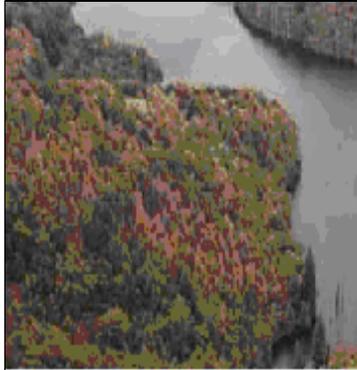




United States Department of Agriculture



2014
Phytophthora ramorum
Inspection and Sampling
Protocol for Nurseries under
DA-2014-02 Compliance



Revised: August 6, 2014

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

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Necessary References:

1) Biology of *Phytophthora ramorum*:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/surveyplan/appendixa.pdf

2) APHIS List of Regulated Hosts and Plants Associated with *P. ramorum*:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf

3) Symptoms Associated with *P. ramorum*: [Appendix D: Symptoms Associated with *P. ramorum*](#)

INSPECTION AND SAMPLING PROTOCOLS FOR NURSERIES UNDER DA-2014-02 COMPLIANCE OBJECTIVES

The objective of the *Phytophthora ramorum* Federal Order DA-2014-02 Compliance Inspection and Sampling Protocol is to detect the presence of *P. ramorum* in interstate shipping nurseries in California, Oregon and Washington which tested positive on or after March 31, 2011, and, any interstate shipping nursery in non-regulated states that tests positive on or after the implementation date of the Federal Order. To detect the presence of *P. ramorum* in the nursery, this protocol describes methods for sampling plants, standing water, drainage water, water for irrigation, container mix, and any other articles designated by an inspector as possible sources of *P. ramorum* inoculum at the nursery.

DEFINITIONS

- Compost pile:** A heap of decaying organic matter layered and mixed together and allowed to compost; used to improve soil structure and provide nutrients (components include plant parts, manure, etc.).
- 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. This final determination of a positive sample allows for federal regulatory action. Report the positive to the APHIS PPQ Operations Manager within 24 hours.
- Container mix:** 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, container mix (growing media), and pots are deposited; also known as a waste or trash pile.
- Final Determination:** The last diagnostic result(s) necessary for a regulatory sample indicating whether *Phytophthora 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, the designated state official reports the result to the APHIS PPQ Operations Manager within 24 hours. Regulatory action may commence on this positive. See 'Confirmed Positive'.
- HAP:** Host and associated host plants (HAP) listed on the official APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*. The current list is on the USDA APHIS PPQ website: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf
- High Risk Genera:** *Camellia*, *Rhododendron*, *Pieris*, *Viburnum* and *Kalmia*. Acronym: HR.
- Infected plants:** Plants officially confirmed as being infected with *P. ramorum*, based on the use of APHIS approved diagnostics, and following the PASS system.
- Non-PASS:** In non-regulated areas, once a nursery is Confirmed Positive by APHIS during a given calendar year, all subsequent samples are considered "Non-PASS", meaning a National Plant Protection Laboratory Accreditation Program (NPPLAP) NPPLAP accredited lab outside of APHIS can make the final determination utilizing APHIS NPPLAP diagnostic work instructions. In the regulated areas, for a nursery that has been historically positive, all samples are "Non-PASS" if tested by the NPPLAP accredited state lab. If the diagnostic result is positive, the

sample is Confirmed Positive and reported to the APHIS PPQ Operations Manager within 24 hours. Regulatory action can commence based on that positive sample.

- Nursery:** Any location where nursery stock is grown, propagated, stored, or sold, or any location from which nursery stock is distributed.
- 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 Dealer:** Nurseries that are resellers – wholesale or retail – of nursery plants.
- Nursery Grower:** Nurseries that grow nursery stock; synonymous with propagator.
- Nursery stock:** Any plants for planting, including houseplants, propagative material that are grown in a nursery and tree seedlings for reforestation. In this and other protocols, the terms nursery stock and “plant” are often used synonymously, but not exclusively.
- PASS:** For non-regulated areas, the Potential Actionable Suspect Sample is the initial sample **Confirmed Positive** by APHIS for a given nursery in the calendar year. More specifically, when a NPPLAP accredited lab detects *P. ramorum* in a sample of a nursery not yet confirmed positive by APHIS during a given calendar year, it is considered a “PASS” and must be routed to APHIS for **final determination**. In the regulated areas, the state NPPLAP accredited labs have final determination authority for any nursery historically positive.
- Sample:** For *P. ramorum* plant sampling, a sample refers to **a single bag** of leaves. The goal is to have at least 2 sq. inches 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 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 the healthy/necrotic margin. 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.
- 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.

