### **PHYTOPHTHORA RAMORUM:**

## A REVIEW OF NORTH AMERICAN AND EUROPEAN ACTIVITY

Spring 2004 Meeting of the California Oak Mortality Task Force Sonoma State University, Rhonert Park March 10, 2004

Sponsored by: The California Oak Mortality Task Force (COMTF) Sonoma State University USDA Forest Service, Pacific Southwest Region California Department of Forestry and Fire Protection University of California, Center for Forestry, Berkeley University of California Cooperative Extension, Marin County Sonoma County Agricultural Commissioner's Office Kendall Jackson Wine Center, Santa Rosa

### TABLE OF CONTENTS

Welcome and Introduction
Mark Stanley, COMTF Chairman
North American Activity and Updates
Spring 2004 Update on Sudden Oak Death/ <i>Phytophthora ramorum</i> in California Susan Frankel, USDA FS State & Private Forestry4
An Update on Sudden Oak Death in Oregon – March 2004 Alan Kanaskie, Oregon Department of Forestry
The Status of <i>Phytophthora ramorum</i> in Washington State Art Wagner, WSDA7
Phytophthora ramorum – An East Coast Update Steve Oak, USDA FS, Southern Region
Sudden Oak Death, an Update from Canada Shane Sela, CFIA9
European Activity and Updates
UK <i>Phytophthora ramorum</i> Research Program Alan Inman, UK DEFRA12
Current status of & regulatory action against <i>Phytophthora ramorum</i> in the UK Stephen Hunter, UK DEFRA
Phytophthora ramorum in the Netherlands - an Update Maarten Steeghs, Plant Protection Service, Netherlands
Investigating the global population structure of <i>Phytophthora ramorum</i> Kelly Ivors, UC Berkeley
Current status of <i>Phytophthora ramorum</i> on trees; Future research direction Joan Webber, UK Forestry Commission
Foliage Susceptibility of UK Trees to <i>Phytophthora ramorum</i> Sandra Denman, UK Forestry Commission14

### Dispersal and Spread

Investigating the Role of Chlamydospores in the Spread of <i>Phytophthora ramorum</i> Nina Shishkoff, USDA ARS
Transmission of <i>Phytophthora ramorum</i> : Practical Applications Jennifer Davidson, USDA FS PSW16
Predicting the Risk of Establishment of <i>Phytophthora ramorum</i> across California Ross Meentemeyer, Sonoma State University17
The Role of Vertebrates as Dispersal Agents of <i>Phytophthora ramorum</i> Hall Cushman, Sonoma State University
Monitoring and Early Detection
The biology of invasive species – what it tells us about <i>Phytophthora ramorum</i> Dave Rizzo, UC Davis
Phytophthora ramorum research at the UC Berkeley Forest Pathology and Mycology Laboratory, and implications for management Matteo Garbelotto, UC Berkeley
Persistence and Spread of <i>Phytophthora ramorum</i> in Oregon Forests Everett Hansen, OSU19

#### SPRING 2004 UPDATE ON SUDDEN OAK DEATH/PHYTOPHTHORA RAMORUM IN CA

Susan J. Frankel, Plant Pathologist, USDA Forest Service, Pacific Southwest Region, State and Private Forestry, Forest Health Protection, Vallejo

Sudden Oak Death-infested forests are scattered along California's central coast from Big Sur (Monterey County) to Southern Humboldt County. Some of the infections are recent, with symptoms appearing as dead tanoaks with crowns comprised of bright, brick-red leaves. Trees in older infestations are slowly deteriorating, with many snapped off near their base. The incidence of *P. ramorum* is increasing in California: the pathogen is common in Sonoma, Marin, Santa Cruz, and the Big Sur area of Monterey County. Over 1,000 *P. ramorum* wildland isolations are on record from laboratories at the California Department of Food and Agriculture, UC Davis, and UC Berkeley.

The California Oak Mortality Task Force's Sudden Oak Death management strategy divides the state into two regions with different objectives:

1. Northern California (Del Norte, Humboldt, and Mendocino Counties) - *P. ramorum* wildland infections are few and isolated from other infestations. Our strategy here is a more aggressive slow-the-spread program. Early detection monitoring is extensive to detect new infestations for treatment. A suppression project was carried out in Southern Humboldt County to remove symptomatic plants in an old growth redwood forest near Redway in February 2004.

2. Central coastal California (Sonoma, Marin, Napa, Santa Cruz, and Monterey Counties) - *P. ramorum* is common so the strategy is containment to keep the pathogen from spreading to new areas. Various efforts have limited pathogen spread including wash stations, regulatory enforcement, best management practices, education and outreach. Details for many of these programs may be found at <u>www.suddenoakdeath.org</u>.



