Citizen science uncovers *Phytophthora ramorum* as a threat to several rare or endangered California manzanita species.

Matteo Garbelotto[1], Tina Popenuck[1], Brett Hall[2], Wolfgang Schweigkofler[3], Francesco Dovana[1,4], Ruby Goldstein de Salazar[1], Doug Schmidt[1] and Laura Lee Sims[1,5]

[1]Department of ESPM, University of California, 54 Mulford Hall, Berkeley, CA 94720, USA

[2] U.C. Santa Cruz Arboretum California Conservation Gardens, 1156 High Street, Santa Cruz, CA 95064, USA

[3]Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, CA 94901, USA

[4]Department of Life Sciences and Systems Biology, University of Torino, Viale P.A. Mattioli 25, I-10125 Torino, Italy.

[5]School of Ag Sciences and Forestry, Louisiana Tech University, 211 Reese Hall, Ruston, LA 71270, USA

Matteo Garbelotto, matteog@berkeley.edu Corresponding author

Abstract

The Sudden Oak Death or SOD Blitzes consist of yearly surveys led by citizen scientists designed to map the distribution of *Phytopthora ramorum*, causing the forest disease called SOD, across Northern California. During the 2017 Santa Cruz County SOD Blitz, six rare or endangered *Arctostaphylos* (manzanita) species were found to be possibly symptomatic for the first time. Symptoms included branch cankers and associated canopy mortality, and affected multiple individuals per species. Isolates of *Phytophthora ramorum* were obtained from each of the six species and through a 30-day long inoculation experiment on live plants, Koch's postulates were completed for each one of them, conclusively determining that they all are hosts of this pathogen. Two additional manzanita species were later found to be apparently symptomatic in Marin County. Inoculations on detached branches using an isolate of *P. ramorum* obtained from one of the six rare species from Santa Cruz County were successful, suggesting these two species may also be hosts of *P. ramorum*. Detached leaves of all eight species were also successfully inoculated at U.C. Berkeley in the Fall of 2018 and then again in the Spring of 2019. In these cases, the same isolate was used for all inoculations, in order to obtain information on the comparative susceptibility of the eight species in question. Both branch and leaf inoculations identified significant interspecific differences in susceptibility. The production of sporangia was low on all species, but it was not zero, suggesting that sporulation may cause within-plant and limited across-plant contagion, especially in rainy years.

45

46

47 **Keywords**: *emergent disease, endemic disease, novel host, Sudden Oak Death,*

48 *threatened plants*

49

Introduction

The pathogen *Phytophthora ramorum* (Werres et al. 2001; Rizzo et al. 2002) is the cause of the notorious deadly forest disease known as Sudden Oak Death or SOD (Garbelotto et al. 2001), and of Ramorum Blight (Grünwald et al. 2008), a less aggressive, but still potentially lethal disease of a range of plants present both in California natural ecosystems and in the ornamental plant industry worldwide. The pathogen was introduced first in ornamental plants sold in Europe and North America, and later it "escaped" infected ornamental plants to invade California and Oregon forests (Croucher et al. 2013) as well as tree plantations and woodlands in the United Kingdom and Ireland (Grünwald et al. 2012). Limited outbreaks have also been reported in France (Schenck et al. 2018), the Netherlands (De Gruyter et al. 2006) and Washington State (USA) (Strenge et al. 2017), while in many other US States and Canada, infestations by the pathogen remain limited to ornamental plant stock or to waterways (Chastagner et al. 2010). Recently, *P. ramorum* was found in Northern Vietnam, where presumably it is native, but the exact sources of North American and European *P. ramorum* populations remain unknown (Jung et al. 2020).

Soon after the isolation of the pathogen from California oaks and tanoaks (Rizzo et al. 2002), a number of other native California plants were found to be hosts for the pathogen, with symptoms ranging from apparently innocuous leaf blotches and spots to progressive die-back of branches, possibly resulting in mortality of infected

plants (Garbelotto et al. 2003). In the majority of cases, these hosts were symptomatic if located in the immediate proximity of California bay laurels and tanoaks, both known to be prolific sporulating hosts for *P. ramorum*. The common manzanita, *Arctostaphylos manzanita*, was listed as one of the hosts for *P. ramorum* in the early paper by Garbelotto et al. (2003) describing the initial discovery of non-oak hosts infected by the pathogen and their confirmation as hosts through greenhouse inoculation experiments. However, later studies of site and climatic factors associated with SOD outbreaks during the initial stages of the invasion by this pathogen, suggested that sites being inhabited by manzanitas (*Arctostaphylos* spp.), being relatively drier and warmer, may not be conducive to pathogen infection (Anacker et al. 2008; Lione et al. 2017; Meentemeyer et al. 2015; Meentemeyer et al. 2004; Venette et al. 2006). As a result, manzanita infection would only be occasional and not widespread within forest ecosystems invaded by the pathogen.

This initial conclusion about the potential impact of *P. ramorum* on species within the genus was important because California is the main center of diversity for the genus, being home to at least 105 species and subspecies of manzanitas (Kauffmann et al. 2015*)*, of which 59 are reported as rare or endangered (Schmid 2002). Many of these manzanita species are present within the *P. ramorum* zone of infestation, spanning 15 California counties (https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/quarantine_map.pdf), thus, these species may currently be at risk of infection.

97

98 In 2017, during the Sudden Oak Death Blitzes, a citizen science program engaging
99 volunteers to map the distribution of *P. ramorum* in California (Meentemeyer et al.
100 2015), many apparently diseased manzanita species present at the arboretum of the
101 University of California Santa Cruz were sampled. Symptoms included leaf spots or
102 blotches and branch dieback. Of eight species tested, six were positive for the
103 pathogen, and all are considered rare or endangered according to the California
104 Native Plant Society inventory of rare and endangered plant species (Schmid 2002).
105 Almost simultaneously, two additional manzanita species were found to be
106 potentially infected by *Phytophthora ramorum* on Mount Tamalpais in Marin
107 County, 130 km north of Santa Cruz.

108

109 This paper describes the experiments conducted to confirm that the six manzanita
110 species found to be infected by the SOD pathogen at the UCSC Arboretum were
111 indeed hosts for *P. ramorum*. The paper describes additional experiments conducted
112 to compare the relative susceptibility and transmission potential of the six UCSC
113 manzanita species and of the two manzanita species from Marin County.
114 Implications are discussed regarding the impact the disease may have on the genus
115 and in particular on endangered coastal manzanita species in California and on
116 other native species.

117

118 **Materials and methods**

119

Collection of plant samples

The 2017 SOD Blitzes included 27 separate surveys conducted at different times across 13 infested (Humboldt, Mendocino, Sonoma, Napa, Solano, Marin, Contra Costa, Alameda, San Francisco, San Mateo, Santa Clara, Santa Cruz, Monterey) and 2 putatively uninfested California counties (Siskiyou and San Luis Obispo) (https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/quarantine_map.pdf). A total of 314 volunteer collectors trained to identify and collect SOD-symptomatic leaves and twigs participated in the program. The number of participants was larger than that, but it is hard to estimate precisely because families and friends usually sign up as single collectors. SOD Blitzes were run between the end of March and mid-June 2017. On March 15th and 16th 2017, 18 volunteers surveyed 555 trees and collected symptomatic leaves from a total of 118 plants in Santa Cruz County, including 22 samples from seven planted manzanita species and 5 samples from native California bay laurels UCSC Arboretum. See Figure 1 for location of plants samples at and near the UCSC Arboretum. Samples were shipped using next day service to UC Berkeley, where they were logged in and processed both using DNA and culture-based testing (see below for diagnostics).

Pathogen diagnostics

Portions of leaf or twig tissue including the transition between lesioned and healthy areas were selected for culturing and DNA testing. Culturing was accomplished by submerging four plant tissue sections about 0.5 x 0.5 cm in size within a Petri dish filled with PARPH *Phytophthora* selective medium (Solel and Pinkas 1984). Plates

were kept in the dark at room temperature (16-22 ^{0}C) and observed after 4, 8 and 12 days for the presence of putative *Phytophthora* colonies. Colonies were identified by their overall morphology and by morphology, clustering and positioning of resting and infectious structures such as chlamydospores and sporangia (Werres et al. 2001). The Internal Transcribed Spacer (ITS) and a portion of the cytochrome oxidase 1 (COX I) gene were also sequenced to confirm the morphological identification and to determine the lineage of the cultures (Rizzo et al. 2002; Kroon et al. 2004). DNA was extracted from lesioned plant tissue using the ROSE extraction protocol (Osmundson et al. 2013). DNA was then diluted 1:10, and PCR was performed using the *P. ramorum* specific Taqman assay described by Hayden et al. (2006).

