

**Eradication Protocol for Propagation
Nurseries Confirmed with *Phytophthora ramorum***

Canadian Food Inspection Agency

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1. Introduction

Phytophthora ramorum is a quarantine pest, regulated by the Canadian Food Inspection Agency (CFIA) in policy directive D-01-01, *Phytosanitary Requirements to Prevent the Entry of Phytophthora ramorum*. The United States Department of Agriculture (USDA) also regulates *P. ramorum*.

In the past several years, incursions of *P. ramorum* have been detected in nurseries as a likely consequence of trade in infested plants. Introductions of the pathogen into cultivated areas of the U.S and Canada have necessitated eradication actions by regulatory agencies in both countries. The USDA and CFIA attempt to harmonize, wherever possible, field level procedures for management of *P. ramorum*. This document outlines Canadian regulatory actions to eradicate *P. ramorum* incursions at commercial nurseries propagating plants.

2. Endorsement

Approved by:

Director
Plant Health Division

3. Definitions

Delimitation survey	A survey done to determine the extent of the infestation within a nursery site. This survey is only deemed complete once results have been received.
Destruction area	A contiguous group of plants to be destroyed which contains one or more plants known to be infested with <i>P. ramorum</i> .
Field	A plot of land with defined boundaries within a place of production on which a commodity is grown. [ISPM No. 5]
Free from	Of a consignment, field or place of production, without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures. [ISPM No. 5].
High risk host plants	Plants of the genera listed in Appendix 1 of this document.

Host plants	Plants listed in Appendix 1 A List of Plant Genera Regulated for <i>Phytophthora ramorum</i> found in CFIA policy D-01-01 A Phytosanitary Requirements to Prevent the Entry of <i>Phytophthora ramorum</i>
Incursion	An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future [ISPM No. 5]
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection [ISPM No. 5]
Low risk host plants	Host plants excluding those plants identified as high risk host plants.
Outbreak	A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area [ISPM No. 5]
Place of production	Any premises or collection of fields operated as a single production or farming unit. This may include production sites which are separately managed for phytosanitary purposes. [ISPM No. 15]
Plants for planting	Plants intended to remain planted, to be planted or replanted [ISPM No. 5]
Propagation nursery	Any place of production where plants for planting are multiplied, excluding those that multiply plants for planting exclusively by seed or tissue culture.
Quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing and/or treatment [ISPM No. 5]
Quarantine area	All plants within a 10 meter radius around the destruction area, used to determine if <i>P. ramorum</i> has spread beyond the destruction area.

4. Goal

The goal of this protocol is to ensure that any infestations at propagation nurseries are consistently and effectively eradicated. Cooperation by nursery management personnel is essential. Early detection and reporting of *P. ramorum* finds are critical to ensure that the infestation is contained and spread minimized. The eradication principles employed in this protocol are similar to those employed by the United States and the European Union.

5. When to use this Protocol

A summary of the measures and activities in this protocol are found in Appendix 10

This protocol shall be implemented when the presence of *P. ramorum* has been confirmed in a propagation nursery. Samples may have been collected as part of a trace forward or trace back activities, as part of the national *P. ramorum* survey or found by other means. Confirmed samples must have been analysed using a methodology approved by the CFIA at a CFIA laboratory.

If *P. ramorum* is detected at a propagation nursery within one year of issuance of a Notice of Release from Quarantine, this may be an indication that previous eradication measures failed. In these cases the propagation nurser must undertake the measures specified in Appendix 9

6. Regulatory Control - Securing the Nursery

Upon detection of *P. ramorum* at a propagation nursery, the entire facility shall be placed under quarantine and all host plants in the propagation nursery shall be held until delimitation sampling and testing within the nursery is complete. The quarantine shall be established using the appropriate notice as specified in Appendix 4.

Quarantine actions shall ensure that cull piles and any potentially infested soils or water used for irrigation are secured until all testing is complete.

Once delimitation surveys are complete and sampling results have confirmed the extent of the infestation, destruction and or treatment areas shall be identified as per Section 8 of this protocol. Once these destruction and quarantine areas have been identified, the facility quarantine may be removed. Destruction and quarantine areas, as identified in Section 10 shall remain under quarantine until all eradication measures have been completed.