Humboldt County – Gathering removed *P. ramorum*infested plant material (Photo by: UCCE/CDF)



Humboldt County – Hauling away *P. ramorum*infested plant material away in contained vehicle (Photo by: UCCE/CDF)

#### AN UPDATE ON SUDDEN OAK DEATH IN OREGON - MARCH 2004

A. Kanaskie<sup>1</sup>, N. Osterbauer<sup>2</sup>, M. McWilliams<sup>1</sup>, E. Goheen<sup>3</sup>, E. M. Hansen<sup>4</sup>, and W. Sutton<sup>4</sup>

<sup>1</sup> Oregon Department Of Forestry, Salem OR USA 97310

<sup>2</sup> Oregon Department Of Agriculture, Salem OR USA, 97310

<sup>3</sup> USDA Forest Service, SW Oregon Forest Insect And Disease Service Center, Central Point OR USA 97502

<sup>4</sup> Department Of Botany And Plant Pathology, Oregon State University, Corvallis OR USA 97331

<u>Oregon Wildlands.</u> Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, was first discovered in Oregon in July 2001. Since then we have been attempting to eradicate the pathogen by cutting and burning all infected host plants and adjacent apparently uninfected plants. Eradication is in progress on approximately 30 sites, totaling 64 acres. The majority of sites are on private land.

The number of infected trees discovered each year has decreased since we first discovered the pathogen in Oregon. Most infected trees discovered in 2003 occurred very near existing eradication sites, except for three that were found 1.8, 0.8, and 0.25 miles from the nearest eradication site. Thus far in 2004 we have found only two new infected trees, both near existing infested sites, and these have been cut and burned. This is a major improvement in the rate of new discoveries compared to previous years, and suggests that the eradication effort is slowing spread of the pathogen.

Monitoring vegetation and stream water within the eradication sites has shown that the pathogen survived cutting and burning on most sites. It survived in stumps and sprouts of host plants. In 2003 and 2004, all stumps and sprouts of host vegetation on private land were treated with herbicide to kill sprouts and prevent future sprouting. On federal lands sprouts are being cut and burned repeatedly in order to keep sites free of host sprouts until the pathogen can no longer be recovered. Ongoing chemical and mechanical destruction of sprouts on all sites will be essential to curtail future spread of the pathogen.

Despite several new occurrences of *P. ramorum* in 2003 and early 2004, distribution of the pathogen in Oregon forests remains limited to a very small area near Brookings. Repeated aerial surveys and ground-checks have failed to detect the pathogen in forests beyond this area. The forested area in Oregon under regulation by the Oregon Department of Agriculture and USDA-APHIS is 11 mi<sup>2</sup>. Efforts to eradicate the pathogen from Oregon forests likely will continue for several years.

<u>Oregon Nurseries.</u> In 2001 and 2002, numerous surveys were conducted and failed to detect *P. ramorum* in Oregon nurseries. In 2003, the Oregon Department of Agriculture (ODA) discovered *P. ramorum* in nursery stock growing in six Oregon nurseries. The pathogen was found infecting *Camellia, Viburnum, Pieris,* and *Rhododendron.* At one nursery both the European and North American strains of the pathogen were found. Most infected plants were traced to a nursery in Stanislaus county California. A definitive source of the plants infected nurseries, and the results indicated that the pathogen did not become established in the natural environment. The ODA was able to find and destroy all of the potentially infected plants sold from four of the six nurseries, including all plants that may have been infected with the European strain of *P. ramorum*.

In late February 2004, the Oregon Department of Agriculture finalized an interim rule requiring that all recipients of tree and shrub nursery stock coming from any out-of-state source notify ODA for possible inspection of the plants. The intent of this rule is to locate any introductions of *P. ramorum* before infected plants can reach consumers or other nurseries.



