# CALIFORNIA OAK MORTALITY TASK FORCE REPORT
# NOVEMBER 2013

## MONITORING

**The Sudden Oak Death (SOD) pathogen continues to spread and intensify in** Humboldt County, with numerous dead tanoak observed less than 1 mile, within meters of the Six Rivers National Forest and Trinity County line. The USDA Forest Service Pacific Southwest Region, Forest Health Protection 2013 aerial survey final flyover was completed at the end of September and covered areas of the Klamath, Shasta-Trinity, and Mendocino National Forests as well as private lands in Humboldt and Mendocino Counties. In total, the 2013 *P. ramorum* aerial survey identified more than 294,000 dead trees on approximately 47,500 acres. While slightly lower than 2012 totals, elevated mortality levels did continue into 2013.

**The United Kingdom (UK) Forestry Commission has updated its *P. ramorum* larch** outbreak map (http://www.forestry.gov.uk/forestry/infd-86ajqa) to include Northern Ireland. The Galloway Red Zone in southwest Scotland has also been added to the map. The Red Zone is the region of Scotland where the rate and severity of disease spread is too intense for control through tree felling; consequently, this region will have requirements put in place regarding the movement of infected timber and bark. Control by statutory plant health notices requiring sanitation felling will continue elsewhere in Scotland. For more information on the status of the situation in Scotland, go to http://www.forestry.gov.uk/forestry/infd-9bglrr.

## RESEARCH

**Fahlgren, N.; Bollmann, S.R.; Kasschau, K.D.; Cuperus, J.T.; Press, C.M.; et al.** 2013. *Phytophthora* Have Distinct Endogenous Small RNA Populations That Include Short Interfering and MicroRNAs. PLoS ONE. 8(10): e77181. DOI: 10.1371/journal.pone.0077181.

Abstract: In eukaryotes, RNA silencing pathways utilize 20-30-nucleotide small RNAs to regulate gene expression, specify and maintain chromatin structure, and repress viruses and mobile genetic elements. RNA silencing was likely present in the common ancestor of modern eukaryotes, but most research has focused on plant and animal RNA silencing systems. *Phytophthora* species belong to a phylogenetically distinct group of economically important plant pathogens that cause billions of dollars in yield losses annually as well as ecologically devastating outbreaks. We analyzed the small RNA-generating components of the genomes of *P. infestans*, *P. sojae*, and *P. ramorum* using bioinformatics, genetic, phylogenetic and high-throughput sequencing-based methods. Each species produces two distinct populations of small RNAs that are predominantly 21- or 25-nucleotides long. The 25-nucleotide small RNAs were primarily derived from loci encoding transposable elements and we propose that these small RNAs define a pathway of short-interfering RNAs that silence repetitive genetic elements. The 21-nucleotide small RNAs were primarily derived from inverted repeats, including a novel microRNA family that is conserved among the three species, and several gene families, including

Crinkler effectors and type III fibronectins. The *Phytophthora* microRNA is predicted to target a family of amino acid/auxin permeases, and we propose that 21-nucleotide small RNAs function at the post-transcriptional level. The functional significance of microRNA-guided regulation of amino acid/auxin permeases and the association of 21-nucleotide small RNAs with Crinkler effectors remains unclear, but this work provides a framework for testing the role of small RNAs in *Phytophthora* biology and pathogenesis in future work.

**Hearst, C.; Nelson, D.; McCollum, G.; Sharma, S.; and Rao, J.R. 2013. Forest Fairy** Ring Fungi *Clitocybe nebularis*, Soil *Bacillus* spp., and Plant Extracts Exhibit *in Vitro* Antagonism on Dieback *Phytophthora* Species. Natural Resources. 4: 189-194. DOI: 10.4236/nr.2013.42025.

