Leachate Testing Instructions

for the Detection of *Phytophthora*

This handout is a summarized version of the leachate testing equipment assembly instructions available at http://phytosphere.com/BMPsnursery/test3_4bench.htm

Figure 1: An in-progress leachate test, setup with a complete leachate collection system, single block of test plants, and a floating pear bait.

Materials:

- Zoospore collection vessel
- Runoff collection sheeting
- Unbruised pear with unbroken Skin
- Watering wand with flow regulation and directed water stream
- Bucket
- 1-2 gal Ziplock Freezer Bags
- Thermometer
- Metronome
- Graduated Cylinder
- Bleach (10% Bleach, approx. 5.25% Sodium Hypochlorite)
- Disposable Gloves

Procedure:

I. Zoospore Collection

1. Sanitize benches with 10% bleach (0.525% Sodium Hypochlorite, 5000ppm available chlorine). Allow contact for at least 1 minute to ensure adequate sanitation time, then rise well (Table 1)
2. Set up Zoospore Collection Vessels (adapted water cooler) and Runoff Collection Sheeting. Collection Vessels should be positioned such that flowthrough leachate water directly fills the vessel without splash or significant loss of water.
3. Select plants for leachate testing. We recommend biased testing based on visual symptoms or relative risk. This includes:
    - Native plants that are known to be particularly susceptible to *Phytophthora* infection, such as *Arctostaphylos*, *Ceanothus*, *Mimulus*/*Diplacus*, etc.
    - Plants showing symptoms of Phytophthora root rot, such as loss of vigor, stunting, leaf browning at blade margins, or death.
    - Plants propagated from high-risk propagules, including rhizomes, tubers, bulbs, collection sites in riparian zones, or tips/seeds collected low to the ground within water splash zones.
    - Plants imported from other nurseries. We recommend leachate testing imported plants before integrating them with your own nursery stock.
4. Based on your selected plants, find your required irrigation volume for each batch based on pot size.
    - Irrigation volumes and flow rates for common pot sizes can be found on the provided tables (see Tables 2 and 3).
    - If your required pot size by volume is not included, you may approximate your appropriate irrigation volume by multiplying the volume of your container (in milliliters) by 17.5%.
5. Arrange plants on the bench directly above the Runoff Collection Sheeting, such that all leachate water flows down the sheeting into the collection vessel (Figures 1 and 2).
    - Note: A maximum of 40 plants per batch may be included in a single test. For larger blocks, select a representative batch of 40 plants per block using biased sampling of symptomatic or poor plants and plants along edges of the block.
6. Using clean disposable gloves, place your fresh pear and thermometer in the leachate collection vessel.
7. Calibrate your watering wand to the appropriate flow rate.
    - Start your metronome. You may use the provided charts as reference or start at 60bpm and adjust accordingly (Table 3).
    - Turn on the water. On the first beat, begin filling the graduated cylinder.

- Note how many beats have passed once your cylinder has filled with the desired volume of water.
- Adjust the bpm of the metronome and/or the flow rate of your watering wand until your desired volume has been standardized to the metronome.
    i. Ex: 45mL of water in 3 beats at 60bpm.

8. Note the start time and begin irrigation, following the metronome to ensure each pot is supplied with the same amount of water. If running multiple tests, continue down the line until each batch has been irrigated.
    - **Note:** We recommend running a maximum of 4 tests at a time in batches with the same required irrigation volume (ie. same pot size), especially if testing is unfamiliar.

Figure 2: Irrigation using a calibrated watering wand. Plants are placed directly about the Runoff Collection Sheeting such that water drains into the Zoospore Collection Vessel. Photo credit: Phytosphere Research

9. After 15 minutes, repeat the irrigation. Continue in this fashion until each batch has received 6 irrigations. Record each irrigation time.
    - **Note:** *The entire applied volume must flow through the pot*. As soil becomes saturated pots with little headspace may begin to overflow. If this occurs, split each irrigation to prevent overflow. For example, if you apply 110ml in 4 beats, you may split this irrigation into 2+2 beats (applying 55 ml in each of the 2 beats) , such that each plant is watered twice per irrigation to apply the total final volume.

10. Allow 15 minutes after the final irrigation, then pull the pear from the leachate water and place in a labeled Ziplock bag. Record the time and the final water temperature.

