



CALIFORNIA OAK MORTALITY TASK FORCE REPORT APRIL 2020

PHYTOPHTHORA RAMORUM ON MANZANITAS



Left. *P. ramorum* caused mortality of *Arctostaphylos montaraensis*. Right. Pallid manzanita with dieback caused by *P. ramorum*. Photos courtesy of Phytosphere Research.

Four more species of manzanita (*Arctostaphylos*, Ericaceae) have been recognized as susceptible to *Phytophthora ramorum*: *A. glauca*, *A. peninsularis*, *A. viridissima*, and *A. viscida*. *Phytophthora ramorum* was first described infecting *Arctostaphylos manzanita* in wildlands in 2003 (Garbelotto et al. 2003, Davidson et al. 2003). At least 18 manzanita species are now known to support *P. ramorum* infections, including several rare, threatened or endangered species. Since 2017, the California Department of Food and Agriculture Plant Pest Diagnostics Center (CDFA) in Sacramento has reported recovery of *P. ramorum* from nine new *Arctostaphylos* hosts, including *A. glandulosa*, *A. glauca*, *A. montaraensis*, *A. pallida*, *A. peninsularis*, *A. rainbowensis*, *A. virgata*, *A. viridissima* and *A. viscida*. The detections were made from native plant nurseries, botanic gardens and natural wildlands. Although symptoms and severity varied among species, most exhibited foliar spotting and necrosis, with or without tip dieback and stem lesions.

USDA APHIS guidelines for *P. ramorum* require that Koch's postulates be completed on live plants before a new species can be added to the official host list. CDFA has completed Koch's postulates on seven of the nine species listed above, with reports pending publication.

The genus *Arctostaphylos* includes over 100 species of evergreen shrubs and small trees (Wahlert et al. 2009). The center of diversity for *Arctostaphylos* is centered about San Francisco, and the geographic range of the current infestation of *P. ramorum* in California overlaps with the area where many endemic manzanita species grow. Nearly half of the California manzanita species are considered rare, threatened, or endangered (CNPS 2020). Their life history and



special status has made acquiring adequate experimental host material to complete Koch's postulates challenging.

It is worth noting that some of the detections were in areas with no associated California bay laurel (*Umbellularia californica* Lauraceae), a common source of the disease. Further work needs to be done to determine if these *Arctostaphylos* species sporulate and can contribute to the spread of *P. ramorum*. For more information contact Suzanne Latham, Suzanne.Latham@cdfa.ca.gov or Cheryl Blomquist, Cheryl.Blomquist@cdfa.ca.gov.

References

CNPS. 2020. California Native Plant Society, Inventory of Rare and Endangered Plants <http://www.rareplants.cnps.org/> [Accessed April 3, 2020].

Davidson, J. M., Werres, S., Garbelotto, M., Hansen, E. M., and Rizzo, D. M. 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Online. Plant Health Progress doi:10.1094/PHP-2003-0707-01-DG.

Garbelotto, M., Davidson, J.M., Ivers, K., Maloney, P.E., Huberli, 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: 18-23.

Wahlert, G. A., Parker, V.T., Vasey, M.C. 2009. A phylogeny of *Arctostaphylos* (Ericaceae) inferred from nuclear ribosomal ITS sequences. J. Bot. Res. Inst. Texas 3(2) 673-682.

NURSERIES

California Department of Agriculture *P. ramorum* Program Update. In the first quarter of 2020, two California nurseries have been detected as positive for *P. ramorum*. One nursery in Santa Cruz County was found to have a *P. ramorum* positive 5-gallon *Camellia japonica* 'Osakazuki' during a pre-shipment inspection in February. The nursery provided shipping records, and 35 counties have been notified that trace-forward inspections are required to determine if nurseries that received high risk host plants have infected stock in their inventory. Due to COVID-19 shelter-in-place orders in multiple counties affected by the trace-forward activity, plants have been put on hold at nurseries in some counties until county agricultural staff can visit the nursery for an inspection.

The other positive nursery is in Contra Costa County where infected *Camellia* and *Loropetalum* were detected during a biannual compliance inspection. No other positive plants were detected in follow-up delimitation surveys. Both nurseries are in quarantined counties and are undergoing the USDA's Official Regulatory Protocol for Nurseries Containing Plants Infected with *P. ramorum*.

