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Phytophthora Now Found in Drier Ecosystems of Marin County, CA: Is Chaparral *Phytophthora*'s Next Target?

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Abstract

Sudden Oak Death (SOD) is a devastating plant disease in California that has killed many native trees. *Phytophthora ramorum*, the causal agent of SOD, belongs to the oomycetes (or 'water molds') and thrives in moist environments. Symptoms similar to those caused by *P. ramorum* were recently detected on manzanitas (genus *Arctostaphylos*) in chaparral communities in drier and sunnier areas in Marin County, CA. To better understand the pathogen's spreading ability, it is important to determine if the symptoms seen in chaparral plants are indeed *P. ramorum.* Is this pathogen spreading into drier environments and if so, what are the potential consequences? During this internship, plant disease symptoms of chaparral and other native plants of Marin County, CA were monitored, with a focus on manzanitas for the National Ornamentals Research Site at Dominican University of California (NORS-DUC). The length of the internship was three semesters, from the summer of 2019 through the spring of 2020. Monitoring included data collection, photo-documentation, and leaf sample collection in natural environments throughout the county. Samples were then brought to NORS-DUC for an immunostrip (Agdia) test that detects *Phytophthora* presence in the leaves. Seven out of forty-eight samples collected over a distance of approximately twenty-two miles were infected (15%). For further characterization, symptomatic leaf tissue was plated on selective media to isolate the plant pathogens. Although results are still preliminary, data collected may provide some evidence that *Phytophthora* might spread to new environments and threaten the predominant plant community of California: chaparral.

Keywords: *Phytophthora*, Chaparral, Sudden Oak Death

1. Introduction

In recent years, Sudden Oak Death has devastated native woody plant species in forests along the coast of Marin County[1] and some non-natives in the nursery industry. Symptoms include leaf spots, dieback and sometimes death of the entire plant. Similar symptoms were discovered recently in chaparral plants. Chaparral is a shrubland or heathland plant community with no or only very few trees, and is found primarily in California. *Arctostaphylos* sp., or manzanitas, are among the most common chaparral plants, with more than sixty species and sub-species. In order to better understand the mechanism of *Phytophthor*a's spreading ability, it is important to determine if the symptoms seen in manzanitas and other chaparral plants are caused by *Phytophthora*. Being an oomycete, or water mold, it is unexpected to see this type of pathogen in chaparral. However, the chaparral of Marin is mostly maritime chaparral which means it is within reach of the fog belt provided by the Pacific Ocean. This consistent moisture from fog combined with the rainier winters of the past few years could provide a well-suited habitat for this pathogen. It is also important to note that similar disease symptoms could be caused by other pathogens or abiotic factors such as drought.

Previously this pathogen has been documented in Marin County in large infections of *Umbellularia californica* (California Bay Laurel), as displayed in maps provided by UC Berkeley (Fig.1)[2]. The health of the California Bay Laurel is not impacted by the pathogen, but this species provides the pathogen with a prime habitat to develop inoculum on its leaves. The inoculum spreads to other native plants, like the more susceptible *Notholithocarpus densiflorus* (Tan Oaks) and *Quercus agrifolia* (Coast Live Oak) that have been devastated by *P. ramorum* in Marin County over the last few decades[3].

