CALIFORNIA OAK MORTALITY TASK FORCE REPORT

AUGUST 2023

Editors' note: This month's report provides updates on a recent detection of the NA2 lineage of *Phytophthora ramorum* near Port Orford, Oregon; a mitigation response plan in Lincoln City, Oregon; *P. ramorum* in Ireland; and the detection of two new U.S. *P. ramorum* hosts, *Cotoneaster pannosus* and *Cornus capitata*.

MANAGEMENT - OREGON

New NA2 *Phytophthora ramorum* infested site found outside the existing quarantine area near Port Orford, Oregon. A third forested area in Oregon has been confirmed infested with NA2 *P. ramorum* north of the original 515-square mile quarantined portion of Curry County. Infected tanoak, *Notholithocarpus densiflorus,* were reported by an Oregon Department of Forestry stewardship forester near Humbug State Park in Curry County. The site is approximately 1.5 miles south of the existing emergency quarantine boundary around Port Orford, 6 miles south of Port Orford, and 28 miles north of Gold Beach. Samples were tested by state and federal diagnostic laboratories which confirmed the NA2 lineage. In Oregon, the NA2 lineage was first detected on forest trees in 2021 near Port Orford. In Pacific Northwest nurseries NA2 infected plants have been reported since 2005.

The newly discovered infestation is in an undeveloped area near Humbug State Park on property managed by the Oregon Parks and Recreation Department (OPRD). The area is being delimited in preparation for eradication treatment. Surveys, being conducted by Oregon Department of Forestry and the USDA Forest Service, include aerial detection flights, ground-based vegetation surveys and stream monitoring. The area, along with a 3-mile surrounding buffer, has been placed under quarantine OAR 603-052-1230 (2)(d) by the Oregon Department of Agriculture (ODA). An emergency quarantine or an amendment to the existing quarantine did not need to be issued. For more information contact Gabriela Ritokova at Gabriela.Ritokova@odf.oregon.gov.

REGULATIONS - OREGON

***P. ramorum* mitigation response plan in Lincoln City, Oregon.** In spring 2022, *P. ramorum* was confirmed in Lincoln City, Oregon from symptomatic rhododendrons that had been submitted for diagnosis from a private residence. The Oregon Department of Agriculture (ODA) responded by delimiting the area and checking surrounding properties. A small botanic garden in the neighborhood and other neighboring properties were found to also harbor symptomatic plants that tested positive for *P. ramorum*. The infestation is not connected to recently acquired plants; it appears to have been present for many years.

ODA and USDA Animal Plant Health Inspection Service, Plant Protection and Quarantine (APHIS-PPQ) conducted a neighborhood survey in Lincoln City, checking 121 parcels and collecting 217 samples. The neighborhood was divided into three sections, running north to south. In Area 1 to the north, 20 plants, found across 14 lots, were confirmed positive for *P. ramorum*. Area 2 had one positive plant, found next to the botanic garden. No positive plants were detected in the southernmost area, Area 3. Infected *Rhododendron*, *Pieris*, *Prunus,*

Gaultheria (salal) and *Polystichum munitum* (western swordfern) were found to be infected with *P. ramorum*. This marked the first detection of *P. ramorum* on western swordfern. The pathogen has not been detected in any waterways or natural forests. Lincoln City is 201 miles north of the *P. ramorum* quarantine boundary in Curry County.

A public meeting was held June 26th in Lincoln City to inform residents of the actions taken thus far and to present a future workplan. The ODA and Oregon State University Forestry & Natural Resources Extension shared plans to remove the known infected plants, destroy the material, and continue to monitor the area. Recommendations to replant with non-hosts are being shared with local landscapers. At the locations where plants will be removed, cedar chip mulch and *Trichoderma* biocontrol may also be applied to the soil. Funds for the eradication are being acquired from the U.S. Forest Service, Pacific Northwest Region. Mitigation plans are slated to begin in August. For more information contact Chris Benemann, chris.benemann@oda.oregon.gov; Kaitlin Gerber, kaitlin.gerber@oda.oregon.gov; or Dan Stark, Dan.Stark@oregonstate.edu.

RESEARCH AND MONITORING

P. ramorum detected on *Cotoneaster pannosus* in Marin County: First detection in the U.S.

Tomas Pastalka, Nilwala Abeysekara, and Wolfgang Schweigkofler, National Ornamental Research Site, Dominican University of California, (NORS-DUC), San Rafael

During a 2022 survey of native and invasive plants in Marin County, necrotic leaf spots were detected on *Cotoneaster pannosus* (silverleaf cotoneaster). The dark brown spots were observed near the tips or margin of the leaves, covering less than half of the leaf surface; no twig dieback or cankers were detected (Fig. 1). Isolations yielded two *P. ramorum* isolates from different leaf samples from the same tree and were confirmed by sequence analysis of the ITS-region. The *Cotoneaster* sampled in 2022 was again symptomatic in spring 2023. Official regulatory samples were taken by the California Department of Food and Agriculture (CDFA) and submitted to USDA APHIS for confirmation. Other symptomatic *Cotoneaster* plants were also observed in 2023. To read more see full report.

