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Proceedings of the Seventh Sudden Oak Death Science and Management Symposium: Healthy Plants in a World With *Phytophthora*

June 25–27, 2019, San Francisco, California, USA

Forest Service
Pacific Southwest Research Station
General Technical Report PSW-GTR-268
July 2020

Technical Coordinators

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Cover: Illustration of leaves and flowers of California bay laurel (*Umbellularia californica*), one of the most common hosts of *Phytophthora ramorum* in California. By Tim Gunther, Gunther Graphics.

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Proceedings of the Seventh Sudden Oak Death Science and Management Symposium: Healthy Plants in a World With *Phytophthora*

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Susan J. Frankel and Janice M. Alexander, Technical Coordinators

U.S. Department of Agriculture
Forest Service
Pacific Southwest Research Station
Albany, California
General Technical Report PSW-GTR-268
July 2020

Abstract

Frankel, Susan J.; Alexander, Janice M., tech. cords. 2020. Proceedings of the seventh sudden oak death science and management symposium: healthy plants in a world with *Phytophthora*. Gen. Tech. Rep. PSW-GTR-268. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 121 p.

The seventh sudden oak death science and management symposium provided a forum for current research concerning sudden oak death caused by the exotic, quarantine pathogen, *Phytophthora ramorum.* Nearly 50 submissions describing papers or posters on the following sudden oak death/*P. ramorum* topics are included: ecology, monitoring, and nursery and wildland management. Abstracts are also provided from sessions on *Phytophthoras* in California native plant nurseries and restoration sites.

Keywords: *Phytophthora ramorum*, invasive species, tanoak, *Notholithocarpus densiflorus*, coast live oak, *Quercus agrifolia.*

Contents

Sudden Oak Death in Oregon

Slowing the Spread of Sudden Oak Death in Oregon Forests, 2001-2018[1]

Sarah Navarro,[2] Randy Wiese,[2] Casara Nichols,[2] Danny Norlander,[2] Alan Kanaskie,[2] Ellen Michaels Goheen,[3] Everett Hansen,[4] Wendy Sutton,[4] Paul Reeser,[4] Nik Grunwald,[4] Jared LeBoldus,[4] Helmuth Rogg,[5] and Elizabeth Savory[5]

Abstract

Sudden Oak Death (SOD), caused by *Phytophthora ramorum,* is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. In July 2001, the disease was first discovered in coastal southwest Oregon forests. Since 2001, an interagency team has been attempting to eradicate and slow the spread of disease through a program of early detection, survey and monitoring, and destruction of infected and nearby host plants. Eradication treatments, totaling approximately 2,550 ha (6,300 ac), eliminated disease from most infested sites, but the disease continued to spread slowly, mostly in a northward direction. From the initial infestations of 2001, the disease has been detected a maximum distance of 30 km (18.5 mi) to the north, 12 km (7.6 mi) to the northeast along the Chetco River, and 15 km (9.3 mi) to the southeast along the Winchuck River.

In early 2015, the EU1 clonal lineage of *P. ramorum* was detected on a single tanoak tree located approximately one mile north of a small private nursery (now closed) near the Pistol River. Genotype comparison of the tanoak and nursery isolates suggests the nursery as the probable source for the forest infestation. This is the first report of the EU1 lineage in US forests. EU1 infested trees have continued to be detected within a small geographic area just north of Pistol River resulting in 190 ha (470 ac) of eradication treatments from 2015 to the end of 2018.

In 2017, an Oregon SOD Task Force convened local, state and federal governments and agencies, local tribes, industry associations, and local residents and environmental groups. The mission of the Task Force was to develop a collaborative-based strategic action plan,

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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including securement of additional resources to contain the NA1 pathogen of *Phytophthora ramorum* and eradicate the EU1 pathogen of *Phytophthora ramorum* in Curry County, Oregon using the best available science.

Comparative Epidemiology of EU1 and NA1 Lineages of *Phytophthora ramorum* in Southwestern Oregon Tanoak Forests[1]

Jared M. LeBoldus[2] and Kelsey L. Søndreli[2]

Abstract

Phytophthora ramorum, cause of Sudden Oak Death (SOD), is an invasive pathogen that infects over 100 species of plants and has been introduced multiple times into the coastal forests of southern Oregon and northern California. In southwestern Oregon forests, tanoak (*Notholithocarpus densiflorus*) is the most susceptible species developing lethal stem cankers and sporulating from infected leaves and branches. The NA1 lineage was first reported in Oregon in the early 2000s and in 2015 the EU1 lineage was discovered infecting tanoak in the South Fork Pistol River drainage in Curry Co., Oregon. Using an approach developed by Garbelotto and others (2017) sporulation of each lineage was compared at six sites (3 NA1; 3 EU1). Sporulation, temperature, and relative humidity were quantified for 5, 2-week intervals in winter 2017/2018 and 2018/2019. In addition, infection frequency of tanoak, Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), western larch (*Larix occidentalis*), and sitka spruce (*Picea sitchensis*) seedlings at the sites was also compared. Preliminary analysis indicates greater sporulation at EU1 sites compared to NA1; however, this did not correspond to increased infection of tanoak seedlings at EU1 versus NA1 sites. In contrast, there were differences in the infection frequency of Douglas-fir (EU1 = 37%; NA1 = 10%), western hemlock (EU1 = 10%; NA1 = 0%); sitka spruce (EU1 = 55%; NA1 = 0%); and larch (EU1 = 90%; NA1 = 13%). Differences in the infection rate of conifer seedlings at EU1 compared to NA1 sites and the implication for the management of SOD will be discussed.

Literature Cited

Garbelotto, M.; Schmidt, D.; Swain, S.; Hayden, K. and Lione, G. 2017. The ecology of infection between a transmissive and a dead-end host provides clues for the treatment of a plant disease. Ecosphere. 8(5): e01815.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Phytophthora ramorum in California

Over Two Decades of Sudden Oak Death in California[1]

Kerri M. Frangioso,[2] Susan J. Frankel,[3] Christopher A. Lee[4]

Abstract

Phytophthora ramorum garnered international attention upon its discovery in the summer of 2000 (Rizzo and others 2002), but thanks to genetic information collected from around the state, we know the pathogen has been in California since the 1980-1990s (Mascheretti and others 2008). As the decades pass and the possibility of large-scale eradication passes solidly out of reach, we continue to witness the transformation of our forests. New tree mortality ebbs and flows as pathogen populations contract and expand during years of severe drought followed by seasons with adequate rainfall. Decades later, millions of trees have been lost, and the pathogen continues to spread and kill trees; yet we still have a lot to lose: habitat, carbon storage, natural beauty, as well as other cultural and ecological values.

From the forests of Monterey County along the Central Coast, to Del Norte County in northern California and into southern Oregon, the pathogen, although a clone, behaves quite differently throughout its range. We have observed wildfire interacting with the pathogen to exacerbate fire severity and create dangerous fire suppression conditions and extreme fuel mitigation issues in Monterey County (Cobb and others 2016, Lee and others 2009, Metz and others 2011). In the greater San Francisco Bay Area there is extensive property damage, and park-land degradation. Along the Sonoma and Mendocino Count coasts, sudden oak death impacts are combining with those of other non-native forest pathogens to unforeseen eventual effect as dominant tree species are eliminated from coastal vegetation. In the farthest north of the pathogen's range, bay laurel (*Umbellularia californica*) trees rarely harbor the pathogen but for the rest of its range, bay is implicated in driving mortality in almost every study.

Many hope that finding both mating types of *P. ramorum* in Vietnam could yield new insight into resistance mechanisms for our local tree species. Meanwhile, another lineage of *P. ramorum* arrived in the Pacific Northwest instilling fear that hybridization or mutation of an already virulent, generalist pathogen could allow for expansion into new ecosystems.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.
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In California forests, cumulative tree mortality levels are at an unprecedented high, as is the loss of life and property due wildfire. For coastal California forests, sudden oak death further compounds the challenge to sustain trees and plants that are integral to the health and well-being of the humans, plants and animals that dwell with them.

Literature Cited

Cobb, R. C.; Meentemeyer, R. K.; Rizzo, D. M. 2016. Wildfire and forest disease interaction lead to greater loss of soil nutrients and carbon. Oecologia. 182: 265–276.

Lee, C.; Valachovic, Y.; Frankel, S. and Palmieri, K. 2009. Sudden oak death mortality and fire: lessons from the Basin Complex. Pages 271 – 279, In, Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M. 2010. Proceedings of the Sudden Oak Death Fourth Science Symposium. Gen. Tech. Rep. PSW-GTR-229. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 378 p.

Mascheretti, S.; Croucher, J.P.; Vettraino, S.; Prospero and Garbelotto, M. 2008. Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology. 17: 2755-2768.

Metz, M.R.; Frangioso, K.M.; Meentemeyer, R.K. and Rizzo, D.M. 2011. Interacting disturbances: wildfire severity affected by stage of forest disease invasion. Ecological Applications. 21: 313–320.

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W. and Koike, S.T. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflora* in California. Plant Disease. 86: 205–214.

Impacts of and Responses to Sudden Oak Death on Marin Watershed Lands[1]

Andrea Williams[2] and Janet Klein[3]

Abstract

The Marin Municipal Water District (MMWD) stewards 22,000 acres of watershed lands in Marin County. Recognized as a biodiversity hotspot, with over 1,000 plant taxa in more than 100 recognized communities, it is one of the first introduction sites of *Phytophthora ramorum* in California. In part to understand and monitor this new threat, MMWD completed its first Vegetation Classification and Map in 2005 with support from California Native Plant Society, California Department of Fish and Wildlife, and Aerial Information Systems (AIS). A 2010 re-map of forested areas with support from the USDA Forest Service and AIS showed the progression of SOD and shift in vegetation types within impacted areas, and a 2015 map tracks increasing canopy gaps and additional impacts to oak woodlands (AIS 2015). Ground sampling and maintenance records reveal new hosts (Rooney-Latham and others 2016), community shifts, threat interactions, and increasing costs and fire danger from dead and downed trees. Responses to forest disease vary based on severity, location, and vegetation type impacted, but include cutting dead and down trees and altering planting palettes.

Literature Cited

Aerial Information Systems, Inc. 2015. Summary report for the 2014 photo interpretation and floristic reclassification of Mt. Tamalpais watershed forest and woodlands project, prepared by Aerial Information Systems, Inc. for the Marin Municipal Water District. Report on file.

Rooney-Latham, S.; Blomquist, C.L.; Williams, A.; Gunnison, E. and Pastalka, T. 2016. Identification of five new hosts of *Phytophthora ramorum* in an infested forest in California. In, Frankel, S.J.; Harrell, K.M., tech. coords. 2017. Proceedings of the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. Pages 83-84.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.
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Status of the *Phytophthora ramorum* Epidemic Across Forests of the East Bay Regional Park District, San Francisco Bay Area, California[1]

Brice A. McPherson,[2] David L. Wood,[2] Greg Biging,[2] Maggi Kelly,[2] and Sylvia R. Mori[3]

Abstract

The East Bay Regional Park District, the largest urban park network in the United States, includes extensive coast live oak (*Quercus agrifolia*)-dominated forests at the urban-wildland interface. Parks that encompass chaparral, grasslands, riparian habitats, and hardwood and conifer forests are adjacent to one of the most heavily-populated urban regions in the country. From 2008 to 2011, we placed 535 randomly assigned, 10 m radius fixed plots in coast live oak-bay laurel (*Umbellularia californica*) stands in each of five parks to establish baseline disease conditions. The random design permits extrapolation to landscape scales. Baseline data included diameter at breast height (DBH) for all woody stems >2.5-cm, disease status of coast live oaks, and woody plant regeneration. Plots were re-assessed between 2015 and 2018 to quantify change and to develop projections for future change.

All parks exhibited increases in infection and mortality levels, with annual infection rates as high as 7.7%. Infections increased markedly following the cessation of the 2011-2015 drought. Despite general similarities in species composition, 2011 infection levels varied from 6.3% (Anthony Chabot) to 14.4% (Wildcat Canyon) and mortality varied from 4.1% (Wildcat Canyon) to 8.7% (Redwood Park). The 2015-2018 evaluations found infection levels between 8.7% (Anthony Chabot) and 27.7% (Wildcat Canyon) and mortality levels from 8.7% (Anthony Chabot) to 29.9% (Wildcat Canyon). Within-park variation in disease and mortality may reflect stand level differences in mean coast live oak DBH, but land-use history also probably affects disease levels. Larger coast live oaks show much higher levels of infection and mortality than the more abundant smaller size classes. The disproportionate loss of the largest mast-bearing trees in these forests will affect wildlife in ways we yet do not understand. In addition, the increase in fuels in these evergreen forests increases the risks of catastrophic wildfire in stands that lie to the east of large population centers.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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We also conducted surveys of three more inland parks; Diablo Foothills and Pleasanton Ridge Regional Parks and Las Trampas Regional Wilderness. Diablo Foothills was assessed using randomly placed plots. Despite having much less coast live oak than blue oak (*Q. douglasii*, not a known host), 34% of the coast live oaks (N = 259) in Diablo Foothills were symptomatic in 2016. The other two parks were surveyed, but not randomly, for presence of symptomatic coast live oaks in 2018. In Las Trampas, 30.6% of coast live oaks (N = 98) were symptomatic, with 10% dead. Pleasanton Ridge showed 10% symptomatic (N = 211), with less than 1% dead.

The continuing epidemic is leading to permanent changes in landscape composition and environmental services, which will require attention to management of these forests adjacent to large populations centers.

P. ramorum Management

Early Host Resistance Selection and Development Should Have Been a Primary Management Response to the Sudden Oak Death Epidemic[1]

Pierluigi (Enrico) Bonello[2] and Richard Sniezko[3]

Abstract

In this presentation I will illustrate the theoretical foundations for a proposed drastic change in how we respond to invasive alien forest pathogens, like *Phytophthora ramorum*. This new framework is the result of an in-depth analysis of the reasons why effective management of invasive alien phytophagous insects and phytopathogens (PIPs) in forest environments remains an elusive aspiration (Showalter and others 2018). A fundamental reason for why we continue failing is that such PIPs encounter evolutionarily naïve host trees in their new environments, which are incapable of mounting adequate resistance responses. However, it is also true that even the most undefended host populations almost always include individuals that are capable of resisting attack. Such resistance need not be absolute (immunity), but sufficient to ensure survival and reproduction of the target host, so that either natural selection can act directionally upon the traits conferring such resistance, or modern approaches can be brought to bear towards tree improvement programs that are increasingly capable of rapidly selecting and augmenting tree defenses. In the latter case, improved trees can then be used for plantings that are capable of withstanding such invasive alien PIPs. Both in-field directional selection and tree-for-planting improvement programs can be accelerated tremendously by using non-destructive resistance screening techniques such as those we have developed for the coast live oak-*P. ramorum* pathosystem. In all cases, however, to be a successful management approach, careful target selection, early implementation and sustained support are fundamental. I will illustrate a simplified proposed framework to guide future responses to invasive alien PIPs like *P ramorum*.

Literature Cited

Showalter, D.N.; Raffa, K.F.; Sniezko, R.A.; Herms, D.A.; Liebhold, A.M.; Smith, J.A. and Bonello, P. 2018. Strategic development of tree resistance against forest pathogen and insect invasions in defense-free space. Frontiers in Ecology and Evolution – Conservation. DOI: 10.3389/fevo.2018.00124.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.
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Incidence and Distribution of Resistance in a Coast Live Oak/Sudden Oak Death Pathosystem[1]

Anna O. Conrad,[2] Katherine M. D'Amico,[2] Pierluigi Bonello,[2] Brice A. McPherson,[3] David L. Wood,[3] and Horacio D. Lopez-Nicora[4]

Abstract

In coastal California, infection by the pathogen *Phytophthora ramorum*, causal agent of sudden oak death, results in extensive mortality of native oak species including *Quercus agrifolia* (coast live oak). However, apparently resistant *Q. agrifolia* have been observed within native populations. In this study (Conrad and others 2019), we monitored disease progression from 2010 to 2017 in *Q. agrifolia* artificially inoculated with *P. ramorum* and disease incidence in *Q. agrifolia* left to become naturally infected in the same stand. After seven years, 61% of artificially inoculated *Q. agrifolia* died while 27% appeared to be resistant (i.e. in remission, no longer showing active symptoms of *P. ramorum* infection) (N = 149). In addition, 13% of non-inoculated *Q. agrifolia* showed symptoms of natural *P. ramorum* infection, e.g. bleeding exudate (N = 423). Canker length measured approximately one year following inoculation was a significant predictor of *Q. agrifolia* resistance and survival ($P < 0.001$). Canker length was also used to examine the distribution of resistant and susceptible *Q. agrifolia* across the landscape using inverse distance weighted analysis. This analysis revealed resistant and susceptible *Q. agrifolia* are aggregated, suggesting resistance is a heritable trait. A better understanding of the amount and distribution of resistant *Q. agrifolia* within native populations can be used to facilitate the restoration of disturbed habitats and identify sources of germplasm for future breeding efforts.

Literature Cited

Conrad, A.O.; McPherson, B.A.; Lopez-Nicora, H.D.; D'Amico, K.M.; Wood, D.L.; Bonello, P. 2019. Disease incidence and spatial distribution of host resistance in a coast live oak/sudden oak death pathosystem. Forest Ecology and Management. 433: 618-624. DOI: 10.1016/j.foreco.2018.11.035.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.
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Long-term Performance of Sudden Oak Death Management Treatments in Northern California Locations[1]

Tedmund J. Swiecki[2] and Elizabeth A. Bernhardt[2]

Extended Abstract

Sudden oak death (SOD), caused by *Phytophthora ramorum*, was first diagnosed as the cause of a lethal canker disease of coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and tanoak (*Notholithocarpus densiflorus*) in 2000. Between 2005 and 2009, we initiated multiple field studies to test strategies for reducing SOD impacts in stands of tanoak and susceptible oaks. In oak forests, California bay laurel (*Umbellularia californica*) is the primary source of *P. ramorum* inoculum that infects oaks, and proximity to bay greatly increases disease risk (Swiecki and Bernhardt 2008). We evaluated the effectiveness of removing California bay, either on an area-wide basis or locally around individual oaks, on the development and progress of SOD in coast live oak, Shreve oak (*Q. parvula* var. *shrevei*), and canyon live oak (*Q. chrysolepis*). Studies were conducted at four Midpeninsula Regional Open Space District preserves (OSP) on the San Francisco Peninsula (https://www.openspace.org/) where *P. ramorum* was present at the study start (fig. 1).

Area-wide removal was used in study areas where bay was present mostly as understory regeneration and small trees, and bay could be readily cleared from the entire plot area. Local bay removal was used to protect large high-value oaks where bay trees were large and abundant. The treatment objective was to eliminate or minimize bay canopy within 2.5 to 5 m of the oak trunk. The minimum horizontal clearance for each oak was measured from its trunk to a vertical plumb line that touched the edge of the nearest bay foliage. The plumb line was determined using a laser pointer attached to an angle gauge. In the Monte Bello OSP area-wide bay removal plot, minimum oak-bay clearance was 5.4 m, but almost all trees had at least 10 m of clearance. Area-wide removal at Rancho San Antonio OSP was conducted in smaller patches, leaving more edges close to bay; 10 of 42 monitored trees in the treated area had clearances less than 5 m. Among trees treated by local bay removal treatment at all locations, bay cover within 5 m of the trunk was always reduced, but 7 of 95 treated trees still had zero clearance to overstory bay canopy, and 7 other trees had clearances between 0.5 and 2.1 m. Remaining treated oaks had clearances of 2.8 m or more, with maximum clearances of 25 to 30 m. At two locations (fig.1, bottom), some oaks with suboptimal bay clearance were also treated with potassium phosphite

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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applied by stem injection (15.3% a.i., Rancho San Antonio only) or by stem spray as described below for tanoaks.

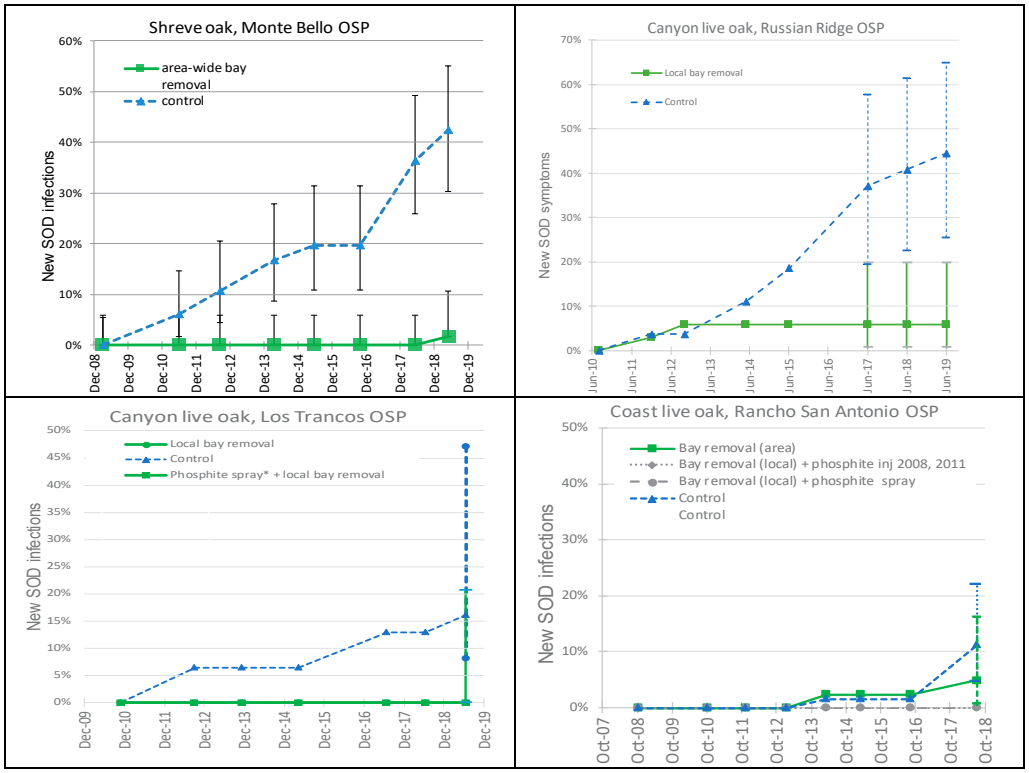

Figure 1—SOD disease progress in untreated control oaks and oaks treated by local or area-wide bay removal at four preserves. Note that scales vary between graphs. Error bars are exact binomial confidence limits. In two locations (bottom) some trees were also treated with phosphite through 2018.

California bay removal treatments around susceptible oaks was effective in preventing new infections compared to matched untreated controls (fig. 1, upper graphs). Early SOD symptoms in canyon live oaks are commonly lacking or very cryptic (Swiecki and others 2016); symptoms on this oak species at Russian Ridge by 2012 (fig. 1, top right) were likely from infections occurring before the start of the study in December 2009. Drought conditions from 2006-2010 and 2011-2016 suppressed disease progress, especially at Rancho San Antonio OSP, the warmest and driest location (fig. 1, bottom right). Disease incidence in control plots increased sharply in years following the wet winters of 2016-17 and 2018-19, whereas only two trees from treated areas developed symptoms over this period. One of these was in dense poison oak (*Toxicodendron diversilobum*), a *P. ramorum* host, and the other was at the base of a steep slope with bay canopy. Adding potassium phosphite treatments to oaks treated with bay removal (fig. 1, lower graphs) provided no additional benefit beyond bay removal alone.

Inoculum from California bay greatly enhances disease development in tanoak stands but *P. ramorum* sporulates readily on tanoak twigs and leaves. Hence, measures beyond bay removal are needed to manage SOD in tanoak stands. Stem application of the systemic chemical

potassium phosphite has been identified as a possible treatment for preventing SOD in susceptible oaks and tanoaks (Garbelotto and Schmidt 2007, 2009). Starting in 2005, we initiated a series of studies to determine if trunk spray applications of this chemical could prevent infection or suppress SOD development to a practical degree in tanoak stands. Phosphite application, which needs to be repeated indefinitely, would need to suppress SOD very substantially to justify the treatment cost. Minor suppression of SOD incidence over short time periods, even if statistically significant, is not likely to be of practical importance in managing affected stands.

At four locations in Sonoma County and two in San Mateo County, plots were established that were initially free of SOD but close to areas with tanoak mortality from *P. ramorum*. California bay was not present in treated or control plots. Trunk spray applications of aqueous potassium phosphite (22.36% a.i.) with Pentra-Bark® surfactant (2.3% v/v) were made at 6-month intervals the first year and annually thereafter. To optimize phosphite activity, spray volumes were scaled to tree diameter. Spray was banded on the trunk starting at a height of about 6 m and applied downward to favor absorption through the thinner bark and maximize potential for absorption as residues were remobilized by rain (Swiecki and Bernhardt 2017). Treating trees in contiguous blocks, especially large blocks, should also optimize disease suppression if phosphite translocated to the canopy suppresses sporulation. The Sonoma County plots (locations SF, BL, PC, and FE) were relatively small, mostly about 30-75 tanoak stems per plot (average area 0.063 ha). The San Mateo plots were much larger, with 233 treated and 243 control tanoaks (1.35 and 1.37 ha, respectively) at Skyline and 159 treated and 166 control tanoaks (0.35 ha for both) at El Corte de Madera OSP. At these locations, groups of trees monitored as controls were distributed around a single large treated plot.

Some of the phosphite treated trees at all locations, have developed SOD, even in plots where annual applications of potassium phosphite were initiated many years before *P. ramorum* was detected in the plots, indicating that the treatment did not completely prevent infection. In two study locations (Skyline and SF), phosphite-treated plots had significantly higher SOD incidence than the adjacent control plots, starting from the time that SOD was first detected in the plots. SOD incidence in the phosphite-treated plots was 32% at both Skyline and SF when phosphite treatments were terminated after 5 and 6 years of applications, respectively. The large Skyline plots were dominated by large-diameter tanoaks (average 45 cm DBH), whereas the SF plots had a mixture of large and small diameter trees (average 24 cm DBH).

Drought conditions that persisted from 2012 through 2016 inhibited the advance of *P. ramorum* into plots at the remaining study locations. SOD incidence at El Corte de Madera and FE plots was between 0 and 5% at their last evaluations in 2018. Following the wet winters of 2016-17 and 2018-19, an increase in SOD was observed at the BL and PC locations in 2019, which are dominated by smaller tanoaks (average DBH in treated plots 15 and 19 cm, respectively). The incidence of SOD in the control plots at BL and PC currently exceeds that seen in the phosphite-treated plots. However, because the initial invasion of tanoak stands by *P. ramorum* is very spotty on a local scale these early differences may not represent actual treatment effects.