QUICK GUIDE FOR CONDUCTING NURSERY INSPECTION AND SAMPLING

1. Determine when to sample each nursery based on the time of year when climatic conditions will be most conducive for *P. ramorum* disease expression (see [Timing Nursery Inspection and Sampling](#), page 8).
2. After you determine when to sample, notify the laboratory beforehand to ensure supplies are available and the lab is prepared to receive the samples (see [Notifying the Lab, page 8](#)).
3. Ensure all supplies and equipment are available for the planned survey and review symptoms before arriving at the nursery [Appendix D: Symptoms Associated with *P. ramorum*](#) (see [Preparing for Nursery Inspection and Sampling, page 9](#)).
4. Prior to inspection day, review: 1) the APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum* (HAP). See: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdap_rlist.pdf 2) obtain and review the nursery inventory, and, 3) any available maps of the nursery to determine areas to inspect and sample (See [Inspecting and Sampling the Nursery](#), Step 1, page 12).
5. Determine the approximate number of plant samples to take from each HR and other HAP taxon (see [Table 1, page 14](#)).
6. On inspection day, begin by conducting a visual of the nursery as a whole. Note topography, water sources, drainage patterns, areas of high risk (cull piles, low vigor plants), etc. (see [Inspecting and Sampling the Nursery](#), Step 2, page 12).
7. Take samples of:
 - a. **Plants:** Sample all or representative amount of symptomatic host tissue. Ensure that at least the minimum samples are collected; see [Table 1 \(page 14\)](#). During this protocol, every plant sampled is on regulatory hold. See Samples definition above, also, [Plants](#) (page 12) and [Plant Symptoms and Sampling for *P. ramorum*](#) (page 10).
 - b. **Water:** Sample water in and around the nursery; each area is a discrete sample from the next area. Fill a 1L bottle per sample. Collect standing water in HAP blocks, and around HAP blocks. Collect from holding ponds, drainage ditches, water around cull piles, etc.) (see [Water, page 15](#)). During this protocol, standing or effluent nursery water is not on hold.
 - c. **Pots and Containers:** If containers are recycled and stored at the nursery or if used pots are purchased, sample residual container mix from pots or other containers. Scrape container mix from pots filling a labeled 1L zip lock. Use the [Soil and Container Mix Protocol](#). During this protocol, pots are not on regulatory hold.
 - d. **Cull Piles:** Examine any area where plants have recently been disposed. If host plants are present, sample symptomatic plant material. Attempt to keep host

genera samples separate from one another. If there is any standing water, take at least one sample from each cull pile area. During this protocol, cull piles are not on regulatory hold.

- e. **Other articles designated by an inspector as possible sources of *P. ramorum* inoculum:** At the inspector's discretion, sample any and all other possible sources of *P. ramorum* inoculum.
- f. **Soil:** Only container mix from used container piles is sampled in this protocol. No soil (nursery substrate) samples are required for this sampling protocol (see [Soil, page 15](#)).

Properly label and store collected samples for shipping to the lab (see [Sample Handling and Submission Protocol, page 17](#)). When sampling, remember to move from low risk to high risk areas to prevent potential spread of the pathogen.

- 8. Sanitize tools and change or sanitize gloves between samples to prevent cross contamination (see [Inspecting and Sampling the Nursery](#), Step 3, page 12).
- 9. As you take samples, flag or mark plants and areas sampled. Also mark areas on a map of the nursery. Take pictures of sampled areas, including areas of standing water (See [Inspecting and Sampling the Nursery](#), Step 4, page 12).
- 10. Forward all samples to the appropriate lab (either NPPLAP accredited or APHIS diagnostic lab (see [Sample Submission Information, page 17](#) and [Points of Contact, page 20](#)). Complete a PPQ Form 391 (or state equivalent) for all samples.

