Phytophthora species and their associations with Chaparral vegetation in Southern California

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ABSTRACT

Invasive *Phytophthora* species can potentially be destabilizing to whole ecosystems with detrimental effects on biodiversity and on ecosystem services. Studies have shown that *Phytophthora* species may be introduced into natural areas through outplanting of infested native plant nursery stock. The Angeles National Forest (ANF), located in Southern California, utilizes thousands of nursery-grown native plants for landscape restoration of heavily disturbed areas. Previous pathogen testing performed on ANF restoration sites detected several *Phytophthora* species. Little is known about the ecology and biology of *Phytophthora* species in drier regions of the world, thus a baseline of *Phytophthora* distribution and diversity is needed in ANF lands. Between 2018-2021 forty sites were selected, and soil samples were taken from plant rhizospheres, riverbeds and off-road vehicle tracks in chaparral and oak woodland areas. From these surveys, fourteen species of *Phytophthora* were detected, including three undescribed species and one hybrid species. *Phytophthora* species were found in both chaparral and oak woodland areas with a higher frequency in riparian areas. Selfing species (homothallic), capable of readily producing oospores, were more abundant in drier chaparral areas. *Phytophthora* species were also detected in off-road tracks, dirt trails, and riverbeds, indicating potential natural and anthropogenic-associated routes of dispersal. Pathogenicity tests were conducted to test the

aggressiveness of detected *Phytophthora* species towards common chaparral plant species. *Phytophthora cactorum*, *P. multivora*, *P. crassamura* and *P. chlamydospora,* were all capable of causing disease on *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Salvia mellifera* and *Eriodictyon crassifolium*. *A. fasciculatum* was determined to be the most susceptible plant species, especially to *P. multivora* and *P. cactorum.* Although the Angeles National Forest is among the driest and most fire prone areas in the United States, these Mediterranean areas harbor a large diversity of *Phytophthora* species indicating a potential risk for the native and endemic Californian chaparral vegetation. The long-term consequences of the presence of *Phytophthora* species in these locations still needs to be understood.

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CHAPTER ONE

Phytophthora surveys of natural areas in Southern California reveal fourteen species associated with Mediterranean chaparral vegetation

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ABSTRACT

In forests and other natural ecosystems, invasive *Phytophthora* species can be destabilizing with detrimental effects on biodiversity and on ecosystem services. *Phytophthora* species may be introduced into natural areas through outplanting of infested native plant nursery stock for restoration. The Angeles National Forest (ANF) utilizes thousands of nursery-grown native plants for landscape restoration of disturbed areas. Previous surveys performed in 2017 in ANF restoration sites detected several *Phytophthora* species. From 2018-2021, baseline *Phytophthora* distribution data was collected on ANF lands to improve restoration outcomes. Surveys were performed on the ANF in Southern California (Los Angeles County), to assess *Phytophthora* species distribution in areas selected for potential restoration. Forty sites were selected, and soil samples taken from plant rhizospheres, riverbeds and off-road vehicle tracks in chaparral and oak woodland areas. From these surveys, fourteen species of *Phytophthora* were detected, including three undescribed species and one hybrid

species: *P. cactorum* (clade 1a), *P. multivora* (clade 2c), *P. inundata* and *P. rosacearum* (clade 6a), *P. crassamura, P. gonapodyides*, *P. lacustris, P. lacustris* x *riparia*, *P. megasperma*, *P*. sp. 'NJB-2015' and *P. riparia* (clade 6b), *P. chlamydospora* (clade 6c), *P*. sp. 'cadmea' (clade 7a) and *P*. taxon 'agrifolia' II (clade 8e). *Phytophthora* species detected in rhizosphere soil were found underneath symptomatic and asymptomatic plants corresponding to fourteen different genera, most frequently associated with *Salvia mellifera*, *Quercus agrifolia* (coast live oak) and *Salix* spp. *Phytophthora* species were found in both chaparral and oak woodland areas primarily in riparian areas. *Phytophthora* species were also detected in off-road tracks, trails, and riverbeds, indicating potential natural and anthropogenic-associated routes of dispersal. Several of the detected *Phytophthora* species have been historically associated with urban gardens, agriculture, restoration areas and nurseries in California. Although these Mediterranean ecosystems on the Angeles National Forest are amongst the driest and most fire prone areas in the United States, they harbor a large diversity of *Phytophthora* species, indicating a potential risk for disease for the native and endemic Californian chaparral vegetation. The long-term consequences of the presence of *Phytophthora* species still needs to be determined.

INTRODUCTION

The oomycete genus *Phytophthora*, family Peronosporaceae and kingdom Straminipila, (Beakes et al. 2014) are amongst the most highly damaging plant pathogens in the world (Hyun and Choi, 2014, Davidson, 2014, Ivanov et al. 2021, Perrine-Walker, 2020). In forest and natural ecosystems, invasive *Phytophthora* species can destabilize ecosystems with detrimental effects on biodiversity and on ecosystem services (Jung et

al. 2016, Boyd et al. 2013, Underwood et al. 2018, Brasier and Jung 2005, Grünwald et al. 2019, Greslebin et al. 2005). Since its detection in the mid-1990s in Northern California (Rizzo et al. 2002), *P. ramorum,* the causal agent of sudden oak death, has expanded its distribution, covering more than 2,229 km^2 and 12.3 km^2 in California and Oregon, respectively, with an estimated death toll of over 50 million trees (Cobb et al. 2020, Grünwald et al. 2019). Western Australia has been severely affected by the "biological bulldozer" *P. cinnamomi*, which is capable of infecting 40% of the 5,710 species of the native flora (Shearer et al. 2004, Burgess et al. 2017). The number of devastating tree and shrub diseases caused by invasive *Phytophthora* species in natural ecosystems in Australia, Europe, and North America has increased exponentially since the 1960s (Jung et. al 2020). The exponential increase in risk of introduction and spread of *Phytophthora* species into native habitats has been associated with a rapid increase in ecosystem disturbance interfaced with population growth and expanded plant cultivation and trade (Brasier 2008, Santini et al. 2013).

Studies have demonstrated that a primary route of unintended introduction of *Phytophthora* species into natural areas is through inadvertent outplanting of infested nursery stock (Jung at al. 2016; Molnar et al. 2020; Parke et al. 2019) with many recorded cases on native plants across diverse ecosystems in California (Bourret, et al., 2018, Garbelotto et al. 2018, Rooney-Latham, et al. 2019, Sims et al., 2019, Swiecki et al. 2018, Swiecki et al. 2021). Restoration projects historically have relied on native plant container nursey stock as a primary source for materials. Many nurseries in California specialize in providing stock for restoration (CALSCAPE, https://calscape.org/plant_nursery.php). However, pathogen surveys in nurseries have

demonstrated that *Phytophthora* species are highly prevalent in horticultural settings, especially when sanitation best management practices (BMPs) are not correctly implemented (Bienapfl and Balci 2014; Ferguson and Jeffers 1999; Guarnaccia et al. 2021; Hardy and Sivasithamparam 1988; Molnar et al. 2020). *P. cactorum*, *P. crassamura*, *P. roseacearum* and *P. xcambivora* are the most frequently detected *Phytophthora* species in Californian restoration areas, with *P. cactorum* amongst the most common in horticultural and forest settings across the state (Bourret et al., 2022).

The risk of introduction is further increasing due to intensifying restoration efforts in response to more wide-spread land degradation due to increased fire frequency. In California, chaparral plant communities account for 9% of the vegetation landcover, with a distribution that extends from the lower elevations of the coastal ranges and western slopes of the Sierra Nevada to the Transverse and Peninsular ranges in the southern part of the state (Parker et al. 2016). Chaparral is comprised of drought-tolerant evergreen sclerophyllous shrubs, with *Adenostoma* spp. (chamise and redshanks, Rosaceae), *Arctostaphylos* spp. (manzanitas, Ericaceae) and *Ceanothus* spp. (Rhamnaceae) as common representatives (Keeley and Zedler, 2009, Kolb and Davis, 1995, Barro and Conrad, 1990). These species are prone to frequent wildfires but have evolved traits that allow for disturbance resiliency through resprouting, germination from persistent seed banks, generation of deep tap roots and other traits (Rapacciuolo 2014, Wolf 2016, Pratt et al., 2007). However, in southern California fire occurrences have increased since the 1930s with decreasing fire return intervals that have caused some chaparral areas to convert to grasslands (Safford 2014, Syphard et al 2008). Fine fuels derived from non-native annual grasses (*Bromus* spp. and *Avena* spp.) can increase the

severity of fires, disproportionally affecting pre-fire shrub dominants like *A. fasciculatum* (Keeley et al. 2005). Alterations to fire regimes can also cause indirect effects on carbon sequestration rates and nitrogen cycling, having consequences in species compositions, soil acidification and eutrophication (Fenn et al. 2003, Rao et al. 2010). Chaparral landscapes severely degraded by fires and/or anthropogenic disturbances tend to recover slowly or are unable to recover completely (Allen et al. 2000, Keeley et al., 2005b). To ameliorate these disturbances, restoration activities are carried out in various landscapes. Herbicide application, weeding, outplanting, seeding or other treatments are used to restore impacted areas (Allen et al. 2000).

These restoration efforts are particularly important because California is one of the five main Mediterranean climate regions in the world, together with the European and north African Mediterranean Basin, southern and southwestern Australia, Cape Province of South Africa, central coastal Chile and Southern California-US and Baja California-Mexico (Allen-Diaz, 2000, Klausmeyer and Shaw, 2009, Myers et al., 2000, Rosenzweig, 1995, Veblen et al., 2007). These areas are biodiversity hot spots of endemic flora and fauna, containing about one sixth of the vascular plant species in the world in just 2.2% of the world's land area (Rundel 2018, Medail and Quezel, 1999, Cowling et al., 1996). Mediterranean climates support vegetation that provides important ecosystem services such as climate amelioration, flood and erosion control, nutrient cycling, carbon sequestration, wildlife habitat, and spiritual renewal. Furthermore, these areas are culturally significant to local indigenous people (Millennium ecosystem assessment 2005).

The Angeles National Forest (ANF), in Southern California, USA utilizes thousands of nursery-grown container native plants for restoration of areas disturbed by special uses, fire and recreation activities (Myer et al., 2021). Previous pathogen testing has shown plants being grown in southern California nurseries and outplanted at ANF restoration sites were contaminated with *Phytophthora* species (Swiecki and Bernhardt, 2017., Fajardo et al. 2020). Baseline pathogen distribution data is needed on ANF lands to characterize *Phytophthora* distribution in different ecosystems and thereby improve the ability to predict harmful invasions (Vitousek et al. 2010). Prompted by the concern of unintentional introduction of *Phytophthora* species into natural areas, a collaborative project between United States Forest Service (USFS) ANF & Pacific Southwest Research Station (PSW), and University California Davis, funded by the National Fish and Wildlife Foundation (NFWF), was initiated with a primary objective to determine patterns of distribution and diversity of *Phytophthora* species on ANF lands that had previously burned and were being considered for restoration. Thus, it was proposed as the main objective to determine the diversity and distribution of *Phytophthora* species present on ANF lands. This study presents the main outcomes of this survey and discusses the potential risks associated with the presence of *Phytophthora* species in these arid lands of Southern California.

MATERIALS AND METHODS

Study area and Sampling

The Angeles National Forest (ANF) is a 283,280 ha. protected area spanning the San Gabriel and Pelona Mountains managed by the United States Forest Service in Los Angeles County, USA (34°32′N, 118°30′W). The primary vegetation types on the ANF

are chamise chaparral and coastal sage scrub, dominated by shrub species including A*. fasciculatum, Eriogonum fasciculatum, Eriodictyon crassifolium, Salvia mellifera***,** which are often surrounded by native and exotic grasses (Figure 1.1.A., B and C). In addition, larger shrubs can be found in this vegetation type, like *Heteromeles arbutifolia* (toyon), *Rhus integrifolia* (lemonade berry) and *Prunus ilicifolia* (hollyleaf cherry). In areas adjacent to natural waterways or rivers *Quercus agrifolia* and the endangered *Berberis nevinii* (Nevin's barberry) grow together with other riparian plants such as *Populus fremontii* (Fremont's cottonwood), *Baccharis salicifolia* (mule fat) and other *Salix* species (Figure 1.1.D). ANF is characterized by having a Mediterranean climate with average temperatures ranging from 8-20 ºC in winter to 15-33 ºC during the dry summer months with a yearly average precipitation of 566 mm. ANF elevations range from 365.76 m to 3067.5 m a.s.l. with Mt. Baldy Notch among the areas with highest elevations where average yearly snowfall is 334 cm.

Three areas across the ANF were evaluated in this study (Figure 1.2): (1) the Northwestern area (NWA), characterized by montane chaparral, (2) the Northeastern area (NEA) where oak woodlands and chamise chaparral dominate, and the (3) Southwestern area (SWA), predominantly chaparral. Recent wildfires in these three areas, including the Copper (2002), Ranch (2006) and Sayre (2008) fires, have affected over 16,187 ha (USFS and NWFW, 2016). Restoration of vegetation and wildlife habitat in these fire scars are the primary goals of the NFWF and USFS. Twenty-eight sites were selected based on USFS criteria used for designation of potential restoration activities. Twelve additional sites were added to the surveyed area to broaden the scope of the study and better represent chaparral and oak woodlands.

Figure 1.1. Examples of areas of the Angeles National Forest from which soil samples were taken to determine the presence of *Phytophthora* species. These include transition areas between chaparral and oak woodland areas (A), montane chaparral areas (B), rip riparian areas with dry and wet riverbeds (C) and oak woodlands (D). *Adenostoma fasciculatum, Eriodictyon crassifolium, Salvia mellifera* and *Quercus agrifolia* were amongst the most sampled native plant species in these areas.

Land disturbances were observed amongst the three areas. Although the presence of barriers and signage of prohibition of off-road vehicle use, vehicles tracks were observed, passing through grasslands and oak woodlands with several going through stream beds. Fencing was also put up to reduce grazing, however evidence of cattle intrusion was observed in many areas. Construction debris and soil movement was also observed in areas where powerlines were erected. Sites were surveyed for *Phytophthora* spp. over five field visits: May 2018, December 2018, March 2019, March

2020, and May 2021. Sampling in the NWA was discontinued in the last three field surveys as requested by the funder due to administrative changes.

Of the NEA sites, two had been outplanted with restoration nursery stock prior to 2018, and three had initiated restoration activities in 2019. Additionally, one restoration site in the SWA and the NWA were sampled. Some sites were repeatedly sampled during the field visits for a total of 576 soil samples. Digital elevation models and flowlines for perennial and ephemeral streams were obtained from the United States Geological Services (USGS) databases, fire perimeters from the Fire and Resource Assessment Program (FRAP) and road and trails from a USFS database.

At each site, 7 to 10 soil samples were taken either from plant-associated soil (associated rhizosphere soil), or bulk soil (from off-road vehicle trails, river basins, or bare-ground). Selected plant species were based on their dominance in the site. Sampled plant individuals were selected if they presented typical *Phytophthora*-associated symptoms (dieback or chlorosis). Healthy individuals were also sampled to determine the possibility of asymptomatic phenotypes despite the presence of *Phytophthora* species. Each sample consisted of approximately 1000 ml of soil collected between 1-30 cm below the soil line (Jung et al 2009). Roots were collected if present. For each sample GPS location, vegetation class context (chaparral or oak woodland), sample category (upland or stream bed) and plant health were recorded. Plant health was assessed for all sampled trees and shrubs by a general rating, ranging from 1 (healthy), 2 (50% or less affected canopy), 3 (75% of canopy affected), and 4 (dead). Samples were maintained in sealed, 1-gallon plastic bags in coolers with ice packs, for transport to the Rizzo laboratory, UC Davis for processing.

