/or Federal 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; (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)
## DEFINITIONS

**Associated plants:** Associated plants are those reported found naturally infected and from which *P. ramorum* has been cultured and/or detected using Polymerase Chain Reaction (PCR). For each of these, traditional Koch’s postulates have not yet been completed or documented and reviewed. See Appendix 1.

**Biosecurity measures:** Actions taken to reduce or mitigate the potential introduction or spread of *Phytophthora ramorum* from one area or site to another area or site of a nursery. See Appendix 9.

**Compost pile:** A heap of mixture of decaying organic matter, as from leaves and manure, used to improve soil structure and provide nutrients.

**Confirmed Positive** The presence of *P. ramorum* is confirmed in a diagnostic test conducted by analysts/labs with federal confirmatory authority by utilizing the APHIS *P. ramorum* Diagnostic Work Instructions. The final determination of one positive sample allows for federal regulatory action utilizing PCR diagnostics as described in the APHIS diagnostic work instructions.

**Container mix** Synonymous with growing media. Materials placed in containers and used to grow plants; usually consisting of bark and peat but also may contain soil, slow-release fertilizer, sand, vermiculite, perlite, etc.

**Cull pile:** An area 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(s) within a nursery site. The quarantine period begins when all delimitation sampling and testing is completed.

**Destruction radii:** The destruction radius is two meters from the edge of the known positive plant(s). The destruction zone around the periphery of positive water is one meter. Short hand is “D radii”. The radii are surrounded by the quarantine radii defined below.

**Emergency Action Notification (EAN)** The PPQ Form 523 or equivalent State document used to specify regulatory requirements and actions within a nursery.

**Federal Confirmatory Authority** The authority to make a final determination on a regulatory sample. 1. National Plant Protection Laboratory Accreditation
Program (NPPLAP) accredited APHIS Labs have authority to make a final determination on any *P. ramorum* regulatory sample. 2. State NPPLAP accredited labs in the three regulated states (CA, OR, WA) have authority to make the final determination on any regulatory sample in a previously positive nursery. 3. State or NPDN NPPLAP accredited labs outside the regulated states have authority for subsequent samples to the initial APHIS Confirmed Positive for a given nursery during the EAN period. Once the EAN period has lapsed and a new positive is detected, it must forwarded to APHIS for final determination.

**Final Determination**

The last diagnostic result(s) necessary for a regulatory sample indicating whether *P. ramorum* is present or not. The final determination test(s) are conducted by analysts/labs with federal confirmatory authority. If the final determination is positive, regulatory action may commence. See confirmed positive.

**Free from:**

Without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures. (ISPM Pub. No. 10, 1999).

**HAP:**

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

**High Priority Trace Forward /Trace Back Target Plants:**

Plants that were shipped (domestic or international) within six (6) months of the first positive detection of *P. ramorum* at a nursery meeting the following criteria: 1) plants of the infected species/cultivar, 2) any HAP which originated in the destruction radii, and 3) plants of the high risk genera: *Camellia, Rhododendron, Pieris, Viburnum* and *Kalmia*. Use the lowest taxonomy possible for each of these criteria (for example, if the plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level).

**High Risk Genera:**

*Camellia, Rhododendron, Pieris, Viburnum* and *Kalmia*.

**Hold block:**

This term is no longer in use; See Quarantine Block.

**Host plants:**

Naturally infected plants verified with completion, documentation, review and acceptance of traditional Koch’s postulates and listed in the “APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*”. 
**Infected plants:** Plants officially confirmed as being infected with *P. ramorum*, based on the use of APHIS approved diagnostics, and following the PASS system.

**Nursery/Facility:** Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed, including locations that grow trees to be sold without roots, such as Christmas trees.

**Nursery block:** A contiguous grouping of plants separated by some distance by a path, preferably at least two meters, from other contiguous groupings of plants.

**Nursery site:** A geographically separate location of a Nursery/Facility that has a distinct physical address and appropriate biosecurity measures (See Appendix 9) to prevent the movement of *P. ramorum* between locations.

**Nursery site quarantine period:** This is a period of time during which host plants and associated plants shall not be moved within or out of the quarantine radii (see Appendix 2). This quarantine period begins when the Nursery Delimitation Survey is completed and lasts for 90 days during which proscribed activities must occur.

**Nursery stock:** Any plants for planting, including houseplants, propagative material that are grown in a nursery and tree seedlings for reforestation.

**Parallel quarantine:** A quarantine or regulation imposed by a State or local plant regulatory authority that is essentially the same as a federally promulgated quarantine. These regulations can be more restrictive for intrastate movement and internal controls.

**PASS (Potentially Actionable Suspect Sample):** A Potentially Actionable Suspect Sample (PASS) is a regulatory sample that contains a plant pest or pathogen of federal regulatory concern and requires confirmation by a lab with federal confirmatory authority. For the *P. ramorum* program, a PASS is a regulatory sample where a preliminary diagnostic test result indicates *Phytophthora sp.* or *P. ramorum* is present and requires confirmation by a lab with federal confirmatory authority.
Presumptive positive: A preliminary diagnostic test result from a laboratory indicating *P. ramorum* is present inferring that a final determination (the confirmatory test) is the next and final step.

Quarantine radii: For plant positives, it is a two meter radius around the destruction radii (see Appendix 2) designed to determine if *P. ramorum* has spread beyond the destruction block. (Use of Quarantine radii 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]). Short hand is “Q radii”.

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. Plants, water, or other articles in quarantine hold radii remain on hold during this period. During the quarantine period, regulatory officials will inspect plants in the quarantine radii and all HAP in the nursery a minimum of two additional 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. All symptomatic plants during these surveys must be sampled and tested. The second inspection can be considered the quarantine release survey at the discretion of the inspector.

Quarantine release survey: This is the second of the two quarantine period inspections. It occurs near the end of the quarantine period. This survey includes inspection of plants in the quarantine radii, and all HAP within the nursery. Sample and test any unhealthy plant tissue. For quarantine hold areas that involved positive plants only, those areas can be released from quarantine if plant inspection, sampling, and testing reveal no further detection of *P. ramorum*. For water and other regulated article positives, see Table 4 for quarantine release instructions. Soil and standing or non-recycled pond water may take longer than 90 days to remediate: an avoidance/exclusion mitigation plan for these positive areas is written into Exhibit D of the compliance agreement prior to the end of the Quarantine Period.

Regulated area: Any state, or portion of a state, in which only nurseries that ship HAP interstate are regulated to prevent the spread of *P. ramorum* and the only regulated article is nursery stock. These areas are
detailed in the regulations posted at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Sample:
For *P. ramorum* plant sampling, a sample refers to a single bag of leaves. The goal is to have at least two sq. inch of symptomatic plant tissue per sample for the diagnostician to test. Therefore, each sample will contain approximately five symptomatic (unhealthy) leaves from medium leaf species and probably five-twelve leaves for small leaf species. For species w/ twig die back as a symptom, include the terminal three inches of a symptomatic branch including one inch of live stem. Regulatory action may commence on one sample confirmed positive. In *P. ramorum* diagnostics, sample may refer to a 25 microliter aliquot of DNA or a culture derived from one plant sample. For container mix and water baiting samples, see Appendix 6: Soil and Container Mix Protocol and Appendix 7: Water Sampling Protocol

Soil:
The loose surface material of the earth in most cases consisting of disintegrated rock with an admixture of organic material. The reference to soil in this protocol is the surface or substrate under plant containers, the bare ground and /or gravel; often with plant debris, peat and bark fines washed from plant containers. Substrates in the field on which potted plants are located; often this consists of peat and bark fines washed from pots, plant debris, soil, gravel, or any combination of these.

SPHD:
The State Plant Health Director of a particular state. Lead APHIS contact in each state responsible for overseeing all Plant Protection and Quarantine activities in that state.

SPRO:
The State Plant Regulatory Official in any given state’s department of agriculture or plant industry. This is the person primarily responsible for plant health programs in that state. SPROs can be found listed at: www.nationalplantboard.org/member/index.html

Trace Back (TB) Plants:
All plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) of the infected plant regardless of size, location or lot, back to the original propagation source if still existing.

Trace Back (TB) Site:
Any location that shipped High Priority Trace Forward/Trace Back Target Plants to a confirmed positive nursery, residence or commercial landscape.

Trace Forward (TF) List/Plants:
A list of High Priority Trace Forward/Trace Back Target Plants that were shipped within 6 months prior to detection at the
nursery. The list includes the shipment date(s), quantities and destination nursery information.

**Trace Forward (TF) Site:** Any location that received **High Priority Trace Forward/Trace Back Target Plants** from a confirmed infested source nursery; including residential or commercial landscapes.

**COMMUNICATE AND NOTIFY**

Prior to the regulatory **confirmation** (final determination) of a positive sample, a State or NPDN lab must communicate suspect positives of any articles 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*)

- In the regulated areas, if the sample is PASS, immediately forward sample/DNA to an APHIS PPQ Laboratory (See Appendix 3, Resource and Contact List). In the non-regulated area, immediately forward all samples as described above with the exception of subsequent samples in a nursery Confirmed Positive. All areas are to 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 or SPRO will notify the *P. ramorum* National Operations Manager. See Appendix 3, Resource and Contact List.

- Laboratories with regulatory confirmation authority must report Confirmed Positives to the SPHD, SPRO, and the National Operations Manager, and the submitter.

- Regarding the initial Confirmed Positive(s) that trigger the Confirmed Nursery Protocol at a nursery, the SPRO (or SPHD, depending on who conducts the *P. ramorum* regulatory program in the state), contacts the Nursery Owner immediately to inform the nursery of the positive and to place a hold on all host plants in the nursery. Concurrently, the SPRO/SPHD informs the National Operations Manager for *P. ramorum*.