Figure 1. Symptomatic leaves of *C. pannosus* sampled in Marin Co. Photo: NORS-DUC

NURSERIES AND MANAGED LANDSCAPES

California Department of Food and Agriculture (CDFA) *P. ramorum* nursery program update. Trace investigations completed and new host record for the U.S. Trace investigations searching for potentially positive plants shipped from the positive nurseries identified in spring 2023 are now complete. Two positive plants from one nursery were detected at two retail nurseries. The USDA Retail Confirmed Nursery Protocol was performed at the two positive retail locations and no additional positive plants were detected.

A *Cornus capitata* "Mountain Moon" dogwood sample from a nursery in a quarantined county tested positive for *P. ramorum* and was confirmed by the CDFA Plant Pest Diagnostic Center and the USDA APHIS PPQ Plant Pathogen Confirmatory Diagnostics Laboratory in April. This is the first record of *Cornus capitata* "Mountain Moon" dogwood in the U.S. It has been previously detected with *P. ramorum* in the U.K. For more information contact Carolyn Lambert, Carolyn.Lambert@cdfa.ca.gov.

Figures 2 & 3. *P. ramorum* leaf spots on *Cornus capitata* nursery stock. Photos: S. Griffard, Humboldt Co. Ag. Dept.

Oregon Department of Agriculture (ODA) *P. ramorum* nursery program update. Currently, there are seven nurseries participating in the Oregon *Phytophthora ramorum* Nursery Program. Five nurseries are interstate shippers under federal compliance agreements (7 CFR 301.92). They are in Washington (2), Columbia (1), and Marion (2) Counties. Two nurseries are intrastate shippers operating under state compliance agreements (both 7 CFR 301.92 and OAR 603-052-1230) in Clackamas and Linn Counties.

Ongoing delimitation inspections at both nurseries where positive plants were detected in the spring finished in early July. At the nursery located in Marion County (Nursery A), four water samples were taken. Three water samples were taken at the nursery in Washington County

(Nursery B). All water samples at both locations tested negative for *P. ramorum*. At Nursery A, 26 additional positive plants were detected during the delimitation inspections. An additional ten positive plants were also detected at Nursery B (Figs 4, 5 & 6). All positive plants at both locations were incinerated to ash on site.

Figures 4, 5 & 6. Rhododendron plants found to be positive for *P. ramorum* during the spring 2023 delimitation inspections in Oregon. The infected plants show damage on leaves. Photos: ODA

To be released from the program, nurseries must achieve six consecutive negative results from compliance inspections over three years. No nurseries were eligible to be released from the program this spring. For more information, please contact Chris Benemann (chris.benemann@oda.oregon.gov) or Kaitlin Gerber (kaitlin.gerber@oda.oregon.gov).

Washington State Department of Agriculture (WSDA) *P. ramorum* program update.
In June, WSDA received trace-forward information on plants that shipped to three nurseries in Washington from a positive out-of-state nursery. Inspectors followed up at each location. Two samples of *Prunus laurocerasus* 'Schipkaensis' were collected and tested negative for *P. ramorum*. For more information contact Scott Brooks, SBrooks@agr.wa.gov.

P. RAMORUM OVERSEAS

Update on *P. ramorum* in Ireland. By R. O'Hanlon, Department of Agriculture, Food and the Marine, Ireland

Phytophthora ramorum was first detected in traded *Rhododendron* and *Viburnum* in Ireland in 2002. In 2010 it was found infecting *Larix kaempferi*, Japanese larch, causing sudden larch death. Sudden larch death has now been diagnosed in several counties in Ireland (Figs. 7 & 8;

Figures 7 & 8. Japanese larch trees infected with *P. ramorum* in Ireland. **Photos: R. O'Hanlon**

for a map follow link). The pathogen has also been detected on *Fagus sylvatica* (European beech) and *Abies procera* (Noble fir). Infection of these tree hosts is often in association with nearby heavily infected *L. kaempferi*.

Samples are collected by field inspectors in horticultural, public amenity, and forest areas and tested in the government laboratories in the Ireland Department of Agriculture, Food and the Marine. In 2020 there were 112 samples tested, with 410 samples in 2021, and 156 in 2022. The EU2 lineage was found in Ireland in late 2021, infecting *L. kaempferi* at one site in County Louth. Up to that time, only the EU1 lineage was known to be present. Epidemiological monitoring in forests indicated that *P. ramorum* was detected in rain splash under infected *L. kaempferi*, and also in streams running near or through infested forests. A number of other *Phytophthora* spp. have been found causing disease on trees, including *P. lateralis* on *Chamaecyparis lawsoniana* (Lawson's cypress, Port Orford cedar) and *P. pseudosyringae* on *L. kaempferi*. *P. ramorum* infections of the invasive *Rhododendron ponticum* have also been detected in forests.