As per Appendix 3, all equipment used on the nursery site is not to be moved from the place of production or an infested field without proper cleaning. The nursery staff shall not be permitted to enter quarantined areas, unless permitted by CFIA. Any additional required sanitary measures as specified in Appendix 3 may be required to prevent spread of *P. ramorum* shall be detailed on the appropriate notice.

7. Survey and Inspection of the Nursery and Perimeter

7.1. Sample Submission

All samples collected shall be submitted per the instructions in Appendix 8 to:

Attn: Plant Pathology

Ontario Plant Laboratories
ADRI-CPOP
Floor 2
3851 FALLOWFIELD RD
PO BOX 11300
OTTAWA ON K2H 8P9

7.2. Delimitation survey

Once a positive plant has been detected, CFIA shall inspect all host plants within the place of production focusing particular attention on high risk hosts. All plants in holding areas, retail sales areas and in propagation areas shall be inspected. Any plants showing symptoms consistent with diseases caused by *P. ramorum*, shall be identified, sampled and the samples submitted according to the protocols detailed in Appendices 7 and 8.

Upon delimitation, any plants confirmed to be infested with *P. ramorum* infestation and adjacent plants as described in Section 8 below shall be placed under quarantine and destroyed.

7.3. Trace Forward and Trace Back Surveys

Where feasible, inspectors shall identify all shipments of infested plants (and/or host plants of same origin of production) moved within the 12 months prior to the first positive detection of *P. ramorum*. Trace-forward activities should focus on plant movements to places of production and places of plant distribution given that these sites represent greater risk of pathogen spread. Where records exist, tracing shipments to landscape and retail locations may also be performed.

Inspectors shall also attempt to determine the origin of infested plants that may have been moved into the facility through trace back activities.

Plants shall be sampled according to the protocols detailed in Appendices 4 and 8

Trace back information relating to infested plants received from outside the local area shall be communicated to the Lead Specialist for *P. ramorum* Management who will relay this information to other areas for inspection or other actions as necessary.

7.4. Soil and Potting Media Sampling

Infested plant material may have contaminated soil or potting media used at the nursery. Inspectors shall sample soil or potting media where infested plants have been found; soils adjacent to infested plants; potting and soil mixing areas and any areas where plants are maintained downhill from infested plants. Inspectors shall also determine the content, origin (composition), storage and handling of soil or potting media used in the facility. Appendix 7 outlines details soil and potting media sampling methods. Soil samples shall be kept separate from potting media samples.

7.5. Water Sampling

The source of water used at the facility to irrigate plants shall be determined. Drainage of water flows, type of irrigation system(s) areas of standing water and any safeguards against water back -flow shall also be identified. If irrigation water is untreated, re-circulated, or there is surface water at risk of contamination by *P. ramorum*, and/or soil contains culled plant material or bark of host plants; soil and water shall be tested.

Appendix 7 outlines a detailed water sampling protocol. Any surface water, irrigation sources or any run off from the nursery should be considered for sampling. Water sampling is not required for irrigation water from municipal water facilities that treat their water prior to release, but any retention pond or area where water collects at the nursery site shall be sampled.

7.6. Cull piles

The location of any cull piles that may be contaminated with infested plant material or associated soil and/or potting media shall be recorded. Cull piles shall be inspected for *P. ramorum* symptomatic plants, and samples collected, if warranted. The disposition of the cull pile shall also be identified (e.g. composted, removed to land fills, etc.) on notices. Soil adjacent to the cull pile shall also be tested for the presence of *P. ramorum*. Particular attention shall be paid to the soil at the edge of the down-slope of the pile.

7.7. Perimeter survey

A perimeter survey shall be conducted of all high risk host plants within 10-meters of the propagation nursery. The purpose of the perimeter survey is to ensure that *P. ramorum* has not spread from the infested nursery, and to verify that the source of the infection on the affected nursery did not originate from the surrounding environment. This survey shall be conducted during the period in which the facility has been placed under quarantine as specified in Section 6 above.

7.8. Survey of Fungicide Use

Determine if fungicides are used on the plants at the nursery. If fungicides were used, copies of records regarding the dates of treatments, the material(s) used, the amounts used and application rates shall be recorded. Inspectors shall determine if any other type of treatments (soil amendments, fertilizers) are applied to the plants, soil or growth media

8. Regulatory Control – Destruction of Infested Material

Destruction areas shall be identified based upon sampling outcomes and all plants identified in Section 8.1 or 8.2 below shall be destroyed. The extent of the order to destroy infested plants is dependant upon whether the infested host plants are high or low risk host plants. Destruction orders shall be provided to the propagation nursery using the appropriate notice, as per the instructions specified in Appendix 4.

