Phytophthora monitoring plan for nursery stock and restoration sites to protect vegetation on the Angeles National Forest – Nursery stock prevention and monitoring
Final performance report
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Contents

Cover image: Sample sites CT18 (dead oak) and CT19 (declining and dead buckwheat and deerweed beyond planting basin edge) at site Chaney Trails/S11BR1. Three *Phytophthora* species were recovered from these two samples.

Summary

We sampled nursery-origin container-stock transplants at restoration sites in the Angeles National Forest in Sept 2016 and May 2021. The majority of the sampled transplants were dead, a few were declining or had extensive dieback. Four to 8 transplants were sampled at each location on each date. *Phytophthora* species were detected both dates on the sampled container stock at 3 restoration sites. At Chaney Trails, *P. cactorum* and *P. mediterranea* were isolated from dead or declining *Quercus agrifolia* transplants on both dates. *P. niederhauserii* was recovered from sampled transplants in 2016 and *P. nicotianae* and *P. quercetorum* were recovered in 2021. At the HAY-1 site, *P. nicotianae* and *P. citrophthora* were recovered from dead or declining transplants of various species in 2016, and *P. cactorum* and *P. serendipita* were recovered in 2021. At WSS28, *P. cactorum* was recovered in 2016 and *P. nicotianae* was recovered in 2021 from dead or declining *Q. john-tuckeri*. We sampled at Pullsite 21 only in 2021 and did not recover , *Phytophthora* from dead *Q. john-tuckeri* or stressed looking *Sambucus* transplants. Spread of *Phytophthora* from the transplants to adjacent vegetation was documented at Chaney Trails and WSS28. At both locations, *Phytophthora* species detected on transplants were detected on dead or declining vegetation on the planting basin rim. In addition, at the Chaney Trails site, *P. cactorum* was detected on a recently dead *Malosma laurina* in an otherwise healthy stand downslope from the planting. This suggests that even in southern California, some *Phytophthora* species are capable of spreading from infected transplants to adjacent natural vegetation in as little as 6 years. We recommend rogueing dead transplants as they occur and discontinuing summer irrigation (observed at

one site in 2021) as mitigation methods that will lessen the reproduction and movement of these destructive plant pathogens into surrounding native vegetation. Any additional use of transplants at these locations should use only nursery stock grown under the Nursery Phytophthora Best Management Practices.

Introduction

Infection of nursery stock by plant pathogens, especially *Phytophthora* species, has been known as a problem of plants produced in nurseries for at least 75 years. *Phytophthora* is a genus of microorganisms with a fungal-like growth habit. They are adapted to reproduce and infect plants in wet conditions and are in a taxonomic group referred to as 'water molds'. Most species of *Phytophthora* are highly destructive on one or more plant species, and some are capable of infecting and killing numerous species across multiple plant families.

In 2014, a quarantine-level *Phytophthora* species, *P. tentaculata*, was found infecting plants in several recently planted restoration plantings and their source nurseries. Subsequent testing showed many restoration nurseries in Northern California were highly contaminated with multiple *Phytophthora* species and these pathogens were being moved into wildlands via restoration plantings. To assess whether the same problem was occurring in southern California, sampling was conducted by Ted Swiecki and Katie VinZant (Forest Service) in September 2016 at three restoration sites planted in 2015 to 2016 in the Angeles National Forest. Nursery stock at three nurseries that supplied container stock for use in ANF restoration plantings was also tested for the presence of *Phytophthora*. This sampling showed that restoration nursery container stock in southern California was also infected with a diverse mix of *Phytophthora* species and that these pathogens were persisting on material planted in the field.

Although *Phytophthora* species are regarded as an important cause of plant losses in irrigated agriculture in southern California, it wasn't known how well these pathogens would survive over a longer time frame in restoration plantings, and if they would pose a threat to nearby native vegetation. This study was undertaken to look at these factors. We cooperated with the Rizzo lab at the Department of Plant Pathology at U.C. Davis for these studies. The Rizzo lab sampled restoration site locations in 2018, 2019, (Bourret and Frankel 2018, Fajardo et al. 2019) and both we and the Rizzo lab sampled locations in spring 2021.