Oregon – Eradication site (Photo by: OSU/ODF)

#### THE STATUS OF PHYTOPHTHORA RAMORUM IN WASHINGTON STATE

Art Wagner, Plant Pathologist, Washington State Department of Agriculture

To date in Washington State, 130 nurseries have been surveyed, and over 17,000 samples processed. Three nurseries have been found infested with P. ramorum. Infected plants include 9 variety-blocks of Rhododendron, 1 Viburnum, and 13 Camellia. All perimeter surveys have been negative. Washington uses an ELISA screen to quickly and cheaply detect Phytophthora spp., allowing far more samples to be collected and processed for a fraction of the cost of other methods. All infected plants in Washington State trace back to non-regulated areas of California, Oregon, British Columbia and Washington. Based on the information gathered so far, the following statements can be made concerning P. ramorum: 1) None of the recent nursery infestations have occurred in the regulated areas of California or Oregon, nor can they be linked to those areas; 2) Nurseries which carry P. ramorum hosts also create micro-climates which are favorable to the survival and spread of the pathogen, regardless of the climatic conditions or susceptibility of the native flora surrounding the nursery; and 3) Current USDA/APHIS regulations focus solely on those geographical areas where native vegetation and climate are likely to support the natural survival and spread of P. ramorum without taking into consideration the "microclimate islands" created by nurseries growing host material. The Washington State Department of Agriculture suggests that, given these facts, it is likely that P. ramorum exists undetected and unlooked for in nurseries outside of the regulated areas of California and Oregon. If regulations and survey priorities are not significantly adjusted to reflect this possibility, it is likely that P. ramorum will continue to spread throughout North America through the movement of infected nursery stock.

#### THE HUNT FOR PHYTOPHTHORA RAMORUM IN THE EASTERN US

Steven W. Oak, Plant Pathologist, USDA Forest Service, Southern Region FHP, Asheville, NC

The known geographic range of diseases caused by *Phytophthora ramorum* in forests in the US is central coastal CA and southwestern OR. However, susceptible hosts and climatic conditions conducive to disease development occur elsewhere. A risk map has been developed that integrates environmental factors (temperature and moisture optima and extremes for the pathogen), host distribution, and proximity to rhododendron nurseries which could conceivably serve as potential pathways of introduction of the pathogen. A large concentration of high-risk area was projected in and around the Southern Appalachian Mountains. This risk projection was the basis for pilot surveys during the growing season of 2003 in forests of seven states comprising the bulk of the eastern high risk area including PA, WV, VA, NC, TN, SC, and GA. Sampling was conducted by cooperating state forestry agencies in two settings: the forested perimeter around rhododendron nurseries and in general forest areas not directly associated with nurseries. A target number of 30 survey locations distributed in high and moderate projected risk polygons in each state was desired with about one-third of these in forested nursery perimeters. Each location was surveyed using four 100 meter transects. Transect width was variable depending on the density of hosts. Target hosts and symptom types were bleeding stem cankers on *Quercus* spp., and leaf and twig blight of *Rhododendron* spp. and *Kalmia latifolia*. Symptomatic foliage samples consisted of 10-leaf composites while bark samples came from single trees. Diagnosis of P. ramorum status was by nested PCR conducted by state university laboratories familiar with the procedures by providing diagnostic services to concurrent surveys of woody ornamental production nurseries. Replicate samples of symptomatic hosts were sent from half of the transects at each location to a separate PCR laboratory as a mutual quality assurance measure.

A total of 172 locations were surveyed with 31 percent falling in forested nursery perimeter settings. Over 77 percent of the projected high-risk polygons had at least one survey location and *P. ramorum* was not detected in any of 1,116 samples submitted for PCR diagnostics. These represented about 100 bark samples and over 10,000 individual symptomatic leaves. *K. latifolia* was the most commonly sampled host. While more work is needed, these results suggest that *P. ramorum* is not endemic to eastern hardwood forests on the sampled hosts.

2004 surveys are planned for a total of 22 states, which will encompass all eastern states with high or moderate projected risk from southern New England to the Gulf Coast and west to the Ozark-Ouachita Mountains. Given the vigorous interstate trade in woody ornamental hosts of *P. ramorum*, it is virtually certain that it will eventually be introduced to the eastern US. Whether introduction will result in pathogen establishment and resource damage is not clear.

#### SUDDEN OAK DEATH, AN UPDATE FROM CANADA

Shane Sela, Canadian Food Inspection Agency, Plant Products Directorate - Western Area

Background: In 1990, infected *Rhododendron* and *Viburnum* began showing signs of infection from an unidentified disease in the EU. In 1995, large numbers of oaks and tanoaks were observed dying along California's central coast. As a result of the increased levels of oak and tanoak mortality, California scientists began to investigate the cause. Shortly thereafter, the USFS, CFS, and CFIA also became involved in analyzing the problem. In November 2000, the link between the unidentified diseases in the EU and US was made, and in January 2001, a new *Phytophthora* was recovered from rhododendron container plants in a Santa Cruz, California nursery.

