CALIFORNIA OAK MORTALITY TASK FORCE REPORT
JANUARY 2015

MONITORING

A second Sudden Oak Death site was discovered in Redwood National Park in mid-September (2014) along the banks of Redwood Creek. The 5-acre site is approximately 2 miles downstream from the Bridge Creek infestation and includes infected tanoak and California bay laurel trees. It is unknown if this outbreak is a separate introduction or a jump from the Bridge Creek site. Park management is currently focused on removal of tanoak and bay to control *Phytophthora ramorum* spread as these two hosts support pathogen sporulation and have canopies up to 40 meters high. For more information, contact Park Vegetation Management Chief Leonel Arguello at (707) 465-7780.

In 2014, a new *P. ramorum*-positive waterway in Kitsap County, Washington was found positive twice from samples collected in March and May. The positive site was downstream from a previously positive nursery. Sixteen waterways in six counties were monitored in all for the pathogen from March to June (2014), with no other confirmations made.

California Stream Monitoring Program, 2014 Summary, By Heather Mehl, UC Davis - In 2014, 146 waterways in Del Norte, Humboldt, Mendocino, Monterey, and San Luis Obispo Cos. were monitored for *P. ramorum.* Overall, recovery from known positive watersheds was low compared to previous years, with *P. ramorum* detected in only 13 (54%) of 24 previously positive sites. This was likely a consequence of low spring rainfall throughout California. Recovery was lowest in Monterey (1 of 5; 20%), followed by Humboldt (6 of 12; 50%), and Mendocino Cos. (6 of 7; 86%). However, even with reduced recovery rates, the pathogen was still detected for the first time in six watersheds (three each in Humboldt and Mendocino Cos.).

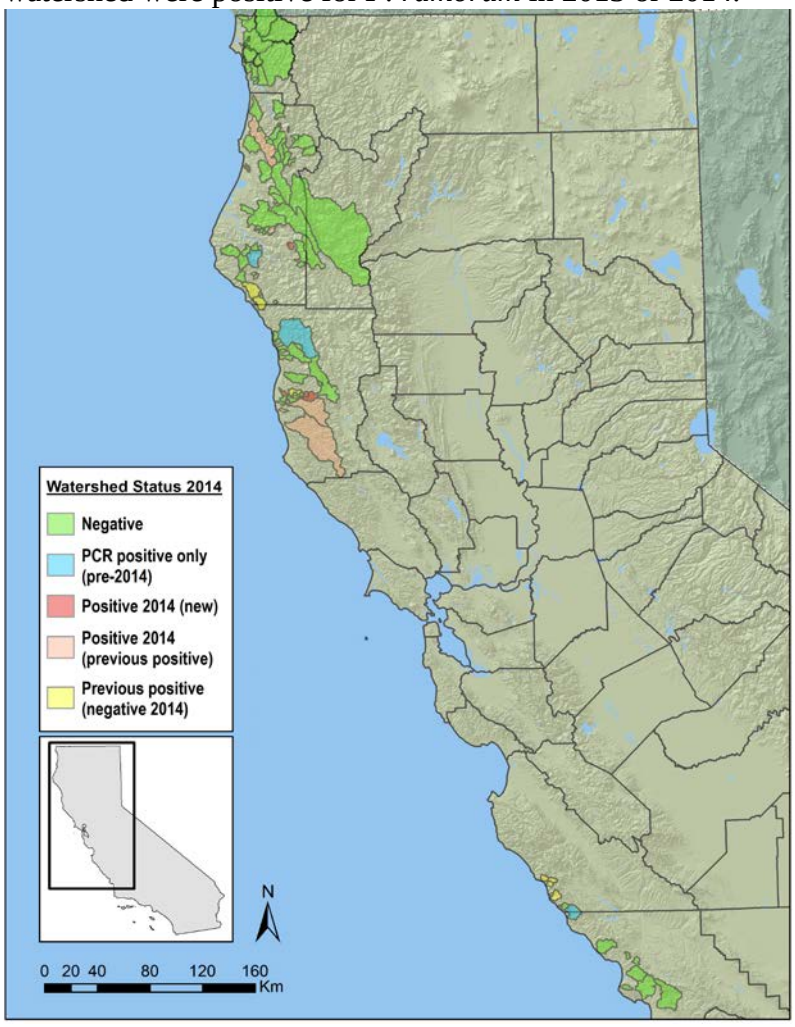
In northern Humboldt Co., *P. ramorum* was detected for the first time in Beaver Creek, a tributary of Redwood Creek (upstream of Cookson Ranch, Redwood Valley). Watersheds monitored on Hoopa Valley and Yurok tribal lands continue to be negative, and there were no new detections in the McKinleyville area. In southern Humboldt Co., two watersheds near the southwest border of the Six Rivers NF were positive for the first time - Butte (Van Duzen watershed) and Cooper (Eel River watershed) Creeks.

