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Citizen Science Uncovers *Phytophthora ramorum* as a Threat to Several Rare or Endangered California Manzanita Species

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Abstract

The Sudden Oak Death (SOD) Blitzes consist of yearly surveys led by citizen scientists designed to map the distribution of *Phytophthora ramorum*, cause of 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 *P. 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 that these two species may also be hosts of *P. ramorum*. Detached leaves of all eight species were also successfully inoculated at the University of California-Berkeley in fall 2018 and then again in spring 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.

Keywords: emergent disease, endemic disease, novel host, sudden oak death, threatened plants

The pathogen *Phytophthora ramorum* (Rizzo et al. 2002; Werres et al. 2001) is the cause of the notorious deadly forest disease known as sudden oak death (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 and Steeghs 2006), and Washington State (United States) (Strenge et al. 2017) while, in many other U.S. 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; however, 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 oak and tanoak (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 dieback 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 laurel and tanoak, 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 article by Garbelotto et al. (2003) describing the initial discovery of nonoak 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 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. 2004; Meentemeyer et al. 2015; Venette and Cohen 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 *Arctostaphylos* 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.

In 2017, during the SOD Blitzes, a citizen science program engaging volunteers to map the distribution of *P. ramorum* in California (Meentemeyer et al. 2015), many apparently diseased manzanita species present at the arboretum of the University of California-Santa Cruz (UCSC) were sampled. Symptoms included leaf spots or blotches and branch dieback. Of eight species tested, six were positive for the pathogen, and all are considered rare or endangered according to the California Native Plant Society inventory of rare

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and endangered plant species (Schmid 2002). Almost simultaneously, two additional manzanita species were found to be potentially infected by *P. ramorum* on Mount Tamalpais in Marin County, 130 km north of Santa Cruz.

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

Materials and Methods

Collection of plant samples. The 2017 SOD Blitzes included 27 separate surveys conducted at different times across 13 infested counties (Humboldt, Mendocino, Sonoma, Napa, Solano, Marin, Contra Costa, Alameda, San Francisco, San Mateo, Santa Clara, Santa Cruz, and 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). In total, 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 15 and 16 March 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 laurel in the UCSC Arboretum. The locations of plant samples at and near the UCSC Arboretum are shown in Figure 1. Samples were shipped using next-day service to the University of California-Berkeley (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 by 0.5 cm in size within a Petri dish filled with pimaricin, ampicillin, rifamycin, pentachloronitrobenzene, and hymexazol (PARPH) *Phytophthora* selective medium (Solel and Pinkas 1984). Plates were kept in the dark at room temperature (16 to 22°C) and observed after 4, 8, and 12 days for the presence of putative *Phytophthora* colonies. Colonies were

Fig. 1. A map showing the exact location, species, and infection status of plants sampled and tested at the University of California-Santa Cruz Arboretum and adjacent wildlands.

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* (*COXI*) gene were also sequenced to confirm the morphological identification and to determine the lineage of the cultures (Kroon et al. 2004; Rizzo et al. 2002). 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, *A. montereyensis, A. silvicola, A. pumila, A. pilosula, A. morroensis*, and *A. hooveri*) 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, reinoculating them on the same host, documenting the development of symptoms similar to the ones observed in nature, and, finally, reisolating 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 regarded as either rare or endangered according to the California Native Plant Society, the number and type of replicates had to be carefully selected to minimize the impact of the experiment on dwindling populations of these species. Details of the inoculations, including isolates employed, number and source of plant replicates, lesion size, and branch diameter, are provided in Table 1.

Inoculations were performed on healthy branches, approximately 1.5 to 2 m long, in 15-year-old bushes on the UCSC Arboretum grounds for the following species: *A. pilosula, A. morroensis*, and *A. hooveri*. On 13 February 2018, four branches of each of the three species above were inoculated with the pathogen and four were mock inoculated using a plug of agar rather than a plug of inoculum. All inoculated branches were bagged in large white trash bags to ensure that no inoculum from the outside would reach the branch, and to ensure that sporangia produced by the inoculation would not infect neighboring branches. Thirty days postinoculation, all inoculated branches were taken to the lab at UC Berkeley still enclosed in their bags. Measurements were taken and pathogen isolations were completed in the lab before autoclaving the plant material and disposing of it. The size of lesions was determined by gently scraping the bark above and below the inoculation point and exposing the necrotic area caused by pathogen colonization under the bark. Scraping was done, moving further away from the inoculation point in all directions, until the edges between the necrotic lesions and healthy tissue were clearly visible. A ruler was used to determine the maximum linear expansion of the lesion starting 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 *A. hooveri* was inoculated with the wrong isolate, the experiment had to be repeated between 13 November and 13 December 2018.