Project activities

Ted Swiecki and Elizabeth Bernhardt sampled four Angeles National Forest restoration sites in southern California on 21 and 22 May 2021. Three of the sites, Chaney Trails (=S11BR1), Aliso HAY-1 (= S6R022); and WSS28 (=S6R029) were previously sampled by Ted Swiecki in September 2016 and *Phytophthora* species had been detected on sampled planted nursery stock at each of these sites. We also sampled one site we had not previously sampled along Angeles Forest Highway, S6R021 (=Pullsite 22 (21)). The selection of sampling locations was worked out in advance with the Rizzo lab and was intended to make the best use of limited sampling time by both teams by dividing up sites to be sampled. The sampling had originally been planned for spring of 2020 but was delayed due to the COVID 19 epidemic.

Methods

We met with Janet Nickerman (USDA-FS) at the Chaney Trails (CT = S11BR1), field site on 20 May 2021 to get access to the site. She joined us for a short time during the sampling and we discussed possible implications of sampling results. On 21 May 2021 we sampled Aliso HAY-1 (H = S6R022), WSS28 (W = S6R029), and Pullsite 22 (21) (AF = S6R021). Abbreviations and alternate names for the sties are shown in Table 1.

Table 1. Sites sampled for this report

Site	Abbreviation	Rizzo lab name
Aliso HAY-1	H	S6R022
Chaney Trails	CT	S11BR1
WSS28	W	S6R029
Pullsite 22 (21)	AF	S6R021

Within the plantings, we mostly collected root samples from recently-killed plants, i.e., plants that died within the past year based on foliage color and deterioration. Sampling was conducted to emphasize the inclusion of live (if present) and dead roots in the sample, including the original container rootball if possible, along with other roots near the center of the planting basins. Some samples contained roots of several different species where plants were growing close together.

Root/soil samples were dug using hand tools (trenching shovels, masonry hammer, trowels, pruning shears). The specific tools had been selected and, in some cases, modified to ensure that they could be thoroughly cleaned and sanitized between samples. Duff and debris on the soil surface were scraped aside and roots and soil were collected to a depth of 10 to 20 cm. Clean or new disposable nitrile gloves were worn for each

sample. For each sample, we collected roots and rootzone soil and placed them into labeled 1-gallon heavy duty zip-closure plastic bag (freezer Ziploc® bag). A total volume of about 1.5 L of roots and soil was collected per sample. After sampling, holes were backfilled with the excavated soil and tools were thoroughly disinfested by brushing off soil into each sampling hole and thoroughly cleaning tools with 70% isopropanol to remove all visible soil. The sample bag was sealed and placed in a larger secondary containment bag and into a shaded container for transport back to our vehicle. Upon return to the vehicle, samples were placed into coolers for transport to the lab.

At each sample location, GPS coordinates were recorded using a Garmin GPSMap 64x high sensitivity GPS. Sample points were photo-documented with digital camera images. Data for each sample location, including plant species sampled, symptoms, and soil characteristics were recorded on datasheets.

Root/soil sample testing

Upon return to the lab at the end of the day, samples were wetted to about field capacity with charcoal-filtered tap water to create favorable environmental conditions for sporangium production. Moistened root/soil samples were incubated for 3 days at 21-24 C (70-75 F) to allow time for sporangia to form. Over this period, samples were misted with additional water as needed to keep roots from drying out and to maintain target moisture levels.

After 3 days, samples were flooded with charcoal filtered tap water and baited with green pears. Flooding stimulates release of zoospores that can infect the pears; sporangia can also continue to form while samples are flooded. Many *Phytophthora* species, readily infect green pears, causing characteristic brown lesions. *Pythium* species may also infect pears, usually starting at a wound. Baited sample bags were incubated at temperatures that fluctuated diurnally between about 21 and 24 C (70-75 F). Pears were removed as soon as *Phytophthora* lesions were evident or after 5 days if no symptoms were seen. Because late symptom development can occur, pears were monitored until at least 8 days after the initial flooding date for the appearance of symptoms.

When removed from the sample bags, pear baits were rinsed with tap water and placed individually on clean paper towels for further incubation. Symptomatic pears were photographed, and notes were taken on the number of lesions observed. To obtain *Phytophthora* isolates, pears were first surface-disinfested by placing them in 0.5% NaOCl (diluted bleach) for 45 seconds. Pieces were cut from the edges of suspect *Phytophthora* lesions using aseptic technique and placed into carrot-cornmeal agar in petri dishes. Mycelium that grew out of the tissue pieces was examined under a

microscope. Initial identification as a *Phytophthora* species was based on morphology of mycelium. Representative cultures for each observed suspected *Phytophthora* morphotype were given to Dr. Tyler Bourret at UC Davis for identification by DNA sequencing of the ITS region. Two morphotypes were sent to Suzanne Latham, Senior Plant Pathologist at the Plant Pest Diagnostics Lab, Plant Health and Pest Prevention Services, California Dept. of Food and Agriculture (CDFA) for identification by DNA sequencing of the ITS region. The CDFA lab also provided ITS sequence species identifications for the 2016 sampling cited in this report.

