

Guidelines to Minimize *Phytophthora* Pathogens for holding (non-production) nurseries at restoration sites

These guidelines present a set of practices to avoid contamination of nursery stock being held for planting at restoration sites. The guidelines are designed to minimize the chance of infection by *Phytophthora* and other plant pathogens.

Definitions:

- **Clean** — Sanitized, heat-treated, or new (e.g., plastic pots), and maintained in a way to prevent subsequent contamination.
- **Clean production area** — Entire nursery area or a fenced, posted, separated area maintained to exclude contaminated materials to the best degree possible.
- **Clean production system** — An integrated system for producing plants that are free of soilborne *Phytophthora* species and other plant pathogens. Plants produced or maintained following these specifications are likely to be free of most significant soilborne pathogens.
- **Contaminated or potentially contaminated** — Any surface or material that is not freshly sanitized, heat-treated or otherwise clean. The ground, soil and potting media that has not been heat treated, used pots, plants not produced following these BMPs (including all plants from other nurseries or in natural or planted landscapes), and anything that has been in contact with these should be considered as potentially contaminated.
- **Cull** — As a verb, to pick out for the purpose of discarding (e.g., plants showing disease symptoms are culled). As a noun, cull refers to the items (e.g., diseased plants) that have been selected for discard.
- **Holding nursery** — A facility where nursery stock is maintained for a short to extended period of time before planting. Plant maintenance activities include irrigation, fertilization or light pruning as necessary. Nurseries that conduct propagation or repotting are considered production nurseries.
- **Nursery stock** — Container or other nursery stock produced for outplanting.
- **Phytosanitary** — Free of plant pathogens, describes techniques or practices that prevent materials from being infected or infested with plant pathogens (e.g., phytosanitary measures).
- **Potting media** — Substrate used for germinating, rooting, or growing plants in containers. Typically a mixture of organic and inorganic materials.
- **Sanitize** — Clean and treat with a sanitizing agent or via a lethal heat exposure to kill plant pathogens present as external contamination.
- **Sanitizing agent** — Materials such as bleach (sodium hypochlorite solutions), alcohol, quaternary ammonium compounds, and peroxides that can directly kill exposed propagules of *Phytophthora* or other plant pathogens when used properly. Most sanitizing agents can also kill a wide variety of bacteria and deactivate many viruses. Note that most fungicides are applied to plants to suppress disease but do not kill the pathogens and are not sanitizing agents.

- **Surface water** – Water from creeks, rivers, ponds, or urban or agricultural runoff, which is commonly contaminated with *Phytophthora*. Water from large reservoirs that is drawn from at least several feet below the water surface and away from the shore has a low risk of significant contamination by *Phytophthora* propagules and is not included in this category.

1. Maintaining nursery stock in a holding facility

Nursery stock should be free of exotic *Phytophthora* to the maximum degree attainable. If plants are held after delivery and before planting, clean nursery practices must be followed to prevent contamination of the nursery stock with *Phytophthora* or other plant pathogens. Container nursery stock has a high risk of infection by *Phytophthora* species if exposed to these pathogenic agents. Exclusion of these pathogens provides the only viable option for maintaining nursery plants free of *Phytophthora*.

A holding nursery or facility is a site outside of the production nursery where plant stock is held for a period of time before being delivered to the planting area. Holding nursery plant maintenance activities normally include irrigation, but fertilization or light pruning may occur. Nurseries involved in other activities, including propagation or repotting shall follow Nursery BMPS.

Because holding nurseries by definition are not involved in plant propagation activities, clean nursery production practices related to planting materials, containers, and potting media do not apply. However, holding nurseries must use non-contaminated water for irrigation and follow a comprehensive program of clean nursery practices to prevent contamination the nursery stock.

2. Clean water specifications

Objective: Use only uncontaminated, appropriately-treated water for irrigation.

Surface waters, including untreated water from streams, ponds and nursery runoff, are known sources of *Phytophthora* contamination. Only uncontaminated water or water that has been effectively treated to remove or kill *Phytophthora* should be used for irrigating plant material.

- 2.1. Water used for irrigation shall be from treated municipal water supplies or wells and delivered through intact pipes with backflow prevention devices. Tertiary-treated municipal recycled water is acceptable.
- 2.2. If well water is used, wellheads shall be protected from contamination by surface water sources.
- 2.3. Untreated surface waters and recycled nursery runoff shall not be used for irrigation, and plants shall not be held where potential contamination from such sources is possible via splash, runoff, or inundation.
- 2.4. Keep irrigation equipment free of contamination that could be transferred to irrigation water or plants. All hose ends, nozzles, emitters, sprinklers, and other irrigation equipment used to apply water to plants must be sanitized before use on plants. Drip lines, hoses, and other irrigation equipment that is suspended over plants or may come in contact with plants must also be sanitized if they happen to

contact the ground or other potentially contaminated surfaces. Sanitize drip irrigation equipment whenever it is moved to a different set of plants.