On 14 February 2018, four plants potted in 1-gal. 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 inoculation on two distinct branches following the inoculation protocol described above for plants in the ground. The experiment was terminated 30 days postinoculation, 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 nonparametric 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 UC Berkeley 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 15 November 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 UC 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

Table 1. Details about branch inoculations of eight California manzanita species with isolates of *Phytophthora ramorum*

Species	Plant type	Treatment	Pathogen isolate code	Pl (*n*)[a]	Br (*n*)[a]	Pathogen reisolation (%)	Lesion (mm) (SE)[b]	Branch (mm) (SE)[c]
Arctostaphylos 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[d]	2[d]	0	3.3 (3)	6.3 (0.3)
A. hooveri	Ground	Inoculated	519.B	3	3	100	134.7 (62.2)	10 (0)
A. hooveri	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)

[a] Number (*n*) of Pl = plants and Br = branches.
[b] Average lesion size. SE = standard error.
[c] Average branch diameter.
[d] One control inoculation had to be excluded because of a natural infection encroaching on one mock-inoculated branch.

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°C and natural lighting. The experiment was completed on 10 December 2018, when each branch lesion was washed with 20 µl of deionized 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 analyses of variance (ANOVAs) and pairwise comparisons were performed using Tukey Kramer tests on JMP v14.

Evaluating the susceptibility and sporulation potential of detached leaves. On 14 November 2018, in total, 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 5×10^3 sporangia/ml for 5 min. 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 s, followed by a 30-s rinse in deionized water. Leaves were then placed on moist paper towels in sealed incubation trays kept at 20°C in the dark for 1 week. Seven days postinoculation, 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 10 of 12 leaves were washed twice using 10 µl 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 ×100 magnification. Metrics analyzed were percent success of pathogen reisolation, lesion size using only inoculated leaves from which the pathogen was reisolated, and number of sporangia in the washes. The experiment was repeated on 29 March 2019 only for the six manzanita species infected at the UCSC Arboretum, with the addition of California bay laurel to provide a valuable term of comparison.

Does weather explain natural infection in manzanita species? To assess possible mechanisms explaining why these manzanita species had suddenly exhibited new symptoms, we analyzed relationships between rainfall and infestation intensity. Several studies have clearly shown a strong correlation between the amount of rainfall and the incidence of disease caused by *P. ramorum* (Eyre et al. 2013; Garbelotto et al. 2017); hence, we calculated average rainfall values for California as follows. Yearly precipitation data between 2008 and 2019 were collected from 28 National Oceanic and Atmospheric Administration (NOAA) weather stations (https://www.ncdc.noaa.gov/) located across the entire SOD zone of infestations in the coastal stretch between Humboldt and San Luis Obispo Counties. Values were averaged and each yearly average value was then expressed as a percentage of the 30-year precipitation average calculated using data from the same 28 NOAA stations. The percentage of *P. ramorum* positives was expressed as the average value over a 12-year period of results from the statewide data collected during the SOD Blitzes (https://nature.berkeley.edu/matteolab/?page_id=148). Percent precipitation and *P. ramorum* positives were calculated for each year between 2008 and 2019. A linear regression was performed using JMP v14 (SAS Institute Inc. 2019) between the percent average precipitation and the percent average *P. ramorum* values for the entire dataset, and then separately for the 2008-to-2013 and the 2014-to-2019 periods, which represent two distinct phases (e.g., arrival versus extensive colonization) in the invasion history of the pathogen in the Greater San Francisco Bay Area (Croucher et al. 2013). Because a 1-year lag was visually noticed when graphing rainfall and *P. ramorum*-positive values together in the 2008-to-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.

Table 2. Species tested at the University of Santa Cruz Arboretum and number of plants that were positive for the pathogen *Phytophthora ramorum*

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

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 2,013 plants. The location of each sampled plant was georeferenced by the volunteers so that results could be mapped (Garbelotto et al. 2014). Overall, 33.8% (range 0 to 75%) of samples were positive.