Completing Koch's postulate

Six manzanita species, namely *Arctostaphylos montereyensis, A. silvicola, A. pumila, A. pilosula, A. morroensis* and *A. hooverii* were reported as hosts of *P. ramorum* for the first time. In order to confirm their status as hosts of the pathogen, Koch's postulates had to be completed. This was accomplished by taking pathogen isolates obtained from each one of the hosts, re-inoculating them on the same host, documenting the development of symptoms similar to the ones observed in nature, and, finally, re-isolating the pathogen from each inoculated plant. Inoculations were performed by gently scraping the bark, placing a plug of inoculum on the wound, securing it with a strip of parafilm wrapped around the inoculation point, and covering it with a strip of aluminum tape for protection. Because all six species are

166 regarded as either rare or endangered according to the California Native Plant
167 Society (CNPS), the number and type of replicates had to be carefully selected to
168 minimize the impact of the experiment on dwindling populations of these species.
169 Details of the inoculations, including isolates employed, number and source of plant
170 replicates, lesion size and branch diameter are provided in Table 2.
171
172 Inoculations were performed on healthy branches, approximately 1.5-2 m long, in
173 15-year-old bushes on the UCSC Arboretum grounds for the following species: *A.*
174 *pilosula, A. morroensis* and *A. hooverii*. On February 13th 2018, four branches of each
175 of the three species above were inoculated with the pathogen and four were mock
176 inoculated using a plug of agar, rather than a plug of inoculum. All inoculated
177 branches were bagged with large white trash bags to ensure no inoculum from the
178 outside would reach the branch, and to ensure sporangia produced by the
179 inoculation would not infect neighboring branches. Thirty days post inoculation, all
180 inoculated branches were taken to the lab at U.C. Berkeley still enclosed in their
181 bags. Measurements were taken and pathogen isolations were completed in the lab
182 before autoclaving the plant material and disposing of it. The size of lesions was
183 determined by gently scraping the bark above and below the inoculation point and
184 exposing the necrotic area caused by pathogen colonization under the bark.
185 Scraping was done moving further away from the inoculation point in all directions,
186 until the edges between the necrotic lesions and healthy tissue were clearly visible.
187 A ruler was used to determine the maximum linear expansion of the lesion starting
188 at the uppermost edge of the exposed and visible lesion and ending at the

lowermost edge of the same lesion. Only lesions that resulted in a positive pathogen reisolation were included in the analyses.

Because *Arctostaphylos hooverii* was inoculated with the wrong isolate, the experiment had to be repeated between November 13th and December 13th, 2018.

On February 14th 2018, four plants potted in one-gallon containers of each of the following three species were inoculated: *A. montereyensis*, *A. silvicola* and *A. pumila*. Plants were approximately 3 years old. Each plant received a pathogen inoculation and a mock one on two distinct branches following the inoculation protocol described above for plants in the ground. The experiment was terminated 30 days post inoculation, when measurements were taken as described above, isolations were performed, and inoculated plants were autoclaved and disposed.

Lesion size, success of pathogen reisolation, and diameter of the inoculated branch were the three metrics recorded and analyzed. Lesion size and branch diameter were compared between treatments and controls using the median non-parametric test. Lesion size was also compared among species that were inoculated at the same time and in the same location, namely between *A. pilosula* and *A. morroensis*, both inoculated in the field at the same time, and among *A. montereyensis, A. silvicola* and *A. pumila,* all inoculated at the UCB greenhouse. All statistical analyses were done using JMP v14.

Confirming the susceptibility to *P. ramorum* of two manzanita species from Mount Tamalpais in Marin County

In late 2017, and especially in 2018, *A. glandulosa* (Rooney-Latham et al., 2017) and *A. canescens* plants growing on the slopes of Mount Tamalpais (Marin County) were reported to be diseased and to display symptoms consistent with those described for Ramorum Blight on ericaceous hosts (Davidson et al. 2003). On November 15th 2018, 24 branches of *A. canescens* and 24 of *A. glandulosa* collected the previous day on Mount Tamalpais (Marin County) were used in an inoculation experiment at U.C. Berkeley. An equal number of bay laurel leaves was also inoculated to provide a term of comparison. Branches were placed with the cut end in water, and 12 of each species were inoculated using isolate 518-leaf.1, originally isolated in 2017 from *A. morroensis* in Santa Cruz County. Inoculations were performed as described above for the other six manzanita species. The remaining branches were instead mock inoculated using agar plugs rather than inoculum plugs. Branches were kept in the greenhouse with temperatures cycling between 19 and 24 ^{0}C and natural lighting. The experiment was completed on December 10th 2018, when each branch lesion was washed with 20 uL of de-ionized water to collect sporangia present on the surface. Bark washes were then stained with Trypan blue and used to count sporangia as described below. After the washes, bark was gently scraped around the inoculation point, the size of the visible under-bark lesion was measured as described above, and isolation of the pathogen was attempted by plating small wood chips excised from the upper and lower edge of the visible lesion on PARPH

medium. Lesion size and number of sporangia from detached branch inoculations were analyzed with ANOVAs and pairwise comparisons were performed using Tukey Kramer tests on JMP v14.

Evaluating the susceptibility and sporulation potential of detached leaves

On November 14th 2018, a total of 20 leaves from each of the six species at the UCSC arboretum and from each of the two Marin County manzanitas were plucked from branches brought to UC Berkeley and inoculated by dipping the tip of each leaf in a solution containing 5 x10^3 sporangia per mL for 5 minutes. Isolate 518-leaf.1 ,originally isolated from *A. morroensis* in 2017 was used for all inoculations. An additional 20 detached control leaves were inoculated using 1% soil tea (Erwin and Ribeiro 1996) instead of a sporangial suspension. All leaves had been previously surface sterilized by dipping them in 70% Ethanol for 30 seconds, followed by a 30-second rinse in de-ionized water. Leaves were then placed on moist paper towels in sealed incubation trays kept at 20 ^{0}C in the dark for one week. Seven days post inoculation, leaves were analyzed for the presence of lesions on the tips of leaves, and length of lesions was measured along the tip-to-petiole axis of each leaf before being plated on PARPH. The presence of preexisting symptoms such as spots and blotches had been previously noted. Lesions of ten out of 12 leaves were washed twice using 10 uL of deionized water. The two washes from the same leaf were then combined before adding the stain Trypan blue and counting the number of sporangia in the wash using a hemacytometer under the compound scope at 100X

257 magnification. Metrics analyzed were % success of pathogen re-isolation, lesion size
258 using only inoculated leaves from which the pathogen was re-isolated, and number
259 of sporangia in the washes. The experiment was repeated on March 29th 2019 just
260 for the six manzanita species infected at the UCSC Arboretum, with the addition of
261 California bay laurel to provide a valuable term of comparison.
262
263 **Does weather explain natural infection in manzanita species?**
264 To assess possible mechanisms explaining why these manzanita species had
265 suddenly exhibited new symptoms, we analyzed relationships between rainfall and
266 infestation intensity. Several studies have clearly shown a strong correlation
267 between the amount of rainfall and the incidence of disease caused by *P. ramorum*
268 (Eyre et al. 2013; Garbelotto et al. 2017), hence we calculated average rainfall values
269 for California as follows. Yearly precipitation data between 2008 and 2019 were
270 collected from 28 National Oceanic and Atmospheric Administration (NOAA)
271 weather stations (www.ncdc.noaa.gov) located across the entire SOD zone of
272 infestations in the coastal stretch between Humboldt and San Luis Obispo County.
273 Values were averaged and each yearly average value was then expressed as a
274 percentage of the 30-year precipitation average calculated using data from the same
275 28 NOAA stations. The percentage of *P. ramorum* positives was expressed as the
276 average value over a 12-year period of results from the statewide data collected
277 during the SOD Blitzes (www.sodblitz.org). Percentage precipitation and *P.*
278 *ramorum* positives were calculated for each year between 2008 and 2019. A linear
279 regression was performed using JMP v14 (SAS 2019) between the percentage

average precipitation and the percentage average *P. ramorum* values for the entire dataset, and then separately for the 2008-2013 and the 2014-2019 periods, which represent two distinct phases (e.g. arrival vs. extensive colonization) in the invasion history of the pathogen in the Greater San Francisco Bay Area (Croucher et al. 2013). Because a one-year lag was visually noticed when graphing rainfall and *P. ramorum* positives values together in the 2008-2015 period, a further regression was performed between the percentage of average *P. ramorum* positives and the percentage of average rainfall of the preceding year for that time period.