Abstract: *In vitro* Kirby-Bauer disc-diffusion assays coupled with bio-imaging software techniques were used to assess native forest dwelling "fairy ring" forming fungi (*Clitocybe nebularis*) and co-habitant forest tree-root colonizing non-pathogenic, antibiotic producing bacteria (*Bacillus licheniformis*, *Bacillus pumilis*) for their antagonism towards Japanese larch dieback oomycetes phytopathogens which also affects ornamental alternative hosts. The aqueous extracts of *C. nebularis* exhibited the highest clearance (inhibitory) zone of 21.4 × 105 pixels = 573%) against *Phytophthora ramorum* than growth/clear zone Cartesian integrates recorded in untreated (control) disc (3.7 × 105 pixels = 100%) over 3-day incubation. The fairy ring fungal extracts also exhibited substantive antagonism against *P. kernoviae* (147%), *P. lateralis* (347%) and a solanaceous crop infecting *P. infestans* (86%). Quite encouragingly, the soil oomycete phytopathogen *P. ramorum* was inhibited strongly (mean ~ 177%) by both forest bacilli. Aqueous extracts of non-forest antifungal herbaceous plants (garlic and elderberry) expressed similar inhibitory effects (mean ~ 70%). A seaweed fungal elicitor component fucoidan showed moderate levels (mean ~ 85%) of antagonism against *P. ramorum*, *P. kernoviae*, *P. lateralis* and *P. infestans*. The results in this *in vitro* study highlight the intensity and vigor of antagonistic forest microflora and non-forest herbaceous antifungal agents such as garlic and other plant extracts as serious candidates for suppression of the oomycete *Phytophthora* pathogenic fungi in forest soils. This study calls for urgent scoping and impact assessment studies in pot experiments and mini-plot forest trials to gauge the fitness of these natural resources for field level potential biotechnological applications to combat the devastating dieback disease in the native woodlands and horticulture.

**McPherson, B.A.; Mori, S.R.; Opiyo, S.O.; Conrad, A.O.; Wood, D.L.; and Bonello,** P. 2013. Association Between Resistance to an Introduced Invasive Pathogen and Phenolic Compounds That may Serve as Biomarkers in Native Oaks. Forest Ecology and Management. *In Press*.

Abstract: California coast live oaks (*Quercus agrifolia* Nee) have suffered large losses from sudden oak death, caused by the introduced oomycete *Phytophthora ramorum*. Infected mature coast live oaks exhibit cankers on the main stem that produce a viscous

sap-derived exudate, referred to as bleeding. Subsequent attacks by ambrosia and bark beetles, followed by the activity of fungi introduced by these insects, have led to mortality levels greater than 50% since the mid-1990s. Despite an infection rate of 5% year$^{-1}$, asymptomatic trees still persist in many heavily infected stands after more than 15 years of exposure to the pathogen. We hypothesized that varying responses to *P. ramorum*, including apparent recovery from infections, reflected phenotypic differences in susceptibility. In this study we evaluated the relationship between the early development of symptoms in mature trees that were experimentally inoculated with *P. ramorum* and long-term survival. A logit model showed that external canker lengths measured 9 months following inoculation predicted survival 7 years later. We defined resistance to *P. ramorum* in the surviving trees as absence or cessation of bleeding after the 7 years of the study and absence of beetle attacks on bleeding trees. Probability of resistance was also predicted by external canker length measured 9 months after inoculation. Canker length distribution was consistent with quantitative resistance to *P. ramorum*. The role of plant chemistry in resistance was examined by quantifying soluble phenolics in phloem methanol extracts prepared from the surviving trees. A logistic regression model found that expression of resistance was associated with four phenolic compounds; ellagic acid and a partially characterized ellagic acid derivative, and two chromatographic peaks that represent two uncharacterized phenolic compounds. Ellagic acid and a crude methanol extract from coast live oak phloem (total phenolics) were fungistatic when assayed in vitro at physiologically relevant levels and total phenolics were fungicidal at the highest concentration tested. The association of certain phenolics with resistance may facilitate the use of biomarkers in minimally invasive assays to predict the response of trees to *P. ramorum*, thereby increasing the options for managing threatened forests.