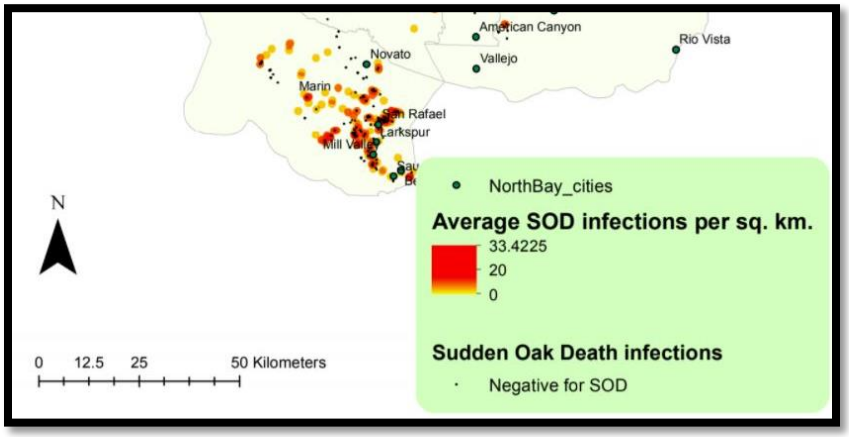

Figure 1. Density map of trees affected by Sudden Oak Death in Marin County. Provided by UC Berkeley SODmaps of 2019[2].

However, little data exists on *Phytophthora* in chaparral plants such as manzanita in Marin County, and there is some doubt as to whether water molds are capable of survival in a chaparral community.

Bolinas Ridge is one location where *P. ramorum* and the related species *P. cinnamomi* were detected in several manzanita species and other native chaparral plants[1]. With this discovery came the question: "Is Sudden Oak Death Becoming a Threat to California's Chaparral Ecosystem?"[5]. The objective of this field monitoring project was to provide further evidence of *Phytophthora*'s presence in the chaparral throughout Marin County, California.

2. Materials & Methods

2.1. Field Monitoring

This year-long field monitoring internship project for NORS-DUC began in May of 2019 and ended in May of 2020. All field-sampling was done between July and November of 2019. Manzanitas (genus *Arctostaphylos*) were the most monitored and sampled plants of the project. A few other plants were sampled that displayed similar symptoms and included: *Chrysolepis chrysophylla* (Golden Chinquapin), *Quercus chrysolepis* (Canyon Live Oak), and *Baccharis pilularis* (Coyote Brush). For identification of particular species Calflora.org[6] was used out of the field, while the Field Guide for Manzanitas was used during field monitoring[7]. Field monitoring sites were chosen by researching where manzanitas grow using Calflora.org. The areas monitored in Marin County include Olompali State Park, China Camp State Park, Tomales Bay State Park, San Pedro Mountain Preserve, Gary Giacomini Open Space Preserve, Cascade Canyon Open Space Preserve, Tennessee Valley of the Golden Gate National Recreation Area (GGNRA), Marin Headlands (GGNRA), and multiple parts of Mount Tamalpais (State Park and Marin Municipal Water District). Symptoms of interest were similar to those shown by plants infected by *P. ramorum*: leaf necrosis, branch dieback, a burnt appearance, and full plant death.

Monitoring was conducted by one person and involved hiking through an area to find manzanita aggregations. Once found, the number of plants was estimated, and the overall health of the plants was noted. First, a small group of plants was counted and assessed for symptom severity. Next, at a high vantage point, an overall estimation of the plants in the area was done using the initial count as a reference. To minimize error, multiple vantage points were used including from within the manzanita patches. Then, a generalized percentage of the severity of symptoms was made (Table 1). The SODmap application provided by UC Berkeley was used for risk assessment for *Phytophthora* presence and

provided the coordinates of where the monitoring took place or samples were taken[8]. A generalized symptom severity ranking system was created by Dr. Schweigkofler for a simplified notation, as can be seen in Table 1.

Table 1. Symptom severity scale associated description.

	Symptom severity scale description
0	Healthy (no necrosis)
1	Limited (symptoms on 1-2 branchlets)
2	$\leq$ 50% of plant = necrotic
3	50-90% of plant = necrotic
4	Dead (few necrotic leaves to no leaves)

Symptomatic plants with necrotic leaves ($\geq$1) were sampled at the transitional branch between living and dead tissue. This means half of the cutting had green foliage and red bark, while the other half had necrotic leaves and stems. Off-trail ($\geq$10 feet) at each location 5 or 6 samples were collected, and during the project 48 samples were collected total. Sterilization of cutting tools was completed between samples with 70% alcohol. In areas where there were many manzanitas, an estimation of the number was recorded with the severity of the plants' symptoms summarized as percentages in the "*Arctostaphylos* Hotspot Summary" described in the first section of the results.