At S11BR1, *Phytophthora* had been detected in some non-planted native vegetation downslope from the restoration planting in sampling by Katie VinZant in 2017 and by the Rizzo lab in 2018 and 2019 (Bourret and Frankel 2018, Fajardo et al. 2019). We collected a number of samples at this site that were outside of the planted area in both uphill and downhill directions to investigate whether *Phytophthora* might be spreading from the planting into adjacent vegetation or vice versa. A few samples of this type were also collected at S6R029. We also collected some samples that were a short distance (0.5-1 m) from installed nursery plants to get some information about short range spread in and adjacent to the planting basins.

Results

In May 2021, *Phytophthora* species were isolated from samples collected at the three sites we had previously sampled in 2016: Chaney Trails, HAY-1 and WSS28. No *Phytophthora* species were detected at Pullsite 21 (22), which we had not previously sampled. Both S11BR1 and S6R029 in particular had an abundance of recently killed and declining oak transplants (*Quercus agrifolia* and *Q. john-tuckeri* at the two sites, respectively). Tables 2 through 6 summarize results of the May 2021 samples collected at these four Angeles NF restoration sites and include results of our 2016 sampling and Rizzo lab sampling in 2018 and 2019 (Bourret and Frankel 2018, Fajardo et al. 2019). Sampling results for each location are discussed below.

Chaney Trails / S11BR1

This is a long, narrow planting running crosswise across a slope and underneath high voltage transmission lines in the mountains north of Altadena (Figure 1). An older set of transmission lines is parallel to this set and just uphill from the restoration site. Native vegetation occurs above and below the planting, and an old foundation and remnant small citrus trees near the parking area suggest a former habitation was in the area. Many native plants originating from the hydroseed mix or other local sources have established throughout the site.

Transplants

In limited sampling of recently dead and declining *Quercus agrifolia* transplants from 2016 to 2021, six different *Phytophthora* species were detected on 13 transplants (Table 2, Figure 2). No *Phytophthora* was detected in five other transplants that we sampled in 2021 (Table 2). The most consistently isolated species were *P. cactorum* (4 transplants) and *P. mediterranea* (5 transplants). *P. niederhauserii, P. nicotianae,* and *P. quercetorum* were found on one transplant each. Two of the samples yielded two different *Phytophthora* species. At this location, the number of *Phytophthora* species detected generally increased with the number of *Q. agrifolia* transplants sampled. These observations suggest that the original *Q. agrifolia* nursery stock was infected with multiple *Phytophthora* species. It is possible that due to limited sampling, some *Phytophthora* species introduced with the 2015-2016 planting stock may not yet have been detected in planted basins. Based on the diversity of *Phytophthora* species detected at this location in 2021, it appears multiple *Phytophthora* species introduced on the nursery stock have persisted at the site and continue to cause mortality in the transplants. All the detected species are known to occur on nursery stock as discussed below.

Other vegetation

Evidence of spread from *P. cactorum*-infected *Q. agrifolia* was detected in two instances at this location. In one instance, *P. cactorum* was detected on a recently dead *Malosma laurina* located at least 7 m from the nearest symptomatic planting basin (CT21) and downslope from the footprint of the planting area (Figure 1, bottom). This indicates that *P. cactorum* was capable of spreading from infected transplants and causing mortality of established native vegetation downslope within about 6 years. In the second instance, *P. cactorum* was found infecting symptomatic plants 30 cm beyond the planting basin rim, 60 cm or more from the original oak transplant, which had died recently (cover image). The affected plants near this basin were on the same elevation contour as the basin. We baited *P. quercetorum* and *P. mediterranea* from the dead *Q. agrifolia* in the basin. Given the prevalence of *P. cactorum* in the stock at this site, it is possible the dead transplant could have had also been infected with *P. cactorum* that we failed to detect. This scenario would suggest that *P. cactorum* spread from the transplant to adjacent plants outside the basin. Alternatively, the *P. cactorum* could have come from other upslope *P. cactorum*-infected planting sites and would represent longer range spread of at least several meters.