Today, the host list for *P. ramorum*, the pathogen known to cause Sudden Oak Death, continues to grow. Current hosts regulated by Canada include: true firs, oaks, tanoak, *Rhododendron*, buckeye, madrone, *Viburnum*, kinnikinnick, honeysuckle, mountain laurel, maple, buckthorn, huckleberry, California bay laurel, Christmas berry, camellia, hazelnut, beech, witch hazel, *Pieris*, Victorian box, Douglas-fir, poison oak, *Rubus*, coast redwood, blueberry, wood rose, and Leucathoe

*P. ramorum*'s distribution continues to expand. Sudden Oak Death was first observed along California's central coastal area in the mid 1990s. It is presently confirmed in 13 California counties and in 1 county in OR. While only several US nurseries have had *P. ramorum* confirmations, there are over 300 nurseries in the EU that have been found to have *P. ramorum* infected plants.

*Quercus* and *Lithocarpus* usually exhibit bleeding cankers as a sign of pathogen presence. When bark is shaved away from the trunks of these trees, a black canker margin can be seen, delimiting the healthy tissue from the diseased tissue. Young tanoak sprouts may also have wilting shoots and flagging. Rhododendron symptoms vary, and include leaf spots and twig dieback. *Vaccinum* symptoms include bark splitting, leaf spots, and twig dieback to the soil line. Other hosts and their symptoms include CA bay laurel leaf spots, *Viburnum* stem cankers, and leaf cankers on madrone.

With *P. ramorum* having been found in nurseries and natural settings, there is great concern that through propagative and non-propagative plant material, this pathogen could spread to areas previously unexposed to it. Areas of concern for new infection in North America include Canada and the east coast, where numerous suitable hosts can be found in natural settings. Additionally, climatic conditions necessary to support *P. ramorum* can be found in areas of Europe as well as N. America, causing even greater concern for pathogen dispersal.

The likelihood of pathogen contamination is relatively high, given numerous commercial pathways for dispersal. Trans-shipment of infected material is a likely pathway, and there are numerous hosts capable of harboring *P. ramorum*. However, the kind of impact a given area may endure if *P. ramorum* were presented into the landscape may vary from insignificant, to the levels of oak mortality seen in California's wildlands, or even greater. Much of the mark made on a given area where the pathogen is introduced will vary depending on the types of hosts present, the economic and ecological significance of the hosts found in the area, and the local climate. Therefore, when assessing risk, we must consider the likelihood of pathogen introduction and its potential impact to a given area.

In response to the threat of *P. ramorum*, Canada established stringent regulatory controls in March, 2001 (D-01-01). The CFIA completed a risk assessment of *P. ramorum* in 2002, and put its current 9<sup>th</sup> regulatory revision into force on Sept. 25, 2003. This new policy establishes certification options for regulated areas, which until this time were under movement prohibitions for regulated articles.

In 2001, CFIA initiated a visual survey of oak woodlands. Additionally, across Canada, a survey of nurseries importing stock from Europe and western California and public green spaces in British Columbia and Ontario was conducted. Sampling in 2001, included 60 sites. In 2002, 328 sites were visited, with 2,800 samples collected, and no *P. ramorum*-positive plants were identified. Finally, in 2003, 211 sites were surveyed, with 6,000 samples taken. A positive nursery location was included in the survey (further details are provided below). Host plants surveyed in 2003 included, but were not limited to: *Acer, Arctostaphylos, Azalea, Camellia, Pieris, Quercus, Rhododendron, Rubus, Vaccinium, Viburnum*.

The Canadian National Surveillance Plan for 2004 will target plants received by importing nurseries in the last 6 years and inspection of public green areas. All hosts will be inspected, but *Rhododendron* will be given the highest priority. CFIA personnel have targeted 160 sites in BC, ON, PQ, NS/NB for the surveys which will be conducted during the fall and spring.

*P. ramorum* was first detected in Canada in a nursery in May 2003, during an investigation initiated in response to US detections of the pathogen in western nurseries. Three shipments had been sent from nurseries in the western US to a nursery in the Lower Mainland of British Columbia. Plants at the nursery were subsequently identified as being infected with *P. ramorum*. CFIA applied quarantine measures at the nursery and established a stakeholder advisory task force consisting of industry, scientists, and others affected to establish a strategy for dealing with the infection.

The management strategies put in place included: destruction of infected plant material; requirements for the maintenance of sanitary practices during production; a 90-day quarantine period during which sampling and testing was used to verify containment and eradication of the infection; and the implementation of trace-out/back of potentially infested plant material.

Sampling is ongoing at Canadian nurseries that received plants from the infected site. To date, 2,844 samples have been collected from 13 nurseries and eight landscape sites. Five *Rhododendrons* have been found *P. ramorum*-positive from survey efforts, all found in nursery settings or downstream from an infected nursery.