Spring compliance inspections are underway at ten previously positive California nurseries as directed by federal and state regulations. These nurseries ship *P. ramorum* host material outside of the quarantined counties so receive biannual inspections. Annual inspections, at other



nurseries and establishments with compliance agreements in quarantined counties, are also on-going. For more information, contact Carolyn Lambert, Carolyn.Lambert@cdfa.ca.gov.

Oregon Department of Agriculture (ODA) *P. ramorum* Program Report. Currently there are 13 Oregon nurseries participating in the *Phytophthora ramorum* Certification Program. Of these, seven are interstate shippers and are regulated at the federal level (DA-2014-02). As intrastate shippers, the other six nurseries are regulated by Oregon state quarantine requirements (both 7 CFR 301.92 and OAR 603-052-1230). The participating nurseries are located in eight counties (Washington, Columbia, Tillamook, Marion, Multnomah, Lane, Lincoln, and Polk).

Field activities from the fall 2019 surveys lasted well through the winter season partly due to the detection of positive plants and soil at an interstate shipper in Washington County. The plants have been destroyed and trace-back activities resulted in no further positive detections. The areas with infested soil (one in a greenhouse and another in a gravel lot) have been taped off to prohibit entry. The nursery chose to steam the soil as a mitigation option and ODA staff will return to steam the area when conditions are appropriate (estimated for July 2020).

To treat a soil infestation, a retail nursery in Multnomah County is currently laying cement over part of the nursery. The nursery destroyed all positive plants as well as those in a quarantine zone. Trace-back investigations from this retail location led to a commercial interstate shipper in Marion County where positive *Camellia*, *Pieris*, and *Rhododendron* plants were found. This was a first detection for this location and the Confirmed Nursery Protocol (CNP) has been implemented. To date, 42 foliar samples, 3 soil samples, and 1 groundwater sample have been confirmed positive. Additionally, as a result of a routine inspection, another interstate shipper in Marion County was confirmed positive in early March 2020. To date, 7 *Rhododendron* plants have tested positive. The find is also a first detection at this nursery; the CNP is underway. Both nurseries have signed federal compliance agreements.

Compliance surveys for the 2020 spring season began on March 10; two nurseries have been sampled with results pending. Surveys are scheduled to be completed by the second week of May. For more information, contact Chris Benemann, sbenemann@oda.state.or.us.

Washington State Department of Agriculture *P. ramorum* Program Update. Spring compliance surveys for *P. ramorum* are underway in Washington. At one wholesale shipping nursery in the compliance program, the spring survey is being conducted in two stages. In March, samples were collected on evergreen *P. ramorum* associated-host plants (HAP). All samples were negative. In May, a second survey will occur for deciduous HAP and all other plants at the nursery. A fall compliance survey will be scheduled as well.

At a regulated botanic garden in Kitsap County, three confirmed water positives have been detected since the beginning of 2020. All positive water baits are from baiting sites previously found positive. The baits are from a pond below previous plant positive sites, and the outflow is less than a quarter mile to the Puget Sound. A two-day vegetation survey will be conducted at the



garden in late spring, or as soon as agency COVID-19 health policies allow. For more information, contact Scott Brooks, SBrooks@agr.wa.gov.

REGULATIONS

USDA APHIS has updated the Potentially Actionable Suspect Sample (PASS) protocol for submission of official, regulatory samples where preliminary diagnostics indicate *P. ramorum* is present and confirmation is required by the USDA APHIS Plant Protection and Quarantine Science and Technology, Beltsville laboratory. Samples now need to include symptomatic plant tissues and DNA. For more information see https://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/passpolicy.pdf.

For federal compliance agreements, USDA-APHIS increased the number of required samples for large nurseries that ship interstate from 199 to 332. The sampling is designed so the minimum number of regulated plants that must be sampled in a confirmed-positive nursery ensures detection at a 1.0% incidence with 95% confidence level. The revised table of sample requirements appears in the new USDA *Phytophthora ramorum* Domestic Regulatory Program Manual for *Phytophthora ramorum*, released in January, available on the USDA APHIS *P. ramorum* [website](#).

FUNDING

Over \$1.2 million for *Phytophthora ramorum* projects has been awarded via the Plant Pest and Disease Management and Disaster Prevention Program (formerly referred to as the Farm Bill) administered by the USDA APHIS. The funds support *P. ramorum* nursery survey and research in 22 states. Nationwide, \$63 million was provided for 357 plant health projects, for further details see the 2020 spending plan at https://www.aphis.usda.gov/plant_health/ppa-7721/fy20/fy20-ppdmdpp-spending-plan.pdf.

