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Phytosanitary Procedures
for CNPS BMPs for Producing Clean Nursery Stock

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This document provides descriptions and details of phytosanitary procedures referenced in the CNPS BEST MANAGEMENT PRACTICES (BMPS) FOR PRODUCING CLEAN NURSERY STOCK**.** Note that alternative methods may be acceptable if they are supported by published data or other valid test results showing that the methods are effective.

1. Sanitizing materials and treatments

This section discusses selected chemicals and treatments that are used to kill propagules of *Phytophthora* and other plant pathogens that are present in or on materials used nursery production. It is important to understand the appropriate uses and limitations of each treatment. Chemicals and heated materials can pose safety hazards to workers. Personnel should identify and observe all necessary safety precautions to protect themselves and others from injury. All chemicals should be handled, stored, and disposed of in accordance with applicable local, state, and federal regulations.

1.1. Chemical sanitizing agents

1.1.1. Aqueous chemical sanitizers

Several types of chemical sanitizing agents are used in aqueous (water-based) solutions. These include:

- chlorine bleach (sodium hypochlorite, NaOCl)
- quaternary ammonium compounds
- hydrogen dioxide (=hydrogen peroxide, formulated with peroxyacetic acid)

These materials can be used to sanitize a variety of materials, including containers, benches, and other surfaces. All these materials are deactivated when they contact soil or organic matter. Therefore, solutions become ineffective with use and over time. The following general procedures apply to all of these materials.

- Sanitizing solutions should be freshly made or tested before use to ensure target concentrations. Test strips are available to check the concentrations of these materials.
- Use only clean water free of organic debris or rust for diluting the chemicals. Contaminants in water can deactivate sanitizing chemicals.
- Before treatment, surfaces should be brushed or rinsed to remove debris, soil, old potting media, etc., to the degree possible. Note that if items are rinsed with water first, they should be allowed to dry to the point that the sanitizing solution is not diluted excessively.
- A sufficient amount of the sanitizing solution needs to be in contact with all portions of the items being treated for at least the minimum specified time. In general, treated items should be fully immersed or flooded with a film of liquid for the duration of the treatment time.
- It may be necessary to dry or rinse treated items after treatment to remove chemical residues for some uses.

Bleach (sodium hypochlorite) solutions. Concentrations of sodium hypochlorite vary in available bleach products, so the concentration in any given product should be checked, and the dilution rate adjusted as necessary before preparing solutions. The following table provides dilutions for several common bleach concentrations. The final sodium hypochlorite concentrations in these diluted bleach solutions (about 0.525%) is equivalent to 5000 ppm (or 0.5%) available chlorine.

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, 100 ml of bleach would be added to 1480 ml of water to make 1580 ml of 0.525% NaOCl.

The sodium hypochlorite in bleach solutions breaks down quickly in contact with soil or organic debris, and is also decomposed more quickly in light, at higher temperatures (above about 75°F = 24°C), in the presence of various metal ions. It is also less active at pH values less than 5 or higher than 7. Because it is difficult to monitor all of these factors, diluted bleach solutions should normally be made up freshly before use and replaced frequently. Chlorine test strips can be used to check chlorine concentrations, but commercial strips vary in the range of concentrations they detect, so check the range before you purchase. The most common and inexpensive strips detect up to 200 ppm chlorine. To use these strips to detect 5000 ppm chlorine requires further dilution of the solution you wish to test. For example, to check whether a diluted bleach solution contains 5000 ppm chlorine, you would need to add 1 part of the solution to 24 parts water and check that the test strip shows 200 ppm chlorine. Test strips that cover higher ranges of chlorine concentrations are available, but are more expensive.

The hypochlorite concentration in new, sealed bleach containers can also diminish over time, so it is better to use bleach that has been stored no longer than 3 to 5 months after purchase. Concentrated bleach solutions are corrosive and can release toxic chlorine gas if mixed with ammonia or acids.