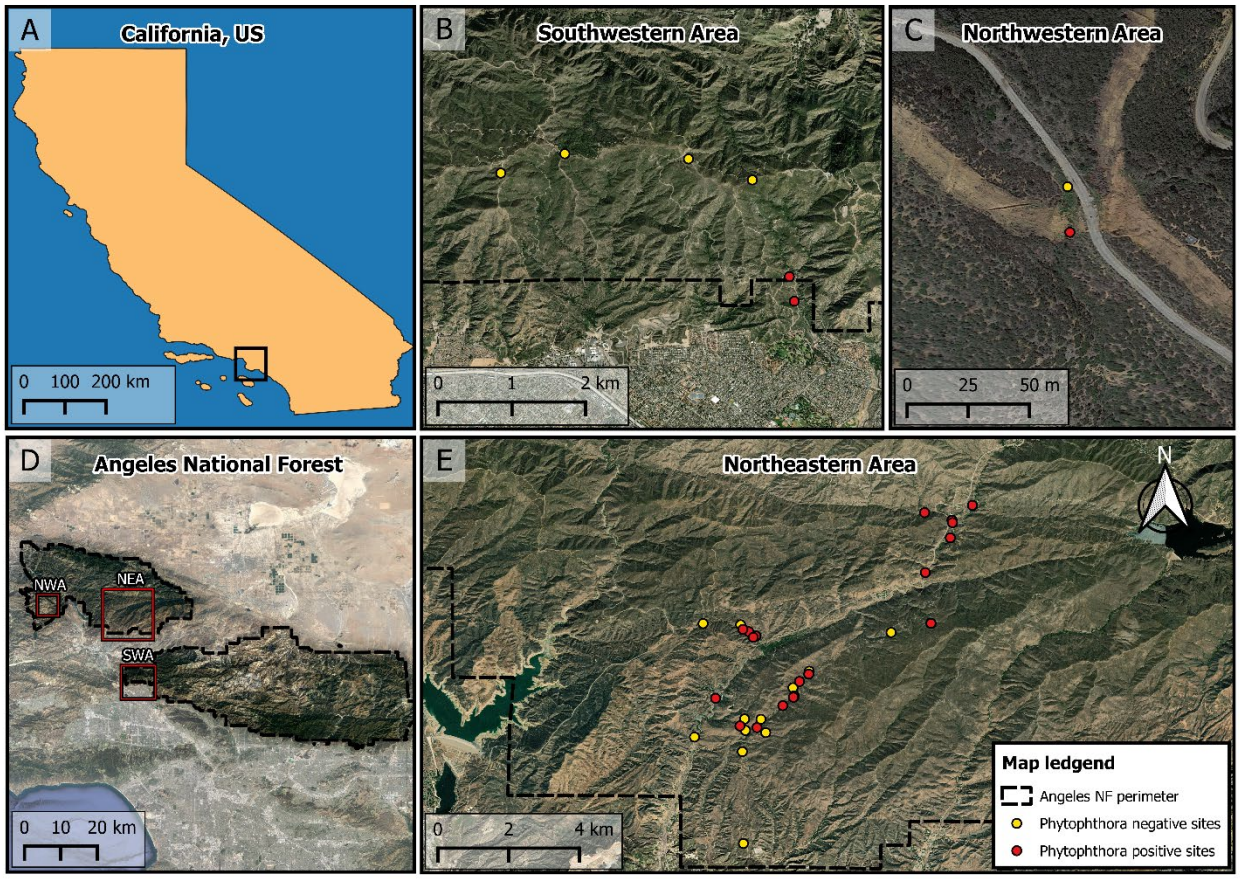

Figure 1.2. Location of the 40 sites sampled in the Angeles National Forest. Three main areas were sampled, Southwestern (SWA, B) six sites, Northwestern (NWA, C) two sites, and the Northeastern (NEA, E) with 32 sites. Red dots indicate Phytophthora positive sites, and yellow indicate *Phytophthora* negative sites (B and D). Site geographical coordinates are listed in table 1.

Baiting, isolation, identification, and isolate storage

Phytophthora species baiting was done according to Erwin and Ribeiro (1996). In each soil sample bag, a depression was made in the soil and a green D'Anjou pear was placed in the depression with pedicel upwards. Double-distilled water (ddH_2O) was added to the sample bags until the water level was in contact with the pear fruit, about 3

cm above the soil surface. A single rhododendron leaf from a confirmed pathogen-free greenhouse plant was placed into the bag. Baited soils were maintained at 20-22 °C with indirect natural light for 6-7 days. Once lesions appeared, pears were removed from the baiting bag and isolations made from lesions. Pear lesions were individually excised and submerged in CMA-PARP (modified from Erwin & Ribeiro [1996]: 15 g/l corn meal agar, 0.025 g/l pentachloronitrobenzene, 0.25 g/l ampicillin, 0.01 g/l rifampicin, 0.01 g/l pimaricin). For rhododendron baits, leaves were surface sterilized for 1 min in 5% bleach and rinsed with ddH_2O. Twelve to fifteen 0.5 cm diameter leaf discs were excised from leaves and plated on CMA-PARP. Isolation plates were incubated in the dark at 18 °C for two weeks and checked periodically for growth.

Phytophthora-like growth from pear lesions and rhododendron leaf disks were subcultured onto CMA-PARP and maintained in the dark at 20 °C. Isolates were morphotyped based on cultural morphology. Indistinguishable isolates from the same sample were “dereplicated” and a representative isolate selected.

Clean growing strains were transferred to a sterile 1 ml pea broth solution and grown at 20 °C for at least 48 hours for DNA extraction (Bourret et al. 2018). A tuft of growing mycelium from the pea broth vials was removed with a sterile needle and placed in 100 µl of PrepMan Ultra kit (Thermo Fisher Scientific, Waltham MA, USA) and DNA extracted according to manufacturer's instructions. The oomycete-specific primer pair FRiz + ITS4TT was used to amplify the internal transcribed spacer (ITS) locus of the isolates (Bourret et al. 2018), and amplification success was checked with agarose gel electrophoresis. Amplifications were carried out in a Eppendorf PCR thermocycler (Eppendorf AG, Hamburg, Germany) with initial denaturation at 94 °C for 4 min,

followed by 35 cycles of 94 °C for 30 sec, 57 °C for 30 sec, and 72 °C for 30 sec and final extension at 72 °C for 10 min. Amplifications consisted of 25-µl reactions containing 0.02 Unit AmpliTaq Taq (Thermo fisher, Waltham, MA, USA), 10X AmpliTaq Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 µg BSA and ~50 ng/µ 1 µl of DNA template. Polymerase chain reaction (PCR) products were prepared for Sanger sequencing with Exo-SAP IT (Thermo Fisher, Waltham, MA, USA) according to manufacturer's instructions, and sequencing performed with PCR primers by the UC Davis College of Biological Sciences, DNA Sequencing Facility. Contigs and finished sequences were formed from the sequencing runs as described by Bourret et al. (2018).

ITS sequences were compared against those available in the GenBank nucleotide collection using BLAST searches. Positive determinations were made based on 100% matches to sequences from strains of verifiable identity (Abad et al., 2019). If an ITS haplotype differed from verifiable strains by a 1-2 base pair (bp) mismatch (either substitutions or indels), distance trees produced by BLAST searches were consulted to assess whether strains with this haplotype could be determined to the closest matching species, or more study was needed. For storage of representative isolates up to 3 years, six to eight 0.5 cm^3 mycelia cubes were transferred from pure culture 1/3 strength clarified V8 agar to duplicate sterile vials containing 1 ml water; the vials were stored at 14 °C and 20 °C.

RESULTS

Phytophthora species incidence

Out of the 40 sites sampled, *Phytophthora* species were detected at twenty-two sites, encompassing all three areas. Out of the 576 samples, 73 samples were positive for

Phytophthora (~12% positivity rate) (full *Phytophthora* species positive data table is displayed in supplementary Table S1.1), 317 samples were positive for *Pythium* spp. *sensu lato* (including *Globisporangium* spp., *Pythium* spp. *s.s.*), 77 for *Phytopythium* spp. and 199 samples were negative for oomycetes. Positive *Phytophthora* samples were collected from 475 m to 1011 m above sea level, and across 5 survey dates with a 5.3% *Phytophthora* positivity rate out of 56 samples in May 2018, 6.2% of 161 samples in December 2018, 18% of 199 in March 2019, 16% of 53 in March 2020, and 14% of 107 (14%) during May 2021. GenBank accessions numbers of ITS sequences of representative isolates of all fourteen species of *Phytophthora* and additional *P. cactorum* ITS haplotypes are given in Table 1.

Phytophthora *community composition.*

From the 73 *Phytophthora*-positive samples, 90 *Phytophthora* isolates were obtained. The most frequently detected *Phytophthora* species were in clade 6b, *P. gonapodyides* (twenty-four isolates matching strain P-1905, Aram and Rizzo, 2019, Gen Bank accession n° MK908981.1), followed by *P. crassamura* (fourteen isolates, strain CPHST BL 151 Gen bank accession n° MG865482.1), and *P. riparia* (Ten isolates, differed in 1bp to strain CPHST ex-type BL 111, MG865583.1) (Table 1.1 and Figure 1.3). In lesser frequency, two ITS haplotypes of *P. cactorum* (clade 1a) were detected. Two *P. cactorum* isolates (CAC1) matched CBS voucher isolate 108.09, Gen bank accession N°KJ128036.1. The other three *P. cactorum* isolates (CAC3) differed in 2 bp from CAC1, with two polymorphisms in positions 616 and 717 (Table S1.2). All seven isolates of *P. multivora,* (clade 2c), differed in 1bp strain Ex-type CPHST BL 104. The clade with most representatives was clade 6, with *P. inundata* (7 isolates, strain CPHST

BL 130, MG865517.1) and *P. rosacearum* (three isolates, diffed in two pb WPC:3315A1962, GU259045.1), from clade 6a, *P. lacustris* (six isolates, missing reference type)*, P. lacustris* x *riparia* (two isolates, strain SM15APR_ARG, MG696509.1, Bourret et al. 2019) and *P. megasperma* (three isolates, strain WPC: 10838A1072, GU258767.1) from clade 6b, and, finally, *P. chlamydospora* (two isolates, strain Ex-type CPHST BL 156, MG865471.1) from clade 6c. Three undescribed *Phytophthora* species were also detected, *P*. sp. 'NJB-2015' (four isolates, strain SM08FEB_FLZ1, MG696351.1) from clade 6b, *P*. sp. 'cadmea'(Two isolates, strain SCVWD235, MG707801.1) from clade 7a and finally a single record was made of *P*. taxon 'agrifolia' II (clade 8e). Out of the fourteen detected *Phytophthora* species, thirteen of them were detected in the sampled sites in the NEA, with the sole exception of *P. multivora* from the SWA. *P. multivora* was only detected in the SWA, together with *P. cactorum*, *P. crassamura* and *P. gonapodyides*. Only two *Phytophthora* positive samples were recorded for the NWA, with both samples positive for *P. crassamura*.

Twenty-two different plant species were sampled from fourteen different plant families in oak woodland areas, primarily *B. salicifolia* (Asteraceae), *Q. agrifolia* and *Populus fremontii* (Salicaceae). Less intensive sampling occurred from *Diplacus auricantus* (Sticky monkey flower, Phrymaceae) and *Hazardia squarrosa* (sawtooth goldenbush, Asteraceae). Out of the 144 samples taken from upland oak woodland areas, 13% were positive for *Phytophthora* species with *P. cactorum, P. crassamura, P. gonapodyides, P. lacustris, P. lacustris x riparia, P. megasperma, P. riparia*, *P*. sp. *cadmea*, and P. sp. Njb-2015. The most common *Phytophthora*-positive associated-hosts were *Q. agrifolia*, from which *P. cactorum*, *P. crassamura*, *P. gonapodyides* and *P. riparia* were found in

the rhizosphere soil, as well as *P. lacustris*, *P. lacustris x riparia* and *P*. sp. NJ-2015 which were detected underneath *Salix* sp. individuals. These clade 6b *Phytophthora* species were also found present in rhizosphere soil of grasslands and from a *B. salicifolia* with mild crown thinning.

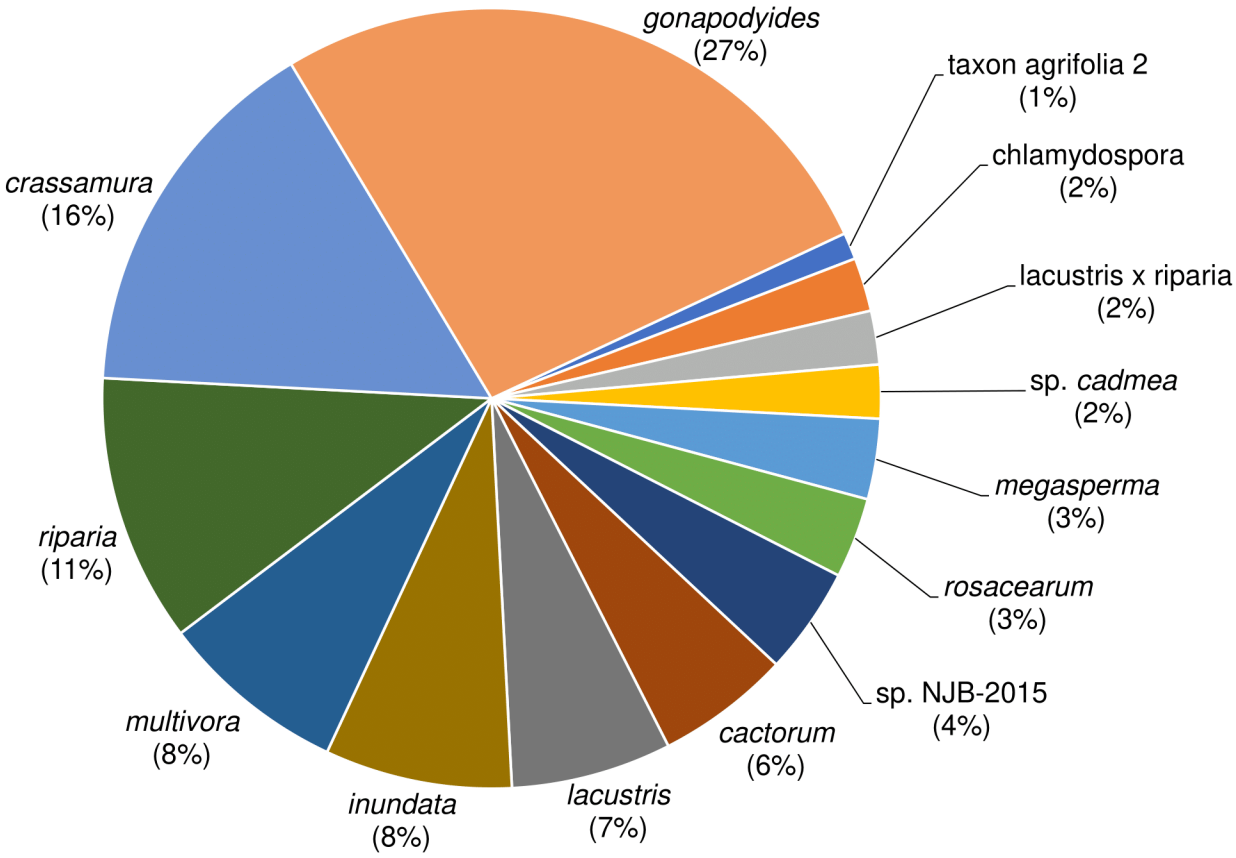

Figure 1.3. Diversity and frequency of *Phytophthora* taxa isolated from surveys of burnt areas of Angeles National Forest during 2018 through 2021. Upland areas and streams beds were sampled in chaparral and oak woodlands including restoration areas. Multiple isolates of a *Phytophthora* taxon from the same sample were considered as one record.