As a result of the *P. ramorum* confirmation, CFIA has begun to review and develop harmonized policies for outbreaks. Pathways for pathogen introduction are being explored and certification options are being analyzed and strengthened. CFIA has also begun harmonization efforts of its nursery management framework with State and APHIS officials.

Canada has also begun reviewing its import policies. An audit of the Dutch plant export stream was conducted by CFIA. It identified some small weaknesses in the program, which are being addressed, but generally found that the program mitigates pathway risks through the use of the Canadian host list as the basis of pathogen control, annual inspections, lab testing, export shipping inspections, strict phytosanitary controls applied at positive nurseries, and positive plants being removed from export stream. Dutch authorities did commence strengthening input controls and increasing inspection frequencies in *P. ramorum* positive blocks at the request of CFIA.

In mid-March the USDA informed CFIA that Monrovia and Specialty Plants, Inc. had infected plant material, and that 11 other nurseries were suspected of infection. Consequently, CFIA engaged its emergency measures developed as a result of the 2003 nursery situation; prohibiting the importation of all plants from Monrovia and Specialty Plants, Inc. as well as all hosts from California. Canada then began to trace all Monrovia material that had entered the country, and began sampling and testing any identified imports. Investigations determined that several BC nurseries had received *P. ramorum*-positive *Camellias* from Monrovia. Canada's next steps will be to eradicate the infected material and test adjacent hosts within the sites. Canada's ultimate goal continues to be protection of Canadian trade forest and agricultural products and the environment.



Canada – *P. ramorum*-infested nursery delimitation area (Photo by: CFIA)



Canada – *P. ramorum*-infested nursery stock bagged for destruction (Photo by: CFIA)

#### UK PHYTOPHTHORA RAMORUM RESEARCH PROGRAM

Alan Inman, Central Science Laboratory, Department for Environment, Food, and Rural Affairs (DEFRA), UK

Research in the UK is currently being undertaken by the Central Science Laboratory and by Forest Research under funding from Defra, Forestry Commission, the Horticultural Development Council and the European Union. The main areas of research until the end of 2003 included: laboratory-based diagnostics (TaqMan<sup>®</sup> PCR; AFLP fingerprinting); field based diagnostics (portable PCR SmartCycler®; *Phytophthora* antibody-based lateral flow devices); comparative behaviour and adaptive differences of European and American isolates; mating type and breeding system; host range and infection studies (tree species; woodland under-storey & hedgerow species; heathland species; ornamentals); sporulation potential of leaf hosts; dispersal and transmission in the UK context; survival of chlamydospores, especially over-wintering; epidemiology through site studies; latent/incubation period on ornamentals; fungicides and sterilants for use with hardy ornamental nursery stock. An EU-funded project (RAPRA), starting January 2004 and co-ordinated by Forest Research, will develop an EU pest risk analysis through experimentation at the European level and will include additional work on aspects of the pathogen's epidemiological and management. Results of CSL studies were presented; work done by Forest Research was presented separately by Joan Webber and Sandra Denman. Diagnostically, TagMan PCR assays have been developed, evaluated for laboratory use and adapted for use in a field portable PCR system (SmartCycler®, Cepheid). Over 75 non-tree plant species (ornamentals, woodland under-storey and heathland plants) have been tested for potential leaf, and in some cases stem, susceptibility. For potentially susceptible woodland under-storey and hedgerow species, their potential contribution to tree epidemics has been assessed based on various factors including their natural susceptibility potential determined by zoospore dipping of unwounded leaves, sporulation potential and phenology. Different species had different inoculum thresholds for infection and wounds were more important for some than for others. Data on the survival of chlamydospores and sporangia in vitro were presented, as well as overwintering in infected lilac and rhododendron leaves in containment cylinders outside at two locations. The approaches and methodologies for studies being done at several large estate garden outbreaks was also presented, including preliminary observations about the potential role of vertebrates in dispersal, and levels of soil inoculum in relation to disease levels and management action.

#### Contacts:

Dr Alan Inman and Dr Judith Turner, Central Science Laboratory, Defra, Sand Hutton, York, YO41 1LZ (<u>a.inman@csl.gov.uk; j.turner@csl.gov.uk</u>)

Dr Joan Webber, Forest Research, Alice Holt Lodge, Wrecclesham, Farnham, Surrey GU10 4LH (joan.webber@forestry.gsi.gov.uk)

#### PHYTOPHTHORA RAMORUM IN THE NETHERLANDS – AN UPDATE

M.H.C.G. Steeghs, Plant protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands

*Phytophthora ramorum* (*P. ramorum*) was found in the Netherlands for the first time in 1993. Awareness of the risks involved came only in 2000 with the large scale oak mortality in California. In 2001 an initial survey was done to get familiar with the problems of detecting *P. ramorum*.

