PhytoFrontiers™
An Open Access Journal from The American Phytopathological Society

Research

Validating and Optimizing a Method for Detecting *Phytophthora* Species by Baiting Leachate from Arrays of Container Nursery Plants

Tedmund J. Swiecki[1,†] | Elizabeth A. Bernhardt[1] | Sean G. McClanahan[2]

[1] Phytosphere Research, Vacaville, CA 95687

[2] Institutional Research, University of California, Riverside, CA 92521

Accepted for publication 20 October 2023.

Abstract

In 2014, Phytophthora root rot was identified as a serious problem in nursery plants grown for habitat restoration in California. To support efforts to produce restoration nursery stock free of *Phytophthora* species, we developed a standardized leachate baiting method that can be conducted by nursery staff. Each plant in an array of up to 42 container-grown nursery plants is irrigated six times at 15-min intervals with a standardized water volume. Irrigation leachate is collected in a specialized vessel containing a green (unripe) pear bait. Test sensitivity was assessed using arrays of *Phytophthora*-free plants containing varying numbers of plants from which *Phytophthora* had previously been baited. The percentage of tests with *Phytophthora* detections increased as the proportion of infested plants in the array increased. In tests of individual inoculated plants, *Phytophthora* detection from a given plant varied over time, especially for less susceptible hosts. This variability likely limits test sensitivity in arrays with few infested containers. Direct probability and Monte Carlo simulation models showed that testing two to three arrays per block and 20 to 40 plants per array provided the greatest increases in detection probability for blocks of 200 to 1,000 plants in which 1 to 5% of the plants were infected. Random sampling had lower detection success than sampling biased to increase the odds of selecting infected plants. Results of extensive testing at a case study nursery were consistent with model predictions. The leachate baiting method has wide applicability for detecting and identifying *Phytophthora* species in nursery stock.

Keywords: container plant nursery, irrigation leachate, Monte Carlo simulation, pathogen detection, pear bait, Phytophthora root rot

† Corresponding author: T. J. Swiecki; phytosphere@phytosphere.com

Funding: Partial funding for this work was provided by the San Francisco Public Utilities Commission through agreements administered through USDA Forest Service, Pacific Southwest Research Station, with matching funds provided by Phytosphere Research. Additional funding was provided by the Santa Clara Valley Water District and the Midpeninsula Regional Open Space District.

e-Xtra: Supplementary material is available online.

The author(s) declare no conflict of interest.

Infected nursery stock is a well-known pathway through which *Phytophthora* species have been introduced into cultivated landscapes and natural habitats (Bienapfl and Balci 2014; Erwin and Ribeiro 1996; Jung et al. 2016; Migliorini et al. 2015; O'Brien et al. 2009; Weiland 2021). Starting in the late 1990s, the sudden oak death pathogen

Phytophthora ramorum caused widespread tree mortality in California coastal forests. This aerially dispersed pathogen was shown to have been introduced on nursery stock growing near susceptible forest trees (Cobb et al. 2020; Croucher et al. 2013; Goss et al. 2011; Grünwald et al. 2012). Invasions of *P. cinnamomi* and other soilborne *Phytophthora* species were subsequently found to be causing severe losses of native plant species in California habitats with Mediterranean climates (Serrano and Garbelotto 2020; Serrano et al. 2019; Swiecki et al. 2003, 2011). These introduced pathogens have severely degraded previously stable natural plant communities and raised awareness among California land managers of the risks that introduced *Phytophthora* species pose to native ecosystems.

Ecological restoration activities in the United States were estimated to produce $9.47 billion in direct economic output during 2014 (BenDor et al. 2015; Kelmenson et al. 2016). As part of ecological restoration in California, large quantities of nursery plants are planted into native habitats each year as required compensatory mitigation for habitat destruction and to enhance natural environments. Most of these plants are grown in nurseries that specialize in producing plants specifically for habitat restoration, but native plants grown in ornamental plant nurseries are also planted in and adjacent to native habitats. In 2014, several large restoration projects were found to have been planted with nursery stock infected with root-rotting *Phytophthora* species, including *P. tentaculata*, which had been identified as a high-priority threat to nurseries and forests by the US Department of Agriculture (Rooney-Latham et al. 2015). In the same year, nursery-grown stock of *Ceanothus ferrisiae*, a rare and endangered California endemic plant, was planted into a pristine native habitat as part of a federally mandated multiyear mitigation project. After plants began to die during the first growing season, it was determined that nearly all of the nursery stock was infected with *P. cactorum.* This pathogen had the potential to spread through the restoration site and jeopardize the entire mitigation project (Frankel et al. 2020). In these and other California habitat restoration projects, use of nursery container stock infected with *Phytophthora* species has led to poor performance and mortality in plantings, pathogen spread into adjacent natural vegetation, and expensive remediation to limit adverse outcomes (Bourret 2018; Frankel et al. 2020; Rooney-Latham et al. 2019; Sims and Garbelotto 2021; Swiecki et al. 2021).

As the extent of *Phytophthora* infection in restoration nursery stock became apparent, land managers wanted to require that restoration nursery stock be free of *Phytophthora* species. To support that goal, comprehensive Nursery *Phytophthora* Best Management Practices (NPBMPs) were developed to exclude soilborne *Phytophthora* species during nursery plant production. These NPBMPs are the basis for Accreditation to Improve Restoration (AIR), a program to accredit nurseries that are capable of consistently producing nursery stock free of *Phytophthora* species (Swiecki et al. 2021). A key part of the NPBMPs and the AIR program is testing stock for the presence of *Phytophthora* species at appropriate stages during production and just before delivery. This required a sensitive, repeatable protocol that could be conducted by nursery personnel. Testing is necessary because visual inspections of aboveground plant parts for disease symptoms are not effective for detecting plants with Phytophthora root rot (Bienapfl and Balci 2014; Osterbauer et al. 2014).

Various methods have been used to detect *Phytophthora* species in surveys of nursery plants, although the efficiency of these tests for detecting infection has generally not been reported (Bienapfl and Balci 2014; O'Brien et al. 2009; Parke et al. 2014; Rooney-Latham et al. 2019; Sims et al. 2019). The incidence of infection by *Phytophthora* species is commonly high in conventional nursery stock (Bienapfl and Balci 2014; Rooney-Latham et al. 2019; Swiecki et al. 2021). Consequently, the presence of *Phytophthora* species can be documented even with inefficient methods if enough plants are sampled. However, in nurseries complying with NPBMPs, *Phytophthora* species should not be present. *Phytophthora* detections are rare and exclusively associated with specific plant blocks in which deviations from NPBMPs occurred. Such accidental contamination events need to be identified when infection levels are low so corrective actions (e.g., delineation and disposal of infected plant blocks) can be taken before contamination can spread further.

Current methods available to test for the presence of *Phytophthora* species did not meet the needs of NPBMP-compliant restoration nurseries. These nurseries needed to be able to detect a wide variety of *Phytophthora* species at relatively low infestation rates. Testing protocols needed to be scalable to a wide range of container volumes and plant lot sizes. Restoration nurseries commonly use containers that range in volume from as little as 19 ml for plug tray cells to 49 liters for large containers. Plant lots range from fewer than 20 to thousands of containers. Nursery plant lots produced under contract for restoration are commonly grown from propagules collected from specific watersheds or habitats and may include a wide range of both collections and species.

Under the NPBMPs, detection of *Phytophthora* species from a contiguous block of plants triggers destruction of the block because these pathogens spread readily between adjacent containers. Because the infection status of individual plants is not needed, tests that assay multiple plants at once can be used to reduce sample processing time. Testing methods that do not generate false positives were imperative to avoid unnecessary destruction of clean stock or delays to tight planting schedules resulting from follow-up testing. Methods that do not require destruction of tested plants were preferable to avoid the need to propagate additional plants solely for testing purposes. Finally, test methods needed to be inexpensive and simple enough that nurseries and their clients could routinely use them to monitor for *Phytophthora*.

Phytophthora species have been detected in nursery irrigation runoff (Bienapfl and Balci 2014; MacDonald et al. 1994; Parke et al. 2014). We initiated tests in October 2015 to determine whether baiting irrigation leachate from arrays of container nursery plants could be an effective way to nondestructively test for the presence of *Phytophthora* species in blocks of container nursery plants (Swiecki et al. 2018b). Container-grown nursery plants are irrigated frequently, which provides consistently favorable conditions for sporangium production by *Phytophthora* species. Baiting only detects living propagules capable of infecting the bait, so the method does not generate false positive results if *Phytophthora* presence is confirmed by culturing from baits. Leachate baiting may be less effective for plants treated with chemicals that interfere with *Phytophthora* sporangium production or zoospore activity (Shishkoff 2014), but the use of such chemicals is prohibited under the NPBMPs (Swiecki et al. 2021).

To be practical for use by nurseries, baits needed to be readily available throughout the year and susceptible to most of the *Phytophthora* species that could be present. Baits used to detect *Phytophthora* spp. in nursery plants have included seedlings, leaves, or fruits of various host plants (Bienapfl and Balci 2014; Erwin and Ribeiro 1996; Migliorini et al. 2015; O'Brien et al. 2009; Parke et al. 2014; Sims et al. 2019). Unripened fruit of European pear (*Pyrus communis* L.) has been used to bait *Phytophthora* species at least since 1964 (McIntosh 1964). In California and many other parts of the United States, the cultivars Bartlett and D'Anjou are available in grocery stores year-round.

European pears are climacteric and are picked in a narrow harvest period at a partially ripe stage based on firmness and other physiological measurements, including the intensity of green coloration (Washington State University Tree Fruit 2023). Before marketing, pears are conditioned by low-temperature storage or ethylene treatment to enable them to ripen at room temperature, but ripening is inhibited by refrigeration. When selected carefully at purchase, these pear varieties serve as readily available and highly uniform baits for detecting *Phytophthora* species.

Phytophthora species vary in virulence to pears and other baits, so false negative results can occur if baits are not readily infected by the species present. However, more than 50 *Phytophthora* taxa have been detected using Bartlett or D'Anjou pears (Bourret 2018; Frankel et al. 2020). All 15 identified *Phytophthora* species detected by Rooney-Latham et al. (2019) in root and crown samples from native plant nurseries using direct isolation and serological methods also infect pear baits.

An advantage of pear baits compared with leaf baits is that infections on pears caused by *Phytophthora* species give rise to distinctive lesions that expand over time. Lesions caused by many *Phytophthora* species on pears are similar in appearance, typically rounded with indefinite margins, medium to dark brown, and initially firm. Lesions need to be cultured or otherwise tested to confirm *Phytophthora* presence and identify species. Isolations from lesions can be made onto general purpose agar rather than semi-selective agar, which is required for leaf baits (Ferguson and Jeffers 1999). *Pythium* (sensu lato) species and fungi can infect pears at wounds, but *Phytophthora* species can cause lesions on nonwounded pear epidermis.

The objectives of this research were to optimize detection of *Phytophthora* species using an irrigation leachate baiting method and estimate the sensitivity of the optimized protocol. No systematic testing has previously been reported on use of irrigation leachate baiting for detecting *Phytophthora* species in container-grown nursery plants. Statistical modeling was used to examine the effects of altering sampling parameters on the likelihood of obtaining a detection from a block of plants containing one or more infected plants. The resulting sampling and testing parameters were used in a commercial restoration nursery to assess how testing performed under actual nursery conditions. A simple but effective leachate baiting method for detecting *Phytophthora* species in existing stock could be useful for many sectors of the container nursery industry.

Materials and Methods

Standard pretest parameters were established to ensure conditions were favorable for detecting *Phytophthora* zoospores via baiting. Plant containers received regular irrigation before testing, daily or less frequently depending on temperature and rainfall, to favor sporangium production within the containers. Plants were also tested when root systems were well established in their containers to provide high root density. No systemic oomycete-suppressing chemicals were used on tested plants.

The temperature within plant containers was recorded at 5- to 10-min intervals using single-channel temperature loggers with external temperature sensors (THC-4; Inkbird, Shenzhen, Guangdong, China or RC-4; Elitech, Milipitas, CA). Sensors were inserted into the center of the root mass of one container per test.