2.2. Immunostrip Test (Agdia)

Manzanita samples were brought to the NORS-DUC lab to be tested for *Phytophthora* presence, using an established immunostrip method[9]. Symptomatic leaves were cut into small pieces and muddled within the mesh lining of the buffer solution packet. The buffer solution helps extract *Phytophthora* antigens from the leaf matter, while the small strip of paper that is inserted into the side of the packet (outside of the mesh lining containing the leaf matter) has antibodies on it that recognize the plant pathogen's antigens. First, a bold red line appears on the strip to show that the test is functioning properly. Then, if positive, a pink line appears below the first line. The thickness of the second line also estimates the amount of *Phytophthora* present, so the thicker the line the more *Phytophthora* present. If negative, no second line appears, and no *Phytophthora* is present. To be clear, the test is for the detection of the genus *Phytophthora* but is not species-specific. Even if the samples are positive for *Phytophthora*, it is not certain that *P. ramorum* (which causes Sudden Oak Death) is present, which is why further characterization is inherent. The test also cross-reacts with some species of *Pythium*, a genus of mainly soil-borne plant pathogens closely related to *Phytophthora*.

2.3. Isolation of Pathogen

Leaf samples were surface-sterilized, placed in a medium, and incubated for 1 week at 20° C before being refrigerated. The process of surface sterilization included submerging each leaf sample for one minute in each of three beakers containing water, 70% ethanol, then water. Between each submersion, excess liquid was removed by gently drying it with a paper towel. For plating, the samples were cut into small pieces (approximately 5 mm by 5 mm) and tucked into the medium in the pattern of a five-sided die.

The positive or negative immunostrip results dictated which medium the leaf samples should be put in. Positive samples were plated in a PARPH-V8 medium, a selective medium for *Phytophthora.* If the test was negative, it did not necessarily mean that *Phytophthora* was absent in the leaves sampled. The pathogen could have been in other parts of the plants other than the leaves: for example, the root system. These samples were then placed in a petri dish filled with the medium PDA, which is a more general medium for the isolation of plant pathogenic microbes.

3. Results

3.1. *Arctostaphylos* Hotspot Summary and Field Data

Hotspots are field monitoring areas that have relatively easy access, with an abundance of plants of interest (*Arctostaphylos*). As mentioned, the number of plants was estimated in these areas. Estimating plants is difficult, but manzanitas present particular challenges because of their intertwining growth pattern and shared root systems. Manzanitas often grow in dense aggregations that are challenging to navigate through while, the sharing of roots brings into question how individual manzanitas are defined, which is why the estimation aspect of this method is emphasized. Each Hotspot Summary had a topographical map that shows how to access the area by hiking trail. Pictures, a generalized percentage of symptom severity, and what other plant species were growing in the immediate area were also included.