In 2002 and 2003 a large-scale survey was executed outside the nurseries. As *P. ramorum* outside the nurseries only was found in *Rhododendron*, *Rhododendron* was the inspection object. Ultimately, *Vaccinium myrtillus* was also inspected after considering its susceptibility. 1400 *Rhododendron* sites and 100 *Vaccinium myrtillus* sites were inspected. Two percent of the sites with *Rhododendron* were found infested. No infection was found in *Vaccinium*.

At the infested sites, the owner had two options - either follow the advice of the Plant Protection Service (PPS) in eradicating the infestation or make sure that the infected plants are isolated. Much effort has been invested to gain support for the measures. At more than 75 percent of the infested sites, eradication measures were taken according the guidelines of the PPS.

Follow-up inspections were held at the sites to detect infestations on other host plants and to assess the effectiveness of the measures taken. At 2 sites an infected *Quercus rubra* tree was found, one tree was also infected by other pathogens, the other one appeared healthy.

At many sites where eradication measures were taken, re-growth was infected. In our trials, we found after 4 months that more than 30 percent of the re-grown plants had infected shoots. The infection in the shoots came from the stem. Also, in many cases the delimitation of the infestation had been too narrow, especially with the larger infestations, where the fungus had spread further than expected.

By the end of 2003, a conclusion was drawn on the basis of the results of the survey and the eradication measures that it is not possible to eradicate the disease from the territory of the Netherlands. It was decided that considering the risks for the indigenous plants, the emphasis of the activities for 2004 outside the nurseries has to focus on the reduction of the inoculum loads and on investigating the spread of the fungus under indigenous plants.

In 2002 the EU decided that the host plants *Rhododendron* and *Viburnum* only could be traded after growing-season inspections and with a plant passport. During the season 2002/03, four percent of the nurseries growing these host plants were found infested. From July to December 2003, the percentage of infested nurseries fell to one and a half percent, despite the intensification of the inspections. The reduction is mainly due to a decrease in the production of *Viburnum x bodnantense dawn* and the awareness of the growers. It can be concluded that current measures at the nurseries are successful.

#### FOLIAGE SUSCEPTIBILITY OF UK TREES TO PHYTOPHTHORA RAMORUM

S. Denman, S.A. Kirk, J. Rose, J.F. Webber and C.M. Brasier. Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK

High mortality of oak trees in California (Sudden Oak Death) due to Phytophthora ramorum together with its presence in the UK and Europe has led to major concern about the damage it could cause to British forests and vegetation. As part of a pest risk analysis leaves of 10 conifer and 23 broad-leaved species important to British forestry were tested for their susceptibility to P. ramorum. Two European isolates and 2 US isolates were used. Wounded and unwounded leaves were dipped in zoospore suspensions during early or late summer and winter. Successful infection of tissue and amount of necrosis were assessed. Highly susceptible broad-leaved hosts included European ash, elm, holm oak, horse-chestnut, sweet chestnut and to a lesser extent sessile and turkey oak, together with bay laurel and rhododendron. Alder, beech, common English oak, cork oak, hazel, hornbeam, red oak, sycamore and wild cherry had consistently low susceptibility. The conifers coastal redwood, Douglas-fir, noble fir, Norway and sitka spruces, and Western hemlock were also susceptible with Douglas-fir and noble fir being severely affected. Corsican, lodgepole and scots pines were virtually resistant while yew foliage was only slightly affected. In general conifers were less susceptible than broad-leaved hosts. The time of year that inoculations were carried out and the treatment applied to the host tissue (wound or nonwound) influenced results. Increased effects of the pathogen were recorded on leaves produced early in the season (summer) and on those that were inoculated through wounds. These results extend the known range of trees that P. ramorum is able to attack and confirm once again its relative host-non-specificity. The pathogen's ability to produce inoculum on foliage of European trees in vitro and in the field is under investigation.