3. Clean nursery practices for holding (non-production) nurseries

Objective: Prevent contamination of plant materials by consistent, comprehensive phytosanitary working practices.

3.1. Workers

- 3.1.1. Nursery workers shall be trained in approved phytosanitary procedures and follow the procedures at all times.
- 3.1.2. Clothing worn in the nursery shall be free of all mud, soil, or detritus. If clothing is not freshly laundered, all debris and adhered soil should be removed by brushing with a stiff brush before entering the nursery. Use a removable outer layer (apron or coveralls) if necessary to provide a clean clothing layer.
- 3.1.3. Footwear should be cleaned and sanitized before entering clean areas of the nursery. Clean off all soil and detritus first and finish by soaking the soles and contaminated portions of the uppers with a disinfectant. Disinfectant options are listed in in Section 4, below.
- 3.1.4. Use waterproof gloves when possible and clean and sanitize regularly (or discard as needed if using disposables). Leather or fabric gloves are hard to sanitize and keep free of soil particles and should be avoided. Where use of these gloves is necessary, use multiple washable pairs and change into clean gloves if gloves become contaminated or when switching between activities. If not using gloves, wash hands thoroughly with soap and water or hand sanitizer (quaternary ammonium or alcohol based), making sure to clean off all adhering soil.

3.2. Nursery design and layout

- 3.2.1. Assess areas adjacent to the nursery to determine whether they could serve as sources of contamination via flowing water, mud flows, blowing soil or debris, or splash from roads or vegetation. Install drainage, fencing, and barriers where appropriate to mitigate contamination from off-site sources. Consider worst-case conditions (heavy rainfall, high winds, etc.) when designing mitigation measures such as drainage to ensure that these measures will be effective across the whole range of weather conditions.
- 3.2.2. Nursery stock needs to be maintained in a clean area identified by signage at all access points. On signs specify decontamination procedures required before entry and required phytosanitary working practices.
- 3.2.3. Provide disinfectant footbaths or other decontamination supplies (brushes and disinfectant sprayers) for sanitizing footwear at all entrances to clean areas. Alternatively, workers may use a separate set of sanitized shoes or boots that are used only in the clean area. Sanitize these at least daily.
- 3.2.4. Designated clean areas of the nursery shall be separated by a buffer of no less than 20 ft from potentially contaminated areas and shall be bounded by a fence or other physical barrier to prevent direct movement of personnel or equipment into the clean area without passing through a designated decontamination area. A buffer of less than 20 ft may be

acceptable if the clean production area is surrounded by a wall or other barrier that prevents contamination via splashing or running water.

- 3.2.5. Contaminated areas (e.g., trash bins, dirty piles of containers) shall not be located where runoff, splash, or wind can move contaminated soil, water, or debris into clean areas. Keep the size of contaminated areas to a minimum. Use solid surfaces, catchments, and drains to capture and remove contaminated soil, debris, and runoff to minimize opportunities for spread into clean areas.
- 3.2.6. Use closed bins or dumpsters for disposal. Areas for handling and discarding dead or diseased plants should be outside of the nursery clean area and should not be located where wind or flowing water could carry contamination into the clean area.
- 3.2.7. If possible, locate delivery and pickup areas at the edge of the clean area so that vehicles and equipment do not need to enter clean production areas. Tires, wheels, and undercarriages of vehicles, equipment, and carts must be cleaned to be free of soil and debris before entering the clean area of the nursery.

3.3. Benches and growing areas

- 3.3.1. Tops of growing benches must be a minimum of 2.5 ft above the underlying ground surface (3 ft is preferable) to minimize the risk that water splashed from the ground can contact containers. If this is not practical due to the use of very tall containers, keep containers as far above the ground surface as possible and modify ground cover materials and irrigation to minimize potential of contamination from ground splash.
- 3.3.2. Benches shall have a surface of expanded wire mesh or similar to prevent water movement between pots. Plywood, wood pallets, or similar solid surfaces that allow water to pool or run laterally are not acceptable. Do not use benches that have wide horizontal surfaces that can catch and hold water or debris.
- 3.3.3. Clean and sanitize benches before placing a new set of nursery stock on them. The bench surface and underlying ground surface beneath any plant testing positive for *Phytophthora* shall be sanitized before any other plant material is placed on the bench.
- 3.3.4. Manage surfaces underneath benches and in walkways and driveways to prevent puddles, eliminate potential for splash, and remain free of weedy vegetation. Maintain adequate drainage and use gravel, landscape fabric, pavers, concrete, or other materials to keep underlying soil covered and avoid having exposed damp soil or mud.
- 3.3.5. Allow as much space as possible between benches and between blocks of plants within benches to minimize the potential for cross contamination via splash. A gap of 3-5 ft is recommended. Note that if contamination is detected, all plants within splash distance of a *Phytophthora*-infected plant or block of plants need to be quarantined for further testing or discarded, so maintaining separation helps localize spot infestations to the fewest plants possible.
- 3.3.6. Maximize spacing between pots within blocks to the degree possible to reduce the potential for pot-to-pot splash.

