CALIFORNIA OAK MORTALITY TASK FORCE REPORT
JULY 2004

MONITORING

A PCR-positive sample for *Phytophthora ramorum* from Nassau County, NY was confirmed by the USDA Animal and Plant Health Inspection Service (APHIS) on 6/25. Follow-up surveys are being conducted by a team from the USDA's Forest Service (FS) and APHIS, as well as the New York Department of Agriculture and Markets. The lone nested PCR-positive mature red oak tree is located in a 192 acre hardwood and oak forest county park. Further surveys are underway to identify other possible infected plants and infested sites. Laboratory work continues in an effort to obtain a *P. ramorum* culture from the tree, soil, or water in the area. The current *P. ramorum* regulations require regulatory action to be taken based on nested PCR results only. The USDA APHIS is finalizing its not yet announced regulatory response to this detection.

LEGISLATION

U.S. Senators Barbara Boxer (D-CA) and Gordon Smith (R-OR) introduced the Sudden Oak Death Syndrome Control Act of 2004 (S. 2575) on June 24, 2004. The legislation authorizes the U.S. Department of Agriculture (USDA) to continue its research program, develop methods to control, manage, and eradicate Sudden Oak Death (*Phytophthora ramorum*). If passed, the bill would require the USDA to conduct management, treatment, and fire prevention activities and establish public education and outreach. Additionally, under the legislation, the USDA would regularly meet with federal, state, tribal, and local government officials to exchange information and recommendations. The Act authorizes $44.2 million annually, including $25 million for USDA research, regulation, and monitoring; $18.5 million for management, treatment, and fire; and $700,000 for education and outreach. For the complete text, see http://thomas.loc.gov/ and query for S. 2575.

U.S. Representatives Max Burns (R-GA) and David Scott (D-GA) introduced legislation (HR 4569) supporting and expanding efforts to halt the spread of the *Phytophthora ramorum* on 6/15. The legislation authorizes the USDA to identify all the possible host plants for this disease; determine how widespread the outbreak has become; research past and current control, quarantine, and hazardous fuel reduction methods; and identify a workable national plan with cost estimates to eradicate the disease. For the complete text see http://thomas.loc.gov/ and query for HR 4569.

NURSERIES

Trace-forward surveys from nursery shipments are still underway, and the national survey continues. Of the trace-forward, national, and other surveys conducted to date, 140 sites in 19 states have had *P. ramorum* detections. Positive findings by state are: CA(45), AL(3), AR(1), FL(6), WA(18), OR(9), TX(10), CO(1), GA(13), LA(6), MD(2), NC(9), NM(1), TN(2), PA(1), NJ(1), NY(1), SC(1), and VA(1).

REGULATIONS

The Oregon Department of Agriculture (ODA) adopted a temporary rule on June 23rd, calling for all Oregon growers and dealers of plants susceptible to *P. ramorum* to be annually inspected, tested, and certified free from the pathogen before host plants are allowed to be sold. The new regulation comes at the request of Oregon´s nursery industry and is in response to the discovery of *P. ramorum* on plants in a Columbia County nursery that were identified in April, 2004 as part of a trace-back survey from Maryland.

The presence of *P. ramorum* at the Columbia County nursery was confirmed on May 18, 2004, following surveying, sampling, culturing, and nested PCR confirmation. Anna Kruschke and Catawbiense Grandiflorum *Rhododendron* cultivars were confirmed positive. Following the confirmation, Oregon officials performed delimitation surveys within (4,240 samples tested) and outside of the Columbia County nursery (720 samples tested). Within the nursery, *P. ramorum* was recovered from a composite potting media sample taken from several plants in one hoop house. Of the samples taken outside of the nursery, *P. ramorum* was confirmed on three *Rhododendron* 'Anna Kruschke' that had been planted four weeks prior to the delimitation survey at the entrance of a neighboring business. The plants had been donated to the business from the nursery.