Phytophthora ramorum was detected in Hollow Tree Creek (an upper tributary of the South Fork of the Eel River), northern Mendocino Co., for the first time in 2013. This detection was substantially north of previously known infestations in the county and south of known infestations in southern Humboldt Co. *Phytophthora ramorum* was also recovered for the first time from several drainages in the South Fork of the Noyo River watershed in Jackson Demonstration State Forest (JDSF), central Mendocino Co. In response to these finds, 2014 monitoring efforts were increased throughout northern and central Mendocino Co., adding three sites in the Angelo Coast Range Reserve and six along Hwy 1, north of Fort Bragg. None of the new sites tested positive. Waterways in JDSF continued to be intensively sampled, and *P. ramorum* was detected for the first

time in the North Fork of the Big River and the west fork of Chamberlain Creek, both near the Camp 20 Day Use Area on Hwy 20, as well as in Hare Creek, a watershed southeast of Fort Bragg.

The Little North Fork of the Big River (LNFB), sampled in Mendocino Woodlands State Park (east of the town of Mendocino), tested *P. ramorum* positive for the first time in 2013. The LNFB watershed spans both Mendocino Woodlands State Park and JDSF. California State Parks personnel conducted ground surveys in this watershed in 2013, but no terrestrial infections were found. In an effort to determine the source of inoculum, two sampling sites were established in 2014 in LNFB tributaries, upstream from Mendocino Woodlands State Park campgrounds. The original site at the mouth of the LNFB tested positive again this year, but the upstream sites did not.

There were no new detections in Monterey Co. and all watersheds monitored in San Luis Obispo Co. continued to be *P. ramorum* negative. In 2012, *P. ramorum* was detected for the first time through PCR-based diagnostics in San Carpoforo Creek, a watershed spanning both Monterey and San Luis Obispo Cos. However, no samples from this watershed were positive for *P. ramorum* in 2013 or 2014.



2014 California *P. ramorum* watershed monitoring results.

NURSERIES

Oregon Department of Agriculture *P. ramorum* Program, 2014 Summary - Fourteen nurseries participated in the USDA Animal and Plant Health Inspection Service *P. ramorum* certification program at its inception in March 2014. During spring, 2,868 samples were collected from plants, used pots, potting media, and water sources. The pathogen was detected in five nurseries infecting: *Gaultheria shallon* (15 plants), *Rhododendron*, and *Rhododendron azalea* 'Hahn's Red' at nursery #1; *Rhododendron* sp. (3 plants) and used potting media at nursery #2; *Pieris taiwanensis*, *Pieris* 'Valley Valentine', *Pieris* 'Valley Rose', and a retention pond at nursery #3; *Rhododendron* sp. (2 plants), *Rhododendron* 'Lost Forest Holding', and used potting media at nursery #4; and used potting media at nursery #5. The USDA Confirmed Nursery Protocol (CNP) was enacted at the nurseries. Delimitation surveys detected additional positives in two *Rhododendron* sp. at nursery #2 and in *Pieris* 'Valley Valentine,' *Pieris* 'Valley Rose,' and soil substrate in two areas at nursery #3.