In the SF and Skyline plots described above, SOD incidence was initially greater in phosphite-treated plots and remained so during the study observation intervals (6 and 4 years, respectively, from first observed trunk cankers). Because phosphite treatment is unlikely to increase SOD incidence, results from these plots illustrate that persistent differences in disease incidence can develop between closely spaced plots due to chance alone. In plots such as these, in which phosphite treatment clearly did not prevent extensive infection, the lack of a treatment effect can be interpreted with confidence over a relatively short time interval. However, because control plots may initially experience much higher levels of SOD than treated plots by chance alone, it is necessary to withhold judgement on potential positive treatment effects in tanoak stands until enough time has elapsed for disease levels to even out within the stand. To provide proof of an actual treatment effect, treated plots need to maintain very low disease levels after multiple years that are favorable for infection while disease levels in adjacent untreated areas continue to increase.

The observed lack of phosphite efficacy may be related to insufficient phosphite accumulation in target tissues (phloem of lower bole) or inadequate uptake. Stem diameter could be a factor influencing phosphite uptake in tanoaks treated with trunk spray applications. Although we scaled the applied phosphite dose by tree diameter and applied phosphite as high as feasible on the stems, the thicker layer of dead outer bark that occurs on larger trees may inhibit phosphite absorption. Phosphite might provide some protection to small diameter tanoaks that have little or no dead outer bark, though this has not yet been demonstrated in our plots. If SOD is partially suppressed in small tanoak, land managers would need to determine if any reduction in mortality of small tanoaks justifies the recurring expense of treatment, especially if the treatment is likely to become ineffective as the trees increase in size.

Acknowledgements

Funding was provided by USDA Forest Service State and Private Forestry, and Pacific Southwest Research Station, Midpeninsula Regional Open Space District, San Francisco Public Utilities Commission, Kashia Band of Pomo Indians of the Stewarts Point Rancheria, and Phytosphere Research. We thank project managers Phil Cannon, Susan Frankel, Cindy Roessler, Coty Sifuentes-Winter, Ellen Natesan, and Veronica Corella for their support. We thank Bill Stringfellow, Quest Products, for donations of potassium phosphite and Pentra-Bark®.

Literature Cited

Garbelotto, M.; Schmidt, D.J. and Harnik, T.Y. 2007. Phosphite injections and bark application of phosphite + Pentrabark™ control sudden oak death in coast live oak. Arboriculture & Urban Forestry. 33: 309–17.

Garbelotto, M. and Schmidt, D.J. 2009. Phosphonate controls sudden oak death pathogen for up to 2 years. California Agriculture. 66: 10-17.

Swiecki, T.J. and Bernhardt, E. 2008. Increasing distance from California bay reduces the risk and severity of *Phytophthora ramorum* canker in coast live oak. Pages 181-194 in: Frankel, S.J.; Kliejunas,

J.T.; Palmieri, K.M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 491 p.

Swiecki, T.J. and Bernhardt, E.A. **2017**. Field studies evaluate methods to prevent sudden oak death in oaks and tanoak. Forest Phytophthoras. 7(1): 15-29. doi: 10.5399/osu/fp.7.1.4024.

Swiecki, T.J.; Bernhardt, E.A.; Aram, K.; Rizzo, D.M.; Kasuga, T. and Bui, M. 2016. *Phytophthora ramorum* causes cryptic bole cankers in canyon live oak. Plant Health Prog. 17: 20-26.

Selective Thinning of California Bay Laurel is a Cost-Effective way to Control *Phytophthora ramorum* in Mixed-Oak Woodlands[1]

Doug Schmidt,[2] Laura Sims,[2] Guglielmo Lione,[2] Melina Kozanitas,[2] and Matteo Garbelotto[2]

Abstract

It has been long determined that *Umbellularia californica* (California bay laurel) is the key transmissive host for the sudden oak death pathogen (SOD, *Phytophthora ramorum*) in mixed-oak woodlands, however the parameters of bay to oak disease transmission have not been fully clarified (Garbelotto and others 2003). Here, we concisely present results of three studies: one clarifying the parameters of bay to oak transmission, and two testing whether two cost-effective approaches involving selective removal of a limited number of California bay laurel trees may reduce disease incidence in naturally infested oak stands.

Study 1 consisted of a multi-year survey of approximately 900 bay laurels and two thousand oaks (mostly *Quercus agrifolia*, coast live oak) across 63 transects in the San Francisco Public Utilities Commission (SFPUC) mixed-oak woodlands in San Mateo County. Results identified an inverse relationship between bay to oak distance and oak infection, with infection levels approaching zero as distance between bays and oaks approaches 20 m. Oak size (stem diameter), was positively correlated with likelihood of SOD infection. Study 1 also identified that bay infection levels dropped dramatically during dry years.

Study 2 (Garbelotto and others 2017) consisted of a stand manipulation trial in which the frequency of Inoculum Pressure Events (IPE) above the threshold levels necessary to infect oaks was monitored every 3 weeks for 7 years in 64 "treatment" and in 64 "control" plots located in the Soquel Demonstration Forest (Santa Cruz County). After 2 years, all bay laurels were removed from "treatment" plots, generating bay-free buffer zones around plots of 10 and 20 m. Results showed that inoculum pressure was strongly affected by rainfall and by bay absence/presence. IPE frequency was reduced to zero during drought years, while in wet years, IPE frequency was significantly lower in treatment than in control plots, with a stronger effect in plots with a 20 m bay-free buffer around them. Results thus provided additional experimental

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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evidence that selectively removing bays 10 and 20 m around oaks reduces the likelihood of oak infection.

Study 3 was performed in 4 “treatment” and 4 “control” plots in SFPUC lands between 2014 and 2015, as follows. Eight plots with low bay disease incidence at the end of a 4-year long drought were identified. In each plot, SOD disease incidence was recorded on 25 bay laurels. The 2014 results identified all bays still carrying SOD infection during a drought, and in 2015 these bays (5 to 8 per plot) were herbicide-killed in the four treatment plots. In 2016, despite extremely high rainfall, disease incidence was significantly lower in treatment than in control plots, and the same result was obtained in 2017. Conversely, in 2018, a dry year, disease incidence dropped to very low levels both in treatment and control plots. These results indicate that selectively removing the small numbers of bay laurels that are infected by *P. ramorum* after a prolonged drought effectively reduces SOD disease incidence. The selection of bays to be removed can be either driven by their proximity to oaks or by their infection status at the end of a drought.
This finding thus provides a cost-effective way to manage SOD in mixed-oak woodlands.

Literature Cited

Garbelotto, M.; Davidson, J.M.; Ivors, K.; Maloney, P.E.; Hüberli, D.; Koike, S.T. and Rizzo, D.M. 2003. Non-oak native plants are main hosts for sudden oak death pathogen in California. California Agriculture. 57(1): 18-23.

Garbelotto, M.; Schmidt, D.; Swain, S.; Hayden, K. and Lione, G. 2017. The ecology of infection between a transmissive and a dead-end host provides clues for the treatment of a plant disease. Ecosphere. 8(5):e01815 10.1002/ecs2.1815.

Diagnostics and Genetics

A High Throughput DNA Fingerprinting Tool for Biosurveillance of the Sudden Oak Death Pathogen *Phytophthora ramorum*[1]

Resmi Radhamony,[2] Arnaud Capron,[2] Nicolas Feau,[2] Richard Hamelin,[2] and Angela Dale[3]

Abstract

Phytophthora ramorum has emerged repeatedly as four distinct clonal lineages in North America (lineages NA1, NA2, and EU1) and Europe (EU1 and EU2). Long-distance migration of *P. ramorum* is known to have occurred via the nursery trade. While most populations sampled in North American forests belong to the NA1 clonal lineage, nurseries have been shown to be infested with three lineages NA1, NA2 and EU1. EU1 and NA2 populations were discovered in environments around nurseries suggesting that nursery infestations could spread in the forest. It is therefore important to monitor populations for clonal lineages and screen for emergence of potential new lineages. To achieve this goal, we are developing a high throughput genomic tool that uses targeted sequencing to accurately identify species and lineage from minute amounts of pathogen material within a 24-hour time frame.

Targeted sequencing of a defined subset of the genome (<5Mb) allows PCR based enrichment of select genomic regions. This technique is powerful for environmental and outbreak samples given its robustness and speed. We selected our target genome regions in a hierarchical fashion using published gene sequences and unique markers generated by genome comparison (Feau and others 2018). Sequencing data from outbreak and survey samples can be used to accurately identify the pathogen, its lineage, and potential sources of introduction using primer panels that target unique regions.

We have developed two detection panels comprising 114 amplicons that can generate genome sequences polymorphic among *Phytophthora* species (panel I) or between *P. ramorum* lineages (panel II). We tested these panels on 28 samples and generated over 500 single nucleotide polymorphisms. We used variant calling, principal component analysis and phylogenetic assignment to accurately assign each sample to its phylogenetic clade and *P. ramorum* samples to the right lineage. This approach is scalable since each panel can be augmented as needed, and high-throughput as 384 samples can be pooled in a single reaction. The assay is suitable for

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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P. ramorum outbreaks to facilitate an understanding of its spread and to enable early detection and control.

Literature Cited

Feau, N.; Beauseigle, S.; Bergeron, M.J.; Bilodeau, G.J.; Birol, I.; Cervantes-Arango, S.; Dhillon, B.; Dale, A.L.; Herath, P.; Jones, S.J.M.; Lamarche, J.; Ojeda, D.I.; Sakalidis, M.L.; Taylor, G.; Tsui, C.K.M.; Uzunovic, A.; Yueh, H.; Tanguay, P.; Hamelin, R.C. 2018. Genome-Enhanced Detection and Identification (GEDI) of plant pathogens. PeerJ-the Journal of Life and Environmental Sciences. 6: e4392.

Dual Transcriptome Analysis Reveals Insights into Innate and Phosphite-Induced Resistance of Tanoak to *Phytophthora ramorum*[1]

Takao Kasuga,[2] Matteo Garbelotto,[3] Catherine A. Eyre,[3] Peter J. P. Croucher,[3] Shannon Schechter,[3] Katherine J. Hayden,[4] and Jessica W. Wright[5]

Abstract

Phosphites have been used in the control of sudden oak death, however, the precise mode of action of these compounds is not fully understood. In order to study the action of phosphites in the context of naturally occurring host resistance, we designed an inoculation experiment on four open-pollinated tanoak families, previously defined as partially resistant. Stems of treatment-individuals were sprayed with phosphite, and 7 days later, distal leaves were inoculated with the sudden oak death pathogen, *Phytophthora ramorum*. Leaves from treated and untreated control plants were harvested for RNA extraction before and 7 days after inoculation, and transcriptomes of both host and pathogen were analyzed. We found that tanoak families differed in the presence of innate resistance and in the response to phosphite treatment. Sets of genes associated with innate resistance and with phosphite-induced resistance showed little overlap among tree families. However, sets of genes associated with innate resistance and with phosphite-induced resistance largely overlapped within a more susceptible but phosphite-treatment responsive tanoak family, supporting the hypothesis that phosphite treatment increases the resistance of susceptible host plants to *Phytophthora* infection. In addition, our dual RNA-Seq enabled us to monitor gene regulation of the pathogen *in planta*. Genes for energy generation such as those in the TCA cycle and genes for amino acid membrane transporters were upregulated, whereas elicitin genes were downregulated when comparing genic expression of *P. ramorum* in tanoak leaves relative to genic expression of *P. ramorum* mycelium in culture. We also found that genes of the pathogen involved in detoxification, such as ATP-binding cassette (ABC) transporters and vitamin B_6 biosynthesis genes, were upregulated in phosphite-treated plants, but not in untreated plants. Upregulation of these genes has been observed for axenic culture of *P. cinnamomi* in the presence of phosphite, indicating these genes responded to the direct toxicity of phosphite. In summary, our dual RNA-Seq supports a dual mode of action of phosphite compounds, including a direct toxic effect on *P. ramorum* and an indirect enhancement of resistance in the tanoak host.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Lightning Talks

Bark Scribing as a Treatment for Sudden Oak Death: A Case Study in Why Controls Matter[1]

Steven Swain,[2] Doug Schmidt,[3] and Matteo Garbelotto[3]

Abstract

Over the past two decades, many treatment methodologies have been proposed to therapeutically treat oaks infected with *Phytophthora ramorum*, causal agent of sudden oak death. Because of the coastal distribution of *P. ramorum*, one of the species at greatest mortality risk is coast live oak, *Quercus agrifolia*. Bark scribing, a treatment technique listed by the University of California Integrated Pest Management program to treat *Phytophthora* infected citrus trees, seemed to show promise, especially as it had a treatment history going back nearly 100 years. We therefore endeavored to test the technique on *Q. agrifolia*.

Detecting lesion size in the bark of infected trees is a challenge without cutting into the bark. An attempt at using thermography to non-invasively detect lesion size in naturally infected oaks failed. Another approach is to introduce live inoculum at known rates into the bark of healthy trees. However, mature trees would be required for this study because young trees differ in their infection response. Finding enough uninfected mature trees to do the work took time, as it is somewhat difficult to find property owners willing to potentially sacrifice enough healthy coast live oaks to be statistically significant. In the interim, uncontrolled bark scribing pilot studies using over 200 naturally infected trees suggested that treated oaks had a better than 80% survival rate in the field.

Controlled, replicated studies done on branches of mature oaks show that the situation is much more complicated than expected. Of the approximately sixty trees used in the study, about one third resisted the pathogen so effectively that no growth occurred in either the treatment inoculations, the control inoculations, or both, and in most of these cases no living isolate of the pathogen could be recovered (though residual pathogen DNA could be detected via PCR in many of these lesions). Another sixth or so of the trees were killed by the inoculation, and therefore could not be used for this study. The remaining half of the oaks showed no significant difference between treatments and controls at the 95% confidence level.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Our interpretation of the results is that oaks are surprisingly good at killing *P. ramorum*, even if the trees do not always survive infection. Our findings have been corroborated by other researchers, using other field techniques. Thus, when practitioners utilize tools such as bark scribing to treat infections, results cannot be reliably interpreted from the survival rates of treated trees alone. Furthermore, our results cast doubt upon the purported efficacy of bark scribing as a treatment technique, even in such "proven" systems as citrus (Grafton-Cardwell and others 2008). It turns out that this technique does not have adequately controlled research studies to back up its purported efficacy in citrus systems.

Literature Cited

Grafton-Cardwell, E.E.; Morse, J.G.; O'Connell, N.V.; Phillips, P.A.; Kallsen, C.E. and Haviland, D.R. 2008. UC IPM Pest Management Guidelines: Citrus. UC ANR Publication 3441. http://ipm.ucanr.edu/PMG/r107100411.html (16 January 2019).

Phytophthora species Can Be Reliably Detected by Dogs Both From Infested Substrates and Infected Plants[1]

Lauralea Oliver,[2] Matt Quinn,[3] Tina Popenuck,[4] and Matteo Garbelotto[4]

Abstract

In recent years, reports of *Phytophthora* detections have increased from plant production facilities supplying plant stock for restoration projects (Garbelotto and others 2018). When introduced into new natural habitats through infected plant stock, the potential for *Phytophthora* infection and spread is high. Monitoring of nursery stock is key to reducing new introductions into wildlands, however, sampling in nurseries is currently considered too expensive and complex to be performed on a large scale.

This study was undertaken to determine if it would be possible to train ecological scent detection dogs to discern *Phytophthora species* and discriminate *Phytophthora* odors from other scents in leaves and soil of infected plants. The U.C. Berkeley Forest Pathology and Mycology Lab teamed with H. T. Harvey & Associates to develop a *Phytophthora* detection dog pilot study (Swiecki and others 2018), starting with a single dog.

The training has occurred in phases, first to expose the dog to recognize *Phytophthora* odor in a range of media. Four species of *Phytophthora* - *P. ramorum, P, cinnamomi, P. nemorosa and P. cactorum* – were grown in four different media – soil-water solution, soil-water-pea broth solution, local soil collected under oak trees, and commercial potting soil. The dog had a 100% detection level in blind testing consisting of 10 trials each.

Phase two of the training employed infected *Rhododendron* plants for the scent trials. *P. ramorum* and *P. nemorosa* were inoculated on leaves, while *P. cinnamomi* and *P. cactorum* were soil inoculated. The dog again had a 100% detection success level in blind testing.

In phase three, discrimination training of *Phytophthora* from co-occurring *Pythium* isolates was performed to ensure that the detection is genus specific. In addition, we tested the dog's ability to correctly identify *Phytophthora* infection in plant species other than *Rhododendron* spp. A fifth species of *Phytophthora – P. tentaculata* – was added to the dog's repertoire during this phase.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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The dog had 100% detection rate in double-blind testing consisting of 10 trials on the new *Phytophthora* species and very promising results during the *Pythium* discrimination trial.

Results from the study so far suggest that ecological scent detection dogs may offer an innovative and reliable method to survey for *Phytophthora* in a variety of settings. Dogs could offer a rapid way to reliably detect the pathogen in a variety of controlled environments, such as nurseries; to prescreen plants before they are installed at habitat restoration sites; and possibly to identify infected naturally occurring plants and soil in the field.

Literature Cited

Garbelotto, M.; Frankel, S.J. and Scanu, B. 2018. Soil- and waterborne *Phytophthora* species linked to recent outbreaks in Northern California restoration sites. California Agriculture. 72(4): 208-16.

Swiecki, T.; Quinn, M.; Sims, L.; Bernhardt, E.; Oliver, L.; Popenuck, T. and Garbelotto, M. 2018. Three new *Phytophthora* detection methods, including training dogs to sniff out the pathogen, prove reliable. California Agriculture. 72(4): 217-225.

Using Citizen Science and Outreach Education to Reduce the Risk of *Phytophthora ramorum* Spread in Oregon Forests[1]

Norma Kline,[2] Sarah Navarro,[3] and Jared LeBoldus[4]

Abstract

Sudden oak death (SOD), caused by a non-native pathogen *Phytophthora ramorum* has killed hundreds of thousands of tanoak (*Notholithocarpus densiflorus*) trees in Curry County, Oregon since it was first detected in 2001. With the expansion of the *Phytophthora ramorum* state quarantine in 2015, more landowners in Curry County are now under regulations to slow the spread of sudden oak death. Some landowners are under a state quarantine for the first time and in some cases are unaware of the state sudden oak death quarantine regulations. Since 2015, the European lineage (EU1) of *P. ramorum* has been detected in 19 infested areas within the SOD quarantine boundary. In Europe, the EU1 lineage kills or damages several conifer tree species and is considered more aggressive than the North American lineage (NA1). These two developments have brought to light an increased need for outreach education of local landowners about SOD, state quarantine regulations, and the new EU1 lineage in southwestern Oregon forests. Additionally, the EU1 lineage has become the highest priority for multiple state and federal agencies, which has led to the opportunity for increased monitoring near the EU1 infestations. Oregon's SOD Program would greatly benefit from a coordinated outreach effort to train citizen scientists about the importance of early detection in order to slow the spread of the disease (Meentemeyer and others 2015). We focused on communities along the leading edge of the disease, and held workshops to teach local residents about disease recognition, early detection methods, and effective treatment options. A citizen science project was piloted and focused on training residents to conduct multiple early detection methods for sudden oak death and to coordinate landscape-level sampling for new SOD infestations. Additionally, focusing on potentially resistant tanoak, we are training residents to identify and report healthy tanoak in infested areas. We will present project design and first year results.

Literature Cited

Meentemeyer, R.K.; Dorning, M.A.; Vogler, J.B; Schmidt, D. and Garbelotto, M. 2015. Citizen science helps predict risk of emerging infectious disease. Frontiers in Ecology and the Environment. 13(4): 189–194.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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The Development and Application of Dynamic, Geospatial *P. ramorum* Spread Models for Oregon[1]

Devon Gaydos[2], Anna Petrasova,[2] Chris M. Jones,[2] Ross K. Meentemeyer,[2] and Richard C. Cobb[3]

Abstract

Dynamic geospatial simulations are valuable assets for exploring how an invasive pest or pathogen is likely to spread given a range of possible scenarios. In particular, they are powerful for conducting computational experiments to assess how management affects spread, as large-scale management experiments may be impractical or unethical. Further, models can be used to generate no-management "control" comparisons, which do not exist with observed datasets. Geospatial simulations such as these have been developed to examine *Phytophthora ramorum* spread and impacts in California (Cunniffe and others 2016, Meentemeyer and others 2011). Yet, models had not been developed for Oregon, where sudden oak death continues to pose a significant economic and environmental threat.

In collaboration with experts from Oregon State University, Oregon Department of Forestry, and the U.S. Forest Service, we have updated these *P. ramorum* models to reflect epidemiological conditions in Oregon. Most notably, evidence suggests that tanoak disproportionately affects disease patterns in Oregon (Hansen and others 2005), so the model was updated to reflect a single-host tanoak system which accounts for disease-induced mortality. Model development is complicated by the presence of two disease strains (NA1 and EU1) and years of intensive management which can obscure natural spread patterns. By simulating both the disease spread and management simultaneously, we were able to parameterize the model for both strains. Using these derived parameters, we generated a hypothetical no-management scenario for 2001-2017 to evaluate how effective Oregon's landscape-scale management efforts were at reducing pathogen spread.

Further, to increase the interactivity and usability of this model, we have linked it with a decision-support system called Tangible Landscape (Tonini and others 2017). This innovative modeling tool allows users to intuitively guide models, regardless of prior experience with code or geospatial software. We present questionnaire results from a modeling workshop with Oregon

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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stakeholders that highlight this tool's potential to engage stakeholders in sudden oak death management and decision-making.

Literature Cited

Cunniffe, N.J.; Cobb, R.C.; Meentemeyer, R.K.; Rizzo, D.M. and Gilligan, C.A. 2016. Modeling when, where, and how to manage a forest epidemic, motivated by sudden oak death in California. Proceedings of the National Academy of Sciences. 201602153.

Hansen, E.M.; Parke, J.L. and Sutton, W. 2005. Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infection. Plant Disease. 89(1): 63-70.

Meentemeyer, R.K.; Cunniffe, N.J.; Cook, A.R.; Filipe, J.A.; Hunter, R.D.; Rizzo, D.M. and Gilligan, C.A. 2011. Epidemiological modeling of invasion in heterogeneous landscapes: spread of sudden oak death in California (1990–2030). Ecosphere. 2(2): 1-24.

Tonini, F.; Shoemaker, D.; Petrasova, A.; Harmon, B.; Petras, V.; Cobb, R.C.; Mitasova, H. and Meentemeyer, R.K. 2017. Tangible geospatial modeling for collaborative solutions to invasive species management. Environmental Modelling & Software. 92:176-188.

Oregon Sudden Oak Death Management Follow-up: Epidemiology[1]

Hazel Daniels[2] and Jared LeBoldus[2]

Abstract

Phytophthora ramorum, the oomycete pathogen responsible for Sudden Oak Death (SOD) in California and Oregon forests, requires constant vigilance to detect and manage. Prior to 2011, the focus of Oregon-based management was complete eradication of all disease centers through removal of infected plant material. Despite intense eradication procedures, the disease continued to spread. Current Oregon management focuses on reducing the spread of SOD within Curry County, and preventing spread to neighboring counties (Goheen and others 2017).

Previous research published in 2015 focused exclusively on the NA1 lineage and its population genomics (Kamvar and others 2015, Manter and others 2010). The EU1 lineage was first confirmed in a forested site in 2015, in Curry County, OR (Grünwald and others 2016). Therefore, there's a need to reassess the effectiveness of management techniques with the discovery of this more virulent lineage. Ongoing research looks at the effectiveness of eradication/suppression efforts, including the newly discovered EU1 strain. Additionally, impacts of natural fire on eradication can be studied through the 2017 Chetco Bar Fire, which swept through portions of the Oregon Quarantine Zone and Generally Infested Area.

In addition to assessing management of SOD, a study of the relation between spatial and genetic distance is nearing completion. Using data collected since 2001, both NA1 and EU1 infections were tracked over time and space. A preliminary computational assessment of subsequent new SOD infections relative to previously eradicated infections revealed candidate samples for sequencing. Whole-genome sequencing of spatially related trees and their neighbors will reveal their level of genetic relatedness.

Literature Cited

Goheen, E.M.; Kanaskie, A.; Navarro, S. and Hansen, E. 2017. Sudden oak death management in Oregon tanoak forests. Forest Phytophthoras.7**:** 45–53.

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Kamvar, Z.N.; Larsen, M.M.; Kanaskie, A.M.; Hansen, E.M. and Grünwald, N.J. 2015. Spatial and temporal analysis of populations of the sudden oak death pathogen in Oregon forests. Phytopathology. 105: 982–989.

Grünwald, N.J.; Larsen, M.M.; Kamvar, Z.N.; Reeser, P.W.; Kanaskie, A.; Laine, J. and Wiese, R. 2016. First report of the EU1 clonal lineage of *Phytophthora ramorum* on tanoak in an Oregon forest. Plant Disease. 100: 1024–1024.

Manter, D.K.; Kolodny, E.H.; Hansen, E.M. and Parke, J.L. 2010. Virulence, sporulation, and elicitin production in three clonal lineages of *Phytophthora ramorum*. Physiological and Molecular Plant Pathology. 74: 317–322.

Fire and Sudden Oak Death's Effect on Species Prevalence in Big Sur, California[1]

Jacqueline Rose[2] and Richard Cobb[2]

Abstract

Sudden Oak Death (caused by *Phytophthora ramorum*) has been present in the Big Sur region since the mid-1990s (or earlier) and is the primary agent of mortality in tanoaks (*Notholithocarpus densiflorus*) there. *Phytophthora ramorum* also causes significant mortality in coast live oak (*Quercus agrifolia*) and Shreve oak (*Quercus parvula* var. *shrevei*) as well as ramorum blight in other species including California bay laurel (*Umbellularia californica*). A body of work has shown this changes forest composition and species abundance in Big Sur (Metz and others 2011).