In addition to the statewide temporary rule, the ODA also adopted an emergency 90-day quarantine on June 6th for all nurseries and compost production facilities in Columbia County. The quarantine requires nurseries in the county to have all *P. ramorum*-susceptible plants tested and found free from the pathogen before they are available for sale. This requirement applies to in-state and out-of-state shipments. The quarantine also prohibits sale or shipment of potting media and compost produced in the county that contains material from susceptible plants, unless the production facility is inspected and found free of *P. ramorum*. Additionally, the composted material must not have originated from areas already generally infested with the pathogen. Finally, commercially produced compost containing susceptible plant material cannot be sold to nurseries unless it is sterilized by a method approved by ODA.

For more information, contact Dan Hilburn, Oregon Department of Agriculture, at dhilburn@oda.state.or.us or (503) 986-4663.

The USDA Animal and Plant Health Inspection Service (APHIS) amended the regulation of *Camellia* on 6/23 to include all species, hybrids, and cultivars. The amendment came as the result of 7 new species (10 total) and 18 new hybrids (19 total) of *Camellia* that have been found to be infected with *P. ramorum.*

APHIS added *Clintonia andrewsiana* (Andrew's clintonia bead lily), *Dryopteris* *arguta* (California wood fern), *Smilacina racemosa* (false Solomon's seal), and *Taxus brevifolia* (Pacific yew) to the *P. ramorum* associated host list on 6/23, as the pathogen has been isolated from each plant type in native California settings. Following the

completion and confirmation of Koch's Postulates, the associated hosts will be reclassified as hosts.

RESEARCH

On June 10, 2004, the US Department of Energy (DOE) Joint Genome Institute (JGI) and the Virginia Bioinformatics Institute (VBI) announced the completion of the DNA sequencing of *Phytophthora ramorum* and *Phytophthora sojae* (a soybean disease). Having sequenced both *Phytophthoras*, researchers will be able to compare the genome of each, gaining a greater understanding of the uniqueness or similarities between these pathogens. The new information will provide a framework for the identification of cellular processes that can be targeted for diagnostics, in addition to safe and effective applications for use in disease treatment and/or control. Currently, the USDA Forest Service, Pacific Southwest Research Station, is funding research at JGI using the sequence information for the development of a rapid, effective field detection system for *P. ramorum* (see DOYLE below.).

The sequencing project for these two *Phytophthoras* received nearly $4 million in support from the U.S. Department of Agriculture (USDA), the National Science Foundation (NSF), and the DOE. The sequences of these organisms can be found on the JGI portal at http://genome.jgi-psf.org/ramorum/ and at http://genome.jgi-psf.org/sojae/.

Funded projects for the FY'04 USDA Forest Service Pacific Southwest Region (PSW), *P. ramorum* Request for Proposals have been selected. Forty-one proposals were submitted in response to the request, for a total of over $4 million. PSW funded 15 proposals, allocating slightly over $1 million during this funding cycle. Projects chosen represent a broad array of needed research, and include research in the eastern US as well as international interests.

The USDA Animal and Plant Health Inspection Service (APHIS) convened a *Phytophthora ramorum* science panel June 29-30 at the Center for Plant Health Science and Technology, North Carolina State University, in Raleigh, NC. The meeting brought together approximately 75 university, federal, and state scientists, as well as regulators from North America and Europe with expertise on *P. ramorum* as well as other *Phytophthora* species. The meeting objective was to compile the best scientific information available in the areas of survey and detection, diagnostics, biology, etiology and epidemiology, and control and risk mitigation.

"Mapping the Risk of Establishment and Spread of Sudden Oak Death in California" by Ross Meentemeyer, David Rizzo, Walter Mark, and Elizabeth Lotz is in press and will be published in Forest Ecology and Management. The abstract follows.