- SPHD’s and/or SPRO’s, shall notify nurseries within their states that are impacted by the trace backs and trace forwards and provide a list of these facilities to the National Operations Manager or the Field Operations Pest Management Programs Director. See “Conduct Investigations” Section.
**TABLE 1** Confirmed Nursery Protocol (CNP) Procedures by Nursery Category

<table>
<thead>
<tr>
<th>If:</th>
<th>Then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The nursery is currently in the DA-2014-02 Compliance Program. That is, a nursery that has had at least one inspection based on the Inspection and Sampling Protocol within the Calendar Year prior to the detection.</td>
<td>Conduct the action listed in Tables 2 and 3.</td>
</tr>
<tr>
<td>The interstate shipping nursery is <strong>not</strong> in the DA-2014-02 Compliance Program. That is, a nursery that has not been inspected based on the Inspection and Sampling Protocol within the Calendar Year prior to the detection.</td>
<td>Conduct the actions listed in Tables 2 and 3 with one addition: for the Delimiting Survey, utilize the Inspection and Sampling Protocol. Meaning, the Inspection and Sampling Protocol for newly positive nurseries is considered by APHIS as the Delimiting Survey. Also, review the DA-2014-02 Work Aid to bring the nursery into the compliance program.</td>
</tr>
</tbody>
</table>

**TABLE 2** CNP STEPS AND PROCEDURES

Nurseries covered by this CNP fall into **two categories** as described above in Table 1. Interstate shipping nurseries that are in the DA-2014-02 Compliance Program and interstate shipping nurseries that have been found positive but not yet in the DA-2014-02 Compliance Program.

In chronological order, the steps are: Communicate and Notify, Secure the Nursery, Delimit, and Disinfest. As concurrently as possible, Conduct Investigations (described on page 17) after notifying the nursery and while securing the nursery.

<table>
<thead>
<tr>
<th>Chronological Steps</th>
<th>Nursery Category</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) <strong>Communicate and Notify</strong> - Initial (first) Confirmed Positive(s)</td>
<td>Both</td>
<td>How the communication flow goes depends on which laboratory (e.g., State lab, NPDN lab, or PPQ lab) has the federal regulatory authority to diagnose a Confirmed Positive, see Communicate and Notify Section above. Once there is the initial Confirmed Positive for a nursery, the regulatory official notifies the nursery owner and puts a stop sale hold on the nursery. Prior to arrival at the nursery, the inspectors review this document in its entirety and acquire the necessary equipment and supplies specified in Appendix 9 on page 40.</td>
</tr>
<tr>
<td>2) <strong>Secure the Nursery</strong></td>
<td>Both</td>
<td>1) Inspector(s) arrive on site as soon as practicable. For any type of positive, hold all HAP within each established Q radii for the entire Quarantine Period (90 days). For any type of positive, hold all HAP in the <strong>remainder</strong> of the nursery (outside each Q radii) until the delimitation survey is complete and test results for a given area is negative*. *Note: during the delimitation survey the inspector may find blocks of plants completely free from symptoms (and, therefore, un-sampled during delimitation), if so, those, and only those specific...</td>
</tr>
</tbody>
</table>
blocks identified by the inspector may be released for shipment.

2) Restrict access to any D radii until the inspector/nursery is prepared to begin disinfestation procedures.

3) The hold may include “any other product or article that an inspector determines to present a risk of spreading *P. ramorum*, if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations” (7CFR part 301.92-2) within the infested nursery site.

4) 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 Sale notices should be placed on the nursery by the appropriate State or Federal Regulatory Official.

<p>| 2) Secure the Nursery – Plant(s) Positive | Both | For positive plants, establish and restrict access to Destruction radii (D radii) by flagging two meters out from the positive plant(s). See Appendix 2 of the schematic diagram of D and Q radii for exact instructions. |
| 2) Secure the Nursery – Standing Water or Soil Positive | Both | For positive standing water or soil, establish and restrict access to the D radii by flagging one meter out from the standing water/soil demarcation. See Appendix 2 of the schematic diagram of D and Q radii for exact instructions. |
| 2) Secure the Nursery - Cull Pile Positive | Both | Cordon area to avoid movement of inoculum by personnel or equipment. Institute business practice to ensure avoidance/containment until remediation measures are determined and written into the compliance agreement. |
| 2) Secure the Nursery – Used Containers Positive | Both | Flag for hold until sanitation is applied. See Appendix 8. |
| 2) Secure the Nursery – Tools or Equipment dirt Positive | Both | Prior to continued use, require sanitation of tools and equipment. See Appendix 8. |
| 2a) Disinfest The Nursery | Both | See Table 3, <strong>Disinfest the Nursery</strong>, for each article or water positive for <em>P. ramorum</em> using one or more techniques detailed in Appendix 8. |
| 2b) Delimiting Survey | Already in Program | Ensure that proper sanitation measures are applied by regulatory officials while in the confirmed nursery (see Appendix 9). No matter what type of article or water is positive, examine all HAP and non-HAP plants held for sale or propagation within the nursery. Sample any symptomatic tissue found. See specific delimiting procedures in 2c- g dependent on the positive article and Appendix 4 for plant positive delimiting. Only after careful scrutiny, the inspector may find some blocks of plants symptomless and may release them from the hold during the delimiting survey. |</p>
<table>
<thead>
<tr>
<th>2b1) <strong>Delimiting Survey</strong></th>
<th>Not in the Program</th>
<th>Depending on what articles are positive follow specific delimiting instructions below. In addition, no matter what articles are positive, utilize the 2014 Inspection and Sampling protocol as the delimiting survey.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2c) Delimiting - Plant Positive</td>
<td>Both</td>
<td>Immediately examine plants in the Q radii, sample any unhealthy tissue found. Sample standing water or soil from positive plant D radii and adjacent down slope area. Utilize Appendix 4 in this document for exact sampling instructions.</td>
</tr>
<tr>
<td>2d) Delimiting - Standing Water or Soil Positive</td>
<td>Both</td>
<td>Examine plants in the Q radii, and within 10 meters of the radii. Sample any unhealthy tissue found.</td>
</tr>
<tr>
<td>2e) Delimiting - Cull Pile Positive</td>
<td>Both</td>
<td>If not sampled in initial inspection, sample standing water or soil adjacent and/or down slope from cull pile.</td>
</tr>
<tr>
<td>2f) Delimiting - Used Containers Positive</td>
<td>Both</td>
<td>Inspect any plants within two meters of pot storage area; sample symptomatic HAP. Sample standing water or soil within the two meter radii.</td>
</tr>
<tr>
<td>2g) Delimiting – Perimeter Survey</td>
<td>Both</td>
<td>The purpose of the perimeter survey is twofold: (1) to ensure that <em>P. ramorum</em> has not spread outside of the infested nursery to the surrounding environment and (2) to verify that the infestation in the nursery did not originate in the surrounding environment. Survey plants of all HAP genera located within 10-meters of the infested nursery for symptoms. Sample all symptomatic plants.</td>
</tr>
<tr>
<td>2+ ) <strong>If Positives Result from Delimiting Survey</strong></td>
<td>Both</td>
<td>If plants, water or other articles are found positive, conduct a second delimiting survey (using 2b) and the 90 Quarantine Period re-starts after the survey is complete and diagnostic results are negative. Use the delimiting procedures in 2c- g above depending on the positive article. For disinfest procedures for these new positives, use Table 3.</td>
</tr>
<tr>
<td>2h) Release of delimiting survey samples as negative results come in.</td>
<td>Both</td>
<td>Delimiting Survey samples taken outside established Q radii: If plants and standing water or soil samples from a specific block or area are diagnosed negative by the delimitation survey, the plants, water and soil in those areas are released. If any plants (HAP or non-HAP) are present in standing water, these plants must remain on hold until water testing is completed.</td>
</tr>
<tr>
<td>2i) Delimiting Survey and Diagnostics End (no further positives)</td>
<td>Both</td>
<td>The 90 Day Quarantine Period begins when all delimiting sample results are negative*. After delimiting results are in, release additional blocks/grids free of <em>P. ramorum</em> for interstate shipping. Begin Critical Control Point (CCP) Assessment (see Appendix 12). *Note: positive standing water/soil may require a longer to disinfest/remediate.</td>
</tr>
<tr>
<td>3) Conduct Investigations (concurrent notifying and securing the nursery)</td>
<td>Both</td>
<td>See details below in Conduct Investigations Section. Conduct trace forward and trace back investigations, determined if nursery stock, equipment or other articles move between multiple nursery sites. If nursery is required to notify, to check compliance, see Appendix 11 for instructions.</td>
</tr>
<tr>
<td>4) Conduct CCP Assessment</td>
<td>Both</td>
<td>See Inspection and Sampling Protocol CCP Assessment Appendix, the Compliance Agreement Exhibits and Conformance Instructions (contact PPQ National Operations Manager).</td>
</tr>
</tbody>
</table>
### TABLE 3 CNP STEPS AND PROCEDURES - Disinfest the Nursery

<table>
<thead>
<tr>
<th>If:</th>
<th>Then:</th>
</tr>
</thead>
</table>
| Plant(s) are positive | 1. Destroy all plants in the D radius (radii) including pots and container mix, per Appendix 8. See Appendix 2 for the Destruction and Quarantine radii schematic.  
  
2. All HAP and non-HAP plant debris including container mix, leaves, stems, flowers, roots, and any other plant parts found within the D radii will be removed and destroyed using one or more of the techniques detailed in Appendix 8. All non-porous surfaces in the D radii shall be disinfested. See Appendix 8 for recommended disinfestation options.  
  