A plot network consisting of 280 plots was established in 2006 and 2007 to study the epidemiology and impacts of *P. ramorum* in Big Sur. The plots were established in forests dominated either by coast redwood (*Sequoia sempervirens*) or by mixed-evergreen species (Metz and others 2011). The purpose of the plot network has shifted focus to include the interaction of *P. ramorum* and fire following the 2008 Basin Complex Fires, in which 97 plots burned; and the 2016 Soberanes Fire, in which 113 plots burned. Previous work has shown that there is increased coast redwood mortality under certain conditions of disease and fire (Metz and others 2013) and that the resprouting patterns of tanoaks and coast redwood following fire are altered by the presence of *P. ramorum* (Simler and others 2018). However, the recovery of species following fire and the role of *P. ramorum* in changing the composition of the plant community is unknown. This analysis uses repeated plot surveys of tree diameter, species composition, and ground fuels (Browns transect fuel measurements), performed between 2006 and 2018, to improve understanding of these interactions.

This talk will explore the recovery of a few key species following fire in plots with or without evidence of *P. ramorum* invasion prior to the 2008 Basin Complex fire. We performed previous analysis on the prevalence of several common species looking at presence or absence of *P. ramorum* and fire return interval. We found a significant difference in the prevalence of California-lilac species (*Ceanothus* spp.), a known soil nitrogen fixing plant, between plots with medium or long fire return intervals where *P. ramorum* tested positive. We expect to see decreased post-fire abundance of tanoak and oak species on plots invaded by *P. ramorum* prior

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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to burning, and that plots on which *P. ramorum* has never been isolated will have greater post-fire abundance of those species. The decrease should be independent of the time since the burn, indicating a possible change in the composition of the plant community when compared with uninfected and unburned plots. These changes should be driven by significantly higher pre-fire fuels accumulations documented at plot establishment and in 2012 and 2013 plot surveys.

Literature Cited

Metz, M.R.; Frangioso, K.M.; Meentemeyer, R.K. and Rizzo, D.M. 2011. Interacting disturbance: wildfire severity affected by stage of forest disease invasion. Ecological Applications. 21(2): 313-320.

Metz, M.R.; Varner, J.M.; Frangioso, K.M.; Meentemeyer, R.K. and Rizzo, D.M. 2013. Unexpected redwood mortality from synergies between wildfire and an emerging infectious disease. Ecology. 94(10): 2152-2159.

Simler, A.B.; Metz, M.R.; Frangioso, K.M.; Meentemeyer, R.K. and Rizzo, D.M. 2018. Novel disturbance interactions between fire and an emerging disease impact survival and growth of resprouting trees. Ecology. 99(10): 2217-2229.

Wildfire Limits the Occurrence, Frequency, and Impacts of *Phytophthora ramorum* in the Coastal Forests of Big Sur, CA[1]

Allison B. Simler,[2] Margaret R. Metz,[2] Kerri Frangioso,[2] David M. Rizzo,[2] and Ross K. Meentemeyer[3]

Abstract

Ecological disturbances have clear potential to shape the dynamics of native and introduced plant diseases, via their impacts on the spatial pattern of hosts, host composition, pathogen survival, and microclimatic conditions. Yet, studies examining potential interactions between disease and disturbance are rare, or primarily focus on pathogen occurrence, rather than the additional processes of spread, infection, and host mortality that determine disease impacts. Given that anthropogenic activities have both altered disturbance regimes across the western United States and introduced ecologically-damaging, non-native plant pathogens, understanding how disease dynamics may interact with or be determined by historical and changing disturbance regimes may be of significant conservation and management importance.

The emerging infectious disease sudden oak death (SOD) impacts fire-prone coastal forests in California and Oregon, and previous studies suggest that historical and recent fire history may influence the occurrence of SOD's causal agent, *Phytophthora ramorum* (Moritz and Odion 2005, Beh and others 2012). In this study, we leveraged a ten-year forest monitoring dataset tracking the impacts of *P. ramorum* across the Big Sur region to explore the mechanisms underlying the relationship between fire and disease. We analyzed how both long-term fire history and recent wildfires influence pathogen presence, but also other metrics of disease, including infestation intensity, re-invasion, and severity of SOD host mortality.

We found that areas that burned more frequently over the last sixty-five years were less likely to contain California bay laurel (*Umbelluria californica*) trees and had reduced SOD host basal area. In turn, more frequently burned plots were less likely to contain *P. ramorum,* had lower rates of host infection, and exhibited decreased rates of host stem mortality. Immediately following the 2008 Basin Complex fire in this region, previous research recorded a reduction in *P. ramorum* occurrence in burned areas, with the pathogen primarily persisting in surviving, intact California bay laurel canopies (Beh and others 2012). Up to 7 years following fire, we find no evidence that regenerating host vegetation plays an epidemiologically significant role and find that infestation intensity is primarily determined by this legacy of surviving hosts. Further,

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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infection by *P. ramorum* does not appear to strongly determine host mortality in these recently-burned areas, suggesting that wildfire may reduce *P. ramorum* propagule pressure or alter microclimates to reduce SOD severity. Overall, these results suggest that severe wildfire reduces not only *P. ramorum* occurrence, but also its spread, infestation intensity, and mortality impacts, which could have significant implications for predictions of future SOD dynamics under climate change and increasing wildfire ignitions from human activities in this region.

Literature Cited

Beh, M.M.; Metz, M.R.; Frangioso, K.M. and Rizzo, D.M. 2012. The key host for an invasive forest pathogen also facilitates the pathogen's survival of wildfire in California forests. New Phytologist. 196: 1145–1154.

Moritz, M.A. and Odion, D.C. 2005. Examining the strength and possible causes of the relationship between fire history and sudden oak death. Oecologia. 144: 106–114.

Focus on Phytophthoras in Restoration Areas

Evaluating Threats Posed by Exotic *Phytophthora* Species to Sensitive Plant Communities in the Santa Clara Natural Community Conservation Plan Area[1]

Tedmund J. Swiecki,[2] Elizabeth A. Bernhardt,[2] and Janell Hillman[3]

Extended Abstract

Root rots caused by exotic *Phytophthora* species have been associated with dying and declining vegetation in multiple native plant communities in the San Francisco Bay Area and elsewhere in northern California. This project focused on detecting *Phytophthora* species that are currently affecting or have the potential to seriously affect populations of covered plants in the Santa Clara Valley Habitat Conservation Plan/Natural Community Conservation Plan (HCP/NCCP) area (Swiecki and Bernhardt 2018). A key component of the Santa Clara Valley HCP/NCCP is the creation of an 18,800 ha reserve network within the plan area to protect and conserve covered species, natural communities, and ecosystem function in perpetuity. We developed a sampling strategy by using GIS data to determine where in the plan area various priority habitat types with *Phytophthora*-susceptible vegetation might be exposed to *Phytophthora* contamination from roads, trails, past restoration plantings, or other known risk pathways. High-priority vegetation included rare and threatened species, as well as vegetation complexes that were poorly represented in the plan area. We also considered proximity to lands enrolled or proposed for enrollment in the HCP/NCCP reserve system, access, and in-field observations of vegetation symptoms and risk factors to determine sampling locations.

Green pear baits were used to detect *Phytophthora* in samples. *Phytophthora* species were cultured from lesions on baits onto cornmeal-carrot agar, and cultures of different morphotypes recovered were submitted to the California Department of Food and Agriculture (CDFA) Plant Pest Diagnostics Lab for identification by nucleotide sequencing of the ITS region of nuclear ribosomal RNA; COX2 was also sequenced for some cultures. Twenty-one water samples (2.5 L of water skimmed from the surface of streams, ponds, or lakes and baited immediately in the field with a green pear in a 3.8 L plastic bag) and 168 soil/root samples (3 to 6 subsamples collected under target plants, 1-1.5 L sample volume, roots collected preferentially by hand when digging samples) were collected. Soil samples that were already moist or saturated and near 20 °C at the time of sampling were baited on the same day they were collected. For dry and/or cold samples, soil moisture was adjusted to near field capacity and samples were maintained at 20-

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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24.5 ºC (night/day) for 3-3.5 days to allow time for sporangia to form before being flooded and baited with green D'Anjou pears. Pear baits were maintained in water samples for 3 days, rinsed lightly, and observed for up to 5 more days for symptom development. Pears remained in soil/root samples until symptoms developed (normally not before day 3) or until 5 days from the start of baiting, rinsed, and observed for symptom development until day 8. Baited samples were incubated at temperatures that fluctuated between 20 ºC (night) and 24.5 ºC (day).

We collected 189 samples from 15 locations in Santa Clara County Parks and Santa Clara County Open Space Authority preserves, and other reserve system areas with high-priority vegetation types. Sixty-eight samples were collected in the three remaining populations of Coyote ceanothus (*Ceanothus ferrisiae*), an endangered species endemic to the county that is a conservation priority in the NCCP. In all, 20 *Phytophthora* taxa (table 1) were recovered from 53 samples (28% of total) from 14 of the 15 sampled locations. Eight *Phytophthora* species (most in *Phytophthora* clade 6) were detected in 13 of 21 water samples (67% of samples) collected in seven sampled locations. Water samples with *Phytophthora* were collected from ephemeral runoff along a road (*P. crassamura*), puddles on unpaved roads (*P. gonapodyides*), seasonal streams (*P. acerina*, *P.* taxon forestsoil-like, *P. gonapodyides, P. lacustris* complex), perennial streams (*P. chlamydospora, P. lacustris* complex, *P. megasperma* complex, *P. ramorum*), and a reservoir (*P. chlamydospora, P. gonapodyides, P. lacustris* complex).

Phytophthora species were recovered from 59% of 44 root/soil samples collected from sites in 13 locations that were periodically flooded, including riparian floodplains, seasonal creeks and ponds that were moist to dry when sampled, and margins of perennial spring-fed ponds. Samples from periodically flooded sites had the greatest diversity of *Phytophthora*; 16 taxa including 5 that were also found in water samples (table 1). *P. crassamura*, *P. gonapodyides*, and *P. inundata* were the most commonly detected species in these sites. Unusual taxa detected in these sample locations included *P. asparagi* and the undescribed taxa *P.* taxon agrifolia (previously known only from a nursery-grown *Quercus agrifolia* seedling planted in San Mateo County) and *P.* taxon europaea-like.

We collected the greatest number of root/soil samples (124) from uplands or flats and lowlands not subject to inundation. Sampled areas including upland *C. ferrisiae* habitat, oak woodlands, and chaparral or other shrublands. Six *Phytophthora* taxa were detected among the 14 positive samples (11%) from these dry sites, which were sampled at all 15 locations. *P. cambivora*, *P. crassamura*, and *P. cactorum* were the only species detected at more than one location (table 1). Another undescribed taxon, *P.* taxon ohioensis-like, was found in association with valley oaks (*Quercus lobata),* in one location. Five samples from transplanted nursery stock (upland or flat/lowland sites) at four reserves yielded two *Phytophthora* detections.

Table 1—Frequency of *Phytophthora* taxa detected in water samples (n=21) and root/soil samples from normally dry uplands and non-flooded lowlands (n=124) and seasonally wet sites (n=44).

Number of samples with detections	*Phytophthora* taxon	Sample site type: dry	seasonally wet	water
11	*Phytophthora crassamura*	3	7	1
10	*Phytophthora gonapodyides*	-	6	4
7	*Phytophthora lacustris*	-	1	6
6	*Phytophthora cambivora*	5	1	-
5	*Phytophthora chlamydospora*	-	2	3
4	*Phytophthora inundata*	-	4	-
3	*Phytophthora cactorum*	2	1	-
3	*Phytophthora pseudocryptogea*	1	2	-
1	*Phytophthora cryptogea* complex	-	1	-
3	*Phytophthora megasperma*	1	1	1
3	*Phytophthora* taxon raspberry	-	3	-
2	*Phytophthora riparia*	-	2	-
2	*Phytophthora* taxon agrifolia	1	1	-
2	*Phytophthora* taxon forestsoil-like	-	-	2
1	*Phytophthora acerina*	-	-	1
1	*Phytophthora asparagi*	-	1	-
1	*Phytophthora* taxon europaea-like	-	1	-
1	*Phytophthora multivora*	-	1	-
1	*Phytophthora* taxon ohioensis-like	1	-	-
1	*Phytophthora ramorum*	-	-	1
	Number of taxa	**7**	**16**	**8**

Phytophthora was not detected in dry upland *C. ferrisiae* habitat in two of the three populations. However, *P. acerina, P. lacustris,* and *P.* taxon forestsoil-like were detected along a seasonal stream below the Kirby Canyon population and *P. inundata* was found on the edge of a spring-fed pond adjoining the Llagas population. An extensive, but still localized, infestation involving multiple *Phytophthora* species near the Anderson Lake reservoir poses the greatest threat to the largest remaining population (fig. 1). At least 2.8 ha of a lower slope area that includes part of this population was apparently infested with multiple species via the planting of infected *C. ferrisiae* nursery stock as part of a 1993 habitat restoration planting. *P. cactorum, P. cambivora, P. crassamura*, *P.* 'kelmania', *P. megasperma* and *P. syringae* have been detected here in recent and prior sampling. No *Phytophthora* was detected in upslope portions of this population, which did not have significant disturbances that might introduce contamination (fig.1). Additional detections of multiple *Phytophthora* species near the reservoir high-water line appear to be

related to contaminated runoff and/or infected debris from landscaped urban development along the lakeshore about 1 km away.

Our baseline sampling indicated that *Phytophthora* infestations were generally uncommon in and near reserve system lands and infestations detected were mostly associated with known risk factors for *Phytophthora* introduction. Most *Phytophthora* detections were associated with common *Phytophthora* sources such as plantings of nursery stock, previous agricultural areas, and contaminated watercourses. Spread from these sources was associated with roads, trails, development and grading activities, livestock grazing, and overland water flow. Some detections were in disturbed areas where the likely source(s) of contamination were less clear, but *Phytophthora* was not detected in any of the relatively remote and undisturbed uplands that we sampled.

Management of the HCP/NCCP reserve system to minimize threats posed by *Phytophthora* should prioritize preventing introduction of additional *Phytophthora* species into habitat areas by excluding likely sources of contamination, such as infested nursery stock. Actions should also be undertaken to minimizing spread from existing infestations. *Phytophthora* management should be considered in conjunction with other management practices, such grazing, construction activities, and trail use. At some reserves, the season of grazing and the direction of livestock movement between pastures may need to be altered to minimize the risk of spreading contamination from known infestations. Additional research will be needed to guide adaptive management of *Phytophthora* infestations.

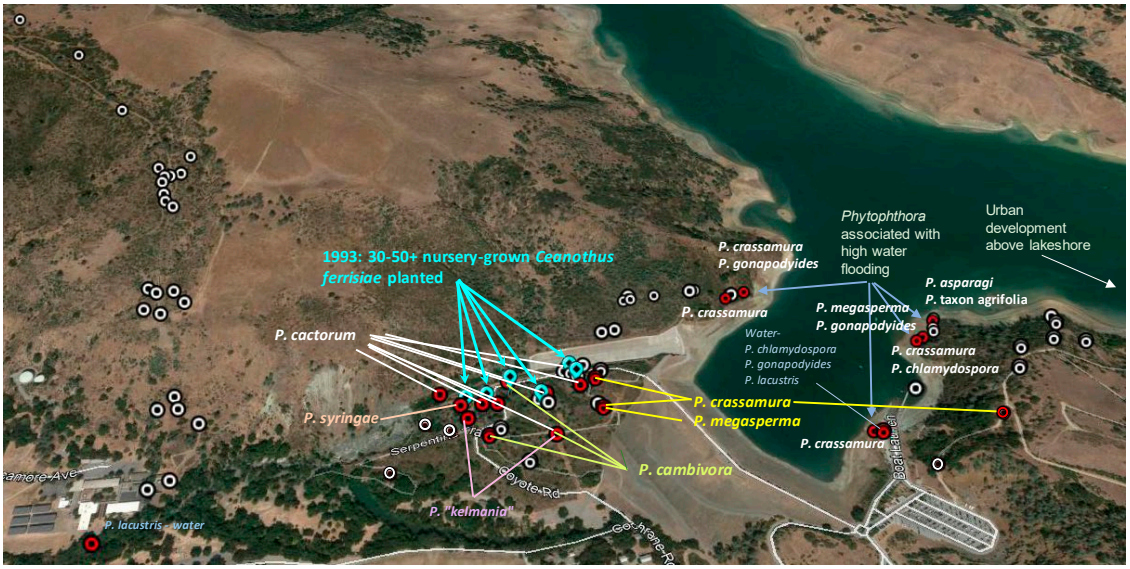

Figure 1—Results from soil and water samples collected in *Ceanothus ferrisiae* habitat near Anderson Lake as part of this and related projects. *Phytophthora* was detected (red symbols) in symptomatic plants on the dam abutment slope where nursery stock was previously planted and in plants along the lakeshore that were inundated briefly in winter 2016-17. White symbols are sample locations where *Phytophthora* was not detected.

Acknowledgements

Funding provided by a Natural Community Conservation Planning Local Assistance Grant from the California Department of Fish and Wildlife to Santa Clara Valley Habitat Agency.

Literature Cited

Swiecki, T.J. and Bernhardt, E.A. 2018. Evaluating threats posed by exotic *Phytophthora* species to endangered Coyote ceanothus and selected natural communities in the Santa Clara NCCP area. Final report. Prepared for Santa Clara Valley Habitat Agency. 135p. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=161911. (12 February 2020).

Exotic *Phytophthora* species Are Being Systematically Introduced in California Wildlands During Restoration Projects[1]

Laura Sims[2] and Matteo Garbelotto[3]

Abstract

This study describes the identification of several *Phytophthora* species previously found in San Francisco Bay Area nurseries (Sims and others 2018a) from multiple sites at the urban wildland interface within the greater San Francisco Bay Area. Isolation success was highest from the three plant species: *Diplacus aurantiacus* (sticky monkey-flower)*, Ceanothus thyrsiflorus* (blueblossum)*,* and *Frangula californica* (California coffeeberry), growing in restoration sites and in adjacent disturbed sites. Isolation success was zero in control undisturbed "natural" areas adjacent to infested sites. In order to confirm plant production facilities were the source of the *Phytophthora* strains isolated from restoration sites, genetic and phenotypic analyses were performed on *Phytophthora crassamura,* one of the most common species identified during the course of this and other studies. Results indicated that field isolates were genetically identical to those found in plant production nurseries. Resistance to fungicides such as phosphites and mefenoxam was identified in a select number of isolates found both in plant production facilities and wildland sites, which further corroborates a "nursery" origin for the wildland infestations (Sims and others 2018b). Spatial landscape-level analysis identified patterns of *Phytophthora* spread consistent with an outward expansion from sites that were planted and with topography, although the presence of some species was not associated with water accumulation patterns. *Phytophthora* species assemblages were site-specific and possibly related to site type, nursery where the plants were grown, and time since planting. To our knowledge, this is one of the first studies providing robust evidence that exotic *Phytophthora* species are being systematically introduced through the use of infected plants in restoration projects.

Literature Cited

Sims, L.; Tjosvold, S.; Chambers, D. and Garbelotto, M. 2018a. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathology. 68: 196–204.

Sims, L.; Chee, C.; Bourret, T.; Hunter, S. and Garbelotto, M. 2018b. Genetic and phenotypic variation of *Phytophthora crassamura* isolates from California nurseries and restoration sites. Fungal Biology. https://doi.org/10.1016/j.funbio.2018.11.012.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Ten New Provisional Species of *Phytophthora* and *Nothophytophthora* From California[1]

**Tyler B. Bourret,[2] Kamyar Aram,[2] Heather K. Mehl,[2] David M. Rizzo,[2]
Suzanne Rooney-Latham,[3] Tedmund J. Swiecki,[4]
Elizabeth A. Bernhardt,[4] and Susan J. Frankel[5]**

Abstract

Phytophthora diseases in landscapes have gained prominence during the 21st century, due to an increase in *Phytophthora* surveys and a greater focus on species-level diagnostics. Concurrently, the number of known *Phytophthora* species has increased from about 60 in 1996 to more than 300 today. In addition to entirely novel species being discovered, many of the most common and well-known 20th century species have been revealed to represent species complexes and are rapidly being divided into new taxa. Beyond the increasingly large *Phytophthora*, two *Phytophthora*-like related genera, *Calycofera* and *Nothophytophthora* were described in 2017.

Herein are presented nine novel species of *Phytophthora* and one novel *Nothophytophthora* species recently isolated from California. Each species is provisionally described by demonstrating it to be phylogenetically distinct from all other named species based on analysis of ITS rDNA and COX1 mtDNA sequences, the two barcoding loci used for *Phytophthora* species-level identification. Some species appear to be cryptic species within well-known complexes; these taxa are also found outside of California based on the geographic sources of publicly deposited sequence data. For other species, there is no evidence that they have ever been previously isolated. Whether these new species represent endemic Californian pathogens, long-term residents or recent invaders is unknown.

Two novel species are presented in *Phytophthora* clade 2: *Phytophthora* sp. *aureomontensis* is a member of the *P. citricola* species complex only known from California and Oregon coastal streams. *Phytophthora* taxon eriodictyon is a member of the *P. citrophthora* species complex that appears to be moving worldwide via the nursery trade; this species is known in California only from restoration outplantings. *Phytophthora* sp. *cadmea* is a novel species in clade 7a also

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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baited from restoration areas. *Phytophthora* taxon wysteria, also a member of clade 7 was isolated from a commonly planted ornamental. *Phytophthora* taxon agrifolia represents a novel sub-clade within the vast clade 8. Two novel species are presented in clade 9, one of which, *Phytophthora* taxon xguadalupesoil, appears to be an interspecific hybrid. *Phytophthora* taxon juncus is closely related to the only other member of clade 11, *Phytophthora lilii*, while *Phytophthora* taxon mugwort represents its own subgeneric clade, clade 13. *Nothophytophthora* taxon umbellularia is known only from a single isolate, baited from a North coast creek with a California bay laurel (*Umbellularia californica*) leaf.

Although provisional, naming these taxa and depositing their barcoding sequences into public databases provides vital information to the worldwide *Phytophthora* diagnostics community about the distribution and movement of these potentially pathogenic and invasive organisms. More systematic work, including morphological characterizations and pathogenicity tests are needed to more fully characterize these provisional species, and live strains will be deposited in culture collections. Nevertheless, because the direct comparison of DNA sequences represents the most tractable and reliable way to compare *Phytophthora* isolates across space and time, this initial step serves to inform the scientific and regulatory communities of the existence of these species.

Assessing the Incidence and Diversity of *Phytophthora* Species in Planned Restoration Areas of the Angeles National Forest[1]

Sebastian N. Fajardo,[2] Tyler B. Bourret,[2] Chris Endelenbos,[2] Evan Lozano,[2] David M. Rizzo,[2] Susan J. Frankel,[3] and Katie VinZant [4]

Abstract

The Angeles National Forest (ANF), located in the greater Los Angeles metropolitan area encompasses approximately 700,000 acres (238,230 ha), comprising a vital biodiversity hot spot. From 2002 to 2008, the ANF was affected by three major fires, the Copper (2002), Ranch (2007) and Sayre (2008). Together, these fires affected approximately 40,000 acres (16,187 ha) of coastal sage scrub, montane chaparral, grassland, riparian corridor, as well as isolated big cone Douglas-fir (*Pseudotsuga macrocarpa)* stands. Droughts and floods in the subsequent years, plus off-highway vehicle use, aggravated erosion and altered chaparral vegetation regeneration. As a mitigation effort, restoration attempts were initiated in these areas including planting native nursery stock.

In 2016 - 2017, prompted by concerns that *Phytophthora* species may have been introduced on restoration plantings, *Phytophthora* surveys were conducted in several restoration locations associated with utility project mitigation on ANF lands. The inadvertent outplanting of infested nursery stock is considered one of the main pathways for exotic *Phytophthora* to enter into natural areas. These preliminary surveys detected numerous *Phytophthora* species associated with outplanted native plants and at the source nurseries. The ANF has a typical Mediterranean climate and averages about 15 – 20 inches (28 to 50 cm) of precipitation per year with long dry periods in late spring into early fall. The ability of *Phytophthora* species to survive and become established under these conditions is not known.

To better understand the *Phytophthora* distribution on arid lands of the ANF, three *Phytophthora* surveys were performed between May 2018 to March 2019 in areas that had burned in the Copper, Sayre and Ranch fires and were prioritized for restoration. From the three areas, a total of 508 soil samples were collected from 27 sites to determine the incidence and distribution of *Phytophthora* pathogens. A range of forest types and conditions were sampled including four sites which had been planted with container nursery stock. Thirteen *Phytophthora* species were

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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detected from 15 sites (*P. borealis, P. cactorum, P. chlamydospora, P. crassamura, P. gonapodyides, P. inundata, P. lacustris, P. riparia, P. lacustris x riparia* hybrid, *P. multivora, Phytophthora* sp. cadmea*, Phytophthora* sp. NJB-2015*,* and an undescribed *Phytophthora* species provisionally named *P*. taxon agrifolia 2). *Phytophthora* was not detected in the outplanted areas. *Phytophthora* detections were primarily associated with dry stream beds. *Pythium* species were recovered from all 27 sites, which suggests that *Pythium* may be resident to ANF lands. Among the 13 detected species*, P. crassamura* was found to be the most widely distributed *Phytophthora* species on ANF lands, present on eight of the 27 sampled sites. Previous studies have associated *P*. *crassamura* with a Mediterranean climate and with dieback during restoration activities, thus indicating the potential danger that this species could have on the ANF, but the pathogenicity of this species is not fully known.

Sampling will be repeated seasonally in all three fires areas to determine what additional factors could be correlated with the incidence of *Phytophthora* pathogens. Further research is on-going to explore the ecological factors affecting the survival and distribution of *Phytophthora* species on arid ecosystems and fire-affected areas of the ANF.

Metabarcoding for *Phytophthora*—Benefits and Limitations[1]

Neelam Redekar,[2] Joyce Eberhart,[2] Ebba Peterson,[3] and Jennifer Parke[2,3]

Abstract

The metabarcoding approach has revolutionized the study of microbial ecology with its ability to detect microbial DNA at an unprecedented depth and coverage. This method exploits high-throughput sequencing and DNA barcoding for identification of microbial species, including novel and uncultivable microbes. The metabarcoding approach is gaining more interest by the *Phytophthora* scientific community for monitoring the existence and spread of *Phytophthora* species in diverse habitats. Understanding and mitigating metabarcoding limitations is crucial for achieving better resolution and accurate determination of the diversity of *Phytophthora* species.