Sudden Oak Death, caused by the recently described pathogen *Phytophthora ramorum*, is an emerging forest disease that has reached epidemic levels in coastal forests of central California. We present a rule-based model of *P. ramorum* establishment and spread risk in California plant communities. The model, which is being used as a management tool to

target threatened forests for early-detection monitoring and protection, incorporates the effects of spatial and temporal variability of multiple variables on pathogen persistence. Model predictions are based on current knowledge of host susceptibility, pathogen reproduction, and pathogen transmission with particular regard to host species distribution and climate suitability. Maps of host species distributions and monthly weather conditions were spatially analyzed in a GIS and parameterized to encode the magnitude and direction of each variable's effect on disease establishment and spread. Spread risk predictions were computed for each month of the pathogen's general reproductive season and averaged to generate a cumulative risk map (Fig. 6a and b). The model identifies an alarming number of uninfected forest ecosystems in California at considerable risk of infection by *Phytophthora ramorum*. This includes, in particular, a broad band of high risk north of Sonoma County to the Oregon border, a narrow band of high risk south of central Monterey County south to central San Luis Obispo County, and scattered areas of moderate and high risk in the Sierra Nevada foothills in Butte and Yuba counties. Model performance was evaluated by comparing spread risk predictions to field observations of disease presence and absence. Model predictions of spread risk were consistent with disease severity observed in the field, with modeled risk significantly higher at currently infested locations than at uninfested locations (p,0.01, n=323). Based on what is known about the ecology and epidemiology of Sudden Oak Death, this model provides a simple and effective management tool for identifying emergent infections before they become established.

MANAGEMENT

Northeastern state foresters met in Windsor Locks, CT on June 30, 2004 to discuss the status of Sudden Oak Death (SOD) and Emerald Ash Borer (EAB) and increase the awareness of potential impacts of these diseases on northeastern forests. The meeting provided a forum for states to share their experiences in dealing with SOD and EAB, as well as assist in identifying local needs, such as response planning, funding, public education and outreach, agency coordination, and restoration efforts. While planning for these exotic pests, the development of an overall emergency response plan for future state forest health emergencies, as well as other natural emergencies, was also addressed.

RESOURCES

The Oregon State University (OSU) Extension and Experiment Station has developed a new website on information about Sudden Oak Death in Oregon. Postings include technical documents, magazine articles and OSU news releases. New information will continue to be added. To view the site, go to: http://extension.oregonstate.edu/emergency/oak_death.php .

National Invasive Species Council has featured Sudden Oak Death as the "Invasive of the month" for July. See http://www.invasivespecies.gov/ismonth/sod.html

PERSONNEL

Dr. Niklaus J. Grünwald, research plant pathologist, recently joined the USDA Agricultural Research Service (ARS) Horticultural Crops Research Laboratory staff, located on the Oregon State University campus. As a Research Plant Pathologist,

Niklaus will conduct research on nursery crop diseases, including the biology, epidemiology, and control of *Phytophthora ramorum*. Grünwald received his Ph.D. from the University of California, Davis, where he worked under the direction of Ariena H. C. van Bruggen, studying the ecology of damping-off pathogens during decomposition of cover crops. He subsequently worked as a post-doctoral scientist with William E. Fry in the Department of Plant Pathology at Cornell University. While at Cornell, Nik worked in Toluca, Mexico, conducting research on the biology, epidemiology, and population genetics of *Phytophthora*. Most recently he worked as a Research Plant Pathologist for the Vegetable and Forage Crops Research Unit, USDA ARS, at Prosser, WA, where he conducted research on soil-borne diseases of edible legumes with emphasis on *Aphanomyces euteiches*, *Fusarium solani*, and *Sclerotinia sclerotiorum*. Nik may be reached at 541/738-4049.

Washington State Department of Agriculture (WSDA) Pathologist Art Wagner will be leaving his position on July 15, 2004. The new WSDA plant pathologist, Jennifer Falacy, started July 1, 2004. Jennifer previously worked in the Gary Grove laboratory at Washington State University. She can be reached via e-mail at jfalacy@agr.wa.gov or by phone at the lab (360) 586-5309.

Kelly Ivors, post-doc in Matteo Garbelotto's lab at UC-Berkeley, has accepted an assistant professor position of plant pathology with North Carolina State University. Kelly will be located at the Mountain Horticultural Crops Research and Extension Center near Asheville, NC and will conduct pathology-related research on the economically important crops of western North Carolina, including vegetables, ornamentals (nursery crops), Christmas tress, and tobacco. While at Berkeley, Kelly investigated the population genetics of European and North American *P. ramorum* isolates, as well as on developing DNA-based techniques to distinguish the two geographical populations. Kelly also developed a molecular test to detect *P. ramorum* in environmental plant extracts, and worked briefly in Peter Bonants' lab in Wageningen, The Netherlands on a collaborative project. She provided genotyping, training, and other services to regulatory agencies, other researchers, nurserymen, arborists, and forest managers. Kelly can be reached after July 20th at: kelly_ivors@ncsu.edu.