Quaternary ammonium compounds: Quaternary ammonium compounds ("quats", "QAs"; search for “quaternary ammonium disinfectants” to find examples) are considered low-level disinfectants by the US Centers for Disease Control and Prevention. Quats are effective against most vegetative bacteria and enveloped viruses and some fungi. Before using a product, check the label and any supplemental materials to determine if the product is suitable for your use situation and whether activity against *Phytophthora* is actually claimed for particular uses. These products vary in composition and concentration and must be used at the concentration and exposure times described on the product label. Some labels may include a range of uses that may have different exposure times and concentrations. Many quaternary ammonium product labels require relatively long contact time (commonly 10 minutes) for disinfecting hard surfaces. Label recommendations for specific uses (e.g., hard surfaces, footbaths, etc.) may not be applicable to all target organisms on the label. One of the most important parameters for nursery use is whether the materials can kill thick-walled, resistant *Phytophthora* spores such as oospores or chlamydospores. Unfortunately, most products do not provide specific information on this parameter.

Another issue is that getting the correct dose is based on concentration and exposure time, but the relationship between these factors and efficacy is not necessarily linear. In one study (Smith and Clements no date, experiment 5 and figure 4) some survival of *P. cinnamomi* was detected in soil treated with the test quat at 0.2% (2000 ppm) for up to 10 min. No survival was detected in soil treated at 1% (10,000 ppm) for as little as 30 sec of contact time, but this concentration is about 5 times the highest labeled use rate for most quats. Beyond this, it is not clear whether the rinsing protocol in this study was sufficient to remove all of the quat from the soil. If it was not completely removed by rinsing, exposure times would have been longer than stated and the presence of residual chemical in the soil could have affected the test assay for pathogen survival. The overall effect would be to overestimate efficacy. Furthermore, no data were provided for concentrations between 2000 and 10000 ppm or exposure times longer than 10 minutes, so it is not really possible to determine what other time × concentration doses might have been effective.

At present, few independent tests of products have been reported by researchers that provide good information about the activity of quats against various *Phytophthora* propagules, especially resistant spores. Given the lack of definitive data, quats are not generally recommended as primary disinfecting agents in critical applications. Efficacy may be adequate for treating hard surfaces that have already been cleaned thoroughly. To provide the best margin for safety, use the highest labeled use rate and use an exposure time that substantially exceeds the recommended minimum time. Concentration of solutions should be tested before reuse (e.g., using commercial test strips) and solutions should be replenished or replaced in accordance with label specifications to maintain the required concentration. Make sure that test strips match the target range of your solution. If necessary, you can dilute the sanitizing solution as needed with water in a small container to adjust the concentration for test strips that cover a lower range of concentrations than your target.

Hydrogen dioxide products. Several related products consisting of hydrogen dioxide (=hydrogen peroxide) and peroxyacetic acid are registered for sanitizing hard surfaces and for disinfesting irrigation systems. These materials also need to be made freshly before use. Some of these formulations are also labeled for post-harvest treatment of fruits and vegetables, and may be of use in surface sterilization for vegetative materials. However, few independent tests of these products are currently available, so more research is needed to assess their efficacy when used as sanitizers to kill resistant *Phytophthora* spores.

1.1.2. Alcohol-based sanitizers

Alcohol-water mixes are available at concentrations of 70% to 99% alcohol. As noted on the CDC Chemical Disinfectants site, 60-90% isopropyl or ethyl alcohol is optimal for killing bacteria; alcohol also has virucidal and fungicidal activity. Some studies have shown better efficacy of 70% alcohol compared with concentrations of 90% or higher. This is related to the fact that lower alcohol concentrations are more likely to penetrate the cells before acting to coagulate and denature proteins, whereas high concentrations cause immediate denaturation of surface proteins and may inhibit further absorption. Highly concentrated alcohol also evaporates more quickly, reducing contact time in spray or dip applications. Alcohol solutions are not corrosive and are stable, although they can evaporate if not tightly sealed. Alcohol is the primary active ingredient in some formulated aerosol products, such as Lysol®

Disinfectant Spray (79 percent ethyl alcohol). Note that if aerosol products are used, the treated surface still needs to be thoroughly wetted, not simply sprayed with a fine mist.

