

STANDARD OPERATING PROCEDURES

Mitigation of *Phytophthora ramorum* on the Bloedel Reserve

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END-OF-DAY CLEAN UP (for non-quarantine area activities)

Goal: Maintain tools as clean and disease-free from day to day as a general disease control measure.

General: All tools used on the grounds (outside of quarantined areas) will be brought up to the shop for cleaning at the end of the work day or once the tools are no longer needed. By the end of the day, all used tools will be washed of dirt, mud, and debris and thoroughly sprayed with an approved disinfectant before being returned to their proper storage location. The Director of Horticulture and Plant Health Manager will maintain signage at the tool wash location to remind employees to properly wash and disinfect tools (see Appendix).

Equipment:

- Approved disinfectant.
 - 70% alcohol solution, or
 - 10% bleach solution (use within 6 hours, not recommended for metal tools), or
 - Lysol, Greenshield, 409, or other quaternary ammonium disinfectant
- Clean water supply for washing tools.
- Stiff bristled brush.

Procedure:

1. Remove dirt, debris, and mud from the tool using clean water.
2. Thoroughly spray the tool with an approved disinfectant so that 100% of the tool surface is covered with disinfectant.
3. Return the tool to its proper storage location.
4. Wash dirt and debris from boots before leaving the grounds at the end of the day.

EMPLOYEE SANITATION WHEN WORKING IN QUARANTINE AREAS

Goal: Prevent the spread of *P. ramorum* out of quarantined areas by employees.

General: All employees performing work within the quarantined areas will adhere to the sanitation procedures specified in this section. A check list, provided in the appendix, will be used upon entry and exit into quarantine areas to document the implementation of these sanitary procedures. All employees working in quarantine areas shall consider all tools, footwear, body parts, clothing, etc. that contacts soil, mulch, plant debris, etc. to be contaminated and must be disinfected with an approved disinfectant upon exiting the quarantine area.

Equipment:

- Large washing bowl or plastic tub.
- Stiff bristled brush.
- Water bucket and clean water.
- Approved disinfectant.
 - 70% alcohol solution, or
 - 10% bleach solution (use within 6 hours), or
 - Lysol, Greenshield, 409, or other quaternary ammonium disinfectant.
- Rubber boots or disposable boot covers.
- Rubber gloves or disposable gloves.
- Rain gear (in wet weather).
- A copy of “Checklist: Quarantine Area Worker Sanitation” (see appendix).

Procedure:

Before entering the quarantine area:

1. Verify that all the above equipment is present in an adjacent vehicle or non-quarantine location where it can be easily accessed.
2. The worker(s) who will be in the quarantine area will wear rubber boots or disposable booties over their regular work shoes. They will also wear rubber or disposable gloves. Both footwear and gloves should either be disposable or able to be disinfected with an approved disinfectant.
3. In conditions (e.g. rain) where foliage is wet, workers in the quarantine area will wear rain gear capable of being disinfected with an approved disinfectant.
4. Check off appropriate boxes on the sanitation checklist.

When exiting the quarantine area (all disinfection occurs INSIDE the quarantine area):

1. Scrub all dirt, mud, and organic matter off footwear using a stiff bristled brush and water bucket. Clear treads of rocks, sticks, and other debris. Dispose of used water, mud, and debris on site.
2. Spray the outer surface of footwear with approved disinfectant.
3. If wearing raingear, spray the outer surface of the raingear with an approved disinfectant.
4. Use hand sanitizer to disinfect hands and arms.
5. See section on disinfecting tools and equipment.
6. Check off appropriate boxes on the sanitation checklist.

SAMPLING POTENTIAL *P. ramorum* POSITIVES

Goal: To observe proper sanitation practices while collecting and testing samples so as to prevent the spread of *P. ramorum*.

General: There are a few guidelines to consider first.