RESEARCH

Anandaraj, M.; Mathew, S.K.; Eapen, S.J.; Cissin, J.; Rosana, B. and Bhai, R.S. 2020. Morphological and molecular intervention in identifying *Phytophthora* spp. causing leaf and nut fall in nutmeg (*Myristica fragrans* Houtt.). European Journal of Plant Pathology. 156(2): 373-386.

Nutmeg, a major crop of India provides two important spices. During the monsoon season of 2011, a serious leaf and nut fall occurred in nutmeg plantations and *Phytophthora ramorum* was reported as the causal organism. *P. ramorum* being an alien species, the aetiology warranted a detailed investigation. *Phytophthora* isolated from different endemic locations were studied for morphological and molecular characterization using ITS, ITS-RFLP, MLST, SSCP and hybrid analysis. The isolates showed papillate, ovoid-obovoid sporangia with pedicel length > 20 µm which is distinct from the semi-papillate, caducous sporangia with short pedicel of <5 µm characteristic of *P. ramorum*. The isolates failed to grow at 10 °C or at 37 °C and chlamydospores were not produced in culture, a characteristic distinct to *P. ramorum*. MLST



analysis placed the isolates in Clade 2 while *P. ramorum* belongs to Clade 8. *P. ramorum* has a unique set of morphological characteristics and a unique molecular sequence at the ITS region which delineate it from other *Phytophthora* species. Among the eight isolates studied, ITS region of six isolates (13–01–13-06) showed similarity to *P. meadii* and two isolates (13–55 and 98–68) to *P. tropicalis*. In SSCP, the isolates formed a separate group distinct from *P. meadii* of cocoa and cardamom. The double peaks in the ITS chromatograms indicated hybridity and further hybrid analysis placed them under *P. citrophthora* and *P. meadii* (Cit-Med) lineage. Thus due to the distinct differences from *P. ramorum* and evidences of shared lineage with *P. citrophthora* and *P. meadii*, we propose to call these isolates as *P. citromeadii* (13–01- 13-06) and *P. citrocaptalis* (13–55 and 98–68).

Dadam, D.; Siasou, E.; Woodward, S. and Clark, J.A. 2019. Migratory passerine birds in Britain carry *Phytophthora ramorum* inoculum on their feathers and “feet” at low frequency. Forest Pathology. e12569.

In this study, we investigated whether birds could be vectors facilitating long-distance spread of *Phytophthora ramorum* in Britain. Migratory bird species associated with the main sporangium-producing host plants and most likely to pick up *P. ramorum* spores were considered. Swabs were taken from the flank and “feet” of 1,014 birds over a 12-month period (April 2011–March 2012) in the west of Britain and subsequently analyzed for the presence of *P. ramorum* using nested PCR. Ten positive samples from 10 birds were identified: three in Cornwall, one in Devon, three in Gloucestershire, two in north Wales and one in Merseyside. *Phytophthora ramorum* was detected on samples from four species of thrushes (Redwing, *Turdus iliacus*; Fieldfare, *T. pilaris*; Blackbird, *T. merula* and Song Thrush, *T. philomelos*) and one species of warbler (Chiffchaff, *Phylloscopus collybita*). All birds that tested positive were sampled in late autumn and winter (October–February), when long-distance movements (over 100 km) would have stopped. The low incidence of *P. ramorum* found using PCR suggests that the incidence of inoculum, whether viable or not, on birds was low. The apparently low incidence of inoculum on birds suggests migratory passerine birds can carry *P. ramorum* inoculum on their feathers and “feet,” albeit at low frequency. The dates of positive samples indicate that birds would not have been moving long distances at the time but further work is needed to estimate the extent of their contribution to the spread of *P. ramorum* in Britain.

Press, C.; Fieland, V.; Creswell, T.; Bonkowski, J.; Miles, L. and Grünwald, N.J. 2020 (First Look). First report of the NA2 clonal lineage of *Phytophthora ramorum* in Indiana. Plant Disease. <https://doi.org/10.1094/PDIS-12-19-2543-PDN>.