3.4. Tools, surfaces, and handling nursery stock

- 3.4.1. Clean and sanitize hands, surfaces, and tools before use on nursery stock and periodically when handling many plants successively in operations such as pruning. Clean and sanitize hands/gloves, tools, etc., when switching between different sets of plants.
- 3.4.2. Items (including workers' gloves or hands) that have been in contact with the ground or other potentially contaminated surfaces or materials must be sanitized before being placed in contact with clean plant materials, pots, soil, or benches.
- 3.4.3. Unsanitized items shall not be placed in contact with or inserted into the plant potting media (this includes checking moisture with a finger). Plant stakes, irrigation emitters, and all other items placed on or in pots shall be new or freshly sanitized before use. Use clean and sanitized tools, fingers, etc., when moving from one plant container to the next if it is necessary to probe multiple pots.
- 3.4.4. Commonly used tools and equipment should be designated for exclusive use in the clean area. Provide clean storage areas where tools can be stored off the ground and away from splashing water. Tools and equipment should be stored clean and sanitized before use.
- 3.4.5. Avoid unnecessary handling, rearranging, and moving of plants. Handling increases chances for contamination. Rearranging plants can obscure patterns that might indicate a disease or pest problem, and can also increase the chances for spread by giving infected plants new neighbors.
- 3.4.6. Nursery stock shall not be placed on the ground or any unsanitized surface at any time. Plants that are potentially contaminated through improper handling should be discarded or moved to a quarantine area. It is better to discard a single potentially contaminated plant than to risk contaminating an entire block.
- 3.4.7. Place plants and other clean items only on clean or sanitized surfaces if it is necessary to move them. Clean intact sheets of plastic or paper may be used as a clean working surface.
- 3.4.8. Remove suspected diseased plants as soon as problems are seen. Transfer to a quarantine area for testing. Note the locations on the bench and make notes indicating date, symptoms, and test results. Monitor and test adjacent plants as appropriate (see Inspection and testing, below).
- 3.4.9. Promptly dispose of culled plants and disposable contaminated materials by placing them in a closed waste container. Do not maintain containers of contaminated waste or culls in the clean area. After use, take them to the waste disposal area and clean and sanitize the container before bringing it back to the clean area. Alternatively, use disposable bags for waste collection, seal, and take directly to the waste disposal area.
- 3.4.10. Maintain general cleanliness in the nursery by removing plant debris and spilled potting media. Avoid creating dust and splash when cleaning.

3.5. Irrigation and other cultural inputs

- 3.5.1. Use low water pressure and small droplet sizes when irrigating to minimize splash between containers.
- 3.5.2. Schedule overhead irrigations to minimize the duration of leaf wetness.

- 3.5.3. Avoid excessive irrigation or stressing plants with inadequate water. Consider water loss from evapotranspiration, inputs from rainfall, plant and pot size and other factors when scheduling irrigations.
- 3.5.4. Irrigation wands, nozzles, hose ends shall be kept free of contamination by being hung on a clean hook or rack at least 3 ft above the ground. The same standard applies to any portion of a hose that may come in contact with or will be held over plants or benches during use. Resanitize these items after any contact with the ground or other potentially contaminated surfaces.
- 3.5.5. Do not apply materials to plants (e.g., organic amendments, organic fertilizers, additional potting media) unless these materials (a) are reliably documented as being free of *Phytophthora* and other pathogens and (b) have been stored and handled in a way to prevent contamination.
- 3.5.6. Do not use fungicides labeled for use against *Phytophthora* or phosphites (including use of fertilizers containing phosphite salts). These chemicals suppress *Phytophthora* symptom expression in the nursery, but do not eliminate infections, and thus can allow introductions of *Phytophthora* into the nursery to be undetected.
- 3.5.7. Avoid using soil biological control agents (e.g., soil bacteria or fungi) or other microbial additives that may interfere with disease monitoring. All such inoculants need to be free of plant pathogens. Do not use biofungicides, since in general they suppress but do not eliminate infections.