For most nursery operations, it is more economical to use hand sprayers filled with 70% isopropanol. Ethanol is most commonly available in the form of denatured alcohol (methylated spirits), which consists of a mixture of ethanol and methanol and/or other solvents added to make the mixture poisonous if consumed. The additives and ratios of ethanol to methanol vary widely between manufacturers and products. Consult the product Safety Data Sheet (SDS, formerly known as Material Safety Data Sheets or MSDS) to determine actual composition, hazards, and precautions. A less toxic alternative to ethanol denatured with methanol is ethanol denatured with isopropanol, which has been marketed as an indoor fuel.

1.2. Heat treatments

Phytophthora species and other plant pathogens can be killed by exposure to high temperatures for a sufficient length of time. The most common methods for applying heat in nursery operations are via:

- steam or aerated steam (steam/air mixtures)
- hot water
- dry heat (e.g., insulated or noninsulated containers heated by electricity or natural gas)
- solarization (solar heating via greenhouse effect under clear plastic or glass)

Materials to be heat-treated should be moist before treatment because target organisms are killed more readily and at lower temperatures if they are hydrated. For 30 minute heat treatments, temperatures had to be increased by up to 36°F (20°C) to kill dry propagules of some plant pathogenic fungi compared to temperatures required for propagules premoistened for 16 hours (van Loenen et al 2003). If materials to be treated (e.g., potting mix, residues on used pots) have not been kept moist for at least 12 hours before treatment, treatment temperature and/or time should be increased well above minimum standards to ensure efficacy. When wetting up dry materials before treatment, the wetting period begins when the driest part has been moistened. For example, dry aggregates of potting media would need to be wetted to their centers before you start timing the wetting period.

Effective treatment times decrease as temperatures increase. For instance, metal tools can be sterilized by exposure to flame for a short period. Standard treatments for killing plant pathogens in water include 203°F (95°C) for 30 seconds and 185°F (85°C) for 3 minutes (Runia and Amsing 2001). However, longer treatment times at lower temperatures are more useful for treating large volumes and bulky materials (e.g., used pots and potting media) because of the time required to uniformly heat the materials to the desired temperatures without overheating. Based on multiple studies, heating of moist materials to 140°F (60°C) or higher for at least 30 minutes will kill propagules of *Phytophthora* and other water molds, as well as most plant pathogenic fungi.

In all heat treatment procedures, the timing of the heat exposure period starts when the coolest portion of the heated material reaches the target temperature. Total heating time can be reduced by ensuring that that treated materials are as warm as possible before treatment; preheating via solarization or simply warming materials in the sun will help reduce energy needs. Total heating time will also be minimized if the heated material (e.g., water, potting media) is agitated and heat of the material is uniform, without cold spots. In all heat treatments, some margin for error should be allowed to account for non-uniform heating. Increase treatment times substantially beyond the minimum requirement if it is difficult to ensure uniform heating.

For materials heated via solarization, temperatures fluctuate based on sun exposure. The treatment duration is related to the total amount of time above target temperatures of about 110-125°F (43-52°C). Typical treatment duration for soil solarization is 4 to 6 weeks at the hottest time of the year, but may be shorter if the coolest portions of the treated material routinely reach 125°F (52°C) or more. A calculator for estimating the minimum solarization times and depth of heating in nursery beds is available online at http://uspest.org/soil/solarize.

Phytophthora species and other water molds are relatively sensitive to heat (Table 2). The differential between the temperatures that are lethal to *Phytophthora* and to plant propagules (seeds, bulbs, cuttings, etc.) provides an opportunity for freeing plant propagules from these pathogens though carefully controlled heat treatments. Vegetative plant materials tend to tolerate heat treatment better if they are in a dormant condition, under slight water stress, and have low nitrogen levels. Plant materials should be selected or preconditioned to be in their most tolerant state before treatment.