Results

The 27 individual 2017 SOD Blitzes had large turnouts, and 314 volunteers surveyed a total of 14,398 trees and collected approximately 10,000 leaves displaying putative *P. ramorum* symptoms from 2013 plants. The location of each sampled plant was geo-referenced by the volunteers so results could be mapped (Garbelotto et al. 2014). Overall, 33.8% (range 0-75%) of samples were positive. At the UCSC Arboretum, 14 out of 22 samples from six manzanita species and all of five California bay leaf samples yielded positive *P. ramorum* isolations and PCR results (Table 1, Figure 1). Cultures from the six manzanita species were sequenced and both ITS (GenBank accessions: MT248335-MT248340) and COXI (GenBank accessions: MT235266-MT235271) sequences were a perfect match for the NA1 lineage of *P. ramorum*.

303 It is noteworthy that all three sampled plants of *A. ohloneana* were negative for *P.*
304 *ramorum* (Figure 1). All manzanitas positive for *P. ramorum* had symptoms
305 including foliar blotches, branch lesions and branch dieback on at least two
306 branches (Figure 2). *P. ramorum* positive bay laurel leaves had the typical spotting
307 associated with infection by this pathogen (Davidson et al. 2003). Symptoms on all
308 *P. ramorum*-positive manzanitas appeared to have developed almost simultaneously
309 on all plants, with all plants displaying recently dead canopies or branches at the
310 time of sampling.
311
312 Koch's postulate was completed for six manzanita species through controlled
313 branch inoculation. At the end of the experiment, branches that were mock
314 inoculated on site at the UCSC Arboretum, although bagged, did not display any
315 obvious sign of withering, leaf burning or wilting that may have been caused by the
316 bag. Lesions on branches inoculated with the pathogen were always larger than
317 those on control mock-inoculated branches (Table 2, Figure 3). The inoculated
318 pathogen was always re-isolated from lesions clearly radiating out from the
319 inoculation point. With the exception of one branch of *A. pumila* in which the lesion
320 of one inoculated branch infected a control branch, no *P. ramorum* was ever isolated
321 from control mock-inoculated branches, both at the UCSC arboretum and at UC
322 Berkeley. Median tests showed that lesions associated with inoculations were
323 always significantly larger than lesions in control branches (Table 2, Figure 3).
324 When lesion size was compared among potted *A. montereyensis*, *A. silvicola* and *A.*
325 *pumila* inoculated in the UCB greenhouse, the only marginally significant (P=0.05)

difference was the one between smaller average lesion size in *A. pumila* and the larger average lesion size in *A. silvicola.* Lesion size was larger in *A. silvicola* in spite of its smaller branch diameter size when compared to branch diameter size of *A. pumila* (P=0.02). When lesion size and diameter of inoculated branches were compared between *A. morroensis* and *A. pilosula*, both tested at the UCSC arboretum, lesions on *A. pilosula* were significant larger then lesions on *A. morroensis* (P=0.045), while branch diameters were not different.

Inoculations on detached branches of *A. glandulosa* and *A. canescens* from Marin County were both successful, the pathogen was re-isolated from 92% of inoculated branches and lesions in inoculated branches were different from lesions in controls with alpha set at 0.05 (Table 2, Figure 3). Inoculations were successful only on 8% of California bay laurel leaves, hence bay laurel data were omitted from the results and discussion. Lesion size values in inoculated branches were only marginally different between the two manzanita species (P= 0.06). Average lesion size caused by *P. ramorum* on *A. glandulosa* was larger than that observed in *A. canescens*, in spite of the fact that branch diameter size was larger (P= 0.0001) in *A. canescens* (mean= 5.29 mm; se= 0.24) compared to branch diameter size of *A. glandulosa* (mean= 3.5; se= 0.24). No differences in branch stem diameter were found between treatments and controls within the same species.

Sporangia counts were zero on mock-inoculated branches of both species, while those counts were 6.3 (SE= 1.4) and 1.9 (SE= 1.4) in inoculated branches of *A. glandulosa* and *A. canescens*, respectively (Table 2). The only significant pair-wise

difference found at alpha= 0.05 was between the mean sporangia count in pathogen-inoculated and mock-inoculated branches of *A. glandulosa*.

Leaf inoculations were performed twice, once in the Fall 2018 and once in the Spring 2019. The Fall 2018 inoculation trial had a success of pathogen reisolation lower than 50% in six out of the eight species tested; however, successful pathogen reisolation (range 5-100%) was obtained from all eight species (data not shown). Furthermore, bay laurel leaves were not inoculated as comparison and for these reasons, analyses from this inoculation trial are not presented in full. The Spring 2019 inoculation trial, instead, had an excellent success of pathogen re-isolation, and thus results are presented in Table 3 and Figure 4, and further analyzed and discussed. Fall results for the two species from Mount Tamalpais, namely *A. glandulosa* and *A. canescens* are included in Table 3 and Figure 4, because these two species were not retested in the Spring; however, reisolation success was low for both species and results may not be reliable, and definitely not comparable to Spring results, because of the confounding effects of varying plant phenology in different seasons (Dodd et al. 2008). Percentage of success of pathogen reisolation in the Spring trial was indicative that in all cases, infection by *P. ramorum* was successful. For all manzanita species inoculated in the Spring, in fact, Chi square tests (results not shown) confirmed there was no significant difference in pairwise comparisons between pathogen reisolation success from each manzanita species and reisolation success from artificially inoculated bay leaves. Additionally, reisolation success was always zero from mock-inoculated leaves, and reisolation

success was always different (P<0.05), when comparing inoculated and control leaves within each species. However, when trying to assess lesion size, ANOVA determined that lesion sizes were significantly different at alpha= 0.05 between inoculated and control leaves only for the following species: *Umbellularia californica* (P<0.0001), *A. canescens* (P<0.0001), *A. pilosula* (P= 0.02). *A. pumila* (P=0.001), *A. morroensis* (P=0.0003) and *A. silvicola* (P<0.0001) (Figure 4). Differences in foliar lesion size were not significant between inoculated *A. glandulosa*, *A. hooverii*, *A. montereyensis* and controls of each species suggesting that measurements of lesion size in these hosts may have been hampered by preexisting conditions. Control leaves of *A. hooverii* and of *A. montereyensis* had in fact the highest incidence of leaf spots prior to inoculation, 60 and 100%, respectively, among the species tested in the Spring, while lesion development in *A. glandulosa* may have been negatively affected by plant phenology as that species was tested in the Fall and not the Spring. An ANOVA comparing lesion size among the species with significant differences in foliar lesion size between treatments and controls, revealed that the largest lesion size was found in *A. morroensis*. Lesion size in this species was larger but not statistically different from lesions in California Bay laurel, while lesion sizes in *A. morroensis* and California bay laurel were significantly different from foliar lesion sizes in all other species (P<0.0001). When an ANOVA was run comparing number of sporangia instead, only California bay laurel was different from the other species (P=0.003).

394 The number of *P. ramorum* positive tree samples in 2017 was the largest value
395 recorded in 10 years of SOD Blitzes and was matched by one of the highest rainfall
396 levels (140% of the 30-year average) during the same time frame (Figure 5). When
397 % yearly precipitation values for 2008-2019 and % *P. ramorum* positives for the
398 same period were regressed against each other using a linear fit, the relationship
399 between the two variables was explained by the equation [% *P ramorum positives] =*
400 *34.597 + 0.583*[% Rainfall]*. R-squared was 0.39 and ANOVA resulted in an F Ratio=
401 6.4 and P=0.03. For the 2014-2019 period, we observed a strong fit between
402 precipitation and *P. ramorum* occurrence (R-squared = 0.87, p=0.006, [% P.
403 *ramorum* positives] =20 + 0.74* [%Rainfall]); however for the earlier samples
404 (2008-2013), precipitation did not explain a large portion of the variation in *P.*
405 *ramorum* positives (R-squared = 0.03). Instead, between 2008-2013, % *P. ramorum*
406 positives were more closely associated with rainfall values from the previous year,
407 suggesting a lagged climate effect [% *P. ramorum positives] = 4.955 + 0.037*[% Rain*
408 *previous year]*. For this linear fit, R-squared= 0.79 and ANOVAs resulted in an F
409 Ratio= 19.26 and P= 0.0071.
410
411 **Discussion**
412
413 The first report of unusual tanoak mortality due to a novel disease goes back to
414 1995 (Svihra 1999). That disease was later dubbed Sudden Oak Death (Garbelotto et
415 al. 2000), and its causal agent *Phytophthora ramorum* was identified in 2000 (Rizzo
416 et al). By 2003, it was recognized that many other plants, besides tanoaks and oaks,

were hosts for the pathogen (Garbelotto et al. 2003). In many of these hosts, the disease was quite different, being characterized by branch die-back and anthracnose-like symptoms developing progressively, rather than causing apparently sudden mortality. This type of disease was dubbed Ramorum Blight, and common manzanita was one of these hosts identified early on as suffering from Ramorum Blight. While discovery of hosts affected by Ramorum Blight happened at a vertiginous rate in the few years after 2000 (Garbelotto and Rizzo 2005), very few new California hosts were described after 2006.