Baits

Pear baits used in all tests were cultivar D'Anjou or Bartlett purchased from local retail groceries 1 to 2 days before use and stored at 4°C. D'Anjou was used in most tests and was preferred because it ripens more slowly than Bartlett at room temperature. Pears were selected to be as unripe as possible based on the intensity of green color and to have few or no visible wounds to minimize risk of infections by nontarget microorganisms. Before use, pear baits were gently washed with a dilute detergent solution, thoroughly rinsed with tap water, and placed in clean water to assess buoyancy. Although most pears floated, those that did not were made to float by attaching a piece of clean closed cell foam (8 to 9 cm^2, 0.7 cm thick) with a rubber band.

Test irrigation regime

We and others (Cameron and Carlile 1977; Khew and Zentmyer 1973) have observed that zoospore release by many *Phytophthora* species typically requires at least 20 to 30 min after a triggering event (e.g., chilling or rinsing with water) and is complete within 1 to 2 h under laboratory conditions. Based on this and the time required to irrigate multiple containers, each tested plant was irrigated six times at 15-min intervals to trigger zoospore release and wash zoospores out of containers. The irrigation leachate may also include other forms of inoculum, including zoospore cysts, chlamydospores, sporangia, and infected root fragments. Water was applied directly to each container so that all applied water flowed through the potting mix. Due to the pretest irrigation regime, potting mix was consistently moist enough that water began to leach from the containers after the first irrigation. After the sixth irrigation, leachate was allowed to drain from the plants for at least 15 min, so the minimum total leaching time was 90 min.

In initial trials, the headspace in the container, defined as the space between the potting mix surface and the container rim, was filled with water at each irrigation (Swiecki et al. 2018b). As the method was tested on additional container sizes that differed with respect to headspace and drainage characteristics, it became apparent that water volumes applied to individual containers needed to be standardized. We compiled data on irrigation volumes used in successful tests on different container sizes commonly used in restoration nurseries, including #5 (14.4 liters), #1 (2.8 liters), and Deepot D40H (0.66 liter) (Stuewe & Sons, Tangent, OR). The volume of water applied per irrigation in these tests was between 15 and 20% of the total container volume. The midpoint of this range (17.5%, about one-sixth of the container volume) was selected as the approximate target irrigation volume to apply during each irrigation. We used this to develop standard irrigation volumes (Table 1) in which the total volume of the irrigation water applied to each container over six irrigations approximated the container volume.

To apply a uniform irrigation volume to each container, water flow from a hose wand was set to a rate that did not cause excessive splash or fill the headspace in less than one to several seconds. For small containers, a precise needle valve was used to adjust water flow delivered through a small-diameter wand tip. Irrigation of each container was timed using an audible electronic metronome typically set near 60 beats/min (range about 55 to 75 beats/min). The water flow rate and metronome speed were adjusted to provide a whole number of beats for each irrigation (Table 2). Water flow rate was calibrated by measuring the water volume delivered during a given number of beats. If drainage was slow or headspace was limited, each irrigation was delivered in two or more increments to prevent water from flowing over the container rim.

Collecting and baiting container leachate

To conduct a test, an array of container nursery plants was set on a clean, elevated, mesh-top bench or cart. Benches and other equipment used for testing were rinsed with tap water to remove debris, treated with 0.525% sodium hypochlorite solution for at

least 5 min (sprayed to runoff or dipped), and rinsed with tap water to remove hypochlorite residues before each test. A collection surface made of one to three overlapped sheets of vinyl flashing (each 0.5 m wide, 1.32 m long, 0.38 mm thick) was suspended under the bench and sloped to drain into a collection vessel (Fig. 1). Two longitudinally slit sections of thin-walled PVC pipe (2.54 cm diameter) were slid over the downslope edge of the lowest sheet and held in place by friction to direct leachate into the collection vessel (Fig. 1C). Leachate flowed into the vessel over a strip of vinyl flashing positioned between the PVC pipes to minimize water turbulence in the collection vessel (Fig. 1A to C). For small containers in racks, a sloped plastic paint tray into which a drain was installed was used as the collection surface (Fig. 1D). A single pear bait was placed at the bottom of the collection vessel before the start of each test.

The insulated 7.6-liter collection vessel (Sport 2 Gallon Cooler/Jug/Beverage container; Igloo, Katy, TX) (Fig. 1) was a narrow cylinder (19 cm diameter, 30 cm height), which served to concentrate upward-swimming zoospores (Cameron and Carlile 1977) in a small area near a pear bait floating at the top of the water column. With the exception of some tests on arrays of small containers, the volume of leachate generated during the test exceeded that of the collection vessel. The collection vessel was modified to drain this excess water through a vertical PVC pipe (2.54 cm inner diameter). Water entered the pipe from the top of an open elbow joint inside the vessel, 7 cm above the bottom (Fig. 1E). Water flowed out of an elbow joint at the top of the pipe when the water level in the vessel rose to within 6 cm of the rim (water depth in vessel 24 cm) (Fig. 1A, C, and E). This prevented zoospores that had concentrated in the upper part of the water column from washing over the rim of the vessel. With this drain configuration, the maximum volume of leachate retained in the vessel was about 6.7 liters. Temperature of the water in the collection vessel was monitored using either an analog dial thermometer with a 30-cm stem (82160-12; Vee Gee Scientific, Vernon Hills, IL) or a digital IR thermometer (62 Max; Fluke Corp., Everett, WA).

Post-test processing of baits

After allowing leachate from the last irrigation to drain into the collection vessel for 15 min, the drainpipe was tilted from its vertical orientation to lower the water level in the vessel. Draining continued until the depth of water remaining in the vessel was 10 cm. The remaining leachate (about 2.7 liters), the pear bait, and debris at the bottom of the vessel were transferred to a 3.8-liter plastic zip-close bag supported in a container (Fig. 1F). Bags were closed during transport but otherwise left open to allow for air exchange. Baits floating in the leachate were incubated at diurnally fluctuating temperatures (18 to 24°C) for 3 days or fewer if symptoms became evident earlier (Fig. 1F and G). Removed baits were rinsed with tap water, placed on paper towels on a laboratory bench at room temperature (18 to 26°C), and observed for symptom development for 8 days from the start of the test (Fig. 1H). If potential *Phytophthora* lesions were observed, pears were surface disinfested with 0.525% NaOCl for 45 s, and pieces from lesion edges were cultured on carrot agar (Hansen et al. 2012) modified by using cornmeal agar (BBL, Becton, Dickinson & Co, Sparks, MD) in place of Bacto agar. Initial identifica-

TABLE 1

Nursery container volumes and corresponding standardized water volumes applied during leachate baiting tests[a]

Container		Water per irrigation		Total irrigation per test	
Name	Volume (liters)	Volume (liters)[a]	Percent of container volume	Volume (liters)	Percent of container volume
SC7R[b]	0.107	0.020	18.7	0.12	112
AB35[b]	0.397	0.070	17.5	0.42	106
D40H[b]	0.655	0.11	16.8	0.66	101
#1	2.80	0.50	17.6	3.0	106
#5	14.4	2.50	17.4	15	104
#15	49.2	8.0	16.3	48	98

[a] During the test, each plant was irrigated six times at 15-min intervals.
[b] Stuewe & Sons, Tangent, OR.

TABLE 2

Examples of water flow rates and metronome-based timings used to apply standardized water volumes per irrigation to individual containers when conducting the leachate test

	Irrigation[a]			Metronome timing	
Container volume (liters)	Volume (ml)	Flow rate (ml/s)[b]	Time/container (s)[c]	Beats/min[d]	Beats/irrigation[e]
0.107	20	10.0	2.0	60	2
0.397	70	22.0	3.2	75	4
0.655	110	22.0	5.0	60	5
2.8	500	83.3	6.0	60	6
14.4	2,500	312.5	8.0	75	10
49.2	8,000	312.5	25.6	75	32

[a] Standardized irrigation volume per container applied at each of six irrigations is scaled to container volume, approximately 1/6 of the container volume for each irrigation.
[b] Maximum usable flow rates are limited by container volume and the amount of headspace in the container. Irrigation flow rate is controlled using a flow-regulating valve attached between a hose and the trigger valve of an irrigation wand.
[c] Number of seconds needed to deliver the target water volume per irrigation at the flow rate shown.
[d] An audible electronic metronome is used to time the irrigation for each container. The metronome rate (beats per min) is adjusted to generate a whole number of beats for each container size. If test arrays with different container volumes sizes are irrigated concurrently, the flow rate and metronome speed selected must be able to deliver each target volume in a whole number of beats.
[e] Number of metronome beats to apply the required water volume at the metronome speed shown.

tion of *Phytophthora* species was based on cultural morphology. Cultures were submitted to the California Department of Food and Agriculture (CFDA) Plant Pest Diagnostics Lab for species identification by sequencing the internal transcribed spacer (ITS) rDNA region (Rooney-Latham et al. 2019).

Timing of inoculum release during leaching

To determine when inoculum was washed out of containers using the irrigation regime described above, studies were conducted in which leachate was collected from irrigations 1 + 2 (0 to 30 min), 3 + 4 (30 to 60 min), and 5 + 6 (60 to 90 min). Leachate

FIGURE 1
The leachate baiting method for detecting *Phytophthora* species from nursery plants growing in containers. **A to C,** Vinyl sheets suspended under benches direct water leaching from irrigated plants into a vessel baited with a floating green (unripe) pear. **D,** For small containers in racks, plastic paint trays fitted with a drain can be used to direct leachate. A to C, Strips of vinyl sheet or D, PVC pipe minimize splash and turbulence in the collection vessel. **E,** The collection vessel drains through a pipe from an intake near the vessel bottom that C, prevents water from flowing over the vessel rim to conserve zoospores. Multiple tests can be set up to run concurrently; A, B, and D, up to six test arrays can be irrigated by one person within the 15-min repeat irrigation interval. **F,** Pears remain floating in 2.7 liters of the collected leachate for 3 days; upper left pear bait was non-buoyant but was floated using a piece of closed-cell foam. **G,** Pear baits on the third day in leachate and **H,** 1 day after removal from leachate (4 days from start of test) show rings of brown *Phytophthora* lesions near the water line.

from each of these three collection periods was separately baited with a pear bait during the 30-min leaching period and for a 3-day incubation period afterward. Pears were then removed and observed on a lab bench for an additional 5 days as described above. The number of *Phytophthora* lesions that developed on the pear during each time interval was used as a relative measure of inoculum density (Matheron and Porchas 2009).

The experiment was conducted six times using plants that were at least 1 year old. Four experiments were conducted using single infected plants. The experiment was conducted twice using a single *Arctostaphylos uva-ursi* plant (D40H container, 0.66 liter) that had been inoculated with zoospores of both *P. cinnamomi* and *P. niederhauserii* 252 days before the first test. The experiment was also conducted with two *Juniperus chinensis* 'Pfitzeriana' plants grown in 2.8-liter (#1) containers. When purchased, one plant was infected with *P. cinnamomi* and the other was infected with both *P. cinnamomi* and *P. niederhauserii*. Each plant was tested once. The experiment was also conducted twice using *Ceanothus thyrsiflorus* plants in 14.4-liter (#5) containers. Those tests were conducted using a test array, defined as a set of plants irrigated within the same 15-min intervals and from which all leachate was collected together (Fig. 1A, B, and D). The *C. thyrsiflorus* test array consisted of 14 noninfected plants and one plant that was infected by *P. cactorum* when acquired.

Timing of bait infection

To determine if pear baits became infected during the 1.5-h irrigation period and/or the subsequent 3 days of incubation in the collected irrigation leachate, separate pear baits were used for each of these periods. Pear baits placed in the collection vessel at the start of leachate tests were removed at the conclusion of the leaching period (15 min after the sixth irrigation), placed directly into a closed plastic bag to maintain high humidity, and incubated at 18 to 24°C (night/day). After 24 h, baits were removed from bags, allowed to air dry on paper towels, and observed for an additional 7 days. To bait leachate after irrigation ended, the water in the collection vessel was drained to 2.7 liters 15 min after the sixth irrigation as described above. The leachate and debris at the bottom of the vessel were transferred to a clean 3.8-liter plastic bag containing a second pear bait. The second bait remained in the collected leachate for 3 days before being removed and observed for an additional 5 days, as described above. This experiment was conducted five times using plants at least 1 year old in 2.8-liter (#1) containers. These plants were infected when obtained from nurseries. One experiment was conducted with a three-plant test array consisting of one *Arctostaphylos patula* plant infected with *P. cambivora* and two noninfected plants. The remaining four experiments were conducted with test arrays of 20 or 42 plants that included one *Juniperus sabina* 'Tamariscifolia' infected with both *P. cinnamomi* and *P. niederhauserii.* The remaining plants in the arrays were noninfected, as described below.