Culture-based methods rely on Sanger sequencing for species identification. This approach is limited to detection of culturable species and is a low throughput method that takes a very long time to process a small number of samples. The metabarcoding approach, on the other hand, relies on a next generation high throughput sequencing platform such as Illumina MiSeq that allows for detection of the entire targeted microbial community in the sample at once. Any environmental sample (soil, water, plant, animal) containing a mixture of microbial populations could be processed with the metabarcoding approach in which DNA is first extracted from the sample, amplified with primers specific to markers loci (such as ITS, COX), and the amplified product is then directly sequenced on the Illumina MiSeq sequencer. These 300 bp long Illumina sequences are clustered into operational taxonomic units (or OTUs) based on sequence identity. The OTU sequences are then compared against the sequences of known species for identification. The availability of 384 unique Illumina sequencing barcodes facilitates sample tagging and pooling ('multiplexing') prior to sequencing, and separation of sequences between different samples ('demultiplexing') post sequencing, making metabarcoding a high throughput approach. We have employed this approach to study *Phytophthora* communities in nursery irrigation water, streams and lakes; at multiple native plant restoration sites; and to test the efficacy of disinfestation methods such as soil solarization and chlorination.

Several universal primers targeting genomic or mitochondrial marker regions are available for *Phytophthora* identification, however these primers have not been tested for every known

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Phytophthora species (Bilodeau and others 2014, Cooke and others 2000, Robideau and others 2011, White and others 1990). Moreover, any mismatch in the priming site could affect the rate of amplification of the target DNA. For example, ITS6 and ITS7 primers are considered to be universal for oomycete amplification (Cooke and others 2000), however, certain *Phytophthora* species with mismatching ITS7 primer binding sites are less likely to be amplified with this set of primers (Redekar and others 2019, Sapkota and Nicolaisen 2015).

Species identification also relies on the availability of an up-to-date and reliable reference sequence database that includes marker sequences from all isolates of known species. There are four reliable reference sequence databases available for *Phytophthora* species identification (Abad and others 2019, Bilodeau and others 2014, Grünwald and others 2011, Robideau and others 2011). While these databases are accurate, they may not include sequences of newly discovered species or new isolates of well authenticated species. The metabarcoding sequences that do not match any known species are often considered to originate from novel species, and a follow-up isolation effort is required to validate such novel species.

Closely related *Phytophthora* species may have identical DNA marker sequences, and the metabarcoding approach may not be able to differentiate between such closely related species. However, such closely related species are grouped into a species complex, where member species of the complex are indistinguishable with the DNA marker. If sequence identity between closely related *Phytophthora* species is only limited to the amplified region in metabarcoding, then such closely related species are grouped into a species cluster. Metabarcoding with ITS6 and ITS7 primers results in 8 *Phytophthora* species complexes and 15 species clusters.

Metabarcoding can detect rare and abundant *Phytophthora* species within a sample but cannot determine absolute abundance of a single species within a community. Despite this limitation, the metabarcoding approach is the most sensitive method currently available for rare species detection, with an exponentially higher limit of *Phytophthora* detection compared to the quantitative real-time PCR assay. The quantitative PCR limit of detection for the quarantine pathogen *Phytophthora ramorum* was 500 femtogram/µl, whereas it was 0.5 femtogram/µl with metabarcoding.

Metabarcoding cannot differentiate between cellular and non-cellular DNA or relic DNA that could originate from dead organisms. However, we can easily eliminate relic DNA in metabarcoding by treating samples with a high affinity photoreactive DNA binding dye, propidium monoazide (PMA), that tags relic DNA and prevents its amplification in metabarcoding. Effectiveness of PMA in metabarcoding was demonstrated on synthetic samples comprised of a serial dilution series of *Fusarium* spores mixed with relic *Rhizopogon* DNA. *Rhizopogon* DNA was completely eliminated with PMA and only *Fusarium* was detected in metabarcoding.

Metabarcoding could be coupled with *Phytophthora* capture methods such as filtration and baiting. We separately filtered and baited stream water with rhododendron leaves and extracted

DNA from both filters and leaf baits. Metabarcoding showed presence of different *Phytophthora* communities in filters vs. leaf baits. Filtration detected the 'total' *Phytophthora* community, while baiting detected *Phytophthora* species associated with leaf lesions. Filtration captured a greater diversity of oomycete species than did baiting. This was expected because baiting selectively increased the number of active plant pathogens. We, therefore, recommend using a combination of *Phytophthora* capture methods prior to metabarcoding.

In summary, metabarcoding is a high throughput and sensitive method to detect the total microbial community in a sample at once. It relies on next generation sequencing technology such as Illumina MiSeq that facilitates sequencing of 384 samples simultaneously and detection of rare, novel and unculturable species. It also requires universal primers efficient in amplifying the targeted microbial community, and a reliable and complete reference sequence database. Shorter Illumina sequencing reads are sometimes incapable of resolving sequences of closely related species, and in such cases species identification is limited to species complexes or clusters. Despite the challenges, the metabarcoding approach continues to be a promising tool for studying *Phytophthora* ecology.

Literature Cited

White, T.J.; Bruns, T.D.; Lee, S.B. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. *In:* PCR protocols A guide to methods and applications. Academic Press, San Diego, CA.

Cooke, D.E.; Drenth, A.; Duncan, J.M.; Wagels, G. and Brasier, C.M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genetics and Biology. 30: 17-32.

Robideau, G.P.; De Cock, A.W.; Coffey, M.D.; Voglmayr, H.; Brouwer, H.; Bala, K.; Chitty, D.W.; Désaulniers, N.; Eggertson, Q.A.; Gachon, C.M.; Hu, C.H.; Küpper, F.C.; Rintoul, T.L.; Sarhan, E.; Verstappen, E.C.; Zhang, Y.; Bonants, P.J.; Ristaino, J.B. and Lévesque, C.A. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular Ecology Resources. 11(6): 1002-11.

Bilodeau, G.J.; Martin, F.N.; Coffey, M.D. and Blomquist, C.L. 2014. Development of a multiplex assay for genus- and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order. Phytopathology. 104: 733-74.

Sapkota, R. and Nicolaisen, M. 2015. An improved high throughput sequencing method for studying oomycete communities. Journal of Microbiological Methods. 110: 33-9.

Redekar, N.R.; Eberhart, J.L.; Parke, J.L. 2019. Diversity of *Phytophthora*, *Pythium*, and *Phytopythium* species in recycled irrigation water in a container nursery. Phytobiomes. 3: 31- 45.

Grünwald, N.J.; Martin, F.N.; Larsen, M.M.; Sullivan, Press, C.M.; Coffey, M.D.; Hansen, E.M. and Parke, J.L. 2011. Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease. 95: 337-342.

Abad, Z.G.; Burgess, T.; Bienapfl, J.C.; Redford, A.J.; Coffey, M.D. and Knight, L. 2019. IDphy: Molecular and morphological identification of *Phytophthora* based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, and Centre for *Phytophthora* Science and Management. http://idtools.org/id/phytophthora/index.php. (25 June 2019).

Soilborne *Phytophthora* Species at Restoration Sites in the Midpeninsula Regional Open Space District[1]

Ebba Peterson,[2] Joyce Eberhart,[3] Neelam Redekar,[3] Jennifer Parke,[2,3] and Amanda Mills[4]

Abstract

The inadvertent spread of *Phytophthora* species from nurseries into native ecosystems has increased interest in assessing *Phytophthora* diversity in native plant communities. Of major concern is the inadvertent movement of *Phytophthora* spp. from native plant nurseries into vulnerable habitats during restoration outplantings. Root-infecting *Phytophthora* spp. are abundant within plant nurseries; their introduction can result in failed plantings and further spread of *Phytophthora* into surrounding habitat. To assess *Phytophthora* diversity, we surveyed restoration sites within the Midpeninsula Regional Open Space District (MROSD) to determine the presence and distribution of *Phytophthora* pathogens.

In December 2017 and 2018, we collected and baited a total of 579 soil samples from the base of native shrubs and seedlings at 30 planted restoration sites, 12 planned restoration sites, and 29 non-planted areas adjacent to restoration projects. We also extracted DNA from each sample and submitted ITS1 amplicons (250 base pairs in length) for sequencing on the Illumina MiSeq platform. Distinct sequences, or operational taxonomic units (OTUs), were assigned to a species when the amplicon was a ≥99% match to known sequences. In many cases, species could not be distinguished over the sequenced region, in which case the OTU was assigned a complex or cluster designation representing multiple potential species. To reduce the inclusion of false-positives we required an individual OTU comprise a minimum of 0.05% of the within-sample relative abundance.

Phytophthora was equally prevalent in planted and non-planted areas, however many species were only found in areas in which nursery plants were introduced. Soil baits yielded 18 *Phytophthora* species (73 samples, comprising 13% of the 562 samples baited). Common species of concern include *P. ramorum*, *P. cinnamomi*, *P. cambivora*, and *P. cactorum*, all of which are associated with plant decline in native plant communities. Metabarcoding revealed a high

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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diversity of *Phytophthora* OTUs; 57% of the 220 samples sequenced contained at least one *Phytophthora* OTU detection above minimum thresholds. This method also detected ITS1 sequences matching the *P. quercina*-cluster (which may be *P. quercina* and/or *P. versiformis* or closely related taxon not in our database) and *P. tentaculata*, species of concern not detected by baiting. While the short read lengths and deficiencies in existing databases make positive identification of OTUs difficult, Illumina MiSeq sequencing is a sensitive tool able to detect prior introductions and describe *Phytophthora* diversity.

Phytophthora spp. are widespread within MROSD preserves, although some preserves had noticeably greater species diversity and detection frequency. Importantly, DNA-only detections may be remnants of prior introductions and may not indicate substantial disease at the site. However, the long-term outlook of *Phytophthora* establishment is poorly understood. *Phytophthora* can persist in soils and may cause disease later or on different hosts; disease development may be slow; and disease may only occur at specific stages like regeneration. For these reasons, future management of MROSD preserves and restoration projects should utilize best management practices to limit the spread of *Phytophthora* to surrounding environs.

Nurseries

Determining the Minimum Treatment Area and Importance of Soil Moisture for Effective Soil Solarization in Nurseries[1]

Logan Bennett,[2] Ebba Peterson,[2] and Jennifer Parke[2]

Abstract

Soil solarization is a low-cost, non-chemical method that can be highly effective in killing soilborne *Phytophthora* species in infested nurseries. This method consists of using a transparent plastic film to trap solar radiant heat. Understanding and implementing the best methods for soil solarization are critical for successful eradication of pathogens. We examined how the size of the solarization treatment area, soil moisture content, and duration of solarization affected the survival of *Phytophthora* inocula buried at 5, 15, and 30 cm.

Research sites were established in Corvallis, OR and at the National Ornamental Research Site at Dominican University of California (NORSDUC) quarantine facility in San Rafael, CA in the summers of 2017 and 2018. At the California site, we tested survival of *P. ramorum*, and *P. pini*; at the Oregon site, we tested only *P. pini*. Each site contained 16 plots, arranged in a randomized block design with four blocks. Each block included three sizes of solarization treatment areas (0.25, 1.0, and 4.84 m^2) and a non-solarized control. Half of the blocks were irrigated before solarization; the other half were not irrigated. Inoculum survival was determined after 2, 4, 6, and 12 weeks.

In 2017, each of the factors (species, depth, irrigation treatment, treatment area, and duration) significantly affected *Phytophthora* survival. Regardless of irrigation treatment, the greatest recovery of inoculum occurred in the non-solarized and 0.25 m^2 plots, and the lowest recovery occurred in the largest (4.84 m^2) plots. At the 30 cm depth, *P. ramorum* was eliminated from both irrigated and non-irrigated treatments by two weeks in the 4.84 m^2 plots, whereas it persisted in 1 m^2 plots for up to six weeks. *P. pini* at the same depth in the 4.84 m^2 plots was eliminated by four weeks of solarization, but it took twelve weeks to kill it in the 1 m^2 plots. The smallest plots (0.25 m^2) used for solarization were ineffective, with inoculum survival no different than in non-solarized soil. Similar trends were observed in 2018 trials. Effects of soil moisture on soil temperature and solarization effectiveness are still being investigated.

The impact of solarization in nurseries and restoration sites could be maximized and the associated costs reduced by optimizing treatment area, soil moisture content and duration of

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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solarization. This research is critical for soil solarization to be an effective management tool in preventing the spread of soilborne *Phytophthora* species.

Accreditation to Improve Restoration Program Shows Promise for Pathogen Prevention[1]

Susan J. Frankel,[2] Tedmund J. Swiecki,[3] Elizabeth A. Bernhardt,[3] Diana Benner,[4] Cheryl Blomquist,[5] and Suzanne Rooney-Latham[5]

Abstract

In 2018, we launched a pilot project, "Accreditation to Improve Restoration and Native Plant Nursery Stock Cleanliness (AIR)" to explore whether an audit-based accreditation program could be used to increase confidence that restoration and native plant nursery stock are not infected with *Phytophthora* species. Due to capacity and cost limitations, we accepted eleven nurseries in the greater San Francisco Bay Area in the initial phase. Each participating nursery completes a self-assessment to confirm that they are producing plants with the practices outlined in the Phytophthoras in Native Habitats Work Group best management practices for restoration nursery stock (BMPs) (see http://www.suddenoakdeath.org/wp-content/uploads/2016/04/Restoration.Nsy_.Guidelines.final_.092216.pdf). These practices require the growers to dedicate a significant amount of time and attention to phytosanitation throughout their operations.

The nursery self-assessment covers all aspects of production divided into twelve categories including layout, water source, growth media (soil), propagation and sanitation. The assessment form is a shared online document that includes both nursery-supplied data and the auditors' responses and risk ratings as well as test results. After the self-assessment is completed, the audit team visits the facilities to check for BMP compliance and assess risk pathways associated with the nursery's infrastructure. The auditors also conduct limited testing of nursery stock for the presence of *Phytophthora* using a standardized irrigation leachate baiting method unless equivalent third-party test results are available.

The audit process helps nurseries validate their existing practices and identify areas for improvement. In general, restoration nurseries that adopted the Nursery *Phytophthora* BMPs in

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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2016 or earlier and had results from internal and/or third-party pathogen testing, showed good BMP compliance. Feedback from periodic testing is critical for identifying and correcting problems before they can spread. Nursery audits have provided insights into both the variety of challenges faced by different nurseries as well as the approaches that can be used to implement the BMPs. Feedback from the audit process have also been used to make AIR program improvements and identify research data gaps. For the program to be more widely adopted, a consistent source of financial support and an organization to act as the auditor need to be identified.

Approaches to Protect Against Phytophthoras at the Presidio[1]

Christa Conforti[2]

Abstract

The Presidio, a 1,500 acre National Park on a former military post at the foot of the Golden Gate Bridge, is a major outdoor and cultural recreation hub in northwest San Francisco. As part of the Golden Gate National Recreation Area, it is among the most visited urban national parks, but also home to twelve species of rare, threatened or endangered plants, many associated with serpentine soils, as well as habitat for over 300 bird species and other wildlife.

In 2015, the Presidio initiated a *Phytophthora* management program to protect endangered plant species and native habitat. Dieback associated with *Phytophthora pseudocryptogea* on Raven's manzanita (*Arctostaphylos hookerii* ssp. *ravenii*), an endangered species with only one known wild individual, underscored the resources at risk given the threat of human-assisted introductions from plantings for large-scale construction and restoration projects. The program is also informed by the Presidio's native plant nursery program and underlying value of natural resource stewardship.

The *Phytophthora* management program includes mapping *Phytophthora*, and pre-plant *Phytophthora* screening of incoming landscape plants. Best management practices are used for fieldwork to promote sanitation and thereby lower the likelihood of *Phytophthora* introductions on imported soil or on workers' or visitors' shoes, and to educate staff and tourists in the role they can play in reducing the spread of plant pathogens. The program represents a significant effort, with 80% time of an IPM specialist and an intern for at least 3 months in the spring and summer and other costs associated with rejected container plant lots, construction delays, etc.

The evaluation of *Phytophthora* species on purchased, incoming landscape plants and determination of resident Phytophthoras in restored areas demonstrates the complexity and difficulty of managing these pathogens. Pre and post restoration sampling on eleven sites indicates that the recovery of *Phytophthora* in some areas is undesirably high. The patterns of species recovery present many questions that propel further adaptive management.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Focus on Wildland Tree Diseases

Common and Uncommon Diseases of Oak, Tanoak and Bay—New Diagnostic Tools Have Led to Important New Discoveries[1]

Suzanne Rooney-Latham[2] and Cheryl Blomquist[2]

Abstract

Although sudden oak death has been the primary concern for California oak woodlands over the last 20 years, other fungal pathogens of oak (*Quercus* spp.), California bay (*Umbellularia californica)*, and tanoak (*Notholithocarpus densiflorus*) also occur in California. Many cosmopolitan Botryosphaeriaceae species with wide host ranges can invade oak and tanoak under stress conditions through wounds, causing perennial cankers in twigs and shoots and large bole cankers. Oak root rot caused by *Armillaria mellea* is widespread in native California soils and infected trees often exhibit cambium death and canopy dieback due to root colonization. White mycelial fans are often seen under the bark and cambium and the distinctive mushrooms may be present during the fall and winter months on the bases of infected trees. Species of *Ganoderma* can also cause root and butt rots of oak and other hardwoods. The large characteristic conks are generally found at or near the base of the tree and usually indicate advanced wood decay (Swiecki and Bernhardt 2006). Although macromorphological characters of some of these fungal pathogens make generic identification possible in the field, species-level identification for many fungi, including those in the Botryosphaeriaceae usually requires culturing onto media followed by DNA sequence analysis.

Our increased awareness of other pathogens associated with hosts of *Phytophthora ramorum* has coincided with the increased use of DNA-based techniques for fungal species identification. More than 2,000 new fungal species were described in 2017, and it is estimated that there are millions yet to be discovered (Niskanen and others 2018). In 2018, a new *Tubakia* species (*Tubakia californica*) causing a foliar disease and twig dieback of Fagaceae species was described in California (Braun and others 2018). Leaves of infected trees do not undergo typical defoliation in the fall. Instead, they remain attached, allowing the fungus to overwinter and release inoculum in close proximity to the new season's spring growth. Confirmed hosts of *T. californica* include California black oak (*Quercus kelloggii*), interior live oak (*Q. wislizeni*),

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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coast live oak (*Q. agrifolia*), chinquapin *(Chrysolepis chrysophylla*), and tanoak. In 2010, a new leafspot disease of bay resembling ramorum blight was documented in a Sonoma County area known to be infested with *P. ramorum*. Subtle differences in symptomology between this disease and those caused by *P. ramorum* led the collector to send the sample to the California Department of Food and Agriculture (CDFA) for identification. Culturing and PCR analysis revealed it to be a novel species of *Cylindrocladium* that has yet to be officially described. New DNA-based diagnostic tools have also detected the presence of many more Botryosphaeriaceae canker pathogens than previously known from oak, tanoak, and bay (Lynch and Eskalen 2014). *Dothiorella iberica, Botryosphaeria dothidea*, and multiple species of *Diplodia* and *Neofusicoccum* have all been confirmed on these hosts. Nearly all these species occur on agricultural and other hardwood hosts and cross infection is likely occurring. As the Plant Pest Diagnostics Lab continues to receive more samples, we are placing greater emphasis on using molecular, sequence-based diagnostic approaches to detect existing and new pathogens. These techniques have not only improved the accuracy and reliability of the Plant Pest Diagnostics Lab's determinations but have also increased our knowledge of the diversity of fungal pathogens in California.

Literature Cited

Braun, U.; Nakashima, C.; Crous, P.W.; Groenewald, J.Z.; Moreno-Rico, O.; Rooney-Latham, S.; Blomquist, C.L.; Haas, J. and Marmolejo, J. 2018. Phylogeny and taxonomy of the genus *Tubakia* s. lat. Fungal Systematics and Evolution. 1(1): 41-99.

Lynch, S.C. and Eskalen, A. 2014. Resource manual: oak woodlands disease management within the Nature Reserve of Orange County and adjacent wildlands. First Edition. University of California Riverside and Nature Reserve of Orange County Joint Publication. 63p.

Niskanen, T. and others. 2018. New discoveries: Species of fungi described in 2017. Pgs. 18–23. In, Willis, K.J. ed., State of the World's Fungi. Report. Royal Botanic Gardens, Kew. 92 pg.

Swiecki, T.J. and Bernhardt, E.A. 2006. A field guide to insects and diseases of California oaks. Gen. Tech Rep. PSW-GTR-197. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture, 151 p.

Phytophthora Species Associated with Decline and Mortality of Native Vegetation in California Wildlands[1]

Chris Lee,[2] Kim Corella,[3] Suzanne Rooney-Latham,[4] Cheryl Blomquist,[4] Tyler Bourret,[5] Tedmund J. Swiecki,[6] and Elizabeth A. Bernhardt[6]

Abstract

Before *Phytophthora ramorum* was identified as the causal agent of sudden oak death in 2000, *P. lateralis,* cause of Port-Orford-Cedar root disease, was the only introduced *Phytophthora* that had been associated with widespread plant decline and mortality in California wildlands. The soil-borne *P. lateralis* is closely associated with riparian or high-rainfall sites with high soil moisture. In 2003, the well-known root pathogen *P. cinnamomi* was found to be causing extensive mortality of manzanita (*Arctostaphylos myrtifolia, A vicida*) in dry upland habitats of the lower Sierra Nevada foothills. This find expanded the search image for *Phytophthora* root diseases in California willdands. Subsequent investigations have associated *P. cinnamomi* with decline and mortality of native trees and woody shrubs in a variety of other California plant communities across a range of climate zones and soil types. As symptomatic native vegetation in additional sites have been assayed for the presence of *Phytophthora,* other soil-borne *Phytophthora* species have been associated with decline and /or mortality of native species in a variety of habitats. *P. cactorum, P. cambivora*, *P. crassamura, P. cryptogea, P. pseudocryptogea, P. pseudotsugae*, and others have been associated with symptomatic native hosts that include conifers, hardwood trees, and woody to nonwoody shrubs and perennials. Some non-*Phytophthora* oomycetes may also be involved in observed declines (e.g., *Elongisporangium (=Pythium) undulatum* and *Phytopythium* spp.).

Establishing a clear connection between pathogen presence and plant symptoms is difficult. Baiting of root/soil samples has been a reliable method for detecting these soil-borne pathogens, but detection efficiency can be low and can vary seasonally. Direct isolation from affected plant tissues often yields false-negative results. Although some highly susceptible hosts die rapidly when infected, other hosts appear to have long latent periods followed by progressive decline.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Dates of pathogen introduction are generally unknown, which complicates the interpretation of symptom progress and the roles of other stressors or secondary agents that may be present. Human activities appear to be associated with introductions of these pathogens to virtually all affected sites, and in some cases, *Phytophthora* species have likely been directly introduced via infected stock used in habitat restoration or forestry activities. Management options for affected stands are few; steps to prevent further spread are the only options in many sites. The examples discussed here illustrate that *Phytophthora* and other oomycete pathogens pose increasing threats to California forest health. Increased efforts are needed to improve detection, impact assessment, and management of these pathogens.

Posters

Observations of *Castanea sativa* as a Host of *Phytophthora ramorum* in England over a Decade[1]

Mick Biddle,[2] Barnaby Wylder,[2] Anna Harris,[3] and Joan Webber[3]

Abstract

Forestry Commission England aerial surveillance operations to detect *Phytophthora ramorum* between 2009 and 2014 were primarily focused on identifying infected larch (*Larix* spp.), although sweet chestnut (*Castanea sativa*) has been recognized as a sporulating host of *P. ramorum* since the mid-2000s (Denman and others 2006). During follow-up ground investigations, infected sweet chestnut was confirmed and considered an incidental host on 23 sites (54 laboratory positives), with individual trees or small discrete stands of sweet chestnut affected but always in close proximity to other infected sporulating hosts (usually *Rhododendron ponticum* and larch). Observed symptoms of sweet chestnut comprised foliar wilting, leaves with blackened petioles, discolored mid-ribs, and/or 'water-soaked' or discolored leaf margins. These symptoms were most common on abundant epicormic growth low on the stems of mature trees. In 2014 an area of sweet chestnut showing general symptoms of decline and crown dieback was noted. The site was in south-west England with known historic *P. ramorum* infection, so survey flights specifically targeting areas of sweet chestnut were added to the surveillance program in 2015. Between 2015 and 2017, 182 sweet chestnut woodland sites (predominantly in south-west England) were identified with crown dieback and mortality, ranging in severity from individual trees through to approximately ≥30% trees affected. Follow-up ground investigations inspected trees for symptoms consistent with *P. ramorum* infection. Many of the sites were found to contain sweet chestnut trees with symptoms which yielded positive lateral flow test results, and laboratory testing of samples from 82 of the sites yielded 150 positive *P. ramorum* results, either based on isolation of *P. ramorum* (EU1) cultures and/or rtPCR confirmation. In addition to foliar symptoms, new symptoms observed included premature abscission of symptomatic leaves from the crown, cankers on epicormic shoots, and in some cases extensive cankers affecting branches and stems of mature trees. An apparent co-occurrence of symptoms with rapid or chronic crown dieback was observed.

In 64 cases, confirmed sweet chestnut infection was in a location with a current or historic presence of *P. ramorum* in larch or rhododendron. Dieback appeared to have progressed in

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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recent years in spite of the removal of the other sporulating hosts on these sites. In a further 18 cases however, infected sweet chestnut trees with crown decline were confirmed in locations where any infected larch and rhododendron could be several km distant (up to 7.5 km in one instance). This suggests long distance aerial transmission of *P. ramorum* to sweet chestnut, and that the disease can also cycle on sweet chestnut in the absence of any other sporulating hosts.

Literature Cited

Denman, S.; Kirk, S.; Whybrow, A.; Orton, E. and Webber, J. 2006. *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin. 36(2): 373-376.

Characterization of Hybrids Between *Phytophthora lacustris* and *P. riparia*[1]

Tyler B. Bourret,[2] Christopher Edelenbos,[2] Sebastian N. Fajardo,[2] Evan Lozano,[2] and David M. Rizzo[2]

Abstract

Studies of hybrid organisms often reveal two types of interspecific hybridization: polyploid hybridization, where the number of chromosomes changes during or immediately following hybridization, and homoploid hybridization, where the number of chromosomes is unchanged. Homoploid hybridization is thought to occur only between very closely-related species, while polyploidy can allow for the persistence of what would normally be a sterile or unstable cross between more distantly related species. Polyploid hybrids may not be able to interbreed with their parents, persisting through time as reproductively isolated species, while homoploids are likely to be able to back-cross, sometimes leading to a "hybrid swarm" of individuals related in varying degrees to the two parent species, blurring the lines between them. Polyploid hybridization has been well-documented in the plant-pathogenic genus *Phytophthora* (Phylum Oomycota), where it can lead to the formation of hybrid species with different host ranges and degrees of virulence than the parent species.