Of the five positive nurseries identified during the spring sampling period, three nurseries (#1, #2, and #5) elected to opt-out of the federal program and ship intrastate only, one nursery (#3) opted to participate in the multistate soil steaming pilot study, and one nursery (#4) had its federal compliance agreement revoked. At Nursery #4, a *Rhododendron* 'Purple Lace' was found infected during the 90-day hold period. Additional positive plants (*Rhododendron* 'Repen x Nygo Chief,' 'Unique,' and 'Scyntillation') and contaminated soil substrate were detected during the second delimitation survey. Nursery #3 also underwent a hazard assessment after eradication activities were completed, which identified mandatory best management practices (BMPs) the nursery was required to include in their federal compliance agreement.

Phytophthora ramorum was also detected infecting two plants (*Rhododendron* 'Roseum Elegans' and 'Roseum 2') at a nursery during its annual general licensing inspection. The USDA CNP was enacted with no additional positive plant, soil, or water samples found. Despite the negative results, the nursery opted to fumigate the soil beneath the infected plants as a precautionary measure. The nursery is now included in the federal certification program.

Ten nurseries were surveyed for *P. ramorum* during the fall sampling period, with 2,016 plant, soil, and water samples collected. The pathogen was detected in two nurseries infecting *Rhododendron* at nursery #7 and *Rhododendron* at nursery #8 as well as infesting water from a retention pond there. The USDA CNP was enacted at the nurseries and additional positives were found. Nursery #7 had infected *Rhododendron* (eight plants), *Rhododendron*'Unique' (two plants), and soil substrate beneath the infected plants. Nursery #8 had infected *Rhododendron* (four plants) and infested stream runoff from the retention pond. After eradication activities were implemented, hazard assessments were conducted at both nurseries with mandatory BMPs included in their federal compliance agreements.

Of the eight positive nurseries identified during federal certification surveys, four opted out of, or were removed from, the federal program. These four nurseries are now subject to Oregon's state quarantine requirements for *P. ramorum*, which includes mandatory testing to ensure there is no intrastate movement of the pathogen from these locations. The other four are currently operating under modified compliance agreements that include mandatory BMPs to address specific hazards at their nurseries. Seven nurseries tested pathogen free and continue to participate in the federal program.

FEATURED RESEARCH

NORS-DUC Investigation into the Epidemiology of *P. ramorum* Inoculum in Nursery Soils. Ebba Peterson and Jennifer Parke, Oregon State University, and Nik Grünwald, USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR.

Phytophthora ramorum is thought to survive in soil via leaf litter infested with the pathogen harboring mycelium, sporangia, and chlamydospores. While the summer survival of *P. ramorum* in leaf debris incorporated into nursery and forest soils is well established, the timing of sporulation and infection, and the relative contribution of inoculum at various depths are unknown. Two aspects of the disease cycle are being investigated at the National Ornamental Research Site at Dominican University of California (NORS-DUC): the infection of leaves at the soil surface, and the survival and sporulation potential of inoculum after incubation in soils. Rhododendron leaf disks infested by *P. ramorum* were introduced into an irrigated, experimental soil plot at two depths, 5 and 15 cm, in June 2014. Periodically a subsample has been baited with non-infested leaf disks placed atop the soil column containing inoculum. In addition to baiting, inoculum has also been recovered for plating in selective media (to assess survival) or placed in filtered, sterilized creek water (to assess sporulation). Recovery from soil-incubated disks remained stable after an initial decline observed within the first 2 weeks. Sporulation has been greatest from inoculum at 15 cm, and declined in summer months for inoculum at both depths. With the onset of cooler temperatures in autumn, an increase in recovery success from incubated disks has been observed. While a moderate increase in sporulation has also been observed, *P. ramorum* has not yet been isolated from baits placed above inoculum introduced in June. Results indicate that recovery of *P. ramorum* from infested leaf disks may overestimate the risk soilborne inoculum poses to infection of plants at the soil-surface interface due to significant decreases in sporulation capacity. While cooler and more constant temperatures and moisture levels present at lower depths may contribute to greater survival and sporulation, dispersal from these depths may be limited. This work will continue through the winter and spring, times classically considered a higher risk for the re-establishment of *P. ramorum* from soilborne sources.