Within the European Union, the phytosanitary status of *P. ramorum* has recently changed. Previously the pathogen was a quarantine pest. However, legislation has now come into effect which changes the regulatory status of *P. ramorum*, based on the origin of the infected material. "Non-EU isolates" of *P. ramorum* are quarantine pests. These pests are treated as the most serious pests in the EU, and if detected, extensive control actions must be taken. The other legislative grouping is for *P. ramorum* "EU isolates" which are now treated as regulated non-quarantine pests (RNQP). The focus of the legislation on RNQP's is to limit the spread of these pathogens on plants for planting only. For a map, more pictures, and more information see link.

RESEARCH

Quiroga, G.B.; Simler-Williams, A.B.; Frangioso, K.M.; Frankel, S.J.; Rizzo, D.M.; Cobb, R.C. 2023. An experimental comparison of stand management approaches to sudden oak death in restoration and prevention contexts. Canadian Journal of Forest Research. https://doi.org/10.1139/cjfr-2022-0328.

Many coastal forests stretching from central California to southwest Oregon are threatened or have been impacted by the invasive forest pathogen *Phytophthora ramorum*, cause of sudden oak death. We analyzed a set of stand-level forest treatments aimed at preventing or mitigating disease impacts on stand composition, biomass, and fuels, using a before-after-control-intervention experiment with a revaluation after five years. We compared the effects of restorative management in invaded stands to preventative treatments in uninvaded forests. The restorative treatments contrasted two approaches to mastication, hand-crew thinning, and thinning with pile burning with untreated controls (N=30) while the preventative treatments were limited to hand-crew thinning (N=10). Half of the restoration treatments had basal sprouts removed two- and four-years after treatment. All treatments significantly reduced stand density and increased average tree size without significantly decreasing total basal area both immediately and five years after treatments. Preventative treatments also significantly increased dominance of timber species not susceptible to *P. ramorum*. Follow-up basal sprout removal in the restoration experiment appears to maintain treatment benefits to average tree size and may be associated with small decreases in stand density five years after initial treatment. Our study demonstrates that for at least five years, a range of common stand management practices can improve forests threatened or impacted by sudden oak death.

Roig-Paul, M. 2023. Development of a diagnostic assay for *Phytophthora ramorum* lineage detection. Undergraduate thesis. Oregon State University. https://ir.library.oregonstate.edu/concern/undergraduate_thesis_or_projects/76537851x.

Phytophthora ramorum is the causal agent of Sudden Oak Death (SOD), which is an economically and environmentally important disease causing up to 80% mortality of tanoaks in Pacific Northwest coastal forests (LeBoldus et al. 2022). There are currently four clonal lineages in the United States and Europe, named North America 1 (NA1), North America 2 (NA2), European 1 (EU1), and European 2 (EU2); only the first three are found in the Pacific Northwest. There is a critical need for fast, accurate, and inexpensive diagnostic probes to identify lineages. Specific High Enzymatic Reporter unLOCKing (SHERLOCK- a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based assay) can be used to identify lineage from a sample based on polymorphisms unique to each clonal lineages' genome. With a detection limit of 100 copies of target DNA region template per µL, the SHERLOCK assay is a specific test that can be used in <1 hour, allowing for field diagnostics. In the current work, we developed an assay specific for the NA1 clonal lineage. DNA from each of the lineages was extracted using a Qiagen DNeasy kit, with concentrations verified using a Nanodrop device. Genomes were scanned to identify diagnostic primers and guide RNA loci for each lineage, using the krisp-vcf algorithm. Krisp-vcf identified a region located on *P. ramorum* Pr102 scaffold 0001, position 225003 to 225626 that contained a unique single nucleotide polymorphism (SNP) within the diagnostic sequence for NA1 as compared to the other three lineages. Results of the NA1 SHERLOCK assay, including NA1 strains as positive controls as

well as other lineages as negative controls, indicated the assay is lineage specific. This work provides a rapid assay for specific identification of samples for lineage NA1, the oldest discovered lineage of *P. ramorum* in the continental United States.

Sondreli, K.L.; Tabima, J.F. and LeBoldus, J.M. 2023. Rapid new diagnostic LAMP (Loop-mediated Isothermal Amplification) assays to distinguish among the four lineages of *Phytophthora ramorum*. Plant Disease. https://doi.org/10.1094/PDIS-08-22-1965-RE.