## INVESTIGATING THE ROLE OF CHLAMYDOSPORES IN THE SPREAD OF *PHYTOPHTHORA RAMORUM*

Nina Shishkoff, USDA Agricultural Research Service – Foreign Disease Weed Science Research Unit

*Phytophthora ramorum* causes cankers, dieback, and foliar symptoms on a number of hosts, but its behavior in soil has not been well examined, either its survival in soil or its ability to infect roots. Infected leaf tissue containing chlamydospores or mycelium that included chlamydospores was buried in mesh bags in pots containing azalea plants growing in potting mix. The pots were kept under greenhouse conditions and duplicate mesh bags were sampled monthly. Individual chlamydospores were picked out and plated on selective media (approx. 100 chlamydospores per bag). Although the percent germination of these chlamydospores decreased over time, germinating chlamydospores were still present 155 days after burial. When roots of rhododendron (rooted cuttings of "Cunningham's White") were dipped in a sporangial suspension (approx. 2,500 sporangia/mL) and then planted in coarse soilless medium, *P. ramorum* could be isolated from washed or 0.5 percent sodium hypochlorite surface-sterilized roots 10-66 days later. These results indicate that *P. ramorum* may be able to persist in nursery container media. [These data were published in Shishkoff, N. and P. Tooley. 2004. Phytopathology 94: S95]



Chlamydospore Germinating (Photo by: Dr. Shishkoff)

# TRANSMISSION OF *PHYTOPHTHORA RAMORUM*: PRACTICAL APPLICATIONS

Jennifer M. Davidson, Pacific Southwest Research Station Current address: University of Hawaii

The objectives of our long-term study on the transmission biology of *Phytophthora ramorum* were to determine the main sources of inoculum, the seasonality of inoculum production, and the transmission pathways of inoculum in California oak woodlands. Determining the sources of inoculum for *P. ramorum* remains challenging, given the large host range of this pathogen. Consequently, we concentrated on dominant hosts in mixed-evergreen and tanoak-redwood forests to test for spore production. Using laboratory moist chambers and detached plant parts, spore production was tested from infected coast live oak (*Ouercus agrifolia*) trunks, tanoak (Lithocarpus densiflorus) trunks, tanoak twigs, tanoak leaves, California bay laurel (Umbellularia *californica*) leaves, and coast redwood (*Seauoia sempervirens*) twigs plus attached needles. No sporangia production occurred from the surface of coast live oak or tanoak trunk bark. However, P. ramorum was able to produce sporangia from tanoak twigs, tanoak leaves, bay laurel leaves, and redwood twigs/needles. The quantities of sporangia produced on all but bay laurel leaves were limited. Therefore, we focused on bay laurel leaves for additional studies. Zoospore production was quantified from infected tips of bay laurel leaves collected at Fairfield Osborn Preserve ~24 hours after the onset of rainstorms. The mean number of zoospores produced from infected bay laurel leaves under natural field conditions during rainstorms was 1173.0 + SE 301.48, and ranged as high as 5200 spores per leaf, demonstrating the importance of this host for transmission of *P. ramorum*. To monitor the seasonality of inoculum production, we tracked the concentration of inoculum in rainwater at Fairfield Osborn Preserve throughout the past three rainy seasons. Considerable variation in inoculum concentration occurred from year to year. Colony-forming units per liter rainwater were two to three orders of magnitude higher in 2002-03 as opposed to 2001-02 and 2000-01. This increase in inoculum production may be due, in part, to the extended duration of the 2002-03 rainy season with rains continuing through April. Concurrent with the increased inoculum production of 2003, we detected a 10.4% increase in infection on coast live oak trees at the Preserve, as well as a surge in infections on Douglas-fir (Pseudotsuga menziesii) terminal branches. Our studies on transmission pathways demonstrated recovery of *P. ramorum* not only from rainwater, but also soil, litter and streamwater. Dispersal distances were investigated for these substrates. In rainwater, inoculum moved 5 m and 10 m into a meadow from a source of infected bay trees at the forest edge. For soil, one-third to one-half of the hikers tested at the Preserve during the rainy season were carrying infested soil on their shoes after walking a 2.4 km trail. In streamwater, P. ramorum was recovered from an unforested site in pasture  $\sim 1$  km downstream of forest with inoculum sources. In total, these studies provide details on the production and spread of *P. ramorum* inoculum to aid in forecasting and managing this environmentally destructive pathogen.

# PREDICTING THE RISK OF ESTABLISHMENT OF *PHYTOPHTHORA RAMORUM* ACROSS CALIFORNIA

Ross Meentemeyer, Sonoma State University, March 2004

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. In response to this threat, the federal and state government of California have assembled task forces to devise strategies for management and prevention of further spread of this pathogen. Because the disease may be too extensive to broadly apply control methods, California has established an active monitoring program focused on early detection of pathogen activity at isolated locations, where it may be possible to apply chemical treatments or attempt eradication. The considerable cost of monitoring necessitates careful targeting and prioritization of early detection efforts given the extensive size (408,512 km<sup>2</sup>), diversity of host species and environmental variability of the state of California.