Plant debris in the destruction area must be collected by raking in from the outer edge in towards the infested plants from a distance of three (3) metres outside the perimeter of the destruction area. Debris must be double bagged and disposed of with the infested plants according to the requirements

8.1. Destruction of High Risk Host Plants

If the infested plants are high risk host plants, a destruction area shall be established which consists of all host plants within a contiguous block of plants until a separation of 2 m is present, as a result of a break in plant material or the presence of non-host plants over the 2 m. Plants shall be destroyed according to the specifications in Appendix 2. Any non-host plants that are present in a destruction area will be held in place, or moved under official supervision to a safeguarded area with a non-porous surface, during the quarantine period and be subject to the same conditions as the host plants in the quarantine area(s).

8.2. Destruction of Low Risk Host Plants

If the infested plants are low risk host plants, a destruction area shall be established which consists of all host plants within 2m of the infected plants. Plants shall be destroyed according to the specifications in Appendix 2.

9. Regulatory Control – Treatment of Infested Soil and Water

Where water, soil or growing media at the facility has been found to be positive for *P. ramorum*, treatment is required in accordance with the specifications in Appendix 2. Additionally, if the infested water was used for irrigation purposes, all plants and growing media in the irrigated area shall be tested and placed under facility quarantine until testing confirms that the plants are free of evidence of *P. ramorum*.

If any plants, plant material, growing media, or soil from a cull pile is found to be positive for *P. ramorum*, the cull pile must be treated or destroyed using one or more of the techniques detailed in Appendix 2. The appropriate notice must be issued as per Appendix 4.

10. Regulatory Control - Quarantined Areas Following Destruction

A quarantine area of 10 m surrounding destruction areas shall be identified using the appropriate notice specified in Appendix 4. All host plants within the quarantine area shall be held for a minimum of 90 days following the date of the first positive test results ensuring that the 90 days is inclusive of environmental conditions conducive to disease expression. Non-host plants in this area may be moved, provided the conditions for movement have been prescribed in writing by a CFIA inspector.

During the quarantine period:

- Host plants may not be moved within or out of the quarantine area.
- The propagation nursery may not use any oomycete specific fungicides for *Phytophthora* control.
- All high risk host plants within the quarantine area shall be inspected and sampled according to the protocol detailed in Appendix 5 at least twice during environmental conditions conducive to expression of the disease. Any other plants showing symptoms resembling *P. ramorum* should also be sampled. Inspection and sampling should occur at about halfway through the anticipated quarantine period and once again near the end of quarantine, with the final test results coinciding with the end of the quarantine.

- If a plant sample tests positive for *P. ramorum*, the destruction area(s) and 10 m quarantine area(s) shall be redefined, and the quarantine period reestablished.
- If water, soil, and/or media samples tested positive for *P. ramorum* during the delimiting survey, samples of the infested water, soil, and/or media material will be re-sampled and tested during each of the two quarantine period plant samplings.
- If a soil sample or potting media sample is found to be positive, then any plants (host or non-host) in the block with the infested soil are to be placed under an appropriate quarantine notice, sampled and tested.

11. Release of the Nursery from Quarantine

There are two options for release from regulatory control:

11.1 Release Prior to the Completion of Quarantine

If water, and soil, potting media and perimeter sampling are negative for *P. ramorum*, a propagation nursery may avoid the 90 day quarantine period specified in Section 10 by voluntarily destroying all host plants and host plant parts in the destruction and 10 m quarantine area specified in Section 10. In addition, any officially required treatments must have been completed. The nursery must, however, be revisited after approximately 90 days of conducive conditions for development of the disease to conduct an inspection and sampling of soil in the destruction area.

OR

11.2 Release Upon Completion of Quarantine

Propagation nurseries that have been placed under quarantine for 90 days as specified in Section 10 may be released by CFIA, if;

- During conditions conducive to the development of the disease, there are no additional detections of *P. ramorum* in the quarantine area specified in Section 10 following destruction of infested plants, and/or treatments of soil or water based on CFIA approved sampling and testing protocols ; and
- Water, soil, and potting media have also tested negative for *P. ramorum* based on CFIA approved sampling and testing protocols.