Sudden oak death (SOD) is caused by *Phytophthora ramorum*, an invasive oomycete pathogen. This pathogen is of major regulatory concern for nurseries, horticulture, and forestry in the U.S. and around the world. Three of the twelve identified lineages of *P. ramorum* currently occur in the U.S. (NA1, NA2, and EU1) impacting in wildland forests and nurseries. Rapid identification and lineage determination is essential to accelerate management decisions, detect introductions of new lineages, and control the spread of SOD. The objective of this study was to develop and validate diagnostic tools to rapidly identify *P. ramorum* and distinguish among the four common lineages of the pathogen and to accelerate management decision making. The Loop-mediated Isothermal Amplification (LAMP) assays developed here are species specific with no cross reaction to common *Phytophthora* species found in Oregon, California, and Washington. The lineage specific assays unambiguously distinguish among the four common clonal lineages. These assays are sensitive and able to detect *P. ramorum* DNA ranging in concentration from 30ng/µl to 0.03ng/µl depending on the assay. These assays work effectively on a variety of sample types including plant tissue, cultures, and DNA. They have been integrated into the SOD diagnostic process in the forest pathology lab at Oregon State University. To date 190 samples have been correctly identified from over 200 field samples tested to date. The development of these assays will help managers in forestry and horticulture identify and rapidly respond to new outbreaks of *P. ramorum.*

YuFang, G.; Xia, H.; Dai, T.; Liu, T.; Shamoun, S.F. and Wu, C. 2023. CRISPR/Cas12a-based approaches for efficient and accurate detection of *Phytophthora ramorum*. Frontiers in Cellular and Infection Microbiology. 13: 1218105. https://doi.org/10.3389/fcimb.2023.1218105.

Methods: In this study, we developed a *P. ramorum* detection technique based on a combination of recombinase polymerase amplification (RPA) with CRISPR/Cas12a technology (termed RPACRISPR/ Cas12a).

Results: This novel method can be utilized for the molecular identification of *P. ramorum* under UV light and readout coming from fluorophores, and can specifically detect *P. ramorum* at DNA concentrations as low as 100 pg within 25 min at 37 °C.

Discussion: We have developed a simple, rapid, sensitive, unaided-eye visualization, RPA CRISPR/Cas12a-based detection system for the molecular identification of *P. ramorum* that does not require technical expertise or expensive ancillary equipment. And this system is sensitive for both standard laboratory samples and samples from the field.

RELATED RESEARCH

Branco, S.; Douma, J.C.; Brockerhoff, E.G. [and others]. 2023. Eradication programs against non-native pests and pathogens of woody plants in Europe: which factors influence their success

or failure**?** NeoBiota. 84: 281-317. doi: 10.3897/neobiota.84.95687. https://neobiota.pensoft.net/article/95687/.

Contreras-Cornejo, H.A.; Larsen, J.; Fernández-Pavía, S.P. and Oyama, K. 2023. Climate change, a booster of disease outbreaks by the plant pathogen *Phytophthora* in oak forests. Rhizosphere. 27: 100719. https://doi.org/10.1016/j.rhisph.2023.100719.

Corcobado, T.; Cech, T.L.; Daxer, A.; Ďatková, H. and others. 2023. *Phytophthora*, *Nothophytophthora* and *Halophytophthora* diversity in rivers, streams and riparian alder ecosystems of Central Europe. Mycological Progress. 22(7): 50.

Marcot, B.G.; Scott, P.; and Burgess. T.I. 2023. Multivariate Bayesian analysis to predict invasiveness of *Phytophthora* pathogens. Ecosphere. 14(6): e4573. https://doi.org/10.1002/ecs2.4573.

Matsiakh, I.; Menkis, A. 2023. An overview of *Phytophthora* species on woody plants in Sweden and Other Nordic Countries. Microorganisms. 11(5): 1309. https://doi.org/10.3390/microorganisms11051309.

Proaño-Cuenca, F.; Espindola, A.S. and Garzon, C.D. 2023. Detection of *Phytophthora*, *Pythium*, *Globisporangium*, *Hyaloperonospora*, and *Plasmopara* species in high-throughput sequencing data by in silico and in vitro analysis using Microbe Finder (MiFi). PhytoFrontiers. 3(1): 124-136. https://doi.org/10.1094/PHYTOFR-04-22-0039-FI.

Serrano, M.S.; González, M.; Romero, M.Á.; Alconero, M.R. and Sánchez, M.E. 2023. Mineral nutrients improve phosphonate effectiveness against cork oak root disease. Forest Ecology and Management. 543: 121152. https://doi.org/10.1016/j.foreco.2023.121152.

Schiffer-Forsyth, K.; Frederickson Matika, D.; Hedley, P.E.; Cock, P.J. & Green, S. 2023. *Phytophthora* in horticultural nursery green waste—A risk to plant health. Horticulturae. *9*(6): 616. https://doi.org/10.3390/horticulturae9060616.