At the UCSC Arboretum, 14 of 22 samples from six manzanita species and all of five California bay leaf samples yielded positive *P. ramorum* isolations and PCR results (Table 2; Fig. 1). Cultures from the six manzanita species were sequenced and both ITS (GenBank accessions MT248335 to MT248340) and COXI (GenBank accessions MT235266 to MT235271) sequences were a perfect match for the NA1 lineage of *P. ramorum*.

It is noteworthy that all three sampled plants of *A. ohloneana* were negative for *P. ramorum* (Fig. 1). All manzanitas positive for *P. ramorum* had symptoms that included foliar blotches, branch lesions, and branch dieback on at least two branches (Fig. 2). *P. ramorum*-positive bay laurel leaves had the typical spotting associated with infection by this pathogen (Davidson et al. 2003). Symptoms on all *P. ramorum*-positive manzanitas appeared to have developed almost simultaneously on all plants, with all plants displaying recently dead canopies or branches at the time of sampling.

Fig. 2. Ramorum blight can cause both a leaf blight and branch anthracnose. **A,** Underbark lesion and **B,** canopy mortality caused by *Phytophthora ramorum* on *Arctostaphylos silvicola* at the University of California-Santa Cruz Arboretum. Photos: Laura Sims

Koch's postulate was completed for six manzanita species through controlled branch inoculation. At the end of the experiment, branches that were mock inoculated on site at the UCSC Arboretum, although bagged, did not display any obvious sign of withering, leaf burning, or wilting that may have been caused by the bag. Lesions on branches inoculated with the pathogen were always larger than those on control mock-inoculated branches (Table 1; Fig. 3). The inoculated pathogen was always reisolated from lesions clearly radiating out from the inoculation point. With the exception of one branch of *A. pumila*, in which the lesion of one inoculated branch infected a control branch, no *P. ramorum* was ever isolated from control mock-inoculated branches, both at the UCSC Arboretum and at UC Berkeley. Median tests showed that lesions associated with inoculations were always significantly larger than lesions in control branches (Table 1; Fig. 3). When lesion size was compared among potted *A. montereyensis*, *A. silvicola*, and *A. pumila* inoculated in the UC Berkeley 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 with 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 significantly 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 reisolated from 92% of inoculated branches and lesions in inoculated branches were different from lesions in controls with α set at 0.05 (Table 1; Fig. 3). Inoculations were successful on only 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; standard error [SE] = 0.24) compared with 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 0 on mock-inoculated branches of both species, whereas 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 1). The only significant pairwise 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 fall 2018 and once in spring 2019. The fall 2018 inoculation trial had a success of pathogen reisolation lower than 50% in six of the eight species tested; however, successful pathogen reisolation (range 5 to 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