12. Post Eradication Monitoring

Nurseries that have been infested will continue to be monitored for two years following release from quarantine. Sampling shall take place during a season in which there are optimal conditions for the growth and development of *P. ramorum* symptoms. These nurseries are not under any quarantine or regulatory action, unless additional outbreaks are detected.

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List of High Risk Host Plants

Plants of the following genera:

- *Camellia*
- *Rhododendron*
- *Pieris*
- *Kalmia*
- *Viburnum*

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Treatments and Disinfections

The following treatments may be ordered by CFIA in the event of detection of *P. ramorum* in plants, soil, media or water.

Alternative treatments may be proposed to CFIA and may be utilized if approved by the CFIA in writing.

1. Infested Plants

Note: host material, including leaf litter, must not be placed in compost piles or be removed from the facility as trash or in debris removal. Host material shall be collected and incinerated or double bagged and deep buried in a site approved by CFIA.

Incineration (burning to ash): Infested 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) if: permitted within provincial and municipal statutes or regulations and acceptable to CFIA. 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 shall be taken. Burning may be through open burning or in an incinerator.

Deep burial: Infested 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 metres. The material must be buried at a CFIA approved site. Every effort to prevent plant debris or soil from being dislodged from the plants shall be taken.

2. Non-Porous Surfaces

Most disinfectants are not labelled 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 practicable, tools such as knives, pruners, water breakers, water wands and other implements used in the quarantine area shall 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 required prior to removal from the quarantine area. Any porous surfaces (soil like) will require treatment as described for soil.

Water

Water shall be treated with enough chlorine that will result in 2mg/litre of active chlorine or greater in samples taken at the emitters/sprinklers. The treatment must be approved by the regulatory official, and must be properly monitored and documented.

3. Soil and Potting Media

Potting media: Potting media must be heated such that the temperature in the centre of the load reaches at least 82 degrees C for 30 minutes. Treatment must be conducted in the presence of an inspector.

Soil: Soil must be heated such that the temperature in the centre of the load reaches at least 82 degrees C for 30 minutes. Treatment must be conducted in the presence of an inspector.

Biosecurity Measures for Nurseries under Regulatory Control

The following measures shall be put in place by affected nurseries and may be ordered by CFIA inspectors:

1. Restricted Access

Access to infested areas and hold areas shall be limited to officials and employees only. Proper control measures shall be used to prevent unauthorized access including the use of physical barriers such as barricade tape, temporary fences, hanging tarps, and the use of additional signage identifying an area as restricted. Where it is necessary that visitors enter the facility, the facility shall ensure that every precaution is taken to prevent the movement of infested plants, contaminated soil or debris with the visitor.

2. Sanitation Practices

Appropriate biosecurity measures must be taken to ensure that *P. ramorum* is not transferred from the destruction area to non-infested areas of the nursery. Work shall be scheduled to enable employees to progress from low risk areas to the higher risk quarantine area over the course of the work day. If possible, no inspector shall work in the destruction area and in the rest of the facility for delimitation surveys on the same day.

3. Vehicles

If possible, vehicles shall be parked on paved, concrete or gravel areas away from the immediate inspection area. The tires (or other parts in contact with the soil) of vehicles must be cleaned of soil before leaving the infested facility. A portion of the vehicle must be designated as a Aclean area@ and another area such as the trunk or a specified enclosed area shall be designated as a Adirty area@ to hold double bagged clothes, equipment or material for disposal.

4. Clothing

Coveralls (cloth or disposable) shall be worn for inspection activities. At the completion of the inspection or prior to working in other areas of the nursery, coveralls must be double bagged for disposal/cleaning and stowed in a designated Adirty area@ of the vehicle.

5. Footwear

Footwear shall be cleaned/disinfested on arrival to mitigate any quarantine pest risks and to provide confidence to the facility operator that CFIA is taking appropriate biosecurity measures. A disinfectant foot bath shall be placed and used by personnel entering and exiting the quarantine area at the infested facility, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant on a daily basis. Disposable shoe covers may be used in lieu of a footbath. Upon completion of the inspection, footwear must be cleaned of soil and disinfested, and disposable boot covers must be double bagged prior to placing into the designated Adirty area@ of the vehicle.