P. sp. kelmania was detected in *Salvia mellifera* just upslope of the current planting in sampling conducted by Katie VinZant in 2017 (Frankel 2018, Tyler Bourret, personal communication 11/10/2021, confirmed identification of CDFA ITS sequence supplied by Suzanne Latham). *P*. sp. kelmania was also detected by the Rizzo lab in the grove of oaks and other native plants that occur in the swale below the planting in 2019 (Fajardo et

al. 2019). These *P*. sp. kelmania detections may be due to an introduction that predates the restoration planting, possibly related to the older transmission line corridor just upslope from the recent planting. We detected the distinct but closely related *P. pseudocryptogea* in the same area in 2021 sampling. Although this apparently mixed species introduction could predate the planting, it could also have been associated with contamination introduced during the recent construction or restoration activities at this site. Although *P*. sp. kelmania and P. *pseudocryptogea* have not been detected elsewhere in the planting, it is possible that all of the species introduced in planting stock have not yet been detected in the limited sampling to date, as noted above.

Figure 1. Chaney Trails restoration site. Top – natural vegetation is located upslope and downslope from the restoration planting, mostly dominated by hydroseeded *Eriogonum fasciculatum* in this view. Note irrigation lines and staked wire mesh baskets around transplants. Bottom – *P. cactorum* was baited from dead *Malosma laurina* (foreground) in natural vegetation downslope form the planting; note mesh baskets upslope.

Table 2. Recovery of *Phytophthora* species (pink highlight) from transplanted container nursery stock, and other vegetation as noted at Angeles National Forest restoration site Chaney Trails in 2016 through 2021. Note that often transplants were infected with multiple *Phytophthora* species. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling data (Bourret and Frankel 2018, Fajardo et al. 2019).

	***Phytophthora* positive samples/total samples *Phytophthora* species detected, # samples with species**			
	September 2016	**March 2018**	**May 2019**	**May 2021**
Transplants				
Quercus agrifolia	3/4 *P. cactorum, 2* *P. mediterranea*, 2* *P. niederhauserii, 1*	0/2	1/2 *P. mediterranea*,* 1	4/5 *P. cactorum, 2* *P. mediterranea*, 2* *P. nicotianae, 1* *P. quercetorum, 1*
Q. berberidifolia				0/3
*Eriogonum fasciculatum***	0/2			
Hydroseed or natural recruits at basin edge of *Phytophthora*-infected *Quercus agrifolia*				
Acmispon glaber, E. fasciculatum				1/1 *P. cactorum, 1*
Within planting - hydroseed or natural recruits				
A. glaber, E.fasciculatum, Hesperoyucca whipplei				0/1
Encelia farinosa, E. fasciculatum				0/1
Ericameria nauseosa			0/1	
Eriogonum			0/1	
Salvia mellifera		0/1		0/1
Upslope from planting – natural vegetation				
S. mellifera		1/1 *P. cryptogea* complex***, *1*		0/3
Bulk composite			0/1	
Downslope from planting - natural vegetation				
Malosma laurina				1/1 *P. cactorum*, 1
Q. wislizeni or hybrid			1/2 *P.* sp. *kelmania*, 1	1/1 *P. pseudocryptogea*, 1
Q. durata		0/1		
S. mellifera				0/1
Total samples	6	5	7	15

*Newly described species (Bregent et al . 2021), in our previous reports referred to as *Phytophthora* sp. nov. or hybrid – *P. cinnamomi / P. parvispora* group. Bregant et al. 2021 was unaware of its occurrence in California nursery stock when they first detected and subsequently named this species.
**salvaged from field, grown in nursery, then outplanted

***Collected by Rizzo lab, processed elsewhere and identified as *P. cryptogea* complex. Same spot sampled by Katie VinZant in 2017 and identified as *P.* sp. *kelmania* by Tyler Bourret based on the ITS sequence from CDFA Plant Pest Diagnostic lab.

Figure 2. Sampling locations and *Phytophthora* detections at Angeles National Forest restoration site Chaney trails / S11BR1 in 2016 through 2021. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling (Bourret and Frankel 2018, Fajardo et al. 2019). Coordinates for some Rizzo lab samples are approximate due to GPS failure.

HAY-1 / S6R022

HAY-1 is a small level site along Aliso Canyon Rd (Figure 3). Most of the planting sites are clustered and many planting sites have several closely spaced plants of different species. Dieback and recent mortality of several transplanted species was noted, particularly *Eriodictyon* and *Adenostoma fasciculatum*. *Phytophthora* species have been detected in each year of sampling on transplants in planting basins, with detections in 14 of 18 samples (78%) taken within planting basins (Table 3). *P. cactorum* and *P. nicotianae* were the most common species detected. *P. citrophthora* and *P. ×serendipita* have also been detected, the latter for the first time in the 2021 sampling. No detections

have been made from the six samples of natural and seeded plants between basins or adjacent to the planting (Table 3).