TIMING NURSERY INSPECTION AND SAMPLING

Nurseries should be inspected and sampled at a time when climatic conditions conducive to *P. ramorum* disease expression are occurring, providing the best opportunity for expression of symptoms due to *P. ramorum*.

- Research suggests that the most favorable climate for the expression of symptoms includes ambient temperatures between 3°C (37.4° F) and 28°C (82.4° F) (optimum 20°C, 68°F) and free moisture present on host tissue for at least twelve hours over ten or more days. In most areas where this inspection and sampling will occur, the timing is primarily in the spring and fall.
- Be aware that greenhouses, hot houses and nursery beds under shade cloth or overhead irrigation should be considered micro-climates where optimum conditions can occur outside of the typical fall/spring window.
- Disease expression typically begins between 30 and 90 days after bud-break. Inspection and sampling should begin after the spring flush is underway, when some of the leaves have fully expanded. Plants can express symptoms throughout the growing season, though isolation of the pathogen may be more difficult during hot and dry periods.
- Plan inspections and samplings when nurseries receive shipments in the spring and fall when host plants will be present and environmental conditions will be optimal.

Notifying the Lab

Notification of an upcoming sampling date, and a request for the bottles and leaves to be used for the water sampling (bottle of bait), need to be made at least two weeks in advance, if possible. This will also ensure that the laboratory is prepared to receive the samples and will be prepared to process them promptly.

PREPARING FOR NURSERY INSPECTION AND SAMPLING

Supplies and Equipment Check List

- APHIS List of Regulated Hosts and Plants Proven or Associated with *Phytophthora ramorum* (October 23, 2013): The current list is found at the USDA APHIS PPQ website at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf
- 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).

PLANT SYMPTOMS AND SAMPLING FOR *P. RAMORUM*

Plant Symptom Resources

Inspectors must be trained to identify symptoms associated with *P. ramorum* on host plants. At a minimum they should review photographs of the wide range of symptoms possible before starting the inspection and sampling. Photographs of typical and atypical symptoms are available in [Appendix D: Symptoms Associated with *P. ramorum*](#). **Remember that symptoms of *P. ramorum* are often not “typical” and over reliance on identification by these symptoms could result in infected plants remaining undetected – the greatest chance of detecting *P. ramorum* infections is through the collection of ANY unhealthy looking plant tissue for laboratory analysis.**

For definition of *P. ramorum* plant sample, see **sample** in definition section above. 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. Inspect the lower, more shaded portions of plants and the interior of the canopy where moisture and high humidity may persist. Pay special attention to leaf areas where water would linger such as the midrib and leaf tips. Check for leaves inside the pot of asymptomatic (*Camellia* and *Rhododendron*) plants. Infection can cause premature leaf drop with symptomatic leaves found only in the pot or on the ground. Remember, many *Phytophthora* spp., other pathogens and environmental stressors can cause symptoms that cannot be distinguished from *P. ramorum* infection by visual inspection. Do not assume you know what all *P. ramorum* symptoms look like; collect samples of leaves that you think may be caused by abiotic stressors. After that, collect samples from healthy, asymptomatic plants only after all unhealthy plants have been sampled and the minimum number of samples has not yet been collected.

Sampling by Symptom Type

Leaf Spots and Lesions

- Collect symptomatic leaves.
 - Some plants, such as *Camellia* or *Loropetalum* may have very small pin point lesions.
 - Some leaves have very subtle symptoms, such as flecking or chlorotic spots.
 - For plants with very small leaves or needles, samples can be submitted as twig sections with the leaves attached. In these cases try to ensure that the sample has a minimum of 2 square inches of symptomatic tissue.
 - If there are not enough symptomatic leaves on the plant, take symptomatic leaves that have dropped into the pot provided they are not exhibiting extensive decay.
 - If necessary, you can composite leaves from up to five plants to make a single sample and get the required amount of symptomatic tissue.

Twig Dieback

- Cut the twigs below the cankered regions (1" into healthy tissue).
- Sterilize pruning equipment between samples using a dilute (10%) bleach solution, or a quaternary ammonium solution at labeled rates, or spray Lysol.