Figure 3. Full Zoospore Collection Vessels after the final irrigation. Incubating pears are floating at the top of the vessel where zoospores are most likely to gather. Photo credit: Phytosphere Research

11. Drain excess leachate water by tilting the overflow spout until the water level reaches the marked line.
    - **Note:** Zoospores collect at the top of the vessel. Emptying the water from the bottom of the vessel thus allows us to concentrate spores in the remaining water.

Figure 4. Proper drainage from the bottom of the Zoospore Collection Vessel. Photo credit: Phytosphere Research

12. Pour the remaining leachate water into the Ziplock bag (Figure 5).

Figure 5: Collected leachate ready to be poured into a Ziplock bag and bucket for transportation and incubation.

- **Note:** A single round of testing will take an hour and a half, not including setup and cleanup time.

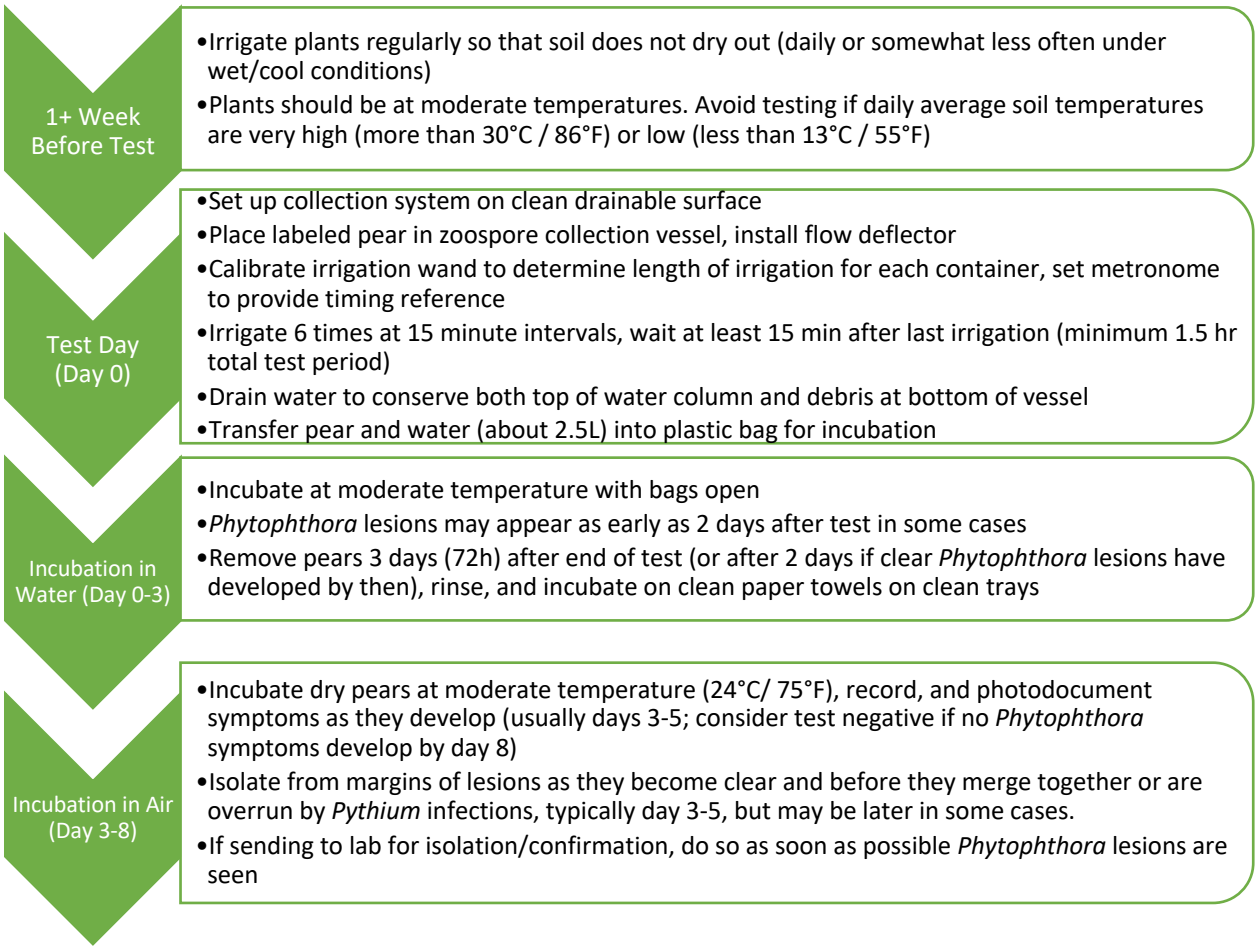

Figure 6. Timeline showing sequence of activities for conducting bench test of leachate for *Phytophthora* species using green pear baits.