The simultaneous discovery of six manzanita species suffering from significant dieback caused by *P. ramorum* occurred after a 12-year hiatus in the discovery of new hosts, and, based on the moderate symptoms observed in the field, it is presumed to have happened rather rapidly after initial infection of all six species. The rapid discovery of a new disease on multiple hosts after many years without many new reports is one of the benefits of having an extensive statewide survey network thanks to the regular and annual involvement of hundreds of volunteers through a structured citizen science program, known as SOD Blitzes. The SOD Blitzes, thus, not only have monitored new SOD outbreaks due to the progressive invasion of California by this exotic pathogen (Meentemeyer et al. 2015), and have documented weather-driven local changes in population size of the pathogen (Lione et al. 2017), but this publication marks the unexpected service provided by the SOD Blitzes in assisting scientists in the timely discovery of new hosts. Due to the endangered or rare status of the six manzanita species, the timely discovery of *P.*

ramorum infection likely increased the likelihood of success of preservation efforts ignited by the discovery. We believe this outcome proves that citizen science is a powerful research approach, capable of achieving results comparable to those obtained by professional scientists and in line with those in other reports about the usefulness of citizen science to track invasive species in both marine and terrestrial ecosystems (Crall et al. 2010; Delaney et al. 2008; Dickinson et al. 2012; Gallo and Waitt 2011).

The endangered status of the six species identified as potential *P. ramorum* hosts, although not yet officially recognized by the legislature for all species (California Department of Fish and Wildlife, 2016), dictated a parsimonious approach in the design of inoculation experiments aimed at satisfying Koch's postulate. These species are not available on the market and in most cases the plants at the UCSC arboretum represented a significant proportion of all surviving individuals. Repeating branch inoculations was simply impossible, but results from our branch inoculation trials were convincing and exhaustive. One hundred percent infection success across all species tested, development of significantly larger lesions in inoculated plants of all six species compared to mock inoculated controls and 100% pathogen re-isolation success, together, provide convincing evidence these species are hosts of *Phytophthora ramorum*. Success of inoculation of detached branches on two species from Marin County, although technically not a proper way to fulfill Koch's postulate, brings convincing evidence that these two species may also be hosts for *P. ramorum.*

463

464 In all cases, symptoms observed in the field matched those reported for common
465 manzanita (Davidson et al. 2003) and for *A. glandulosa* (Rooney-Latham et al, 2017)
466 and were fully recreated with artificial inoculations, e.g. foliar blight and die-back of
467 twigs and branches. Pathogen isolations from naturally infected plants were
468 successful from withering branches, being girdled by the pathogen and
469 characterized by a brown under-bark lesion (Figure 2A). Pathogen re-isolation was
470 successful both from underbark lesions in artificially inoculated branches and from
471 leaf blotches in artificially inoculated leaves. These findings place all of these
472 manzanitas in a group of hosts that includes most ericaceous host species in which
473 disease causes a progressive die-back by girdling individual branches, besides
474 potentially causing foliar spots and blotches. Conversely, in the case of California
475 bay laurel, Ramorum Blight never infects branches but it is exclusively a foliar blight
476 without associated anthracnose symptoms (Davidson et al. 2003).

477

478 While we recognize that more exhaustive experimentation is needed to determine
479 the relative susceptibility and disease transmission potential of the eight manzanita
480 species tested in this study, some comparisons among individual species were
481 legitimate, at least when the same type of plant or plant tissue was tested (i.e. potted
482 plants vs. plants in the ground vs. detached branches). Additionally, we did
483 inoculate detached leaves of all eight species in the same trials. Based on branch
484 inoculations on live plants, two species emerged as being potentially more
485 susceptible: *A. silvicola* and *A. pilosula*. To these two species, we may want to add *A.*

glandulosa, which developed larger lesions when detached branches were inoculated at UC Berkeley, and *A. hooverii*, which developed the largest lesions among all species tested, even if inoculated a few months after the other species. We place in a potentially less susceptible group the remaining four species, namely *A. morroensis*, *A. montereyensis*, *A. pumila* and *A. canescens* (but see below for changes in grouping). One additional noteworthy result emerged from the field sampling: in spite of the location of three *A. ohloneana* plants, which were closer to the natural source of inoculum (see below) than other manzanita species, none of them were found to be infected, suggesting they may be potentially less prone to infection than the other species studied here.

Susceptibility was also studied by means of leaf inoculations. When leaves were inoculated in a relatively natural way by dipping them in a suspension of sporangia (Widmer 2009), the pathogen could be re-isolated from foliar lesions of all 8 species a week after inoculation. This result confirms all 8 species are hosts for *P. ramorum*, and suggests that initial infection may occur both on branches and on leaves. However, when attempting to compare susceptibility among species using lesion size, this proved to be an impossible task for some species, due to the presence of lesions caused by other pathogens, in most cases recorded as being already present at the time of inoculation. This result informs future research on the limitation of using leaf inoculations to study susceptibility of manzanita species to *P. ramorum,* and possibly to other foliar pathogens as well. *Arctostaphylos morroensis* was the only species characterized by foliar lesions that were significantly larger than foliar

lesions in all other species, hence we place this species in an intermediate category of susceptibility. In summary, and considering only species that were artificially inoculated, we suggest manzanita species may be placed in three groups: very susceptible species, namely *A. silvicola*, *A. pilosula*, *A. glandulosa* and *A. hooverii*; species with intermediate susceptibility, namely A morroensis; species with lower susceptibility, namely *A. montereyensis*, *A. pumila* and *A. canescens.* It would be interesting to study if A. *ohloneana* may be included in a fourth more resistant group of species. As of March 2020, all *A. ohloneana* plants at the arboretum are still healthy.

All naturally infected manzanitas were growing in an open meadow bordering on one side the more formal planted beds of the UCSC Arboretum and several buildings and greenhouses (Figure 1). However, patches of woodland were adjacent the meadow on the opposite side. All five California bay laurels sampled in these woodlands and located at the very edge of the meadow tested positive for *P. ramorum*. As shown in Figure 1, all 10 manzanita species sampled were no further than 200 m from an infected bay laurel, and this distance has been determined as the most likely dispersal range of sporangia during a wet year (Eyre et al. 2013). Given symptoms severity was comparable among the various Manzanita species, it is likely they were all infected by inoculum produced on California bay laurel leaves. It has also been reported that in drier years dispersal range of *P. ramorum* sporangia may be significantly lower, and it may be almost nil during a drought (Eyre et al. 2013): thus manzanita plants would often be out of the dispersal range of the

pathogen. For this reason, we were extremely interested in determining whether manzanita species could themselves support sporulation by the pathogen. If that were the case, inter-manzanita contagion could lead to the spread of Ramorum Blight even in areas not contiguous to woodlands containing infectious hosts such as California bay laurel.

This study provides novel information on the production of sporangia by eight manzanita species. Although a few sporangia were counted in foliar and branch washes, the numbers were low, more than one order of magnitude lower than counts from California bay laurel leaves and other ericaceous hosts (Tooley et al. 2004). Only exception was the count from the Fall inoculation of *A. hooverii*. Our cautious interpretation of the data is that, in general, manzanitas may not support significant sporulation by *P. ramorum*. Nonetheless, sporangia counts were not zero. A previous study has shown successful infection with rather low inoculum density (Tooley et al. 2013) suggesting that intra –manzanita contagion may occur among branches of the same plant, and inter-manzanita contagion may occur periodically in particularly rainy springs if the pathogen was capable of surviving dry spells on this host. The exception of *A. hooverii* requires further investigation, but given the fact this species was also included in the most susceptible group of manzanitas based on branch lesion size, it is plausible that Ramorum Blight may represent a threat to this species.