Hands

Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or disinfectant immediately after completion of task. In addition, hands shall be thoroughly washed with soap and water (rubbing hands together with soapy water for a minimum of 20 seconds) before entering and after leaving the inspection site. If a hand wash station is not available, antiseptic rubs/gels/rinses must be used (with a minimum of 70% ethyl alcohol). Disposable gloves may be used, and must be double bagged for disposal.

Equipment

Tools such as knives, pruners, water breakers, water wands and other implements used in the quarantine area shall only be used in the quarantine area. Otherwise, any equipment used at a work site must be disinfested prior to leaving the work site. Where practical, equipment shall be disinfested as frequently as possible at each work site. If equipment must leave work site for disinfection, it must be double bagged before being placed in the designated Adirty area@ of the vehicle.

General Recommendations for the Use of Forms Used under the Plant Protection Act and Plant Protection Regulations

1. Notice of Quarantine (CFIA/ACIA 0106 (2001/10) - Informed Filler - NOTICE OF QUARANTINE 0106.ITP)

Issued when the inspector requires a period of time to determine if the thing is or could be infested with a pest. Authority - Section 11, Plant Protection Regulations.

Usage notes:

Ending on (date)

Any practical date, including A indefinite@. Presently, Informed Filler does not allow use of A indefinite@. An expected date of ending the quarantine, but the notice will have to be reissued if the expiry date is approaching and the Quarantine issue has not been resolved (e.g. testing results are not available)

Description of Things (recommended wording)

Plants and plants parts and growing media at the location described below. If the space in the form is insufficient, an attachment may be used, indicating the serial number and date of issue. (Caution, Informed Filler does not warn that the maximum number of characters have been exceeded in this field)

The thing(s) described herein . . .

All details must be filled. A local street address is insufficient,. (Presently Informed requires n/a to be placed in all the fields prior to printing or saving) A reference to a map can be made in this section, this makes the location of the Quarantine as clear as possible to the grower.

Conditions of Quarantine (recommended wording)

Plants and plant parts and growing media are to be preserved, safeguarded or stored at the location described.@ (or *A at the affected premises@ as appropriate if there is field grown stock*)

Delivered at

Identify the regional location such as county, city, township, regional district, etc. This may be left blank until actual delivery and hand written if the grower is likely to have the notice delivered at a different location.

2. Notice of Prohibition of Movement (CFIA/ACIA 0113 (2000/01) - Informed Filler - PROHIBITION OF MOVEMENT 0113.ITP)

Issue when the inspector has reasonable grounds(e.g. diagnosis is confirmed, ready recognition of the symptoms of the disease, etc.) to believe that a thing infested.

Authority - Section 6(2), Plant Protection Act

Check boxes

Check boxes shall be consistent between documents. Use the check box described as follows - A. . .described below at the place described below . . . :is (are) infested or could be infested . . .@

Description of Thing(s)

Plants and parts of plants and growing media in association with plants in the affected location described below.

Description of Place

Complete as directed. A map is recommended indicating the location of the greenhouse or any material grown outdoors

3. Movement Certificate (CFIA/ACIA 0108 (2000/06) - Informed Filler - C0108EV2.itp)

Authority - Section 45, Plant Protection Regulations

This document must exactly describe the things to be moved. CFIA must be able to verify that the material being shipped is identifiable at destination. The use of labels etc. is recommended. There is an associated responsibility of the grower to maintain identification of things to be moved.

4. Notice to Dispose (CFIA/ACIA 0107 (2000/06) - Informed Filler - C0107e.itp)

Check Boxes

Check boxes shall be consistent between documents. Use the check box described as follows:

- AI hereby advise you that the thing(s) located and described herein . . .:is (are) infested with a pest . . .@

Manner of Disposition

Identify that the infested articles are to be disposed of in a manner that prevents the entry or introduction of pests into Canada by incineration or deep burial to a depth of no less than 2 metres in an area where the soil will not be disturbed following burial. The non-compliant materials must be immediately covered with soil/dirt. The grower must maintain identification of things to be disposed through the disposal process to allow CFIA to know what has been moved and can track the affected material through the process.

Place of Disposition

This description shall be in accordance with the disposal method used, ie if deep burial, then the location of sanitary land fill shall be noted. In this case, a **Movement Certificate** would also be necessary.

Location of the Things

The location of where the things are presently located prior to disposal.