Leachate method test sensitivity for multi-plant arrays

To determine the practical limits of detection in test arrays containing a low percentage of infected plants, we conducted a series of experiments in which the numbers of containers with *Phytophthora* source plants (defined below) and noninfested turfgrass were varied to produce test array infection rates (TAIRs) ranging from 2 to 33% in arrays of 3 to 49 containers. The TAIR is defined as the number of containers with *Phytophthora* source plants divided by the total number of containers in the test array. Most tests were conducted for a 2.4 to 5% TAIR, which corresponds to one *Phytophthora* source plant in 42 to 20 plants, respectively. All plant containers in each test array were the same size.

Tests were conducted over several years on plants maintained in a lathhouse on wire mesh benches in Davis, CA, which has a Mediterranean climate with a hot, dry summer and cool, wet winter. Over this period, monthly average high/low air temperatures were 35/15°C in August and 13.9/3.3°C in December, with an overall daily maximum of 43.3°C and minimum of −2.7°C. The rootzone temperature of a representative container plant was recorded at 15-min intervals using a single-channel temperature logger with an external temperature sensor as described above. Plants were irrigated up to two times daily during summer and two to three times weekly during winter, depending on rainfall and temperatures. Rainfall was variable but common between November and March; 25 mm or less occurred in the months of September, October, April, and May, and none occurred from June through August.

Phytophthora source plants were plants in containers that were used as sources of *Phytophthora* inoculum in these tests. All were at least 1 year old. *Phytophthora* source plants that were infested or infected with one or more *Phytophthora* species at the time they were acquired from nurseries were used in about half of the test arrays (Table 3). *Phytophthora* species associated with these plants were identified by using pears to bait leachate from individual plants or by placing a pear bait in a depression in the top of the potting mix and flooding the container for 24 h. *Phytophthora* species were isolated from lesions that developed on pear baits, and isolates were identified as described above. *Phytophthora* source plants used in the remaining test arrays (Table 3) were inoculated with zoospore suspensions of identified *Phytophthora* isolates as described below.

Noninfested containers used in the test arrays were filled with pasteurized potting mix seeded with a *Lolium multiflorum–Festuca arundinacea* turfgrass mix. These grasses have not been reported as hosts of any *Phytophthora* species (Farr and Rossman 2023). Turfgrass roots stabilized the potting mix to prevent it from washing out of containers during repeated irrigations. Grass was clipped periodically to maintain a height of about 10 cm above the potting mix surface. Turfgrass controls were maintained away from *Phytophthora* source plants to prevent possible splash contamination.

For these multi-plant test arrays and the single-plant tests discussed below, we isolated from lesions that developed on pear baits after each leachate baiting test. Morphological characteristics of isolates were used to confirm that the *Phytophthora* species recovered matched those previously identified in the *Phytophthora* source plants.

Detection of *Phytophthora* species in single-plant tests

To characterize the variation in inoculum production by individual infected plants over time, we also conducted individual leachate tests on single plants 4 to 312 days after inoculation. One- to two-year-old plants of 10 species grown in 397- or 655-ml containers (AB35 and D40H, respectively; Stuewe & Sons) were inoculated with one (or rarely two) of nine *Phytophthora* species as described below. For each inoculation date and method, four replicate plants were used for each combination of plant species and *Phytophthora* species, except in a few instances (Fig. 2). Pathogenicity had been previously demonstrated for 2 of the 19 host-pathogen combinations in these tests: *P. cactorum* on *Quercus agrifolia* (Mircetich et al. 1977) and *Heteromeles arbutifolia* (Keim et al. 1976). *Q. lobata* was moderately resistant to stem inoculation with *P. cactorum* (Smith 1937). Irrigations were applied to each container using a calibrated beaker. Because the total volume of leachate from these containers was small (Table 1), all leachate from each plant was collected directly in a 1-liter plastic vessel containing a pear bait.

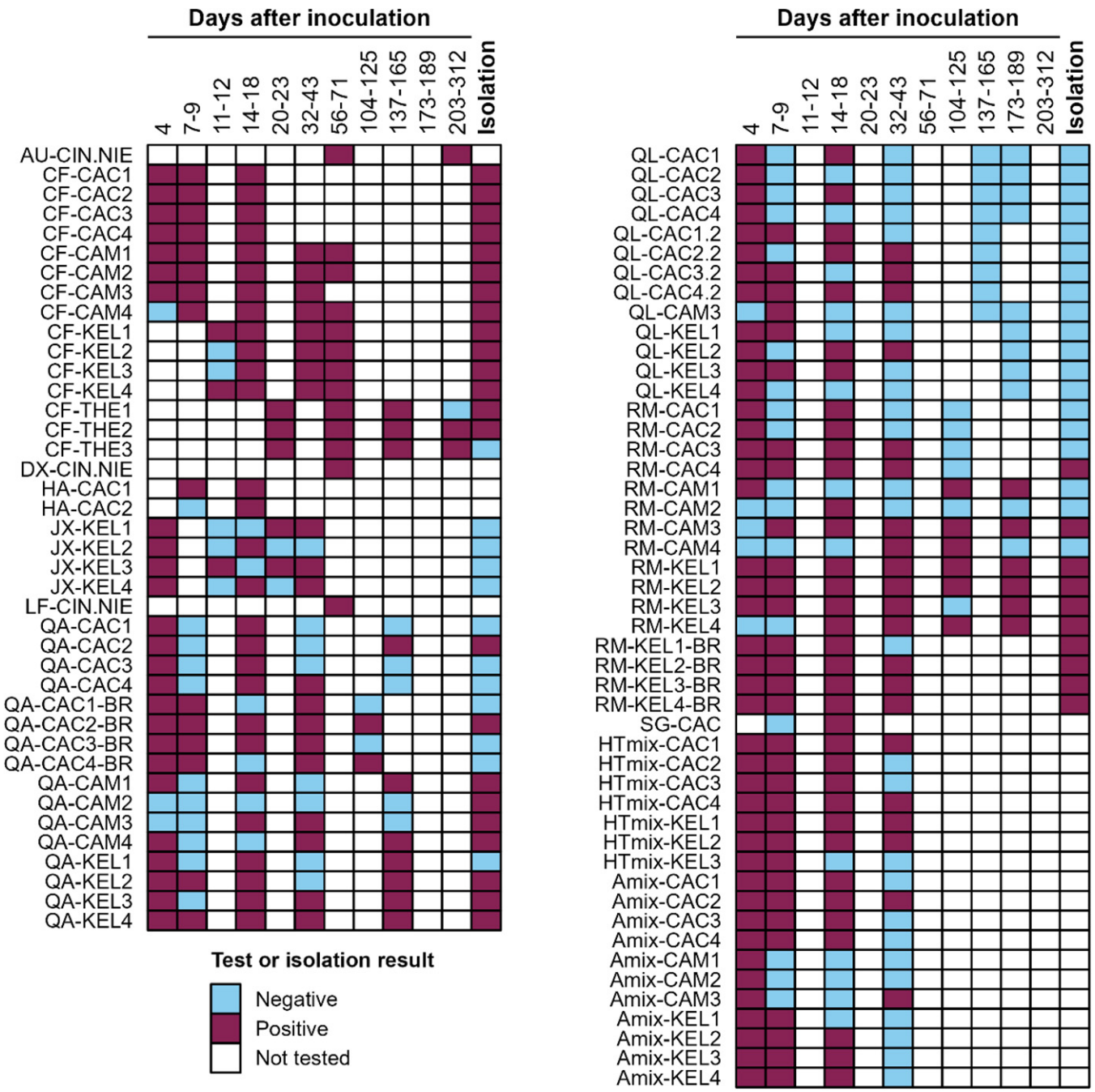

FIGURE 2

Phytophthora detections from single containers tested with the leachate baiting method at various times after inoculation. Each row represents an individual plant tested one or more times; row labels indicate plant species or type of plant-free control, *Phytophthora* species, and container number for multiple plants inoculated on the same date. A second set of plants inoculated with the same *Phytophthora* species using the same method on a different date is denoted by a suffix (0.2). Suffix -BR denotes inoculations conducted on bare root systems in zoospore suspensions. For all other plants and plant-free controls, zoospore suspensions of *Phytophthora* species (10^5 zoospores total) were added to water-saturated potting mix. Plant abbreviations: AU = *Arctostaphylos uva-ursi*, CF = *Ceanothus ferrisiae*, DX = *Diplacus* hybrid, HA = *Heteromeles arbutifolia*, JX = *Juncus xiphioides*, LF = *Lyonothamnus floribundus* ssp. *aspleniifolius*, QA = *Quercus agrifolia*, QL = *Q. lobata*, RM = *Ribes malvaceum*, SG = *Salvia greggii*. *Phytophthora* species: CAC = *Phytophthora cactorum*, CAM = *P. cambivora*, CRY = *P. cryptogea*, CIN = *P. cinnamomi*, HED = *P. hedraiandra*, KEL = *P. kelmanii*, NIE = *P. niederhauserii*, PIN = *P. pini*, THE = *P.* taxon thesauri. Plant-free controls: HTmix = Heat-treated potting mix moistened with water before zoospores were added, Amix = Heat-treated potting mix "aged" by irrigating with leachate from 2-year-old *Phytophthora*-free plants for 2 weeks before zoospores were added. Days after inoculation columns are the number of days (grouped in ranges) between inoculation and leachate tests. The isolation column indicates whether the inoculated *Phytophthora* species was recovered by isolation from roots after the last baiting test or when the plant died. Replicates 1, 2, and 4 of QL-CAM had no detections by baiting or isolation and are not shown or included in data analyses.

Root isolations were made after the final leachate test for surviving plants or at the time that the plant shoot died. From each plant, eight symptomatic 1-cm-long root pieces 1 mm in diameter or less were selected based on symptoms, surfaced disinfested with 0.525% NaOCl, and placed individually under a flap of epidermis of a surface-disinfested pear (two rows of four flaps per pear). Inoculated epidermal flaps were closed by wrapping with Parafilm. Resulting lesions were cultured to confirm the *Phytophthora* species present.

Inoculation methods. Inoculum was produced by placing mycelium from the margins of actively growing agar cultures under flaps of epidermis cut into green, surface-disinfested D'Anjou pears. Inoculated flaps were covered with Parafilm. Once lesions had expanded, zoospore suspensions were made by floating 7-mm-diameter disks of infected pear epidermis on water in Petri plates that were incubated at room temperature (18 to 26°C) until sporangia formed (usually 2 to 7 days, depending on species). Zoospore release was triggered by chilling plates at 4°C for 15 min. Resulting zoospore suspensions were filtered through 450-µm mesh polyester fabric and quantified with a hemocytometer. Plants in 397-ml (AB35) containers were inoculated individually by placing each one in an 800-ml vessel and adding activated charcoal-filtered water to a depth of 2 to 4 mm above the potting mix surface. Approximately 10^5 zoospores (12 to 29 ml depending on zoospore concentration) were applied to the water surface over the potting mix with a syringe. The container was then lifted halfway out of the water to allow the surface water to drain into the potting mix, and an additional 30 to 40 ml water was poured on the potting mix surface to move the zoospores downward. The container was lowered back into the water until the potting mix was saturated to just below the surface. Containers remained flooded for 3 to 6 h, after which they were removed from the water and allowed to drain.

We also inoculated *Ribes malvaceum* and *Quercus agrifolia* plants using a root dip method that avoided adding zoospore inoculum directly to the potting mix. Each plant was carefully removed from its container, and potting mix was gently removed and rinsed from the root systems. The bare root system of each plant was inoculated separately by submerging it for 5 h in 100 ml of zoospore suspension containing 10^5 zoospores (10^3 zoospores/ml). Upon removal from the suspension, each plant was transplanted into heat-treated potting mix in a clean 397-ml container.