3. Sample standing water or soil underneath D and Q radii. Utilize Appendix 6 and 8 of this document. |
| Standing water positive | Locate demarcation of standing water area. Flag for quarantine hold and avoidance. If plants are within this positive standing water, see next step below. Take photos of area for the use of the nursery owner and CCP assessment team. Site-specific conditions may apply depending on CCP assessment. Maintain flagging for avoidance until remediation or permanent exclusionary/avoidance mitigation is chosen by the nursery owner with approval of the regulatory inspector and written into Exhibit D of the Compliance Agreement. |
| Plants sitting in positive standing water. | All plants, container mix, and pots sitting in positive water have been exposed to water containing a quarantine organism and must be destroyed as well as all plants within a 1 meter buffer. Additional site-specific conditions may apply depending on CCP assessment. The 90 day quarantine is still applicable. |
| Irrigation water is positive | Cease using positive irrigation source until treated. Irrigation water sources must be free from *P. ramorum* as determined by water testing protocols described in Appendix 7. If irrigation water was sampled and tested positive for *P. ramorum* during the survey and delimitation of the infestation at the nursery, mitigation of the water must occur so that *P. ramorum* can no longer be detected. See Appendix 8 for more details on procedures and treatment options. |
| Cull pile is positive | Immediately demarcate for avoidance (quarantine hold). If any plants, plant material, water, growing media or soil from a cull pile is positive for *P. ramorum*, if possible, all material in the cull pile shall be properly disposed of by incineration or deep burial as described in Appendix 9. Utilize the CCP Assessment to address site specific conditions and determine appropriate mitigation measures, see Exhibit A and D of the Compliance Agreement and Compliance Agreement Conformance Conditions for further instructions. |
| Effluent water (culvert/ditch, stream, non-recycled retention pond) is positive | Immediately demarcate for avoidance. Identify remediation, mitigations or business/cultural practices via the CCP assessment w/ Nursery Owner. Utilize the CCP Assessment, Exhibit D of the Compliance Agreement and Compliance Agreement Conformance Conditions for further instructions. |
| Soil (nursery substrate) is positive | Locate and re-establish boundary demarcation, place barrier mitigation and/or avoidance practices while determining the disinfestation/remediation strategy. Utilize CCP assessment, Appendix 8 and mitigation options available from nursery associations, county extension, and state nursery practices manuals. Utilize the CCP Assessment, Exhibit D of the Compliance Agreement and |
Tools or Equipment dirt is positive  

Disinfest using options in Appendix 8. Choose and institute cultural practice to ensure future sanitation. Utilize the CCP Assessment, Exhibit D of the Compliance Agreement and Compliance Agreement Conformance Conditions for further instructions.

Next Steps (Step 4 in Table 2)  

As stated above in Table 2, for each positive, after delimiting is complete and disinestation procedures are implemented or planned in writing, conduct the Critical Control Point (CCP) Assessment. Then, utilize the CCP assessment and reference material for remediation, mitigation, business/cultural practices and bmp options for the nurseries site-specific plan to address *P. ramorum* in the nursery. See Exhibit A and D of the Compliance Agreement and Compliance Agreement Conformance Conditions document for further instructions.

<table>
<thead>
<tr>
<th>TABLE 4 CNP STEPS AND PROCEDURES – Quarantine Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronological</strong></td>
</tr>
<tr>
<td><strong>Steps (continued from Table 2)</strong></td>
</tr>
</tbody>
</table>
| 5) Quarantine Period Activities | Both | The Quarantine Period, a minimum of 90 days, begins when the Nursery Delimitation Survey(s) is/are complete and all test results are negative. Plants, water, or other articles in Q radii remain on hold for the full period.  

During the Quarantine Period:  
1) No fungicides registered for *Phytophthora* sp. will be used in the plant Q radii.  
2) Regulatory Officials will inspect plants in the Q radii and host plants in the nursery a minimum of two times, once about half way through the anticipated quarantine period and once near to the end to have test results coincide with the end of the period. All symptomatic HAP must be sampled and tested. The second inspection can serve as the quarantine release survey (see Step 6).  
3) If positives result from quarantine period surveys, return to Steps 2-4, the quarantine remains in place, and the quarantine period starts over. |
| 6) Quarantine Release Survey | Nursery Already in Program | The Quarantine Release Survey is the second of the two quarantine period inspections. It occurs near the end of the quarantine period. This survey includes inspection of plants in the Q radii, and all HAP within the nursery. Sample and test any unhealthy plant tissue. |
| 6) Quarantine Release – Transition from EAN to new or revised Compliance Agreement | Q radii: after 90 days, if the quarantine release survey reveals no symptomatic plants or further positive plants in Q radii, and reveals no further positive standing water or soil in Q radii, release Q radii.  

Note: Standing water, effluent water (culvert, ditch and non-recycled pond water), soil, or cull piles may take longer than 90 days to disinfest/remediate; ensure that avoidance/exclusion mitigations are in place for these positive areas. This short term mitigation and the
longer term remediation plan needs to be written into Exhibit D of the compliance agreement prior to the end of the Quarantine Period.

The end of the Quarantine Period infers the close of the EAN and the beginning of the compliance agreement or the modification of the compliance agreement to contain measures to address *P. ramorum* in the nursery. Close EAN after these four conditions are met:

1) Tests of all symptomatic plants from the quarantine release survey are negative.

2) If standing water, effluent water (culvert, ditch and non-recycled pond water), soil, or cull pile is still positive awaiting remediation, ensure exclusionary/avoidance mitigation is in place, the specific temporary mitigation and the permanent remediation plan is written into Exhibit D of the compliance agreement prior the end of the quarantine period. Contact PPQ National Operations Manager for template and Conformance Instructions.

3) If the irrigation source water was positive, it has been treated and found negative prior to the quarantine release survey, or, an alternate source is in place until the water tests negative. The alternative source and/or sanitation/mitigation plan is written into Exhibit D of the compliance agreement.

4) The new/revised compliance agreement is signed.

| 6) Release at the end of the Quarantine Period (min 90 days) | Nursery Not in the Program | To retain interstate shipping status, or to otherwise distribute plants for interstate shipment (brokered), the nursery must enter into the DA-2014-02 compliance program and follow step 6 above. |
CONDUCT INVESTIGATIONS

Concurrent to notifying and securing the nursery, inform the nursery to research shipments of the past 6 months for the trace forward and trace back investigation.

Nursery Sites:

Determine whether additional locations (nursery sites) are maintained by the same nursery owner, or if HAP moved to other sites or between sites during the 6-month time period preceding the positive detection.

- **Equipment or other article:** Determine if equipment or other articles used at the site is shared with other nursery sites or field areas. Document any shared equipment, used containers, tools, etc., in different nursery sites or field areas. Equipment movement without appropriate biosecurity measures (See Appendix 9) between nursery sites requires that all nursery sites utilizing the equipment be included under this protocol.

- **Plants:** Determine if HAP moved between sites. If so, then all sites receiving HAPs must be included under this protocol.

Trace Forward Investigation:

Once *P. ramorum* is confirmed in a nursery, it is necessary to determine if the nursery has shipped plants that could be potentially infected. The first step of the trace forward investigation is to determine if the nursery is required to notify under Federal Order DA-2012-53, see Appendix 11 for further instructions.

Initiate the trace forward investigation by identifying all interstate and international shipments in the six (6) months prior to the 1st confirmed plant positive. Identify shipments of the following plants: 1) the infected plant species, variety or cultivar, 2) all HAP which originated in the destruction block, and, 3) any plants of the High Risk Genera: *Camellia, Rhododendron, Pieris, Viburnum* and *Kalmia* regardless of their location in the nursery. This combination of shipped plants is referred to as the **High Priority Trace Forward/Trace Back Target Plants**. These plants, shipment date(s), quantities and respective destinations make up the **Trace Forward List.** Identify these High Priority plants using the best available information and to the lowest taxonomy possible (for example, if the plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level).

Forward the Trace Forward List(s) to PPQ’s National Operations Manager within ten business days. Reporting trace forwards within ten days is essential. If requested or necessary, Smuggling Interdiction and Trade Compliance (SITC) or Investigative and Enforcement Services (IES) may be asked to assist in the information gathering, as appropriate.

The National Operations Manager will forward domestic lists to the States that have received plants. The National Policy Manager will inform international trading partners of shipments to their countries. The plants sent to the receiving States are to be inspected at the receiving nurseries (trace forward sites). Once the trace forward information is collected and communicated to the
receiving states (or counties), the receiving regulatory officials will implement the Trace Forward Protocol, see
protocol.pdf

Trace Back Investigation

Once *P. ramorum* is confirmed in a nursery, determine if the positive plant(s) was shipped in from another nursery. Trace Back plants include all plants of the same taxon (i.e., species, hybrid, variety, or cultivar) of the infected plant regardless of size, location or lot. The trace back should continue to the original propagation source, if still existing. Forward the Trace Back List(s) to PPQ’s National Operations Manager within ten business days. For the Trace Back Protocol, see
ocol-10-09.pdf

Alternative Quarantine Release Strategy:

A nursery may avoid a quarantine period, through a voluntary management decision. This can occur if the following conditions are met:

1) Steps 2-4 in Table 2 (CNP procedures) and all steps in Table 3 (Disinfest the Nursery) above are complete
2) All plants, pots, media, debris, etc. in the D radii and all HAP and their pots, media, debris, etc., in the Q radii are destroyed
3) The CCP Assessment is complete
4) Standing water, effluent water (culvert, ditch and non-recycled pond water), soil, or cull piles may take some time to disinfest/remediate; ensure that avoidance/exclusion mitigations are in place for these positive areas. The short term mitigations and the longer term remediation plan are written into Exhibit D of the compliance agreement.
5) Compliance Agreement contains measures to address *P. ramorum* in the nursery, and the agreement is signed.