**Osmundson, T.W.; Eyre, C.A.; Hayden, K.M.; Dhillon, J.; and Garbelotto, M.M.** 2013. Back to Basics: An Evaluation of NaOH and Alternative Rapid DNA Extraction Protocols for DNA Barcoding, Genotyping, and Disease Diagnostics From Fungal and Oomycete Samples. Molecular Ecology Resources. 13: 66–74. DOI: 10.1111/1755-0998.12031.

Abstract: The ubiquity, high diversity and often-cryptic manifestations of fungi and oomycetes frequently necessitate molecular tools for detecting and identifying them in the environment. In applications including DNA barcoding, pathogen detection from plant samples, and genotyping for population genetics and epidemiology, rapid and dependable DNA extraction methods scalable from one to hundreds of samples are desirable. We evaluated several rapid extraction methods (NaOH, Rapid one-step extraction (ROSE), Chelex 100, proteinase K) for their ability to obtain DNA of quantity and quality suitable for the following applications: PCR amplification of the multicopy barcoding locus ITS1/5.8S/ITS2 from various fungal cultures and sporocarps; single-copy microsatellite amplification from cultures of the phytopathogenic oomycete *Phytophthora ramorum*; probe-based *P. ramorum* detection from leaves. Several methods were effective for most of the applications, with NaOH extraction favored in terms of success rate, cost, speed and simplicity. Frozen dilutions of ROSE and NaOH extracts

maintained PCR viability for over 32 months. DNA from rapid extractions performed poorly compared to CTAB/phenol-chloroform extracts for TaqMan diagnostics from tanoak leaves, suggesting that incomplete removal of PCR inhibitors is an issue for sensitive diagnostic procedures, especially from plants with recalcitrant leaf chemistry. NaOH extracts exhibited lower yield and size than CTAB/phenol-chloroform extracts; however, NaOH extraction facilitated obtaining clean sequence data from sporocarps contaminated by other fungi, perhaps due to dilution resulting from low DNA yield. We conclude that conventional extractions are often unnecessary for routine DNA sequencing or genotyping of fungi and oomycetes, and recommend simpler strategies where source materials and intended applications warrant such use.

**Preuett, J.A.; Collins, D.J.; Luster, D.G.; and Widmer, T.L. 2013. Screening** Selected Gulf Coast Forest Species for Susceptibility to *Phytophthora ramorum*. Online. Plant Health Progress. DOI: 10.1094/PHP-2013-0730-01-RS.

Abstract: *Phytophthora ramorum*, the causal agent of sudden oak death, poses a threat to woody plants in the rest of the United States. Several plant species native to Gulf Coast and southeastern US forests were tested for reaction to *P. ramorum*, including eastern baccharis (*Baccharis halmifolia*), spicebush (*Lindera benzoin*), yaupon (*Ilex vomitoria*), southern magnolia (*Magnolia grandiflora*), sweetbay magnolia (*M. virginiana*), Virginia creeper (*Parthenocissus quinquefolia*), black willow (*Salix nigra*), and baldcypress (*Taxodium distichum*). The foliage of each species was inoculated with a zoospore suspension and placed in a dew chamber for 5 days. The average percentage of leaf area necrosis was 0.2, 4.9, 27.9, 32.1, 8.6, 1.5, 1.1, 0.2, and 5.0% for inoculated eastern baccharis, spicebush, yaupon, southern magnolia, sweetbay magnolia, Virginia creeper (Louisiana), Virginia creeper (Maryland), black willow, and baldcypress, respectively. Comparison of the percent necrotic leaf area between inoculated and non-inoculated plants showed significant differences ($P \leq 0.05$) for yaupon ($P = 0.0008$), southern magnolia ($P = 0.001$), and sweetbay magnolia ($P = 0.0009$). The other species did not show significant differences although infection was confirmed on spicebush, Virginia creeper, and baldcypress. This is a first report of yaupon, sweetbay magnolia, and baldcypress being hosts of *P. ramorum*.