Cankers on Boles and Branches of Certain Trees

- Some trees do not have foliar symptoms but get cankers on boles or branches. Follow procedures in your state for inspection and sampling these trees in nurseries. Bole or branch cankers consistent with *P. ramorum* disease must be sampled.
- In some states, nursery inspectors may sample trees while in other states forestry or other officials may be asked to sample trees.

INSPECTING AND SAMPLING THE NURSERY

- 1) Before the inspection season begins, review the APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum* (HAP). See: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf. If possible, time nursery inspection to periods when standing water is likely to be present. This could be after a rain event or after irrigation occurs.
- 2) Prior to inspection day, obtain and review a nursery plant inventory, a plant location map, an aerial map and a topographic map, if possible, of the nursery to determine areas to sample. Create a sampling plan based on the number of host plants present in the nursery and plan the areas to visit within the nursery (See Tables 1 and 2). Inspect the non-host and lower risk HAP areas first, the high risk hosts next, and the cull piles last.
- 3) Begin the inspection by conducting a visual assessment of the nursery as a whole. Identify cull piles, 'plant hospitals' or low vigor plants reduced for sale, and areas that may include host plant returns. Determine source of irrigation water (well, municipal, treated and recycled). Note topography of the nursery, nursery drainage patterns and systems. Confirm low lying areas, standing water, nursery layout, source of water, the general condition of the plants and nursery environment. Use this information to help guide your plant, water, and other article sampling.
- 4) Decontaminate personnel, tools, and equipment between blocks in the nursery, between host genera within a nursery, and between nurseries. Wear rubber boots or other water proof 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
- 5) Indicate on the map of the nursery areas inspected and sampled. Flag/mark plants sampled, standing water areas sampled, cull piles sampled, etc. Take pictures of sampled areas, including areas of standing water. Sampling instruction for each article provided below.

Plants

Inspect all plants within a nursery with careful attention paid to plants on the official APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum* (HAP). See: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf You should consult it for the latest list of plants before beginning your inspections. There are five plant genera most frequently reported to be infected by *P. ramorum* in U.S. nursery settings. These hosts are considered "high risk": *Camellia*, *Rhododendron*, *Viburnum*, *Pieris*, and *Kalmia*. **Collect samples from all symptomatic HAP**, and at the inspector's discretion, all other non-host plant tissue with symptoms suggestive of *P. ramorum*.

Here are two basic principles that should govern the inspection and sampling processes.

- 1) *Phytophthora ramorum* cannot be diagnosed by a visual inspection of symptoms alone; only laboratory testing can provide a definitive diagnosis.
- 2) If there is any doubt as to whether the symptoms observed could be caused by *P. ramorum*, collect a sample.

Read the PLANTS SYMPTOMS Section above and view [Appendix D: Symptoms Associated with *P. ramorum*](#) photos prior to entering the nursery. Each sample should consist of at least 2 square inches of symptomatic plant tissue. It is strongly encouraged that each sample is from one plant, however, if there are not enough symptomatic leaves on the one plant, collect symptomatic leaves (if present) from within the pot of that plant or adjacent plants (i.e. same cultivar/variety) to help make up the sample. If the inspector is certain that leaves on the ground adjacent to the pot is from that plant, they can be used to complete the sample. Unless the inspector is absolutely certain, leaf debris from the ground should be a separate sample and labeled as such. Remember the more plants composited into one sample, the larger the destruction radii if found positive.

Determine the minimum number of samples of hosts and associated plants to take within a nursery: Using the nursery inventory, determine the number of HAP in the nursery. Add all plants of each high risk genera together (*Camellia*, *Rhododendron*, *Pieris*, *Viburnum* and *Kalmia*) plus all other HAP. Find this total in Table 1 for the corresponding minimum number of samples to take in that nursery. See:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf

If the number of HAP in a nursery is greater than one number (ex. 405 is greater than 400), move to the next greater number listed (500). If there are more symptomatic plants, always take more samples than the minimum calculated to 1) pinpoint *P. ramorum* in the nursery, and, 2) To lessen the potential regulatory impact on the nursery.

Table 1. Minimum Number of Plant Samples to take based on the number of HAP within a nursery

Host and Associated Plants Per Nursery	Minimum Number of Samples to Take (95% Confidence Limit of Detecting 2.0% Disease*)
25	25
50	50
100	100
200	140
300	156
400	165
500	172
600	176
700	179
800	181
900	183
1,000	184
2,000	191
3,000	193
4,000	195
5,000	196
6,000	196
7,000	196
8,000	196
9,000	197
10,000	197
20,000	197
30,000	197
40,000 +	199

*Numbers are the minimum number of host and associated host plants that must be sampled in a nursery 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.