Table 2. Target temperatures needed to kill specific organisms in moist soil or potting medium heated to the target for a minimum of 30 minutes (based on Baker, 1957.)

Moist soil, 30 minutes at	**Organisms killed**
120°F (49°C)	Water molds (oomycetes, including *Phytophthora* and *Pythium*), nematodes
145° F (63°C)	most plant pathogenic fungi, bacteria, and viruses, worms, slugs, centipedes
160° F (71°C)	plant pathogenic bacteria, soil insects
180° F (82°C)	weed seeds
212° F (100°C)	heat resistant plant viruses and weed seeds

2. Applications of phytosanitary procedures

This section includes methods for sanitizing and disinfesting items, including propagules, potting media, and hard surfaces or objects.

2.1. Disinfesting outer surfaces of vegetative materials (stems, rhizomes, roots, divisions)

Chemical treatments with sanitizing agents such as diluted bleach can remove external contamination, but will not affect pathogens that have infected plant parts and are growing in plant tissues.

Important: Chemical or heat treatments of propagules should be tested on a small set of plant material to ensure that the plant propagules will tolerate the treatment without significant damage or loss of viability. Viability of treated propagules may also decrease over time. Alternative durations, concentrations, or methods may be needed to prevent damage.

2.1.1. Sodium hypochlorite dip

Propagules should be thoroughly brushed and/or rinsed to remove soil, dead tissue layers or roots, and other surface contaminants. Allow to dry if rinsed. Immerse propagules in a freshly-made diluted bleach solution followed by a rinse with clean noncontaminated water. Immersing materials in 0.525% sodium hypochlorite (Table 1) for a minimum of 1 minute is typically sufficient to eliminate surface contamination by *Phytophthora* and other oomycetes, most fungal pathogens, and many bacteria, but not all plant propagules will tolerate this time and concentration. Using the same concentration for a shorter duration (30 sec) or using lower concentrations (no less than 0.26% sodium hypochlorite) for 1 minute or more may provide similar levels of decontamination with less damage to more sensitive plant propagules.

2.1.2. Hot water treatment

Although hot water treatment (typical temperature ranges of about 120-125°F [49-52°C] for 30 minutes) can be effective for killing both surface contaminants and internal infections, insufficient research is available to make specific recommendations for California native plant materials at present. See Baker (1957) (section 13, starting p. 223 https://archive.org/details/ucsystemforprodu23bake) for a detailed discussion of heat treatment of vegetative propagules.

2.2. Sanitizing recycled containers

2.2.1. Chemical sanitizers

- Containers should be brushed or rinsed to remove as much potting media as possible before treatment.
- Containers must be unstacked or loosely stacked so that the solution can circulate freely to all portions of the pots.
- Sanitizing solutions shall be freshly made or tested to ensure target concentrations.
- Containers should be fully immersed in the sanitizing solution for at least the minimum specified time before removing to rinse or dry. Some agitation may be necessary to break up air bubbles that may keep surfaces from being wetted.

Sodium hypochlorite: Immerse containers in a fresh solution of diluted bleach (0.525% sodium hypochlorite, Table 1) for a minimum of 5 minutes. Solutions must be made fresh and replaced if contaminated with substantial amounts of organic debris. Chlorine concentrations should be tested before reuse (e.g., using commercial test strips) and should be no lower than 5000 ppm in the solution being used.

Quaternary ammonium compounds: As discussed in section 1.1.1., these materials must be used at the concentration and exposure time described on the product label. Concentration should be tested before reuse (e.g., using commercial test strips) and replenished or replaced in accordance with label specifications to attain the required concentration. Exposure times listed on labels are minimums and may not be sufficient to kill all types of *Phytophthora* spores; check the label for specific efficacy claims.

2.2.2. Heat treatment

If residues on containers are moist, heating to a temperature of at least 122°F (50°C) for at least 30 minutes will kill propagules of many, but possibly not all, *Phytophthora* species. To provide for a margin of safety and to obtain kill of other plant pathogenic fungi, containers with moist residues should be heated to 140°F (60°C) or higher for at least 30 minutes. If residues are dry, treatment temperature should be at least 160°F (71°C). All portions of the treated containers must be maintained at the target temperature for 30 minutes. Note that timing should start when the coolest portions of the treated containers have reached the target temperature.