Table 1.1. *Phytophthora* species isolated from the Angeles National Forest, including sample source or host, sample type and a representative isolate of each *Phytophthora* species with its corresponding Genbank accession number.

ANF area	Vegetation type	Type of sample	ITS Clade	*Phytophthora* species	No. of isolates	Representative isolate	Sample source/host species
NEA	Oak woodland	Rhizosphere	1a	*cactorum (CAC3)*[1]	3	LA123_R1	*Quercus agrifolia*, *Hazardia squarrosa*
NEA, SWA	Chaparral	Rhizosphere	1a	*cactorum (CAC1)*[2]	2	LA266_L3	*Salix sp.*, *Eriodictyon crassifolium*
NEA, SWA	Chaparral	Bulk	6c	*chlamydospora*[3]	2	LA340_L5	Riverbed
NEA, SWA, NWA	Chaparral, Oak woodland	Rhizosphere, Bulk	6b	*crassamura*[4]	14	LA196_L4	*Artemisia californica*, *B. salicifolia*, Grass[16], OHV tracks[17], *Q. agrifolia*, *Salvia mellifera*, Riverbed[18],
NEA, SWA	Chaparral, Oak woodland	Rhizosphere, Bulk	6b	*gonapodyides*[5]	24	LA140_L5	*Adenostoma fasciculatum*, *B. salicifolia*, Grass, OHV tracks, *Populus fremontii*, *Q. agrifolia*, Riverbed
NEA	Chaparral, Oak woodland	Rhizosphere, Bulk	6a	*inundata*[6]	7	LA316_L3	*B. salicifolia*, Riverbed,
NEA	Chaparral, Oak woodland	Bulk	6b	*lacustris*[7]	6	LA330_L1	Riverbed, *Salix* sp.
NEA	Oak woodland	Rhizosphere, Bulk	6b	*lacustris x riparia*[8]	2	LA140_L2	*B. salicifolia*, Riverbed

SWA	Chaparral	Rhizosphere, Bulk	2c	*multivora*[10]	7	LA377_R1	*E. crassifolium*, *Eriogonum fasciculatum*, Riverbed, *S. mellifera*, *Toxicodendron diversilobum*
NEA	Oak woodland	Rhizosphere, Bulk	6b	*riparia*[11]	10	LA140_L4	Grass, OHV, *Q. agrifolia*, Riverbed, *Salix* sp.
NEA	Chaparral	Rhizosphere, Bulk	6a	*rosacearum*[12]	3	LA615	Grass, Riverbed
NEA	Chaparral, Oak woodland	Rhizosphere, bulk	7a	*sp. cadmea*[13]	2	LA412_L4	Grass, Riverbed
NEA	Chaparral, Oak woodland	Rhizosphere, Bulk	6b	*sp. NJB2015*[14]	4	LA180_L8	*B. salicifolia*, OHV, Riverbed
NEA	Oak woodland	Bulk	8e	*tax. agrifolia 2*[15]	1	LA202_L1	OHV

GenBank accession numbers:[1]MW260162.1, MW260167.1[2] [3]MW260177.1, [4]MW260212.1, [5]MW260184.1, [6]MW260257.1, [7]MW260241.1, [8]MW260242.1, [9]NA, [10]MW260173.1, [11]MW260245.1, [12]NA, [13]MW260176.1, [14]MW260180.1, [15]NA

[16] Native and exotic grasses (*Bromus* spp., *Avena* spp.)

[17] Tracks left by Off Highway Vehicles (OHV)

Figure 1.4. *Phytophthora* positive plants and streams in fire areas of Angeles National Forest. (A) *Eriodictyon crassifolium* positive for *P. cactorum*. (B) Dead *Quercus agrifolia* restoration plant positive for *P. gonapodyides*. (C) Dead *Salvia mellifera* positive for *P.*

multivora. (D) *E. crassifolium* with mild symptoms of crown thinning, close to a *P. crassamura* positive stream. (E) Thinning *Adenostoma fasciculatum* from which *P. gonapodyides* was isolated. (F-I) Sites positive for *P. crassamura* and other clade 6 *Phytophthora* species.

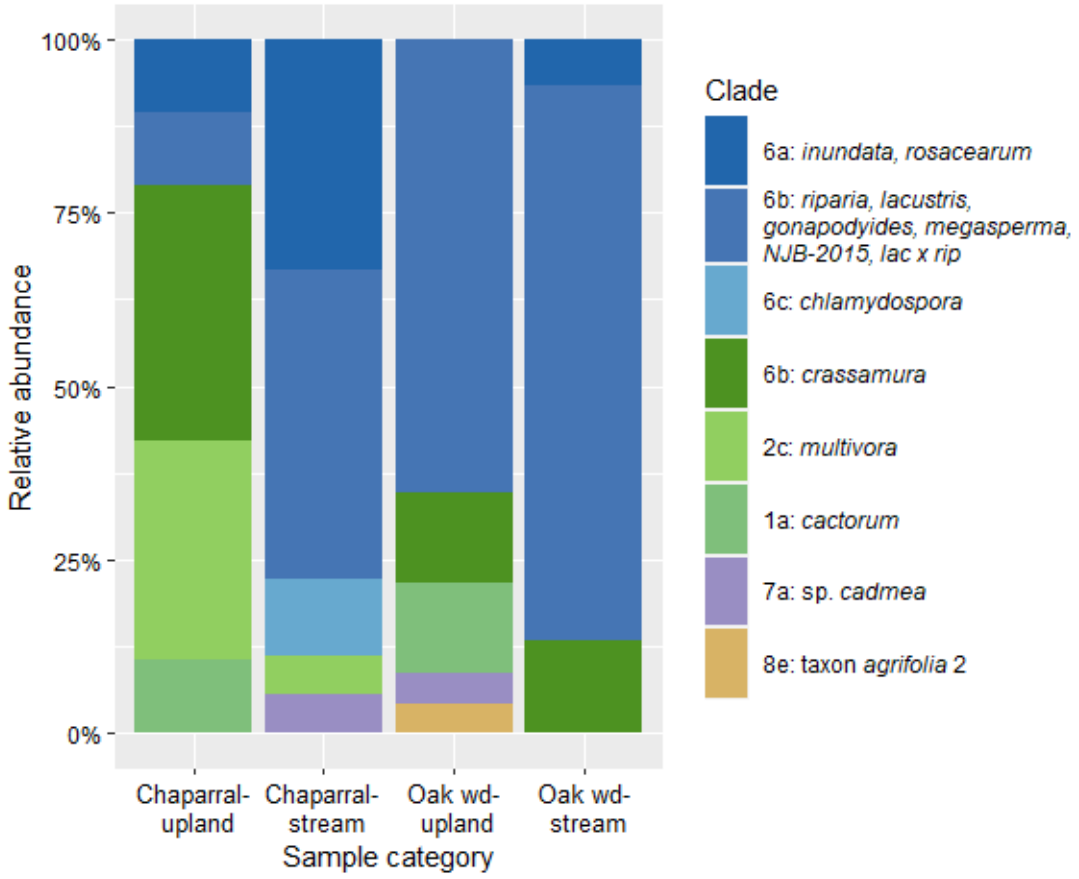

Figure 1.5. Relative abundance of *Phytophthora* species grouped according to ITS clade found in soil samples taken in each habitat category.

Off highway vehicle track* Phytophthora *species.

Across all three fire areas, off highway vehicle (OHV) tracks were observed in twenty sites. OHV tracks varied in the amount of disturbed area which depended primarily on

the vehicles used to make the tracks and amount of use (motorbikes, heavy machinery, or trucks). In total eighty-one samples were taken from OHV tracks, sixty-seven from within chaparral and nineteen from oak woodlands. These tracks had a 6.48% positivity with eight *Phytophthora*-positive samples. *P. crassamura* was the most frequently isolated, followed by *P. gonapodyides*, *P*. NJB-2015, *P. riparia* and the only detection of *P*. taxon Agrifolia 2 (Figure 1.4.H).

Association between* Phytophthora *presence in the rhizosphere and disease symptoms.

In total, 450 soil samples were taken from underneath plants rated for overall health: from 48 plant species, 24 plant families, and from 149 samples taken from underneath plants rated with a "1" (appeared healthy), 127 rated with a "2" (50% or less affected canopy), 78 with a "3" (75% of canopy affected), and 96 with a "4" (dead).

Twenty-nine samples, from thirteen plant species, were positive for *Phytophthora* species and were associated with plants from all health ratings. Plants rated with a "1 - healthy" had higher *Phytophthora* species diversity when compared with symptomatic plants. *P. cactorum, P. crassamura, P. gonapodyides, P. lacustris x riparia, P. multivora, P. riparia, P. sp. cadmea and P. NJB-2015* were all found under asymptomatic individuals from seven sites, mostly in oak woodland areas. Asymptomatic *Q. agrifolia* from two sites harbored *P. crassamura* and *P. cactorum*, while other clade 6 species were found associated with *Salix* sp. In the SWA, *P. multivora* was isolated from two healthy appearing individuals of *T. diverisfolia*.

The most common symptoms observed across sampled plant species were crown thinning and branch dieback, with some individuals presenting yellowing and leaf tip

necrosis. From rhizosphere soil samples, under symptomatic plants (nine plants species), *P. cactorum, P. crassamura, P. gonapodyides, P. inundata, P. lacustris, P. megasperma, P. multivora,* and *P. riparia* were detected. In total 78 dead plants were sampled, from which four individuals were positive for *Phytophthora* species, detecting only *P. crassamura, P. multivora* and *P. gonapodyides* were isolated. Symptomatic *Phytophthora*-associated plants were observed at twelve sites, eight from oak woodland areas and four from chaparral areas. *P*. *cactorum* was isolated underneath a *H. squarrosa* with several leaves presenting tip necrosis and *E. crassifolium* with severe yellowing, especially from its bottom branches (Figure 1.4.A). Additionally, *P. cactorum* was isolated underneath a *Q. agrifolia* with clear symptoms of dieback of the upper crown. *P. crassamura* was detected underneath a *B. salicifolia* with sparse branches and thinning and from two dead *S. mellifera* plants. *P. gonapodyides* was the only *Phytophthora* species found in a restoration basin across the three areas. This basin had previously been planted with *Q. agrifolia* which had a dead crown with brown leaves still attached (Figure 1.4.B). Adjacent to this restoration basin, *P. gonapodyides* was again isolated from a cluster of thinning *A*. *fasciculatum* plants (Figure 1.4.E). At other sites, *P. gonapodyides* was isolated from *B. salicifolia* and *Q. agrifolia* with mild crown dieback. Other clade 6 species were also found underneath plants with mild symptoms, with the following host associations: *P*. *lacustris*-*Salix*. sp., *P. inundata*-*B. salicifolia*, *P. megasperma*-*D. auricanticus*, and *P. riparia*-*Q. agrifolia.* Additionally, *P. multivora* was isolated underneath dead *S. mellifera* and *E. crassifolium*, and also detected underneath *S. mellifera* and *E. fasciculatum* with some branch dieback.

DISCUSSION

This survey represents the first extensive *Phytophthora* survey of Southern California natural areas, and amongst the few studies of *Phytophthora* diversity in Mediterranean regions. Across the forty selected sites in both chaparral and oak woodlands areas, which included riparian areas and streams, fourteen *Phytophthora* species were detected. Considering the relatively low number of sampled areas and the arid nature of the region, *Phytophthora* diversity was higher than expected. The three most frequently detected species in this survey, *P. gonapodyides*, *P. crassamura* and *P. riparia,* were found associated with streams in both chaparral and oak woodlands. These species in addition to *P. cactorum* and *P. multivora,* are also commonly encountered in forest and restoration scenarios in California (Aram et al 2018, Bourret et al. 2017, Bourret et al., 2018, Bourret et al., 2022, Sims et al. 2020) and in natural areas in other states in the US (Brazee et al. 2016, Hansen et al. 2012, Hong et al. 2012, Hwang et al, 2011, Reeser et al, 2011, Sims et al. 2015, Stamler et al 2016, Bily et al., 2018, Hulvey et al. 2010).

A comparable *Phytophthora* survey of Mediterranean-type plant communities in the National Park of La Maddalena archipelago (northeast Sardinia, Italy) recovered nine *Phytophthora* species (Scanu et al. 2015). Of the *Phytophthora* species associated with the Italian maqui vegetation, two were found in the current ANF survey: *P. gonapodyides,* found in Sardinia waterways, and *P. crassamura* detected in the rhizosphere soil of *Juniperus phoenicea* (Phoenicean juniper, Cupressaceae). Although different flora was sampled in the two surveys, a comparison of *Phytophthora* positive plant families can be made. In the Anacardiaceae, *T. diversifoli*a in ANF chaparral areas

and *Pistacia lentiscus* (mastic) from the La Maddalena archipelago were *Phytophthora* positive and *Rhus intergrifolia* on the ANF was negative. Only *P. multivora* was found underneath *T. diversifolia*, and *P. gonapodyides*, *P. ornamentata*, and *P. bilorbang* were all associated with *P. lentiscus* in Sardinia. In the Scanu et al. (2015) study, *Phytophthora* species were also isolated from symptomatic and declining *Asparagus albus* (Asparagaceae), *J. oxydecrus* (Cupressaceae) and *Rhamnus alaternus* (Rhamnaceae). Species in the same plant families were sampled on the ANF, but no *Phytophthora* species were detected. These ANF plant species included *Hesperoyucca whipplei* (Asparagaceae), *Calocedrus decurrens* (Cupressaceae) and *Ceanothus* sp. (Rhamnaceae). Although in ANF these species were *Phytophthora* negative, their phylogenetic relationship could indicate potential host affinity for other *Phytophthora* species.