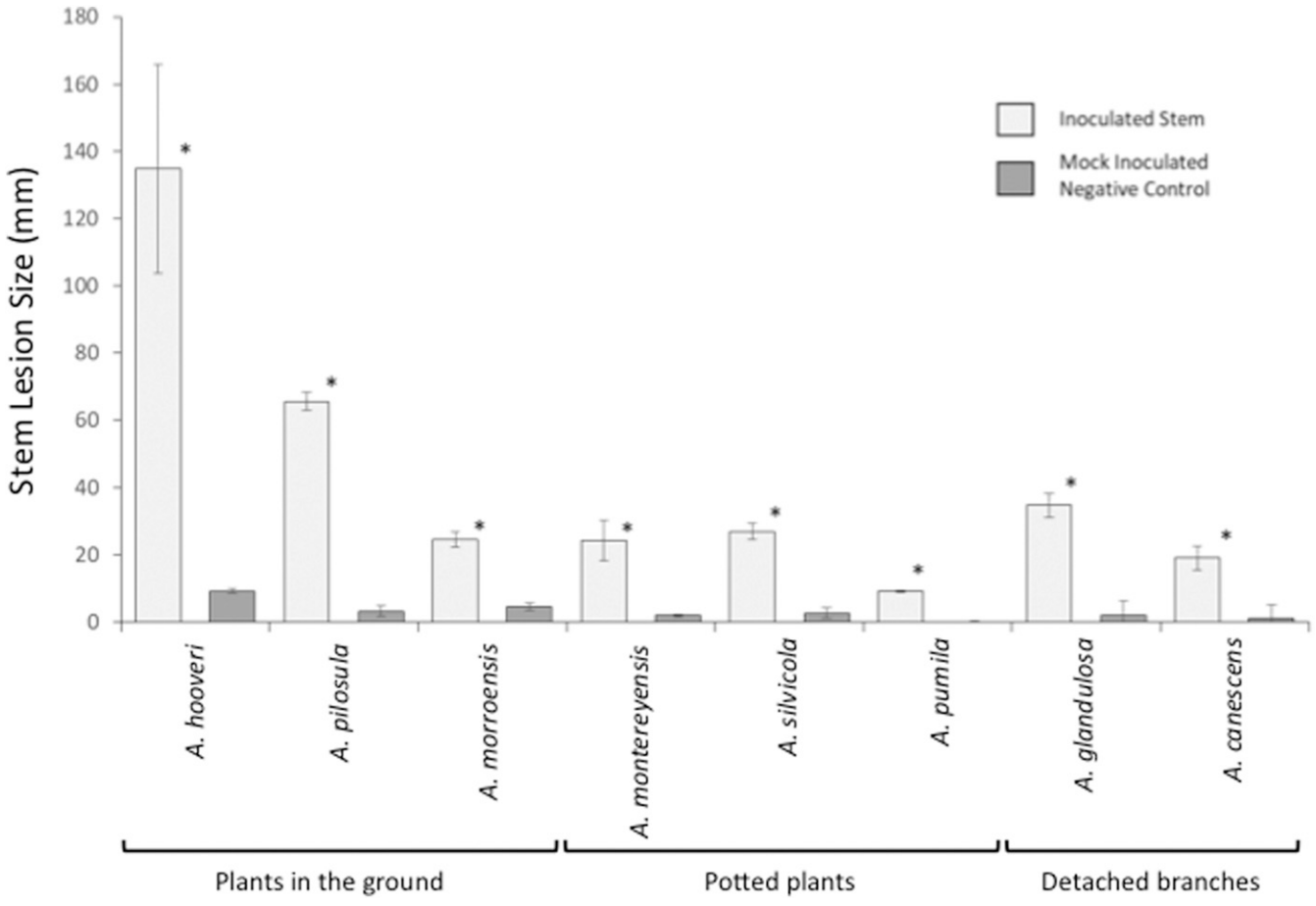

Fig. 3. Length of lesions in stems of eight *Arctostaphylos* spp. inoculated with the pathogen *Phytophthora ramorum* or mock-inoculated as controls. Inoculations were performed using agar plugs colonized by the pathogen for about a week. Mock inoculations were performed using uncolonized agar plugs instead. 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. 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 that 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.

pathogen reisolation 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 with 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, χ^2 tests (results not shown) confirmed that 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* ($P < 0.0001$) (Fig. 4). Differences in foliar lesion size were not significant between inoculated *A. glandulosa*, *A. hooveri*, *A. montereyensis*, and controls of each species, suggesting that measurements of lesion size in these hosts may have been hampered by preexisting conditions. In fact, control leaves of *A. hooveri* and *A. montereyensis* had 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 because 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, whereas 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 ($P = 0.003$).

The number of *P. ramorum*-positive tree samples in 2017 was the largest value recorded in 10 years of SOD Blitzes and was matched by one of the highest rainfall levels (140% of the 30-year average) during the same time frame (Fig. 5). When percent yearly precipitation values for 2008 to 2019 and percent *P. ramorum* positives for the same period were regressed against each other using a linear fit, the relationship between the two variables was explained by the equation % *P. ramorum* positives = 34.597 + 0.583 × % Rainfall. R^2 was 0.39 and ANOVA resulted in an F ratio = 6.4 and $P = 0.03$. For the 2014-to-2019 period, we observed a strong fit between precipitation and *P. ramorum* occurrence ($R^2 = 0.87$, $P = 0.006$; % *P. ramorum* positives = 20 + 0.74 × % Rainfall); however, for the earlier samples (2008 to 2013), precipitation did not explain a large portion of the variation in *P. ramorum* positives ($R^2 = 0.03$). Instead, between 2008 and 2013, percent *P. ramorum* positives were more closely associated with rainfall values from the previous year, suggesting a lagged climate effect (% *P. ramorum* positives = 4.955 + 0.037 × % Rainfall previous year). For this linear fit, $R^2 = 0.79$ and ANOVAs resulted in an F ratio = 19.26 and $P = 0.0071$.