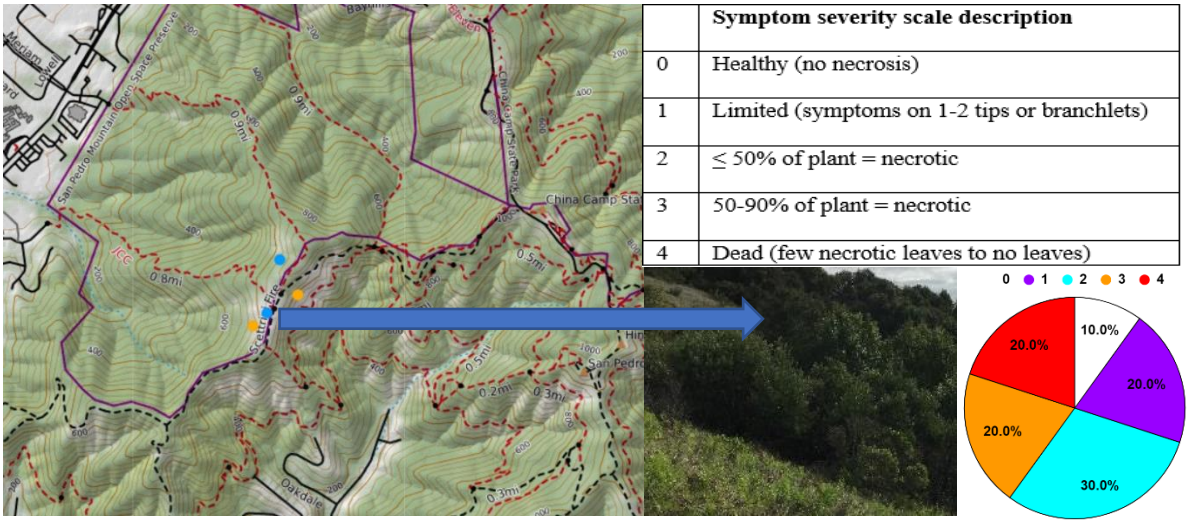

Figure 2. San Pedro Mountain Open Space Preserve *Arctostaphylos* Hot Spot Summary. Left = monitoring location map. Upper right = symptom severity scale. Middle = photo-documentation of the overall monitoring site. Bottom right = generalized percentage of 1000+ manzanitas in the area related to symptom severity scale (0-4).

Although there are 18 total *Arctostaphylos* Hotspot Summaries, for brevity only one example of what they describe is provided in Fig. 2.

Table 2. Data collected from selected Marin County sites with samples & immunostrip method results; summer-fall 2019.

Sample #	Date	Location	Species	Symptom Severity (0-4)	Agdia Test (+/-)
1	7/31/19	China Camp State Park	*Arctostaphylos manzanita*	2	-
2	7/31/19	China Camp State Park	*A. glandulosa* ssp. *cushingiana*	2	-
3	7/31/19	China Camp State Park	*A. manzanita*	2	-
4	7/31/19	China Camp State Park	*A. manzanita*	2	-
5	7/31/19	China Camp State Park	*A. manzanita*	2	-
6	7/31/19	China Camp State Park	*A. manzanita*	2	-
7	8/1/19	Mt. Tam Rock Springs Trailhead	*A. glandulosa*	3	-