## FUNDING

**The Scottish government recently allocated nearly $1.5 million to help tackle the** outbreak of *P. ramorum* on Japanese larch in Scotland, which has progressed so rapidly in the southwest that most of the Japanese larch trees in Galloway will likely need to be felled over the next 2 to 3 years. Aerial surveys in May and early June 2013 revealed a major expansion of the pathogen in the Dumfries and Galloway area, where 9,800 – 14,800 acres of larch are now likely infected. Eradication in Scotland is no longer considered achievable; therefore, focus has turned to containment and slow the spread efforts.

## RELATED RESEARCH

**Carmichael, P. and Tscholl, M. 2013. Cases, Simulacra, and SemanticWeb** Technologies. Journal of Computer Assisted Learning. 29(1): 31–42.

Sudden oak death in California is presented as an example of an interactive exhibit-based SemanticWeb application displaying distribution of affected species, possible co-factors, and climate data. A 'pop-up' allows users to introduce further data from other online sources and see them displayed on the map.

**Gao, R. and Zhang, G. 2013. Potential of DNA Barcoding for Detecting Quarantine** Fungi. Phytopathology. 103(11): 1103-1107.

**Kasuga, T. and Gijzen, M. *In Press.* Epigenetics and the Evolution of Virulence.** Trends in Microbiology. DOI 10.1016/j.tim.2013.09.003.

The paper reviews progress in understanding genome-embedded transposable elements for *Phytophthora ramorum* and other *Phytophthora* species.

**Mazur, R.; Klimley, A.P.; and Folger, K. 2013. Implications of the Variable** Availability of Seasonal Foods on the Home Ranges of Black Bears, *Ursus americanus*, in the Sierra Nevada of California. Animal Biotelemetry. 1:16. DOI: 10.1186/2050-3385-1-16.

Although the paper is on wildlife food sources in the Sierra Nevada, sudden oak death is discussed as a potential threat to black bear food sources.

**Parnell, S.; Gottwald, T.R.; Riley, T.; and van den Bosch, F. *In press*. A Generic** Risk-Based Surveying Method for Invading Plant Pathogens. Ecological Applications. http://dx.doi.org/10.1890/13-0704.1.

**Quinn, L.; O'Neill, P.A.; Harrison, J.; Paskiewicz, K.H.; McCracken, A.R.; Cooke,** L.R.; Grant, M.R.; and Studholme, D.J. 2013. Genome-wide sequencing of *Phytophthora lateralis* Reveals Genetic Variation Among Isolates From Lawson Cypress (*Chamaecyparis lawsoniana*) in Northern Ireland. FEMS Microbiology Letters. 344: 179–185. DOI: 10.1111/1574-6968.12179.

**Science for Environment Policy (a journal of the European Commission), Invasive** Alien Species special issue. September 2013. Issue 41. Available online at http://ec.europa.eu/environment/integration/research/newsalert/pdf/41si.pdf.

**Voggesser, G.; Lynn, K.; Daigle, J.; Lake, F.K.; and Ranco, D. 2013. Cultural** Impacts to Tribes From Climate Change Influences on Forests. Climatic Change. 120:615–626. DOI: 10.1007/s10584-013-0733-4.

Sudden oak death's impact on Native American tribes is discussed as an example of the effects of climate change on cultural values.

## EDUCATION AND OUTREACH

**In its sixth year, the 2013 Sudden Oak Death Blitzes were the largest to date, with** over 500 participants surveying over 13,000 trees and collecting samples from over 2,000 of them in 16 regions. This year's findings include identifying new outbreaks in southern Mendocino County, northern Sonoma County, and Golden Gate Park (near AIDS Memorial Grove) as well as significant increases in bay infection in Santa Cruz and San Matteo Counties (around South Skyline Blvd). Oak infections were also detected in the East Bay hills on eastern and western slopes, with the infection on the western slopes initially detected on bay during the 2011 SOD Blitz. This year, blitz participants tagged bay trees to track infection status over time on individual trees that are continuously infected (especially in dry years), as they are a key source of sustainable pathogen inoculum.