554 Finally, it may be legitimate to wonder what changed between 2003 and 2017
555 leading to the infection of multiple manzanita species in a habitat, an open meadow,
556 traditionally not regarded as ideal for the spread of Sudden Oak Death or Ramorum
557 Blight. The most conservative explanation is that a combination of at least two
558 prerequisites resulted in conditions particularly favorable to contagion by *P.*
559 *ramorum*. First: the pathogen has been colonizing new sites at a relatively low
560 spread rate (Mascheretti et al. 2008), and it may have been originally absent or rare
561 from the woodland patches at the edge of the UCSC Arboretum. It is important to
562 note this is not just an issue of presence/absence, but it is also an issue of disease
563 incidence and establishment in ideal microhabitats. Our survey showed that all out
564 of 5 bay laurels sampled were infected, thus disease incidence was high at that
565 particular site in 2017. Second, as shown by our SOD Blitz and precipitation data
566 (Figure 5), there is a strong positive correlation between rainfall and percentage of
567 trees infected by *P. ramorum*. The extremely high levels of precipitation recorded in
568 2017 resulted in high infection levels across the State and in Santa Cruz County as
569 well. High infection levels were both cause and effect of the production of a large
570 numbers of sporangia, effectively incrementing the range of their dispersal.
571 Comparable levels of rainfall were recorded in 2011, but it is plausible that the
572 pathogen may not have been that widespread near the UCSC Arboretum at that time
573 or may not have been inhabiting the most suitable niches. Although crowdsourced
574 data has the potential to support applied environmental research (Conrad et al.
575 2011), its use is not risk-free. In fact, the constraints related to equipment
576 availability combined with haphazard sampling schemes may lead to procedural

errors and biases potentially affecting data quality (Conrad et al. 2011; Dickinson et al. 2012). However, our approach to use the entire California statewide dataset obtained from the SOD Blitzes, and not the Santa Cruz subset by itself, can be regarded safe for two important reasons. First, it has been shown repeatedly that larger crowdsourced datasets are subject to fewer biases and provide more reliable information (Foody et al. 2015; Steinke et al. 2017). Second, the specific statewide SOD Blitz dataset employed in this study has already been tested and found to provide reliable information that is well correlated with climatic cycles (Lione et al. 2017).

We also note that our statewide average precipitation levels vs. statewide % P. *ramorum* positive samples graph shows that in 2011, *P. ramorum*, still in its establishment phase, experienced a one year lag between the increase in rainfall levels and an increase in percentage of pathogen positives. The presence of this lag was confirmed by an extremely poor linear fit (R-squared=0.03) when fitting % *P. ramorum* positive values against % rain values of the same year, contrasted by a much higher R-squared value of 0.79 when % *P. ramorum* positive values were fit against % rain of the previous year.

However, starting in 2015, that lag disappears and a linear fit correlating precipitation values with % of *P. ramorum* positives shows a much higher R-squared value for 2014-2019 than the R-squared value obtained when performing the same linear fit for 2008-2013 data. We believe that the faster and more synchronized demographic response of *P. ramorum* populations to changing rainfall levels starting in 2015 was determined by the fact that by that date the pathogen was well

established in areas where it was introduced, it had infested its ideal microhabitats, and had become truly endemic. Lags in responses have been documented for many invasive species, and when dealing with adaptive responses, decreased lag time is usually a result of increased adaptation (Crooks 2005). In the case of *P. ramorum*, a shorter lag between variations in rainfall and transmission rates would increase the synchronicity between the weather and the biology of the pathogen (Dodd et al. 2008; Huberli et al. 2012), thus effectively increasing its dispersal rate.

While we understand this is only a plausible working hypothesis requiring more data in its support, the work implies that as the pathogen becomes widely established and truly endemic across Coastal California, it may be able to infect new hosts, as it was the case for the eight manzanita species here studied.

Acknowledgements

We thank Kerri Frangioso, U.C. Davis, for collecting and analyzing precipitation data. Funding for the SOD Blitzes was provided by Phil Cannon, USFS Region 5, State and Private Forestry, by the PG&E Foundation and by MidPen Open Space, and by the UCSC Arboretum. Funding for inoculation experiments was in part provided by the SF Public Utility Commission and by in kind contributions by the US Forest Service, Region 5. Thanks to Mia Ingolia (SFPUC), Susan Frankel (USFS) and Brett Hall (UCSC) for assisting with funding.

References

Anacker, B.L., Rank, N.E., Hüberli, D., Garbelotto, M., Gordon, S., Harnik, T., Whitkus, R. and Meentemeyer, R., 2008. Susceptibility to *Phytophthora ramorum* in a key infectious host: landscape variation in host genotype, host phenotype, and environmental factors. New Phytol. 177(3): 756-766.

Conrad, C.C.; Hilchey, K.G. 2011 .A review of citizen science and community-based environmental monitoring:Issues and opportunities. Environ. Monit. Assess. 176:273–291

Crall, A.W., Newman, G.J., Jarnevich, C.S., Stohlgren, T.J., Waller, D.M. and Graham, J., 2010. Improving and integrating data on invasive species collected by citizen scientists. Biol. Invasions *12*(10): 3419-3428.

Crooks, J.A., 2005. Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. Ecoscience 12(3): 316-329, DOI: 10.2980/i1195-6860-12-3-316.1 .

Croucher, P.J., Mascheretti, S. and Garbelotto, M., 2013. Combining field epidemiological information and genetic data to comprehensively reconstruct the invasion history and the microevolution of the sudden oak death agent *Phytophthora ramorum* (Stramenopila: Oomycetes) in California. Biol. Invasions 15(10): 2281-2297.

Davidson, J.M., Werres, S., Garbelotto, M., Hansen, E.M. and Rizzo, D.M., 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Plant Health Prog. 4(1): 12.

De Gruyter, J. and Steeghs, M.H.C.G., 2006. Managing *Phytophthora ramorum* in the Netherlands 1. EPPO Bulletin 36(2): 399-401.

Delaney, D.G., Sperling, C.D., Adams, C.S. and Leung, B., 2008. Marine invasive species: validation of citizen science and implications for national monitoring networks. Biol. Invasions 10(1): 117-128.

Dickinson JL, Shirk J, Bonter D, et al. 2012. The current state of citizen science as a tool for ecological research and public engagement. Front. Ecol. Environ. 10: 291–97.

Dodd, R.S., Hüberli, D., Mayer, W., Harnik, T.Y., Afzal-Rafii, Z. and Garbelotto, M., 2008. Evidence for the role of synchronicity between host phenology and pathogen activity in the distribution of sudden oak death canker disease. New Phytol. 179(2): 505-514.

Erwin, D.C. and Ribeiro, O.K., 1996. *Phytophthora diseases worldwide*. American Phytopathological Society (APS Press).

Eyre, C.A., Kozanitas, M. and Garbelotto, M., 2013. Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California forest under different climatic conditions. Phytopathology 103(11): 1141-1152.

Foody, G.M., See, L., Fritz, S., Van der Velde, M., Perger, C., Schill, C., Boyd, D.S. and Comber, A., 2015. Accurate attribute mapping from volunteered geographic information: issues of volunteer quantity and quality. Cartogr. J 52(4), pp.336-344.

Gallo, T. and Waitt, D., 2011. Creating a successful citizen science model to detect and report invasive species. BioScience 61(6): 459-465.

669 Garbelotto, M., Schmidt, D., Swain, S., Hayden, K. and Lione, G., 2017. The
670 ecology of infection between a transmissive and a dead-end host provides clues for
671 the treatment of a plant disease. Ecosphere 8(5): p.e01815.
672 Garbelotto, M., Maddison, E.R. and Schmidt, D., 2014. SODmap and SODmap
673 Mobile: two tools to monitor the spread of Sudden Oak Death. Forest Phytophthoras
674 4(1).
675 Garbelotto, M. and Rizzo, D.M., 2005. A California-based chronological review
676 (1995–2004) of research on *Phytophthora ramorum*, the causal agent of sudden oak
677 death. Phytopathol. Mediterr. 44(2): 127-143.
678 Garbelotto, M., Davidson, J., Ivors, K., Maloney, P., Hüberli, D., Koike, S.T. and
679 Rizzo, D., 2003. Non-oak native plants are main hosts for sudden oak death
680 pathogen in California. Calif. Agric. 57(1): 18-23.
681 Garbelotto, M., Svihra, P. and Rizzo, D., 2001. New pests and diseases: Sudden
682 oak death syndrome fells 3 oak species. Calif. Agric. 55(1): 9-19.
683 Grünwald, N.J., Garbelotto, M., Goss, E.M., Heungens, K. and Prospero, S., 2012.
684 Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends
685 Microbiol. 2*0*(3): 131-138.
686 Grünwald, N.J., Goss, E.M. and Press, C.M., 2008. *Phytophthora ramorum*: a
687 pathogen with a remarkably wide host range causing sudden oak death on oaks and
688 ramorum blight on woody ornamentals. Mol. Plant Pathol. 9(6): 729-740.
689 Hayden, K., Ivors, K., Wilkinson, C. and Garbelotto, M., 2006. TaqMan chemistry
690 for *Phytophthora ramorum* detection and quantification, with a comparison of
691 diagnostic methods. Phytopathology 96(8): 846-854.