Date of disposal

The date of disposal shall take the grower=s ability to complete job into consideration along with the need to limit exposure to infested material when setting the date. A time limit is not mandated by legislation

Description of the Thing(s)

The description of the material to be disposed off shall be specific. A map of the location of the material to be disposed off would be an asset in ensuring good communication with the grower.

5. **Notice of Release from Quarantine (CFIA/ACIA 0109)**

Authority - Section 15, Plant Protection Regulations

Form is straightforward and shall be delivered promptly once the quarantine process is complete.

Description of Thing(s)

Shall include all items noted on the **Notice of Quarantine**, less those items which have been destroyed through treatment or disposed.

6. **Notice Prohibiting/Restricting the use of a Place (CFIA/ACIA 0120)**

Issue upon completion of the quarantine period. Authority - Section 20, Plant Protection Regulations.

Usage notes:

Check boxes

The first box shall be checked- Ais infested or suspected of being infested with a pest. @

Ending on (date)- two years from the date of issuance of the notice.

Description of the Place

Complete as directed. Include GPS reading and description of infested area. A map may be attached in addition to the GPS reading(s) indicating the location of the infested plant(s) on the property. All attachments must be referred to in the notice.

Prohibitions and Restrictions

Suggested wording: Plants of host genera of *Phytophthora ramorum* listed in Appendix 1 of directive D-01-01 titled A Phytosanitary Requirements to Prevent the Entry of *Phytophthora ramorum*, @ as amended from time to time, must not be replanted within a the infested area described herein.

Delivered at

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Identify the regional location such as county, city, township, regional district etc. This may be left blank until actual delivery if the owner or the occupier of the place(s) is likely to have the notice delivered at a different location.

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Delimitation Sampling for the Confirmed Nursery Protocol

This procedure is implemented at a site when a sample has tested positive for *P. ramorum*. If a positive sample can be identified to a single Destruction area, further sampling in that block is not necessary and the block can be destroyed. However, if a positive sample was a composite made from more than one block, the blocks from which the positive was drawn must be re-sampled according to **Method 1** below. For the remaining negative blocks at the positive site, apply **Method 2** below.

In both methods the sample unit is a host species *within* a block. A block in this case follows the definition for >Destruction area= in the definitions section of the *P. ramorum* Confirmed Nursery Protocol.

Sampling Method 1

Use this method when a positive sample cannot be identified back to a single block. Table 1 is used to determine the number of host plants to be randomly inspected for symptoms in the target blocks associated with a positive sample. Sample plant tissue from all visually inspected plants that show symptoms characteristic of *P. ramorum*. For example, in a block of 5,000 host plants, if 5% of plants show symptoms of *P. ramorum* then 42 samples would be taken. Each sample consists of symptomatic tissue from one symptomatic plant. The sample shall consist of at least 7 symptomatic leaves, if present. A physical sample of each plant inspected is only to be taken if plant tissue is symptomatic for *P. ramorum*. If, however, only one leaf is symptomatic include only the one leaf with lesions. Each nursery plant from which a sample was drawn must be marked with the appropriate sample number using a label, flagging tape or stake.

Table 1. Delimitation Sampling Schedule for *P. ramorum* in Positive Blocks

Number of Host Plants in Block	Number of Plants to Inspect
#500	all
1000	632
5000	792
10000	815
>50000	839

Sampling Method 2.

Use this method for blocks that tested negative in the National or Trace Forward surveys at a site where a positive was found. Attempt to inspect the host material at the site in a consistent pattern representative of the physical layout of the blocks. For each genus within each block at the site, sample symptomatic plants according to Table 2. This will provide one composite sample per block derived from the symptomatic plants in the block. In most instances, only a few leaves per symptomatic plant shall be collected as part of the composite sample.

The number of plants to be selected (as per Table 2) is a minimum. However, if the number of symptomatic plants found in a block is less than the numbers listed in Table 2, then sample up to the minimum number. If the number of symptomatic plants within a block is unusually large, then a larger number could be sampled, not to exceed twice the values given in Table 2.