The continuous and increasing SOD Blitz volunteer effort has helped improve SOD spread prediction accuracy and an understanding of which factors most affect spread. Additionally, the large blitz database has facilitated the creation of SODmap (a detailed disease distribution map) and SODmap Mobile (a smart phone app used to identify trees infected at the time of sampling and to determine risk of oak infection). Continuing this effort will further improve successful infection prediction, determining which trees may be *P. ramorum* carriers during dry seasons, and early identification of new outbreaks. For more information on the SOD Blitzes and SODmap, go to www.sodblitz.org and www.sodmap.org.

## MEETINGS

**"Visualizing Sudden Oak Death," to be held in February 2014, is an e-conference** that will provide attendees with updates on research and management while also illustrating disease impacts and ecology. A SOD status webinar, Google Hangout with SOD experts, videos, and photo essays will be featured. The e-conference will be hosted at the COMTF website and is being co-organized by the California Oak Mortality Task Force and the USDA Forest Service, Pacific Southwest Research Station. SOD art and the fourth "Art of Saving Oaks" online gallery will display how artists have interpreted SOD and the beauty of California coastal forests. More details will be available in January 2014. Please check the COMTF website for updates (www.suddenoakdeath.org) or contact Katie Palmieri at kpalmieri@berkeley.edu.

## CALENDAR OF EVENTS

**11/9 - SOD Treatment Training Workshop; Foothills Park; 3300 Page Mill Road,** Los Altos Hills; 10:00 a.m. – 12:00 p.m.; For more information, or to register, contact Sue Welch at sodblitz09@earthlink.net.

**11/12 - SOD Treatment Training Workshop; San Francisco Presidio; Location to be** Determined; 8:30 – 10:30 a.m.; For more information, go to http://nature.berkeley.edu/garbelotto/english/sodblitzfollowup.php.

**11/12 - SOD Treatment Training Workshop; Fort Bragg Town Hall, Fort Bragg;** 6:30 – 8:30 p.m.; For more information, or to register, contact Lori Hubbart at lorih@mcn.org.

**11/13 - SOD Treatment Training Workshop; Santa Lucia Preserve; Time and** Location to be Determined; For more information, go to http://nature.berkeley.edu/garbelotto/english/sodblitzfollowup.php.

**11/16 - SOD Treatment Training Workshop; Dominican University, 155 Palm Ave.,** Joseph R. Fink Science Center, Room 102, San Rafael; 10:00 a.m. – 12:00 p.m.; For more information, or to register, contact Kristin Jacob at kristinjakob@att.net.

**11/16 - SOD Treatment Training Workshop; Pelusi Building, 2296 Streblow Drive** at Kennedy Park, Napa; 1:00 – 3:00 p.m.; For more information, or to register, contact Bill Pramuk at info@billpramuk.com.

**11/17 - SOD Treatment Training Workshop; Cal Fire Saratoga Summit Fire Station** 21; 12900 Skyline Blvd, Los Gatos; 10:00 a.m. – 12:00 p.m.; For more information, contact Jane Manning at skyline_sod@yahoo.com.

**11/23 - SOD Treatment Training Workshop; Montalvo Arts Center, 15400** Montalvo Road, Saratoga; 10:00 a.m. – 12:00 p.m.; For more information, contact Kelly Sicat at KSicat@montalvoarts.org.

**11/24 - SOD Treatment Training Workshop; Joaquin Miller Park, Oakland; 1:00 –** 3:00 p.m.; For more information, or to register, contact Kimra McAfee at coordinator@sausalcreek.org.

**11/10 – 11/14/14 - Seventh meeting of the IUFRO Working Party 7.02.09** "*Phytophthora* in Forests and Natural Ecosystems;" Esquel, Argentina. For more information, registration, or abstract submission details, go to http://www.iufrophytophthora2012.org/.