In California, two closely-related species of *Phytophthora*, *P. lacustris* and *P. riparia* are commonly isolated from freshwater environments, along with individuals that appear to be hybrids between the two species. Using a population of stream isolates obtained during sudden oak death monitoring activities, a traditional cloning approach was employed in an attempt to characterize the nature of these *P. lacustris* X *riparia* hybrids and to determine if a hybrid swarm is present. Results and their implications are discussed.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Intraspecific Diversity of Californian Clade 3 *Phytophthora* Isolates[1]

Tyler B. Bourret,[2] Kamyar Aram,[2] Christopher Edelenbos,[2] Sebastian N. Fajardo,[2] Evan Lozano,[2] Heather K. Mehl,[2] and David M. Rizzo[2]

Abstract

Within the large plant-pathogenic genus *Phytophthora,* lies an enigmatic phylogenetic cluster of five species known as "clade 3." Conflicting studies have suggested that this group of species may be native to Europe or North America. At the turn of the 20th century, clade 3 was known only from a single species, *P. ilicis*, which had been isolated in North America and Europe. In 2002, a second species, *P. psychrophila* was described from declining European oak forests. In 2003, two additional species were introduced, with *P. nemorosa* found only in North America and *P. pseudosyringae* from both Europe and North America. In 2013, *P. pluvialis*, another North American species was added.

Despite being distantly related to *P. ramorum*, the sudden oak death (SOD) pathogen, *P. nemorosa* and *P. pseudosyringae* cause indistinguishable symptoms on native Californian hosts, albeit with less frequency and virulence. Because SOD is an emerging disease caused by a non-native pathogen, this led to speculation that *P. nemorosa* and *P. pseudosyringae* were also introduced to North America. In 2009, a study of genome-wide diversity of the two species suggested highly clonal populations, and (for *P. pseudosyringae*), that the North American isolates were derived from the European population. The 2009 study, combined with a lack of association with landscape-level disease in Europe and a lack of aggressiveness on native European hosts led to a 2015 assessment that *P. ilicis, P. pseudosyringae* and *P. psychrophila* were the only species of *Phytophthora* (out of about 60 categorized) native to Europe.

Phytophthora nemorosa and *P. pluvialis* have still never been documented in Europe. Originally described only from Oregon, *P. pluvialis* was found causing significant disease in *Pinus radiata* (Monterey or radiata pine) plantations in New Zealand as well as native stands of *Pseudotsuga menziesii* (Douglas-fir) in Oregon; this New Zealand occurrence represented the first documentation of clade 3 outside of North America or Europe. A worldwide collection of *P. pluvialis* strains from Oregon, California and New Zealand suggested that the New Zealand

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population was derived from North America, although these results do not necessarily indicate this is the native range of *P. pluvialis*. Most recently, *P. pseudosyringae* was isolated from South America.

Another source of evidence regarding geographic origins is the diversity of sequences derived from *Phytophthora* clade 3 isolates available in public databases, the expectation being that the greatest genetic diversity will be found in the native range. So-called "barcoding sequences" including the ITS rDNA and various sections of the mitochondrial cox2-cox1 region are commonly deposited for *Phytophthora* isolates, allowing for the identification and comparison of isolates across space and time. A 2017 study demonstrated that all five clade 3 species are either common or uncommon but consistent in *Phytophthora* surveys of Oregon natural ecosystems, and that more intraspecific diversity can be found across Oregonian than European isolates. This evidence is consistent with the notion that at least some species in clade 3 may, in fact, be native to North America.

We obtained barcoding sequences from more than one hundred Californian isolates in *Phytophthora* clade 3, comprising four of the five species. These isolates were obtained from various UC Davis, Rizzo Lab projects sampling California natural ecosystems for purposes of research and management over the course of more than a decade. Preliminary results suggest in regards to *Phytophthora* clade 3 that California, like Oregon, is a source of great intraspecific diversity.

Genomic and Metagenomic Exploration of Microbial Endophytes and a new Potential *Phytophthora* Species in the Monkey Puzzle Tree *Araucaria araucana* in Chile[1]

Sebastián Márquez,[2] Francisca Venegas,[2] Gabriela Jiménez,[2] Guus Teunisse,[2] Freddy Boehmwald,[2] Pablo Zamora,[2] Álvaro Castro,[2] Jaime Alarcón,[3] and Eduardo Castro-Nallar[3]

Abstract

Araucaria araucana (Araucaria or monkey puzzle tree; Class = Pinopsida; Family = Araucariaceae) is an endangered conifer with a fragmented and relict distribution in southern Chile and Argentina. Araucaria is the type species of a genus of relatively old conifers with an ancient distribution that covered the continent of Gondwana, and that now comprises 19 extant species distributed in Oceania and South America.

Araucaria has been historically threatened by logging (banned in 1990), wildfires, overgrazing, invasive trees, and extensive human harvesting of Araucaria seeds. More recently, the Chilean forest authority reported extensive damage spread throughout its geographic distribution in Chile and Argentina, which is characterized by browning of branches and needles following a "bottom-up" pattern and radiating from the trunk to the tip of the branches. While 90% of *A. araucana* population is affected there is only a 2% mortality rate in Chile, according to the Corporación Nacional Forestal; Chilean National Forestry Corporation, CONAF. The disease was dubbed DFA as "foliar damage of the Araucaria tree" for its acronym in Spanish. While there are several hypotheses regarding the cause of DFA including approximately a 10-year drought in the region, the widespread nature of the disease which covers all of its geographic distribution at various intensities, plus gardens, nurseries, and public squares, suggests the influence of a pathogen, opportunistic or otherwise.

Here, we use amplicon sequencing targeting the 16S rRNA and ITS taxonomic marker genes to reveal the structure and composition of Araucaria's microbial communities throughout its geographic distribution (n > 600). Community analyses suggest that Araucaria's microbial communities are structured primarily within tree by tissue, and secondarily by sampling site, i.e., Andes or Nahuelbuta mountain ranges and north/south gradient.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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To get a better view of Araucaria's microbial communities, we complement these analyses with shotgun metagenomic sequencing, which revealed that up to 20% of the endophytic communities are dominated by a *Phytophthora* lineage closely related to subclade 8c species that include *P. ramorum*, *P. lateralis*, *P. hibernalis*, and *P. foliorum*. We extracted contigs from this new lineage and were able to reconstruct a preliminary phylogeny. Ongoing efforts include culturing and isolation of this *Phytophthora* member, as well as PCR screening over 300 Araucaria samples (healthy and infected) from its entire geographic distribution. We discuss our results and future experiments in the light of testing whether this lineage of *Phytophthora* is the causal agent of DFA.

Acknowledgements

Funded by CONAF (Corporación Nacional Forestal; Chilean National Forestry Corporation), executed by UC Davis Chile Life Sciences Innovation Center, and supported by Universidad Andrés Bello, Center for Bioinformatics and Integrative Biology (CBIB) and Fundación Mar Adentro.

Management of *Phytophthora ramorum* at a Botanical Garden in Washington State, USA[1]

Marianne Elliott[2] and Gary Chastagner[2]

Abstract

In March 2015 *Phytophthora ramorum* was detected at The Bloedel Reserve, a 150 acre botanical garden in Kitsap County, WA. Infected plants were destroyed and the soil in an area surrounding these plants was steam-sterilized to a depth of 15 cm during the summer of 2015. An IPM program was developed in an effort to control the spread of *P. ramorum* and other *Phytophthora* species in the garden, reduce the risk of *P. ramorum* spread to the surrounding landscape, and minimize additional destruction of valuable plants and visual impacts to the garden. Several treatments were employed, including the use of *Phytophthora*-specific fungicides, removing host vegetation, soil steaming, replanting affected areas with non-host or host plant species that have shown some resistance to *P. ramorum*, and the use of *Trichoderma* biocontrol agents and mulch to reduce spread of disease from soil to plants. Surveys in the *P. ramorum* positive areas and perimeter were done during 2015-2018. Symptomatic foliage was collected and tested for *Phytophthora* using ELISA. Any ELISA positive samples were tested for *P. ramorum* with PCR. Isolates of *P. ramorum* were genotyped using microsatellite markers.

Many of the *P. ramorum* positives were detected on certain native hosts. In February 2016, the IPM strategy was therefore modified to include the removal of native host vegetation within the positive areas. Fungicides were applied in the positive areas during 2016-2018. The rate of ELISA+ plants was between 37% - 90% during this time period. The rate of ELISA+ samples has decreased in the positive areas since the peak of 90% in October 2016 and has stayed below the initial 72% measured in January 2016.

Seven NA1 microsatellite genotypes of *P. ramorum* were detected at Bloedel between March 2015 – February 2016. The two most commonly found genotypes were identical to the genotypes of *P. ramorum* from two nurseries in Washington State. The remaining five genotypes have only been detected at Bloedel and are very similar to the nursery genotypes. These are probably derived from the nursery genotypes rather than being new introductions.

Fungicide applications and long term continual removal of native host plants in the positive areas, and the reapplication of Plant Helper (*Trichoderma atroviride*) in areas that were identified as higher risk due to slope and proximity of prior positive sites has been continued

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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until the present. The Plant Helper is applied as a soil drench and then covered with a mulch that is made from chipped alder wood and other vegetation, but not containing host plant material, on the Reserve. Expanding the use of Trichodermas in the prior positive areas of the Reserve to include areas further from these known positive sites is being considered. Soil steaming was effective in the immediate areas of the positives that were detected, but there are extensive areas that should be protected from potential infestation, and there may be undetected infestations that could be mitigated by added populations of *Trichoderma* in the soil.

A Technique for Treating Contaminated Soil with Steam for Eradication of *Phytophthora*[1]

Marianne Elliott[2] and Gary Chastagner[2]

Abstract

During a series of large riparian restoration projects in the San Francisco Bay Area, Phytophthoras on infected planting stock were inadvertently introduced into a number of sites. There are many planting basins at some of these sites where hosts from a nursery that had a high rate of *Phytophthora* positives were planted. These basins are scheduled for treatment once a potential mitigation approach to kill any introduced Phytophthoras is decided upon. Steaming has been shown to be an effective mitigation treatment to eliminate Phytophthoras from infested soil. In nursery and landscape sites where *P. ramorum* has been detected, steaming soils to reach a temperature of 50 ºC at 30 cm for 30 minutes is an accepted USDA APHIS mitigation treatment. In this project, two steaming techniques were tested. In addition, thermal cover materials for retaining heat in steamed soil were compared. Testing was done at a restoration site in California (CA) and at WSU Puyallup (WSUP).

At the CA site, a 24" diameter steam auger was attached to a hydraulic-powered shaft that passed through a transfer case welded to an excavator bucket. Steam was delivered to the auger via a 2-inch diameter hose that connected a steam generator to the transfer case. Steam was introduced through the auger during soil mixing. In another set of trials the soil was first augured to 24" depth, then a 1.5” diameter injector was used to introduce steam at the bottom of the hole. Temperatures were measured at several depths along the edges of the holes during and after steaming. Testing in a similar soil type at WSUP under several moisture conditions was done using the injector and temperature sensors mounted on a grid inside the hole. The temperature at each point on the grid was measured during and after steaming.
Preliminary results from auger and injector field tests in CA were not conclusive due to saturated soil conditions. Although a steam auger was not tested at Puyallup, the results with the steam injector indicate that the results of at least the steam injector tests at the CA site would have likely been acceptable if they had been done under dryer soil conditions. In the silt loam soil at WSUP, the conditions for killing *P. ramorum* (50 ºC for 15 minutes) and *P. pini*, which has a heat resistant spore stage (50 ºC for 40 minutes) were reached over most of the soil volume when soil at field capacity was steamed for 5 or 10 minutes.

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Data collected during the steaming at both sites indicated that there is little risk of a negative impact to organisms in the bulk soils adjacent to augured and steamed planting basins unless the soil has larger channels for the steam to move outside of the soil in the augured hole.
A thermal cover used after steaming will retain heat in the soil after 10 minutes of steaming when sufficient heat has accumulated. There was little difference between the steamed, uncovered plots and plots with some type of thermal cover after 5 minutes of steaming. After 10 minutes, the differences between thermal cover treatments were significant at all depths. The materials that prevented the most heat loss from the soil were an insulated metal drain pan, rubber floor mats, and denim insulation.

Exploring Interactions Among Disease, Fuel Loads, and Fire Intensity in Sonoma County Oak Woodlands[1]

Manuel Hernandez[2] and Lisa Patrick Bentley[2]

Abstract

Understanding the impacts of disease and fire on forested ecosystems is a major challenge facing scientists, land managers, policy makers and landowners. Due to difficulty in predicting wildfire, few studies of ecological effects of fire are based on both pre- and post-fire data. The goal of our research is to use a large scale and long-term plot network with both pre- and post-fire data in eastern Sonoma County to determine the ecological impacts of an exotic pathogen (*Phytophthora ramorum*) and wildfire on oak-woodland forest communities.

We aim to answer the following questions: 1) Do plots with higher inoculum loads and disease prevalence have greater pre-fire fuel loads? 2) Do plots with greater mortality due to disease have greater burn severity? and 3) How do disease and wildfire restructure community composition and vegetation recovery post-fire?

In 2003, 197 15 x 15 plots were established within a 275 km^2 heterogeneous region in eastern Sonoma County to study *P. ramorum* (Meetenmeyer and others 2008). Every two years until 2016, measurements of microclimate, disease prevalence, tree growth, mortality and survival of the three most abundant host species, coast live oak (Quercus *agrifolia*), California black oak (*Quercus kelloggii*), and California bay laurel (*Umbellularia californica*) were taken. In 2016, microclimate and fuels loads were quantified using standard forestry protocols (Brown 1974). In 2017, the Central LNU (Lake Napa Unit) Complex fires burned 44,806 ha in Sonoma, Napa and Lake Counties during which approximately half of these study plots burned. In 2018, microclimate data, tree mortality and survival, and fuel loads were quantified across 95 plots (51 burned, 44 unburned). In burned plots, we observed a higher level of root sprouting in *U. californica* post-fire than in oak species. Preliminary results exploring fuel loads and fire indicate that duff depth has a significant effect on fire intensity (LRX^2 = 4.15, p = .0416). In addition, the interaction between *U. californica* disease prevalence and total downed woody debris is also significant (LRX^2 = 4.397, p = .0360). Current and future work will continue to explore these relationships and assist with management of oak-woodlands in light of future drought and increased fire risk.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Literature Cited

Brown, J.K. 1974. Handbook for inventorying downed woody material. Gen. Tech. Rep. INT-16. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 24 p.

Meentemeyer, R.K.; Rank, N.E.; Anacker, B.L.; Rizzo, D.M. and Cushman, J.H. 2008. Influence of land-cover change on the spread of an invasive forest pathogen. Ecological Applications. 18(1): 159–171. https://doi.org/10.1890/07-0232.1.

Rapid Recovery and Detection of *Phytophthora ramorum* Propagules in Nursery Water[1]

Douglas Luster,[2] Karen Suslow,[3] Supriya Sharma,[3] Wolfgang Schweigkofler,[3] and Vernon Huffman[3]

Abstract

Phytophthora ramorum, causal agent of sudden oak death, continues to threaten U.S. forest ecosystems and the nursery industry. Currently, USDA APHIS's protocol (2014) utilizes the Bottle of Bait (BOB) recovery method for *P. ramorum*, which requires collecting water from a source, baiting with healthy rhododendron leaves for a 3-day incubation period, followed by plating on semi-selective media. Rapid methods are needed for recovery and detection of *P. ramorum* propagules from water sources. Working at the National Ornamentals Research Site at Dominican University of California (NORS-DUC), we are developing rapid water filtration and flocculation methods for recovery and detection of *P. ramorum* propagules from nursery irrigation water. A mock irrigation pond was established with flow from a *P. ramorum*- infested plot into an adjoining plot. Antibodies raised against *P. ramorum* -specific secreted proteins were applied for detection of zoospores and sporangia from 1 L samples in filter extracts or alum flocculates using standard immunoassay procedures. Results with spiked samples indicate that propagules of *P. ramorum* recovered by filtration or flocculation from spiked nursery water samples can be detected in 24 h or less.

Introduction

Presently, USDA APHIS (2014) relies upon water baiting for diagnosis and confirmation of *Phytophthora ramorum* in nurseries inside the boundaries of the *P. ramorum* regulated areas. As of March 31, 2014, the Confirmed Nursery Protocol utilizes the Bottle of Bait (BOB) technique for recovery of *P. ramorum* from standing water on nurseries and from water sources such as container runoff, irrigation retention ponds, etc. The process, as described in the Official Regulatory Protocol for Nurseries Containing Plants Infected with *Phytophthora ramorum,*

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Confirmed Nursery Protocol: Version 8.2 (see: https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/ConfirmedNurseryProtocol.pdf) can take as long as two weeks to obtain results.

We have been developing methods for rapid concentration, recovery, and detection of *P. ramorum* propagules from nursery water sources. To this end we have tested rapid microfiltration and flocculation techniques combined with antibody detection to reduce the time required to detect and identify *P. ramorum.* Microfiltration of nursery water sources has been used to recover pathogens for detection (e.g Ali-Shtayeh and others 1991, Hwang and others 2009). Flocculation is a standard practice in municipal drinking water treatment for removal of human pathogens (Andreoli and Sabogal-Paz 2019, EPA 2008, Engelhardt 2010), and has been tested in nurseries for removal of waterborne plant pathogens (Machado and others 2013, Majsztrik 2017). Both microfiltration and flocculation are rapid and inexpensive and hold promise to reduce the time required to detect and identify *P. ramorum* and other waterborne plant pathogens in nursery water sources.

In this study, we used monoclonal and polyclonal antibodies generated against *P. ramorum* secreted proteins and used them in ELISA immunoassays of filtrates and flocculates captured from a simulated nursery retention pond containing *P. ramorum* propagules, generating results in 24 hrs. or less.

Methods and Materials

Retention Pond Construction and infestation with P. ramorum

An open retention pond was constructed at NORS-DUC using existing facilities, in plots covered with mesh screening but open to receive rainfall (fig. 1A). The retention pond consisted of a raised bed plot with pool liner. An adjacent plot was infested with 6 mesh bags each containing 6 Rhododendron ‘Cunningham’s White’ leaves inoculated with *P. ramorum* NA1. Overhead irrigation was provided to the infested plot over the infested leaf bags for 5 minutes, twice daily, and the irrigation/rainfall runoff was captured and diverted to the retention pond. New inoculum bags were added to the plot monthly. The objective was to generate *P. ramorum* propagules in the adjacent plot and flush them into the retention pond, simulating runoff from an infested nursery.

Baiting and sampling

Water was sampled every two weeks between January- May 2019. Three 1 L plastic bottles were filled at the location where irrigation/rainfall runoff entered the retention pond. The three bottles from each sampling were analyzed independently. Baiting of the retention pond was conducted using mesh bags containing 6 Rhododendron ‘Cunningham’s White’ leaves (fig .1B). Bait bags were replaced every month. Leaf discs from baited leaves were plated on PARPH-V8 medium (Ferguson and Jeffers 1999) and examined microscopically for *P. ramorum* growth.

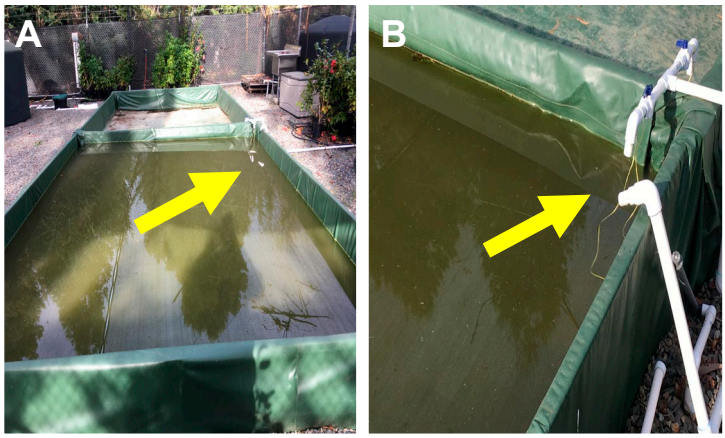

Figure 1. Arrows point to bait bags in mock retention pond placed at inflow from adjacent plot; water samples were collected there. A. Mock retention pond configuration in NORS-DUC plot. B. Close-up of inflow from adjacent plot and sampling point.

Microfiltration

Water samples from the runoff pond were used to test microfiltration protocols, refining methods from Ali-Shtayeh and others (1991) and Hwang and others (2009). Laboratory trials were conducted to trap zoospores from 1.0 L batches of retention pond water. A subset of the samples were spiked with known quantities of zoospores, produced from sporangial samples generated from cultures of *P. ramorum* NA1 (Widmer 2009). When necessary, samples were pre-filtered through 149-53-20 µ nylon mesh macro filters (Spectrum, New Brunswick, NJ) to clarify the sample and remove silt and debris. Samples were then filtered through 5µ polyvinylidene fluoride (PVDF) membrane filters (MilliporeSigma, Rockville, MD). Filters were incubated overnight (15-18 h) at 20 ºC in moist petri dishes to encourage zoospore encystment and germination. After incubation, filters were extracted in plant extraction buffer bags using GEB2 buffer (Agdia, Elkhart IN). After overnight incubation, filters were placed in bags with 3 mL of buffer, rubbed vigorously with the blunt end of a felt-tip marker pen, and extracts were frozen for ELISA assays.

Flocculation

Three 1 L water samples from the runoff pond, spiked and unspiked with known quantities of zoospores, were used to test flocculation protocols for collection and concentration of *P. ramorum* propagules. Water samples were transferred to clear plastic 500 mL bottles containing a 5 cm stir bar and stirred at 125 rpm at room temperature. The pH was measured with test strips; samples were consistently pH 6.5-7. While stirring at 125 rpm, 50 mL of fresh 1 mg/mL AlSO4 ("Alum", Sigma Chemical Co., St. Louis, MO) was slowly added to a final concentration of 50 mg in 500 mL and the solution was stirred for 10 min. The stir plate was turned off after 10 minutes and the resultant fluffy flocculant allowed to settle for 60 minutes until the supernatant was clear (fig. 2A). The clear supernatant was slowly pipetted into a beaker and the flocculant (ca. 25 ml) was removed to a 50 mL, then to a 15 mL disposable plastic centrifuge tube and allowed to continue to settle (fig. 2B). An aliquot of the resulting flocculant (ca 5 mL) was

dilution plated on PARPH-V8 to calculate recoveries and the remainder frozen for ELISA assays.

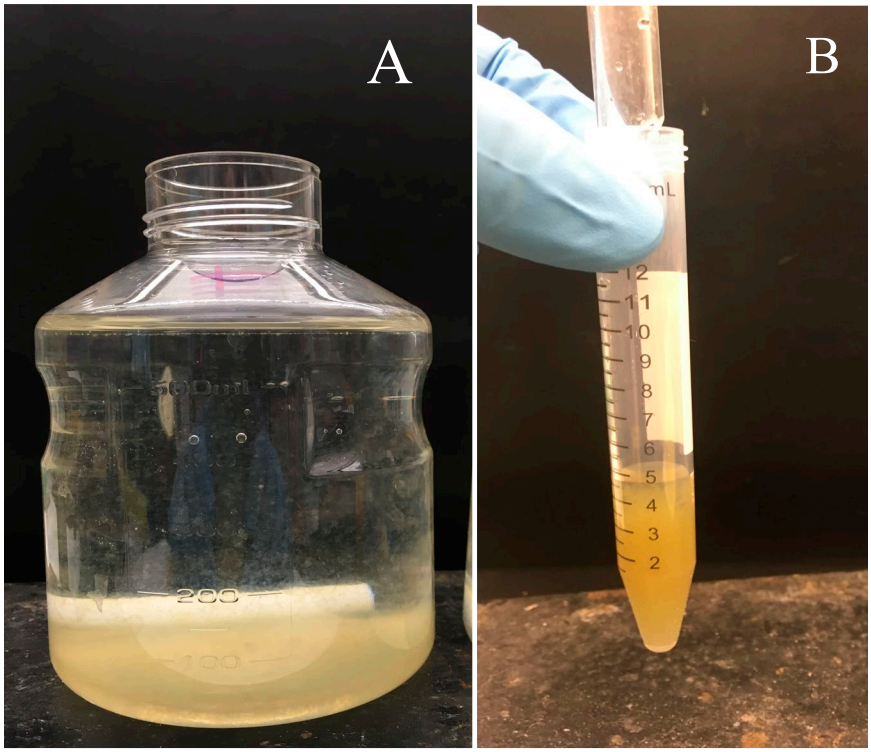

Figure 2. A. Nursery water sample 60 minutes after 50 mg alum flocculant was added with stirring and the flocculate allowed to settle. B. Concentrated flocculant from a 1L nursery water sample.

P. ramorum *antibodies*

Antigen targets for antibody development were identified using a mass spectrometry proteomic approach to identify proteins secreted by encysting zoospores in culture, referencing an annotated *P. ramorum* NA1 genome. Proteins with high antigenicity scores were BLASTed against all *Phytophthora* genomes in GenBank to identify those unique to *P. ramorum*, and unique proteins were selected for recombinant protein or peptide generation and antibody production in mice (monoclonal antibodies, mAbs) or rabbits (polyclonal antibodies, pAbs). One secreted protein unique to *P. ramorum* ("H3N7") and found at the highest titer in secreted fractions on encysting zoospores, was selected for assays. Inclusivity testing was conducted against encysting zoospores of 14 NA1, NA2, EU1 and EU2 *P. ramorum* isolates. In laboratory tests, we have determined the sensitivity of these antibodies to be on the order of 10^2 to 10^3 *P. ramorum* propagules (data not shown). Exclusivity testing against near neighbor *Phytophthora spp.* is still in progress to demonstrate specificity.

ELISA

ELISA assays were conducted on 100 µL samples of filtrate extracts or concentrated flocculate using the method described by Baysal-Gurel and others (2008), substituting ABTS (KPL, Gaithersburg, MD) as the enzymatic peroxidase conjugate substrate, reading absorbance at 405 nm.

Results

As presented in table 1, the recovery of sporangia in flocculates from spiked samples was 60-70%, while recovery of zoospores from similarly spiked samples was 40-60%. The *P. ramorum* mAb was able to detect both sporangia and zoospores in flocculates, while the pAb was less effective (Agdia *Phytophthora* immunostrips were used as a check). We observed clumping of encysting zoospores and sporangia in flocculants which may have reduced the observable number of colony-forming units (CFU) on culture plates and inhibited detection of propagules to microtiter well plates. We are currently testing mild surfactants and chaotropes on flocculates to reduce aggregation and provide more reliable results.