692 Hüberli, D., Hayden, K.J., Calver, M. and Garbelotto, M., 2012. Intraspecific
693 variation in host susceptibility and climatic factors mediate epidemics of sudden oak
694 death in western US forests. Plant Pathol. 61(3): 579-592.
695 Jung, T., Scanu, B., Brasier, C.M., Webber, J., Milenković, I., Corcobado, T.,
696 Tomšovský, M., Pánek, M., Bakonyi, J., Maia, C. and Bačová, A., 2020. A survey in
697 natural forest ecosystems of Vietnam reveals high diversity of both new and
698 described *Phytophthora* taxa including *P. ramorum*. Forests *11*(1): 93.
699 Kauffmann, Michael; et al. (2015). "Field Guide to Manzanitas". Backcountry
700 Press.
701 Kroon, L. P. N. M., Verstappen, E. C. P., Kox, L. F. F., Flier, W. G., and Bonants, P. J.
702 M. 2004. A rapid diagnostic test to distinguish between American and European
703 populations of *Phytophthora ramorum*. Phytopathology 94: 613-620.
704 Lione, G., Gonthier, P. and Garbelotto, M., 2017. Environmental factors driving
705 the recovery of bay laurels from *Phytophthora ramorum* infections: an application of
706 numerical ecology to citizen science. Forests *8*(8): 293.
707 Mascheretti, S., Croucher, P.J.P., Vettraino, A., Prospero, S. and Garbelotto, M.,
708 2008. Reconstruction of the sudden oak death epidemic in California through
709 microsatellite analysis of the pathogen *Phytophthora ramorum*. Mol. Ecol. 17(11):
710 2755-2768.
711 Meentemeyer, R.K., Dorning, M.A., Vogler, J.B., Schmidt, D. and Garbelotto, M.,
712 2015. Citizen science helps predict risk of emerging infectious disease. Front. Ecol.
713 Environ. 13(4): 189-194.

714 Meentemeyer, R., Rizzo, D., Mark, W. and Lotz, E., 2004. Mapping the risk of
715 establishment and spread of sudden oak death in California. For Ecol. Manage.
716 200(1-3): 195-214.
717 Osmundson, T.W., Eyre, C.A., Hayden, K.M., Dhillon, J. and Garbelotto, M.M.,
718 2013. Back to basics: an evaluation of N a OH and alternative rapid DNA extraction
719 protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and
720 oomycete samples. Mol. Ecol. Resour. 13(1): 66-74.
721 Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W. and Koike, S.T., 2002.
722 *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and
723 *Lithocarpus densiflorus* in California. Plant Dis. 86(3): 205-214.
724 Rooney-Latham, S.; Blomquist, C.L.; Williams, A.; Gunnison, E.; Pastalka, T.
725 2017. Identification of five new hosts of *Phytophthora ramorum* in an infested forest
726 in California. In: Frankel, Susan J.; Harrell, Katharine M., tech. coords. Proceedings of
727 the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255.
728 Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest
729 Research Station: 83-84.
730 SAS Institute Inc. 2019. JMP (14.0.0).
731 Schenck N, Saurat C, Guinet C, Fourrier-Jeandel C, Roche L, Bouvet A, Husson C,
732 Saintonge FX, Contal C, Ioos R. First report of Phytophthora ramorum causing
733 Japanese larch dieback in France. Plant Dis 2018 102(10):2045
734 Schmid, R., 2002. California Native Plant Society's Inventory of Rare and
735 Endangered Plants of California.

Solel, Z. and Pinkas, Y., 1984. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. *Phytopathology*, *74*(4): 506-508.

Steinke, J., van Etten, J. and Zelan, P.M., 2017. The accuracy of farmer-generated data in an agricultural citizen science methodology. Agron. Sustain. Dev. 37(4), p.32.

Strenge, D., Elliott, M., Chastagner, G. and Sclar, C., 2017. Managing *Phytophthora ramorum* at Bloedel Reserve. In Proceedings of the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255. Albany, CA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station: 43-44.

Svihra, P., 1999. Sudden death of tanoak*, Lithocarpus densiflorus.* Pest Alert, 1: 1-2.

Tooley P.W., Browning M., Leighty R.M., 2013. Inoculum density relationships for infection of some Eastern US forest species by *Phytophthora ramorum*. *J. Phytopathol.* 161(9): 595-603.

Tooley, P.W., Kyde, K.L. and Englander, L., 2004. Susceptibility of selected ericaceous ornamental host species to *Phytophthora ramorum*. Plant Dis. 88(9): 993-999.

Venette, R.C. and Cohen, S.D., 2006. Potential climatic suitability for establishment of *Phytophthora ramorum* within the contiguous United States. For. Ecol. Manage. 231(1-3): 18-26.

Werres, S., Marwitz, R., In't Veld, W.A.M., De Cock, A.W., Bonants, P.J., De Weerdt, M., Themann, K., Ilieva, E. and Baayen, R.P., 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycolo. Res. 10*5*(10): 1155-1165.

759 Widmer, T.L., 2009. Infective potential of sporangia and zoospores of
760 *Phytophthora ramorum*. Plant Dis. 93(1): 30-35.
761
762
763

764 **Table 1.** Species tested at the University of Santa Cruz Arboretum and
765 number of plants that were positive for the pathogen *Phytophthora ramorum.*
766

Species Sampled	*Number of plants*	*P. ramorum positive*
Arctostaphylos hookeri	1	0
Arctostaphylos hooverii	2	1
Arctostaphylos montereyensis	4	2
Arctostaphylos morroensis	1	1
Arctostaphylos ohloneana	3	0
Arctostaphylos pilosula	2	1
Arctostaphylos pumila	1	1
Arctostaphylos silvicola	8	8
Umbellularia californica	5	5

767

769 **Table 2.** Details about branch inoculations of eight California manzanita species

770 with isolates of *Phytophthora ramorum*

771

Species	*Plant type*	*Treatment*	*Pathogen isolate code*	*No. plants*	*No. branches*	*% pathogen reisolation*	*Average Lesion size in mm (SE)*	*Avearge Branch Diameter in mm (SE)*
A. montereyensis	Potted	Inoculated	841-Leaf.1	4	4	100	24.2 (12)	3.75 (0.48)
A. montereyensis	Potted	Control	Mock	4	4	0	2 (1)	4 (1)
A. silvicola	Potted	Inoculated	ARSI-780-TDC1.1	4	4	100	27 (5)	2.75 (0.25)
A. silvicola	Potted	Control	Mock	4	4	0	2.7 (3.2)	2.5 (0.29)
A. pumila	Potted	Inoculated	ARPU-846-CANKER.1	4	4	100	9.2 (0.5)	4.5 (0.5)
A. pumila	Potted	Control	Mock	4	4	0	4,3 (0.5	3.5 (0.5)
A. morroensis	Ground	Inoculated	518-leaf.1	1	3	100	24.6 (4.8)	7.3 (0.88)
A. morroensis	Ground	Control	Mock	1	3	0	4.6 (2.3)	8 (1)

A. pilosula	Ground	Inoculated	851-leaf.1	3	3	100	65.5 (5.5)	10.3 (0.9)
A. pilosula	Ground	Control	Mock	2*	2*	0	3.3 (3)	6.3 (0.3)
A. hooverii	Ground	Inoculated	519.B	3	3	100	134.7 (62.2)	10 (0)
A. hooverii	Ground	Control	Mock	3	3	0	9.3 (1.5)	10.7 (0.3)
A. glandulosa	Cut Branch	Inoculated	518-leaf 1	12	12	92	34.8 (7)	3.5 (0.3)
A. glandulosa	Cut Branch	Control	Mock	12	12	0	2.2 (8.2)	3.5 (0.25)
A. canescens	Cut Branch	Inoculated	518-leaf 1	12	12	92	19.1 (7)	5.3 (0.2)
A. canescens	Cut Branch	Control	Mock	12	12	0	1.1 (8.2)	5.1 (0.3)

772 * One control inoculation had to be excluded because of a natural infection encroaching one mock-
773 inoculated branch.
774

776 **Table 3.** Details about leaf inoculations of eight manzanita species with *P. ramorum.*
777 A total of 20 leaves for each treatment and species were inoculated using isolate
778 518-leaf.1 originally obtained from an *Arctostaphylos morroensis* shrub growing in
779 Santa Cruz County.