Post CNP Monitoring

Nurseries under this protocol will be required to meet the requirements of the DA-2014-02 Inspection and Sampling protocol and obtain or retain a compliance agreement. Monitoring is set forth in the Inspection and Sampling Protocol and is agreed to within the compliance agreement. Under the compliance program, if a nursery in the regulated or non-regulated area tests negative after three years (all samplings (six or >) during conducive time), it is no longer regulated. For nurseries in the Quarantine area, the sampling returns to the 7 CFR 301.92 regime.
Appendix 1- APHIS List of Regulated Hosts and Associated Plants
Revised August, 2013

A current list may be found at the USDA APHIS PPQ website at

You should consult it for the latest list of plants before beginning any survey, inspection or
delimitation. There are five plant genera most frequently reported to be infected by \textit{P. ramorum} in U.S. nursery settings. These hosts are considered “high risk”; be sure to inspect
these five genera while sampling HAP in the nursery. The high risk genera are: \textit{Camellia},
\textit{Rhododendron}, \textit{Viburnum}, \textit{Pieris}, and \textit{Kalmia}. \textbf{Collect samples from all symptomatic HAP},
and at the inspector’s discretion, all other non-host plant tissue with symptoms suggestive of
\textit{P. ramorum} (see Appendix D: Symptoms Associated with \textit{P. ramorum}).

For the Biology of \textit{Phytophthora ramorum}:
a.pdf
Appendix 2 - Schematic of Destruction and Quarantine Radii

D and Q radii for positive plants, positive standing water, or positive soil or other articles as appropriate.

<table>
<thead>
<tr>
<th>Area</th>
<th>Name</th>
<th>Once D and Q radii are flagged, then:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red (*1)</td>
<td>Destruction radii</td>
<td>Destroy all plants, containers, and leaf debris</td>
</tr>
<tr>
<td>Yellow (*2)</td>
<td>Quarantine radii</td>
<td>Hold HAP from sale for 90 days; if there is non-HAP mixed in it can be taken out and moved. For water or soil positive, the Q radii is 1 meter.</td>
</tr>
<tr>
<td>Red (*3)</td>
<td>Destruction radii</td>
<td>Destroy all plants; non-HAP nursery stock could still move the pathogen</td>
</tr>
<tr>
<td>Yellow (*4)</td>
<td>Quarantine radii in Non-Hap Block</td>
<td>Release non-HAP for sale if found symptomless during the delimiting survey</td>
</tr>
<tr>
<td>Blue (*5)</td>
<td>The rest of the HAP block</td>
<td>Release all plants for sale only found symptomless during the delimiting survey</td>
</tr>
<tr>
<td>White (*6)</td>
<td>The rest of the non-HAP block</td>
<td>Release all plant material for sale if found symptomless during the delimiting survey</td>
</tr>
</tbody>
</table>
Appendix 3 - Contact List for APHIS-PPQ

Prakash Hebbar
National Policy Manager
USDA APHIS PPQ
Riverdale, MD 20737
Tel: (301) 851-2228
Fax: (301) 734-8584
Email: prakash.hebbar@aphis.usda.gov

Stacy Scott
National Operations Manager
USDA APHIS PPQ
Fort Collins, CO 80526
Tel: (970) 494-7575
Fax: (970) 494-7501
stacy.e.scott@aphis.usda.gov

Russ Bulluck
Associate Director, Science & Technology
Center for Plant Health Science and Technology
USDA APHIS PPQ
Raleigh, NC 27606
Tel: (919) 855-7646
Fax: (919) 855-7480
russ.bulluck@aphis.usda.gov

<table>
<thead>
<tr>
<th>PPQ Diagnostic Labs</th>
<th>Sample Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dr. Craig Webb</strong></td>
<td><strong>USDA-APHIS-PPQ-CPHST</strong></td>
</tr>
<tr>
<td>USDA APHIS PPQ</td>
<td><strong>BARC-East, Bldg. 580</strong></td>
</tr>
<tr>
<td>Diagnostic Laboratory</td>
<td><strong>Powder Mill Road</strong></td>
</tr>
<tr>
<td>Kansas State University</td>
<td><strong>Beltsville, MD 20705-2350</strong></td>
</tr>
<tr>
<td>4024 Throckmorton Plant Sciences Center</td>
<td><strong>(301) 313-9204</strong></td>
</tr>
<tr>
<td>Manhattan, KS 66506</td>
<td><strong><a href="mailto:APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov">APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov</a></strong></td>
</tr>
<tr>
<td>(785) 532-1349</td>
<td></td>
</tr>
<tr>
<td><a href="mailto:craig.a.webb@aphis.usda.gov">craig.a.webb@aphis.usda.gov</a></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 4 – Delimitation Sampling for Plant Positives

The delimitation survey begins once the laboratory reports positive results to the Inspector. Conduct delimitation inspection and sampling after (on the same day(s)) the D and Q radii are established. The delimitation survey instructions for all other articles besides plants are provided in Table 2 above.

Reminder: Decontaminate personnel, tools, and equipment between blocks in the nursery, between host genera within a nursery, and between nurseries. Wear rubber boots or other waterproof boots without crevices. Sanitize or change gloves between samples. Use a spray bottle containing a dilute (10%) bleach solution, a quaternary ammonium solution at labeled rates, or spray Lysol (with ETOH) to treat all tools between samples. Brush loose dirt from boots then spray boots with disinfection solution in spray bottle, or use foot bath, between nursery blocks. Decontaminate all equipment you use between each sample and before leaving a nursery.

When delimiting due to positive detection in plants, focus on sampling the individual blocks where the positive plants were found. Follow the sampling method below for each individual block that had a positive(s). Inspectors should sample all symptomatic material present. Each sample should consist of a minimum of 2 in² of symptomatic plant tissue. The number of plants that need to be sampled will depend on the total number of plants in the positive nursery block.

Collect samples from all symptomatic HAP, and at the inspector’s discretion, all other non-host plant tissue with symptoms suggestive of P. ramorum (see Appendix D: Symptoms Associated with P. ramorum). Foliar symptoms of P. ramorum infection are highly variable and can range from pinpoint discolorations on the leaf surface to large “V” shaped lesions along the leaf mid-vein. Include inspection of the lower portions of plants where conditions favoring P. ramorum would be present. Moisture will tend to be present for longer periods of time on the plant surfaces on the lower portions of the plants also resulting in higher humidity. Shading on the lower portions of the plants can promote cooler temperatures and offer protection from the effects of UV rays on spores. Pay attention to leaf areas where water would run off or persist the longest such as the midrib and leaf tips. In some hosts (Camellia and Rhododendron) low rates of infection can cause premature leaf drop, yielding infected plants that appear to be asymptomatic. As a result, leaves found in the pot should also be checked for possible symptoms and collected for laboratory analysis.

Determine the minimum number of symptomatic plant samples of HR and other HAP to take within a positive nursery block:

Samples should be targeted, not random. Inspectors should sample all symptomatic plant material present, including leaf tissue from the pots. Or, if necessary, from the ground immediately adjacent to a given plant if the inspector is absolutely certain the leaves are from the plant.
Table A4. Minimum number of samples to take based on the number of HAP within a positive nursery block.

<table>
<thead>
<tr>
<th>HAP in Nursery Block</th>
<th>Minimum Number of Samples to Take in Block (95% Confidence Limit of Detecting 2.0% Disease*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>140</td>
</tr>
<tr>
<td>300</td>
<td>156</td>
</tr>
<tr>
<td>400</td>
<td>165</td>
</tr>
<tr>
<td>500</td>
<td>172</td>
</tr>
<tr>
<td>600</td>
<td>176</td>
</tr>
<tr>
<td>700</td>
<td>179</td>
</tr>
<tr>
<td>800</td>
<td>181</td>
</tr>
<tr>
<td>900</td>
<td>183</td>
</tr>
<tr>
<td>1,000</td>
<td>184</td>
</tr>
<tr>
<td>2,000</td>
<td>191</td>
</tr>
<tr>
<td>3,000</td>
<td>193</td>
</tr>
<tr>
<td>4,000</td>
<td>195</td>
</tr>
<tr>
<td>5,000</td>
<td>196</td>
</tr>
<tr>
<td>6,000</td>
<td>196</td>
</tr>
<tr>
<td>7,000</td>
<td>196</td>
</tr>
<tr>
<td>8,000</td>
<td>196</td>
</tr>
<tr>
<td>9,000</td>
<td>197</td>
</tr>
<tr>
<td>10,000</td>
<td>197</td>
</tr>
<tr>
<td>20,000</td>
<td>197</td>
</tr>
<tr>
<td>30,000</td>
<td>197</td>
</tr>
<tr>
<td>40,000+</td>
<td>199</td>
</tr>
</tbody>
</table>

*Numbers are the minimum number of host and associated host plants that must be sampled in a positive nursery block to ensure detection at a 95% confidence level when the disease is present in 2.0% of the plants, when 75% of infected plants express disease symptoms.