The plant and *Phytophthora* species combinations used in single-plant tests (Fig. 2) included a slow-growing, undescribed species in clade 1 originally recovered from nursery stock and designated *P.* taxon thesauri. After 8 days of incubation, production of sporangia and zoospores by this species from floated pear epidermis was poor, so plants were inoculated by cutting the floated pear epidermis into small pieces (1 to 2 mm^2) and incor-

TABLE 3

Detection of *Phytophthora* species using leachate baiting on multi-plant test arrays with known test array infection rates (TAIRs)

Test arrays					*Phytophthora* source plants[a]		
TAIR (%)[b]	Array size	Arrays tested	*Phytophthora* detected (% of tests)	Container volume (liters)	Count	Host[c]	*Phytophthora* species[d]
2.0	49	2	0	0.11	1	HA[e]	CAC
2.4	42	4	100	0.11	1	HA[e]	CAC
2.4	42	1	0	0.66	1	LF	CAM, HED
2.4	42	2	50	2.80	1	TC	CAM, CRY, HED
2.4	42	2	50	2.80	1	JP	CIN, NIE
2.4	42	2	100	2.80	1	JS	CIN, NIE
2.4	42	1	0	2.80	1	RO	PIN
2.5	40	2	100	0.66	1	HA[e]	CAC
2.5	40	1	0	0.66	1	AU[e]	CIN, NIE
4.8	21	3	100	2.80	1	TC	CAM, CRY, HED
4.8	21	3	0	2.80	1	AP	CAM, HED
4.8	21	3	67	2.80	1	JP	CIN
4.8	21	2	50	2.80	1	JS	CIN
4.8	21	3	33	2.80	1	JP	CIN, NIE
5.0	20	4	100	0.11	1	HA[e]	CAC
5.0	20	3	100	0.66	1	HA[e]	CAC
5.0	20	2	50	0.66	1	AU[e]	CIN, NIE
5.0	20	2	0	0.66	1	LF[e]	CIN, NIE
6.6	15	3	100	14.40	1	CT	CAC
7.0	14	1	100	14.40	1	CT	CAC
7.5	40	1	0	0.66	3	AU[e], DX[e], LF[e]	CIN, NIE
9.5	21	3	67	0.66	2	LF, RO	CAM, HED, PIN
10.0	40	1	100	0.40	4	QA[e]	CAC
10.0	40	2	100	0.40	4	RM[e]	CAM
10.0	40	2	50	0.40	4	RM[e]	KEL
20.0	15	1	100	0.40	3	QA[e]	CAC
20.0	20	1	100	0.40	4	QA[e]	CAC
20.0	20	3	100	0.40	4	RM[e]	KEL
33.0	3	1	100	2.80	1	AP	CAM, HED

[a] Container with either an infected or infested plant used as a source of *Phytophthora* inoculum.

[b] Percent TAIR = (number of *Phytophthora* source plants/total number of containers in the test array) × 100. Noninfested containers in the test array contained *Phytophthora*-free plants.

[c] Plant abbreviations: AP = *Arctostaphylos patula*, AU = *Arctostaphylos uva-ursi*, CT = *Ceanothus thyrsiflorus*, DX = *Diplacus* hybrid, JP = *Juniperus* × *pfitzeriana* 'Old Gold', JS = *Juniperus sabina* 'Tamariscifolia', HA = *Heteromeles arbutifolia*, LF = *Lyonothamnus floribundus* ssp. *aspleniifolius*, QA = *Quercus agrifolia*, RM = *Ribes malvaceum*, RO = *Rhus ovata*, SG = *Salvia greggii*, TC = *Torreya californica*.

[d] *Phytophthora* species used to inoculate source plants or determined by baiting after the plant was acquired: CAC = *Phytophthora cactorum*, CAM = *P. cambivora*, CRY = *P. cryptogea*, CIN = *P. cinnamomi*, HED = *P. hedraiandra*, KEL = *P. kelmanii*, NIE = *P. niederhauserii*, PIN = *P. pini.*

[e] Inoculated plant (10^5 zoospores per plant).

porating about 0.4 g into the upper 5 cm of the potting mix of each 655-ml (D40H) container. The dilute zoospore suspension from the plates ($<10^3$ zoospores/ml) was also added to the containers (11 ml/container). Each container was placed in a tight-fitting plastic bag, and water was added to saturate the potting mix to the surface. Plants were kept flooded for 24 h and then drained. A second 24-h flood event was conducted in the same way 13 days after inoculation.

Plant-free controls. Two types of plant-free controls were used to assess whether applied inoculum that had not infected roots could persist in potting mix and contribute to infection of baits in leachate tests. Stored potting mix that had been heat-treated at 60°C for a minimum of 30 min was used for the first set of controls. Containers with heat-treated potting mix were thoroughly irrigated with tap water and allowed to drain before suspensions containing 10^5 zoospores were added as described above.

Although the stored heat-treated potting mix in these controls was not sterile, it would have had low populations of microorganisms at the time of inoculation. In contrast, plants used in single-plant tests were at least 1 year old when inoculated, and the containers would likely have been populated with a diverse mix of microorganisms. To mimic this situation, a second type of plant-free control was used. These "aged" plant-free controls contained heat-treated potting mix to which irrigation leachate from 2-year-old container plants known to be free of *Phytophthora* was applied on alternate days over a 2-week period. Suspensions containing 10^5 zoospores were then added as described above.

Statistical analyses

Nonparametric recursive partition analyses were conducted using the partition platform in JMP (version 9.0, SAS Institute) to explore relationships between detection of *Phytophthora* spp. and various interacting explanatory variables in multi-plant and single-plant tests. Logistic regression was used to assess the effects of temperature and other continuous variables on the detection of *Phytophthora* species in these tests. Boschloo's unconditional exact test for proportions in 2×2 tables (R package Exact, version 2.1) was used to test for significance of differences in proportional data.

Statistical modeling of sampling scenarios

Detection probability function. Data from the multi-plant test arrays were used to develop an estimated detection probability function for the detection of *Phytophthora* species in test arrays with differing percentages of infected plants. A logistic model was fitted using the GLM function in R. The lower bound of the curve (0% detection) was set at a 2% TAIR, the lowest level tested. The upper bound was based on the assumption that *Phytophthora* will be detected more than 99% of the time when all plants in a multi-plant test array are infected (100% TAIR). This function was used to model detection probability for different sampling strategies.

Statistical models. Direct probability and Monte Carlo simulation models were developed to investigate interactions between the test array size, number of arrays, block size (where block is a discrete population of plants being tested), block infection rate (number of infected plants/total number of plants in block), and sampling bias. For the unbiased (random) sampling condition, models assumed that the number of infected plants in a given test array followed a hypergeometric distribution with parameters corresponding to the block size, block infection rate, and array size. For the biased sampling condition, Wallenius' noncentral hypergeometric distribution was assumed. This is an extension of the hypergeometric distribution that includes an odds ratio parameter controlling the relative weights of the sampled units (plants). The weights were used to model intentional bias toward sampling infected plants. For example, at a 2:1 bias ratio, a given infected plant present in the block was twice as likely to be selected for the test array relative to a given noninfected plant.

Direct probability models. For single tests (array count = 1), the probability distributions of TAIR values generated by random or biased sampling without replacement were calculated based on fixed values of array size (20 or 40), block size (100, 500, or 1,000), block infection rate (0 to 25%), and sampling bias (1:1 [random sampling], 2:1, 5:1, or 10:1). The detection probability function was then used to calculate the detection probability for each resulting TAIR value.

Monte Carlo simulations. Monte Carlo simulations were used to compare the added effect of running multiple tests (array count > 1) on a block of plants. Simulations used sampling without replacement at various bias levels (1:1 [random sampling], 2:1, 5:1, 10:1), block sizes of 200 and 1,000 plants, and block infection rates of 1 to 5%. First, the test arrays were populated with infected and noninfected plants from the block using either random or biased sampling from the hypergeometric distribution corresponding to the bias level. *Phytophthora* detection from a test array was assumed to follow a binomial distribution with success probability determined by the TAIR from the detection probability function. The binomial distribution based on the TAIR was randomly sampled to generate the detection outcome for each test array. A detection was scored for a set of multiple arrays if a detection occurred in at least one array of the set. For each combination of variables, 10^6 arrays or sets of arrays and their corresponding detection outcomes were simulated to minimize Monte Carlo error.

Case study

To test model performance under actual nursery conditions, we designed and executed a testing plan based on the results of model predictions for a temporary nursery that was producing approximately 34,500 container plants under contract for a single large project. The nursery, with about 870 m^2 of growing area, was established 3 years earlier over turf and concrete adjacent to a vacant office building where the risk of preexisting contamination by soilborne *Phytophthora* species was low. The plant stock included 53 plant species native to California in 10 different container sizes that ranged in volume from 49.2 (#15) to 0.164 liters (SC10R, Stuewe & Sons), including some species in more than one container size. Plants were in 96 blocks, defined as contiguous groups of one plant species in a single container size that originated from the same propagation batch and had a common risk profile with respect to potential for contamination after planting.

The nursery was aware of the NPBMPs and made some efforts to comply with them, but the degree of NPBMP compliance varied among blocks. The potting mix used was not heat treated, and efforts to sanitize reused containers were unlikely to have been fully effective. A few of the plant blocks had also been acquired from other noncompliant nurseries. The nursery did not report any use of chemicals that would suppress *Phytophthora* species.

To assess the prevalence of *Phytophthora* species on container stock in the nursery, we conducted 128 leachate tests on plants by block. With limited time and budget to conduct testing, we developed a plan to test as many blocks as possible but minimize testing of some species, primarily grasses, which had not been shown to be *Phytophthora* hosts in previous testing. Testing older plants, mostly in larger containers, was prioritized because they had more opportunities to become infected over time. Most of the larger stock was maintained on wooden pallets about

12 cm above ground level. This practice is not permitted under the NPBMPs due to the high potential for contamination from water splashed from the ground during overhead irrigation or rainfall. Eight blocks were small enough that all plants in each block were tested. For other blocks, ranging from fewer than 100 to over 2,000 plants, sampling was used to select plants for testing. Blocks spanning larger areas were divided into smaller sub-blocks to spatially stratify sampling. Sampling was biased to include plants with symptoms that could be related to root disease, including wilting, leaf scorching, stunting, off-color, or poor growth. In blocks that showed no apparent symptoms, plants were selected based on whether their position could increase their risk of contamination (e.g., adjacent to an aisle or other blocks). Once *Phytophthora* was identified within a block through testing, later tests were also biased to include plants close to infested blocks.

Because a *Phytophthora* detection from a single test triggered the destruction of the tested block, we conducted multiple tests on a block on different dates where feasible. If *Phytophthora* was detected in a test from the block, subsequent planned tests were omitted. Because concurrent sets of test arrays were grouped by container size to simplify irrigation, it was sometimes more efficient to test multiple arrays from a given block on the same day. Testing was conducted over 9 days between late May and mid-August 2021. Due to the proximity of the nursery to the San Francisco Bay, temperatures over this period were cool to moderate. Leachate water temperature measured at the end of tests ranged from 14 to 23°C (mean 17.8°C, SD = 1.84, $n = 128$).

Baiting of root systems was conducted on dead plants observed within 10 blocks. Up to 1.5 liters of roots and associated potting mix from dead plants were placed in a 3.8-liter plastic bag. A green pear bait was added, and activated charcoal-filtered tap water was added to a depth of about 2 to 3 cm above the roots and potting mix. Baits were removed when lesions became apparent or after 5 days. Baits with no symptoms were observed for at least 3 more days for possible lesion development. Culturing and identification of isolates was conducted as described above.

Results

Timing of inoculum release during leaching

In the six experiments conducted to determine when inoculum was leached from containers, *Phytophthora* was consistently detected in leachate from irrigations 3 + 4 (30 to 60 min) and 5 + 6 (60 to 90 min) (Supplementary Table S1). *Phytophthora* was not detected in leachate from irrigations 1 + 2 (0 to 30 min) in two of the six experiments. In the remaining four tests, lesion densities for irrigations 1 + 2 were similar to or lower than those for irrigations 3 + 4 and 5 + 6. Results from these experiments supported the collection of leachate from all six irrigations.