Table 1. Recovery of *P. ramorum* zoospores and sporangia in flocculates of 2019 nursery water samples spiked with the indicated propagules. ELISA Results Symbol Key: O.D. above background: < 0.1 = (-), 0.3-0.1= (+), 0.5-0.3 = (++), > 0.5 = (+++)

Sample Date/Type	Description	Agdia Strip	ELISA (pAb)	ELISA (mAb)	CFU (% Recovery)
Feb 20	+Zoospores	+	+	+	70%
Mar 14	+Zoospores	+	+	+	70%
Mar 22	+Zoospores	+	+	+	70%
Apr 1	+Zoospores	+	+	+	70%
Apr 25	+Zoospores	+	++	+	60%
May 8	+Zoospores	+	++	+	60%
Feb 20	+ Sporangia	+	++	++	40%
Mar 14	+ Sporangia	+	+	+	60%
Mar 22	+ Sporangia	+	-	+	60%
Apr 1	+ Sporangia	+	-	+	50%
Apr 25	+ Sporangia	+	-	+	40%
May 8	+ Sporangia	+	-	+	40%

Bait bag sampling in plot 11 was positive for *P. ramorum* in samples collected in February, March, April and May 2019 (table 2). This indicated that our mock retention pond design and operation was effectively generating *P. ramorum* propagules and flushing them from the infested

source plot into the mock retention pond. Bait samples were not collected in January, and in some samples other *Phytophthora spp*. were present.

Phytopthora ramorum was detected by immunoassay with mAbs and pAbs in micro-filtered and flocculant samples from January, February, March, April and May 2019, indicating successful recovery and detection of *P. ramorum* propagules on filters. We also detected *P. ramorum* by immunoassay in flocculant samples from January, February, March, April and May 2019. The *P. ramorum* mAb was again able to both detect sporangia and zoospores in flocculates, while the pAb was slightly less effective in some cases.

Table 2. Results of 2019 baiting and immunoassays on nursery water sample microfiltrate extracts. N.D. = Not Determined. ELISA Results Symbol Key: O.D. above background: < 0.1= (-) , 0.3-0.1 = (+), 0.5-0.3 = (++), > 0.5 (+++)

Sample Date/Type	Baiting*	Agdia Strip	ELISA (pAb)	ELISA (mAb)
Jan 23 Bottle 1	**N.D.**	**+**	**++**	**+++**
Jan 23 Bottle 2	**N.D.**	**+**	**+**	**+**
Jan 23 Bottle 3	**N.D.**	**+**	**+**	**++**
Feb 7 Bottle 1	*P.ramorum* +	**+**	**++**	**++**
Feb 7 Bottle 2	*P.ramorum* +	**+**	**+**	**+++**
Feb 7 Bottle 3	*P.ramorum* +	**+**	**+**	**++**
Feb 19 Bottle 1	**N.D.**	**+**	**+**	**+**
Feb 19 Bottle 2	**N.D.**	**+**	**-**	**+**
Feb 19 Bottle 3	**N.D.**	**+**	**+**	**+**
Mar 14 Bottle 1	*P.ramorum* +	+	++	++
Mar 14 Bottle 2	*P.ramorum* +	+	+	+
Mar 14 Bottle 3	*P.ramorum* +	+	+	++
Mar 22 Bottle 1	*P.ramorum* +	+	+	++
Mar 22 Bottle 2	*P.ramorum* +	+	+	++
Mar 22 Bottle 3	*P.ramorum* +	+	+	+++
Apr 1 Bottle 1	*P.ramorum* +	+	++	+++
Apr 1 Bottle 2	*P.ramorum* +	+	+++	+++
Apr 1 Bottle 3	*P.ramorum* +	+	+++	++
Apr 25 Bottle 1	*P.ramorum* +	+	+	++
Apr 25 Bottle 2	*P.ramorum* +	+	++	++
Apr 25 Bottle 3	*P.ramorum* +	+	+	++
May 8 Bottle 1	*P.ramorum* +	+	+	+
May 8 Bottle 2	*P.ramorum* +	+	++	+
May 8 Bottle 3	*P.ramorum* +	+	++	+

Discussion

Microfiltration and flocculation are effective methods for concentration of microbes from water samples, and when combined with immunoassays provide a rapid means of detection, with advantages over the baiting and culturing methods currently employed in detection of *P. ramorum* in nursery water sources. Filtration and flocculation are sampling methods that rely on detectable numbers of propagules in the water sample captured at a single time point, while baiting has the advantage of a retrieval method based upon zoospore chemotaxis and thus has a much larger effective sample volume. The tradeoff is thus time of sampling to detection vs. sensitivity. In this study we did not quantify *P. ramorum* propagules, but set the detection limit in ELISA at a low level of absorbance above controls/background.

Microfiltration can be a less useful method for propagule concentration when samples contain excessive sediment or algal growth, causing slow filtration or complete clogging of filters. In such cases flocculation may be the preferred method. Because flocculation has been demonstrated to be effective in removal of bacterial and protozoan pathogens from municipal water sources, we assume that our methods can be improved to demonstrate effective recoveries (or recovery quantification).

We have demonstrated that propagules of *P. ramorum* recovered by filtration or flocculation from spiked nursery water samples can be detected in 24 h or less. With improvements, these methods may provide alternatives to the current protocols required by regulatory agencies for detection of *P. ramorum* in surface waters.

Acknowledgements

Funding for this project was provided by USDA APHIS 10007 Farm Bill # 16-8130-0652-CA, #AP18PPQS&T00C174 and USDA-ARS NACA #58-8044-8-001-N.

Literature Cited

Ali-Shtayeh, M.S.; MacDonald, J.D.; Kabashima, J. 1991. A method for using commercial ELISA tests to detect zoospores of *Phytophthora* and *Pythium* species in irrigation water. Plant Disease. 75: 305-311.

Andreoli, F.C. and Sabogal-Paz, L.P. 2019. Coagulation, flocculation, dissolved air flotation and filtration in the removal of *Giardia spp*. and *Cryptosporidium spp*. from water supply. Environmental Technology. 40: 654-663.

Bush, E.A.; Hong, C.X.; Stromberg, E.L. 2003. Fluctuations of *Phytophthora* and *Pythium spp*. in components of a recycling irrigation system. Plant Disease. 87: 1500-1506.

Engelhardt, T.L. 2010. Coagulation, flocculation, and clarification of drinking water. Drinking water sector, Hach Company Technical Bulletin.
https://pdfs.semanticscholar.org/41df/6382693e015bb060f5effd76301bc2fcdd81.pdf

EPA Report 2008. Large-Volume Sample Preparation for Waterborne Pathogens, EPA, Office of Water Research and Development, Cincinnati, OH. https://www.epa.gov/water-research/development-and-evaluation-large-volume-sample-preparation-techniques-microbial.

Ferguson, A.J. and Jeffers, S.N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Disease. 83: 1129-1136.

Hwang, J.; Oak, S.W.; Jeffers, S. 2008. Detecting *Phytophthora ramorum* and other species of *Phytophthora* in streams in natural ecosystems using baiting and filtration methods. In: Frankel, S.J.; Kliejunas, J.T. and Palmieri, K.M., tech. coords. Proceedings of the Sudden Oak Death Third Science Symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 55-58 p.

Machado, P.D.S.; Alfenas, A.C.; Coutinho, M.M.; Silva, C.M.; Mounteer, A.H.; Maffia, L.A.; de Freitas, R.G. and Freitas, C.D.S. 2013. Eradication of plant pathogens in forest nursery irrigation water. Plant Disease. 97: 780-788.

Majsztrik, J.C.; Fernandez, R.T.; Fisher, P.R.; Hitchcock, D.R.; Lea-Cox, J.; Owen, J.S.; Oki, L.R. and White, S.A. 2017. Water use and treatment in container-grown specialty crop production: a review. Water, Air, and Soil Pollution. 228:151.

USDA APHIS 2014. Official Regulatory Protocol (Confirmed Nursery Protocol: Version 8.2) for Nurseries Containing Plants Infected with *Phytophthora ramorum.* http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/ConfirmedNurseryProtocol.pdf

Widmer, T.L. 2009. Infective potential of sporangia and zoospores *of Phytophthora ramorum*. Plant Disease. 93: 30-35.

A Survey of *Phytophthora*, *Pythium,* and *Phytopythium* Species in Soil from Upland Prairie Restoration Sites in Western Oregon[1]

Jennifer Parke,[2] Erika Mittermaier,[2] Neelam Redekar,[2] Joyce Eberhart,[2] and Teresa Matteson[3]

Abstract

Native upland prairie and oak savanna habitats were once widespread in the Willamette Valley of western Oregon, but have been diminished by conversion to other land uses. These threatened habitats are considered essential for rare and endangered species such as the Fender's blue butterfly. Restoring native upland prairie habitats is a major goal of wildland restoration in Oregon.

The inadvertent spread of *Phytophthora* species from nurseries into native ecosystems can have long-term environmental and economic impacts, as has been seen with *Phytophthora ramorum*, *P. lateralis*, *P. cinnamomi, P. tentaculata*, and other species. The risk may be particularly great when nursery-grown plants infested with *Phytophthora* spp. are planted in restoration sites, introducing pathogens directly into native habitats (Garbelotto and others 2018). The objectives of this study were to survey the distribution of *Phytophthora*, *Pythium,* and *Phytopythium* spp. in upland prairie restoration sites in western Oregon and to determine if they are detected at greater frequency in planted vs. non-planted sites.

We collected soil samples (0-20 cm depth) from 55 upland prairie/oak savanna sites in the Willamette Valley in western Oregon between November 2016 and May 2017. Of these, 27 sites had been planted with seeds, bulbs, or live plants within the last 5 years, and 28 sites had not been planted. Soil was sieved (2-mm) and split into two homogenized subsamples. One set of subsamples was baited with pears. Pear lesions were plated to obtain pure culture isolates. DNA was extracted from cultures and species were identified based on Sanger sequencing of the ITS region. From the second set of subsamples, DNA was extracted directly from 10 g of soil. PCR was performed with these extracts using oomycete-specific ITS primers before sequencing on the Illumina MiSeq platform. Samples with low DNA (< 2ng/µl) were excluded. Species abundance was normalized across all samples and adjusted for the total amount of DNA recovered from the soil. A species was considered present in soil if its abundance was ≥1% of the sample DNA.

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[1]A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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We detected 13 *Pythium* species, 5 *Phytophthora* species, and 1 *Phytopythium* species with the pear baiting and metabarcoding approaches (fig. 1). *Pythium* species were nearly ubiquitous, detected in 46 of the 55 sites (84%). *Pythium attrantheridium* was the most frequently detected species with the metabarcoding approach. It was not recovered from pear baits. Other *Pythium* species included the *glomeratum-complex, pectinolyticum, paroecadrum, macrosporum, ornacarpum*-complex, *volutum, pachycaule*-complex, *parvum, mamillatum*-complex, *terrestris*-complex, and *ultimum*. *Phytophthora* species were detected, but in only 7 of 55 sites (13%), and included the *P. cactorum* cluster, *P. megasperma* complex, *P. cambivora* complex, *P. fragariae* complex, and a *P. ramorum*-like species. A complex consists of closely related species that are indistinguishable based on the ITS1 region. A cluster consists of closely related species that are identical between the priming sites (Redekar and others 2019). Only two of the five *Phytophthora* species were recovered from pear baits. A quantitative real time PCR assay confirmed that the *P. ramorum*-like species has an ATP9-NAD9 region different than *P. ramorum*, despite an identical ITS1 region. There was no statistically significant relationship between the presence of *Phytophthora* (p=0.389) or *Pythium* (p=0.503) species and planting history based on contingency tables using a Pearson's test for independence (data not shown).

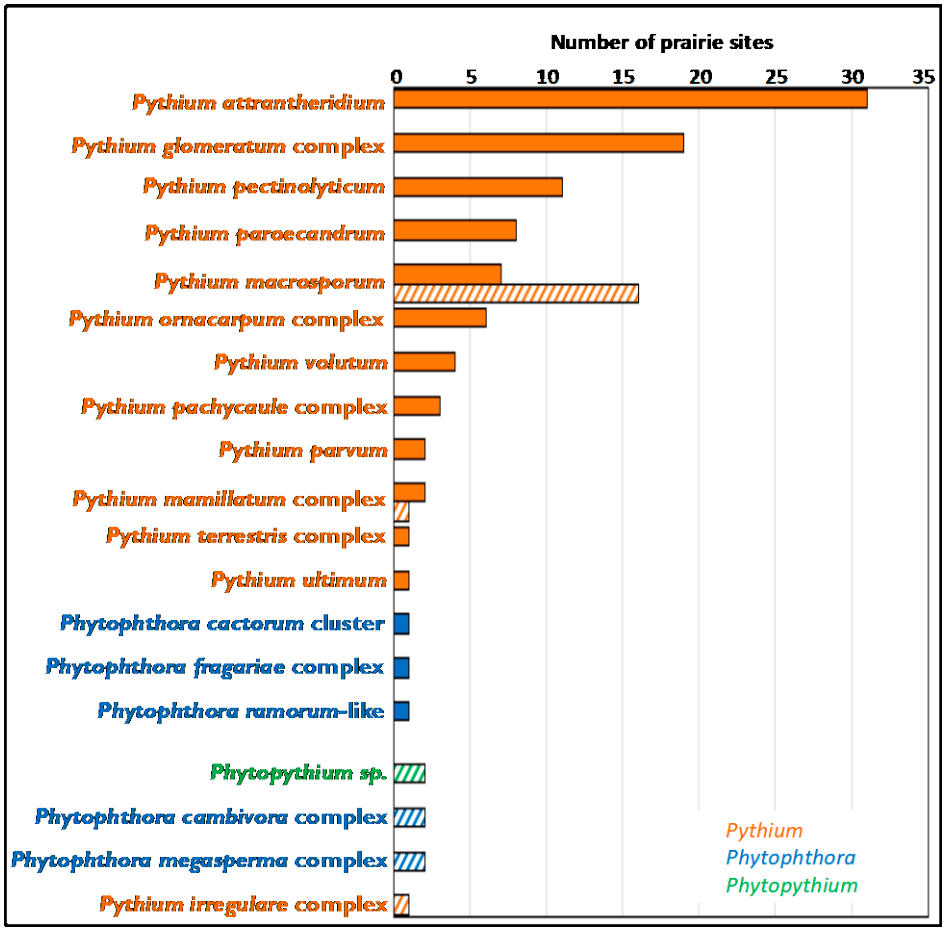

Figure 1—Frequency distribution of *Pythium, Phytophthora*, and *Phytopythium* species detected in soil from 55 upland prairie sites in western Oregon using pear baiting (striped bars) and Illumina MiSeq sequencing (solid bars).

Pythium appears to be nearly ubiquitous in upland prairie soils of western Oregon. The ecological role(s) of *Pythium* in this habitat is not known but *P. attrantheridium* has been shown to affect the distribution of native plants elsewhere (Allain-Boulé and others 2004, Packer and Clay 2000). In contrast, *Phytophthora* species were detected relatively infrequently in upland prairie soils but do include plant pathogenic species clusters or complexes (*P. cactorum* and *P. cambivora*) of potential concern to wildlands. Pear baiting resulted in detection of only 6 species. Illumina MiSeq appears to be a more sensitive method of detection, expanding the number of species or species complexes detected to 15.

Results of this study provide a snapshot of the current distribution of *Phytophthora* and *Pythium* species in restoration sites in western Oregon and can serve as a baseline for recognizing future introductions.

Literature Cited

Allain-Boulé, N.; Tweddell, R.; Mazzola, M.; Bélanger, R.; Lévesque, A.C. 2004. *Pythium attrantheridium* sp. nov.: taxonomy and comparison with related species. Mycological Research. 108(7): 795–805.

Garbelotto, M.; Frankel, S.J.; Scanu, B. 2018. Soil- and waterborne *Phytophthora* species linked to recent outbreaks in Northern California restoration sites. California Agriculture. 72: 208-216.

Packer, A. and Clay, K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature. 404: 278–281.

Redekar, N.R.; Eberhart, J.E.; Parke, J.L. 2019. Diversity of *Phytophthora, Pythium*, and *Phytopythium* species in recycled irrigation water in a container nursery. Phytobiomes Journal. 3: 31-45.

Comparative Epidemiology of NA1 and EU1 *Phytophthora ramorum* Isolates from Curry County, OR[1]

Ebba Peterson,[2] Jennifer Parke,[2,3] and Sarah Navarro[4]

Abstract

The 2015 detection of the *Phytophthora ramorum* EU1 lineage in Oregon forests poses a new threat to sudden oak death management in Curry County. EU1 may be more aggressive and spread at a faster rate than has been observed for NA1 over the 17 years it has been managed in Oregon forests. EU1 may also infect some hosts, notably conifers, at a greater frequency. To assess any additional risk posed by EU1, we performed field surveys assessing the distribution and frequency of understory infection surrounding SOD-infested trees. We also conducted laboratory assays testing for epidemiologically relevant differences between Curry County NA1 and EU1 isolates.

To determine if the EU1-infested sites were larger upon detection, or if EU1 was infecting hosts at a greater rate, we established transects 20 m uphill, downhill and perpendicular to a confirmed, SOD-infected tanoak tree presumed to be the primary inoculum source contributing to understory infection at a site (7 sites per lineage). In 5 m^2 blocks we recorded the presence of understory hosts and collected samples for plating in selective media to confirm infection by *P. ramorum.* Recovery of *P. ramorum* from understory vegetation declined with distance from the primary source of inoculum in both EU1 and NA1 sites. EU1 sites were the same size as NA1 sites upon detection, having similar disease incidence at a given distance from the site center (Wilcoxon rank-sum test; $p = 0.38$). Tanoak was abundant and was the most commonly infected host at both NA1 and EU1 sites. There was no difference between the recovery rates of either lineage for all hosts (Pearson's test for independence at $\alpha = 0.05$). *P. ramorum* was not recovered from conifers.

To complement field studies investigating rates of spread of the NA1 and EU1 lineages, we tested for epidemiologically-relevant differences between Curry County NA1 and EU1 isolates, with the following objectives:

1) Assess variation in isolate aggressiveness.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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2) Determine sporangial concentrations needed for 50% disease incidence on epidemiologically important hosts and conifers as an indication of greater dispersal capacity or infection rates.
3) Determine optimum temperatures for sporulation from rhododendron as an indication of potential for earlier sporulation.

For objective 1, we artificially inoculated tanoak, *Notholithocarpus densiflorus,* stems and rhododendron and bay laurel, *Umbellularia californica,* leaves with mycelial plugs. Leaves and stems were incubated at 20 °C for 7 days before measuring lesion area (leaves) or length (stems). Consistent with prior studies, EU1 isolates were, on average, more aggressive on rhododendron, however some isolates were notably less or more aggressive than others within their lineage. Lesion size on rhododendron was positively correlated with lesion size on bay laurel leaves and tanoak stems.

For objective 2, non-wounded leaves, tanoak sprouts, or conifer seedling-tips (3 per host per concentration) were dipped tip-down into mixed-isolate sporangial suspensions diluted to various concentrations. These were then incubated for 7 weeks prior to plating in selective media to confirm infection. Two sets of assays were performed: one in the spring using Douglas-fir (*Pseudotsuga menziesii*), Japanese larch (*Larix kaempferi*), and rhododendron; and one in the summer using tanoak, bay laurel, and rhododendron. There was no difference between the two lineages in the number of sporangia needed to cause 50% disease incidence on tanoak, bay laurel, or rhododendron. High infection rates of Japanese larch and rhododendron were observed at relatively low concentrations in the spring; greater concentrations are needed for infection on Douglas-fir, though results were too variable to analyze. Preliminary results of a new assay indicate no difference between the two lineages in the number of zoospores needed to produce infection on conifers.

For objective 3, plug-inoculated rhododendron leaves were incubated for 7 days at 20 °C to allow for infection establishment. Leaves were then rinsed and placed in growth chambers set between 4 and 20 °C for one week. Sporangia were rinsed, filtered and counted; the leaves were scanned and lesion area was assessed. Lowest sporulation was observed at 4 and 20° C, with greatest sporulation being observed at 8 °C for some isolates. On average, EU1 isolates sporulated at cooler temperatures, however this was highly dependent upon the isolate used.

Overall, greater lesion sizes and greater capacity to produce sporangia at lower temperatures indicates the EU1 lineage may pose a greater threat to Oregon forests, however some isolates performed more similarly to the opposing lineage.

Distribution of *Phytophthora quercina* and Other Oak-root *Phytophthora* Pathogens in the Midpeninsula Regional Open Space District[1]

Ebba Peterson,[2] Joyce Eberhart,[3] Neelam Redekar,[3] Jennifer Parke,[2,3] and Amanda Mills[4]

Abstract

Surveys of native wildlands worldwide to determine *Phytophthora* diversity have found a surprisingly large assortment of root disease-causing species, many of which may contribute to the phenomenon of oak decline. Many species of concern, for example *P. cinnamomi*, are widely distributed throughout the San Francisco Bay Area. Others, notably *P. quercina* and *P. uliginosa*, may additionally contribute to oak decline in Europe (Jung and others 1999, 2002), but are not thought to be widely distributed in the western United States.

To determine *Phytophthora* diversity and distribution in the Midpeninsula Regional Open Space District (MROSD), we collected soil from 30 planted restoration sites, 12 planned restoration sites and 29 adjacent, minimally disturbed non-planted areas in December 2017 and 2018. In addition to baiting, we extracted DNA from a 10 g subsample of each soil. The ITS1 region was amplified and PCR products were submitted for Illumina MiSeq high-throughput sequencing. During the 2018 sampling, we additionally returned and re-sampled sites with strong DNA-only detections of the *P. quercina*-cluster (which may be *P. quercina* and/or *P. versiformis*) and the *P. uliginosa*-cluster (which may be either *P. uliginosa* and/or *P. europaea*) in an attempt to bait these species from soils.

Phytophthora was detected at all 9 MROSD preserves sampled. The *P. quercina*-cluster and the *P. uliginosa*-cluster were widespread, being detected via Illumina MiSeq in either 6 or 5 preserves, respectively. Nearly all detections were from non-planted areas, found in association with overstory oak or tanoak.

We were unable to obtain any isolates matching *P. quercina* or closely related species. To confirm the identity of *P. quercina* in the DNA extracts, we additionally sequenced these extracts

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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with the MinION sequencer, which provides longer (1,000 bp) read lengths. This revealed this OTU was an approximate 90% match to the *P. quercina*-cluster and likely represents a taxon not present in our database. In 2018, we recovered three isolates from two preserves with ITS1 sequences poorly matching to *P. europaea.* Subsequent sequencing of the COX region revealed these isolates are *P.* sp. 'cadmea' which was only recently recovered by Bourret (2018) in a neighboring county. This new taxon has not been evaluated for its risk to native flora.

Illumina MiSeq high-throughput sequencing is a useful tool to study the distribution of hard to bait taxa; however, DNA-only detections are difficult to interpret without isolates to confirm their identity, viability, and pathogenicity.

Literature Cited

Bourret, T. **2018**. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD dissertation. UC Davis.

Jung, T.; Hansen, E.M; Winton, L; Oswald, W. Delatour, C. **2002**. Three new species of *Phytophthora* from European oak forests. Mycological Research. 106(4): 397-411.

Jung, T.; Cooke, C.E.L.; Blaschke, H.; Duncan, J.M. and Oßwald, W. 1**999**. *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycological Research. 103(7): 785-798.

Phytophthora Diversity in Lake Mathews, the Irrigation Water Source for a Southern California Nursery[1]

Neelam Redekar,[2] Joyce Eberhart,[2] and Jennifer Parke[2,3]

Abstract

Lake Mathews is a 182,000 acre-feet capacity reservoir located in Riverside County, California. It is the western terminus of the Colorado River Aqueduct that serves as the main source of irrigation water for several horticultural nurseries in southern California. Some of these nurseries do not disinfest Lake Mathews water before using it for irrigation. This could increase disease pressure at these nurseries and facilitate survival, propagation and spread of important waterborne plant pathogens such as *Phytophthora* spp. on infested nursery stock.

We examined the diversity of *Phytophthora* species detected in this water over the span of 30 months to determine what risks it poses to the container nurseries that use this water source for irrigation. Metabarcoding approach: We periodically collected two 1-L water samples from Lake Mathews as supplied by the Western Municipal Water District to a large container nursery in southern California. One set of samples was filtered through 5µm Millipore nylon membranes to physically capture all *Phytophthora* species present in the water. Another set of samples was baited using rhododendron leaves to detect viable *Phytophthora* species in the water. In both cases, the ITS1 region was first amplified from the filter and leaf bait DNA using ITS6 and ITS7 primers (Cooke and others 2000), and then sequenced with high-throughput Illumina MiSeq 250PE sequencing for metabarcoding. The paired-end Illumina sequences were first cleaned, quality filtered and queried against a custom oomycete reference ITS database (Redekar and others 2019) using a nucleotide megablast search. Operational taxonomic units (OTUs) or taxa were identified based on percent sequence similarity to the reference sequence of known *Phytophthora* species.

In some cases, the Illumina MiSeq sequencing approach was incapable of differentiating between sequences of closely related species. Such closely related species were either classified as species complex or cluster, depending whether they share sequence identity across an entire ITS1 region, or across a shorter amplified ITS1 region, respectively.

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[1] A version of this paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Twenty-two *Phytophthora* taxa were detected on filters; of these, 9 taxa colonized leaf baits (Figure 1). The ITS1 sequences originating from this study showed over 99% similarity to some of the important *Phytophthora* taxa including: *P. lateralis, P. citricola*-complex, *P. capsici*-cluster, *P. cryptogea*-complex, *P. citrophthora*-cluster, *P. tropicalis*, and *P. amaranthi.* We also detected ITS1 sequences that matched *P. ramorum* and *P. kernoviae*. Species-specific quantitative real time PCR assays (Bilodeau and others 2014) confirmed them to be similar to but not identical to *P. ramorum* and *P. kernoviae*, respectively. There were no seasonal trends in the occurrence of *Phytophthora* species detected in Lake Mathews water. Detection of most species was limited to a particular sampling time, where it was detected in greater abundance.