Using a permanent marker, label the sample bag: date, sample number, genus species cultivar, collector’s identification, location of sample collection, etc. Mark the sampled plant with flagging tape, stake, etc., and label with the corresponding sample number. This will facilitate any additional work in the event of a positive or the need for a second sample. See sample handling and submission instructions below.
SAMPLE HANDLING AND SUBMISSION PROTOCOL

• Always write out the identifying label remarks on the outside of the bag with a permanent marker.
  o Attach labels on the outside of bags since labels inside the bag may deteriorate due to moisture and become illegible.
  o Include on all labels with a permanent marker: time, date, collector’s identification number, location of sample site, sample number.
• Do not add extra moisture to the sample to keep it fresh. Do not wrap leaves in paper towels when shipping. Extra moisture/paper towels speed deterioration of the sample. Sanitize or remove gloves and place sample bag in a second protective bag.
• To provide extra insurance against accidental release during shipping, the labeled specimen bags should be double-bagged – i.e. first place the specimen in a self-locking labeled plastic bag and then place that labeled specimen bag(s) within a second self-locking plastic bag. **The Form 391 (or state equivalent) should be placed inside the outer bag**
• Samples should be placed in a cooler out of the sun as soon as possible. When sampling large areas, coolers should be brought out to the sampling areas. Samples can heat up quickly when placed in plastic bags in sunlight for even short periods of time. If it is not possible to have coolers in the area of sampling, place the samples in a shaded area until they can be collected and placed in a cooler as soon as possible.
• Refrigerate samples while awaiting shipment. Place double-bagged samples in a sturdy cardboard box or heavy Styrofoam container so that the samples are not damaged during shipping and handling. Ship with an ice pack with some buffer between ice and leaves. Thoroughly seal all seams on the container with shipping tape. Mail or deliver the sample to the laboratory as soon as possible to preserve freshness (if mailing use overnight mail). Do not ship on Fridays. It is better to hold them in the refrigerator over the weekend than to have them sit over the weekend in unknown environmental conditions.

Sample Submission Information

Follow the laboratory’s standard operating procedure (SOP). Typically, have ready the required information: 1) tracking number, 2) number of samples being shipped, 3) the disease being tested for. All samples must have either a completed PPQ Form 391 or equivalent state documentation. The lab may be your NPPLAP accredited State Lab, cooperating NPDN lab, or an APHIS PPQ lab (see Contact List at the end of this document). If submitters aren’t sure which lab to send samples to, contact your State Plant Health Director.

If the PPQ391’s are electronic, they can be emailed when notifying the lab about the pending shipment, but attach a hardcopy to the sample. Fill out blocks 1-5, 7, 10, 11, 16, 22, 23 (see green circled items below) in the PPQ Form 391 instruction sheet.
Sample Forwarding and Reporting Under DA-2014-02

Nursery plant samples within the *Phytophthora ramorum* program that are ELISA or ImmunoStrip positive for the genus *Phytophthora*, must be forwarded to your cooperating NPPLAP accredited lab and/or to an APHIS diagnostic lab to determine if the species is *P. ramorum*. Every initial sample from nurseries in non-regulated areas must be forwarded to APHIS for confirmatory testing. If that sample is Confirmed Positive by APHIS, all subsequent samples may be diagnosed by any NPPLAP accredited lab.

For labs with federal confirmation authority, the lab must report positives to the SPRO/SPHD, then to the *P. ramorum* Field Operations Manager, within 24 hours of the diagnostic result. All subsequent positive samples taken at a Confirmed Positive Nursery must be reported to the SPRO/SPHD, then to the *P. ramorum* Field Operations Manager, within 24 hours of the diagnostic result.
Instructions

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

<table>
<thead>
<tr>
<th>BLOCK</th>
<th>INSTRUCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. Assign a number for each collection beginning the year, followed by the collector’s initials and collector’s number.</td>
</tr>
<tr>
<td></td>
<td><strong>EXAMPLE</strong> In 2031, Brian K. Long collected his first specimen for determination of the year. His first collection number is D1-BLK-001</td>
</tr>
<tr>
<td>2</td>
<td>2. Enter the collection number</td>
</tr>
<tr>
<td>3</td>
<td>Enter date</td>
</tr>
<tr>
<td>4</td>
<td>Check block to indicate Agency submitting specimens for identification</td>
</tr>
<tr>
<td>5</td>
<td>Enter name of sender</td>
</tr>
<tr>
<td>6</td>
<td>Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)</td>
</tr>
<tr>
<td>7</td>
<td>Enter address</td>
</tr>
<tr>
<td>8</td>
<td>Enter name and address of property owner</td>
</tr>
<tr>
<td>9</td>
<td>BA-3L Check all appropriate blocks</td>
</tr>
<tr>
<td>10</td>
<td>Leave Blank</td>
</tr>
<tr>
<td>11</td>
<td>Enter scientific name of host, <strong>Genus</strong> Species, particularly <strong>Cultivar</strong> name</td>
</tr>
<tr>
<td>12</td>
<td>Enter quantity of host and plants affected</td>
</tr>
<tr>
<td>13</td>
<td>Check block to indicate distribution of plant</td>
</tr>
<tr>
<td>14</td>
<td>Check appropriate blocks to indicate plant parts affected</td>
</tr>
<tr>
<td>15</td>
<td>Check block to indicate pest distribution</td>
</tr>
<tr>
<td>16</td>
<td>Enter sampling method</td>
</tr>
<tr>
<td>17</td>
<td>Enter type of trap and lure</td>
</tr>
<tr>
<td>18</td>
<td>Enter trap number</td>
</tr>
<tr>
<td>19</td>
<td>Enter X in block to indicate isolated or general plant symptoms</td>
</tr>
<tr>
<td>20</td>
<td>Enter X in appropriate block for weed density</td>
</tr>
<tr>
<td>21</td>
<td>Enter X in appropriate block for weed growth stage</td>
</tr>
<tr>
<td>22</td>
<td>Provide a brief explanation if Prompt or URGENT identification is requested</td>
</tr>
<tr>
<td>23</td>
<td>Enter a tentative determination if you made one</td>
</tr>
<tr>
<td>24</td>
<td>Leave blank</td>
</tr>
</tbody>
</table>

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:
1. Send original along with the sample to your Area Identifier.
2. Retain and file a copy for your records.
Appendix 5 - Diagnostics

For questions about diagnostic work instructions, contact Dr. Mark Nakhla at USDA-APHIS-PPQ-CPHST Laboratory in Beltsville, MD. BARC-East, Bldg. 580, Powder Mill Road Beltsville, MD 20705-2350 (301)313-9204 Mark.K.Nakhla@aphis.usda.gov

For the National Plant Pathogen Laboratory Accreditation Program (NPPLAP), contact Dr. Patrick Shiel, (919) 855-7416. Shiel, Patrick J - APHIS Patrick.J.Shiel@aphis.usda.gov
Appendix 6 - Soil and Container Mix Protocol

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

See the *P. ramorum* PPQ Website for latest approved Soil and Container Mix Protocol.
Appendix 7 - Water Sampling Protocol
Revised March 22, 2014

See the Inspection and Sampling Protocol APHIS Phytophthora ramorum Program Response page for the latest approved protocol. Or here:
Appendix 8 - List of Treatment and Disinfection Options

The following techniques have been determined to be efficacious for control of *P. ramorum* in nurseries found to contain sample sites positive for *P. ramorum*. Any of these options may be required to mitigate *P. ramorum* infestations, providing that the options are appropriately labeled for use in the state. A systems approach to treatment should be considered rather than relying on just one method. Always follow label directions when applying any chemical treatment.

**Infected Plants and associated potting mix and containers:**

*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, double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority, or steam sterilized. All tools or materials used for cleanup or movement of material should be properly disinfected. All personnel involved with cleanup should follow appropriate procedures to prevent unintentional movement of inoculum. All movement of material on the nursery site should follow appropriate procedures to safeguard movement to prevent contamination of other areas and methods of conveyance.

**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 mm 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:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored should be treated with steam or dry heat commonly heated to internal temperatures of 176°F (80°C) for 60 minutes (steam) or 120 minutes (dry heat), or as otherwise detailed in the USDA Treatment Manual for “plant pathogenic fungi and bacteria”, Schedule T521.


*Note:* Associated containers (i.e. pots and trays) may be treated for re-use. Guidelines for safeguarding should be determined and approved to prevent movement or potential contamination of *P. ramorum* to non-infected areas of the nursery operation. All potting mix should be removed to the extent possible prior to treating. Dispose of the potting mix using one
of the above methods. Containers may be sterilized by treatment with steam at or above 122°F (50°C) for 30 minutes or more (most containers will withstand 140 to 160°F) or other approved disinfectant methods for the correct contact time.

**Disinfection of 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://www.ehs.columbia.edu/decon.html](http://www.ehs.columbia.edu/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. The contact time for the products must be followed to ensure efficacy. If the surface dries before the contact time is reached, the surface should be rewetted until the contact time is achieved.

### Summary of Disinfectant Activities

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

Other systems which can be approved for treating water can include one or a combination of the following: Bromine, Chlorine, Sodium hypochlorite, Calcium hypochlorite, Chlorine dioxide, Ozone, activated peroxygen, ultraviolet radiation, copper ionization, heat treatment/pasteurization, filtration.

Disinfestation of Soil and Container mix:

**Container mix:** Container mix must be heated such that the temperature in the center of the load reaches at least 60°C (140°F) for 30 minutes. Heat treatment must be conducted in the presence of an inspector.

Fumigation may be the most efficacious and economical option to disinfect container mix.

**Soil/Container mix in pots:** Soil must be heated such that the temperature in the center of the load reaches at least 60°C (140°F) for 30 minutes. Heat treatment must be conducted in the presence of an inspector.