The oomycete pathogen *Phytophthora ramorum* is the causal agent of ramorum leaf blight and sudden oak death. *P. ramorum* is known to cause leaf blight and stem dieback on nursery hosts including Rhododendron. The disease typically results in dark, brownish lesions on leaves or stems which can cause wilting and death of the plant. Since the emergence of the disease, a federal quarantine was established to prevent the spread of the pathogen and nurseries that export *P. ramorum* hosts are subject to a federally mandated certification program for interstate export. This disease has had a significant impact on the US nursery industry via quarantine regulations



imposed on nurseries infested with the pathogen. The pathogen has been introduced at least 3 times into the Western US as clonal lineages NA1, NA2, and EU1. In the spring and summer of 2019, USDA-APHIS reported that a shipment of potentially *P. ramorum* infested plants was delivered to several Eastern and Midwestern states. Rhododendron leaves from numerous counties in Indiana, showing characteristic necrotic leaf blight symptoms, were sampled by Indiana Department of Natural Resources (IDNR) nursery inspectors between April 18 and June 3, 2019. These samples were initially screened for the presence of *Phytophthora* at the Purdue Plant and Pest Diagnostic Laboratory using an ELISA test. Sub-samples from tissue producing a positive ELISA result were forwarded to the Michigan State University Plant & Pest Diagnostics lab for *P. ramorum*-specific PCR testing, which was later confirmed by USDA CPHST. ... All 26 of the Indiana isolates were unambiguously classified as belonging to the NA2 clonal lineage. This is the first report of the NA2 clonal lineage outside of British Columbia, Washington, and California indicating that this pathogen was most likely moved West to East. Prior documented lineages detected obtained from *P. ramorum*-infected plants in Eastern receiving states have only harbored the NA1 clonal lineage. As a result of these detections the IDNR ordered destruction of more than 6,100 Rhododendron plants at retail outlets in Indiana.

[Due to its length, this abstract was edited. To view the full abstract check the link above.]

Srivastava, S.K.; Abad, G.; Knight, L.M.; Zeller, K.; Mavrodieva, V.A. and Nakhla, M.K. 2020. (First Look.) Draft genome resource for the Ex-types of *Phytophthora ramorum*, *P. kernoviae* and *P. melonis*, species of regulatory concern, using ultra long-read MinION Nanopore sequencing. Molecular Plant-Microbe Interactions.

Phytophthora ramorum, *P. kernoviae* and *P. melonis* are each species of current regulatory concern in United States, United Kingdom and other areas of the world. Ex-type material are cultures, and duplicates of the Type that was used to describe each species, and that are deposited in additional culture collections. Using these type specimens as references is essential to designing correct molecular identification and diagnostic systems. Here we report Whole Genome Sequence (WGS) for the Ex-type material of *P. ramorum*, *P. kernoviae* and *P. melonis* generated using High Throughput Sequencing (HTS) via the MinION third generation (3G-HTS) platform from Oxford Nanopore Technology. We assembled the quality filtered reads into contigs for each species. We assembled the continuous contigs of *P. ramorum*, *P. kernoviae* and *P. melonis* (1322, 545 and 2091 contigs respectively). The Ab initio prediction of genes from these species reveals that there are 16,838, 12,793 and 34,580 genes in *P. ramorum*, *P. kernoviae* and *P. melonis*, respectively. Of the 34,580 *P. melonis* genes, 10,164 genes were conserved among all three of these *Phytophthora* species which may include pathogenicity genes.

Wong, B.; Leal, I.; Feau, N.; Dale, A.; Uzunovic, A. and Hamelin, R.C. 2020. Molecular assays to detect the presence and viability of *Phytophthora ramorum* and *Grosmannia clavigera*. PLoS ONE 15(2): e0221742. <https://doi.org/10.1371/journal.pone.0221742>.

Wood and wood products can harbor microorganisms that can raise phytosanitary concerns in countries importing or exporting these products. To evaluate the efficacy of wood treatment on the survival of microorganisms of phytosanitary concern the method of choice is to grow