Table 2. Delimitation Sampling for *P.ramorum* in Negative Blocks at a Positive Site

Number of Plants per Genus per Block	Minimum Number of Symptomatic Plants per High Risk Plants per Block to Select in forming the Composite Sample
#150	3
151-500	5
501-1200	8
1201-10000	13
10001-35000	20
35001-500000	32
>500000	50

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Inspector=s Checklist and Record

Name of Business:	
Manager name:	
Owner name:	

Site address:	

Mailing address:	

Contact phone number:		Contact fax number:	
Type of facility:	<input type="checkbox"/> Retail Nursery	<input type="checkbox"/> Propagation Nursery	<input type="checkbox"/> Retail Greenhouse
	<input type="checkbox"/> Propagation Greenhouse	Other:	

GPS Coordinates:		Size of site:	
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Site Information

What is the water source?	
Is the water re-used/re-circulated? If yes, explain system.	
Is there treatment of the water (detail treatment if applicable)?	
Are there established biosecurity measures in place to deal with equipment use and personnel? Explain	

Is soil/media sourced off site? If yes, what is the source?	
Is composted material or bark added to the soil mix? If, yes, what is the source of this material?	
List the locations of cull piles and debris piles on the site:	

Attach a sketched map of the facility (include shipping areas)

Chemical Use	
Are fungicides used on the facility?	
When were plants that have tested positive last treated with fungicides?	
What product was used, when and at what application rate?	

Condition of plants	
Have the suspect plants been trimmed or pruned?	
How are the trimmings disposed of?	
Did the plant material come in pots?	
Were pots disposed of or re-used?	
If re-used, how were they handled?	
Has the producer noticed any problems with plants on the property? If yes describe.	

<p>Has the producer moved any plants to a different location?</p> <p>What types/varieties were moved?</p> <p>How long ago?</p> <p>What is the location of the moved plants?</p>	
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Trace Back Activities	
<p>What is the origin (to the point of propagation) of the infested material?</p> <p>Plants were propagated</p> <p>Plant types</p> <p>Plant numbers</p>	
<p>Obtain invoices, packing slips and other relevant documentation for the past 12 months</p>	

1. Soil and Potting Media Sampling

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

1.1 Preparing for sampling

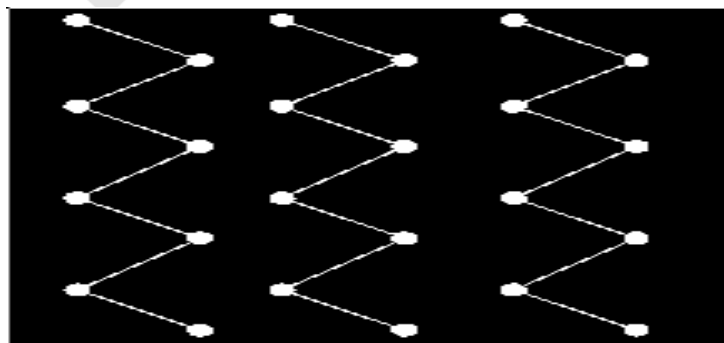
Soil and growing media samples shall be collected as composite samples. Composite samples of growing media shall be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or pot)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

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

Size of Treated Sites (hectares)	Sq Metre	No. of Soil and Growing Samples Collected (total)
0.00<n<0.1	n>1012	5 (10)
0.1<n<0.2	1012<n<2023	10 (20)
0.2<n<0.4	2023<n<4046	20 (40)
n>0.4	n>4046	30 (60)

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

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



2. Water Samples

2.1 *In Situ* Water Sampling With Leaf Baits

1. Prepare the leaves (contact the Central Plant Health Lab for appropriate leaves) as bait by cutting the leaves in a herringbone pattern into (but not through) the mid-vein or by trimming off the petiole end of each leaf.
2. Place 3-4 cut leaves into a mesh bag. Label the bag with a plastic tag listing the date, water source (location), and nursery (i.e., nursery license number).
3. Place the mesh bag into the water source for a minimum of 48-h to 1-wk (preferable).
4. Do not leave the bait in the water source for longer than 1-wk as the bait will begin to decompose.
5. Place the bags such that the leaves will remain submerged the entire time (i.e., even if water levels fluctuate within the water source). If possible, place the bait near the influent coming from the area closest to or containing the infested plants.
6. Remove the bait from the water source and transfer to a sealable bag and follow instructions in Appendix 7

2.2 Water Sampling for Filtration

1. Water samples shall be collected in a sterile wide-mouth bottle and kept at 5 – 10 C.
2. Water samples shall be taken from the surface to increase the likelihood of obtaining zoospores of *Phytophthora*.
3. Sample size shall be approximately 1000 ml. Number of samples is determined by the size of the nursery pond to be sampled (Table 1)
4. Sample shall be processed within 48 hours of collection. samples shall be submitted according to the protocols established in Appendix 7.