Five distinct ITS haplotypes of *P. cactorum* are present across California (Bourret et al. 2022b). Of those five, two of them were detected among the *P. cactorum* isolates of the ANF survey, CAC1 and CAC3. CAC1 was found in a single site in an oak woodland upland area in NEA, underneath a mildly symptomatic *Q. agrifolia* tree and *H. squarrosa* shrub. CAC3 was found in the NEA and SWA, underneath a yellowing *Er. crassifolium* and a thinning *Salix* sp. In California, CAC3 is the most commonly encountered ITS haplotype, being present in restoration outplantings in both northern and southern California, and frequently detected in native plant nurseries (Bourret et al. 2022b). CAC1 has a more limited distribution, being only found in natural areas and streams of California. In Bourret et al., (2022a), these isolates were put into a 74-isolate, multi-locus maximum likelihood tree of *Phytophthora* subclade 1a, inferred from ITS rDNA, mt

cox2+spacer and mt cox1 loci. ANF CAC3 isolates belonged to a larger worldwide linage related with an apple-oak linage, present in Czechia, Germany, Japan, The Netherlands, New Zealand, Russia, the UK and the USA. This suggests that the apple–oak lineage is moving locally and worldwide through horticulture (Bourret, et al. 2022a). While isolates of CAC1, did not fit in any linage within the multilocus analysis, thus more reference isolates are needed to obtain a better understanding of the distribution of this isolate in a worldwide context and in California. The presence of *P. cactorum* across many locations in the ANF and the widespread distribution of certain ITS haplotypes, could be seen as a potential problem because of its adaptability and ease to establish in different environments. *P. cactorum* is known to kill or damage forest trees, horticultural and agricultural plants (Hudler, 2013).

On the ANF, *P. multivora* was only found in the SWA, detected in two upland chaparral sites with detections from rhizosphere samples of a healthy *T. diverisfolia* and *E. fasciculatum* and from a dead *Er. crassifolium* and *S. mellifera.* In California, *P. multivora* is considered to have a more clonal population, with a single ITS haplotype encountered in nurseries, restorations and in natural areas (Bourret et al. 2022b). P. multivora is distributed globally in natural areas and streams. In Australia, it is associated with numerous dead and dying native hosts (Burgess et al. 2009; Hüberli, et al. 2013, Scott et al. 2009) and in Europe it is associated with the plant trade and outplantings (Jung et al. 2020). For example, in a small Italian nature reserve, *P. multivora* is associated with vegetation types that include plant genera similar to those encountered on the ANF, *Salix* spp, *Quercus* spp, and *Populus* spp. (Riolo et al. 2020).

Of the fourteen detected *Phytophthora* species on the ANF, *P. crassamura* was the only species that was found in all three geographic areas, in both vegetation types, in both upland areas, in streams and on OHV tracks. *P. crassamura* was also found beneath asymptomatic and symptomatic plants from five different plants species, including a dead *S. mellifera. P. crassamura* is among the most common *Phytophthora* species encountered in California with strong associations with agriculture and restoration areas and to a lesser extent with nurseries (Bourret et al., 2022). Reviewing historical records, considering the recent description of *P. crassamura* from Maqui vegetation in Italy and with the possibility of previous *P. megasperma* isolates have been misidentified (Scanu et al 2015, Bourret et al. 2022), *P. crassamura* may have been in the US for at least 100 years (Bourret et al. 2022). Sims et al. (2019) conducted a phenotypic and genotypic analysis, using cytochrome c oxidase subunit 1 (COX 1) sequences, oogonia size and mefenoxam resistance variation and concluded that *P. crassamura* lineages found in Northern California restoration areas had originally come from nurseries and was being spread into wildlands (Sims et al. 2019). Follow-up analysis will be conducted to determine the lineage of the ANF *P. crassamura* isolates and potential source of introduction.

Eleven out of the fourteen species detected (79%) and 84% of the total *Phytophthora* isolates recovered from the ANF survey corresponded to ITS clade 6 *Phytophthora* species. The majority belonged to clade 6b and were more frequent in streams and oak woodlands. Except for *P. crassamura*, most clade 6 species were associated with healthy or asymptomatic plants. Only three plants from which *P. gonapodyides* was isolated showed symptoms of crown thinning. Only one was dead, a *Q. agrifolia* plant

from a restoration plant basin. In California, clade 6 species are common in natural areas and streams and potentially indigenous based on high levels of intraspecific diversity (Bourret et al. 2022). Clade 6 species also are found in streams and riparian areas in many areas of North America and Europe (Hansen and Delatour 1999; Reeser et al. 2011, Riolo et al. 2020). They are mostly associated with a saprophytic lifestyle (Brasier et al. 2003; Hansen et al. 2012). However, when inoculated or present in disease conducive conditions, these species can initiate disease and damage trees (Hansen and Delatour 1999; Greslebin et al. 2005; Smith et al. 2009; Randall 2011, Reeser et al. 2011; Nechwatal et al. 2012, Brasier et al. 2003b; Brown and Brasier 2007; Jung 2009). Among clade 6 species around the world, *P. gonapodyides* and *P. inundata* have been commonly detected in Mediterranean areas (Scanu et al. 2015, Stukely et al. 2007, Bose et al. 2018) and in other parts of California (Garbelotto et. al., 2018). This predominant aquatic and saprophytic lifestyle in natural areas needs to be further investigated to understand the role of these species in these dry and arid areas.

The widespread distribution of many *Phytophthora* species on the ANF is of concern as their origin or source of introduction is still unknown. Periodic or seasonal *Phytophthora* sampling is recommended for monitoring potential undetected *Phytophthora* spp. introductions. Detection of *Phytophthora* species in OHV tracks is also of concern, indicating a potential route of dispersal through human-associated soil movement (Shearer et al 1989). Similarly, monitoring of waterways is also recommended to assess a baseline of *Phytophthora*-diversity, to determine which species are present in the areas and which ones can potentially be dispersed into adjacent areas. Despite the absence of positive detections in established restoration areas, caution and monitoring

should be prioritized by nursery and restoration managers to prevent further introduction as evidence of these events are becoming more common in California.

This survey was performed in a variety of ecological niches with a goal of determining the distribution and ecology of *Phytophthora* species. Amongst the fourteen detected species, certain combinations of niches resulted in different *Phytophthora* species compositions and shifts in their frequency. Certain areas harbored a larger *Phytophthora* diversity while others had only a subset of the encountered species. Streams and oak woodland areas had the highest diversity of *Phytophthora* species, with clade 6 most prevalent. In drier areas, the upland chaparral areas, homothallic species *P. cactorum, P. multivora* and *P. crassamura* were more common. This species can readily produce thick-walled oospores allowing them to potentially survive adverse environmental conditions. It is unclear why *P. multivora* had such a narrow distribution in the ANF compared to other homothallic species being limited to a few sites and only in the SWA. This could be due to many factors. For example, waterways in SWA are fed by the Upper Los Angeles River watershed, while in the NEA the Bouquet Canyon and Upper Santa Clara are the dominant watersheds indicating potential different introduction points and/or dispersal patterns through the various waterways. Further sampling is required to determine if this corresponds to a recent introduction into the area and assess the real distribution of this species in ANF and in other areas of Southern California.

In recent decades interest in *Phytophthora* sampling has increased in areas historically considered unfavorable for their dispersal and pathogenicity (Scanu et al., 2015, Scanu et al. 2010, Jung et al. 2013, Peréz-Sierra et al. 2013). Surveys have increased the

number and diversity of detected *Phytophthora* species in dry and fire-prone Mediterranean areas as well as discovered novel hosts. These plant communities have unique ecological characteristics and support a large diversity of flora and fauna, and often, large human populations, making them more vulnerable to ever increasing disturbances (Allen et al. 2001, Galatowitsch et al. 2012). *Phytophthora* species in the ANF were found more widely distributed than anticipated, not only in dry and wet streams, but also in seasonally dry uplands areas. Soil compaction and poor drainage conditions created by OHV tracks can potentially create ideal soil conditions for proliferation and spread of *Phytophthora* species. Studies are needed to understand the ecological role these *Phytophthora* species pose to the local and native flora, and which *Phytophthora* species characteristics allow for their establishment and dispersal.

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CHAPTER TWO

Selfing and oospore production of *Phytophthora* species associated with establishment in Southern California Chaparral landscapes.

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ABSTRACT

Phytophthora species, known as water molds, thrive in water and soil environments, requiring high humidity or availability of free water as disease conducive conditions. Several *Phytophthora* surveys have been performed in tropical and temperate areas of the world; however, little is known about the ecology and biology of *Phytophthora* species in drier regions. Climatic conditions and other abiotic factors have been shown to influence the establishment of *Phytophthora* species. Between 2018-21 *Phytophthora* surveys were performed in Angeles National Forest, Southern California, encountering fourteen species across many ecological niches. Based on these results, the analysis of the *Phytophthora* community composition indicated that *Phytophthora* species shift in their abundance and diversity based on the sampling context in which they were detected. When comparing chaparral areas with oak woodland areas, similar species composition was observed, but relative abundance of species varied. *Phytophthora* species traits were also assessed, observing Clade 6 species were mostly abundant in streams, while selfing species (homothallic), capable of oospores production were more

abundant in drier chaparral areas. Uncovering the ecological roles and functions of various *Phytophthora* taxa can help landscape restoration efforts.

INTRODUCTION

The oomycete genus *Phytophthora*, belonging to the family Peronosporaceae and kingdom Straminipila (Beakes et al. 2014) are amongst the most highly damaging invasive plant pathogens in the world (Hyun and Choi, 2014). In forest and natural ecosystems, invasive *Phytophthora* species can potentially be destabilizing to whole ecosystems with detrimental effects on biodiversity and on ecosystem services (Jung et al 2016, Boyd et al. 2013, Underwood et al. 2018).

Phytophthora species, known as water molds, thrive in water and soil environments, in most cases requiring free water for extensive production and dispersal of zoospores and infection of plant hosts (Erwin & Ribeiro, 1996). Surveys in recent years of tropical or temperate rain forest have yielded a remarkable diversity of *Phytophthora* species, including descriptions of new species and sister genus like *Nothophytophthora* species (Jung et al. 2017a, Jung et al. 2017b, Jung et al. 2020). However, little is known about the ecology and biology of *Phytophthora* species in drier regions of the world. With only a few *Phytophthora* species surveys in natural Mediterranean areas, the understanding of the distribution and dispersal of these plant pathogens in these types of areas is limited (Scanu et al. 2015, Burgess et al. 2009; Hüberli, et al. 2013, Scott et al. 2009).

Climatic conditions and other abiotic factors have been shown to influence the establishment of *Phytophthora* species (Browning, et al. 2008; Redondo et al. 2018;

Sanchez-Cuesta, et al., 2020, Corcobado et al. 2020, Firester et al. 2018, La Manna et al. 2008). Production of oospores, chlamydospores and hyphal aggregations have been associated with survival of *Phytophthora* species under dry conditions that are not conducive to multiplication, dispersal and infection by the pathogen (Jung et al., 2013, add more citations).

The alarming increase of *Phytophthora* species detections in natural and ecologically diverse areas of the world (Jung et. al., 2018; Jung et al. 2020; Bourret et al., 2017; Perez-Sierra 2022; Burgess et al. 2022), has risen questions regarding the invasion process of forest *Phytophthora* and the potential for ecological adaptions that may allow for establishment in novel environments (Caballol et al. 2021, Redondo, et al. 2018, Redondon et al. 2017; Loiola et al. 2018). For example, phenotypic traits may allow for diverse methos of dispersal, presence, or absence of resistant structures, broad or narrow host ranges, or environmental factors that could be acting as filters for the establishment of different species (Cushman & Meentemeyer, 2008; Ellis, et al., 2010; Jules et al., 2002; Schoebel, et al., 2014). These filters can act as barriers for newly introduced species, preventing their dispersal and potential establishment (Kivlin, et al., 2014; Kolar & Lodge, 2001). Only ecologically compatible species with determined phenotypes can overcome these barriers or filters (Mayfield, et al., 2009). Traits not only determine which filters species can overcome, they also determine the impact of plant pathogens on new environments (Ordonez, et al., 2010; Philibert et al., 2011; Violle et al., 2007).

The exponential increase in risk of introduction and spread of *Phytophthora* species into native habitats has been associated with a rapid increase in ecosystem disturbance

interfaced with population growth and expanded plant cultivation around the world (Brasier, 2008; Santini et al., 2013). Studies have demonstrated that a primary route of unintended introduction of *Phytophthora* species into natural areas is through inadvertent outplanting of infested native plant nursery stock (Jung at al. 2016; Molnar et al. 2020; Parke et al. 2019) with many recorded cases across diverse ecosystems in California (Bourret, et al., 2018, Garbelotto et al. 2018, Rooney-Latham, et al. 2019, Sims et al., 2019, Swiecki et al. 2018, Swiecki et al. 2021). The Angeles National Forest (ANF), a federally protected area in Southern California, utilizes thousands of nursery-grown native plants for the restoration of areas disturbed by special uses, fire and recreation activities (Myer et al., 2021). Due to the concern of potential *Phytophthora* spp. introductions, surveys were performed in ANF between 2018-2021, detecting in total fourteen *Phytophthora* species. This current study analyzes the encountered *Phytophthora* diversity and proposes to identify differences in *Phytophthora* community composition amongst areas of ANF. This study also determines which species are dictating the differences amongst *Phytophthora* communities and identifies species-specific traits that can potentially be associated with establishment in ANF areas. The ANF may serve as a great case study to help elucidate *Phytophthora* ecology in Mediterranean arid lands.

METHODS AND MATERIALS

Study area

The Angeles National Forest (ANF) is a protected area managed by the United States Forest Service area, and it located in northern Los Angeles County in Southern California, USA (34°32′N, 118°30′W), immersed in the San Gabriel and Pelona

Mountains. The ANF is dominated by Chamise chaparral and coastal sage scrub vegetation types, with shrub species like *Adenostoma fasciculatum, Eriogonum fasciculatum, Eriodictyon crassifolium, Salvia mellifera* which are often surrounded by native and exotic grasses. In addition, larger shrubs can be found in this vegetation type, like *Heteromeles arbutifolia*, *Rhus integrifolia* and *Prunus ilicifolia*. In areas adjacent to natural waterways or rivers *Quercus agrifolia* and the endangered *Berberis nevinii* grow together with other riparian plants such as *Populus fremontii*, *Baccharis salicifolia* and other *Salix* species. ANF is characterized by having a Mediterranean climate with average temperatures ranging from 8-20ºC in winter to 15-33ºC during dry summer months with a yearly average precipitation of 566 mm. Mt. Baldy Notch is amongst the areas with highest elevations of the ANF, which can receive some up to 334 cm of average yearly snowfall (ANF elevations range from 365.76 m to 3067.5 m a.s.l.).

Figure 2.1. Examples of areas of the Angeles National Forest from which soil samples were taken to determine the presence of *Phytophthora* species. These include

chaparral areas (A), transition areas between chaparral and oak woodland areas (B), oak woodlands (C) and riparian areas with dry and wet riverbeds (D). *Adenostoma fasciculatum*, *Eriodictyon crassifolium*, *Salvia mellifera* and *Quercus agrifolia* were amongst the most sampled native plant species in these areas.

Sampling,* Phytophthora *isolation and identification.