Species	*Inoculation Date*	*Treatement*	*Reisolation success (%)*	*Average lesion size in mm (SE)*	*Mean number sporangia (SE)*	*% leaves with preexisting spots*
A. montereyensis	3/29/19	Inoculated	100	10 (1.5)	0 (7.5)	10
A. montereyensis	3/29/19	Control	0	8.3 (1.5)	0 (7.5)	100
A. silvicola	3/29/19	Inoculated	70	7.6 (1.75)	0.2 (7.5)	15
A. silvicola	3/29/19	Control	0	1.75 (1.5)	0 (7.5)	40
A. pumila	3/29/19	Inoculated	65	8.75 (1.9)	5.8 (7.5)	0
A. pumila	3/29/19	Control	0	2.3 (1.5)	0 (7.9)	0
A. morroensis	3/29/19	Inoculated	85	19.8 (1.6)	0.3 (7.5)	0
A. morroensis	3/29/19	Control	0	8 (1.5)	0 (7.5)	5
A. pilosula	3/29/19	Inoculated	85	11.6 (1.6)	0.3 (7.5)	20
A. pilosula	3/29/19	Control	0	6.2 (1.5)	0 (7.5)	10
A. hooverii	3/29/19	Inoculated	85	14.4 (1.6)	2.3 (7.5)	20

A. hooverii	3/29/19	Control	0	18.2 (1.5)	0 (7.5)	60
Umbellularia californica	3/29/19	Inoculated	45	13.7 (2.2)	63.5 (7.5)	5
Umbellularia californica	3/29/19	Control	0	0 (1.5)	0 (7.5)	0
A. glandulosa	11/21/18	Inoculated	15	6.3 (3.8)	0.3 (7.5)	10
A. glandulosa	11/21/18	Control	0	2 (1.5)	0 (10.6)	5
A. canescens	11/21/18	Inoculated	25	7.6 (2.9)	1.5 (7.5)	10
A. canescens	11/21/18	Control	0	0.9 (1.5)	0 (10.6)	15

780

782 **Figure 1**. A map showing the exact location, species and infection status of plants
783 sampled and tested at the UCSC Arboretum and adjacent wildlands.
784

785
786

787
788
789
790
791

793 **Figure 2.** Ramorum blight can cause both a leaf blight and branch anthracnose. As
794 shgown in this picture Underbark lesion (A) and canopy mortality (B) caused by
795 *Phytophthora ramorum* on *Arctostaphylos silvicola* at the UCSC Arboretum. Photos:
796 Laura Sims
797

798
799

Figure 3. Length of lesions in stems of 8 *Arctostaphylos* species inoculated with the pathogen *Phytophthora ramorum*, or mock-inoculated as controls. Inoculations were performed using agar plugs colonized by the pathogen for about a week. Mock inoculations were performed instead using uncolonized agar plugs. Inoculations of stems in live plants of six species, whether potted or in the ground, were meant to complete Koch's postulate for each species, and were performed using pathogen isolates that had been originally obtained from the species they were inoculated onto (see Table 2). Inoculations of detached stems from two additional manzanita species were instead performed using isolate 518-leaf.1 obtained from a third species, and were meant to test susceptibility of the two species to the pathogen. Median tests were used to compare lesion sizes between inoculated branches and controls of each species. A single asterisk indicates sizes were different with a P=0.05. Interspecific comparisons could only be done for species inoculated using the same type of plant material, and are presented in the text.

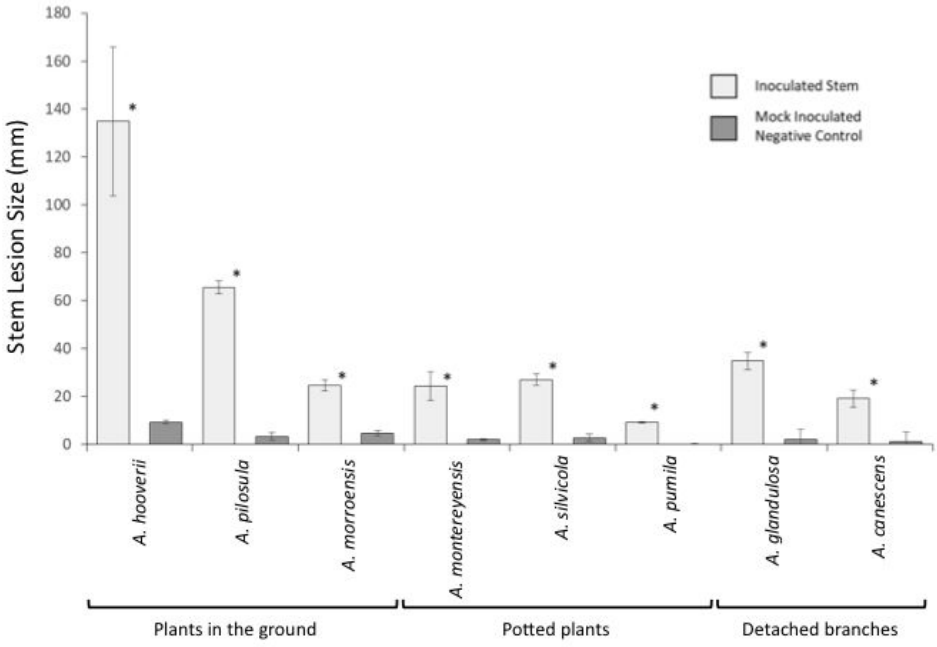
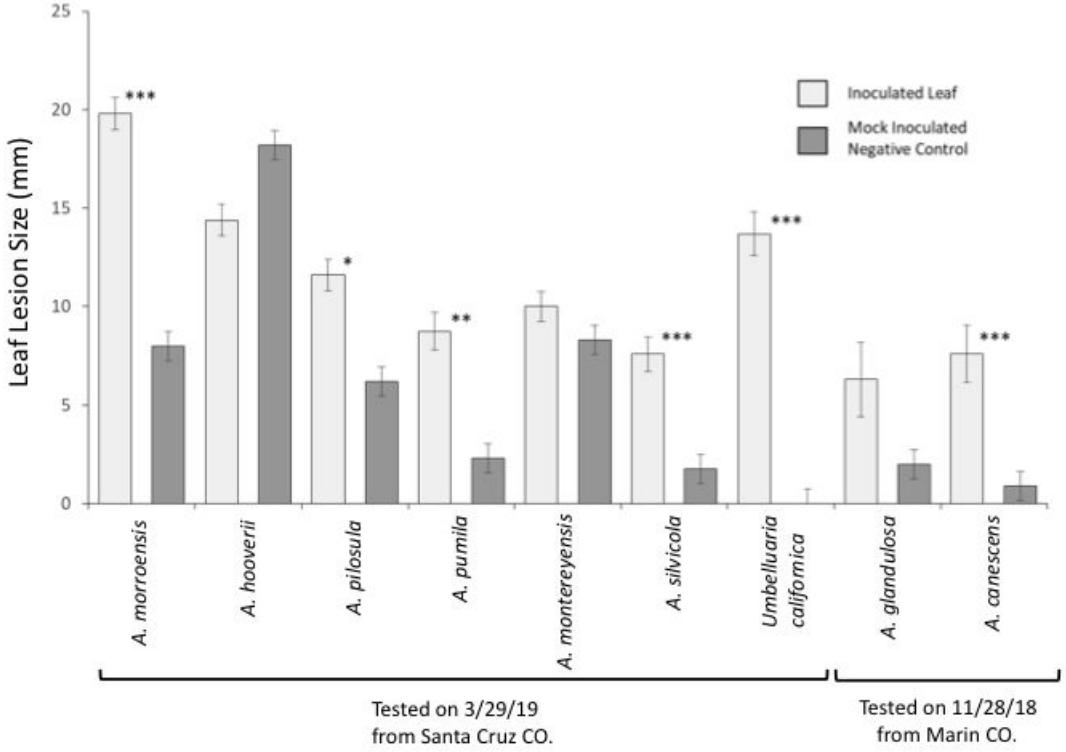
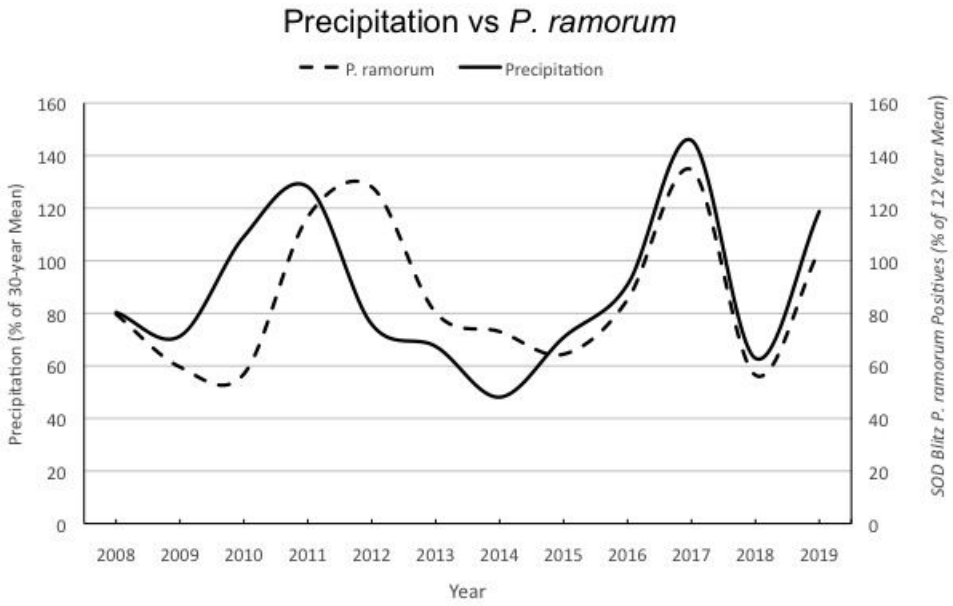


Figure 4. Length of lesions in leaves of 8 *Arctostaphylos* species and of *Umbellularia californica* (California bay laurel) inoculated with the pathogen *Phytophthora ramorum*, or mock-inoculated as controls. Inoculations were performed using zoospore suspensions of the pathogen using isolate 518-leaf.1. Mock inoculations were performed instead using a sterile suspension. For each species, lesion size in inoculated leaves was compared to lesion size in controls using Student's t tests (* = 0.01<P<0.05; ** = 0.01<P<0.001; *** =P<0.001). Data was generated with trials performed in two different dates, and interspecific comparisons were made only among species inoculated on the same date (see text for results).