After the survey, results from the lab will be reported to the inspector. The inspector may release all ELISA or PCR negative plants upon receiving these results from the lab (unless within the Q radii or D radii of a positive detection). See sample handling and submission instructions below.

Water

Examine all areas within the nursery for standing water. This could be after a rain event or after irrigation occurs. Demarcate each area where water is collected with pin flags. If not possible because it's a roadway, draw a sketch, take photos, and flag near areas. Label the water container and the flagging with corresponding numbers so that any positive samples can be located within the nursery. If helpful, take photos of each area where water is collected. Areas of water sampling are not on hold awaiting diagnostic results but must be durably marked in case of detection.

1. Irrigation Water. Sample all types of irrigation water except from a municipal source. Do not sample at source pipe if possible, but at end dispensers (sprinklers, nozzles, drip, etc.). Do sample retention ponds regardless of source, since they likely contain run off from production areas. If the retention pond is treated, it must be sampled to determine the efficacy of the treatment. Collect a minimum of one 1L water sample per sample site and from each recycled holding pond.
2. Standing Water. Sample standing water in and around blocks of HAP and the drainage from HAP blocks as the first priority. Drains in greenhouse, hoop house systems containing HAP material can be accessed for sample collection. This could be after a rain event or after irrigation occurs. Collect a minimum of one 1L water sample from each general area where standing water occurs, a minimum of one sample from each drainage ditch where runoff from host plant blocks collects. Sample any water that is around or drains from cull piles as well.
3. Non-recycled retention ponds. Collect a minimum of one 1L water sample per sample site and from each recycled holding pond.

See Water Sampling Protocol located at:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/surveyplan/appendixI.pdf

Soil

Standing water will be sampled in place of soil sampling. No soil samples are required for this sampling protocol.

Pots and Containers

If containers are recycled and stored at the nursery, or if used pots are purchased, sample pots or other containers with residual container mix. Scrape container mix from pots into a labeled 1L zip lock. During the Inspection and Sampling Protocol, there are no holds on the nursery or container pile associated with sampling containers while waiting for diagnostic results. Use the Soil and Container Mix Protocol; see:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/soil_protocol_11-5-2010.pdf

Cull Pile

Examine any area where plants have **recently** been disposed. If host plants are present, sample unhealthy looking plant material. Attempt to keep host genera samples separate from one another. If there is any standing water, take at least one sample from each cull pile area. Since

the material collected is symptomatic plant tissue, demarcate the cull pile or that area of the cull pile for avoidance waiting for diagnostic results.

Other articles designated by an inspector as possible sources of *P. ramorum* inoculum

At the inspector's discretion, sample any and all other possible sources of *P. ramorum* inoculum.

SAMPLE HANDLING AND SUBMISSION PROTOCOL

- Always write out the identifying label remarks on the outside of the bag with a permanent marker.
 - Attach labels on the **outside** of bags since labels inside the bag may deteriorate due to moisture and become illegible.
 - 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 are unsure

about which lab to utilize, please 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).

BLOCK	INSTRUCTIONS
1	1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number EXAMPLE In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001 2. Enter the collection number
2	Enter date
3	Check block to indicate Agency submitting specimens for identification
4	Enter name of sender
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)
6	Enter address
7	Enter name and address of property owner
8A-8L	Check all appropriate blocks
9	Leave Blank
10	Enter scientific name of host,  Genus Species, particularly Cultvar name
11	Enter quantity of host and plants affected
12	Check block to indicate distribution of plant
13	Check appropriate blocks to indicate plant parts affected
14	Check block to indicate pest distribution
15	<ul style="list-style-type: none"> • Check appropriate block to indicate type of specimen • Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Enter X in block to indicate isolated or general plant symptoms
20	Enter X in appropriate block for weed density
21	Enter X in appropriate block for weed growth stage
22	Provide a brief explanation if Prompt or URGENT identification is requested
23	Enter a tentative determination if you made one
24	Leave blank

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.

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