This study focused on the results obtained by *Phytophthora* surveys performed over five field visits between May 2018 to 2021 on Angeles National Forest lands. Main results and outcomes of the survey results are presented and discussed in Chapter one. Forty sites were sampled across diverse ecological niches including oak woodlands and chamise chaparral areas. A total of 576 samples were collected which were categorized by: the type of sample, that is taken from rhizosphere soil (considered as plant-associated soil in this study) or bulk soil (off road vehicle trails, river basins, or bare-ground areas); sample vegetation type, either chaparral or oak woodland; sample context, upland (samples taken outside stream beds) or stream bed (soil collected from the center of the river basin). Most of the sampled stream beds run dry during the season, however, if water was present, soil was taken from the bottom of the river basin, attempting to leave in the sample bag more soil than water. Selected plant samples were annotated as either being asymptomatic (healthy) or symptomatic with *Phytophthora* associated symptoms: crown thinning, canopy yellowing and defoliation (Jung et al. 2018). Digital elevation models and flowlines for perennial and ephemeral streams from were obtained from the United States Geological Services (USGS) databases, fire perimeters from the Fire and Resource Assessment Program (FRAP) and road and trails from USFS database. Samples were later transported to the UC

Davis Rizzo laboratory in Davis, CA, for *Phytophthora* species baiting, done according to Erwin and Ribeiro (1996) and Jung et al (2009).

After subculturing baited isolates resembling *Phytophthora* species. DNA was extracted from mycelium of representative isolates. The extractions used the PrepMan Ultra kit (Thermo Fisher Scientific, Waltham MA, USA). PCR was performed with oomycete-specific primer pair FRiz + ITS4TT (Bourret et al. 2018) to amplify the internal transcribed spacer (ITS). Sequencing was performed with PCR primers by the UC Davis College of Biological Sciences, DNA Sequencing Facility. Contigs and finished sequences were formed from the sequencing runs as described by Bourret et al. (2018). ITS sequences were compared against those available in the GenBank nucleotide collection using BLAST searches. Positive determinations were made based on 100% matches to sequences from strains of verifiable identity (Abad et al., 2019).

Diversity analysis

Pairwise Sørensen similarity coefficients were calculated between the pooled communities from each vegetation type using the R package vegan (Oksanen et al. 2019). *Phytophthora* species β-diversity was analyzed at community level diversity by determining the relative abundance (number of isolates) and richness (number of different species) of each pooled community by using adespatial and ade4 packages in R.123.4. Jaccard and Sørensen index was also used to break down components of β-diversity into Turnover/replacement or richness differences, and to determine the contribution of each category and species to β-diversity.

Sample association with Multiple Correspondence Analysis (MCA)

All samples were pooled together and categorized by Sample type (rhizosphere or bulk, Sample context (Upland or stream), and Vegetation type (chaparral or oak woodland). To observe additional clustering between categories in the MCA analysis, sample context and vegetation type were combined to create the category Vegetation context (Oak woodland-stream, Oak woodland-upland, Chaparral-stream, and Chaparral-upland). Other sample associated information was incorporated in the analysis: the season in which the sample was taken, early-spring (March), late-spring (May) and Winter (December); Ground cover above the sample soil line (Bare ground, Grass, Leaf litter and water). Based on GPS points additional metadata was obtained, extracting slope, aspect and elevation. Soil properties were categorized by soil family complexes (soil units) extracted by georeferencing each sample location on the interactive UC Soil web map available at https://casoilresource.lawr.ucdavis.edu/gmap/ (Walkinshaw et al. 2022). To determine associations between variable categories for each sample the MCA() function was used from the FactoMineR package available in R.123.4. Full data set used for MCA analysis is displayed in Table S2.1.

Table 2.1. Categorical environmental variables analyzed to determine patterns of landscape distribution associated with positive *Phytophthora* samples obtained in areas of Angeles National Forest.

Environmental variables	Levels
Aspect	Asp-I (0-90°); Asp-II (91°-180°), Asp-III (181°-270°), Asp-IV (271°-360°)
Elevation	Ele-I (475-609m); Ele-II (610-743m), Ele-III (744-877m), Ele-IV (878-1011m)
Ground cover	Bare ground; Grass; Leaf Litter; Water;

Sample context	Stream; upland
Sample type	Bulk; Rhizosphere
Season	Early-Spring; Late-Spring; Winter
Slope	Slp-I (0.72-6.07°); Slp-II (6.08°-11.41°), Slp-III (11.42°-16.76°), Slp-IV(16.76°-22.10°)
Soil units (soil family complexes)	Soil units: 8, 24, 26, 36, 54, 74, and 115.
Vegetation context	Chaparral-upland; Chaparral-stream; Oak woodland-upland; Oak woodland-Stream
Vegetation type	Chaparral; Oak woodland

Identified *Phytophthora* species were placed into functional categories: homothallism/ potentially undergoing sex in nature and clade placement. Based on information available on IDphy webpage https://idtools.org/*Phytophthora*/ (Abad et al. 2022), each species was categorized based on their reproductive mode: homothallic or heterothallic, persistence of sporangia: persistent or caducous, and the presence of asexual reproductive structures: no structure, chlamydospores or hyphal swellings according to literature, and ITS clade (Table 2.2).

Table 2.2. Evaluated *Phytophthora* species traits to determine sample association.

Trait	Trait level
Clade	1a; 2a; 6a,b,c; 7a, 8e.
Reproductive mode	Homothallic; Heterothallic.
Persistence of sporangia	Persistent; Caducous
Asexual survival structures	No structures; Chlamydospores; Hyphal swellings

RESULTS

Sampling and diversity in Chaparral, Oak woodlands and Streams

In summary, and as presented in chapter one with more details, from the five field surveys fourteen species of *Phytophthora* were detected, including three undescribed species and one hybrid species (Table 2.3): *P. cactorum* from clade 1a, *P. multivora* from clade 2c, *P. inundata* and *P. rosacearum* from clade 6a, *P. crassamura*, *P. gonapodyides*, *P. lacustris, P. lacustris x riparia*, *P. megasperma*, *P*. sp. 'NJB-2015' and *P*. *riparia* from clade 6b, *P. chlamydospora* from clade 6c, *P*. sp. 'cadmea' from clade 7a and *P*. taxon 'agrifolia' II from clade 8e. Chaparral area samples had a 5% *Phytophthora* positivity rate, from a total of 377 samples. *P. cactorum*, *P. crassamura*, *P. inundata*, *P. multivora, P. rosacearum* were all isolated from chaparral areas, with *P. cactorum, P. crassamura* and *P. multivora* as the most repeatedly encountered species. Out of the 144 samples taken from upland oak woodland areas, 13% were positive for *Phytophthora* species with *P. cactorum, P. crassamura, P. gonapodyides, P. lacustris, P. lacustris x riparia, P. megasperma, P. riparia*, *P*. sp. *cadmea*, P. sp. Njb-2015 all found to be associated with these areas.

Most of the streams across the fire areas run dry during the great portion of the year, with water flowing during the winter and early spring seasons. In the sampled areas, only a single perennial stream was present, which was sampled in two sites. The remaining sampled waterways corresponded to ephemeral streams. Using the USGS hydrology database, twenty-two streams (based on flowlines) were selected in accordance with site locations, with two sites having perennial streams and twenty with ephemeral streams. Out of the 22 streams, 17 of them were positive for *Phytophthora*

species, taking in total 86 samples with a 45% positivity rate. *P. gonapodyides*, *P. riparia*, and *P. inundata* were isolated in higher frequency. Other clade 6 species like *P. chlamydospora*, *P. crassamura*, *P. lacustris, P. lacustris x riparia*, *P. megasperma*, *P. rosacearum* and *P*. NJB-2015 were also encountered but in lesser frequency. *P. multivora* was the only non-clade 6 species isolated from streams, detected from a single sample from an ephemeral stream. In the sampled perennial stream only clade 6 species were encountered, including *P*. *gonapodyides*, *P. inundata*, *P. lacustris x riparia*, *P. megasperma, P. riparia* and *P*. sp. NJB-2015.

MCA analysis

Sample environmental variables data were analyzed to examine if environmental variables explain variation in *Phytophthora* species composition. MCA indicated that variables of vegetation type (Chaparral v Oak woodland), sample type (Rhizosphere v Bulk) and sample context (stream v upland) categories had the largest contribution to the observed variation (Figure 2.2.A). Clustering for these variables was observed on MCA plots. The vegetation context category (Chaparral-upland; Chaparral-stream; Oak woodland-upland; Oak woodland-Stream) showed the strongest contribution and clustering in the MCA plots (Figure 2.2.B).

Diversity analysis

Frequency of *Phytophthora* positive samples and *Phytophthora* community composition differed across vegetation types and comparing upland areas and streams. For both chaparral and oak woodland areas, frequency of *Phytophthora* positive samples increased when samples were taken from stream beds passing through those areas

(Table 2.6). *Phytophthora* species diversity was similar for both vegetation types, with ten species for Chaparral areas and eleven in oak woodlands.

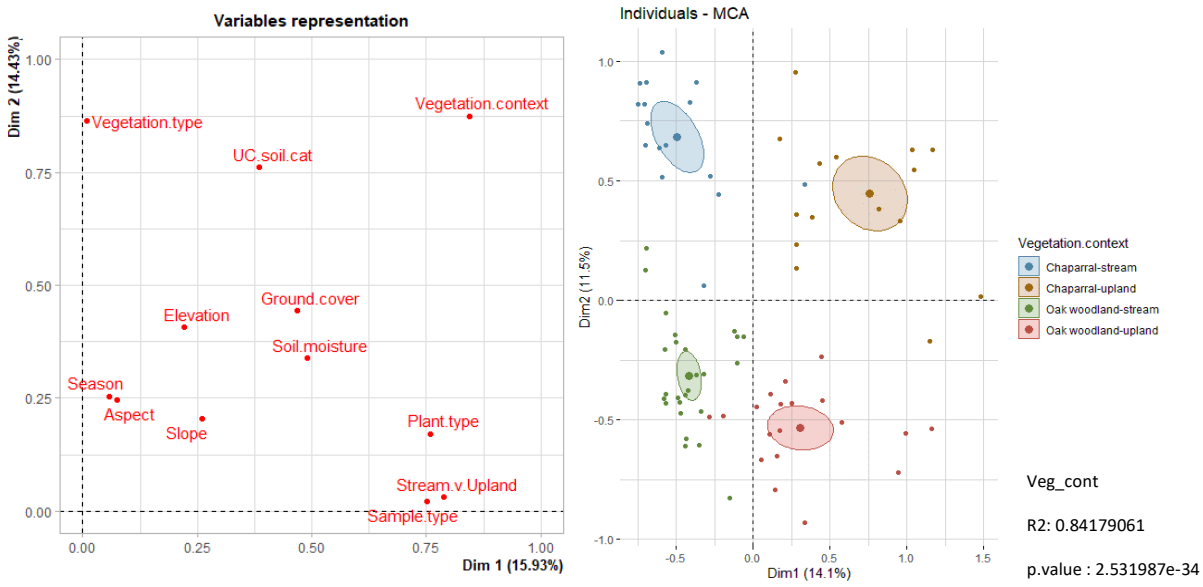

Figure 2.2. Distribution of variables according to their contribution for Dim 1 and 2 (A). MCA showing clustering of samples according to category of Vegetation context (B).

Figure 2. Distribution of variables according to their contribution for Dim 1 and 2 (A). MCA showing clustering of samples according to category of Vegetation context (B).

Similarly, when comparing streams with upland areas, the number of species did not differ, with a total of twelve and thirteen species, respectively (Table 2.6). However, when comparing diversity of vegetation types in contexts of stream or upland, Chaparral upland areas had the lowest diversity, with seven species, and oak upland had the highest diversity with ten *Phytophthora* species.

The pairwise Sørensen similarity measures were highest between the upland and streams of oak woodland areas and with a coefficient of 0.77, and upland chaparral

areas with streams passing through those areas (0.66) (Table 2.3). The lowest Sorensen similarity values occurred between upland oak woodlands and upland chaparral areas (0.48), followed by the streambeds of chaparral and streambeds of oak woodlands (0.50), and streambeds of oak woodlands and upland chaparral areas (0.53).

Table 2.3. Sørensen similarity measurements between the *Phytophthora* communities based on vegetation and sampling context.

Vegetation context	Chaparral-upland	Oak woodlands-upland	Oak woodlands-streams
Oak woodlands-upland	0.47	-	-
Oak woodlands-streams	0.53	0.77	-
Chaparral-streams	0.66	0.44	0.5

To obtain a better understanding of which species were dictating the clustering (i.e. that in figure 2.B), Sørensen and Jaccard β-diversity analysis were performed, and β-diversity was resolved into species richness and replacement or turnover components. A total of 0.31 and 0.38 of total β-diversity was obtained for Sørensen and Jaccard analysis, respectively (Table 4). Seventy-six percent of the β-diversity was due to replacement, and only approximately 23% was explained by richness difference (Table 4).

Table 2.4. Total beta diversity of *Phytophthora* positive samples, partitioned into Richness and replacement/turn over.

B-Diversity	Sorensen	Jaccard
Total BD	0.31	0.38
Replacement	0.23	0.28
Richness difference	0.07	0.09
Replacement/ βD-total	0.76	0.76
Richness Difference/ βD-total	0.23	0.23

When local contribution to β-diversity of the vegetation context were analyzed, the majority of the replacement was observed in streams and upland areas of Chaparral areas (table 5).

Table 2.5. LCBD, local contribution to beta diversity. Partitioned into replacement and turn over.

Vegetation type context	N° of isolates	N° of species	Frequency of isolation (%)	Jaccard replacement	Jaccard richness difference
Chaparral-upland	19	7	5	0.34	0.11
Chaparral-stream	18	8	43.7	0.3	0.18
Oak woodland-upland	22	10	13.5	0.22	0.16
Oak woodland-stream	31	8	48.8	0.12	0.53

Total oak woodland	53	11	17.8	-	-
Total chaparral	37	10	8.2	-	-
Total stream	48	12	46.7	-	-
Total	90	14	12.7	-	-

Local contribution of each species was also determined, observing that *P. multivora*, *P. riparia*, *P. crassamura* and *P. cactorum* cumulatively contributed with at least 50% to β-diversity. Relative abundances of *Phytophthora* species were compared across vegetation context types. Abundances of *P. crassamura*, *P. cactorum* and *P. multivora* were increased in upland areas, compared to their abundance in streambeds of vegetation types. The other clade 6 species (not including *P. crassamura*) were higher in streams and in particular oak woodland streams (Table 6).

Traits variables were incorporated into the MCA analysis together with environmental variables. Reproductive mode was indicated as one of the variables with highest contribution. Clustering was observed with samples positive with either homothallic or heterothallic *Phytophthora* species (Figure 2.3.A). When comparing abundance of species according to Reproductive mode and in which context they were detected, a higher abundance of homothallic species was observed in upland areas and with the highest relative abundance (75%) in chaparral upland areas (Figure 3B).