827 **Figure 5.** Graph showing precipitation values and number of plant samples positive
828 for *Phytopthora ramorum* for the zone of infestation by the pathogen in California.
829 Precipitation was expressed as percentage of 30 year period average, while number
830 of samples positive for *P. ramorum* was expressed as percentage of the 12-year
831 (2008-2019) average.
832



833

834

Figure 1. A map showing the exact location, species and infection status of plants sampled and tested at the UCSC Arboretum and adjacent wildlands.

254x190mm (96 x 96 DPI)

Figure 2. Underbark lesion (A) and canopy mortality (B) caused by Phytophthora ramorum on Arctostaphylos silvicola at the UCSC Arboretum. Photos: Laura Sims

254x190mm (72 x 72 DPI)

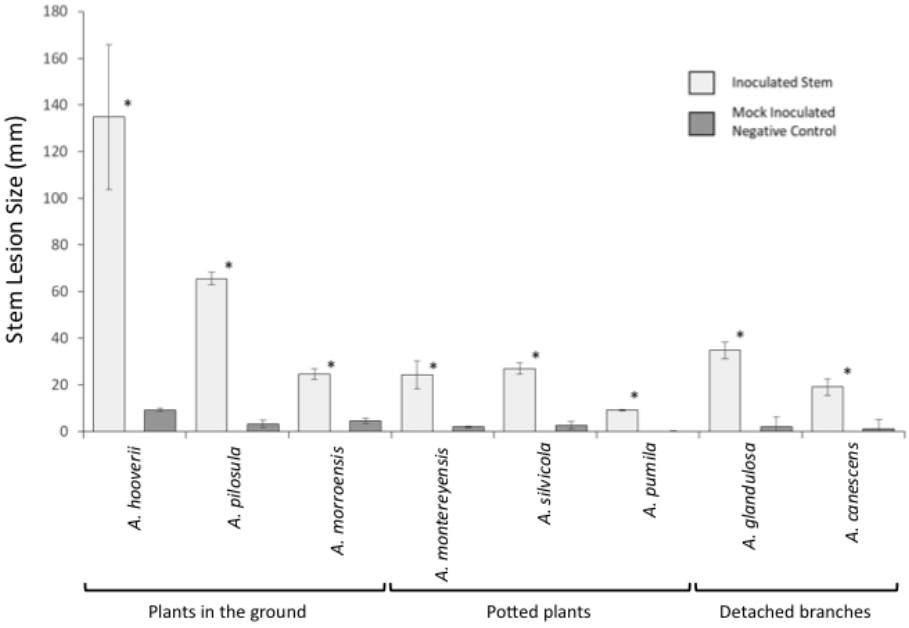
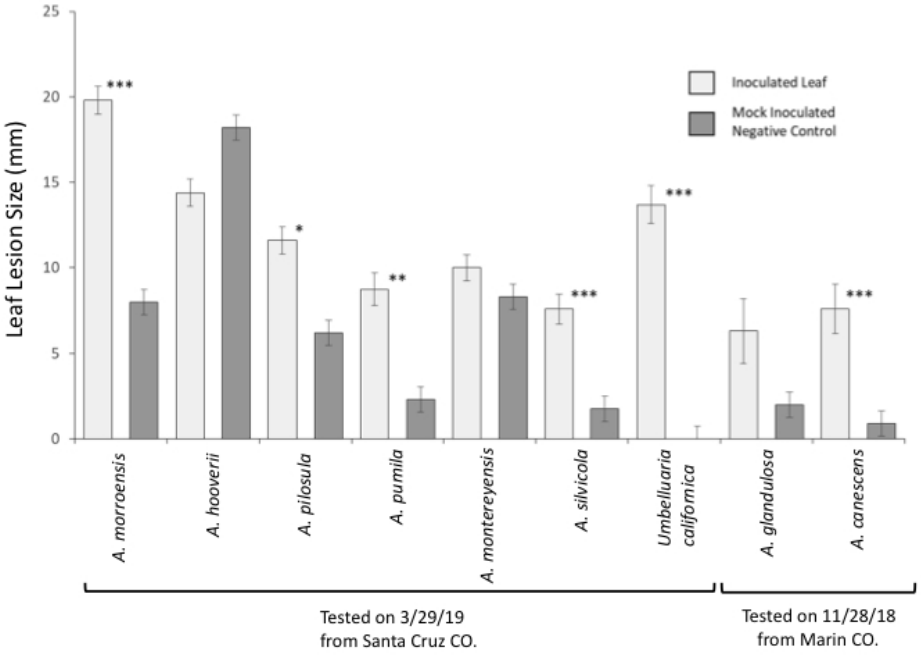


Figure 3. Length of lesions in stems of 8 Arctostaphylos species inoculated with the pathogen Phytophthora ramorum, or mock-inoculated as controls. Inoculations were performed using agar plugs colonized by the pathogen for about a week. Mock inoculations were performed instead using uncolonized agar plugs. Inoculations of stems in live plants of six species, whether potted or in the ground, were meant to complete Koch's postulate for each species, and were performed using pathogen isolates that had been originally obtained from the species they were inoculated onto (see Table 2). Inoculations of detached stems from two additional manzanita species were instead performed using isolate 518-leaf.1 obtained from a third species, and were meant to test susceptibility of the two species to the pathogen. Median tests were used to compare lesion sizes between inoculated branches and controls of each species. A single asterisk indicates sizes were different with a P=0.05. Interspecific comparisons could only be done for species inoculated using the same type of plant material, and are presented in the text.

254x190mm (72 x 72 DPI)



Length of lesions in leaves of 8 Arctostaphylos species and of Umbellularia californica (California bay laurel) inoculated with the pathogen Phytophthora ramorum, or mock-inoculated as controls. Inoculations were performed using zoospore suspensions of the pathogen using isolate 518-leaf.1. Mock inoculations were performed instead using a sterile suspension. For each species, lesion size in inoculated leaves was compared to lesion size in controls using Student's t tests (* = 0.01<P<0.05; ** = 0.01<P<0.001; *** =P<0.001). Data was generated with trials performed in two different dates, and interspecific comparisons were made only among species inoculated on the same date (see text for results).

254x190mm (72 x 72 DPI)

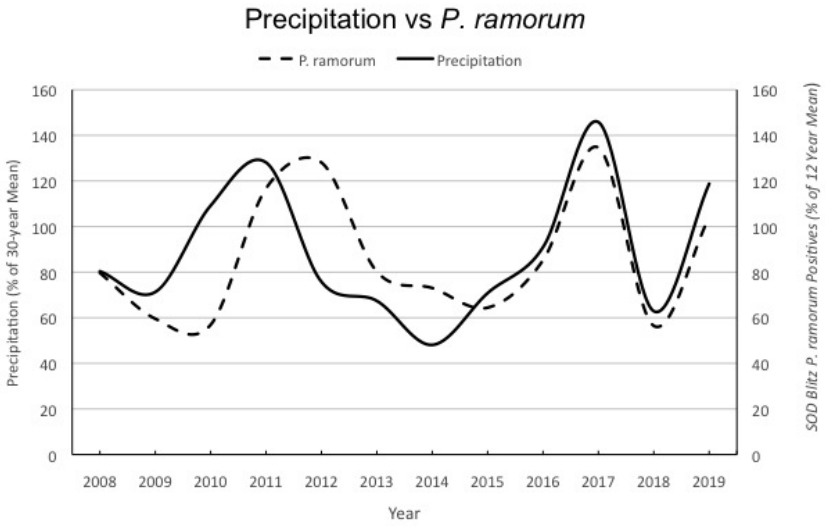


Figure 5. Graph showing precipitation values and number of plant samples positive for Phytopthora ramorum for the zone of infestation by the pathogen in California. Precipitation was expressed as percentage of 30 year period average, while number of samples positive for P. ramorum was expressed as percentage of the 12-year (2008-2019) average.

254x142mm (72 x 72 DPI)