Discussion

The first report of unusual tanoak mortality due to a novel disease goes back to 1995 (Svihra 1999). That disease was later dubbed SOD (Garbelotto et al. 2001), and its causal agent, *P. ramorum*, was identified in 2000 (Rizzo et al. 2002). By 2003, it was recognized that many other plants, in addition to tanoak and oak, were hosts for the pathogen (Garbelotto et al. 2003). In many of these hosts, the disease was quite different, being characterized by branch dieback 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 the hosts identified early on as suffering from ramorum blight. Although 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 annual involvement of hundreds of volunteers through a structured citizen science program, known as SOD Blitzes. The SOD Blitzes, thus, not only

Table 3. Details about leaf inoculations of eight manzanita species with *Phytophthora ramorum*[a]

Species	Date[b]	Treatment	Reisolation success (%)	Average lesion size (mm) (SE)	Mean number sporangia (SE)	Leaves with preexisting spots (%)
Arctostaphylos montereyensis	Mar	Inoculated	100	10 (1.5)	0 (7.5)	10
A. montereyensis	Mar	Control	0	8.3 (1.5)	0 (7.5)	100
A. silvicola	Mar	Inoculated	70	7.6 (1.75)	0.2 (7.5)	15
A. silvicola	Mar	Control	0	1.75 (1.5)	0 (7.5)	40
A. pumila	Mar	Inoculated	65	8.75 (1.9)	5.8 (7.5)	0
A. pumila	Mar	Control	0	2.3 (1.5)	0 (7.9)	0
A. morroensis	Mar	Inoculated	85	19.8 (1.6)	0.3 (7.5)	0
A. morroensis	Mar	Control	0	8 (1.5)	0 (7.5)	5
A. pilosula	Mar	Inoculated	85	11.6 (1.6)	0.3 (7.5)	20
A. pilosula	Mar	Control	0	6.2 (1.5)	0 (7.5)	10
A. hooveri	Mar	Inoculated	85	14.4 (1.6)	2.3 (7.5)	20
A. hooveri	Mar	Control	0	18.2 (1.5)	0 (7.5)	60
Umbellularia californica	Mar	Inoculated	45	13.7 (2.2)	63.5 (7.5)	5
U. californica	Mar	Control	0	0 (1.5)	0 (7.5)	0
A. glandulosa	Nov	Inoculated	15	6.3 (3.8)	0.3 (7.5)	10
A. glandulosa	Nov	Control	0	2 (1.5)	0 (10.6)	5
A. canescens	Nov	Inoculated	25	7.6 (2.9)	1.5 (7.5)	10
A. canescens	Nov	Control	0	0.9 (1.5)	0 (10.6)	15

[a] In all, 20 leaves for each treatment and species were inoculated using isolate 518-leaf.1 originally obtained from an *A. morroensis* shrub growing in Santa Cruz County. SE = standard error.

[b] Inoculation date: Mar = 29 March 2019 and Nov = 21 November 2018.

have monitored new SOD outbreaks due to the progressive invasion of California by this exotic pathogen (Meentemeyer et al. 2015) and documented weather-driven local changes in population size of the pathogen (Lione et al. 2017) but also have provided an unexpected service by assisting scientists in the timely discovery of new hosts, as described in this publication. 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 that this outcome proves that citizen science is a powerful research approach, capable of achieving results comparable with 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, 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. The 100% infection success across all species tested, development of significantly larger lesions in inoculated plants of all six species compared with mock-inoculated controls, and 100% pathogen reisolation success, together, provide convincing evidence that these species are hosts of *P. 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.*

In all cases, symptoms observed in the field matched those reported for common manzanita (Davidson et al. 2003) and *A. glandulosa* (Rooney-Latham et al. 2017) and were fully recreated with artificial inoculations (e.g., foliar blight and dieback of twigs and branches). Pathogen isolations from naturally infected plants were successful from withering branches, being girdled by the pathogen and characterized by a brown underbark lesion (Fig. 2A). Pathogen reisolation was successful both from underbark lesions in artificially inoculated branches and from leaf blotches in artificially inoculated leaves. These findings place all of these manzanitas in a group of hosts that includes most ericaceous host species in which disease causes a progressive dieback by girdling individual branches, in addition to potentially causing foliar spots and blotches. Conversely, in the case of California bay laurel, ramorum blight never infects branches but is exclusively a foliar blight without associated anthracnose symptoms (Davidson et al. 2003).