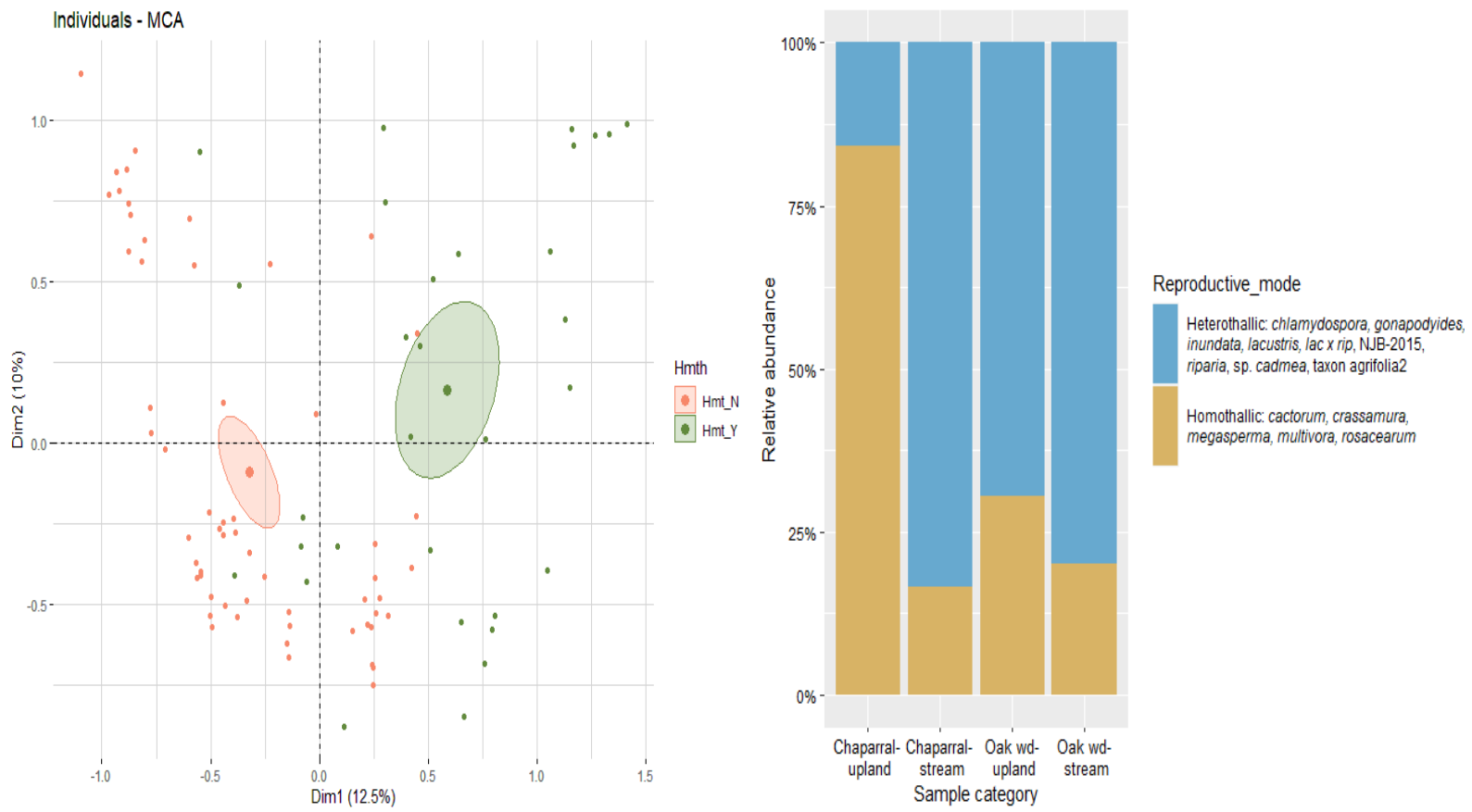

Figure 2.3. Clustering of homothallic and heterothallic *Phytophthora* species according to sample distribution (A). Relative abundance of homothallic and heterothallic species according to vegetation context categories (B).

Table 2.6. *Phytophthora* species detected amongst isolates (n=90) encountered in sampled areas of Angeles National Forest, Los Angeles, California, US. Species contribution to total beta diversity is also shown together with their frequency in the different vegetation contexts. Darker orange indicates higher frequency. No color indicates zero detections for that category

***Phytophthora* spp.**	**ITS Clade**	**Reproductive mode**	**Species contribution to β-diversity**	**Vegetation context**			
				Chaparral -upland	**Chaparral-Stream**	**Oak-upland**	**Oak-Stream**
cactorum[1]	1a	Homothallic	0.09	2	0	3	0
chlamydospora[2]	6c	Heterothallic	0.06	0	2	0	0
crassamura[3]	6b	Homothallic	0.14	7	0	3	4
gonapodyides[4]	6b	Heterothallic	0.07	1	4	7	12
inundata[5]	6a	Homothallic	0.08	1	4	0	2
lacustris[6]	6b	Heterothallic	0.06	0	3	1	2
lacustris x riparia[7]	6b	Heterothallic	0.03	0	0	1	1
megasperma[8]	6b	Homothallic	0.04	0	0	1	2
multivora[9]	2c	Homothallic	0.16	6	1	0	0
riparia[10]	6b	Heterothallic	0.14	0	0	3	7
rosacearum[11]	6a	Homothallic	0.06	1	2	0	0
sp. cadmea[12]	7a	Heterothallic	0.03	0	1	1	0
sp. NJB2015[13]	6b	Heterothallic	0.0014	1	1	1	1
tax. agrifolia 2[14]	8e	Heterothallic	0.02	0	0	1	0

GenBank accession numbers:[1]MW260162.1, [2]MW260177.1, [3]MW260212.1, [4]MW260184.1, [5]MW260257.1, [6]MW260241.1, [7]MW260242.1, [8]NA, [9]MW260173.1, [10]MW260245.1, [11]NA, [12]MW260176.1, [13]MW260180.1, [14]NA

DISCUSSION

The genus *Phytophthora* includes a group of oomycetes predominantly habituating water and/or soil, with higher predominance in wetter environments. However, recent reports in Mediterranean areas (Burgess et al. 2009; Hüberli, et al. 2013, Scott et al. 2009, Scanu et al. 2015, Riolo et al. 2020, Sims, et al. 2020, Swiecki and Bernhardt, 2017) show that some *Phytophthora* species seem to be adapted to drier environments, indicating a need to understand their ecology and potential effect on local and native flora. Patterns of distribution were elucidated in the ANF based on results of a survey of the area that indicated the presence of fourteen species of *Phytophthora*. Different ecological niches were sampled, including chaparral and oak woodland areas in addition to twenty-two streams that passed through these areas. Results indicated that these areas shared many species, however, the frequency of the most common *Phytophthora* species shifted according to ecological niche that was sampled.

The increase in frequency detection in streams and oak woodlands compared to chaparral areas, could be a result of propagule pressure, having better conditions to thrive and increase inoculum in relative wetter areas, thus easier to be baited (Lockwood et al. 2005, Stephens & Sutherland, 1999). The predominance of clade 6 species in these areas could be due to ability of these species to predominantly undergo asexual reproduction, increasing inoculum exponentially, especially during wetter seasons (Brasier et al. 2003; Hansen et al. 2012). Clade 6 species play more of a saprophytic role, breaking down the remainder of any plant tissues in leaf litter and detritus (Brasier et al .2003). Although stream beds in ANF run dry during most of the year, during winter and early spring, these areas have substantially more amount of

water than upland areas, creating ideal conditions for *Phytophthora* spp. for a relatively longer time.

Homothallic species, indicating the capability of selfing and without the need for a compatible mating type to produce sexual reproductive structures (oospores), were more predominant in drier areas like upland chaparral areas. Although, *P. cactorum*, *P. crassamura* and *P. multivora* were either detected in streams or oak woodlands, these species had the highest abundance in upland chaparral areas and are considered homothallic (Scanu et al. 2015, Scott et al. 2009, and Erwin, Ribeiro 1996). Viable sexual structures are associated with the ability of *Phytophthora* to overcome harsh climatic conditions (Erwin and Ribeiro 1996, Jung et al. 2013). Asexual structures such as chlamydospores, hyphal aggregations or hyphal swellings determine the capacity of *Phytophthora* to survive periods of adverse weather conditions (Crone et al., 2013; Jung et al., 2013). In the current study, only *P. cactorum*, and *P. chlamydospora* possessed the ability to potentially produce chlamydospores, encountering both species found in chaparral areas.

Considering previous cases of *Phytophthora* species introduction and establishment through outplanting of contaminated nursery stock in Northern and Southern California (Swiecki et al. 2017; Garbelotto et al. 2018), the detected species in the ANF could have potentially entered through this route. Although, no *Phytophthora* species were detected in the sampled restoration areas in this current study, many other *Phytophthora* species have been detected previously in restoration areas adjacent to the current sampled areas (Swiecki et al. 2017). So, it cannot be discarded that the potential dispersal of the detected *Phytophthora* species could be carried out by other

anthropogenic means, carrying inoculum from urban areas to remote forests or animal-mediated soil and water movements (Cushman & Meentemeyer, 2008; Jules et al.,2002; Webber & Rose, 2008). The relatively high frequency of *Phytophthora* species in streams and in disturbed soil in off road vehicle tracks indicate that in the ANF *Phytophthora* dispersal and distribution is associated with soil and water movement naturally and anthropogenically.

Amongst the top ten most frequently detected *Phytophthora* species in California, seven of these species are homothallic, including*, P. multivora, P. cactorum* and *P. crassamura*, all three detected species in ANF (Bourret et al. 2022). In California, Australia and Europa, *P. multivora* is mostly genetically clonal and strongly associated with the nursery trade and outplantings (Bourret et al. 2022b; Jung et al. 2020), thus being catalogued as a cosmopolitan pathogen with unknown origin (Tsykun et al. 2022). *P. cactorum* in California are composed of five distinct ITS haplotypes (Bourret et al. 2022), with CAC1 and CAC3 detected in the current ANF survey (further descriptions provided in chapter one). In California, CAC3 is commonly encountered in restoration outplantings in both northern and southern California, with higher frequency in native plant nurseries. ANF CAC3 isolates belong to a larger worldwide linage related with an apple-oak linage, present in Czechia, Germany, Japan, The Netherlands, New Zealand, Russia, the UK and the USA, suggesting anthropogenic associated movement (Bourret et al. 2022). CAC1 on the other hand is limited to natural areas and streams of California, detected with much less frequency, limiting the understanding and the distribution of this isolate across California and in the world. *P. crassamura* is amongst the top five most common *Phytophthora* species encountered in California, with strong

association to agriculture and restoration areas and to a lesser extent with nurseries (Bourret et al., 2022). *P. crassamura* lineages found in Northern California restoration areas had originally come from nurseries and were being spread into wildlands (Sims et al. 2019). P. crassamura was first described in 2015, following Phytophthora surveys in declining maqui vegetation in Italy (Scanu et al. 2015), so information regarding is genetic variability is just now being unraveled. Linages detected in Northern California are different from those found in Italy (Sims et al 2019), with a few of them encountered in the current ANF study (unpublished data). Follow-up analysis will be conducted to determine the linage of the ANF *P. crassamura* isolates and to determine their origin and potential source of introduction. Due to the worldwide distribution of these plant pathogens and their association with plant trade and simultaneously encountering them in natural environments, the determination of the potential origin of the detected *Phytophthora* species in ANF cannot be definitively determined. However, the presence of *Phytophthora* species across many contexts in ANF and the widespread distribution in California, it could be seen as a potential problem because of its adaptability and ease to establish in different environments.

The results from this current study suggest that the likelihood of the presence or absence of *Phytophthora* species in the ANF is context dependent. Depending on the vegetation type or if samples are taken from uplands areas or stream beds, *Phytophthora* communities shift in their abundance and composition. In drier chaparral upland areas, there is greater likelihood of detecting *Phytophthora* species mainly associated with the ability to create sexual structures (oospores). However, further studies and sampling are needed to determine the actual extent of *Phytophthora*

distribution and biology in ANF. The ability to tolerate harsher environments may benefit *Phytophthora* species to establish in new environments (Redondo et al. 2017). Special interest and caution should be put on homothallic *Phytophthora* species that can readily produce oospores, or other resistance structures, which can perhaps increase their chances of introduction and establishment from nursery settings to restoration areas, and potentially into natural environments (Redondo et al. 2017). Pathogenicity tests of the most commonly encountered *Phytophthora* species in the ANF should be tested to fully assess the risk of the presence of these phytopathogens towards these unique and fragile Mediterranean ecosystems (Marques, et al 2023). Increasing the understanding of the *Phytophthora* invasion process is crucial to predict new outbreaks caused by these forest pathogens. Uncovering the ecological roles and functions of various *Phytophthora* taxa can help landscape restoration efforts.

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CHAPTER THREE

Pathogenicity of *Phytophthora* species associated with Southern California Chaparral

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ABSTRACT

Pathogen surveys in nurseries have demonstrated that *Phytophthora* species are highly prevalent in horticultural settings. A primary route of unintended introduction of *Phytophthora* species into natural areas is through inadvertent outplanting of infested native plant nursery stock. The Angeles National Forest (ANF), a federally protected area in Southern California, utilizes thousands of nursery-grown native plants for restoration projects. Fourteen *Phytophthora* species have recently been detected in ANF lands, indicating a potential risk for native plants species of the area. Pathogenicity tests were conducted to test the aggressiveness of detected *Phytophthora* species towards common chaparral plant species, which are commonly used for restoration purposes. *P. cactorum*, *P. multivora*, *P. crassamura* and ITS clade 6 species, *P. chlamydospora,* were all capable of infecting disease on *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Salvia mellifera* and *Eriodictyon crassifolium*. *A. fasciculatum* was determined to be the most susceptible plant species, especially towards *P. multivora* and *P. cactorum. S. mellifera* and *Er. crassifolium* showed only slight or no symptoms, but pathogen was frequently isolated from root and above ground tissues. Caution should be placed when dealing with these pathogens if detected in nursery and

restoration scenarios, which could potentially put in risk the Southern California chaparral vegetation.

INTRODUCTION

In recent decades, pathogen surveys in nurseries have demonstrated that *Phytophthora* species are highly prevalent in horticultural settings, especially when best management practices (BMPs) are not correctly implemented (Bienapfl and Balci 2014; Ferguson and Jeffers 1999; Guarnaccia et al. 2021; Hardy and Sivasithamparam 1988; Molnar et al. 2020). Landscape restoration projects historically have used native plants nursey stock as a primary source for their outplanting goals, with many nurseries having the sole objective of providing material for restoration purposes. A primary route of unintended introduction of *Phytophthora* species into natural areas is through inadvertent outplanting of infested native plant nursery stock (Jung at al. 2016; Molnar et al. 2020; Parke et al. 2019) with many recorded cases across diverse ecosystems in California (Bourret, et al., 2018, Garbelotto et al., 2018, Rooney-Latham, et al. 2019, Sims et al., 2019, Swiecki et al. 2018, Swiecki et al. 2021).

It has been estimated that *Phytophthora* spp. can cause over 66% of all fine root diseases and more than 90% of all collar rots on woody plants around the world (Tsao et al. 1990, Kroon et al. 2012). Like many other plant pathogens, predisposing abiotic factors can increase the severity of oomycetes diseases. Extreme droughts reduce vitality of many plant species and decrease their capacity for defending against pathogens (Bostock et al. 2015). Excess soil moisture, caused by heavy rains or a change in river course, can create disease conducive conditions for the pathogen (Brasier 1996).

In California, the dominant Mediterranean native vegetation, “Chaparral”, accounts for 9% of the vegetational landcover, with a distribution that extends form the lower elevations of the coastal ranges, and western slopes of the Sierra Nevada, to the Transverse and Peninsular ranges in the southern part of the state (Parker et al. 2016). Chaparral are drought-tolerant evergreen sclerophyllous shrubs, with *Adenostoma* spp. (Rosaceae), *Arctostaphylos* spp. (Ericaceae) and *Ceanothus* spp. (Rhamnaceae) as common representatives (Keeley and Zedler, 2009; Kolb and Davis, 1995).