Table 3. Recovery of *Phytophthora* species (pink highlight) from transplanted container nursery stock and other vegetation as noted at Angeles National Forest restoration site HAY-1 in 2016 through 2021. Note that some plants were infected with more than one *Phytophthora* species. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling data (Bourret and Frankel 2018, Fajardo et al. 2019).

	***Phytophthora* positive samples/total samples** ***Phytophthora* species detected, # samples with species**			
	September 2016	**March 2018**	**May 2019**	**May 2021**
Transplants				
Adenostoma fasciculatum	1/2 *P. nicotianae*, 1	1/2 *P. cactorum*, 1		0/1
Eriodictyon crassifolia or tomentosum	3/3 *P. citrophthora*, 1 *P. nicotianae*, 2	1/1 *P. cactorum*, 1 *P. citrophthora*, 1	1/1 *P. cactorum*, 1	
Malacothamnus fasciculatus	1/1 *P. nicotianae*, 1			
Eriogonum fasciculatum + *A. fasciculatum*			1/1 *P. cactorum*, 1	1/1 *P. cactorum*, 1
E. fasciculatum + *A. fasciculatum* +*Eriodictyon*			1/1 *P. cactorum*, 1	1/1 *P. cactorum*, 1 *P. ×serendipita*, 1
Eriodictyon sp.+ *A. fasciculatum*		1/1 *P. nicotianae*, 1		0/1
Eriodictyon sp.+ *A. fasciculatum* + *Acmispon glaber*			1/1 *P. nicotianae*, 1	
Within planting				
Eriodictyon sp.				*0/1*
E. fasciculatum, Hesperoyucca whipplei				*0/1*
Bulk composite			0/1	
Upslope from planting				
A. fasciculatum	0/1		0/1	
Bulk composite			0/1	
Downslope from planting				
A. fasciculatum		0/1		
Total samples	7	5	7	6