To better understand and manage the threatened habitats, we have developed a rule-based model of *P. ramorum* establishment and spread risk in California plant communities. The model, which is being actively used 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. 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.

#### The Role of Vertebrates as Dispersal Agents of Phytophthora ramorum

J. Hall Cushman, Department of Biology, Sonoma State University, Rohnert Park, CA 94928

Determining how *Phytophthora ramorum* is dispersed across the landscape is critical for understanding the ecology and epidemiology of this influential pathogen. To date, researchers have shown that abiotic factors – such as rain-splash, wind-blown rain and down-stream transport of inoculum – are important in the dispersal of this pathogen. In contrast, very little research has focused on the potential for human dispersal of *P. ramorum*, although work on other pathogens – such as *P. lateralis* attacking Port Orford cedar – suggests that humans can play an influential role in disease spread. Here, I provide a progress report for a study in eastern Sonoma County that is addressing three research questions: 1) Do humans and other vertebrates disperse *P. ramorum* in natural landscapes? 2) How far do vertebrates disperse *P. ramorum*? and 3) Do areas visited intensively by humans have a greater proportion of foliar and terminal hosts showing symptoms of infection than areas visited less frequently?

To address the ability of humans to disperse *P. ramorum* throughout natural landscapes, my collaborators and I conducted a study during the spring of 2003 within different habitat types at Sonoma State University's Fairfield Osborn Preserve in eastern Sonoma County. We assessed soil samples for the presence or absence of *P. ramorum* on the trail surface and at adjacent locations 2 meters off trail in three habitat types that we hypothesized differed greatly in the amount of pathogen inoculum present in the soil: woodlands dominated by infected California bay laurel (*Umbellularia californica*) and coast live oak (*Quercus agrifolia*), open grassland lacking any foliar or terminal hosts, and stands of white oak (*Quercus garryana*; a non-hosts species). As expected, we found that *P. ramorum* was equally common in soil on and off trail from infected bay/coast live oak woodlands. However, for grasslands and white oak woodlands – habitats that lack *P. ramorum* hosts – our data indicated that the pathogen was commonly found in soil samples collected on trail, while being virtually absent off trail. These data suggest that hikers are important dispersal agents of *P. ramorum* and are able to transport the pathogen into areas that lack a local source of inoculum.

In subsequent field seasons, we will greatly expand our work on biotic dispersal of *P. ramorum* in the following ways. First, we will determine whether our 2003 results can be replicated in different years and study sites. Second, we will compare the ability of hikers versus mountain bikers to disperse the pathogen. Third, we will determine how far humans can disperse *P. ramorum* out of infested forests. And fourth, we will assess the possibility that other vertebrate taxa – such as black-tailed deer, squirrels, turkeys, and scrub jays – play similar roles as dispersal agents of *P. ramorum*.

To determine whether SOD symptom levels are greater in areas with high human activity, we will use 202 plots in eastern Sonoma County, approximately half of which occur in areas experiencing high visitation rates by humans, whereas the other half occur in areas that have low visitation rates. During the spring and early summer of 2004, we will sample all foliar and terminal hosts in these plots for symptoms of infection by *P. ramorum*. After taking into account the influence of elevation, precipitation, solar radiation and topography, we will assess whether the proportion of symptomatic hosts is significantly greater in plots experiencing high levels of human activity than those with low activity levels. Collectively, these data will address the degree to which there are conflicts between recreation and disease spread, and whether more active management of human activity is required.

#### Persistence and Spread of Phytophthora ramorum in Oregon Forests

Everett Hansen, Oregon State University

We have been studying how and where *P. ramorum* survives on Oregon eradication sites, and how it is spreading to new trees. Overall inoculum levels are low in Oregon, and we seldom recover the pathogen from rainwater and only occasionally from soil. We have found, however, that tanoak stump sprouts in the eradication areas were very susceptible, presumably to inoculum splashed by rain from the soil. Examination of the upper crowns of trees with bole cankers, and of adjacent trees without bole cankers revealed twig infections on most cankered trees, and on only a few adjacent trees. Infection of understory hosts rhododendron and huckleberry is found only in the immediate vicinity of cankered trees, suggesting top down spread, presumably in rain drip and splash from the infected overstory. The pattern of new infections on the landscape suggests the classical dispersal gradient of an infectious disease spreading from distinct sources of inoculum. There is a long tail to the distribution, suggesting occasional dispersal in storm winds, but most new infection occurs very close to previously infected trees.



Oregon - Burning felled P. ramorum-infested plant material (Photo by: OSU/ODF)