The Angeles National Forest (ANF), a federally protected area in Southern California, utilizes thousands of nursery-grown native plants for the restoration of areas disturbed by special uses, fire and recreation activities (Myer et al., 2021). Recent surveys of the ANF area have detected fourteen *Phytophthora* species associated with natural areas (for detailed list of species check Chapter 1). Amongst the fourteen species, *P. cactorum*, *P. multivora*, and *P. crassamura* were the most widespread and present across oak woodlands and chaparral areas. However, the risk towards the native flora due to the presence of these historically known plant pathogens is unknown. In ANF, fluctuating conditions between drought and floods occur seasonally, creating an unquantified risk of the presence or movement and disease severity of *Phytophthora* species.

The pathogenicity of stem and/or root inoculations are the two most frequently used approaches to confirm pathogenicity of soilborne *Phytophthora* species (Aghighi et al. 2016; Jung et al. 2017). Thus, the main objective of this study is to assess the aggressiveness to main woody Chaparral species of common *Phytophthora* species obtained in Angeles National Forest. For this, the pathogenicity of *P. cactorum* and *P.*

multivora and two ITS clade 6 representatives, *P. crassamura* and *P. chlamydospora* were tested for their pathogenicity towards commonly used restoration and native nurseries plant species, *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Eriodictyon crassifolium* and *Salvia mellifera*.

METHODS

Plant production

The following plants were considered for pathogenicity tests based on their prevalence in our previous surveys: *Eriogonum fasciculatum* (California buckwheat), *Eriodictyon crassifolium* (thick leaf yerba santa), *Adenostoma fasciculatum* (chamise), *Salvia mellifera* (black sage). For plant production seeds were purchased from S&S seeds (Carpinteria, CA, USA). To induce germination, *S. mellifera* seeds were soaked in gibberellic acid (50 ppm) for 12 h, and *E. fasciculatum, E. crassifolium and A. fasciculatum* seeds were manually scarified before sowing. Seeds were later sown onto tray flats containing a sowing mix of 1 part screened peat moss, 1 part #12 sand, 2-parts perlite (recipe provided by Rancho Santa Ana Botanic Garden (RSABG) in Claremont, CA and soil mix by UC Davis Orchard Park facilities). Additionally, after sowing trays with *A. fasciculatum* seeds were sprayed with 1:100 diluted solution of smokey water (Wrights Liquid Smoke Hickory) to simulate post-fire requirements in order to increase germination rate.

Pathogenicity tests

After two to three months germinated seedlings were transplanted into 700 ml pots that contained a potting mix of 1-part screened peat moss: 1-part cement sand: 2-parts coarse perlite plus ½ tablespoon of Osmocote (recipe provided by RSABG and soil mix

by UC Davis Orchard Park facilities) (Figure 3.1.A.). To avoid root damage during inoculation, two 20 ml tubes cups were inserted 4 cm apart on opposite sides adjacent to the transplanted seedling. Plants were grown in previously mentioned greenhouse conditions for one year, switching from two daily fertigation (Hoaglands Fertilizer, 1:295 ppm) during fall and winter and to three irrigations daily during the summer months by using drip wands (Figure 3.1.B). On inoculation day, tubes were removed, and the hole was filled with inoculum.

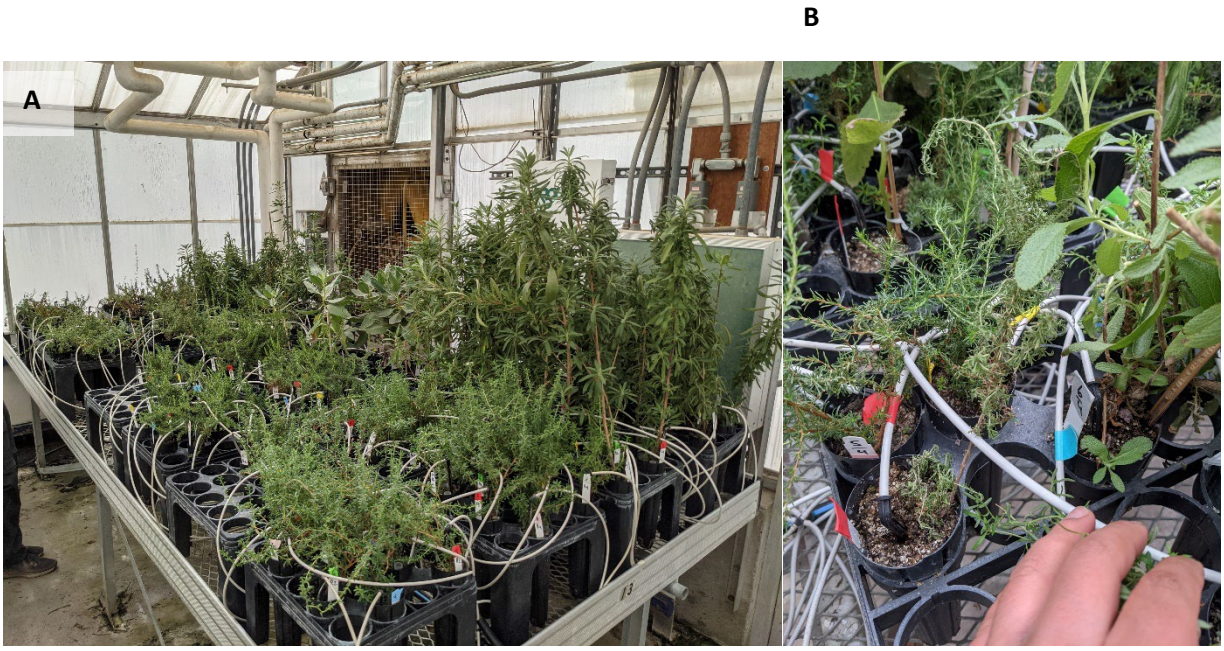


Figure 3.1. Chaparral plants being grown at UC Davis Orchard Park facilities for pathogenicity tests with *Phytophthora cactorum, P. multivora, P. crassamura, P. chlamydospora* and mock control (A). Plants were in a complete randomized test and fertirrigated (Hoaglands Fertilizer) with drip wands (B). Plants were irrigated daily until soil was at field capacity.

Inoculum consisted of 3- to 4-week-old cultures of individual isolates of *Phytophthora* spp. grown at 23°C in spawn bags filled with a substrate mixture per liter of: 500 ml of

fine vermiculite and 40 ml of whole oat-grains or millet seeds thoroughly moistened with 350 ml of V8 juice broth (Scanu, et al 2015, Jung et al, 2009). Trials were conducted on *S. mellifera*, *E. crassifolium*, *A. fasciculatum* and *E. fasciculatum* inoculated five isolates of *Phytophthora* species: *P. cactorum* (CAC1), *P. cactorum* (CAC3), *P. crassamura*, *P. multivora* and *P. chlamydospora*. Each plant received in total 14 ml of inoculum, filling the pre-made holes adjacent to the plant crown. Controls received sterile mixture. Five plant repetitions per plant species were selected per treatment, using the same number for controls. In total 105 plants were used in this trial. To stimulate formation of sporangia and favor disease development, soil irrigation was set up to maintain the plant substrate at field capacity during the duration of the trail.

In addition to assessing mortality incidence, *Phytophthora* species isolations were attempted for each plant by plating, if present, three 2 cm fine root segments, three 2 cm primary root segments, 1 cm of the crown and 1 cm of stem segment. Before plating onto CMA-PARP, plant tissues were surface sterilized by submerging for one minute in 5% bleach solution, three consecutive washes in sterile distilled water and finally dry blotting with paper towels. After processing, dry above ground and root biomass was weighed.

Statistical analysis

Above and below dry biomass data were analyzed by one-way analysis of variance (ANOVA) using Tukey's HSD test (Honestly Significant Difference) as a post-hoc test (R software). Differences at $P < 0.05$ were considered significant.

RESULTS

Disease symptoms and mortality

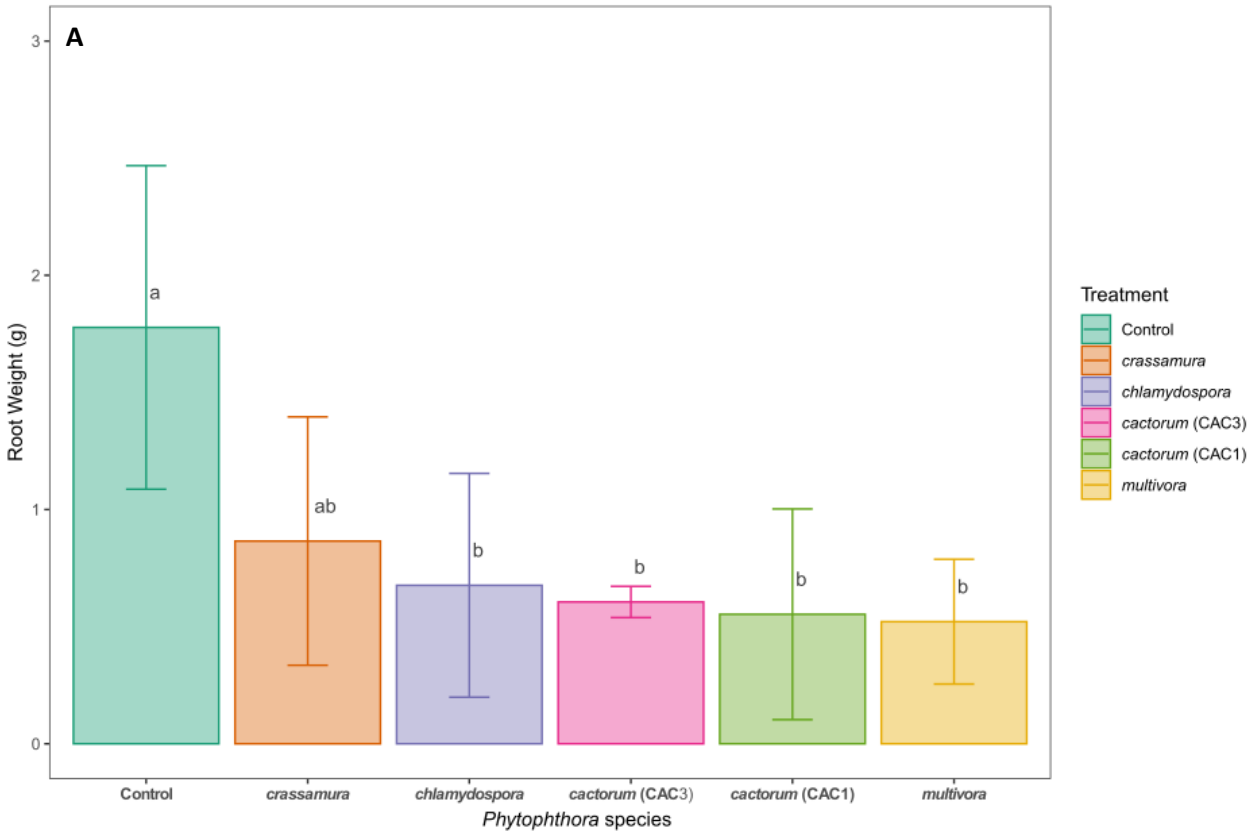
The trial was initiated in November 2021 and was taken down 6 months later. Mortality was observed in the first four months of the trail and was only observed for *A. fasciculatum* and *E. fasciculatum*. None of the tested *Phytophthora* spp. were capable of killing *S. mellifera* and *E. fasciculatum* plants, only observing slight chlorosis and wilting on a few individuals inoculated with *P. multivora* and both haplotypes of *P. cactorum*. *P. multivora* was the most aggressive pathogen, especially on *A. fasciculatum* with 100% mortality rate and 20% on *E. fasciculatum* plants, observed in the first month of the trial. Variability in mortality was observed between ITS haplotypes of *P. cactorum*. *P. cactorum* CAC1 had a mortality rate of 100% on *A. fasciculatum* and 20% in *E. fasciculatum*. While haplotype CAC3, had a mortality rate of 50% in *A. fasciculatum* and 30% in *E. fasciculatum*. The least aggressive pathogen was *P. chlamydospora* with mortality rates of 40% and 20% on *A. fasciculatum* and *E. fasciculatum*, respectively. Overall, *A. fasciculatum* was the most susceptible tested plant species (Figure 3.2).

No significant differences were observed for above ground biomass amongst the treatments for all plant species. However, mean significant differences were observed for dry root biomass in *E. fasciculatum* and *A. fasciculatum*. Treatments with *P. cactorum* (CAC1 and CAC3), *P. multivora* and *P. chlamydospora* showed significant differences with mock inoculated controls for *A. fasciculatum* (Figure3. 2.A).

For *E. fasciculatum* plants inoculated with CAC3 and *P. chlamydospora* showed significant differences with the control. For both plant species, individuals treated with

Figure 3.2. One-year old *Adenostoma fasciculatum* plant soil inoculated with *Phytophthora multivora* (A). Severe chlorosis and mortality was observed one month post-inoculation (B). The root system of mock inoculated control (C) did not show any symptoms, while the inoculated plant root system was severely

Variability was also observed in the frequency of *Phytophthora* species isolations from plant tissue and soil baiting post inoculation (Figure 3.3). The highest frequency was observed for *P. multivora* in *A. fasciculatum*, isolating 100% of the times from crown and stems, and 80% from primary roots. Although no mortality was observed in *S. mellifera* and *E. crassifolium*, *P. cactorum* (CAC1 and CAC3), *P. crassamura* and *P. multivora* were all isolated from at least one plant tissue source. *P. chlamydospora* had the lowest frequency of isolation, mostly being isolated from fine and primary roots on *Er. crassifolium* and *S. mellifera*.