Timing of bait infection

In the five experiments that compared bait infection during and after the leachate period, *Phytophthora* infections developed in all pear baits removed from the collection vessel after the 90-min leaching period. All pears added after the leachate collection period and incubated in collected leachate for 3 days also became infected. Based on these results, baiting during leachate collection and extended baiting of the collected leachate with the same pear for 3 days afterward were maintained in the standardized test protocol.

Leachate method test sensitivity for multi-plant arrays

Results from 61 leachate tests conducted with known numbers of infected plants in multi-plant test arrays are shown in Table 3. Tests included a variety of hosts, *Phytophthora* species, container sizes, array sizes, and TAIRs. *Phytophthora* was detected more consistently as the TAIR increased (Table 3). *Phytophthora* was detected in 67% of 46 tests with TAIRs from 2 to 7%, all of which used a single *Phytophthora* source plant. In most of these tests ($n = 40$), the TAIR was between 2.4 and 5%. *Phytophthora* was detected in 79% of 14 tests that used multiple *Phytophthora* source plants to generate TAIRs from 7.5 to 20%. Isolations confirmed that the *Phytophthora* species detected in each test matched the species known to be in the *Phytophthora* source plants.

Many of the factors that could influence detection, including host and *Phytophthora* species, array size, container volume, time since infection, and TAIR, were nonorthogonal in these tests. In exploratory recursive partition analyses, the TAIR was the most important explanatory variable associated with detection of *Phytophthora*. *Phytophthora* was detected in 80% of the tests with a TAIR of 5% or more compared with 55% of tests with a TAIR of less than 5% ($P = 0.042$). Within the TAIR of less than 5% subgroup, *Phytophthora* detection was lower for plants that were tested at least 60 days after acquisition (for plants infected in source nurseries) or inoculation. We repeatedly observed that the leachate test was inefficient at detecting *Phytophthora* from entirely dead plants compared with standard baiting of the entire root system with pear baits for 5 days (data not presented).

No significant relationships were detected between temperature variables and the detection outcome in these tests. The average temperature in the center of containers for the 7 days prior to leachate tests averaged 17.5°C (range 7.8 to 26.1°C, $n = 36$) in tests where *Phytophthora* was detected compared with 16.6°C (range 7.8 to 23.9°C, $n = 21$) for tests with no detections. Only the TAIR was a significant predictor of the *Phytophthora* detection outcome at a P value less than 0.05 in multivariate logistic regression models that also included 7-day pretest temperature, days after inoculation or acquisition, container volume, and several other tested predictor variables.

Detection of *Phytophthora* species in single-plant tests

In repeated leachate tests on individual inoculated plants, *Phytophthora* detection varied over time (Fig. 2). *Phytophthora* was detected in 64% of the 331 single-plant leachate tests. Even among plants inoculated on the same date, tests resulting in detections were not synchronous, and the duration between skips in detection varied widely. *Phytophthora* detection generally became more variable over time. *Phytophthora* was detected in 70.4% of tests ($n = 267$) conducted from 4 to 71 days after inoculation but in only 39.1% of tests ($n = 69$) conducted at least 104 days after inoculation (difference between proportions $P < 0.0001$).

Phytophthora species were detected more consistently in single-plant tests on highly susceptible hosts. *Ceanothus ferrisiae* was highly susceptible to *P. cactorum* and *P. cambivora*; 4 of 4 and 1 of 4 plants died within 35 days of inoculation, respectively. *C. ferrisiae* plants inoculated with either *P. kelmanii* or *P.* taxon thesauri also developed substantial root rot and some stunting but did not die or develop obvious shoot symptoms over the postinoculation period (69 and 203 days, respectively). Overall, these four *Phytophthora* species were detected in 58 of 62 (93.6%) single-plant leachate tests and reisolated from 14 of 15 inoculated plants.

In comparison, detection rates for leachate tests of inoculated *R. malvaceum* were more variable. *P. kelmanii* was detected in 90% of 40 leachate tests and was isolated from all eight inoculated *R. malvaceum* plants (Fig. 2). Compared with *P. kelmanii*, *P. cactorum* and *P. cambivora* were detected less frequently by leachate baiting (60% of 20, $P = 0.013$, and 46% of 24 tests,

$P < 0.001$, respectively) and isolation (1 of 4 for both). *R. malvaceum* plants inoculated with *P. kelmanii* developed severe root rot, stunting, chlorosis, and leaf drop. Symptoms were most severe in plants inoculated using the zoospore dip method. Root rot severity was somewhat lower in *R. malvaceum* inoculated with *P. cactorum* and even lower in plants inoculated with *P. cambivora*.

The lowest overall *Phytophthora* detection rate in single-plant tests was for *Q. lobata* (37.2%, $n = 78$). In tests conducted before winter dormancy (up to 35 days postinoculation), the overall detection rate for inoculated *Q. lobata* was 55.8% (29 of 52). We were unable to reisolate any of the inoculated *Phytophthora* species from *Q. lobata* in attempts made 137 to 189 days after inoculation (Fig. 2).

Based on recursive partition models, host species, days since inoculation, and *Phytophthora* species within host were the best predictors of *Phytophthora* detection by single-plant leachate baiting. As with multi-plant tests, temperature variables were not significant predictors of detection.

Plant-free controls. The applied *Phytophthora* species were detected from all plant-free containers with potting mix 4 days after zoospore suspensions were added, but detection declined thereafter (Fig. 2). *Phytophthora* species were detected more frequently from the heat-treated mix (81% of 21 tests) than from the aged mix (52% of 33 tests, $P = 0.013$) in combined results for leachate tests conducted 7 to 9, 14 to 18, and 32 to 43 days after inoculation.

Compared with inoculated plants, plant-free controls showed a more rapid and consistent decline in *Phytophthora* detection by baiting. At 32 to 43 days after zoospore application, the applied *Phytophthora* species were detected in 65% of inoculated plants ($n = 54$) compared with 33% of all plant-free infested containers ($n = 18$) and 18% of the plant-free containers with aged potting mix ($n = 11$, $P = 0.023$ and 0.0047, respectively).

Statistical modeling of sampling scenarios

Given the positive association between the TAIR and detection probability, varying array size could affect the likelihood of *Phytophthora* detection. The TAIR varies in a discrete stepwise fashion that is a function of test array size and the number of infected plants in the array (Supplementary Fig. S1). For example, one infected plant results in a 10% TAIR in a test array of 10 plants but only a 1% TAIR in a test array of 100 plants. For a given number of infected plants in the test array, smaller array sizes have a greater TAIR and consequently higher detection probabilities. However, in opposition to this effect, small test arrays have a lower probability of including at least one infected plant if the tested block has few infected plants. This disadvantage may be countered by testing multiple arrays per block to increase the chance that at least one array will have one or more infected plants. Modeling was used to evaluate how varying these test parameters affected overall testing efficiency (positive tests/total tests run on blocks with *Phytophthora*) for varying block sizes and block infection rates.

Direct probability models. For a single test array selected from a block of plants, *Phytophthora* detection probability was calculated directly using the estimated detection probability function (Fig. 3). Curves from blocks of 100, 500, or 1,000 plants were similar; data for 500-plant blocks are shown in Figure 4. Compared with random sampling (bias 1:1), biasing plant selection to preferentially select infected plants increased detection probability, with greater benefit seen at bias ratios of 5:1 or higher (Fig. 4). The detection advantage related to biased sampling (bias Δ) was greatest for block infection rates of less than about 10%. As block infection rates increased, bias Δ decreased because one or more infected plants were likely to be included in the test array even with random sampling. For all block sizes, detection probability was greater for an array size of 40 compared with 20 due to the likelihood that at least one infected plant would be included in the larger array. For the same reason, the smaller array size (20) showed greater benefit from sample bias. The model indicates that irrespective of array size, biased sampling is more efficient than random sampling for detecting *Phytophthora* for block infection rates up to at least 25%.

Monte Carlo simulations. Monte Carlo simulations were used to compare the added effect of testing multiple arrays from a block of plants. As expected from the direct probability models, Monte Carlo models showed that *Phytophthora* detection probability increased with increasing block infection rate and sampling bias in both large (1,000) and small (200) blocks of plants (Fig. 5). For both block sizes, detection probability was greater for test arrays of 20 to 40 plants compared with 10 plants. Detection generally increased as more arrays per block were tested (higher array count). However, array counts of two to three provided a greater relative advantage than four, especially at a 5% block infection rate and high sample bias levels (Fig. 5). When the sampling bias ratio was high, most or all *Phytophthora*-positive plants were included in the first three test arrays, so testing additional arrays did not provide a substantial further increase in detection probability.

Case study

At the case study nursery, *Phytophthora* was detected in 16% of the test arrays overall. *Phytophthora* species were detected in 24% of the 46 tested plant species and 20% of 81 tested blocks. Seven plant species and 16 blocks were not tested. No *Phytophthora* species were detected from monocots (14 species) or ferns (3 species) (Table 4). *Phytophthora* was detected in 1

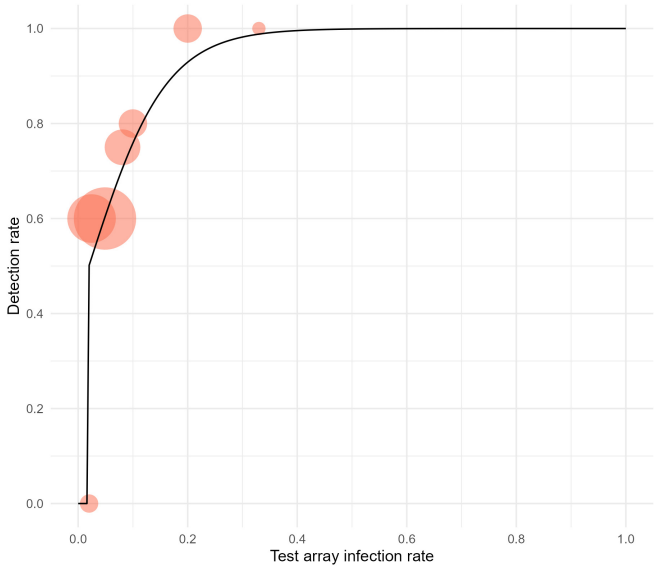

FIGURE 3

Observed ($n = 61$ tests) and modeled *Phytophthora* detection as a function of the test array infection rate (TAIR) for multi-plant test arrays. Results for tests were grouped for TAIR values of 2.4 to 2.5%, 4.8 to 5%, and 6.7 to 9.5% and are plotted at the midpoints of each TAIR range. Circle areas are proportional to the number of tests. The black curve is the fitted estimated detection probability function. Model for TAIR $\geq$ 0.02 is P(Detection | TAIR) = $\text{logit}^{-1}(-0.2804943 + 14.2735191*\text{TAIR})$, where $\text{logit}^{-1}(x)$ denotes $\exp(x)/[\exp(x) + 1]$. P(Detection | TAIR) was set at zero for TAIR < 0.02 and at 1 for TAIR $= 1$.

of the 12 tested species of herbaceous dicot perennials (*Fragaria chiloensis*). The remaining detections were from semi-woody dicots (three of eight tested species) and woody dicot shrubs or trees (seven of nine tested species) (Table 5). Higher detection rates were associated with plant groups considered more likely to be infected, primarily woody dicots grown on pallets (12 cm high) within range of water splash from the ground. *Phytophthora* was detected in all three tests (two blocks) of woody dicots acquired from other commercial nurseries that did not comply with the NPBMPs but not on ferns from a noncompliant nursery (two tests on one block).