Lake Mathews water harbors plant pathogenic *Phytophthora* species that could pose disease risks at the nursery. It should be disinfested before use in irrigation. The metabarcoding approach allowed detection of *Phytophthora* species that were not recovered by baiting. Illumina MiSeq amplicon sequencing technology is very effective and sensitive for describing community composition, however its shorter sequences do not permit differentiation between closely-related species in some cases, resulting in unresolved species complexes or clusters.

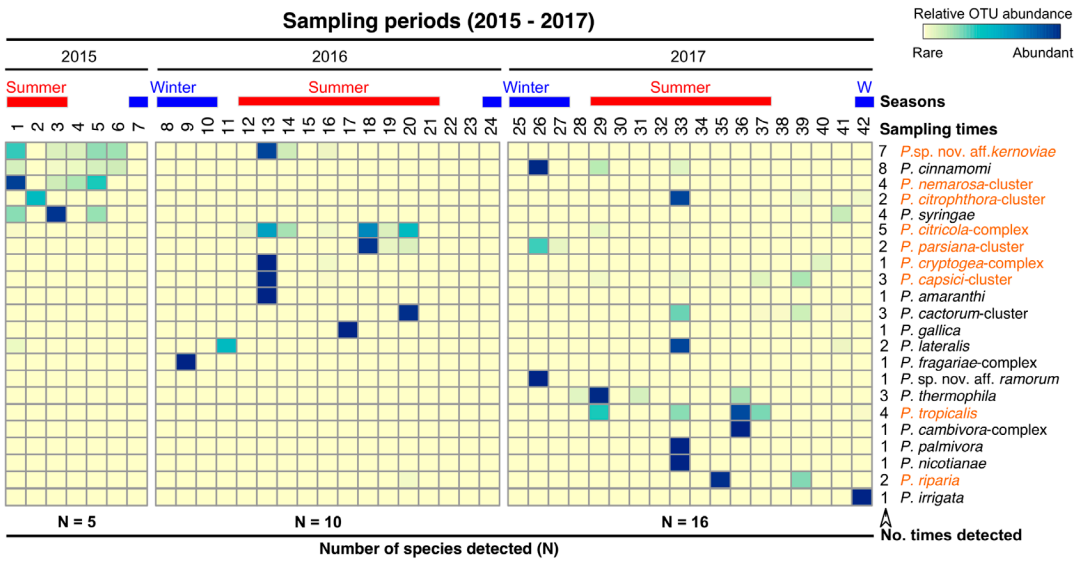

Figure 1 – Detection of Phytophthora in Lake Mathews water with metabarcoding. Species also detected on rhododendron leaf baits are indicated in orange.

Literature Cited

Cooke, D.E.; Drenth, A.; Duncan, J.M.; Wagels, G.; Brasier, C.M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genetics and Biology. 30: 17-32.

Redekar, N.R.; Eberhart, J.L.; Parke, J.L. 2019. Diversity of *Phytophthora*, *Pythium*, and *Phytopythium* species in recycled irrigation water in a container nursery. Phytobiomes. 3: 31- 45.

Bilodeau, G.J.; Martin, F.N.; Coffey, M.D.; Blomquist, C.L. 2014. Development of a multiplex assay for genus- and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order. Phytopathology. 104: 733-74.

Is Sudden Oak Death Becoming a Threat to California's Chaparral Ecosystem? First Indications for *Phytophthora ramorum* Moving into Drier and Warmer Habitats[1]

Wolfgang Schweigkofler,[2] Tomas Pastalka,[2] Karen Suslow,[2] Tina Popenuck,[3] and Matteo Garbelotto[3]

Abstract

Since its introduction into California, *Phytophthora ramorum* was found predominately on a rather narrow band along the coast characterized by mild temperatures and abundant year-long moisture (in the 'fog belt'). The presence of foliar hosts, especially California bay laurel (*Umbellularia californica*), common in this ecosystem, is an essential driver for the spread of the disease to 'dead-end hosts', such as coast live oak (*Quercus agrifolia*). Recently, *P. ramorum* was detected on several plants typical for the chaparral plant community (manzanita, *Arctostaphylos spp.;* chaparral pea, *Pickeringia montana*) on a high, sun-exposed ridge in Marin County (Rooney-Latham and others 2017). During 2018, a severe outbreak of disease was observed on chaparral plants on Mt. Tamalpais in Marin Co., with symptoms including wilting, branch dieback and occasionally plant death. Leaves and branches of several plants showed a positive reaction for *Phytophthora spp*. using immuno-strips; and *P. ramorum* was detected using PCR from a manzanita stem. In addition, *Neofusicoccum australe* (Botryosphaeriaceae) was isolated from a symptomatic plant. The infested area is on a southern slope with no apparent presence of California bay laurel or tanoak (*Notholithocarpus densiflorus*). Potted rhododendron plants were placed near symptomatic plants on Mt. Tamalpais to monitor the possible spread of airborne inoculum during winter 2018/19 and the effect of environmental parameters such as rainfall on the timing and appearance of disease symptoms. Inoculation experiments using *P. ramorum* on several *Arctostaphylos* species are on-going. While it is still unclear whether the observed symptoms are caused by a disease complex, and which role *P. ramorum* has in it, mounting evidence indicates that *P. ramorum* is expanding its host range and moving into new environments.

Literature Cited

Rooney-Latham, S.; Blomquist, C.L.; Williams, A.; Gunnison, E.; Pastalka, T. 2017. Identification of five new hosts of *Phytophthora ramorum* in an infested forest in California. In: Frankel, S.J.; Harrell, K.M., tech. coords. Proceedings of the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Pages 83-84.

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[1]A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Susceptibility of Canadian Flora to EU2 Lineage of *Phytophthora ramorum* and Pathogen Sporulation Potential[1]

Simon Francis Shamoun,[2] Grace Sumampong,[2] Robert Kowbel,[2] Katherine Bernier,[2] Marianne Elliott,[3] Danny Rioux,[4] Martine Blais,[4] and Alexandra Schlenzig[5]

Abstract

Phytophthora ramorum is an oomycete pathogen and causal agent of a disease commonly referred to as sudden oak death (SOD). The pathogen also causes foliar blight and shoot dieback of nursery plants, including *Rhododendron* and *Viburnum*. It is responsible for the widespread mortality of tanoak (*Notholithocarpus densiflorus)* and coast live oak (*Quercus agrifolia*) in coastal California and southwestern Oregon, as well as Japanese larch (*Larix kaempferi*) in the U.K. Thirty-three plant host species commonly found in eastern (8) and western (25) Canadian landscapes and forest sites were selected for this study. Detached leaves/needles were inoculated with *P. ramorum* EU2 lineage mycelia which was isolated from a stream bait near an infected larch plantation in Scotland, U.K. There was a large variation in aggressiveness and sporulation potential among the evaluated hosts. Among the non-conifer species, the EU2 isolate produced the largest lesions on Pacific dogwood (*Cornus nuttallii), Camellia japonica* (western species); red oak (*Quercus rubra*), yellow birch (*Betula alleghaniensis*), and white ash (*Fraxinus americana*) (eastern species). For conifer hosts, we found that the EU2 isolate was most aggressive on both balsam fir (*Abies balsamea*) in the east and grand fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), and western larch (*Larix occidentalis*) in the west. As for sporulation potential, red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllum*) in the west produced significantly more sporangia than California bay laurel (*Umbellularia californica*). Sugar maple (*Acer saccharum)* in the east was a potential spore producer but not significantly different from California bay laurel. Among the conifer species, western hemlock needles in the west were asymptomatic but produced a small amount of sporangia. The conifer host that produced the most sporangia/mm^2 lesion area was white spruce (*Picea glauca*) in the east and Sitka spruce (*Picea sitchensis*) in the west, which produced significantly more sporangia than

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[1] A version of this paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27 2019, San Francisco, California.

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California bay laurel in trial 1 but not in trial 2. Lesion area was biggest on grand fir (*Abies grandis*), balsam fir, and western larch. These results confirm the potential threat of EU2 lineage of *P. ramorum* to Canadian flora.

Introduction

Phytophthora ramorum is an oomycete pathogen and causal agent of a disease, commonly referred to as sudden oak death (SOD). The pathogen also causes foliar blight and shoot dieback of nursery plants, including *Rhododendron* and *Viburnum*. The pathogen is responsible for the widespread mortality of tanoak and coast live oak in coastal California and southwestern Oregon, USA, as well as, Japanese larch in the U.K. There are four distinct clonal lineages of *P. ramorum*, one originally discovered in Europe, but also detected in nurseries and one forest location in western North America (EU1), a lineage recently detected in Europe (EU2), and two lineages present in North America (NA1 and NA2) (Shamoun and others 2018). The host range of *P. ramorum* is very broad (more than 120 host plants) (Shamoun and others 2018). Many of the host species are present in forested and urban areas in the west coast of the US and Canada. To better assess the risk posed by an exotic pathogen such as *P. ramorum*, it is often a good strategy to evaluate its capacity to infect plants prevalent in the area of interest. This approach has been used with success where potential hosts were identified by artificial infections before being found naturally infected by *P. ramorum*. For instance, *Kalmia latifolia* was first identified as highly susceptible to *P. ramorum* under laboratory conditions (Tooley and others 2004) and was thereafter found as a host in the U.K. [(DEFRA 2008, Plant Health Portal. www.defra.gov.U.K./planth/pra/sudd.pdf]. We published similar results when a larch species (*Larix laricina*) was found susceptible for the first time after artificial inoculations (Jinek and others 2008) before another larch (*L. kaempferi*) was reported heavily infected in plantations in the U.K. in 2009 (Webber and Brasier 2010, Webber and others 2010) (figs. 1 and 2).
In the U.K., the known distribution of the EU2 lineage is limited to southwest Scotland and Northern Ireland (NI) (King and others 2015). The EU2 lineage is almost exclusively the only lineage of *P. ramorum* in NI. The EU2 lineage has not been found in England and Wales. Only the EU1 lineage is present there (fig. 3). The objectives of the present study are to determine the susceptibility of selected Canadian flora to EU2 lineage, investigate pathogen sporulation potential, and its threat to Canadian flora as well as its impact on the nursery industry and on forest ecosystems.

Figure 1 (left) —Needle symptoms of *P. ramorum* infection on Japanese larch (Courtesy: Mick Biddle, U.K. Forestry Commission). Figure 2 (right) —Crimson staining around *P. ramorum* infection of Japanese larch stem. Resin on the bark indicates stem infection (Courtesy: Mick Biddle, UK Forestry Commission).

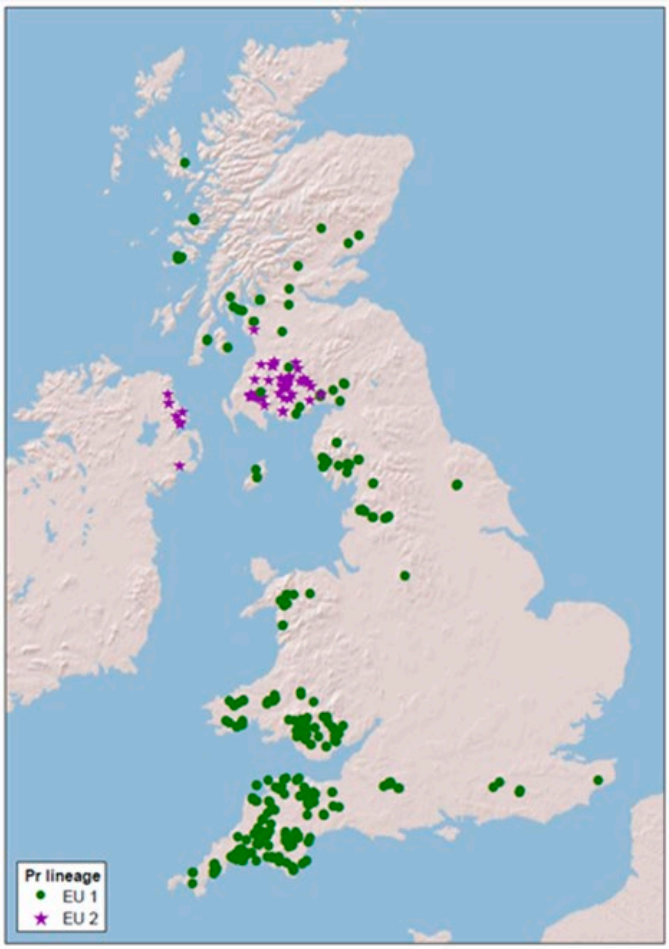

Figure 3 — Distribution map of *P. ramorum* lineages EU1 and EU2 in the U.K. (Courtesy: Dr. Joan Webber, U.K. Forestry Research).

Materials and Methods

Plant Species Tested

Thirty-three species of Canadian plants were selected from eastern and western Canada (tables 1 and 2). Mature, fully expanded foliage was collected in June 16 and 22, 2015, and July 11 and 25, 2016. Every attempt was made to collect from the same host plant to reduce variability due to host genetics and other factors.

Culture and Detached Leaves /Needles Inoculations

Detached leaves/needles representing eastern and western Canadian regions (tables 1 and 2) were inoculated with mycelia from a single isolate of *P. ramorum* EU2 (PFC5414), acquired under Canadian Food Inspection Agency (CFIA) Permit #P-2013-03068, from Dr. Alexandra Schlenzig (Scottish Agriculture and Rural Delivery Directorate, U.K.). This isolate was recovered from a stream bait near an infected larch plantation in 2012. Inoculation methods, assessment of lesion area and sporulation potential were conducted using the methods of Shamoun and others 2017, and the modified protocol of Harris and Webber 2016, respectively.

Table 1—Canadian host species collected from the Western Canadian region.

Scientific name	Common name
Arbutus menziesii	Madrone
Quercus garryana	Garry oak
Acer macrophyllum	Bigleaf maple
Alnus rubra	Red alder
Populus trichocarpa	Poplar
Cronus nuttallii	Pacific dogwood
Betula papyrifera	Paper birch
Camellia japonica	Camellia
Gaultheria shallon	Salal
Mahonia nervosa	Oregon grape
Rhododendron caucasicum	Rhododendron
Ribes spp.	Currant
Rubus discolor	Himalayan blackberry
Arctostaphylos spp.	Manzanita
Umbellularia californica	California bay laurel
Rubus idaeus	Raspberry
Vaccinium corymbosum	Blueberry
Vitus vinifera	Grape
Tsuga heterophylla	Western hemlock
Pinus contorta	Lodgepole pine
Larix occidentale	Western larch
Pseudotsuga menziesii	Douglas fir
Picea sitchensis	Sitka spruce
Abies grandis	Grand fir
Thuja plicata	Western red cedar

Table 2—Canadian host species collected from the Eastern Canadian region.

Scientific name	Common name
Betula alleghaniensis	Yellow birch
Acer saccharum	Sugar maple
Quercus rubra	Red oak
Fraxinus Americana	White ash
Gaultheria procumbens	Wintergreen
Rhus typhina	Sumac
Abies balsamea	Balsam fir
Picea glauca	White spruce

Healthy looking leaves and needles were collected, rinsed in sterile distilled water (sdH2O) twice and blotted with a paper towel to remove excess moisture prior to wounding and inoculation with an agar plug. Ten leaves were wounded next to the midrib using forceps, whereas conifer needles were cut at their base with a surface-sterilized scissor. After wounding, a 5 mm plug of *P. ramorum* EU2 lineage inoculum or blank V8 agar plug was placed mycelium side down over the wounded area on the abaxial side of the leaf or on three needles joined together at their base. After 10 days, leaves were photographed on a scanner and lesion size on each leaf caused by *P. ramorum* EU2 lineage was measured using ASSESS software (Lamari 2002) or measured with a ruler for needles. Lesion area was adjusted for the lesion caused by wounding in the blank (no inoculum) treatments and considered to be zero if the lesion is equal to or less than that caused by wounding alone.

Sporulation

The inoculated area of the leaf or needle surface and any visible necrosis beyond this was gently scraped with a rounded scalpel blade to free all sporangia from the same side where the leaf had been inoculated. A 200 uL droplet of sdH_2O was placed on the inoculated area to suspend the scrapings and then transferred to a 1.5 mL microtube to which 5 uL cotton blue (lactophenol blue) was added. Microtubes were centrifuged for 10 min at 6000 g at 4 °C. The total number of sporangia was counted in a pellet resuspended in 20 uL sdH_2O under the microscope.

Data Analysis

Trials 1 and 2 were analyzed separately since the homogeneity of variance test failed. T-tests between lesion area on inoculated and water-inoculated foliage were done for each host. A host was considered to be asymptomatically infected if $p > 0.05$. There was sufficient power to do 1-way ANOVA on lesion area and sporangia/unit lesion area. When ANOVA was significant Tukey's HSD and Dunnett's post-hoc tests were done. Lesion area was evaluated on foliage in five size class categories (table 3), since differences in lesion area relative to leaf area can be confounding. Sporangia per unit lesion area was analyzed for broadleaf and conifer hosts separately.

Table 3 —Classification of foliar hosts by relative leaf area.

Group	Hosts
Group A: Conifer needles	White spruce, western larch, Sitka spruce, Douglas fir, balsam fir, grand fir, lodgepole pine
Group B: Small size broadleaf	Western red cedar, wintergreen, manzanita
Group C: Medium size broadleaf	Currant, paper birch, Oregon grape, blueberry
Group D: Large size broadleaf	Sugar maple, Camellia, Sumac, Garry oak, rhododendron, yellow birch, salal, Himalayan blackberry, raspberry, madrone, white ash, Pacific dogwood, red alder, red oak
Group E: Extra large size broadleaf	Bigleaf maple, poplar, grape

Results and Discussion

There was a large variation in aggressiveness and sporulation potential among the evaluated hosts. Among the non-conifer species, the EU2 isolate produced the largest lesions on Pacific dogwood, (*Cornus nuttallii),* and *Camellia japonica*, on the western species (fig. 4); and red oak (*Quercus rubra)*, yellow birch (*Betula alleghaniensis)*, and white ash (*Fraxinus americana*) on the eastern species (fig. 5). For conifer hosts, we found that the EU2 isolate was most aggressive on balsam fir (*Abies balsamea*) (eastern species) and grand fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), and western larch (*Larix occidentalis*) (western species). As for sporulation potential, red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllum*) (west species) produced significantly more sporangia than California bay laurel (*Umbellularia californica*). Sugar maple (*Acer saccharum)* (eastern species) was a potential spore producer but not significantly different from California bay laurel. For the sporangia per unit lesion area, Himalayan blackberry (*Rubus discolor*), raspberry (*Rubus idaeus*), and Garry oak (*Quercus garryana*) were significantly higher than California bay laurel, although both Himalayan blackberry and raspberry were asymptomatic. These results confirm the potential threat of EU2 lineage to Canadian flora.

Foliar hosts were classified by relative leaf area into five groups and these groups were analyzed separately (table 3).

Group A, Conifer needles: Western hemlock needles were asymptomatic but produced a small amount of sporangia. The conifer hosts that produced the most sporangia/mm² lesion area were Sitka spruce and white spruce, which produced significantly more sporangia than California bay laurel in trial 1 but not in trial 2. Lesion area was biggest on grand fir, balsam fir and western larch.

Group B, Small size broadleaf: This group included manzanita and wintergreen, both evergreen broadleaf plants. Lesion area was similar for both hosts, which tended to cover approximately 50% of the leaf area on average (data not shown). Both hosts produced fewer sporangia than California bay laurel. Western red cedar was in this size class but was asymptomatic. Sporangia production was highly variable on western red cedar and ranged from 0-157 sporangia/mm².

Group C, Medium size broadleaf: Blueberry and Oregon grape had smaller lesion area than paper birch and currant. The only host that produced abundant sporangia per mm² lesion area was Oregon grape, but was not significantly more than California bay laurel.

Group D, Large size broadleaf: The largest lesions were formed on yellow birch, which had similar sporangia/lesion area to California bay laurel. The average total sporangia per lesion produced by yellow birch was 21 and California bay laurel 186, respectively.

Group E, Extra-large size broadleaf: Lesion area on these three hosts tended to be small, but significantly more sporangia were produced on big leaf maple when compared to California bay laurel.

Figures 6 and 7 show results for lesion area of broadleaf foliage and conifer needles hosts inoculated with the EU2 lineage of *P. ramorum*, respectively. Figures 8 and 9, represent sporulation potential of EU2 lineage of *P. ramorum* on broadleaf foliage and conifer needles hosts, respectively.

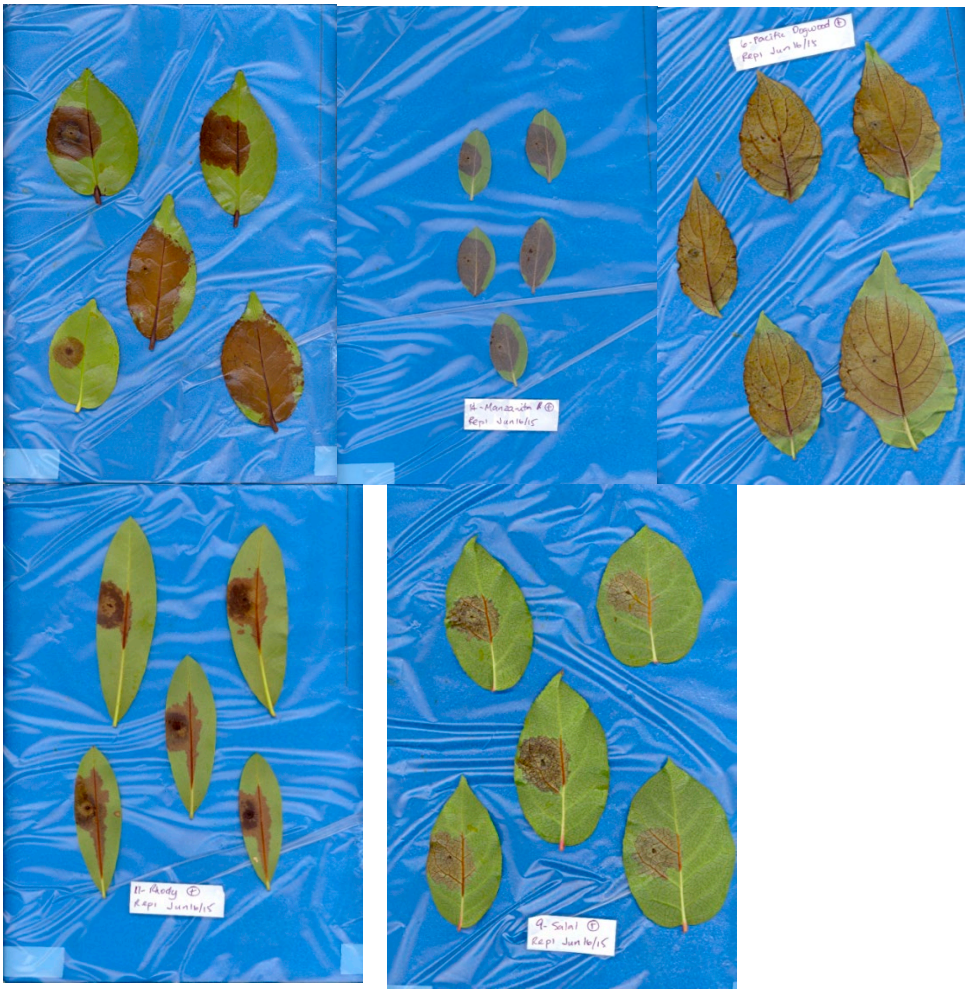

Figure 4 — Lesions formed by *P. ramorum* EU2 lineage on detached leaves of highly susceptible Western Canadian hosts. The upper 3 photos from left to right are camellia, manzanita, and Pacific dogwood. The bottom two photos from left to right are rhododendron and salal.

Figure 5 — Lesions formed by *P. ramorum* EU2 lineage on detached leaves of susceptible Eastern Canadian hosts: from left to right: white ash, wintergreen, and yellow birch.

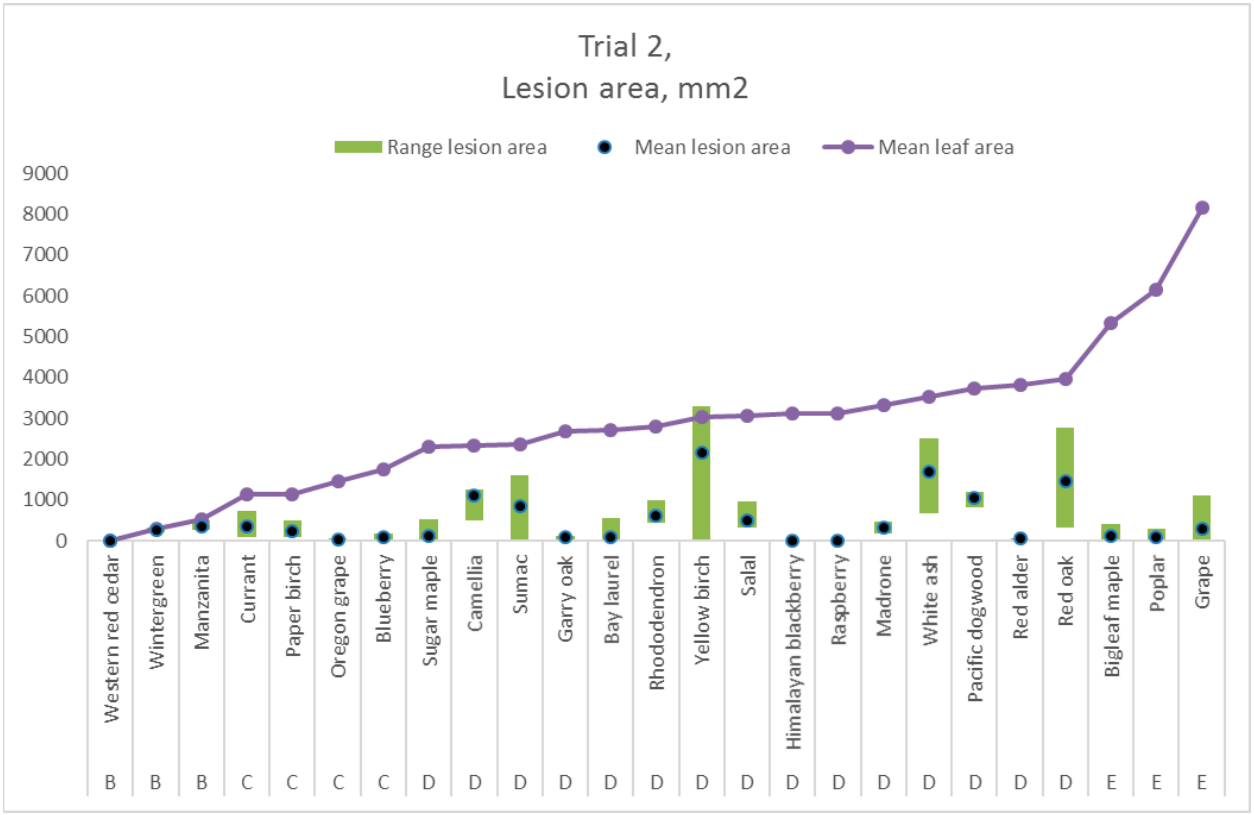

Figure 6 — Lesion area measured on broadleaf hosts inoculated with EU2 lineage of *P. ramorum*. Host foliage was separated into classes depending on total leaf area.