- A suspected positive plant is one that has symptoms resembling a *P. ramorum* infection.
- Until testing produces a negative result, suspected positive plants will be treated as if they are positive for *P. ramorum*.
- The area within a 2 meter radius of a suspected positive plant will be regarded as the “hot zone” for that plant.
- Workers entering, exiting, and exiting this “hot zone” will follow proper sanitation procedures listed in the previous section of this SOP (this includes using the checklist provided in the Appendix).
- Used tests envelopes and test sticks from positive results will be disposed of by being double bagged and place into the trash.

Procedure:

Before entering the 2 meter radius zone around the suspected positive:

1. Gather sanitation supplies (one of the ready-made sanitation kits we have on hand is appropriate).
2. Record appropriate information in the *Phytophthora* Testing log book.
3. Prepare flagging for the suspected positive (write sample number on the ribbon).
4. Look to see that you have all the sample collection/testing kit supplies with you.
5. Wear rubber boots and disposable sampling gloves (vinyl or nitrile).
6. Place sanitation equipment and supplies within easy reach.
7. Prepare sample kit. Cut top off with scissors.

Collecting the sample:




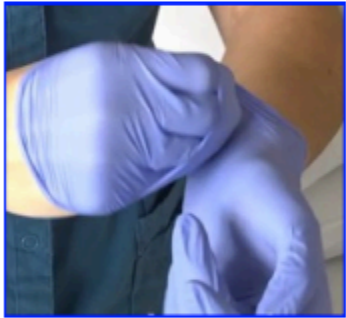


1. Take a one gallon Ziploc bag (labeled “sample bag” with the sample id number on it) and fold back the top edges. Place this just outside of the “hotzone”.
2. Do the same with another Ziploc bag marked “Ramorum/Dirty”.
3. With your left gloved hand, hold open the test kit envelope. With the right gloved hand, take a sample of diseased tissue (approximately 1 square inch) and place it into the test kit envelope.
4. Continue holding the test envelope with your left hand. Using your fingers or a blunt tool, rub the envelope sides to mildly macerate the leaf tissue.
5. With your right hand, place the test strip into the test envelope. Watch the strip until the solution creates the control band on the test strip. If the phytophthora indicator band does not appear within one minute of the control band forming, place the test envelop into the Ziploc bag from step (1), taking care not to contact the Ziploc bag with your gloves.

6. Place tools and scissors into a bucket of freshly made 10% bleach solution. Same one you will step into to decontaminate your boots.

Exiting the Sample Collection Site

1. Step into the bleach bucket and then back into the “hot zone”. This is just to get your boots wet to make brush scrubbing easier.
2. With a brush, scrub rocks, debris, etc off your boots, being careful to keep all of it within the “hot zone”.
3. Put the brush into the bleach bucket.
4. Step into bleach bucket.
5. While standing in the bleach bucket, remove your gloves properly (see picture) and place them into the bag marked “Ramorum/Dirty”.
6. Step out of the bleach bucket. You are now okay to be outside of the hot zone.
7. Repeat these steps for every sample plant.

Photo: Sterile technique for glove removal.

“Beak Method” Glove Removal Steps		
		
<p>STEP 1: Using one gloved hand, pinch and pull the base of the other gloved hand.</p>	<p>STEP 2: Use the middle finger to scoop the cuff of the glove.</p>	<p>STEP 3: Pull the glove inside out over all the fingers and thumb to form a “beak.”</p>
		
<p>STEP 4: With the beaked hand, pinch the opposite glove at the base and pull the cuff.</p>	<p>STEP 5: Roll the glove inside out and off the hand.</p>	<p>STEP 6: With the ungloved hand, use the index finger to pull the beaked glove off at the base of the beak and dispose into the appropriate waste container.</p>
		<p>Always wash your hands after glove removal.</p>

REMOVING MATERIAL FROM QUARANTINE AREAS

Goal: Properly dispose of potentially diseased material while preventing the spread of *P. ramorum* to other areas of the grounds.

General: Plant material that is known to be infected with *Phytophthora ramorum* or is within a designated eradication zone must be destroyed by either burning or deep burial (i.e. the top of the debris is 2 meters below the top of the hole). Removal and destruction of known *P. ramorum* positives must be observed by a USDA or WSDA employee. In some cases, leaving the material within the quarantine area is possible with permission from the Director of Horticulture or the Plant Health Manager. Otherwise, material must be transported to the burn/burial site.