Plant appearance was not a good predictor of *Phytophthora* detection, particularly because many herbaceous plants were going into summer dormancy. Dead plants were uncommon and limited

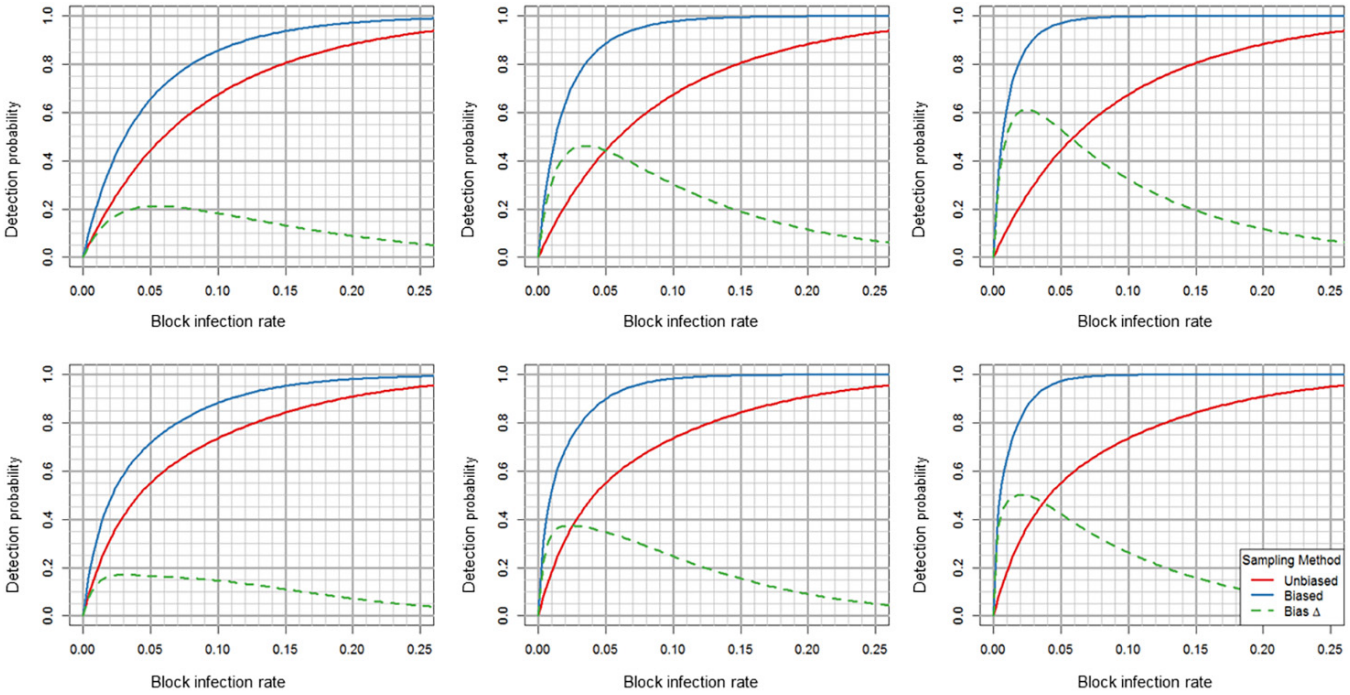

FIGURE 4

Modeled *Phytophthora* detection probability for test arrays selected from blocks (block size = 500) with 0 to 25% of plants infected by *Phytophthora*. Models selected plants using unbiased (random) sampling (red lines) or sampling biased to increase the likelihood of including infected plants (blue lines). Dashed green lines plot the difference in detection probability (Δ) between unbiased and biased sampling. Three sampling bias ratios (2:1, 5:1, 10:1, left to right in each row) at test array sizes of 20 (top row) and 40 (bottom row) are shown. Bias ratios indicate the likelihood of selecting a *Phytophthora*-infected plant to include in a test array compared with random sampling. For example, at bias 5:1, an infected plant is 5 times more likely to be selected for inclusion in a test array compared with unbiased sampling. Modeled *Phytophthora* detection probability was calculated using the function shown in Figure 3.

TABLE 4

Results of leachate baiting tests at the case study nursery

		Total tested			*Phytophthora* detected	
Plant group	Location[a]	Blocks[b]	Arrays[c]	Plants	Blocks (%)	Test arrays (%)
Ferns	Pallet	3	4	118	0	0
	Pallet[d]	1	2	64	0	0
Monocots	Bench	16	18	728	0	0
	Pallet	2	2	63	0	0
Herbaceous perennial dicots	Bench	5	9	352	0	0
	Pallet	14	30	843	7.1	6.7
Semi-woody dicots	Bench	7	10	274	14.3	10.0
	Pallet	8	14	358	37.5	21.4
Woody dicots	Pallet	23	36	407	39.1	30.6
	Pallet[d]	2	3	45	100	100
Totals						
All dicots	Bench	12	19	626	8.3	5.3
	Pallet	47	83	1,653	31.9	22.9
All dicots	Both	59	102	2,279	27.1	19.6
All groups	Both	81	128	3,252	19.8	15.6

[a] Location where plants were maintained prior to testing: bench = nursery benches, pallet = wooden pallets on the ground.
[b] Blocks were contiguous groups of one plant species in a single container size that originated from the same propagation batch. The number of plants per block varied.
[c] Each leachate baiting test used a single test array of plants.
[d] Plants that were acquired from other nurseries rather than propagated by the case study nursery.

to 10 of the tested blocks. We conducted 11 root/potting mix baiting tests on a total of 51 dead or nearly dead plants of eight species removed from these 10 blocks (two tests for one block). Leachate baiting was conducted on 22 test arrays (570 total plants) chosen from the remaining plants in these same blocks. No *Phytophthora* was detected by either test method in four blocks. In 3 of the 10 blocks, *Phytophthora* was detected only by leachate baiting of test arrays. In the three remaining blocks, *Phytophthora* was detected by both methods. For these, the same *Phytophthora* species were detected by each method in two blocks, whereas different *Phytophthora* species were detected by each method in the third block.

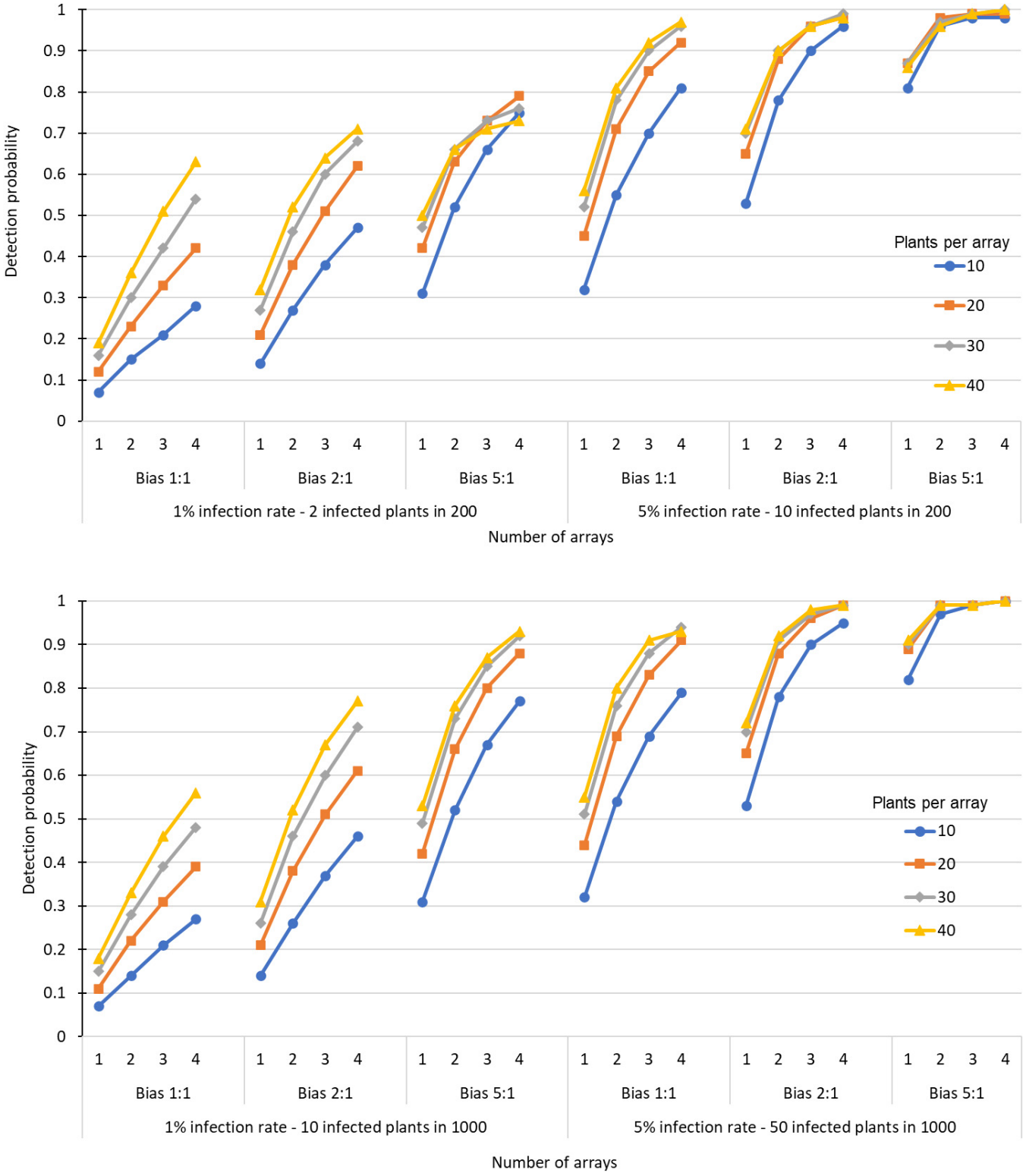

FIGURE 5
Phytophthora detection probability modeled by Monte Carlo simulation as a function of test array count (1 to 4) for arrays of 10 to 40 plants at three sampling bias ratios (1:1 [random sampling], 2:1, 5:1), two block infection rates (1%, 5%), and two block sizes (200 [top], 1,000 [bottom]). Each data point is based on 10^6 simulations.

Overall, *Phytophthora* was detected in the first array tested in 12 of the 81 tested blocks (Tables 4 and 5). After an initial negative test, additional test arrays were scheduled for blocks with high numbers of plants, species that were more likely to be *Phytophthora* hosts, and/or large containers for which few plants could be included per array. Only four more *Phytophthora*-infested blocks were identified by testing additional arrays per block (Table 5). *Phytophthora* was detected in two of the second test arrays from 26 blocks that were negative in the first test. Eleven of the blocks that were negative in a second test array were tested a third time, which yielded one additional detection. *Phytophthora* was detected in one of three blocks tested a fourth time after three negative tests. All plants in the positive fourth array had been included in previous negative test arrays. No *Phytophthora* was detected in seven test arrays from a block of 680 *Diplacus aurantiacus* plants, the only block tested using more than four arrays. This was a large block of a species known to be susceptible to multiple *Phytophthora* species (Rooney-Latham et al. 2019; Sims et al. 2019). For 28 blocks, two (or rarely more) arrays from the same block were tested on the same date to simplify irrigation. In four of these blocks, *Phytophthora* species were detected from both arrays tested on the same day.

Eight *Phytophthora* species were detected across all tests (Table 5). *P. cactorum* was detected most frequently (18 tests), but three different genotypes were represented based on ITS sequences (type plus two haplotypes). Multiple *Phytophthora* species were detected in both blocks of woody dicots acquired from other nurseries, with the highest *Phytophthora* diversity (five species, with two *P. cactorum* haplotypes) detected in *Ceanothus maritimus* (Table 5).

Lesions caused by *Pythium* and *Phytopythium* species can develop at wounds on pear baits, and such lesions were observed on about 90 (70%) of the leachate test pear baits. Species identified included *Pythium cylindrosporum*, *Py. intermedium*, *Py. ultimum*, and *Phytopythium vexans*. In 47 leachate baiting tests, including eight *Phytophthora*-positive tests, the abundance of *Pythium* and *Phytopythium* lesions on pear baits was rated to be extensive enough to potentially reduce the likelihood of detecting slower-growing *Phytophthora* lesions.