Although we recognize that more exhaustive experimentation is needed to determine the relative susceptibility and disease transmission potential of the eight manzanita species tested in this study, some comparisons among individual species were legitimate, at least when the same type of plant or plant tissue was tested (i.e., potted plants versus plants in the ground versus detached branches). Additionally, we did inoculate detached leaves of all eight species in the same trials. Based on branch inoculations on live plants, two species

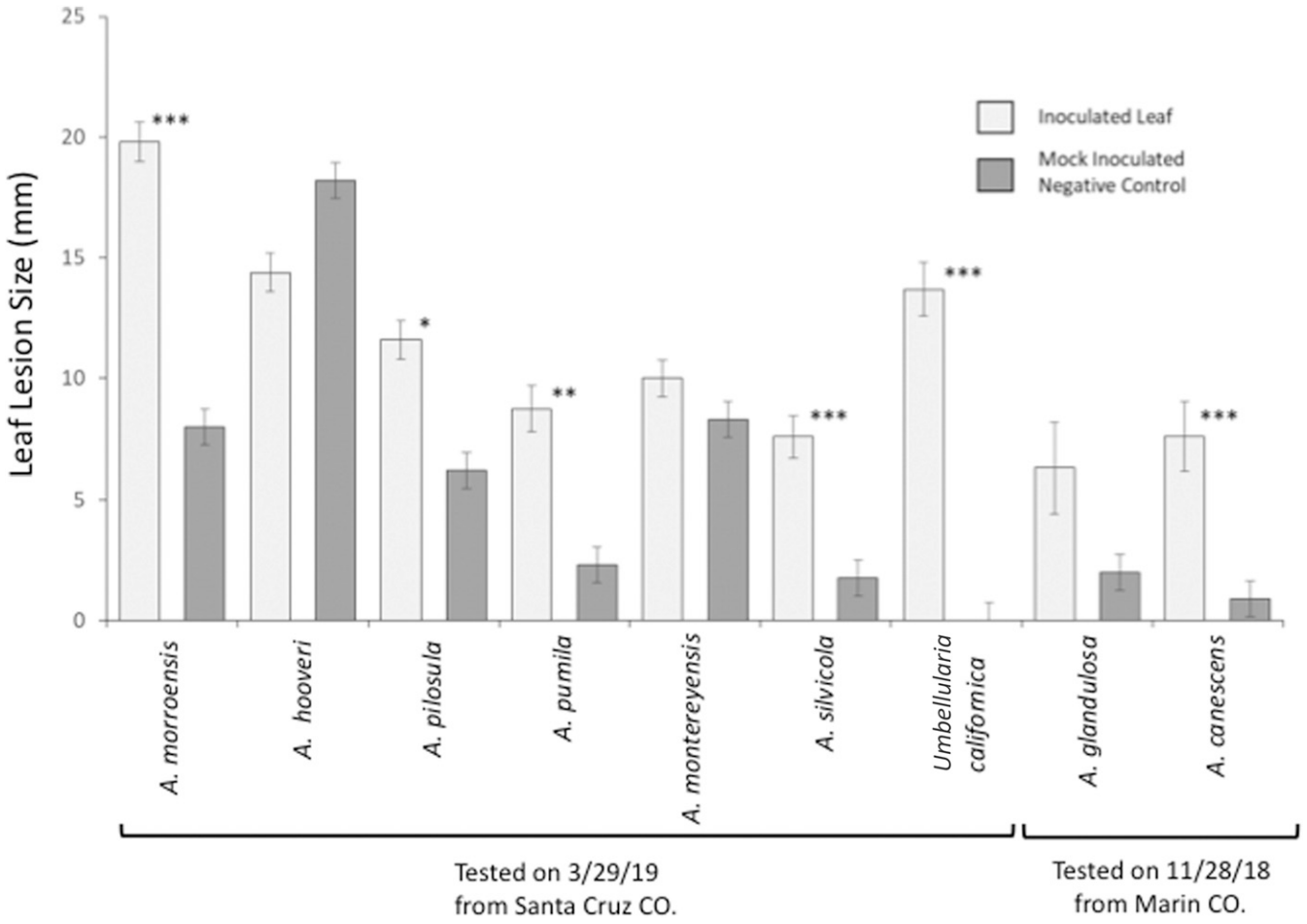

Fig. 4. Length of lesions in leaves of eight *Arctostaphylos* spp. and *Umbellularia californica* (California bay laurel) inoculated with the pathogen *Phytophthora ramorum* or mock-inoculated as controls. Inoculations were performed using zoospore suspensions of the pathogen using isolate 518-leaf.1.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

emerged as being potentially more 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. hooveri*, 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 that 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 reisolated from foliar lesions of all eight species a week after inoculation. This result confirms that all eight 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. *A. 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 that manzanita species may be placed in three groups: very susceptible species (namely, *A. silvicola*, *A. pilosula*, *A. glandulosa*, and *A. hooveri*) species with intermediate susceptibility (namely, A morroensis), and species with lower susceptibility (namely, *A. montereyensis*, *A. pumila*, and *A. canescens*). It would be interesting to study whether *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 (Fig. 1). However, patches of woodland were adjacent to the meadow on the opposite side. All five California bay laurel plants sampled in these woodlands and located at the very edge of the meadow tested positive for *P. ramorum*. All 10 manzanita species sampled were no further than 200 m from an infected bay laurel (Fig. 1), and this distance has been determined as the most likely dispersal range of sporangia during a wet year (Eyre et al. 2013). Given that symptom severity was comparable among the various manzanita species, it is likely that they were all infected by inoculum produced on California bay laurel leaves. It has also been reported that, in drier years, the 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, intermanzanita 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 about 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). The