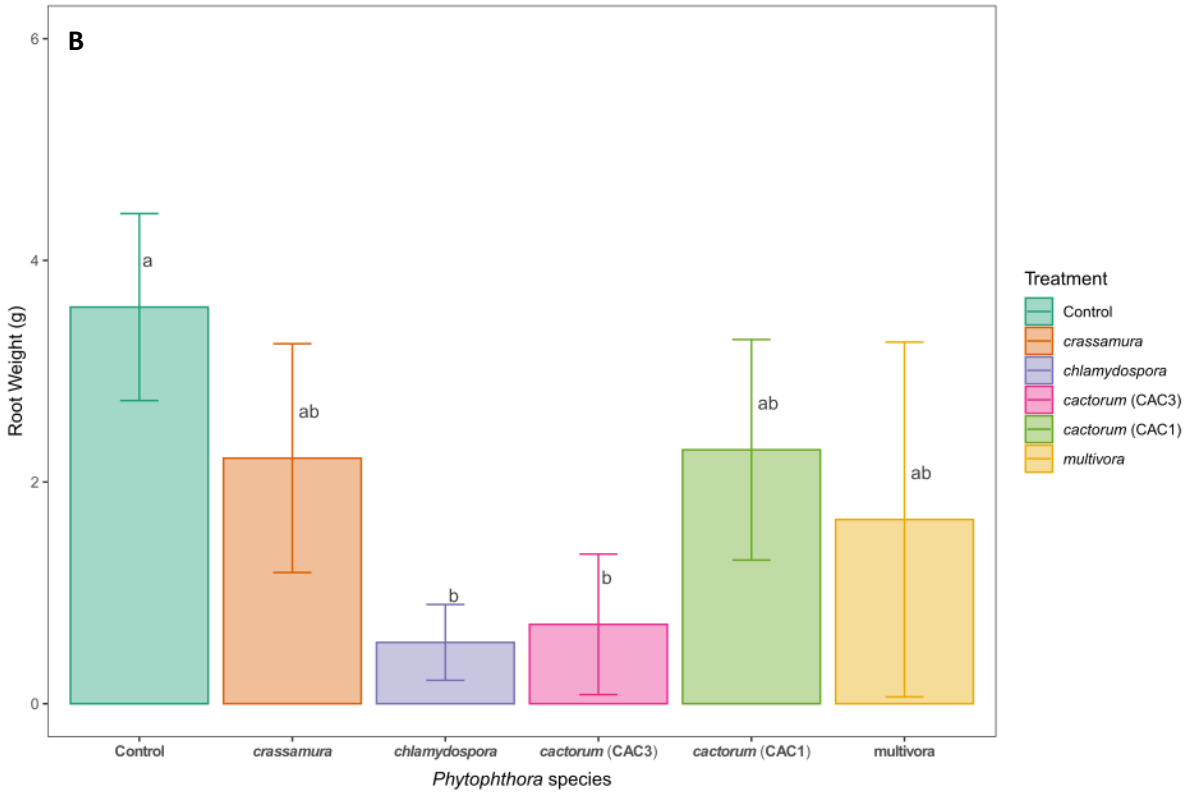


Figure 3.3. Mean total dry root biomass of 1-year-old seedlings of *Adenostoma fasciculatum* (A) *Eriogonum fasciculatum* (B) after 4 months growth in soil infested with *Phytophthora* spp. obtained in this study. Different letters above bars indicate significant differences based on Tukey's HSD test (P = 0.05). Bars represent standard errors

Across all plant species, *P. multivora* had on average the highest frequency of isolations and was retrieved with baiting from all the inoculated individuals (Table 1). CAC3 was isolated in a higher frequency than CAC1, with both haplotypes being retrieved more frequently from crown and stems. *P. chlamydospora* had the lowest isolation frequency of all the *Phytophthora* spp. from plant tissues. However, *P. chlamydospora* was baited from all plant species, with the highest frequency on *E. fasciculatum*, although it was not retrieved from any plant tissue.

Despite the absence of mortality in inoculated *S. mellifera* plants, *Phytophthora* spp. were relatively frequently isolated from fine and primary roots (Table 3.2). On the other hand, *A. fasciculatum* and *E. fasciculatum* had a higher frequency of isolations from fine and primary roots. *Er. crassifolium* had the lowest observed frequency amongst all inoculated plant species but *Phytophthora* was isolated from baits 73.33% of the time. The lowest isolation frequency for all four plant species were from fine roots.

Table 3.1. Cumulative percent recovery of *Phytophthora* spp. from different plant tissues post inoculation according to species; *P. cactorum* (CAC1 and CAC3), *P. chlamydospora*, *P. crassamura*, and *P. multivora.* Inoculations were made on one-year old plants of S*alvia mellifera, Adenostoma fasciculatum, Eriogonum fasciculatum* and *Eriodictyon crassifolium*.

***Phytophthora* species**	**Fine roots (%)**	**Primary roots (%)**	**Crown (%)**	**Stem (%)**	**Bait (%)**
cactorum (CAC1)	46.38	62.91	67.91	62.91	86.66
cactorum (CAC3)	40.55	66.041	80.41	72.08	80
chlamydospora	25	37.5	45	20	80.41
crassamura	33.33	40	35	25	64.58
multivora	47.5	67.08	78.33	72.08	100

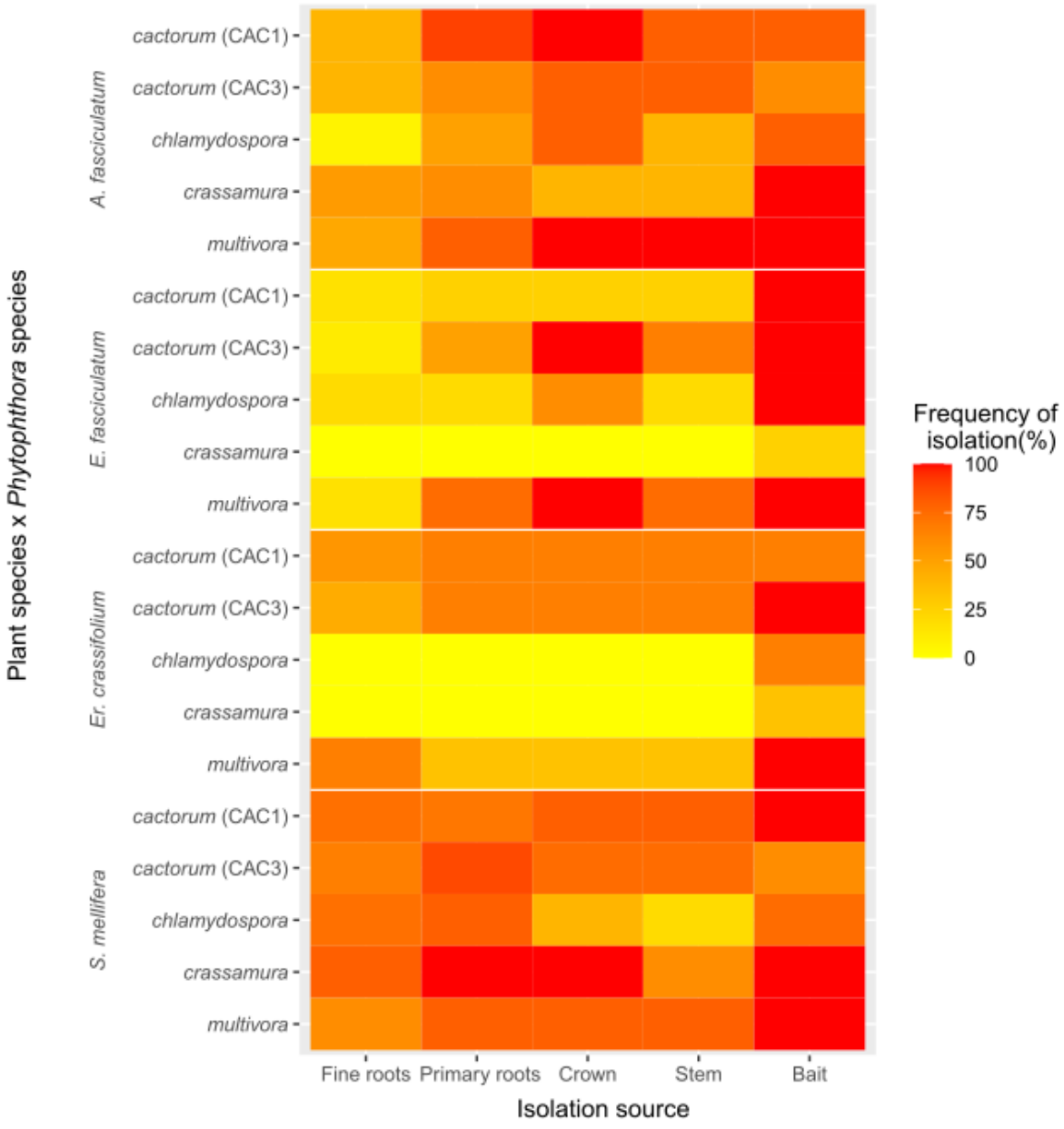


Figure 3.4. Heat map of isolation frequency of *Phytophthora* species six-month post inoculation of 1-year-old seedlings of *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Eriodictyon crassifolium* and *Salvia mellifera*. Isolations were attempted from fine roots, primary roots, crown, and stem tissue. Baiting with pears were also

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attempted at the end of the trail to determine infection. Darker orange colors indicate a higher frequency rate.

Table 3.2. Cumulative percent recovery of *Phytophthora* spp. from different plant tissues post inoculation according to inoculated plant species S*alvia mellifera, Adenostoma fasciculatum, Eriogonum fasciculatum* and *Eriodictyon crassifolium*. Inoculations were made by using *P*. *cactorum* (CAC1 and CAC3), *P. chlamydospora*, *P. crassamura*, and *P. multivora.*

Plant species	Fine roots (%)	Primary roots (%)	Crown (%)	Stem (%)	Bait (%)
Adenostoma fasciculatum	37.33	68	80	68	84
Eriodictyon crassifolium	33.33	33.33	33.33	33.33	73.33
Eriogonum fasciculatum	12.88	34	57	37.33	85
Salvia mellifera	70.66	83.5	75	63	87

DISCUSSION

In the current study it was demonstrated that isolates of two ITS haplotypes of *P. cactorum*, *P. multivora*, *P. crassamura* and *P. chlamydospora*, all isolated from the arid lands of ANF, were able to cause disease on plants of *A. fasciculatum*, *E. fasciculatum*, *Er. crassifolium* and *S. mellifera* under greenhouse conditions. This is the first report of pathogenicity of *Phytophthora* spp. towards representatives of the chaparral community in California and one of the few reports of plant pathogens in this Mediterranean vegetation type.

P. crassamura was amongst the most widespread *Phytophthora* species detected in the ANF in the 18-21 surveys. Despite the wide distribution of the tested *Phytophthora* species, it was amongst the least aggressive. Compared to *P. multivora* and *P. cactorum*, and across all four tested plant species, *P. crassamura* has significantly lower root severity and lower frequency of recovery from fine roots, primary roots, crown and stem tissue. *P. crassamura* is a recently described *Phytophthora* species, associated as one of the causal agents of the Maqui decline in Sardinia Italy (Scanu et al., 2015, Bourret et al. 2022). *P. crassamura* was previously identified as *P. megasperma*, an older described species, with a long history in California agriculture, with estimations that it may have been in the State for at least 100 years (Bourret et al. 2022). Recent detections have associated this species with nurseries and restoration areas in Northern California (Sims et al. 2019), and as an aggressive root pathogen of *Diplacus aurantiacus* (Marques et al. 2022). Although in northern California *P. crassamura* is considered a primary pathogen in failed restoration areas (Sims and Garbelotto, 2021), the role of *P*. *crassamura* in drier Southern California landscape is still unknown, but should be further investigated due to its low, but still present ability to develop resistant structures and cause root damage.

Not only *P. crassamura* has a historical association with California, but also *P. cactorum* and *P. multivora* are amongst the most detected *Phytophthora* species in the state, with *P. cactorum* amongst the most frequent in horticultural and forest settings (Bourret et al., 2022). In this trial, *P. cactorum and P. multivora* were the most aggressive tested species, with *A. fasciculatum* being highly susceptible to both species, causing a significant loss of root biomass and 100% mortality rate. Recovery post inoculation had

a higher frequency on crown and stems, rather than finer and primary roots. In the 2018-21 ANF surveys, these species were also strongly associated with upland chaparral areas, capable of establishing in drier areas thanks to their homothallic nature, capable of producing oospores, a sexual resistance structure (for more details review chapter two).

Five distinct ITS haplotypes of *P. cactorum* are present across California (Bourret et al. 2022). Of those five, two of them were detected amongst the *P. cactorum* isolates of the ANF survey, CAC1 and CAC3. CAC3 isolates belonged to a larger worldwide linage related with an apple-oak linage, present in Czechia, Germany, Japan, The Netherlands, New Zealand, Russia, the UK and the USA. This suggests that the apple–oak lineage is moving locally and worldwide through horticulture (Bourret, et al. 2022). CAC1 has a more limited distribution, being only found in natural areas and streams of California. In the current study, while both tested haplotypes did not vary in the amount of root biomass loss of *A. fasciculatum*, root mass from plants inoculated with CAC3 was significantly lower than plant CAC1 in *E. fasciculatum*. CAC3 had a higher isolation frequency in both stems, crowns and stem, thus overall this haplotype is more aggressive than CAC1. Hinting to a possibility that that CAC3, as a more cosmopolitan world linage, is an introduced haplotype in California (Bourret et al. 2022). While CAC1, with more mild symptoms and aggressiveness, is a haplotype that has been present in the state for longer and potentially native to the California (Bourret et al. 2022).

Marques et al. (2022) tested pathogenicity of *P. multivora* on *Ceanothus thyrsifloris* and *Frangula californica,* finding both plant species susceptible to this pathogen and with significantly more aggressive in stems of *F. californica*. Although mortality was only

observed on *A. fasciculatum* and *E. fasciculatum*, across all plant species, isolations were more predominant from crown tissue, result that are in accordance with Marques et al. (2022), which indicated that *P. multivora* is more aggressive as a stem pathogen than a root pathogen. Isolations frequency of *P. multivora* from fine roots and primary roots in the current study were lower than *P. crassamura* and *P. chlamydospora*, probably due to this species being more aggressive and killing root tissue faster and thus lowering the possibility of isolation due to the low saprophytic ability of *Phytophthora* spp. (Jung et al. and Erwin and Ribeiro, 1996). Due to the aggressiveness of *P. multivora* towards *A. fasciculatum* and *E. fasciculatum*, and the potential asymptomatic nature of this species on *S. mellifera* and *E. crassifolium*, native plant nurseries and restoration managers should consider this species as a high risk for their outcome goals.

Several ITS clade 6 species were retrieved from the ANF sampled areas, with the majority detected in riparian areas and *P. gonapodyides* and *P. crassamura* as the most frequent *Phytophthora* species in the survey (Chapter one). In California, clade 6 species are common in natural areas and streams, and potentially indigenous, based on high levels of intraspecific diversity (Bourret et al. 2018 Bourret et al. 2022) and with a behavior mostly associated with a saprophytic lifestyle (Brasier et al. 2003; Hansen et al. 2012). *P. chlamydospora,* a member of clade 6, was also one the few species, in addition to *P. crassamura*, that was detected in drier chaparral areas of ANF. Thus, it is important to confirm pathogenicity of species mostly associated with a saprophytic lifestyle but are still encountered proximate to chaparral plant species. *P. chlamydospora*, of all the tested *Phytophthora* species, had the lowest mortality

frequency on both *E. fasciculatum* and *A. fasciculatum*. Additionally, *P. chlamydospora* had the lowest stem and crown isolations, but was still recovered over 30% of the time from root tissues, even from 75% of the inoculated *S. mellifera* plants. Relatively, this species was the least aggressive amongst the tested *Phytophthora* species, but still proved that chaparral members are potentially susceptible to *P*. *chlamydospora* and can still reside in the soil without causing symptoms. On worldwide scenario, detections of *P. chlamydospora* have been associated with nursery settings and plantations (Jung and Blaschke 2004, Yakabe et al. 2009m, Brasier et al. 1993), but in the majority of the cases as a member of the *Phytophthora* communities present in natural areas (Bregant et al. 2016, Jung et al. 2004, Jung et al. 2018) with a few cases of being associated with disease, recovered from cankers and roots of native (Reeser et al. 2008, Navarro et al. 2014, Sims et al. 2014) and nut trees (Browne et al. 2020, Türkölmez et al., 2016).

Due to the arid climatic conditions present in ANF, typical of a Mediterranean region, the presence of disease conducive conditions for oomycetes are rare. However, considering the current results, that four of the most common associated *Phytophthora* species detected in chaparral upland areas can cause disease on main representatives of the chaparral community, there is a risk to local endemic flora and potentially affect restoration efforts in the area. The fact that these species can reside in rhizosphere soil without causing disease, further increases the risk undetected movement of *Phytophthora* species in nurseries, and the potential inadvertent introduction into natural areas.

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