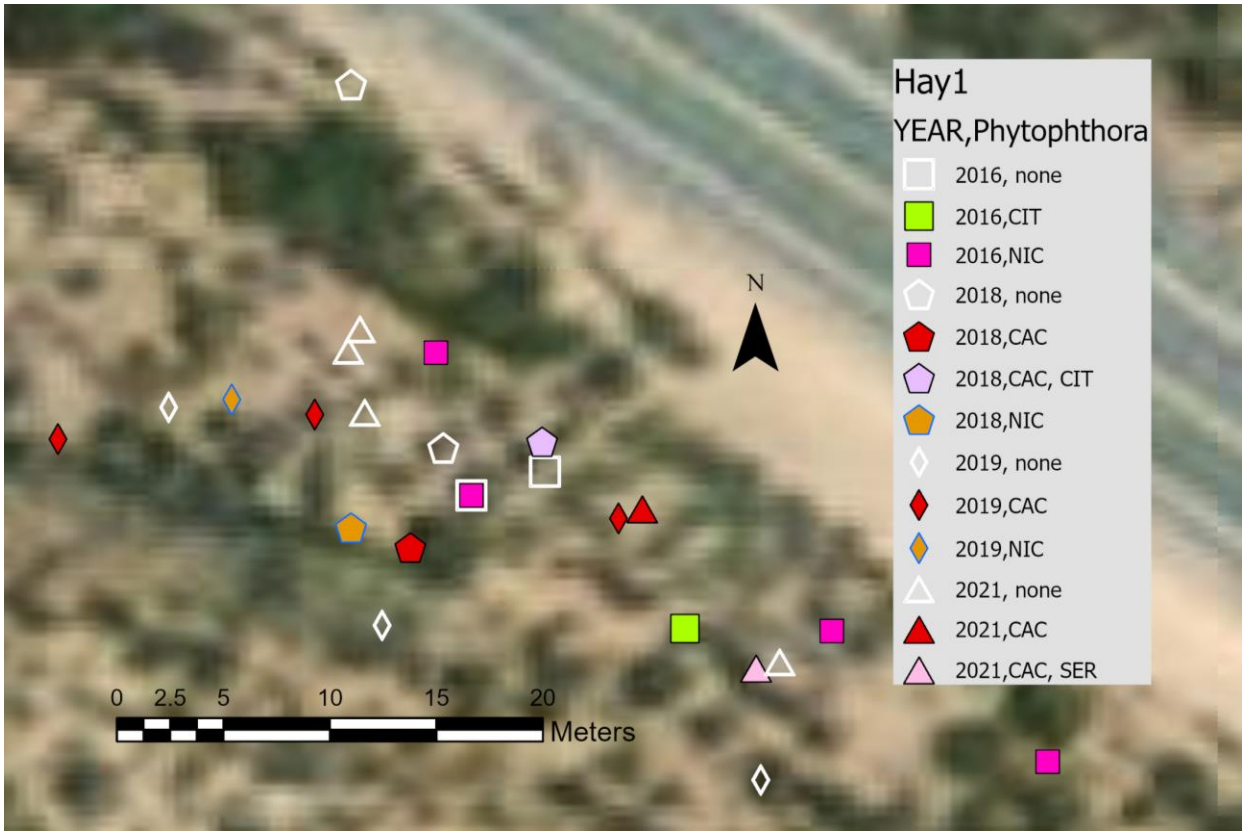

Figure 3. Sampling locations and *Phytophthora* detections at Angeles National Forest restoration site HAY-1 in 2016 through 2021. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling (Bourret and Frankel 2018, Fajardo et al. 2019). Coordinates for some Rizzo lab samples are approximate due to GPS failure.

WSS28 / S6R029

This is large sloped planting site with a natural stand of *Q. john-tuckeri* at the bottom of the slope. *Eriogonum fasciculatum* and *Eriodictyon* were common between the planting basins, presumably resulting from seed. At the time of our visit in May 2021, many planting sites were damp (Figure 4); irrigation had been applied within the last 24 hours before our visit. Most of the sites that had been irrigated were planted with oaks (*Q. john-tuckeri*), and many of these were recently dead or showed substantial foliar necrosis and dieback. Sites with older dead plants and blank sites were also common. *P. cactorum* had been baited from oak planting sites at this location in 2016, 2018, and 2019, and *P. quercetorum* was also detected in 2018 (Table 4, Figure 5).

In 2021, we detected *P. nicotianae* in one of four sampled *Q. john-tuckeri* planting basins. *P. nicotianae* was also baited from a recently-killed *Eriodictyon* on the edge of a planting basin, about 0.5 m from the *P. nicotianae*-positive oak (Figure 4). This detection indicates that some local spread had occurred away from the oak rootball, quite likely

facilitated by irrigation. Due to time limitations only two samples were taken from other symptomatic plants well beyond planting sites. *Phytophthora* was not detected in these two samples.

Figure 4. At site WSS28 in May 2021, we baited *Phytophthora nicotianae* from both the recently dead *Q. john-tuckeri* at the center of this planting basin and from the recently dead *Eriodictyon* located on the outer edge of the planting basin berm. Note the area of moist soil due to recent irrigation.

Table 4. Recovery of *Phytophthora* species (pink highlight) from transplanted container nursery stock and other vegetation as noted at Angeles National Forest restoration site WSS28 / S6R029 in 2016 through 2021. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling data (Bourret and Frankel 2018, Fajardo et al. 2019).

	***Phytophthora* positive samples/total samples** ***Phytophthora* species detected, # samples with species**			
	September 2016	**March 2018**	**May 2019**	**May 2021**
Transplants				
Quercus john-tuckeri	1/3 *P. cactorum*, 1			1/4 *P. nicotianae*, 1
Quercus sp.		2/3 *P. cactorum*, 1 *P. quercetorum*, 1		
Q. chrysolepis		0/1		
Blank oak site			1/2 *P. cactorum*, 1	
Eriogonum fasciculatum	0/1		0/1	
Within planting				
Eriodictyon sp.			0/1	0/2
Eriastrum + *Corethrogyne filaginifolia*			0/1	
Bulk composite			0/1	
Basin edge of *Quercus john-tuckeri* transplant *P. nicotianae* positive				
Eriodictyon sp.				1/1 *P. nicotianae*, 1
Upslope from planting				
Q. wislizeni		0/2		
Bulk composite			0/1	
Downslope from planting				
Q. wislizeni		0/1		
Bulk soil		0/1		
Total samples	**4**	**8**	**7**	**7**

Figure 5. Sampling locations and *Phytophthora* detections at Angeles National Forest restoration site WSS28 / S6R029 in 2016 through 2021. 2016 and 2021 = Phytosphere Research sampling. 2018 and 2019 = Rizzo lab sampling data (Bourret and Frankel 2018, Fajardo et al. 2019). Coordinates for some Rizzo lab samples are approximate due to GPS failure.