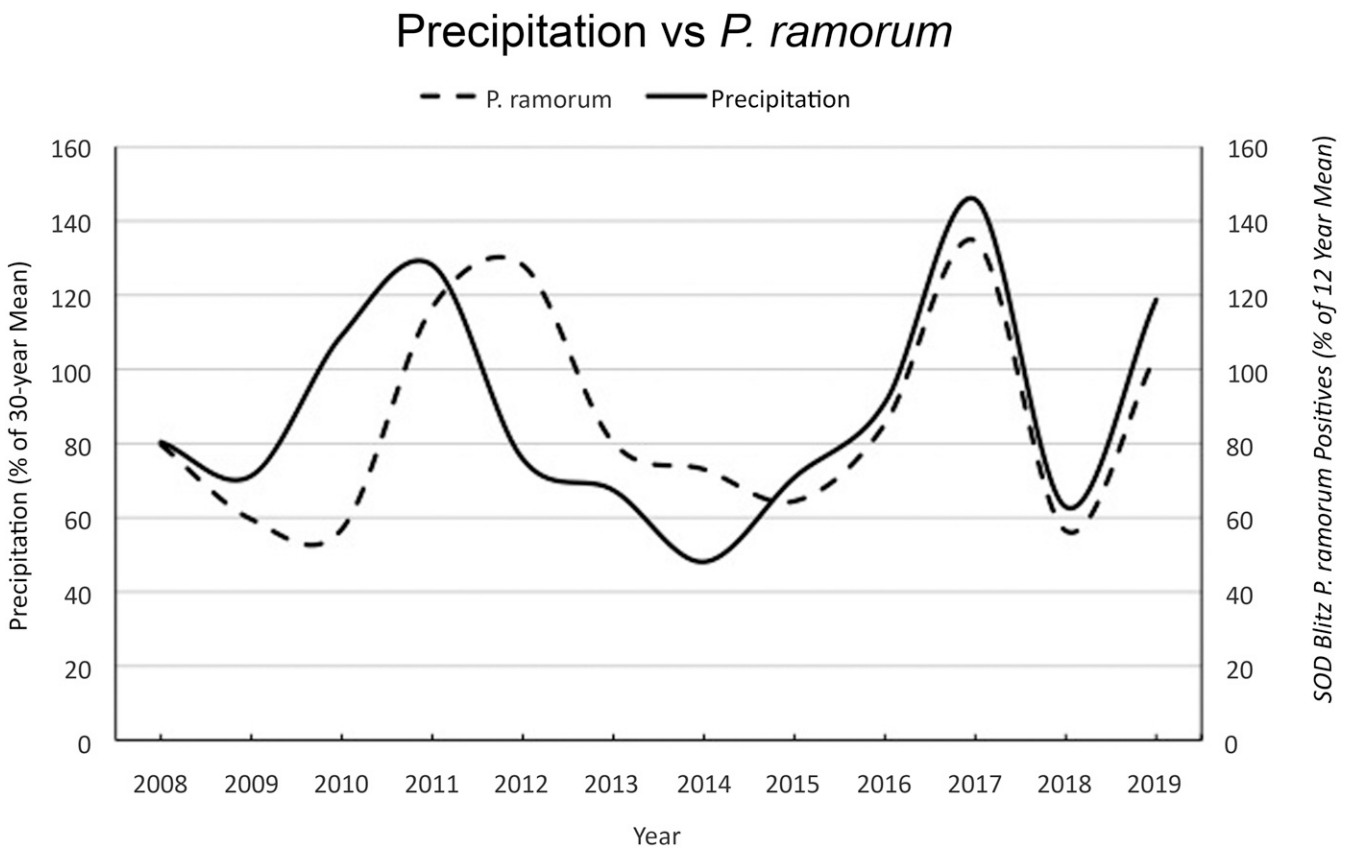

Fig. 5. Graph showing precipitation values and number of plant samples positive for *Phytophthora ramorum* for the zone of infestation by the pathogen in California. Precipitation was expressed as percentage of 30-year period average, while number of samples positive for *P. ramorum* was expressed as percentage of the 12-year (2008 to 2019) average. SOD = sudden oak death.

only exception was the count from the fall inoculation of *A. hooveri*. 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 intramanzanita contagion may occur among branches of the same plant, and intermanzanita contagion may occur periodically in particularly rainy springs if the pathogen was capable of surviving dry spells on this host. The exception of *A. hooveri* requires further investigation but, given the fact that 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.

Finally, it may be legitimate to wonder what changed between 2003 and 2017 leading to the infection of multiple manzanita species in a habitat, an open meadow, traditionally not regarded as ideal for the spread of SOD or ramorum blight. The most conservative explanation is that a combination of at least two prerequisites resulted in conditions particularly favorable to contagion by *P. ramorum*. First, the pathogen has been colonizing new sites at a relatively low spread rate (Mascheretti et al. 2008), and it may have been originally absent or rare from the woodland patches at the edge of the UCSC Arboretum. It is important to note that this is not just an issue of presence or absence but is also an issue of disease incidence and establishment in ideal microhabitats. Our survey showed that all of the five bay laurel plants sampled were infected; thus, disease incidence was high at that particular site in 2017. Second, as shown by our SOD Blitz and precipitation data (Fig. 5), there is a strong positive correlation between rainfall and percentage of trees infected by *P. ramorum*. The extremely high levels of