Currently, the burn site is located in the old orchard area near the well house. To burn at that site, a burn permit must be issued by the Bainbridge Island Fire Department.

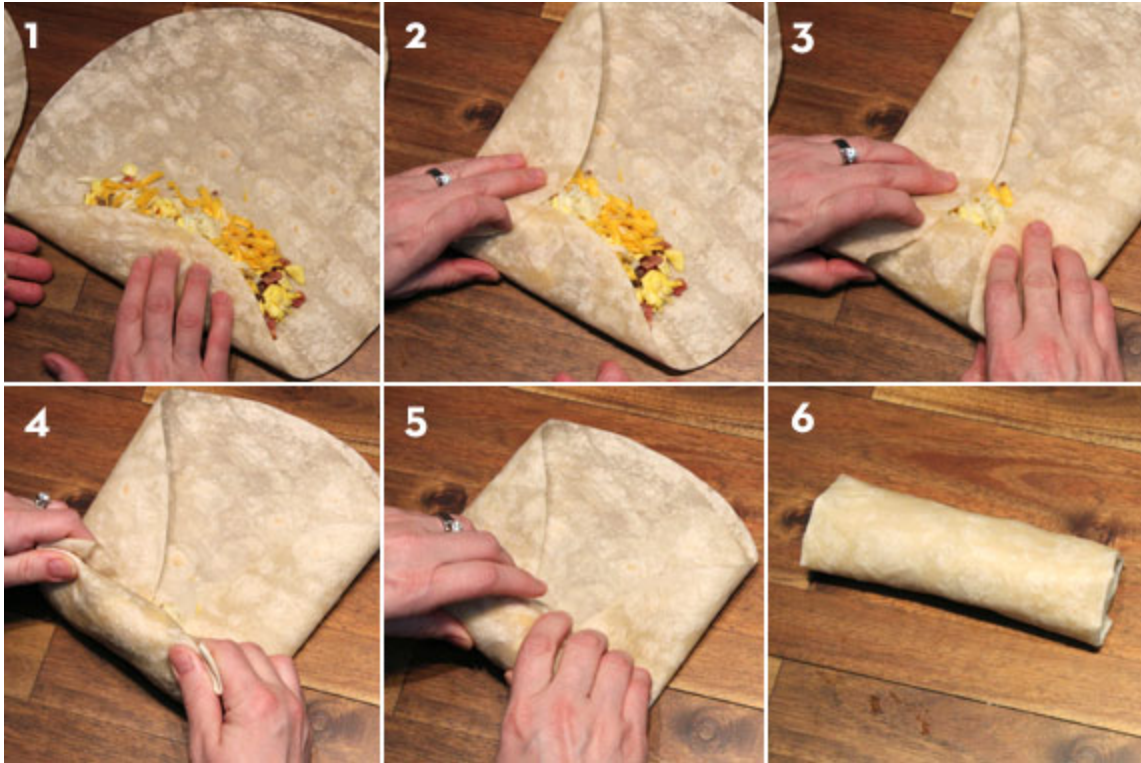
We don't do deep burial unless burning of material is not possible within the USDA-specified time limit .

Equipment:

- All equipment required for worker sanitation (see page 5).
- Clean, undamaged polypropylene tarp (preferably brand new).
- 4mil (or 3mil doubled up) garbage bags.
- Tyvek suit for each worker who enters the quarantine zone (not needed if the site has previously been cleared of debris).
- Duct Tape.