Pullsite 21(22) / S6R021

This is a large restoration site sloping downhill in two direction from a hilltop. Parts of the site have irrigation pipes but planting of the site was stopped early. With the exception of some cacti on the upslope side of the planting, only the lower slope had been planted. Many planting sites had recent or older dead *Q. john-tuckeri*.

One detection of *P. nicotianae* was made in 2017 sampling of this site conducted by Katie VinZant (Bourret and Frankel 2018). We have not located the coordinates of this sample. No *Phytophthora* detections were made at this site from a total of 12 samples collected by the Rizzo lab in 2018 and 2019. In May 2021, we sampled 4 dead *Q. john-tuckeri* and a somewhat wilted *Sambucus* transplant but did not detect *Phytophthora* by baiting (Table 5, Figure 6). We found that it was very difficult to get satisfactory samples at this site because the soil was very dry, hard, and exceptionally difficult to dig. Many of the plants at this site had either settled after planting or had been set quite deep in the soil originally, so it was not possible to dig deep enough to intercept the original container

rootball. Hence, the lack of *Phytophthora* detections at this site since 2017 may be related to the difficulty of obtaining good samples at this site rather than indicating that *Phytophthora* is absent.

Table 5. Recovery of *Phytophthora* species from transplanted container nursery stock at ANF restoration site Pullsite 21(22) (AF) in 2021. 2017=Katie VinZant sampling data. 2018 and 2019 = Rizzo lab sampling data (Bourret and Frankel 2018, Fajardo et al. 2019). 2021 = Phytosphere Research sampling.

	***Phytophthora* positive samples/total samples**			
	March 2017	**March 2018**	**May 2019**	**May 2021**
Transplants				
Unknown	*P. nicotianae*			
Quercus john-tuckeri		0/2	0/2	0/4
Sambucus nigra				0/1
Within planting				
Bulk soil		0/1		
Bulk composite			0/1	
Eriodictyon			0/1	
Eriogonum			0/1	
Prunus sp.			0/1	
Outside planting				
Adenostoma fasciculata		0/2		
Upslope from planting				
Bulk composite			0/1	
Total samples	unknown	5	7	5

Figure 6. Sampling locations at Angeles National Forest restoration site Pullsite 21(22) in 2016 through 2021. No *Phytophthora* species were detected from transplanted container nursery stock in sampling by the Rizzo lab (2018, 2019) (Bourret and Frankel 2018, Fajardo et al. 2019) or Phytosphere (2021), but *P. nicotianae* was detected in a 2017 sample collected by Katie VinZant (not plotted due to lack of coordinates). Coordinates for some Rizzo lab samples are approximate due to GPS failure.

Phytophthora species detected in southern Californian nurseries

In 2016, we sampled three southern California restoration plant nurseries that had current and or past batches of plants slated for planting in ANF sites (Swiecki and Bernhardt 2016). We were not allowed to sample at one nursery that supplied most of the transplants for the restoration plantings in Tables 2-5. *P. nicotianae* was detected at all 3 nurseries and *P. cactorum* was detected at 2 nurseries. *P. niederhauserii,* and *P*. sp. *kelmania* (previously reported as *P. cryptogea* complex) were detected at one nursery each. In tests conducted in 2017 and 2019 (Swiecki and Bernhardt 2019) at another nursery on plants that were to be used in restoration plantings on ANF lands, *P. cactorum, P. citrophthora, P. nicotianae, P. niederhauserii,* and *P. quercetorum* were also detected.

As shown in Table 6, all of the species detected in these limited tests of four restoration nurseries were also found in samples collected at the ANF restoration planting sites.

Table 6. *Phytophthora* detections and associated plant host species from testing at four southern California restoration nurseries and in the field sampling reported here. Yellow highlighting indicates host-pathogen combinations observed in basin-edge spread samples. Orange highlighting indicates host-pathogen combinations observed in samples collected beyond plantings.