8	8/1/19	Mt. Tam Rock Springs Trailhead	*A. montana* ssp. *montana*	1	-
9	8/1/19	Mt. Tam Rock Springs Trailhead	*A. montana* ssp. *montana*	2	-
10	8/1/19	Mt. Tam Rock Springs Trailhead	*Chrysolepis chrysophylla*	1	-
11	8/1/19	Mt. Tam Rock Springs Trailhead	*A. montana* ssp. *montana*	1	-
12	8/5/19	Olompali State Park	*A. manzanita* ssp. *manzanita*	2	-
13	8/5/19	Olompali State Park	*A. manzanita* ssp. *manzanita*	1	-
14	8/5/19	Olompali State Park	*A. manzanita* ssp. *manzanita*	3	-
15	8/5/19	Olompali State Park	*A. manzanita* ssp. *manzanita*	3	-
16	8/5/19	Olompali State Park	*A. manzanita ssp. manzanita*	2	-
17	8/5/19	Olompali State Park	*A. manzanita ssp. manzanita*	2	-
18	9/6/19	Mt. Tam Troup 80 & TCC Trails	*A. glandulosa*	3	-
19	9/6/19	Mt. Tam Troup 80 & TCC Trails	*Chrysolepis chrysophylla*	3	-
20	9/6/19	Mt. Tam Troup 80 & TCC Trails	*A. nummularia*	3	-
21	9/6/19	Mt. Tam Troup 80 & TCC Trails	*Quercus chrysolepis*	1	-
22	9/6/19	Mt. Tam Troup 80 & TCC Trails	*A. glandulosa*	3	-
23	10/3/19	Tennessee Valley/Oak Springs Trail	*A. glandulosa*	2	-
24	10/10/19	Tennessee Valley/Fox Trail	*Baccharis pilularis*	2	-
25	10/10/19	Tennessee Valley/Fox Trail	*Baccharis pilularis*	3	-
26	10/18/19	Marin Headlands/Bobcat Trail	*A. glandulosa ssp. glandulosa*	2	-
27	10/18/19	Marin Headlands/ Bobcat Trail	*A. glandulosa ssp. glandulosa*	3	-
28	10/18/19	Marin Headlands/ Bobcat Trail	*A. glandulosa*	3	-
29	10/20/19	Santa Venetia/ Woodoaks Trail	*A. manzanita ssp. manzanita*	2	-
30	10/20/19	Santa Venetia/ Woodoaks Trail	*A. manzanita ssp. manzanita*	2	-
31	10/24/19	Santa Venetia/ Woodoaks Trail	*A. manzanita ssp. manzanita*	1	+
32	10/24/19	Santa Venetia/ Woodoaks Trail	*A. manzanita ssp. manzanita*	3	+
33	10/24/19	Santa Venetia/ Woodoaks Trail	*A. manzanita ssp. manzanita*	2	-
34	10/25/19	Inverness/ Jepson Trail Loop	*A. virgata*	3	-
35	10/25/19	Inverness/ Jepson Trail Loop	*A. virgata*	3	-
36	10/25/19	Inverness/ Jepson Trail Loop	*A. virgata*	2	-
37	10/25/19	Inverness/ Jepson Trail Loop	*A. virgata*	2	-
38	10/25/19	Inverness/ Jepson Trail Loop	*A. virgata*	2	+
39	11/7/19	Mt. Tam So. Eastern Ridge	*A. canescens*	2	-

40	11/7/19	Mt. Tam So. Eastern Ridge	*A. canescens*	3	+
41	11/7/19	Mt. Tam So. Eastern Ridge	*A. glandulosa*	3	-
42	11/7/19	Mt. Tam So. Eastern Ridge	*A. glandulosa*	2	+
43	11/7/19	Mt. Tam So. Eastern Ridge	*A. glandulosa*	3	+
44	11/8/19	Lagunitas/San Geronimo Fire Road	*A. virgata*	3	-
45	11/8/19	Lagunitas/San Geronimo Fire Road	*A. virgata*	3	-
46	11/8/19	Lagunitas/San Geronimo Fire Road	*A. glandulosa*	3	+
47	11/8/19	Lagunitas/San Geronimo Fire Road	*A. virgata*	2	-
48	11/8/19	Lagunitas/San Geronimo Fire Road	*A. virgata*	2	-

3.2. Immunostrip Test (Agdia)

Positive results for *Phytophthora* using the immunostrip method were found in samples from the southeastern ridges of Mount Tamalpais in Mill Valley; Santa Venetia on the Woodoaks Trail; and Inverness on the Jepson Loop Trail near Heart's Desire beach[9]. 7 out of 48 immunostrip test results were positive (Figure 3).

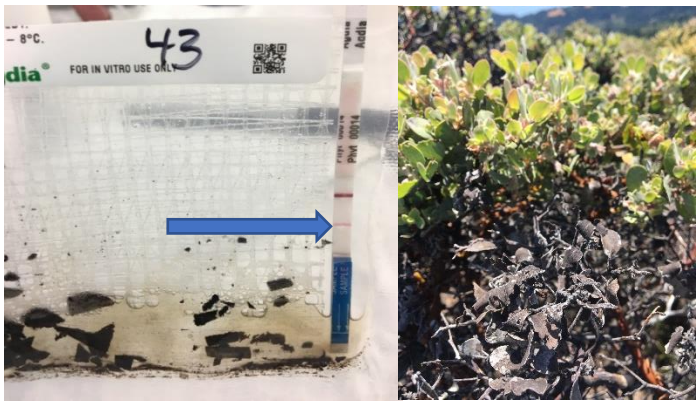

Figure 3. Left = Positive immunostrip test for *Phytophthora*. Right = Sample 43 collected from manzanita on the southeastern ridges of Mt. Tamalpais.