**Soil in situ or in nursery beds**
Nursery beds can be treated with steam following specific protocols to ensure heating of soil to 50°C (122°F) for 30 minutes. Soil water content should be checked prior to steaming to avoid uneven heating. Monitor treatment with thermocouples placed in locations throughout the bed (30 cm deep) with sensors placed in locations most likely to reach temperature the slowest. A steam sock is placed on the nursery bed and covered with a tarp which is sealed on the edges with sand snakes. Concrete blocks can be used to support the tarp off of the bed surface. Timing of treatment begins when the last sensor reaches the target temperature of 50°C. Steam treatment must be conducted in the presence of an inspector.

Steam sock in place on soil surface

Concrete blocks used to support tarp from surface to allow steam distribution from steam sock. Tarp with sand snakes in place to secure edges.

(Treatment of soil through the utilization of solarization is being developed. Consult the regulatory agencies in your state for further information and guidance if you are considering solarization treatment of infested nursery beds.)

Fumigation may be the most efficacious and economical option to disinfect soil. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of Phytophthora spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of Phytophthora cinnamoni in citrus groves.
However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is currently being evaluated as an option for soil treatment.

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**

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

**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. When soil treatment is absolutely impossible due to human health issues, certain soil hydrologic conditions or due to city, county, or state regulations, avoidance and exclusionary methods may be used on a case by case basis for nurseries under a compliance agreement.

**Equipment and Personnel (Inspectors and employees):**

Rubber boots or other very smooth and crevice-free waterproof boots are strongly encouraged. Access to infested areas and hold areas should be limited, as much as possible, to officials and necessary employees. Rubber boots are strongly encouraged, other footwear is too porous. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block and disinfect footwear. Those working with, or in contact with suspected infested material (including plants), must wear gloves and remove them or rub/wash them with...
an approved disinfectant between samples and between blocks. 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 D radii and Q radii 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 and in accordance with label directions.

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

In the course of daily work, nursery personnel are frequently required to visit a number of different nurseries sites, greenhouses, fields, and facilities. These actions could potentially provide a pathway for transferring quarantine organisms from one work site to another during the work day. It should also be recognized that even if a single work site is visited per day, precautions must be taken to avoid contaminated clothing and equipment from being used at a new site the following day. Further, visitors to these same facilities present the same risks and additionally could vector disease-causing-organisms from other sites.

Biosecurity measures must be taken by nurseries and be required of nursery personnel and visitors to avoid and mitigate the spread of *P. ramorum*. The biosecurity measures described here are the minimum measures to be taken by the nursery.

**Communications**

All nursery personnel should be trained and visitors informed of these biosecurity requirements that have been put in place by the facility. As new scientific data and technology is learned, the facility needs to update their biosecurity requirements and retrain their personnel.

**Vehicles**

Vehicles can become contaminated with soil; a primary vector for quarantine pests. The following guidelines seek to reduce the likelihood of this pathway.

**Avoidance:**

Once at the inspection site, if possible, the vehicle should only be driven and parked on paved, concrete or gravel areas to avoid contact with soil and organic matter. Visitors should consider requesting a facility employee to drive them to their designated location with one of the nursery’s vehicles. Loading of nursery stock onto other than the nursery’s vehicles should be done in an area with concrete or asphalt pad located near the gate and not in the interior of the nursery.

**Cleaning:**

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

Where it is not possible to avoid the vehicle going onto the fields, the vehicle must be driven to the edge of the facility where the tires, wheel wells and accessible areas of the undercarriage of the vehicle must be cleaned of soil and organic matter with a brush or a water hose followed by a spray down with a suitable disinfectant. In situations where the undercarriage has been coated with soil it is recommended that after cleaning and disinfecting at the work site an effort be made to go through a car wash that has the ability to clean the undercarriage before proceeding to another work site. If a car wash is not available, avoid driving onto the next work site. To ensure
the entire surface of the tires are cleaned it will also be necessary to move the vehicle forward a foot or so to permit cleaning of the portion of the tire in contact with the ground.

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

A portion of the vehicle must be designated as a “clean area” where clean work supplies and equipment can be kept. A designated “dirty area” of the vehicle such as the trunk of the car or a specified enclosed area of a truck bed must also be identified for use to hold double bagged clothes or dirty equipment that require cleaning. In situations where pool vehicles are used, the work site should adopt a set procedure for all personnel.

Nursery Personnel

Nursery personnel routinely come in contact with potentially contaminated soil, plants and organic matter and this requires the personnel to address a number of biosecurity measures. If the inspection site has distinct levels of biosecurity for different areas in the nursery, it is necessary to be aware of this situation. Work should always be completed working from the areas of lowest to highest risk.

Access:

Access to infested areas and hold areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block and use a disinfection spray on foot wear or use a foot bath. Those working with, or in contact with suspected infested material (including plants), must wear gloves and rub them with an approved disinfectant between samples and between blocks. There are no products currently labeled for use on porous materials for Phytophthora control.

- Personnel should not have access to production areas of the nursery after entering the destruction block on the same day.

- A disinfectant foot bath should be placed and used by personnel entering and exiting the quarantine area and entering and exiting the destruction block at the infested nursery site, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with dirt or debris, in accordance with label directions.

- Do not visit other nursery sites in potentially contaminated work clothing and footwear.

Boots:

Rubber boots are strongly encouraged when working in the infested area(s). Water proof, smooth boots which can be disinfected must be worn. As the last choice, disposable water proof
boot covers can be worn over work boots. 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 between the infested block and other blocks, and prior to placing them into the vehicle area designated as a “clean area”. Dispose of used boot covers by double bagging and place into the designated “dirty area” of the vehicle for proper disposal. After removing boot covers, the soles of the work boots must be inspected for soil and if present, must be cleaned of soil and treated with disinfectant.

**Hands:**

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

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

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

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

If antiseptic rubs/gels/rinses are used, avoid formulations with moisturizers as they leave a gummy residue. Disposable gloves may be used, however they have the tendency to rip and become uncomfortably wet after a short period. Rubber gloves which withstand more abuse than disposable gloves have the same drawbacks as disposable gloves; however will be more practical when handling materials that are sharp or jagged. If rubber gloves are used in cold weather it is recommended to purchase rubber gloves with cotton or acrylic liners. Both disposable and/or rubber gloves must be double bagged after use if working in an infested area and placed into the “dirty area” of the vehicle for disposal or cleaning. If on-site disposal of the gloves are available this option should be chosen. After disposal of gloves, hands must be washed or sanitized. To avoid cross contamination, disinfection of hands must take place after handling any plants or other contaminated matter in the infested area.

**Equipment**

Any equipment used (pruners, measuring tapes, clipboards, pens, etc.) at a work site must be
disinfected prior to leaving the work site. Where practical, equipment should be disinfected as frequently as possible at each work site. Where equipment must leave the work site for disinfection it must be double bagged and place in the designated “dirty area” of the vehicle.

**Sampling Supplies and Equipment Check List**


- Nursery maps and nursery inventory
- Clipboard or PDA, PPQ 391 forms (or state equivalent) nursery inspection and sampling forms, paper, etc.
- Camera
- Writing pen
- Permanent marking pen
- Disposal gloves
- Hand sanitizer to sanitize gloves between samples
- Rubber boots
- Pruners to sample twigs and branches
- GPS (optional)
- Spray bottle of an approved disinfectant for *P. ramorum*
- Re-sealable plastic sample bags
- Bigger collection bag to carry samples in while inspecting and sampling
- Cooler, coolant, and newspapers to keep samples cool until mailed
- Larger bags for mailing samples (must arrive in lab double-bagged)
- Box for mailing samples
- Flagging, pin flags, or label sticks to mark sampled plants/blocks
- Foot bath bin
- Quaternary ammonium solution or other approved disinfectant, at labeled rates 1” deep in bath.
- Toilet brush or other stiff brush for scrubbing dirt off shoes

**Supplies and equipment checklist for Water Samples**

**Bottle of Bait Materials List**

- 1-liter bottles per sample site (Nalgene wide mouth polypropylene provided by diagnostic laboratory).
- Multiple 100-ml plastic measuring cup or beaker and large capacity (100ml) syringe per water sample site. Disposable paper cups are also suitable for collecting water
- Ice chest cooler
- Rhododendron leaves provided by laboratory.
- Hand held paper hole-punch (heart shape preferred).
Appendix 10 – Reserved
Appendix 11 – Notification Requirements

Notification Federal Order DA-2012-53 Instructions for Regulated and Quarantine Areas

The Notification Federal Order of December 10, 2012 remains in effect and is slightly altered by Federal Order DA-2014-02. What has changed is what is meant by the terms “positive, negative” and “release from CNP”. The FO reads: a nursery in the regulated area that is under the Confirmed Nursery Protocol, or within two years of release, must notify for interstate shipments of the high risk genera. Once samples test negative for two years, the nursery is released from the requirement. Under DA-2014-02, the term positive refers to any positive article in the nursery. Negative for two years means all articles are negative*. The two year clock for release from the notification requirement starts when all articles test negative* from the latest sampling session.

*Note: If the nursery has positive soil (standing water in lieu): Eradication options must be documented and attempts must be made if possible. In cases where chemical/steam treatments are not possible or effective due to physical/hydrological conditions, or due to environmental or human health regulations, the nursery owner must take exclusion/avoidance measures of a permanent nature to mitigate the presence of _P. ramorum_ in the soil. The inability to disinfect soil for these reasons must be documented. The alternative solution must be written into the compliance agreement and implemented. After three years, the inspector will assess the efficacy of the exclusionary mitigation measures, i.e., that the nursery system permanently works to exclude all articles and equipment associated w/ nursery stock from the infested area. Nurseries in this situation would have to notify into perpetuity due to positive soil; after 3 years, if the exclusionary measures remain efficacious, and are of a permanent nature, the nursery may be released from the notification requirement by the inspector.
Appendix 12 – Critical Control Point (CCP) Assessment Procedures  
For *P. ramorum* Positive Nursery Sites

Following the positive confirmation of *P. ramorum* at a nursery site, and the completion of the delimiting survey, a Critical Control Point assessment of the nursery operation will be conducted by a team composed of state, federal, and other subject matter experts. CCPs are points in a nursery process or procedure which can result in the spread or introduction of the pathogen *P. ramorum*. Through determination of CCPs, applicable BMPs and/or mitigations to implement can be provided to the nursery. By addressing CCPs, nurseries can reduce the risk of a potential hazard, and take corrective steps which lead to mitigation and/or avoidance of *P. ramorum*.