RESEARCH

Eyre, C.A. and Garbelotto, M. 2015. Detection, Diversity, and Population Dynamics of Waterborne *Phytophthora ramorum* Populations. Phytopathology. 105(1): 57-68.

Abstract: Sudden oak death, the tree disease caused by *Phytophthora ramorum*, has significant environmental and economic impacts on natural forests on the U.S. west coast, plantations in the United Kingdom, and in the worldwide nursery trade. Stream baiting is vital for monitoring and early detection of the pathogen in high-risk areas and is performed routinely; however, little is known about the nature of water-borne *P. ramorum* populations. Two drainages in an infested California forest were monitored intensively using stream-baiting for 2 years between 2009 and 2011. Pathogen presence was determined both by isolation and polymerase chain reaction (PCR) from symptomatic bait leaves. Isolates were analyzed using simple sequence repeats to study population dynamics and genetic structure through time. Isolation was successful primarily only during spring conditions, while PCR extended the period of pathogen detection to most of the year. Water populations were extremely diverse, and changed between seasons and years. A few abundant genotypes dominated the water during conditions considered optimal for aerial populations, and matched those dominant in aerial populations. Temporal patterns of genotypic diversification and evenness were identical among aerial, soil, and water populations, indicating that all three substrates are part of the same epidemiological cycle, strongly influenced by rainfall and sporulation on leaves. However, there was structuring between substrates, likely arising due to reduced selection pressure in the water. Additionally, water populations showed wholesale mixing of genotypes without the evident spatial autocorrelation present in leaf and soil populations.

Garbelotto, M.; Maddison, E.R.; and Schmidt, D. 2014. SODmap and SODmap Mobile: Two Tools to Monitor the Spread of Sudden Oak Death. Forest Phytophthoras 4(1). DOI: 10.5399/osu/fp.4.1.3560. http://journals.oregondigital.org/ForestPhytophthora/article/view/3560/3335.

Abstract: Sudden oak death (SOD) is caused by *Phytophthora ramorum*, an exotic pathogen introduced multiple times in California, Oregon and Washington. The pathogen has been spreading in California and Oregon forests at relatively modest rates from the initial introduction points, covering mostly distances in the range of hundreds of meters, and only occasionally spreading 3-5 km away from established infestations. In California, oak (*Quercus* spp.) and tanoak (*Notholithocarpus densiflorus*) are infected only when infected California bay laurel (*Umbellularia californica*) trees are within tens of meters, hence, a fine-scale knowledge of infected bays is important to assess the risk for oak infection at any given location. Since 2008, the University of California-Berkeley Forest Pathology Laboratory has been enlisting and training volunteers to survey woodlands for the presence of SOD. Symptomatic leaves are collected by volunteers and tested at U.C. Berkeley. Since 2012, results of these citizen-science efforts have been combined with results of surveys by researchers and government agencies and displayed jointly in the web-based SODmap. In 2013, a mobile app was launched to view SOD distribution in the

field and to determine the risk for oak infection at any given location. This is one of the first large-scale joint efforts between volunteers, government agencies and academia resulting in valuable management tools for a forest disease.

Miles, T.D.; Martin, F.N.; and Coffey, M.D. 2015. Development of Rapid Isothermal Amplification Assays for Detection of *Phytophthora* spp. in Plant Tissue. Phytopathology. 105(2): 265-278.