Procedure:

1. Unfold a clean (preferably new) tarp outside of the quarantine area. It should be close enough that workers inside the area can place loose or bagged debris onto it without spilling onto the ground outside of the area. This tarp will not be reused after the plant material is destroyed.
2. Workers inside the work area may deposit plant material/debris onto the tarp. Care must be taken not to let material land outside of the tarp. **VERY IMPORTANT:** Put only as much material on the tarp as will allow it to be folded up completely around the material and securely taped up for transport to the destruction site.
3. **Photo: Properly folding the tarp.** Once the tarp is full, fold it up securely. A recommended method is to fold it similar to how a burrito is folded:



4. Tape up the tarp adequately to prevent it from unfolding during transport.
5. Transport the secured tarp and debris to the destruction site to be stored until it can be destroyed in an approved manner (burning or deep burial).
6. The stored material must be covered with a clean tarp that is weighted to prevent wind from blowing it off. Also, the material must be ribboned off and prominently marked with signage that reads "Do not disturb – per USDA ".

GENERAL GREENHOUSE AND NURSERY PREVENTION

Goal: To prevent the spread of *Phytophthora ramorum* and other plant pests and disease through proper sanitation practices.

General: The approach to preventing the spread of disease in the nursery and propagation area will involve education, exclusion, eradication, and sanitation.

Education: There are two forms of education to be used.

1. Posted informational material. Relevant information will be posted at appropriate locations around the nursery area to remind and educate workers and volunteers about proper phytosanitation practices.
2. Educational training of workers and volunteers. This training may take the format of a classroom session, a meeting, “on-the-job” training, or another method approved by the Director of Horticulture or the Plant Health Manager.

Exculsion: Infected material will be *excluded* from the nursery area by means of a quarantine holding zone located no less than 2 meters from non-quarantined areas. A greater separation distance should be used when space permits. New plants brought to the grounds will be held in the quarantine zone for 6-8 weeks. Longer quarantine durations are preferred when possible. If at the end of that period, the plants are symptom free, they will be released to the nursery area or for planting on the grounds. Plants producing potential *P. ramorum* symptoms will be tested by immunoassay. Positive immunoassay samples will be referred to the WSDA/USDA for confirmation of *P. ramorum*. Plants with positive WSDA results will be destroyed by an approved method under WSDA/USDA supervision. Symptom free plants may only be released from the quarantine zone with permission from the Director of Horticulture or the Plant Health Manager.

Eradication: Plants testing positive for *P. ramorum* by the WSDA/USDA will be destroyed by an approved method under WSDA/USDA supervision.

Sanitation: Steps will be taken to reduce spread of *P. ramorum* and other plant pests and diseases in the nursery area:

- Debris that could potentially harbor pests and spores will not be allowed to accumulate on the ground.
- Weeds (potential hosts) will be removed regularly.
- Surrounding meadow areas will be kept cut short.
- Water will not be allowed to puddle.
- Aisles will be 3-4 feet wide to allow for ease of movement and to prevent physical contact between blocks.
- See the next two sections on new plant quarantine guidelines.

- Plants will not be set under benches.

Watering: Since *P. ramorum* is a water mold, control of water availability is a significant component of control.

- As much as possible do watering in the morning so standing water has more day time to speed evaporation.
- Do not place plants underneath tables where excess water can drip down from plants on top.
- Nursery ground should be as flat as possible with good drainage to avoid puddling.
- Avoid high pressure irrigation heads that produce mist. Larger drop size is preferable.

QUARANTINE GUIDELINES FOR PURCHASED OR DONATED PLANT MATERIALS

Goal: To prevent the introduction of *P. ramorum* via plant material brought in from outside the Reserve.

General: Ready-to-plant materials will be isolated in a quarantine area at the nursery and observed for SOD symptoms for 6-8 weeks to determine suitability for planting on the grounds. Plants with SOD-like symptoms will be tested via immunoassay for *Phytophthora*. Plants testing positive for *Phytophthora* will be referred to the WSDA/USDA for further testing. Positive plants will remain in the quarantine area until the USDA issues a destruction order or clears them for use on the grounds.