***Phytophthora* species detected**	**Associated plant species in nursery testing**	**Associated plant species in field samples of nursery transplants or other vegetation (highlighting)**
P. cactorum	*Quercus agrifolia* *Quercus berberidifolia*	*Adenostoma fasciculatum* *Eriodictyon* sp. *Quercus agrifolia* *Quercus john-tuckeri* *Eriogonum fasciculatum* + other spp *Malosma laurina*
P. x serendipita		*Eriogonum fasciculatum* + other spp
P. citrophthora	*Quercus agrifolia* *Sambucus nigra* ssp. *caerulea*	*Eriodictyon* sp.
P. sp. *kelmania*	*Eriogonum fasciculatum* + *Salvia mellifera*	*Quercus wislizeni* *Salvia mellifera*
P. pseudocryptogea		*Quercus wislizeni*
P. mediterranea		*Quercus agrifolia*
P. nicotianae	*Sambucus nigra ssp. caerulea* *Salvia mellifera* *Quercus berberidifolia*	*Adenostoma fasciculatum* *Eriogonum fasciculatum* + other spp *Eriodictyon* sp. *Eriodictyon* sp. *Malacothamnus fasciculatus* *Quercus john-tuckeri*
P. niederhauserii	*Quercus agrifolia* *Salvia mellifera* *Cercocarpus betuloides*	*Quercus agrifolia*
P. quercetorum	*Quercus agrifolia* *Quercus wislizeni*	*Quercus agrifolia* *Quercus* sp.

Discussion

In 2021, we detected one or more *Phytophthora* species in each of the locations we originally sampled in 2016, indicating that various *Phytophthora* species have persisted at these sites for more than five years. Furthermore, several samples indicate that *Phytophthora* species introduced in nursery stock have spread beyond the original infested nursery stock rootballs in some areas. Short range spread of up to about 1 m was documented at or beyond the edges of some planting basins where plant species other than the original nursery plant hoist were affected. This is not surprising, given that many of the *Phytophthora* species detected have wide host ranges and are widely adapted pathogens.

However, we did not expect to see evidence of native plant mortality related to long range spread (many meters) from the plantings at such a short interval since planting, especially in a dry site like Chaney Trails. This indicates that significant mobilization of inoculum from the planting occurred early on, since mortality of large woody plants like *Malosma laurina* typically does not occur within a single year. The evidence of both persistence and spread of *Phytophthora* from infected nursery stock shows that this route of pathogen introduction can function even in dry southern California habitats.

The *Phytophthora* species detected at the various sites differ in some cases over the years of sampling. This result was expected given that few individual sites were resampled. Given that only a small number of unique plants were sampled each year, random fluctuations in the species recovered each year at each location can be expected if a diverse mix of *Phytophthora* species was present in the original planting material. With only a few exceptions, we did not resample previously sampled planting sites in 2021 because most or all of the rootball of the original nursery stock was removed in the sampling process. Previously sampled sites that have not had any live host material for multiple years tend to have low inoculum levels and a low likelihood of *Phytophthora* detection.

Although we were not allowed to conduct testing at the main nursery that contributed plants to the sampled restoration sites, all six *Phytophthora* species detected in limited sampling at three southern California nurseries in 2016 and an additional nursery in 2018 and 2019 were also found in transplanted material at one or more of the field sites (Table 6). *P. mediterranea* was found only on *Q. agrifolia* transplants at Chaney Trails and *P. ×serendipita* was only found in a mixed set of transplants (*Eriogonum fasciculatum , Adenostoma fasciculatum, Eriodictyon* sp.) at HAY-1. Although these species were not detected in our 2016 nursery sampling, they are is known to occur in the nursery trade (Bregant et al. 2021, Parke et al. 2014).

There are limited options for dealing with *Phytophthora* introductions in wildlands once the introduced species become widespread. Sampling to date provides evidence that some spread of introduced *Phytophthora* spp. from infested planting basins has occurred at Chaney Trails and HAY-1, but does not indicate that all of the introduced species are widespread at present. Therefore, measures to minimize the risk of further spread should include actions to minimize primary spread from infested planting basins. Rogueing of dead and dying transplants, taking care to remove the remove the rootball and associated soil without spreading contamination, would help eliminate some active sources of sporulation in the planting. We observed that the original planting sites were still being irrigated at one location during our 2021 visit. We recommend that irrigation be discontinued at all of the original planting sites to reduce the reproduction and movement of these destructive plant pathogens into surrounding native vegetation. Any additional use of transplants at these locations should use only nursery stock grown in full compliance with the Phytophthora Working Group's nursery *Phytophthora* Best Management Practices (BMPs) (Swiecki et al. 2021). To ensure compliance, nurseries providing plant material should be accredited by the AIR program (Swiecki et al. 2021) and stock should be tested by the FS or a qualified third party prior to acceptance for planting. In addition, all personnel accessing these sites should be informed of the infestations and should follow appropriate phytosanitary BMPs to minimize inadvertent pathogen spread.

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