In the 9 days of testing conducted at the case-study nursery, the 128 test arrays on 3,252 containers required about 76 h of nursery staff time, mainly for moving plants, conducting test irrigations, and sanitizing equipment (0.6 h/test, 1.4 min/plant). These tests also required planning, preparation, selection of plants to be included in tests, photodocumentation, training and supervision of participating nursery staff, data collection and entry, cleanup, sample processing (including isolation, subculturing, and submitting cultures for sequencing), and reporting. These activities were carried out by a plant pathologist and required 205 h (1.6 h/test, 3.8 min/plant) excluding travel time to the nursery. The sampling and root baiting of 51 dead plants described above is included in the overall time but not in the number of containers tested. Test planning at this nursery required considerable effort, and the number of *Phytophthora* detections increased processing time.

In comparison, 47 leachate tests on 1,248 containers were conducted in 2 days of leachate testing in 2022 at an NPBMP-compliant nursery that had been previously tested. These tests required about 18 h of nursery staff time (about 0.4 h/test, 0.9 min/plant) and 37.5 h of time by a plant pathologist (0.8 h/test, 1.8 min/plant). Time requirements were much lower because personnel at this nursery were already familiar with the test process, test planning was less complicated, container volumes were 2.8 liters or less, few *Pythium* lesions developed on pears, and no *Phytophthora* species were detected.

Discussion

Improved and consistent detection of *Phytophthora* in nursery stock is critical for the production of healthy transplants and reducing spread of *Phytophthora* into native habitats. NPBMP-compliant nurseries have an acute need to be able to detect *Phytophthora* infestations at the earliest possible stage because the NPBMPs are based on complete exclusion of *Phytophthora* from the nursery. Plants with Phytophthora root rot often lack obvi-

TABLE 5

Leachate baiting test parameters for 16 blocks in which *Phytophthora* was detected at the case study nursery[a]

Plant species	Container		Test arrays		*Phytophthora* species detected[d]
	Name	Volume (liters)	First detection[b]	Plants[c]	
Ceanothus maritimus[e]	#1	2.8	First	21	CAC1&2, CIT, MUL, NIC, OCC
Ceanothus thyrsiflorus	TP49	1.64	First	27	CAC1
C. thyrsiflorus Block A	#15	49.2	First	3	HED
C. thyrsiflorus Block B	#15	49.2	Second	3, 6	HED
C. thyrsiflorus Block D	#15	49.2	First	3	CACt, NIE
Eriogonum latifolium	TP49	1.64	Third	32, 27, 32	CAC1
Fragaria chiloensis	AB45	0.71	First	36	CACt
Frangula californica	#15	49.2	First	3	HED
Heteromeles arbutifolia	TP49	1.64	First	32	CAC1
H. arbutifolia	#5	14.4	First	12	CAC1
Lupinus arboreus	TP49	1.64	Fourth	32, 30, 30, 30	CAC1
Monardella villosa	D16	0.26	Second	40	MUL
M. villosa	#1	2.8	First	18	CACt
Morella californica[e]	#15	49.2	First	3	CAC, NIE
Oemleria cerasiformis	#5	14.4	First	15	CAC2
Quercus agrifolia	TP1018R	17.8	First	10	CAC1

[a] Blocks were contiguous groups of one plant species in a single container size that originated from the same propagation batch. The number of plants per block varied.

[b] Test arrays from each block were selected and tested sequentially. First detection is the lowest numbered test in the sequence that provided a *Phytophthora* detection.

[c] The number of plants in test array. If more than one array was tested before a *Phytophthora* detection, the number of plants in each array is listed.

[d] *Phytophthora* species detected: CAC = *P. cactorum* - suffix indicates ITS match to type isolate (t), haplotypes (1,2), or not determined (no suffix); CIT = *P. citrophthora*; HED = *P. hedraiandra*; MUL = *P. multivora*; NIC = *P. nicotianae*; NIE = *P. niederhauserii*; OCC = *P. occultans*.

[e] Denotes plants that were acquired from other nurseries rather than propagated by the case study nursery.

ous shoot symptoms (Bienapfl and Balci 2014; Migliorini et al. 2015; O'Brien et al. 2009; Osterbauer et al. 2014), especially at early stages of disease development. In woody or drought-tolerant species, obvious shoot symptoms such as wilt or dieback may not develop until a high percentage of the roots are decayed (Balci et al. 2008; Standish et al. 1982; Swiecki et al. 2018a). Subtle symptoms that may be associated with early stages of Phytophthora root rot, such as reduced growth or vigor, can also be caused by other factors and may not be evident in blocks with high infection rates. In both controlled tests and the case study nursery, *Phytophthora* was detected in many leachate baiting tests from plants that were either completely asymptomatic or had only nonspecific symptoms, such as reduced growth or chlorosis.

Overall, *Phytophthora* was detected in 67% of leachate tests with a single *Phytophthora* source plant in multi-plant test arrays (2 to 7% TAIR) and 66% of single-plant tests. This suggests that variation in inoculum produced by a single *Phytophthora* source plant in a multi-plant array could account for most or all of the observed variability in *Phytophthora* detection at a low TAIR (7% or less). In single-plant tests, detection was less variable in more susceptible host-pathogen combinations. Across the range of host-pathogen combinations used in our studies, the success of *Phytophthora* detection using leachate baiting was comparable to that reported for leaf baits (Ferguson and Jeffers 1999; O'Brien et al. 2009) using different inoculum sources and host-pathogen combinations.

For some host-pathogen combinations, detection efficiency decreased with increasing time after inoculation (Fig. 2). Most of the zoospores detected by leachate baiting likely arise from sporangia on infected roots. Sporangium and zoospore production will decrease as these roots die unless additional healthy roots are available to become infected. Especially in small containers, the supply of roots with sporangia may fluctuate over time and can be affected by host susceptibility, resulting in reduced detection efficiency over time.

Phytophthora species were detected in leachate of host-free potting mix for more than a month after zoospores were added (Fig. 2). Zoospore cysts leached from the potting mix may have been the source of these detections. Zoospore cysts of some *Phytophthora* species are known to persist in soil for extended periods (MacDonald and Duniway 1979) and may produce zoospores by repeated emergence or the production of microsporangia (Erwin and Ribeiro 1996). Zoospore cysts, along with oospores and chlamydospores that germinate indirectly, may sometimes be a source of detectable inoculum in containers even if sporangia are sparse or lacking at the time of the test.

To detect *Phytophthora* using methods such as direct isolation or molecular methods, infected roots or infested debris must be included in the sample and in the material that is processed for detection. It may be necessary to screen and sample large numbers of plants to locate those with detectable *Phytophthora* if only a small percentage of plants in the sampled population are infected, especially if the infected plants are asymptomatic. When testing multi-plant arrays using the leachate test, *Phytophthora* can be detected from inoculum derived from any of the plants in the test array and any portion of the roots and potting mix within the containers. The ability to assay the entire container contents from multiple plants in a single test provides a significant advantage for detecting *Phytophthora* when few plants are infected. *Phytophthora* was detected from sets of inoculated nursery plants using the leachate baiting test at a higher rate compared with sampling roots and testing by commercial immunoassay strips or direct isolation on selective media (Del Castillo Munera 2021).

The leachate baiting method has proven to be effective across a wide range of container sizes. *Phytophthora* was detected in single-plant tests of 107-ml containers. These tests generated only 120 ml of leachate, all of which was captured and baited. At the opposite extreme, *Phytophthora* detections were made from test arrays of six 49.2-liter containers, which generated 288 liters of leachate. These extremes represent nearly a 2,360× range in leachate volume and a 2,760× range in root/potting mix volume. Up to about 6.5 liters of leachate is retained in the collection vessel during the leachate test, and 2.7 liters are transferred along with the pear bait for the subsequent 3-day incubation. These volumes represent 2.25 and 0.94%, respectively, of the total leachate volume from a test of six 49.2-liter containers. These results suggest that scaling of irrigation volume to container size (Table 1) helps avoid excessive dilution of inoculum, and the design of the collection vessel (Fig. 1E) is effective at concentrating negatively geotactic zoospores (Cameron and Carlile 1977) near the floating bait.

Direct probability (Fig. 4) and Monte Carlo models (Fig. 5) both showed that detection efficiency is greatly improved by biasing sampling, especially if block infection rates are low. If sampling bias is low, detection efficiency can be maximized by using larger arrays (up to 40 plants) and multiple test arrays per block. A test array size of 40 to 42 is close to the maximum practical test array size due to irrigation time and bench space considerations. This also represents the maximum array size for which a detection of a single infected plant was likely based on our empirical results (Table 3).

As sampling bias and block infection rate increase, increasing from one to two or three test arrays provided the greatest relative improvement in the probability of detection (Fig. 5). Monte Carlo modeling indicated that with the combination of small block size, low block infection rate, and high sampling bias, "oversampling" can occur with use of large arrays and high numbers of arrays per block (Fig. 5, bottom). If sampling is successfully biased toward selecting infected plants, the first few test arrays are likely to include most or all of the infected plants in the block. Oversampling can lower overall testing efficiency because more tests are conducted than are needed to obtain a detection. Oversampling can be avoided by testing arrays sequentially after previous test results are known, but this strategy is only practical if tests are conducted over a period of weeks.

If only a single *Phytophthora*-infected plant is present in a test array, the amount of inoculum released by that plant clearly limits test sensitivity. However, under typical nursery conditions, *Phytophthora* contamination in most blocks is unlikely to be limited to a single plant (Bienapfl and Balci 2014; Molnar et al. 2020; Parke et al. 2014; Rooney-Latham et al. 2019). Many sources of contamination in nurseries, such as inadequately sanitized containers or inoculum inadvertently introduced during propagation, are likely to affect multiple plants within a block rather than a single container. Even if a single infected plant is initially present in a block, *Phytophthora* can quickly spread by water splash between closely packed containers. If sample biasing based on various risk factors is successful, multiple infected plants are likely to be included in most test arrays, increasing the likelihood of detection.

At the case study nursery, *Phytophthora* was detected with leachate tests in 16 blocks. In 12 of these blocks, *Phytophthora* was detected in the first array tested. The relatively high detection efficiency for these blocks could be due to high TAIRs (due to high block infection rates and/or effective sample biasing) and/or susceptible host-pathogen combinations that produced high levels of detectable inoculum. The true number of infected plant blocks at the case study nursery is unknown and could be higher than the number detected. As with most tests, positive results from leachate testing are more definitive than negative results.

The leachate test has been used to document the presence of *Phytophthora* contamination in multiple nurseries. Due to the possibility of false negatives, leachate testing is generally not appropriate for attempting to identify noninfected plants, either in groups or individually, in a generally infested nursery.

Compliance with NPBMPs, rather than testing, provides the primary confidence that *Phytophthora* contamination has been avoided during plant production (Swiecki et al. 2021). However, testing plays an important role in quality control. Based on our results, an efficient testing program for quality control testing in an NPBMP-compliant nursery should include testing dead or nearly dead plants by destructive root/potting mix baiting or other means whenever such plants are present. Leachate baiting of test arrays from nursery blocks should initially focus on generally more susceptible plant groups (e.g., woody dicots) and any blocks with known risk factors for contamination due to known or suspected deviations from NPBMPs. For restoration nurseries, higher-risk propagative materials, such as field-collected cuttings originating close to or in contact with soil (Swiecki et al. 2021), also have a higher priority for testing. The leachate baiting test has detected *Phytophthora* infestations associated with unintentional NPBMP deviations or high-risk propagative material in specific plant blocks in NPBMP-compliant nurseries.

False positive results from tests can be problematic for any nursery but are not tolerable in NPBMP-compliant nursery production in which spurious detections would trigger costly and unnecessary destruction of stock. Leachate baiting only detects live and active *Phytophthora*, so any confirmed detection is meaningful. NPBMP-compliant nurseries routinely treat potting mix, reused containers, irrigation water, and contaminated surfaces with heat or chemicals before use to kill *Phytophthora* propagules that may be present. DNA that may be present in killed propagules or released into the extracellular medium might be detectable when highly sensitive molecular methods are used that do not distinguish between viable and non-viable DNA (Khaliq et al. 2018; O'Brien et al. 2009). The high sensitivity of molecular methods can be a liability for testing in NPBMP-compliant nurseries unless the potential for false positives can be eliminated.

