

CDFA Flow Through Protocol for Baiting of potted plants to detect presence of *Phytophthora* spp.

Modified June 15, 2015

This sampling protocol, the flow through method, can be used to test representative samples of lots of plants or to test individual plants. Below we describe one method to test the soil and roots of potted plants, referred to as the Bait in the Tub method. As an alternative to this method, one can employ the Bottle of Bait (BOB) method at step 7.

1. Plants to be tested and distilled water should be warmed up to room temperature or 65-75 °F for at least 24 hours prior to testing. You will need enough distilled water per plant for at least one half liter (a little more than a pint) of flow through. Plants should be watered at least 24 hours before testing.
2. Use an aluminum cake pan or tub-like container large enough to hold the potted plant in one liter of water with the pot(s) in the tub.
3. Each tub should be surface sterilized prior to each use and between each sample. Dip or spray tub with 10% household bleach solution or with a spray disinfectant containing at least 50% ETOH; allow for thorough saturation, wait 30 seconds then rinse with tap water and dry with clean towel or air dry.
4. Lay out clean tubs or pans on a cleaned greenhouse bench.
5. Place one plant in the pan. (If using small plugs or containers, more than one plant can be placed in the tub).
6. Irrigate the soil surface of each plant with purified water until 1-1.5 inch (3cm) of standing water is left in each pan. Insure that one half liter of runoff water has collected in the tub. Allow plant to sit in tub for 30-60min. Remove potted plant.
7. Wash hands with soapy water, rinse, dry on clean towel and put on Nitrile exam gloves.

A. BOB Method: Collect 500 ml of the runoff water in a sterile, 1 liter Nalgene bottle. Using a paper hole-punch, cut discs (or other shape, ie. heart-shaped) from clean, unblemished and undamaged bait leaves. Punch them directly into the bottle of water, or into a small, clean dish then transfer to the bottle. [NOTE: To test for *P. ramorum*, use rhododendron leaves. (See CDFA for possible source of clean leaves.) To test for *P. tentaculata* and *P. cactorum*, use entire oregano or celery seedlings, unpunched.] See Appendix 7 of the USDA Water Sampling Protocol. Lay bottles horizontally on their sides to increase water/air surface area.

B. Bait in the Tub Method: Using a paper hole-punch, cut discs (or other shape, ie. heart-shaped) from clean, unblemished and undamaged bait leaves. Punch them directly into the tub of water, or into a small, clean dish then transfer to the tub. [NOTE: To test for *P. ramorum*, use rhododendron leaves. (See CDFA for possible source of clean leaves.) To test for *P. tentaculata* and *P. cactorum*, use entire oregano or celery seedlings, unpunched.]



8. Ideal bench heat or ambient temperature is around 65-75 °F.
9. All effort must be made to prevent disturbance to the water in the trays and movement of the disks and seedlings. This may include turning off fans, if inside a greenhouse. Greenhouse must be clean to prevent contamination of the samples. If outdoors, tubs must be protected from outside contamination, ie. covered.
10. Leaf discs and seedlings must incubate for three days in the still water, preferably in dark conditions. Following 72 hours of incubation, disks are removed and put in the baggie with the number corresponding to the pot(s) in that tray. Use sterilized tweezers to remove the disks and seedlings from each tub. This can be done by dipping the tweezers in 70% (or higher) alcohol and flaming off the residual or by rinsing with sterile water.
11. Test leaves and/or seedlings using the Agdia ImmunoStrip, culture onto PARP or PARPH, or send leaves or BOB's via overnight mail to CDFA lab on that same day.