Abstract: Several isothermal amplification techniques recently have been developed that are tolerant of inhibitors present in many plant extracts, which can reduce the need for obtaining purified DNA for running diagnostic assays. One such commercially available technique that has similarities with real-time polymerase chain reaction (PCR) for designing primers and a labeled probe is recombinase polymerase amplification (RPA). This technology was used to develop two simple and rapid approaches for detection of *Phytophthora* spp.: one genus-specific assay multiplexed with a plant internal control and the other species-specific assays for *Phytophthora ramorum* and *P. kernoviae*. All assays were tested for sensitivity (ranging from 3 ng to 1 fg of DNA) and specificity using DNA extracted from more than 136 *Phytophthora* taxa, 21 *Pythium* spp., 1 *Phytopythium* sp., and a wide range of plant species. The lower limit of linear detection using purified DNA was 200 to 300 fg of DNA in all pathogen RPA assays. Six different extraction buffers were tested for use during plant tissue maceration and the assays were validated in the field by collecting 222 symptomatic plant samples from over 50 different hosts. Only 56 samples were culture positive for *Phytophthora* spp. whereas 91 were positive using the *Phytophthora* genus-specific RPA test and a TaqMan real-time PCR assay. A technique for the generation of sequencing templates from positive RPA amplifications to confirm species identification was also developed. These RPA assays have added benefits over traditional technologies because they are rapid (results can be obtained in as little as 15 min), do not require DNA extraction or extensive training to complete, use less expensive portable equipment than PCR-based assays, and are significantly more specific than current immunologically based methods. This should provide a rapid, field-deployable capability for pathogen detection that will facilitate point-of-sample collection processing, thereby reducing the time necessary for accurate diagnostics and making management decisions.

RELATED ISSUES

Native plant and restoration nurseries are joining the California Department of Food and Agriculture (CDFA) Best Management Practices (BMP) Program in response to detection of *Phytophthora tentaculata,* a newly introduced pathogen in several California native plant nurseries and restoration sites. Outreach to the native plant community through the "Responding to an Expanding Threat: Exotic *Phytophthora* Species in Native Plant Nurseries, Restoration Plantings, and Wildlands" symposium, held 12/2/14 in San Francisco, provided an excellent springboard to encourage nurseries to employ clean stock production practices and participate in the CDFA BMP Program. Numerous nurseries are now implementing BMPs under the guidance of CDFA and NORS-DUC (partners in the statewide program) as well as receiving guidance from

consultants in the field. Plant samples are being collected or submitted voluntarily to the CDFA Plant Pest Diagnostic Lab for disease diagnosis, and customized BMPs are being developed for each nursery. A BMP Manual is under development to provide general guidance to the native plant industry.

RELATED RESEARCH

Weiland, J.E. 2015. First Report of *Phytophthora cactorum* and *P. citrophthora* Causing Root Rot of *Ribes lobbii* in Oregon. Plant Disease. 99(1): 157-157.

Widmer, T.L. 2015. Species Profiles. *Phytophthora palmivora.* Forest Phytophthoras 4(1). DOI: 10.5399/osu/fp.4.1.3557. http://journals.oregondigital.org/ForestPhytophthora/article/view/3557/3332.

OUTREACH

The Fourth Art of Saving Oaks invitational art show will end in February 2015. The show debuted in February 2014 as a featured part of the COMTF "Visualizing Sudden Oak Death" online conference and has been presented exclusively online at www.suddenoakdeath.org. During the course of the year-long exhibit, the works of nine artists have been used to educate and inspire people about SOD. The first "Art of Saving Oaks" was held in Sausalito (2002). Subsequent shows were held at Filoli Gardens, Woodside (2003) and at the UC Santa Cruz Arboretum (2004). This fourth exhibit includes pieces by Stephen Joseph (photography), Anna Dal Pino (oil painting), Kelly Taylor Starr (photography), Jeanne Lotta-Sellars (watercolor), Kathy Kleinsteiber (watercolor and acrylic), Lillian Murphy (watercolor), Bonnie Bonner (watercolor), Katie Bertsche (ink illustration), and Eliza K. Jewett (watercolor and digital).

RESOURCES

The Forest Phytophthoras of the World website has a redesigned home page that makes it easier to navigate, including links to SOD and IUFRO Forest *Phytophthora* conference proceedings and meeting abstracts. The program/abstracts for the IUFRO "Phytophthoras in Forests and Natural Systems" November 2014 meeting in Esquel, Argentina has been posted. The full conference proceedings will be available at this site later this year.