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

Size of pond (acres)	No. of water samples collected (liters)
0.00 - 0.25	1
0.26 - 0.5	2
0.50 - 1.0	4
1.01 - 2.50	8
>2.51	12

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Sample Handling and Laboratory Submission

Critical Instructions for Sample Submission:

1. Complete a Pest Interception Report (PIR) Form CFIA/ACIA 1303 which is available on Informed Filler.
2. Submitters shall give an estimate to the lab of how many samples will be sent and when in advance of shipment.
3. Each sample must be separately bagged (in the case of water sample bottles, place these inside a bag).
4. On each sample bag record, in permanent marker: the sampling location, block/area ID=s (if possible), plant genus (where possible), sampling date, PRI #.
5. Place all of the individual sample bags into a large plastic bag for shipping
6. Ship all samples by express courier in Styrofoam coolers containing ice packs. Samples shall not directly contact the ice packs
7. Separate sample bags with layers of newspaper or other insulation.
8. At the end of each sampling day, samples must be stored in a cold room or refrigerator at 4EC ∇ 2EC prior to shipping.
9. Be sure to include a copy of all PIR(s) inside the box. Indicate *AP. ramorum*@ on the outside of the sample box
10. Send all samples by courier.

Measures Required of Propagation Nurseries with Positive Detections of *P. ramorum* within One Year of Receiving a Notice of Removal of Quarantine

In addition to the measures prescribed in this protocol, the following additional measures shall be implemented in a propagation nursery that has been found to be positive for *P. ramorum* within one year of receiving a Notice of Removal of Quarantine. CFIA inspectors shall order these measures on the appropriate notice prescribed in Appendix 4.

1. During the quarantine period an additional inspection of all host plants at the facility shall be conducted. Any plants demonstrating signs or symptoms of *P. ramorum* infection should be sampled and tested according to the protocols established in Appendix 7 and 8.
2. The biosecurity measures prescribed in Appendix 5 shall be ordered by the CFIA on an appropriate notice as prescribed in Appendix 3. Inspectors shall routinely verify that these measures are being observed by the nursery. These measures shall remain in place for a period of at least two years following release of the nursery from quarantine.
3. All fallen leaves of high risk host plants shall be removed and destroyed from around these plants on a quarterly basis to prevent infestations entering soil or water.
4. All high risk nursery stock entering the propagation nursery is to be inspected in a secure area by trained personnel at the nursery before being moved to production areas. Any plants having symptoms of the disease are to be held in the secure area at least 10 m away from other high risk hosts for inspection by CFIA. Should these plants be found positive, these shall be ordered destroyed according to this protocol and all other hosts imported with the positive plants should be sampled and tested according to the protocols established in Section 7.2 above.

Detection of <i>P. ramorum</i> at a Propagation Nursery	
Trigger	Positive host plant.
Quarantine Action Resulting From Trigger	<p>All plants at the facility are held pending sampling and testing. This facility quarantine may be removed once areas of infestation and destruction actions have been identified.</p> <p>A quarantine area of 10 m. surrounding the destruction area is held until released (see below).</p>
Eradication Action Resulting from Trigger	<p>Where an infested high risk host plant is identified, all host plants in a contiguous block are destroyed until a 2m separation of no plants or non-hosts is present, or</p> <p>Where a low risk host plant is identified as infested, all hosts within 2m of the infested plants are destroyed.</p>
Surveillance Action Resulting From Trigger	<p>All symptomatic plants, particularly high risk hosts are sampled.</p> <p>Soil/water/cull piles also sampled and tested.</p> <p>In 10m perimeter outside the nursery of high risk hosts are sampled and tested.</p>
Trace-out Activities	<p>All shipments of related host plants moved to other propagation nurseries in the past 12 months are identified and tracked.</p> <p>Origin of infested plants is tracked</p>
Subsequent Surveillance Required for Release From Quarantine	<p>Option 1: Completion of destruction of hosts in eradication block and quarantine area or</p> <p>Option 2: Completion of destruction of hosts in destruction area and testing of plants after 90 days quarantine.</p>