Infected samples showed a pink line below the initial red line that appears on the immunostrip when placed in the muddled leaf and buffer solution as seen in Figure 3. Samples 40, 42, and 43 were positive and displayed a dark pink line which could potentially mean that the infection is more developed in these plants. Samples 31, 32, 38, 46 were also positive but less *Phytophthora* was present since only a light pink line was visible.

3.3. Isolation of Pathogens

Phytophthora isolation from manzanita is a difficult process, and *P. ramorum* was not visibly present in any of the growth. Six negative yet symptomatic samples from China Camp State Park had negative immunostrip (Agdia) test results; yet, in each petri dish there is a variety of color, number, and stage of development of the microbe colonies. Figure 4 shows pictures of the diversity of microorganisms growing *within* the different leaf samples, considering the surfaces were sterilized.

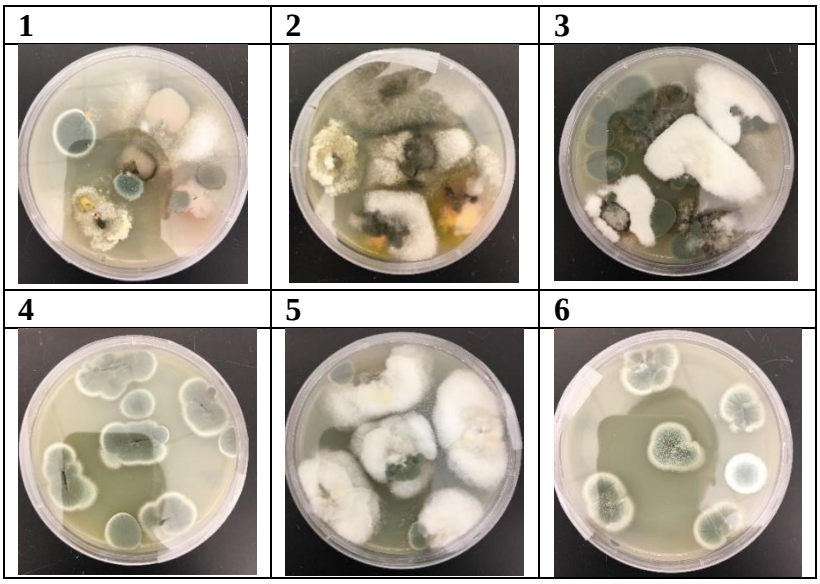

Figure 4. Microbe colonies developed from a manzanita leaf sample in a PDA medium. Numbers 1-6 relate to samples collected from China Camp State Park noted in Table 2.

Although microbial colonies were not identified to species level, the colors represent the following general classification: *pink* is yeast, *green and white* is a *Penicillium*-type mold, and *black* is also a mold. The most important thing to note here is that any *Phytophthora*, if present, was overgrown by a variety of other microbes.

4. Discussion

This project provided a generalization of plant disease symptoms found in chaparral areas—focusing on manzanitas—in Marin County, Ca. Monitoring plant disease symptoms of chaparral in Marin County is important in maintaining California's native natural environments. *Phytophthora* is a genus of interest due to its devastation in natural environments and the nursery industry. Approximately 22 miles were covered throughout the year-long internship. Surprisingly, 7 out of 48 samples collected were infected (14.6%). Each of those infected samples was collected during fall between October and November of 2019, which could relate to *Phytophthora'*s ability to sporulate in wet conditions and spread through water splash and wind. The infected samples were also collected within range of the coastal fog, which increases humidity and therefore improves conditions for water molds such as *Phytophthora* sp.. This concept is most interesting in the context of samples 40, 41, and 43 because they were collected the furthest off-trail on an exposed ridgeline on the southeast side of Mt. Tamalpais. Despite collecting all of the samples off-trail, the further distance off-trail further minimized the potential of anthropogenic influence. Also, this area does not have any California Bay Laurel growing above the ridgeline or among them, which is interesting, because *P. ramorum* growing on Bay Laurel leaves is considered the most important source of inoculum for the spread of this pathogen. Below the