microbes in petri dishes for subsequent identification. However, some plant pathogens are difficult or impossible to grow in axenic cultures. A molecular methodology capable of detecting living fungi and fungus-like organisms in situ can provide a solution. RNA represents the transcription of genes and can become rapidly unstable after cell death, providing a proxy measure of viability. We designed and used RNA-based molecular diagnostic assays targeting genes essential to vital processes and assessed their presence in wood colonized by fungi and oomycetes through reverse transcription and real-time polymerase chain reaction (PCR). A stability analysis was conducted by comparing the ratio of mRNA to gDNA over time following heat treatment of mycelial cultures of the Oomycete *Phytophthora ramorum* and the fungus *Grosmannia clavigera*. The real-time PCR results indicated that the DNA remained stable over a period of 10 days post treatment in heat-treated samples, whereas mRNA could not be detected after 24 hours for *P. ramorum* or 96 hours for *G. clavigera*. Therefore, this method provides a reliable way to evaluate the viability of these pathogens and offers a potential way to assess the effectiveness of existing and emerging wood treatments. This can have important phytosanitary impacts on assessing both timber and non-timber forest products of commercial value in international wood trade.

RELATED RESEARCH

Maass, D.I.; Irland, L.C.; Anderson, J.L.; Laustsen, K.M.; Greenwood, M.S. and Roth, B. E. 2020. Reassessing Potential for Exotic Larch in Northern United States. Journal of Forestry. <https://doi.org/10.1093/jofore/fvz066>.

Roberts, M.; Gilligan, C.; Kleczkowski, A.; Hanley, N.; Whalley, E. and Healey, J.R. 2020. The effect of forest management options on forest resilience to pathogens. Frontiers in Forests and Global Change. 3, 7. <https://doi.org/10.3389/ffgc.2020.00007>.

Wan, J.S. and Liew, E.C. 2020. Genus-level change in aggressiveness with continuous invasions: a phylogenetically-informed Bayesian quantile regression. Biological Invasions. 1-16. doi.org/10.1007/s10530-020-02229-1.

RESOURCES

A preview of the papers in the Proceedings of the Seventh Sudden Oak Death Science and Management Symposium, Healthy Plants in a World with *Phytophthora*, held June 25-27, 2019 in The Presidio, San Francisco is available at the conference website [HERE](#). The Proceedings will be published in the next few months (online only) by the USDA Forest Service, Pacific Southwest Research Station.

The National Ornamental Research Site at Dominican University of California has a new website (<https://www.dominican.edu/directory/national-ornamentals-research-site-nors-duc>) featuring publications, presentations and summaries of current research on *P. ramorum* and other priority pests.



A special Issue of *Sibbaldia* featuring *Phytophthora*, plant health and pathogen management is now available. The year 2020 has been designated the International Year of Plant Health, to celebrate *Sibbaldia*, The Royal Botanic Garden Edinburgh's journal, has published its first ever Special Issue focusing on Plant Health. Several papers discuss *Phytophthora* and all papers discuss plant health and management of pests including pathogens, <https://journals.rbge.org.uk/rbgesib/index>.

EVENTS

Spring 2020 Sudden Oak Death (SOD) Blitzes are scheduled for April through June throughout much of central coastal California. Community members living near areas impacted by sudden oak death are encouraged to participate in a SOD Blitz – a citizen science SOD survey. The training for identification and collection of symptomatic bay and tanoak samples will be provided online and collection materials handled under strict sanitary measures to protect against transmission of COVID-19. Please bring an iPhone or Android smartphone for use with the free SODMAP app. Survey samples will be analysed at the Forest Pathology and Mycology lab, UC Berkeley to determine the presence or absence of the pathogen. Results will be published in the fall at www.sodblitz.org. For details on SOD Blitz locations and times, see https://nature.berkeley.edu/matteolab/?page_id=816.

Oak health workshops originally scheduled for April 20 in Mendocino County and April 21 in Sonoma County are now being offered in a virtual format. In these workshops, oak researchers and educators will present the latest information on a variety of oak-related topics ranging from oak woodland conservation to sudden oak death to a new pest of valley oak (*Quercus lobata*) and blue oak (*Quercus douglasii*). The workshops are now being posted as a series of short recorded talks that will be followed up for meeting registrants by a live, online Q&A session. The talks will be posted by April 14 with the Q & A session to take place at 10 am on April 21. These workshops, organized by UC Cooperative Extension and CAL FIRE, are intended for anyone interested in working with and learning more about California's native oaks and tanoaks, including private landowners, public and private land managers, agency personnel, and the general public. The workshops are free but require registration. To register, go to <http://ucanr.edu/OakHealth>. Further information is available via a link from that page or by contacting Kerry Wininger (kwininger@ucanr.edu, 707-888-5616) or Chris Lee (christopher.lee@fire.ca.gov, 916-203-0856).