Nursery Quarantine Area:

- **Location:** The nursery quarantine area will be separated from other nursery areas by a minimum of 2 meters. A greater distance is preferred. The 2 meter distance reflects the USDA's requirement that plants located within 2 meters of a SOD-positive plant must be destroyed.
- **Blocks:** Plants from the same order will be grouped together into blocks separated by 2 meters to assist in possible trace-backs and to prevent contamination of orders from other nurseries.
Isolation Period: Orders will be kept in the quarantine area for 6-8 weeks. During periods less optimal for symptom development (i.e. summer and winter), the longer end of the range will be preferred to allow extra time for symptoms to appear.
- **Observation:** Plants will be observed twice a week for development of symptoms of *P. ramorum*.
- **Testing:** Plants exhibiting potential symptoms will be tested by immunoassay for *Phytophthora*. Plants that are immunoassay-positive will be retested by the WSDA/USDA to determine if *P. ramorum* is present.

QUARANTINE GUIDELINES FOR IN-HOUSE PROPAGATION

Goal: To prevent the introduction of *P. ramorum* via propagation materials from external sources.

General: Since propagation materials are not ready to plant and usually require different handling than nursery-bought materials, they are handled differently than ready-to-plant materials. The guidelines given below apply to vegetative material, not seeds.

Guidelines:

- **Containers:** All growing containers used will be either previously unused or used and disinfected according to the procedure given in the next section, “Nursery Container Management.”
- **Growing Media:** Only new growing media will be used. Previously used media will be dumped into the cull pile.
- **Propagation Surfaces:** Surfaces used for vegetative propagation (mist benches, heating pads, potting benches, etc) will be disinfected after use with an approved disinfectant.
- **Handling during propagation:** Externally sourced plant materials should not share propagation benches with internally sourced plant materials. They should either have their own dedicated benches or their benches should be properly disinfected once they have been moved to the quarantine site. Benches containing externally sourced materials should be kept a minimum of two meters from other plant materials.
- **Testing:** Plants exhibiting potential symptoms will be tested by immunoassay for *Phytophthora*. Plants that are immunoassay-positive will be retested by the WSDA/USDA to determine if *P. ramorum* is present.
- **Quarantine:** After materials are potted up, they are to be moved to the designated quarantine area where they will remain for 6-8 weeks provided they do not develop symptoms. Time spent in mist beds, heat beds, etc, does not count toward quarantine time.

NURSERY CONTAINER MANAGEMENT

Goal: To prevent contamination of new plant material from old containers and growing media. Used media will not be reused! Used pots will be properly sanitized prior to reuse according to the procedure given in this section.

General: Prevention of disease spread will be achieved by two methods: Separation and disinfection. Plant cull, disinfection, and potting areas will be separated by distance to prevent contamination of new material by contact with old pots and growing media. The storage area for used, non-disinfected pots will be separated by distance from the storage area for ready-for-use pots. Old pots will be disinfected with an approved disinfectant prior to being reused for new plant materials.

Areas:

1. **Cull Pile/Un-sanitized Pot Storage/Pot Washing Area:** This area will be located away and preferably downhill from the nursery. Its purpose is to provide an area dedicated to disposing of old growing media and storing used pots prior to disinfection for reuse. The pot storage location shall be marked with a placard indicating that the pots are not to be used until disinfected.
2. **Disinfection Area:** Pots that have been rinsed will be moved here for disinfection. Until rinsed pots are disinfected, they will still be regarded as potentially infested with disease. A tank will be located in this area for a disinfectant soak. Post a placard indicating that pots stored in this area have not yet been disinfected and are not ready for use in the nursery.
3. **Potting Area/Clean Pot Storage:** This area will be located away from the cull pile area to prevent accidental contamination of new plant material. Both new and disinfected used pots will be stored here. The clean pot area will be marked with a placard indicating the pots may be used.

Procedure: Disinfecting Used Pots:

1. Prior to disinfection, pots will be stored in the unsanitized pot storage area.
2. Debris will be washed off the pots into the cull pile using pressurized water and a nozzle to remove as much debris as possible. Alternatively, they may be soaked overnight in water in a tank located by the cull pile and then rinsed the next day. Soaking aids in removal of debris. Debris on the pots can reduce the efficacy of disinfectants so the pots should be as clean as possible.
3. After rinsing, pots will be transported and stored at the disinfection area.
4. To disinfect pots, fill the container with enough disinfectant fluid to cover the pots completely. A 10% bleach solution is recommended. The bleach solution must be used fresh each time! Never reuse old bleach solution. A quaternary ammonium disinfectant may work but out moderately hard water may adversely affect the efficiency of a quaternary ammonium product.
5. Wear eye protection and rubber gloves (bleach is corrosive).