Planning the CCP Assessment

The CCP Assessment Team is to include at least two members that participated in the inspection and sampling event that detected a positive in the nursery. Coordinate the scheduling of the assessment with the nursery owner and team well in advance.

In order to initiate the assessment, the team will consider any available information prior to arrival. It may be necessary to gather certain information prior to walking the site or other clarification may be necessary following examination of the positive areas. Once at the site, the assessment team will gather additional information through discussions with nursery owners and managers, the inspection sampling team, and potentially county, state and federal personnel with knowledge of the nursery. After deliberating on an approach, the assessment team will walk the nursery site focusing on the known positive sample areas. At the time of the site visit, not all nursery processes or situations which may be seasonal in nature or scheduled may be present.

CCP Assessment Day

Initially, the CCP assessment will focus on the areas of the nursery associated with positive *P. ramorum* samples and the types of samples which were positive. Areas which have been shown to deserve focus from previous research of nursery pathogens include plants (source, movement, propagation, and management), water, soil, and pots. Based on positive samples, divide the nursery into grids or zones to identify CCPs specifically for these areas of the nursery. However, the assessment of the nursery operations may identify CCPs which relate to larger areas or processes of the nursery beyond the zones associated with positive samples.

Information obtained prior to or early during the CCP assessment. Some of these data are obtained prior by the inspection and sampling team. Confer with those personnel. As each situation is unique, this list is not meant to be inclusive of information to consider.

1) Maps of the nursery, with bed layouts if possible
   a. Maps of the surrounding area for assessment planning purposes

2) Site assessment
   a. Perimeter: Look for possible sources of inoculum (water or hosts)
   b. History of site:
      i. Prior ownership
ii. Prior crops on site
iii. Plants grown in ground previously
iv. Failed crops or plants (specifically hosts)
v. History of prior positive detections
vi. History of pesticide use
vii. History of flooding on site
viii. Weather patterns, rainfall, etc.
ix. Location of high risk areas

3) Access to property
   a. Nursery rules for people entering the nursery site
   b. Public vs. Landscapers access
   c. Logs or records of who has been on site
   d. Movement of plants and equipment between properties

4) Production practices/standard operation procedures
   a. Crop rotation practices
   b. Tracking movement of hosts on site
   c. Are high-risk hosts treated or handled differently?
   d. Are holdovers mixed in with new stock or plants moved to fill in blocks?

5) Training
   a. Staff
      i. Has there been any training for P. ramorum symptomology, scouting?
      ii. Are workers equipped with flagging as standard equipment to mark plants after scouting

Figure 1 is the checklist for an assessment or walk through of a positive nursery site. This checklist may be used by the assessment team or may be used prior to arrival of the assessment team for information gathering purposes to focus the efforts and optimize the time of the assessment team.
Figure 1. Assessment Checklist for determining CCPs of *P. ramorum* positive nursery sites

<table>
<thead>
<tr>
<th>Nursery Name</th>
<th>Nursery License Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location:</th>
<th>Phytophthora <em>ramorum</em> hosts present:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐ Rhododendron ☐ Camellia ☐ Kalmia ☐ Viburnum</td>
</tr>
<tr>
<td></td>
<td>☐ Pieris ☐ Other</td>
</tr>
</tbody>
</table>

### I. HOST PLANT BUY-INS AND SALES

- Records kept for sales:
  - ☐ Plants shipped/received
  - ☐ Origin of plants
  - ☐ Received contact info
  - ☐ Shipment date(s)
  - ☐ Invoice number
  - ☐ Number of plants shipped
  - ☐ Fungicide records
  - ☐ Kept 24-months
- BMP for buy-ins:
  - ☐ Inspected on arrival
  - ☐ Isolated for 30-days
  - ☐ Trucks cleaned on arrival
  - ☐ Loading dock cleaned after delivery
  - ☐ Returns treated like buy-ins
  - ☐ Other
- Comments:

### II. GENERAL SANITATION

- Loading dock:
  - ☐ Clean
  - ☐ Muddy
  - ☐ Debris
  - ☐ Other
- Plant debris present, pathways, beds:
  - ☐ Heavy
  - ☐ Medium
  - ☐ Light
  - ☐ Other
- Cull pile:
  - ☐ Isolated
  - ☐ No drainage
  - ☐ Off-site
  - ☐ Other
- Comments:

### III. POTTING MEDIA

- Sourced from:
  - ☐ Commercial
  - ☐ In-house
  - ☐ Commercial/In-house
  - ☐ Other
- Storage:
  - ☐ Concrete
  - ☐ Asphalt
  - ☐ Soil
  - ☐ Other
- Components:
  - ☐ Bark
  - ☐ Used media
  - ☐ Pumice
  - ☐ Peat
  - ☐ Other
- Treated:
  - ☐ Yes
  - ☐ No
  - If treated, type:
- Comments:

### IV. POTTING AREA

- How potted:
  - ☐ Machine
  - ☐ Hand
  - ☐ Both
  - ☐ Other
- Sanitation:
  - ☐ Clean tools
  - ☐ Limit traffic
  - ☐ Dedicated tools
  - ☐ Other
- Pots:
  - ☐ New
  - ☐ Cleaned
  - ☐ Sanitized
  - ☐ Other
- Comments:

### V. PLANTS GROWN IN CONTAINERS

- Pots placed on:
  - ☐ Gravel
  - ☐ Native soil
  - ☐ Benches
  - ☐ Other
- Gravel depth:
  - ☐ ≥ 4”
  - ☐ ≤
  - ☐ Compacted
  - ☐ Other
- Sanitation:
  - ☐ Plant debris
  - ☐ Weeds
  - ☐ Re-used pots
  - ☐ Other
- Comments:

### VI. IRRIGATION WATER*

- Source:
  - ☐ Well
  - ☐ Municipal
  - ☐ River
  - ☐ Recycling pond
  - ☐ Other
- Type:
  - ☐ Overhead
  - ☐ hose
  - ☐ Microsprinkler
  - ☐ Drip
- Treatments received:
  - ☐ Yes
  - ☐ No
  - If yes, type:
- Comments:

### VII. SOIL DRAINAGE

- Standing water present:
  - (check all that apply)
  - ☐ Plant blocks
  - ☐ Greenhouses
  - ☐ Roadways
  - ☐ Media piles
  - ☐ None
  - ☐ Other
- Comments:
After the information gathering and site assessment are completed, the team will identify the CCPs associated with the known positive sample sites and may include other components of the nursery operation. Remediation measures, mitigations and BMP options which correspond to the CCPs will be researched by the nursery owner with the assistance of the local County or university extension, state or nursery association nursery practices manuals and the inspector. The state and PPQ regulatory officials/subject matter experts will provide as much information as possible about such measures to assist the nursery owner. Note to CCP Assessment Team: Standing water, effluent water (culvert, ditch and non-recycled pond water), soil, or cull piles may take longer than 90 days (The Quarantine Period) to disinfect/remediate; if the CCP assessment team makes specific mitigation recommendations, identify short term mitigation versus longer term remediation. Both short term and long term measures chosen by the nursery owner/inspector will be written into Exhibit D of the compliance agreement with appropriate time periods.

After consideration of the recommended measures, the nursery and Inspector will determine which measures to implement to specifically address the known positive sample sites. The nursery may also suggest its own measures for consideration to address specific CCPs. The agreed upon measures will become part of a compliance agreement to take specific actions, with timelines and outcomes. Additional BMPs and mitigations may be implemented by the nursery over time as is feasible for the nursery.
Figure 1. Examples of Critical Control Points (CCPs) where *P. ramorum* has been detected and example remediation, mitigations, Best Management Practices (BMPs), and changes to business/cultural practices