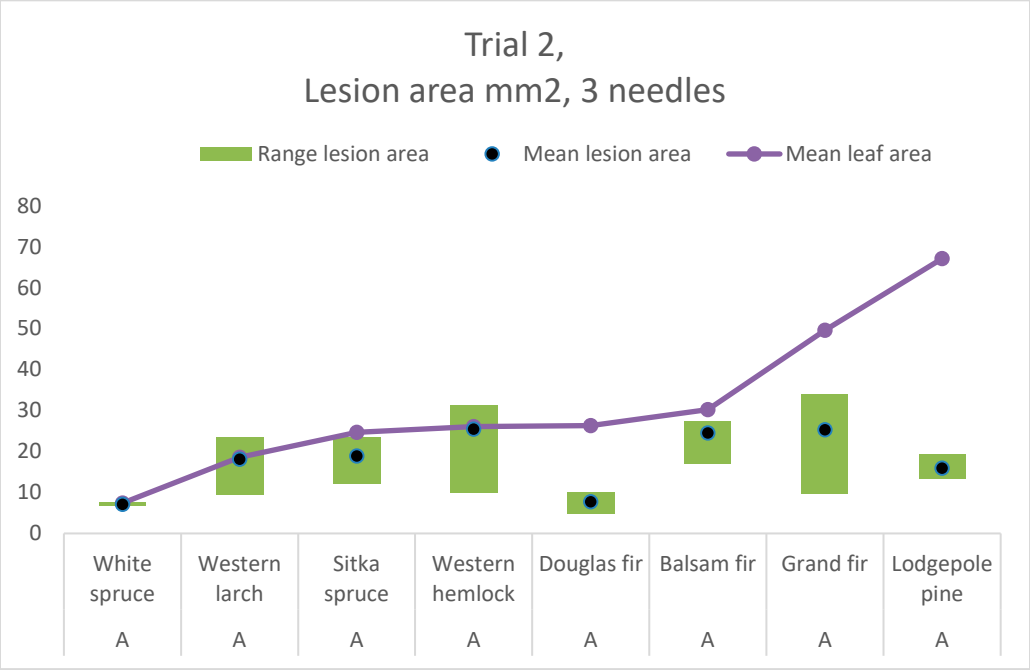

Figure 7 — Lesion area measured on conifer needles hosts inoculated with EU2 lineage of *P. ramorum*. Host foliage was separated into classes depending on total leaf area.

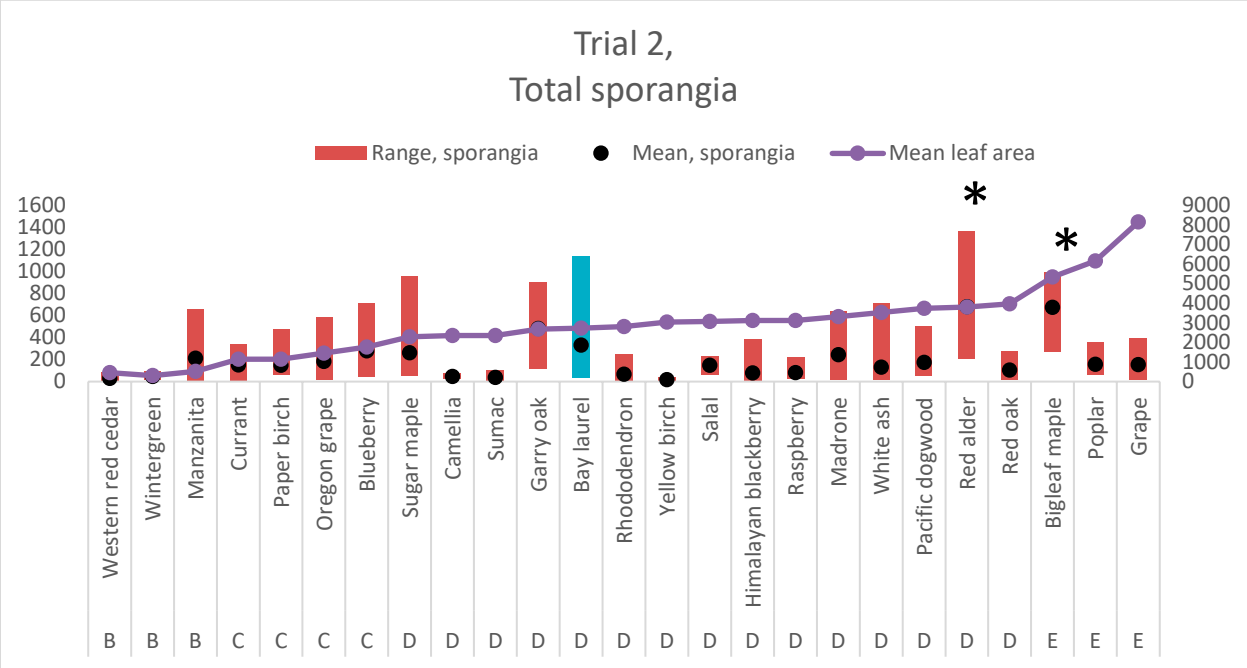

Figure 8 — Sporulation potential of EU2 lineage of *P. ramorum* on Canadian broadleaf host plants as determined by the total number of sporangia per lesion.

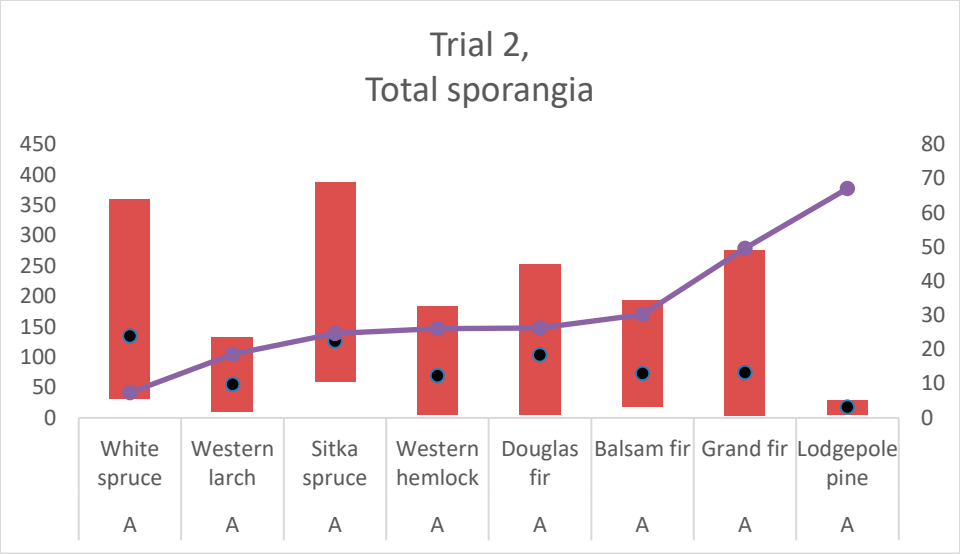

Figure 9 — Sporulation potential of EU2 lineage of *P. ramorum* on Canadian conifer hosts needles as determined by the total number of sporangia per lesion.

These results extend the known potential host range for *P. ramorum* EU2 lineage. The detached, *in vitro*, leaf/needle inoculation method of Elliott and others (2011) was used in the current investigation, as this method and other published SOD research work elsewhere are well established and applied as an approved APHIS/CFIA protocols for testing *P. ramorum* host range throughout North America (O'Hanlon and others 2017, Tooley and others 2004). The natural hosts for EU2 lineage include Japanese larch, European larch, hybrid larch, beech, grand fir, noble fir, western hemlock, rhododendron, red oak and *Vaccinium* (King and others 2015, Personal communication Dr Joan Webber). Molecular (Elliott and others 2009, King and others 2015, Van Poucke and others 2012) and phenotypic (Elliott and others 2011, Franceschini and others 2014, O'Hanlon and others 2017) characterizations and protocols have been designed to objectively discriminate the *P. ramorum* lineages, and these should be made routine for national and international regulatory agencies (e.g., APHIS, CFIA) and plant protection organizations in order to identify if different lineages are spreading.

Previous studies reported the EU2 lineage from Japanese larch, bilberry (*Vaccinium myrtillus*), rhododendron and non-native oak in the U.K. Furthermore, several scientists have provided the first confirmed findings of natural infection by EU2 lineage on European larch, hybrid larch, beech, noble fir and western hemlock (Franceschini and others 2014, King and others 2015, Van Poucke and others 2012). In our present study we have confirmed additional Canadian host plants to the potential host range for *P. ramorum* EU2 lineage. These observations suggest that EU2, like EU1 and NA2, is able to colonize a wide range of host species that may contribute to an increased risk of EU2 spread. Moreover, comparative experiments have shown that the EU2 lineage is more aggressive than EU1 lineage at colonizing larch bark tissue and is therefore likely to kill affected trees more rapidly (McCracken and others 2015, Webber and others 2014). Given these findings on *Larix*, prioritization of the eradication of EU2 lineage may be justified in order to contain the epidemic and protect forest/plant health ecosystems in the U.K. In addition,

our study confirms the potential threat of the EU2 lineage of *P. ramorum* if it becomes established in Canadian nurseries and wildlands.

A draft genome assembly for the *P. ramorum* EU2 lineage has been collected from isolates from outbreak sites in Scotland (Sambles and others 2015). This information will enhance our understanding of the infection biology of the pathogen. Also, it will assist researchers worldwide in accelerating our knowledge of relationships between the four known lineages of *P. ramorum* (NA1, NA2, EU1 and EU2), as well as, the development of molecular diagnostic assays for detection and field monitoring of the EU2 lineage (King and others 2015, Sambles and others 2015). Furthermore, EU2 lineage will have a potential impact on the Canadian horticultural industry, biodiversity, and sustainability of forest ecosystems. Ongoing research is focused on further evaluation of sporulation potential of the EU2 lineage of *P. ramorum* on a subset of selected Canadian flora. The Canadian Forest Service mandate is to monitor the status of the EU2 lineage in the U.K. and work closely with the CFIA to update the existing Canadian Pest Risk Assessment (PRA) to address new relevant *P. ramorum* information as it arises.

Conclusions

1. For broadleaf species, Pacific dogwood and camellia, in the west; and sumac, yellow birch, red oak, and white ash in the east, were the Canadian flora most susceptible to infection by the EU2 lineage.

2. For conifer hosts, we found both balsam fir in the east and grand fir, western hemlock, and western larch in the west to be the most susceptible to EU2 lineage infection.

3. These results extend the known potential host range of the EU2 lineage of *P. ramorum;* the known host range includes Japanese larch, grand fir, noble fir, rhododendron, red oak and *Vaccinium* in the U.K.

4. There was high variability in sporulation potential within and among hosts. Ongoing research is focused on further evaluation of sporulation potential of the EU2 lineage on Canadian flora (i.e., to discover the "spore pump" host or hosts). Preliminary results indicate that maples and spruces have high sporulation potential and these groups will be investigated further.

5. The Canadian Forest Service is closely monitoring the status of the EU2 lineage in the U.K. and working with the CFIA to update the existing Canadian Pest Risk Assessment (PRA) and address new relevant *P. ramorum* information as it arises.

Acknowledgments

We acknowledge the financial support for this project by Natural Resources Canada, Canadian Forest Service, Forest Pest Risk Management- Forest Invasive Alien Species (FIAS) Program. The authors are grateful to Dr. Joan Webber, Principal Plant Pathologist and Head, Tree Health, Forest Research, U.K. for providing us with the epidemiology information and distribution maps of *P. ramorum* EU1 & EU2 lineages in the U.K. In addition, we acknowledge Mick Biddle, Tree

Health Officer, Forestry Service, Forestry Commission England for providing images of symptoms of sudden larch death of *P. ramorum* on Japanese larch.

Literature Cited

Elliott, M.; Sumampong, G.; Varga, A.; Shamoun, S.F.; James, D.; Masri, S.; Brière, S.C. and Grünwald, NJ. 2009. PCR-RFLP markers identify three lineages of North American and European populations of *Phytophthora ramorum*. For. Path. 39: 266-278.

Elliott, M.; Sumampong, G.; Varga, A.; Shamoun, S.F.; James, D.; Masri, S. and Grünwald, NJ. 2011. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. For. Path. 41: 7-14.

Franceschini, S.; Webber, J.F.; Sancisi-Frey, S. and Brasier, C.M. 2014. Gene x environment tests discriminate the new EU2 evolutionary lineage of *Phytophthora ramorum* and indicate that it is adaptively different. For. Path. 44: 219-232.

Harris, A.R. and Webber, J.F. 2016. Sporulation potential, symptom expression and detection of *Phytophthora ramorum on larch needles and other foliar hosts. Plant Pathology* 65(9): 1441-1451.

Jinek, A., Simard, M.; Brière, S.C.; Watson, A.K.; Teweddell, R.J. and Rioux, D. 2008. Susceptibility of six eastern Canadian forest species to *Phytophthora ramorum*. Phytopathology 98: S75 (Abstract).

Jinek, A.; Simard, M.; Brière, S.C.; Watson, A.K.; Teweddell, R.J. and Rioux, D. 2011. Foliage susceptibility of six eastern Canadian forest tree species to *Phytophthora ramorum. Canadian Journal of Plant Pathology. 33: 26-37.*

King, K.M.; Harris, A.R. and Webber, J.F. 2015. *In planta detection used to define the distribution of the European lineages of Phytophthora ramorum on larch (Larix) in the U.K. Plant Pathology 64: 1168-1175.*

Lamari, L. 2002. ASSESS 1.0 Image analysis software for plant disease quantification. APS Press, Saint Paul, MN., USA, 125 pp.

McCracken, A.R.; Quinn, L.M.; Wilson, M.A. and Webber, J.F. 2015. The comparative pathogenicity of two *Phytophthora ramorum* lineages, EU1 and EU2, on a range of hosts. In: Sutton, W., Reeser, P. and Hansen, E.M, eds. Proceedings of the 7th Meeting of the International Union of Forest Research Organizations *Phytophthora* in Forests & Natural Ecosystems. Esquel, Argentina: IUFRO Working Party 7– 02-09.

O'Hanlon, R.; Choiseul, J.; Grogan, H. and Brennan, J.M. 2017. *In vitro* characterization of the four lineages of *Phytophthora ramorum.* Eur. J. Plant Path. 147(3): 517-525.

Sambles, C.; Schlenzig, A.; O'Neill, P.; Grant, M. and Studholme, D.J. 2015. Draft genome sequences of *Phytophthora kernoviae* and *Phytophthora ramorum* lineage EU2 from Scotland. Genomics Data. 6: 193-194.

Shamoun, S.F.; Sumampong, G.; Rioux, D. and Schlenzig. A. 2017. Potential susceptibility of Canadian flora to EU2 lineage of *Phytophthora ramorum*. In: Frankel, S.J.; Harrell, K.M., tech. coords. Proceedings of the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. Pages 91-98.

Shamoun, S.F.; Rioux, D.; Callan, B.; James, D.; Hamelin, R.; Bilodeau, G.; Elliott, M.; Levesque, C.A.; Becker, E.; McKenney, D.; Pedlar, J.; Bailey, K.; Briere, S.; Niquidet, K. and Allen, E. 2018. An overview of Canadian research activities on diseases caused by *Phyophthora ramorum*: Results, progress and challenges. Plant Disease. 102(7): 1218-1233.

Tooley, P.W.; Kyde, K.L. and Englander, L. 2004. Susceptibility of selected Ericaceous ornamental host species to *Phytophthora ramorum.* Plant Disease. 88: 993-999.

Van Poucke, K.; Franceschini, S; Webber, J.F.; Vercauteren, A.; Turner, J.A. and McCracken, AR. 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum* : EU2. Fungal Biology. 126: 1178-1191.

Webber, J. and Brasier, C. 2010. Plant Pathology: sudden larch death. Nature. 466: 825.

Webber, J.F.; Mullet, M.; Brasier, C.M. 2010. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum.* New Disease Reports. 22: 19.

Webber, J.; Turner, J.; Thorpe, G. and McCracken, A. 2014. Differences between genotypes of *Phytophthora ramorum* in the U.K. in relation to risk and disease management. Forest Research final project. Report number FFG1142.

Variation in Susceptibility to the EU1 and NA1 Lineages of *Phytophthora ramorum* among Southern Oregon Tanoak Families[1]

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Abstract

Phytophthora ramorum, the cause of Sudden Oak Death (SOD), is an oomycete pathogen that has invaded coastal California and southern Oregon mixed-hardwood forests. In southern Oregon forests, tanoak (*Notholithocarpus densiflorus*) is the most susceptible species developing lethal stem cankers and sporulating from infected leaves and branches. Two lineages (NA1 and EU1) of *P. ramorum* occur in Oregon forests. The first step in a successful tanoak breeding program is to determine if variation in resistance to these two lineages exists. The objectives of this study are to: (i) characterize the variability in resistance of *N. densiflorus* among families using lesion length; and (ii) determine whether lineage, isolate, family, or their interactions significantly affect variation in lesion length. In a growth chamber experiment approximately 1,000 seedlings from 14 tanoak families were inoculated with 3 isolates of the NA1 lineage and 3 isolates of the EU1 lineage. Stem lesions were measured seven days after inoculation.

Averaged across all tanoak families, there were no significant differences in lesion length between the EU1 and NA1 lineages; however, there were significant differences among the six isolates tested. The averages for each family by isolate combination show an overall pattern of increased average lesion length for EU1 isolates. The majority (89%) of the variation in lesion length is explained by isolate (lineage). The family by isolate (lineage) interaction suggests that more than one isolate may be needed in order to screen for resistance to *P. ramorum.* In addition, for a resistance screening program to be successful, a reliable method to vegetatively propagate tanoak needs to be developed.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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Role of Herbivorous Insects on California Bay Laurel in Sudden Oak Death Disease Dynamics[1]

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Abstract

In California, leaves of California bay laurel (*Umbellularia californica*) are considered the primary naturalized source of inoculum for the devastating forest disease sudden oak death (caused by *Phytophthora ramorum*), and yet this plant and insects associated with its leaves remain understudied. Previous studies have considered the role of insects as synergistic factors on trees that die of *P. ramorum* such as oaks, but none have related disease prevalence to insect presence on bay laurel. Indeed, joint effects of insects and pathogens on plants have been investigated surprisingly rarely, and to our knowledge this is the first such study on bay laurel and *P. ramorum*. Insect attack may prime bay laurel leaves for *P. ramorum* infection by damaging the leaf surface to allow entry of pathogen hyphae. On the other hand, literature on aphids suggests that plant defenses against aphids are similar to those against pathogens. Thus, aphid attack may activate the plant's immune response and suppress susceptibility to a pathogen. In addition, infestation by plant enemies may occur when a plant's defenses are reduced due to environmental stress.

We studied interactions between insects, *P. ramorum,* and bay laurel in a region that has been a hotbed of sudden oak death infection since the early 2000s. In two observational studies, we documented the abundance of scale insects and aphids on bay laurel trees. The first study showed that abundance of the armored scale insect *Aspidiotus nerii* (oleander scale, family Diaspididae) on bay laurel leaves at Fairfield Osborn Preserve related negatively to disease expression of *P. ramorum*. The second study sampled trees across a broad geographic area in eastern Sonoma County. Here, we found that the most abundant insects belonged to the suborder Sternorrhyncha, which includes aphids, scale, whiteflies, and other sessile insects. Across this region, abundance of the California laurel aphid (*Euthoracaphis umbellulariae)* was negatively related to *P. ramorum* disease expression. These studies suggest that plant defense was primed by the insects and that they may have reduced disease levels in nature.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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In spring 2016, we conducted an insect-removal study on trees in multiple localities in Fairfield Osborn Preserve. We compared the progress of insect population change and disease expression for leaves on undisturbed control branches and branches that were subjected to three different removal treatments. The most abundant insects were the same sessile insects that we had discovered in our observational field studies. California laurel aphid and oleander scale abundance increased with increasing leaf age. Aphid abundance showed a negative relationship with pretreatment disease levels at the outset of data collection. Insect abundance on control branches peaked early in the season, and disease expression increased just as insect abundance declined in the third month of the experiment. Throughout the season, control branches showed statistically significantly larger numbers of insects present than branches that underwent insect removal treatments. Data analysis of the season-long relationship between insect abundance and *P. ramorum* prevalence is still underway. Results suggest that insects play a role in dynamics of disease expression in oak woodlands.

Spread the Word, Not the Disease! Sudden Oak Death Outreach and the UC Master Gardener Program of Sonoma County SOD Specialists[1]

Kerry E. Wininger[2]

Abstract

Sonoma County has more sudden oak death (SOD) than any other county in California and the disease is still spreading. At least 163,000 acres of Sonoma County's forests are affected by SOD based on USDA Forest Service aerial surveys, and tanoak (*Notholithocarpus densiflorus*) deaths attributed to the disease in the Northern California Shared Service Area increased almost eight-fold between 2016-2017. SOD Blitz data showed a nine-fold increase in estimated true infection rate of trees in some areas of Sonoma County from 2015-2017 and new outbreaks continued to appear in 2018.

Thanks to funding from the USDA Forest Service, the Sudden Oak Death Outreach Program of Sonoma County provides practical, evidence-based information to a diverse audience. The goal of the program is to understand the impact of SOD in Sonoma County, promote forest health to preserve wildlife habitat and save high value trees, and to prevent spread into disease-free areas via community education and citizen science research. Many homeowners, tree care professionals, and land managers rely upon University of California Cooperative Extension (UCCE) Sonoma's SOD Outreach Program for up-to-date information about disease biology, diagnosis, spread, and management options.

Master Gardener SOD Specialists are volunteer educators who receive specialized training in SOD in order to work with homeowners, community groups, college students, and public parks users. They do so through various educational events such as library presentations, displays at farmers' markets and community festivals, an information desk staffed 35 hours/week, and leading six SOD Blitz events throughout the county each year. The program coordinator, Kerry Wininger, works with landowners, tree-care professionals, tribal groups, educators, and natural resource managers on disease detection and management through site visits, phone and email, and educational meetings. She also provides Master Gardener trainings, supervises interns, and creates visibility in the press, media, and online. The Program Advisor, Steven Swain, supplies expertise in adult education, helps develop workshops, and gives scientific direction to the overall program.

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[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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By empowering knowledgeable and passionate volunteers to interact with the public, the Sudden Oak Death Outreach Program of Sonoma County helps spread the word about SOD to the wider community at a very low cost, helping to mitigate the many impacts of this disease.

A Healthy World and Plants with *Phytophthora*? Multiple Introductions of Tree Pathogens to a Newly Established Woodland[1]

Barnaby Wylder,[2] Mick Biddle,[2] Ana Perez-Sierra,[3] and Joan Webber[3]

Abstract

Established between 1996 and 2010, a site in Dorset was the largest newly-created mainly broadleaved woodland in England. It covered 202 ha (499 acres) and the planted tree species were mostly native, intermixed with small components of non-native species. Historically the site had been managed as farmland for centuries (predominantly grassland but also arable crops), divided by undisturbed hedgerow systems.

The site came to attention in 2011 when the site manager reported dieback of alder and ash (*Fraxinus excelsior*) trees. In an area of around 1 ha, more than 100 ash trees were observed with aerial stem cankers. *Phytophthora syringae* was identified as the causal agent, of which ash was a previously unknown host (Webber and others 2014). During further site visits, grey alder (*Alnus incana*) was observed with dieback and bleeding stem cankers, and both the symptoms and on-site diagnostic tests indicated the causal agent was a root-attacking *Phytophthora* sp. In 2013, laboratory testing identified *Phytophthora siskiyouensis* from the alder stem and root cankers, and also from associated soil samples. It was estimated that 10% of *ca.* 1000 *A. incana* trees planted on the site were affected (Perez-Sierra and others 2015). Follow-up investigations in 2014 which established the distribution of disease also yielded further isolations of *P. siskiyouensis.* Since then, gradual felling of *A. incana* under biosecurity restrictions has been ongoing. The most recent site investigation in 2018 recorded only a very small number of *A. incana* trees remaining, with a few of these still exhibiting symptoms. The decline of affected alder trees has been very gradual, and the site remains the only European record of *P. siskiyouensis*. Other findings from the site include the confirmation of *P. plurivora* from the rhizosphere soil of a healthy common alder (*Alnus glutinosa)*, and *P. cambivora* causing bleeding stem cankers in small-leaved lime (*Tilia cordata*). Following the first UK identification of ash dieback caused by the fungus *Hymenoscyphus fraxineus* in planted ash trees in Leicestershire in 2012, the Dorset site was also found to have accepted a significant proportion of its ash planting material from the same supplier. Subsequent investigations in 2013 concluded

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

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that *H. fraxineus* was likely to have been introduced and causing symptoms on ash trees on the site as early as 2007 - 2008.

Although the site for this new woodland could not be described as pristine undisturbed land, the communities of *Phytophthora* species attacking the recently established trees were varied and even novel. There is a strong likelihood that all were introduced on infected planting stock, which clearly illustrates the importance of careful selection of species, consideration of supply origin and the need for improved biosecurity practices in nurseries supplying planting stock.

Literature Cited

Perez-Sierra, A.; Kalantarzadeh, M.; Sancisi-Frey, S. and Brasier, C.M. 2015. *Phytophthora siskiyouensis* causing stem lesions and cankers on *Alnus incana*. New Disease Reports. 31, 17. dx.doi.org/10.5197/j.2044-0588.2015.031.017.

Webber, J.; Wylder, B.; Harris, A.R. and Brasier, C. 2014. Aerial stem cankers associated with *Phytophthora syringae* on *Fraxinus*: how and why? Page 25, in: Proceedings of the 7th meeting of the IUFRO Working Party S07.02.09: Phytophthoras in forests and natural ecosystems. http://forestphytophthoras.org/sites/default/files/proceedings/IUFRO_Proceedings_2014.pdf.

This publication is available online at www.fs.fed.us/psw/.

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