Temperature should be monitored using thermometers or thermocouples placed among the parts of the containers that will be slowest to heat. Heat may be applied by any of several methods as long as the target temperatures can be maintained for the necessary duration. Loosely stacked containers may be treated using steam or aerated steam in a closed container. Direct solar heating can be used by wrapping moistened containers in clear plastic and laying them horizontally on a clean surface to maximize solar exposure. Greater solar heating may be obtained in a simple solar oven, i.e., a closed, insulated container with a glass or clear plastic top. More uniform heating will be obtained if hot air can circulate around the containers.

Containers can also be heat treated by submerging them in water that is maintained at 160°F (71 C) or higher for a minimum of 30 minutes. Once containers are submerged, timing of the treatment period should begin when water temperature throughout the treatment tank has reached the target temperature. Container stacking should be loose enough to allow all surfaces to become fully wetted and avoid air pockets.

2.3. Sanitizing tools and surfaces

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, all soil and organic material (roots, sap, etc.) should be removed from the surface. If necessary, use a detergent solution and brush to scrub off surface contaminants.

The following materials can be used to sanitize tools or surfaces that are clean and free of surface water. As noted above, if treated surfaces are wet, the sanitizing solution will be diluted:

- 70-90% ethyl or isopropyl alcohol - spray to thoroughly wet 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)
- quaternary ammonium disinfectants - use according to manufacturer recommendations - freshly made or tested to ensure target concentrations
- for hydrogen dioxide products, follow label instructions for treating greenhouse surfaces and equipment, allowing sufficient contact time for disinfecting.

2.4. Heat treatment of potting media

Potting media should be heat treated using steam, aerated steam, or other moist heat applications. If using a dry heat source, media should be moistened to near field capacity. Heat potting media until the temperature of the coolest portion of the treated soil has maintained a temperature of at least 140 F (60 C) for at least 30 minutes. This heat treatment regime is lethal to most plant pathogenic fungi and oomycetes such as *Phytophthora* (Table 2) but does not kill all soil microorganisms and will not result in “sterile” soil.

Excessive heating at high temperatures (generally above 180-212° F [82-100°C]) can increase the potential for phytotoxicity. Potting media, especially those containing readily-decomposed organic matter, can develop levels of ammonium, manganese, or other compounds that are phytotoxic to some plants. Phytotoxicity is usually temporary and is reduced over time or with leaching.

Solarization. Heat-treating nursery potting media via solarization requires sufficient direct sunlight and relatively warm ambient temperatures. Solarization is most practical for treating nursery potting media in situations where the coolest portions of the treated soil mass can sustain a minimum temperature of 113°F (45°C).

In a static solarization system, potting media should be piled no more than 6-10 inches (15-25 cm) deep to facilitate heating to the bottom of the pile. Media should be moistened to near field capacity before solarization. Solarization should continue until the coolest portion of the potting media has been heated to a temperature of 113°F (45°C) for at least 15 hours or a temperature of at least 122°F (50°C) for at least 2 hours.

It may take from two days to more than a week to attain these time/temperature thresholds depending on the weather and your solarization setup. If you want to use plastic sheeting, 6 mil clear thermal anti-condensate greenhouse film is preferable. This material has efficient thermal qualities and a long service life. In cooler areas, using a double layer of plastic film separate by an air gap reduces heat loss. Using a layer of insulation (e.g., a foam insulation panel) beneath the media will also reduce heat loss. Alternatively, soil can be heated more efficiently in an insulated solar oven. Heating will be more efficient and uniform if hot air can circulate beneath and around soil container(s) within the solar oven.

As noted in the BMPs, care must be taken to avoid contamination of potting media after heat treatment. Heat treated material should only be transferred into sanitized containers using sanitized tools by workers with clean gloves following phytosanitary working practices.

3. References

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