field monitoring site is a mixed woodland containing California Bay Laurel, and the fog often moves up the ridgeline which is capable of spreading the inoculum up the ridgeline with it as seen in Fig. 5.

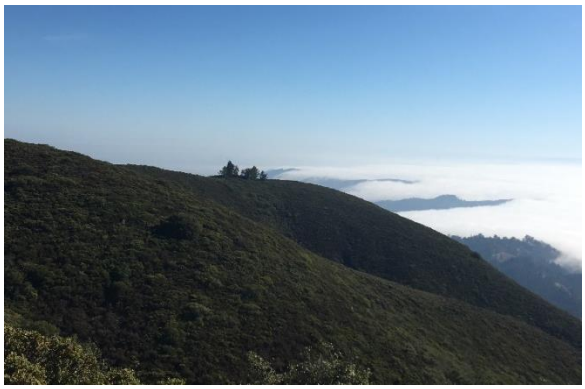

Figure 5. A southeastern ridge of Mt. Tamalpais taken November 7, 2019. Maritime chaparral with heavy fog.

Plant samples were collected when symptoms were similar to those shown in plants infected with *P. ramorum*, and symptom severity appeared greater than one (Table 1). For further characterization, symptomatic leaf tissue was plated on selective media to isolate the plant pathogens. Among 6 symptomatic leaf samples from China Camp State Park inoculated in a PDA plate medium a variety of microbial colonies was isolated, although most likely endophytic or opportunistic fungi not primarily responsible for the symptoms shown. Endophytes are fungi growing in plant tissue without causing disease and are commonly isolated from leaves. Although results are still preliminary, I was able to provide some evidence that *Phytophthora* sp. may spread to chaparral and threaten the native plant community of California. However, it remains unknown whether the infections seen were caused by *P. ramorum*.

A potential consequence of this pathogen moving into chaparral environments is the loss of biodiversity, loss of carbon sequestration by this plant community, and adding potential fuel for wildfires. The loss of carbon sequestration of plant hosts of *Phytophthora* has already been studied: "…carbon sequestration can be threatened by regional scale disturbances including insect and pathogen outbreak…potential [negative] impacts of climate change on the severity and frequency of outbreaks and the acceleration of exotic insect and pathogen introduction resulting from global trade…" [10]. This research did not include manzanita and chaparral plants which may need reconsideration, since chaparral covers an estimated five percent of California. Therefore, a further investigation of the impact of *Phytophthora* on manzanitas and other chaparral plants should be considered.

5. Recommendations

Future monitoring of chaparral ecosystems for plant disease is needed in Marin County, California. Monitoring other abiotic factors of chaparral should also be considered. The addition of more monitoring locations throughout the county is also necessary. Returning to the locations where *Phytophthora* infected manzanitas were found is suggested after the rainy season, which is the time of the year when most of the transmission of oomycetes happens. It is possible that the seasonal dryness during the time of sample collection was the reason for the failed attempt to isolate *Phytophthora* from manzanita samples in the lab, adding to the already difficult identification of this pathogen. DNA analysis using BLAST, or other similar sequencing methods is also suggested for the identification of symptoms seen in manzanitas and other chaparral plants. Phylogenetic analysis of the origin of the *Phytophthora* found in this plant community would develop an epidemiological explanation for how the future spread of this pathogen can be expected. Plant identification accuracy is difficult with *Arctostaphylos*, or manzanitas. Further development of these skills or collaboration with a botanist will be necessary for continuation of this project.

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