II. Pear Bait Incubation

1. Allow the pear to incubate in the leachate water for up to 3 days. Leave the bag open to allow aerobic conditions. Lesions may form at this stage in some cases.
2. After 3 days, remove the pear and allow to incubate at room temperature for up to 5 days. Lesions may develop as soon as half an hour after removal from the water, or they may take several days.
    a. If left too long, secondary infections or *Pythium* may overtake the pear and mask positive results.

Figure 7. Pears showing lesions due to *Phytophthora* infection.

3. Pears with distinctive lesions should be considered suspect. To confirm *Phytophthora* detection pears should be sent for diagnostics to a plant pathology laboratory. The Del Castillo Lab is available to process pear lesions for *Phytophthora* identification. For sample submission please email the Del Castillo Lab at jdelcastillo@ucdavis.edu or send us a message through the AIR Program website https://airnursery.ucdavis.edu/.

Figure 8. Pear lesion tissue assay showing growth of *Phytophthora*.

Note: A negative test means that *Phytophthora* was not detected by the current round of testing. However, keep in mind that **negative detection does not *necessarily* mean that plants are *Phytophthora*-free!** If plants are destined for use in highly sensitive restoration projects, for example, a second round of testing should be considered before out planting.

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

Table 2: Standardized volumes of water for bench testing of leachate from container plants. The irrigation volume listed should be applied to each container for each of the six irrigations used in the standard protocol. The total amount of water applied for a test (all 6 irrigations) is approximately equal to the nominal volume of the container. Note that the container volumes are based on data from suppliers and may not be exact.

Container type	Container volume, ml	Irrigation volume per irrigation, ml	Percentage of container volume per irrigation
LT6 / RCL4	65.5	12	18.30%
RP (Rose pot 2.25x3.25 inch)	100	18	18.00%
SC7 Supercell Stubby	107	20	18.70%
SC10 Supercell / LT8	164	30	18.30%
DP16 (Deepot 16)	262	45	17.20%
"TB2" / AB35 (Treeband 2x5 inch)	397	70	17.80%
DP40 (Deepot 40)	655	110	16.80%
DP60 (Deepot 60)	983	165	16.80%
"TB46" / AB46 (Treeband 3.6x6 inch)	1,098	200	18.30%
"TB410" / AB410 (Treeband 4x10 inch)	2,340	400	17.10%
"TP413" / CP413CH (Treepot 4x13.5 inch)	2,650	450	17.00%
"TP414" / TP4 (Treepot 4x14 inch)	2,830	500	17.70%
#1 ("1 gal")	2,839	500	17.60%
#5 ("5 gal")	14,385	2500	17.40%
#15 ("15 gal")	49,210	8000	16.30%

Table 3: Metronome timings for single irrigation events for various nursery container sizes and irrigation wand flow rates. Maximum flow rates are limited by container size and the amount of head space in the container. If it is necessary to run tests on containers of different sizes concurrently, it is most convenient to select a flow rate and metronome speed that generates an even number of beats for each container size.

Container type	Irrigation volume, ml	Metronome setting, beats/min	Irrigation duration, beats	Irrigation duration, sec	Flow rate, ml/sec
SC7	20	60	2	2	10
SC10	30	60	3	3	10
SC10	30	60	2	2	15
DP16	45	60	3	3	15
DP16	45	80	3	2.3	20
TB2	70	60	3	3	23.3
TB2	70	69*	4	3.5	20
TB2	70	88*	3	2	34.375
DP40	110	75	4	3.2	34.375
DP60	165	75	6	4.8	34.375
DP60	165	68*	3	2.6	62.5
TB46	200	75	4	3.2	62.5
TP414, #1	500	75	10	8	62.5
TP414, #1	500	75	5	4	125
#5	2500	75	15	12	250
#15	8000	75	48	38.4	250
#5	2500	90	10	6.7	375
#15	8000	90	32	21.3	375

Source Materials: Phytosphere.com, http://phytosphere.com/BMPsnursery/test3_4bench.htm