Leachate baiting also provides a relatively efficient way to obtain isolates of *Phytophthora* species present in a nursery. Besides confirming the presence of active *Phytophthora*, isolates provide opportunities to conduct other epidemiologically important studies. At the case study nursery, three haplotypes of *P. cactorum* were identified, suggesting that this species may have been introduced several times through multiple pathways. Furthermore, *P.* taxon thesauri would not have been identified without obtaining a culture. It is not distinguishable from *P. cactorum* based on ITS sequence alone, even though it differs in growth characteristics and morphology.

The controlled studies reported here and extensive use of the leachate baiting test in the case study and many other nurseries (Swiecki et al. 2021) confirm that this test fulfills the major needs identified for testing in NPBMP-compliant nurseries. Information on how to conduct the test and construct testing apparatus has been provided via in-person trainings and online with written resources and videos. Trained nursery personnel have successfully conducted all aspects of the nursery testing and post-test incubation. Because most nurseries lack facilities and expertise to isolate from lesions on baits, they submit pear baits with suspect lesions to a qualified diagnostic laboratory to confirm the presence of *Phytophthora* and identify species. The leachate test has been an important component in establishing and assessing the performance of a nursery accreditation program based on the NPBMPs (Swiecki et al. 2021). This test also has wide applicability to sectors of the nursery industry where *Phytophthora* infections are of concern.

Acknowledgments

We thank David Rizzo, University of California, Davis, Department of Plant Pathology, for arranging space for experiments. We thank Susan J. Frankel, US Forest Service, Pacific Southwest Research Station, for facilitating funding and supporting efforts to develop *Phytophthora*-free nursery stock. Thanks are extended to the nurseries that cooperated with this research. Thanks to Cheryl Blomquist and Suzanne Rooney Latham, California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Plant Pest Diagnostics Lab, Sacramento, for sequencing of isolates.

Literature Cited

Balci, Y., Balci, S., MacDonald, W. L., and Gottschalk, K. W. 2008. Relative susceptibility of oaks to seven species of *Phytophthora* isolated from oak forest soils. For. Pathol. 38:394-409.

BenDor, T., Lester, T. W., Livengood, A., Davis, A., and Yonavjak, L. 2015. Estimating the size and impact of the ecological restoration economy. PLoS One 10:e0128339.

Bienapfl, J. C., and Balci, Y. 2014. Movement of *Phytophthora* spp. in Maryland's nursery trade. Plant Dis. 98:134-144.

Bourret, T. B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. Chapter 2. Restoration outplantings of nursery-origin Californian flora are heavily infested with *Phytophthora*. Ph.D. Thesis. University of California, Davis, CA. ProQuest Dissertations Publishing. 10746957.

Cameron, J. N., and Carlile, M. J. 1977. Negative geotaxis of zoospores of the fungus *Phytophthora*. J. Gen. Microbiol. 98:599-602.

Cobb, R. C., Haas, S. E., Kruskamp, N., Dillon, W. W., Swiecki, T. J., Rizzo, D. M., Frankel, S. J., and Meentemeyer, R. K. 2020. The magnitude of regional scale tree mortality caused by the invasive pathogen *Phytophthora ramorum*. Earth's Future 8:p.e2020EF001500.

Croucher, P. J. P., Mascheretti, S., and Garbelotto, M. 2013. Combining field epidemiological information and genetic data to comprehensively reconstruct the invasion genetics of the sudden oak death agent *Phytophthora ramorum* (Stramenopila: Oomycetes) in California. Biol. Invasions 15:2281-2297.

Del Castillo Munera, J. M. 2021. Evaluating the efficacy of *Phytophthora* detection methods to support restoration nurseries best management practices. Phytopathology 111:S1.37.

Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.

Farr, D. F., and Rossman, A. Y. 2023. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/ (accessed February 1, 2023).

Ferguson, A. J., and Jeffers, S. N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83:1129-1136.

Frankel, S. J., Conforti, C., Hillman, J., Ingolia, M., Shor, A., Benner, D., Alexander, J. M., Bernhardt, E., and Swiecki, T. J. 2020. *Phytophthora* introductions in restoration areas: Responding to protect California native flora from human-assisted pathogen spread. Forests 11:1291.

Goss, E. M., Larsen, M., Vercauteren, A., Werres, S., Heungens, K., and Grünwald, N. J. 2011. *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. Phytopathology 101: 166-171.

Grünwald, N. J., Garbelotto, M., Goss, E. M., Heungens, K., and Prospero, S. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol. 20:131-138.

Hansen, E. M., Reeser, P. W., and Sutton, W. 2012. *Phytophthora borealis* and *Phytophthora riparia*, new species in *Phytophthora* ITS Clade 6. Mycologia 104:1133-1142.

Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A. G., Aguín Casal, O., Bakonyi, J., Cacciola, S. O., Cech, T., Chavarriaga, D., Corcobado, T., Cravador, A., Decourcelle, T., Denton, G., Diamandis, S., Doğmuş-Lehtijärvi, H. T., Franceschini, A., Ginetti, B., Green, S., Glavendekić, M., Hantula, J., Hartmann, G., Herrero, M., Ivic, D., Horta Jung, M., Lilja, A., Keca, N., Kramarets, V., Lyubenova, A., Machado, H., Magnano di San Lio, G., Mansilla Vázquez, P. J., Marçais, B., Matsiakh, I., Milenkovic, I., Moricca, S., Nagy, Z. Á., Nechwatal, J., Olsson, C., Oszako, T., Pane, A., Paplomatas, E. J., Pintos Varela, C., Prospero, S., Rial Martínez, C., Rigling, D., Robin, C., Rytkönen, A., Sánchez, M. E., Sanz Ros, A. V., Scanu, B., Schlenzig, A., Schumacher, J., Slavov, S.,

Solla, A., Sousa, E., Stenlid, J., Talgø, V., Tomic, Z., Tsopelas, P., Vannini, A., Vettraino, A. M., Wenneker, M., Woodward, S., and Peréz-Sierra, A. 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. For. Pathol. 46:134-163.

Keim, R., Mock, T., and Guenther, H. 1976. Phytophthora crown rot of toyon. Plant Dis. Rep. 60:88-90.

Kelmenson, S., BenDor, T., and Lester, T. W. 2016. The economic impacts of the US ecological restoration sector. Federal Reserve Bank of Boston, Communities & Banking, Summer 2016:25-27.

Khaliq, I., Hardy, G. E. S. J., White, D., and Burgess, T. I. 2018. eDNA from roots: A robust tool for determining *Phytophthora* communities in natural ecosystems. FEMS Microbiol. Ecol. 94:fiy048.

Khew, K. L., and Zentmyer, G. A. 1973. Chemotactic response of zoospores of five species of *Phytophthora*. Phytopathology 63:1511-1517.

MacDonald, J. D., Ali-Shtayeh, M. S., Kabashima, J., and Stites, J. 1994. Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. Plant Dis. 78:607-611.

MacDonald, J. D., and Duniway, J. M. 1979. Use of fluorescent antibodies to study the survival of *Phytophthora megasperma* and *P. cinnamomi* zoospores in soil. Phytopathology 69:436-441.

Matheron, M. E., and Porchas, M. 2009. Impact of different preplant cultural treatments on survival of *Phytophthora nicotianae* in soil. Plant Dis. 93: 43-50.

McIntosh, D. L. 1964. *Phytophthora* spp. in soils of the Okanagan and Similkameen valleys of British Columbia. Can. J. Bot. 42:1411-1415.

Migliorini, D., Ghelardini, L., Tondini, E., Luchi, N., and Santini, A. 2015. The potential of symptomless potted plants for carrying invasive soilborne plant pathogens. Divers. Distrib. 21:1218-1229.

Mircetich, S. M., Campbell, R. N., and Matheron, M. E. 1977. Phytophthora trunk canker of coast live oak and cork oak trees in California. Plant Dis. Rep. 61:66-70.

Molnar, C., Nikolaeva, E., Kim, S., Olson, T., Bily, D., Kim, J.-E., and Kang, S. 2020. *Phytophthora* diversity in Pennsylvania nurseries and greenhouses inferred from clinical samples collected over four decades. Microorganisms 8:1056.

O'Brien, P. A., Williams, N., and Hardy, G. E. S. 2009. Detecting *Phytophthora*. Crit. Rev. Microbiol. 35:169-181.

Osterbauer, N., Lujan, M., McAninch, G., Lane, S., and Trippe, A. 2014. Evaluating the efficacy of the systems approach at mitigating five common pests in Oregon nurseries. J. Environ. Hort. 32:1-7.

Parke, J. L., Knaus, B. J., Fieland, V. J., Lewis, C., and Grünwald, N. J. 2014. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. Phytopathology 104: 1052-1062.

Rooney-Latham, S., Blomquist, C.L., Kosta, K.L., Guo, Y.Y., and Woods, P.W. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. Plant Dis. 103: 448-455.

Rooney-Latham, S., Blomquist, C. L., Swiecki, T., Bernhardt, E., and Frankel, S. J. 2015. First detection in the US: New plant pathogen, *Phytophthora tentaculata*, in native plant nurseries and restoration sites in California. Native Plant J. 16:23-27.

Serrano, M. S., and Garbelotto, M. 2020. Differential response of four Californian native plants to worldwide *Phytophthora cinnamomi* genotypes: Implications for the modeling of disease spread in California. Eur. J. Plant Pathol. 156:851-866.

Serrano, M. S., Osmundson, T., Almaraz-Sánchez, A., Croucher, J. P., Swiecki, T., Alvarado-Rosales, D., and Garbelotto, M. 2019. A microsatellite analysis used to identify global pathways of movement of *Phytophthora cinnamomi* and the likely sources of wildland infestations in California and Mexico. Phytopathology 109:1577-1593.

Shishkoff, N. 2014. Growth-inhibiting fungicides affect detection of *Phytophthora ramorum* from infected foliage and roots. Plant Health Prog. 15:36-40.

Sims, L., Tjosvold, S., Chambers, D., and Garbelotto, M. 2019. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathol. 68:196-204.

Sims, L. L., and Garbelotto, M. 2021. *Phytophthora* species repeatedly introduced in Northern California through restoration projects can spread into adjacent sites. Biol. Invasions 23:2173-2190.

Smith, C. O. 1937. Inoculation of some economic plants with *Phytophthora cactorum* and *P. citrophthora*. Phytopathology 27:1106-1109.

Standish, E. D., MacDonald, J. D., and Humphrey, W. A. 1982. Phytophthora root and crown rot of junipers in California. Plant Dis. 66: 925-928.

Swiecki, T. J., Bernhardt, E., Garbelotto, M., and Fichtner, E. 2011. The exotic plant pathogen *Phytophthora cinnamomi:* A major threat to rare *Arctostaphylos* and much more. Pages 367-371 in: Proceedings of the CNPS Conservation Conference: Strategies and Solutions. J. W. Willoughby, B. K. Orr, K.A. Schierenbeck, and N. J. Jensen, eds. 17–19 Jan 2009, California Native Plant Society, Sacramento, CA.

Swiecki, T. J., Bernhardt, E. A., and Frankel, S. J. 2018a. Phytophthora root disease and the need for clean nursery stock in urban forests: Part 2. *Phytophthora* and nurseries. Western Arborist 44:38-45.

Swiecki, T. J., Bernhardt, E. A., Frankel, S. J., Benner, D., and Hillman, J. 2021. An accreditation program to produce native plant nursery stock free of *Phytophthora* for use in habitat restoration. Plant Health Prog. 22: 348-354.

Swiecki, T. J., Bernhardt, E. A., and Garbelotto, M. 2003. First report of root and crown rot caused by *Phytophthora cinnamomi* affecting native stands of *Arctostaphylos myrtifolia* and *A. viscida* in California. Plant Dis. 87:1395.

Swiecki, T. J., Quinn, M., Sims, L., Bernhardt, E., Oliver, L., Popenuck, T., and Garbelotto, M. 2018b. Three new *Phytophthora* detection methods, including training dogs to sniff out the pathogen, prove reliable. Cal. Agric. 72:217-225.

Washington State University Tree Fruit. 2023. Pear Harvest. Available at: https://treefruit.wsu.edu/web-article/pear-harvest/ (accessed June 16, 2023).

Weiland, J. E. 2021. The challenges of managing Phytophthora root rot in the nursery industry. Plant Health Prog. 22:332-341.