3.6. Inspection and testing

- 3.6.1. All plants shall be visually inspected weekly for poor plant growth or appearance.
- 3.6.2. Dead, dying, or poorly performing plants shall be inspected and possible cause(s) shall be identified to the extent feasible. Dead and dying plants and any other plants with possible root disease symptoms shall be removed, tested, and subsequently destroyed. Patterns of symptoms in the block that may suggest spread from one or more infected plants should be noted. Positions of culled plants on a bench should remain unoccupied at least until testing has been completed so that spatial patterns of disease can be determined. The number and locations (bench, block) of affected plants shall be recorded.
- 3.6.3. Remove suspected diseased plants from the clean production area in a manner that will prevent contamination of other remaining plants. Do not allow water or potting media from removed containers to fall into other containers or onto clean surfaces. Place pots directly in a plastic bag or clean container before moving.
- 3.6.4. Root systems of poor performing, dying, or dead plants shall be inspected for evidence of root or crown decay. If possible root decay is detected, plants shall be tested for the presence of *Phytophthora*, or other pathogens.
- 3.6.5. If symptomatic plants are observed or a positive result is obtained from testing, impose an in-place quarantine of the block tested as well as any other stock within 5 ft. Follow-up with testing of quarantined blocks of plants and other plants from the same and related batches.
- 3.6.6. Upon a single positive *Phytophthora* detection within a block, all pots within a 1 ft radius of the edge of the affected container shall be removed from the block and subjected to further

testing. If additional detections are made in this sample, all containers within 5 ft from the edge of the detection shall be removed from the clean area and discarded.

- 3.6.7. The quarantine of plants adjacent to a detection will be lifted if negative results (no *Phytophthora* detections) are obtained in two successive tests conducted at least 2 weeks apart following an approved testing protocol. Additional testing may be required before final plant use if material is held for an extended period after testing.

3.3. Documentation

Nurseries shall record data and maintain records needed to verify compliance with clean production BMPs. Logged entries shall include dates and employee initials. All records shall be available for review. At a minimum, records should cover the following areas.

- 3.7.1. **Water:** Record the water supply used, including practices used for maintaining wellhead integrity, if applicable. If using municipal sources, note any maintenance that has been performed, such as broken pipe repair, etc.
- 3.7.2. **Nursery practices:** Compliance with phytosanitary procedures shall be documented with dated log sheets. Logs shall include records for mixing or refreshing of disinfectant solutions, plant health inspections, checklists, and other records used to maintain clean conditions.
- 3.7.3. **Testing:** Records shall show which batches or individual plants have been tested, where they were located in the holding nursery, and dated test results. Record the disposition of tested plants and corresponding blocks within the nursery, follow-up testing, and determinations as to the source(s) of detections.
- 3.7.4. **Worker training:** Document worker instruction and training and retain copies of written materials used for training.

4. Procedures for sanitizing tools, surfaces, and footwear

Surfaces and tools should be clean and sanitized before use. Tools and working surfaces (e.g., potting benches) should be smooth and nonporous to facilitate cleaning and sanitation. Wood handles on tools should be sealed with a waterproof coating to make them easier to sanitize.

Before sanitizing items, remove all soil and organic material (roots, sap, etc.) from their surfaces. If necessary, use a detergent solution and brush to scrub off surface contaminants. The sanitizing agent may also be used as a cleaning solution. Screwdrivers or similar implements may be needed to clean soil out of crevices or shoe treads. Brushes and other implements used to help remove soil must be visibly clean and sanitized after use.

After surface soil and contamination are removed, treat the surface with one of the following sanitizing agents, allowing the appropriate contact time before rinsing. If surfaces are clean and dry, wet surfaces thoroughly and allow for the appropriate contact time listed. If the sanitizer has been used to help clean the surface, use fresh sanitizer to rinse off any dirty solution and then allow the required contact time. If treated surfaces are wetted with water, the sanitizing solution will become diluted. Apply enough sanitizer to completely displace the water film and then allow the required contact time. Sanitizing agents may be applied with spray bottles to thoroughly wet the surface. Observe all appropriate safety precautions to prevent contact with eyes or skin when using these solutions.

- 70-90% ethyl or isopropyl alcohol - spray to thoroughly wet the surface and allow to air dry before use
- freshly diluted bleach solution (0.525% sodium hypochlorite, Table 1) for a minimum of 1 minute (due to corrosivity, not advised for steel or other materials damaged by bleach)
- 2000 ppm quaternary ammonium disinfectant for 1 min (or according to manufacturer recommendations) - freshly made or tested to ensure target concentrations

Table 1. Dilutions of commonly available bleach products needed to obtain approximately 0.525% sodium hypochlorite concentrations (5000 ppm available chlorine).

Percent sodium hypochlorite in bleach	Parts bleach	Parts water	Diluted bleach percent sodium hypochlorite
5.25%	1	9	0.525%
6.0%	1	10.4	0.526%
8.25%	1	14.6	0.529%
8.3%	1	14.8	0.525%

For example, adding 100 ml of 5.25% bleach to 900 ml of water will make 1000 ml of 0.525% NaOCl solution. If using 8.3% bleach, add 100 ml of bleach to 1480 ml of water to make 1490 ml of 0.525% NaOCl.