<table>
<thead>
<tr>
<th>Identified CCP associated with <em>P. ramorum</em> positive sample sites</th>
<th>Mitigations under the CNP which may have occurred prior to CCP assessment</th>
<th>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host material</td>
<td>Double bag identified material in (2 mm) plastic bags and deep burial (&gt;2m) burial in a site approved by regulatory authorities. Incineration at a site approved regulatory authorities. Heat Sterilization: Dry heat or steam (see USDA Treatment Manual for “insect pests and pathogens in garbage”, Schedule T415b).</td>
<td>Purchase from nurseries that are licensed or certified under all phytosanitary laws and applicable federal and state regulations. If possible, designate an area for unloading and holding HAP material for 30 days monitoring. Do not mix incoming crops with existing HAP material. Designate or assign specific personnel to work with host material for monitoring purposes as well as management. Avoid accepting returned plant material. Destroy or dispose of any host material returns. Train staff and inspect HR plants monthly. Do not allow plant foliage to be in contact with the ground.</td>
</tr>
<tr>
<td>Identified CCP associated with <em>P. ramorum</em> positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>General sanitation of operation</td>
<td>Disinfestation of non-porous surface concrete floors, benches, and plastic sheeting, tools.</td>
<td>Prevent buildup of fallen leaves and plant debris from HAP plants in production areas and monitor with every crop rotation or quarterly, whichever is more frequent.</td>
</tr>
<tr>
<td></td>
<td>Adequately control weeds on the nursery site as they may potentially harbor the pathogen.</td>
<td>After every crop rotation, disinfect propagation mist beds, sorting area, cutting benches, machines, and tools to minimize spread or introduction of pathogens.</td>
</tr>
<tr>
<td></td>
<td>Do not allow trucks to sweep out debris on site.</td>
<td>Do not allow trucks to sweep out debris on site.</td>
</tr>
<tr>
<td></td>
<td>Routinely clean loading areas and shipping areas following shipment arrivals, or after loading activities.</td>
<td>Routinely clean loading areas and shipping areas following shipment arrivals, or after loading activities.</td>
</tr>
<tr>
<td></td>
<td>Develop or review processes of cleaning carts or trailers used in moving plant material, along with tires.</td>
<td>Develop or review processes of cleaning carts or trailers used in moving plant material, along with tires.</td>
</tr>
<tr>
<td></td>
<td>Develop process for ensuring the clothing of workers is clean every day and tools used for management are cleaned and sanitized routinely.</td>
<td>Develop process for ensuring the clothing of workers is clean every day and tools used for management are cleaned and sanitized routinely.</td>
</tr>
<tr>
<td></td>
<td>Properly dispose of any leaves or plant debris resulting from nursery operations or cleanup of areas or beds.</td>
<td>Properly dispose of any leaves or plant debris resulting from nursery operations or cleanup of areas or beds.</td>
</tr>
<tr>
<td></td>
<td>Install foot baths in all high risk areas and for visitors to the production areas.</td>
<td>Install foot baths in all high risk areas and for visitors to the production areas.</td>
</tr>
<tr>
<td>Identified CCP associated with <em>P. ramorum</em> positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Potting Media</td>
<td>Double bag identified material in (2 mm) plastic bags and deep (&gt;2m) burial in a site approved by regulatory authorities.</td>
<td>Ensure that growing container mix and/or components are from an area known to be free from <em>P. ramorum</em>.</td>
</tr>
<tr>
<td></td>
<td>Incineration at a site approved by regulatory authorities.</td>
<td>Ensure that cull piles are clearly separated from container mix components.</td>
</tr>
<tr>
<td></td>
<td>Heat Sterilization: Dry heat or steam (see USDA Treatment Manual for “insect pests and pathogens in garbage”, Schedule T415b).</td>
<td>Do not reuse container mix from HAP plants.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any container mix that is reused should be steam sterilized or composted according to strict national standards.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Move container mix piles away from potential contamination sources of <em>P. ramorum</em> through plant material or water drainage.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Container mixes should be in containers, bins, or on hard surfaces which can be cleaned, and not in contact with site soil.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pasteurize potting media.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purchase components from suppliers that have the ability to supply media that is free from plant pathogens and pests and meets quality requirements.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sample and test media and media components at delivery or before use.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage areas must be raised above the level of the surrounding level of the surrounding land to a height of 10-12 cm to prevent all runoff water from entering the area or surround site with surface drains or diversion banks.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The area on which the growing media sits should drain freely.</td>
</tr>
<tr>
<td>Identified CCP associated with <em>P. ramorum</em> positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Potting Area</strong></td>
<td>Disinfestation of non-porous surface concrete floors, benches, and plastic sheeting, tools.</td>
<td>Use clean equipment to mix or load planting media.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schedule specific times to pot host plants and clean equipment and area before and after potting operations.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regularly clean and disinfect potting facilities.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regularly clean up and discard spilt media around potting facilities.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staff regularly wash hands and maintain good hygiene.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All equipment used to transport media, for example front-end loader buckets, wheel barrows, mobile bins, trolleys or plastic containers need to be cleaned and disinfected between uses.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limit traffic or divert traffic through soil mixing area.</td>
</tr>
<tr>
<td><strong>Nursery Beds</strong></td>
<td>To avoid contact between infested soil/standing water and host plants, install permanent impermeable, non-porous barriers. Steam soil Soil fumigation (e.g. Basamid, McBr) Solarize soil (under development)</td>
<td>Maintain substrate, whether this is through additional gravel, repairing or replacing landscape cloth or covering, leveling to improve or increase drainage.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevent buildup of fallen leaves and plant debris from HAP plants in production areas and monitor with every crop rotation or quarterly.</td>
</tr>
<tr>
<td>Identified CCP associated with <em>P. ramorum</em> positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Irrigation Water</td>
<td>Treat recycled water or water used for irrigation water with chlorine levels of 2ppm or 2mg/liter or greater.</td>
<td>Treat water used for irrigation through the use of system which utilizes one or a combination of the following: Bromine, Chlorine, Sodium hypochlorite, Calcium hypochlorite, Chlorine dioxide, Ozone, activated peroxygen, ultraviolet radiation, copper ionization, heat treatment/pasteurization, and filtration. Avoid overhead irrigation of HR plants or irrigate in a manner to avoid prolonged leaf wetness and splash. During periods of active use, monitor and test, quarterly at a minimum, untreated irrigation water from any source other than a well or a municipal water supply to conform that is free from the pathogen. Monitor water treatment systems to verify that appropriate treatment measures are being applied. Eliminate accumulations of standing surface water, improve drainage of areas designated for HR, HAP plants Prevent standing water by not overwatering or irrigate host plants based on water needs. Irrigate high risk host plants around dawn when possible to prevent extended periods of leaf wetness which is a favorable condition for pathogen proliferation.</td>
</tr>
<tr>
<td>Identified CCP associated with \textit{P. ramorum} positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Water Drainage</td>
<td>Divert soil and water movement from adjacent properties that are populated with \textit{P. ramorum} host plants to prevent contamination of the nursery.</td>
<td>Insert a well-drained physical barrier (e.g. raised benches, effective gravel layer, asphalt, or concrete) between native soil and containers to prevent splash dispersal of pathogen from potentially infested ground. Ensure run off from all cull piles is directed away from media components, media mixing area, growing beds, nursery roadways or paths, and irrigation water to prevent contamination.</td>
</tr>
<tr>
<td>Pots/Containers</td>
<td>Sterilization by steam or disinfestation by alcohols, chlorine, quaternary ammonium, or phenolics.</td>
<td>Use pots that are (1) new, (2) clean and properly disinfested, or (3) sanitized by steam sterilization or hot water dip for HAP production. Establish a procedure for cleaning, sanitizing pots with clear separation of clean pots from dirty pots. Store pots on a barrier that effectively separates them from underlying substrate. Do not store used pots in areas where water drainage would flow or splash into HAP beds. Store new and clean disinfested containers above ground level. Regularly control weeds in and around container storage facilities.</td>
</tr>
<tr>
<td>Identified CCP associated with <em>P. ramorum</em> positive sample sites</td>
<td>Mitigations under the CNP which may have occurred prior to CCP assessment</td>
<td>Example remediation, mitigations, BMPs, changes to business/cultural practices for selection by nursery</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Roads/Pathways</td>
<td>Maintain substrate, leveling to improve or increase drainage. Prevent buildup of fallen leaves and plant debris. Cover pathways and roads adjacent to beds and benches with materials to reduce contamination with soil and water. Roads will be maintained to avoid standing water. Potholes will be filled and drainage will be maintained such that water will be carried away.</td>
<td></td>
</tr>
<tr>
<td>Conveyance</td>
<td>Unload incoming deliveries onto a hard, impermeable surfaced area that is clean and free of any debris. Collect all debris from plants, surface of area, and delivery truck. Require pick-up and delivery trucks to properly clean and sanitize truck bed, undercarriage, and tires prior to entering nursery operations. Do not allow trucks to sweep out debris into nursery. If a known <em>P. ramorum</em> infested area has been visited, wash and sanitize shoes, tools and vehicles that may have contacted contaminated soils before traveling to disease free areas. Develop or review processes of cleaning carts or trailers used in moving plant material, along with tires. Regularly clean and disinfect transport equipment and staff.</td>
<td></td>
</tr>
</tbody>
</table>

*Disclaimer: Although this appendix tries to cover all common potential hazards and the appropriate rectifications, it is not likely to cover all of them. Hazards, CCPs, and potential responses will differ between nurseries.*

---

Confirmed Nursery Protocol: Version 8.2   Revised: August 6, 2014   53
For reference, here are examples of programs which are based on assessments to identify CCPs leading to BMPs and mitigations to address the associated risks include:

United States Nursery Certification Program (USNCP), National Plant Board

Approach to Nursery Certification (SANC) – Nursery Practices

Oregon Grower Assisted Inspection Program (GAIP)

Nursery Industry Best Management Practices for P. ramorum to prevent the introduction or establishment in California Nursery Operations

Washington State University Extension, Nursery Guidelines for Exclusion and Management of P. ramorum in Nurseries

Australia BioSecure HACCP Guidelines, and Nursery Industry Accreditation Scheme, Australia

Example:    | Hazard – Water
| CCP – Recycled irrigation water or standing water

Once the hazards and CCPs are determined through the assessment, best management practices (BMPs) and mitigations will be identified to address the CCPs.

Example:    | CCP – Recycled irrigation water or standing water
| BMP – Test water for pathogens on a routine basis

| CCP - Standing water
| BMP – Monitor and adjust watering cycles
| BMP – Grading to provide better drainage, etc.


Figure 1 above is an example of identification of CCPs, and examples of practices for the nursery to select to mitigate the risk associated with the positive sample sites. All required mitigations related to positive plants will have taken place prior to the assessment. Other required mitigations based on positive samples will not be a component of the CCP assessment recommendations.