6. Submerge pots in the disinfectant solution making sure the entire surface of the pot contacts the solution. Remove pots and shake off excess solution over the disinfection tank.
7. Immediately move pots to the clean pot storage area. Once there, the pots are ready for use in the nursery area.

APPENDIX

The following pages include information, checklists, etc. relevant to these SOP's.

Checklist: Quarantine Area Worker Sanitation

Date of Work _____

Pre-entry Equipment Check

- Large washing bowl or plastic tub.
- Stiff bristled brush
- Water bucket and clean water
- Approved disinfectant
 - 70% alcohol solution, or
 - 10% bleach solution (use within 6 hours), or
 - Lysol, Greenshield, 409, or other quaternary ammonium disinfectant
- Rubber boots or disposable boot covers.
- Rubber gloves or disposable gloves
- Rain gear (in wet weather).
- Spray bottle of non-corrosive approved disinfectant.
- 4mil or doubled 3mil garbage bag(s).
- A copy of "Checklist: Quarantine Area Worker Sanitation" (see appendix)

Site Pre-entry Worker Check

- All disinfection equipment is on site and easily accessible.
- Worker(s) wearing approved footwear.
- Worker(s) wearing approved gloves.
- Worker(s) wearing raingear (under wet foliage conditions).

Site Exit Worker Check

- Sterilize tools with an approved disinfectant (preferably not bleach).
- Scrub and rinse dirt and debris from the bottom of your boots (while in quarantine area).
- Step out of quarantine area into basin filled with fresh 10% bleach solution.
- If wearing raingear, spray down raingear with an approved disinfectant.
- Remove and dispose of work gloves into garbage bag.
- Apply hand sanitizer to hands and arms

Site Lead Signature _____ Date _____

Inspector Signature _____ Date _____

Checklist: Quarantine Area Worker Sanitation

Date: _____ Positive Site (if applicable) # _____

Reason required (check each as applies)

- Work in *P. ramorum* positive or previously positive area.
- Leaving a work site.
- End of work day.
- ELISA positive plant being tested.
- Footwear is dirty/muddy

Footwear sanitation (check to verify completed)

- Footwear treads cleared of rocks, sticks, and all other debris.
- Dirt, mud, and organic matter scrubbed off footwear using a stiff brush and water.
- Used water, mud, and debris disposed of on site.
- Outer surface of footwear sprayed with approved disinfectant.
- Bleach solutions (e.g. other than Lysol cans) made the same day or more frequently if necessary.
- Hand sanitizer used to disinfect hands and arms.

Sanitizing work tools and equipment (check to verify completed, write NA if not applicable)

- Plant debris, soil, mud, and organic matter scrubbed from all tools and equipment.
- Disinfectant sprayed on all surfaces of equipment, including tool handles.
- Water drains away from host plants and any foot/vehicle traffic.
- High pressure hose used to remove organic matter from machinery.
- Under carriage and tire treads inspected to insure all debris removed.
- Disinfectant sprayed on all surfaces including seats and steering wheels.
- Hand sanitizer used at the completion of the sanitation work.

Additional (check to verify completed, write NA if not applicable)

- Collect plant material and debris onto tarps.
- Tarps sanitized before exiting (if they are inside positive sites).
- Tarps sanitized after use or properly disposed of.

Employee Name and Signiature _____

Supervisor Name and Signature _____

Signage: Tool Wash Reminder

End-of-day Tool Cleanup

- 1. Rinse all mud, dirt, and debris from tool surfaces.**
- 2. Completely cover tool surfaces with disinfectant spray.**
- 3. Return tools to their proper storage location.**