precipitation recorded in 2017 resulted in high infection levels across the state and in Santa Cruz County as well. High infection levels were both cause and effect of the production of a large numbers of sporangia, effectively incrementing the range of their dispersal. Comparable levels of rainfall were recorded in 2011 but it is plausible that the pathogen may not have been that widespread near the UCSC Arboretum at that time or may not have been inhabiting the most suitable niches. Although crowdsourced data has the potential to support applied environmental research (Conrad and Hilchey 2011), its use is not risk free. In fact, the constraints related to equipment availability combined with haphazard sampling schemes may lead to procedural errors and biases, potentially affecting data quality (Conrad and Hilchey 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 as 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 versus statewide percent *P. ramorum*-positive sample graph shows that, in 2011, *P. ramorum*, still in its establishment phase, experienced a 1-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 ($R^2 = 0.03$) when fitting percent *P. ramorum*-positive values against percent rain values of the same year, contrasted by a much higher R^2 value of 0.79 when percent *P. ramorum*-positive values were fit against percent rain of the previous year.

However, starting in 2015, that lag disappears and a linear fit correlating precipitation values with percentage of *P. ramorum* positives shows a much higher R^2 value for 2014 to 2019 than the R^2 value obtained when performing the same linear fit for 2008-to-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 it 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; Hüberli et al. 2012), thus effectively increasing its dispersal rate.

Although we understand that 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 was the case for the eight manzanita species here studied.

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