CALENDAR OF EVENTS

7/7/04 - San Joaquin Valley training session on "*Phytophthora ramorum* in Nurseries: Diagnosis and Control." This free one-day class in Modesto will be dedicated to pathogen recognition, regulations, and other *P. ramorum* nursery topics. For more information, contact Karl Buermeyer, southern COMTF outreach coordinator, at (831) 763-8012 or krbuermeyer@ucdavis.edu. Further details are posted to the COMTF website at www.suddenoakdeath.org.

10/1/04 – Submission deadline for second Sudden Oak Death Science Symposium abstracts of proposed papers or posters. For more information, contact Joni Rippee, UC Berkeley Center for Forestry at rippee@nature.berkeley.edu or http://nature.berkeley.edu/forestry/sodsymposium.

1/18 – 21/05 - Second Sudden Oak Death Science Symposium, Marriott Hotel, Monterey, CA. For Symposium program content, contact Rick Standiford, UC Berkeley Center for Forestry, at standifo@nature.berkeley.edu or Pat Shea, USDA Forest Service Pacific Southwest Research Station, at pjshea@davis.com. Updates on the meeting will be posted at http://nature.berkeley.edu/forestry/sodsymposium.

FEATURED RESEARCH

Developing Tools for Field Detection of *Phytophthora ramorum*, the causative agent of Sudden Oak Death - Sharon A. Doyle, DOE Joint Genome Institute (sadoyle@lbl.gov)

The goal of this project is to use genomic information to develop reagents for a rapid and sensitive field test for *Phytophthora ramorum*. This test could be used to diagnose infected plants, soil, and water, and to mitigate the spread of *P. ramorum*.

The basis of this rapid test is the production of reagents that will detect specific proteins, called elicitins, secreted by *Phytophthora ramorum*. These proteins are abundant at the plant-pathogen interface. Identification of proteins instead of the genes allows for the in-field aspect of the system, since secreted or extra-cellular membrane proteins can be detected directly and do not require extensive laboratory manipulation, such as extraction. In addition, protein detection may distinguish between live or dead *P. ramorum*, which is not possible when DNA detection methods are used.

The reagents that will be generated are called DNA aptamers. Aptamers are short, single-stranded DNA molecules that fold into 3D structures and bind very selectively and specifically to their selected proteins. The use of DNA aptamer reagents instead of traditional immunological (antibody-based) methods for protein detection provides key advantages. DNA-based reagents offer optimal stability, affinity and specificity, and are optimal for use in a field tool.

The first step in developing such a test is to search the genome of *P. ramorum* for biomarkers, or genes that encode secreted proteins with unique sequences. Once these sections of the genome are identified and isolated, they are cloned to produce recombinant proteins in the lab.

The next step is to generate DNA aptamers that bind the target proteins and signal the presence of *P. ramorum*. The aptamers will first be tested in the laboratory to determine whether they specifically recognize proteins from *P. ramorum*. Then the aptamers will be tested with field samples containing *P. ramorum* from plant material.

The final step is to use these reagents to create a diagnostic tool for use in the field. The DNA aptamers generated by this project will be transferred to engineers for development of a rapid field diagnostic kit. It is unsure exactly how such a field kit would be designed, but the benefit of a rapid field diagnostic tool for *P. ramorum* is obvious.

The first step of this process is underway, and hopefully the second step will take place in the next 12-24 months. Once the information is handed off to the engineers for the development of the kit, the turnaround time will depend on how complicated and technical the kit may be. Similar ideas are being developed by government and commercial labs as well, so the ultimate time until a field kit is available may be shorter.

The project is funded by the USDA Forest Service, Pacific Southwest Research Station.