Sudden Oak Death, a Science Symposium

The State of Our Knowledge

December 17 - 18, 2002
Marriott Hotel
Monterey, California

Conference Purpose

A Symposium designed to bring together a broad array of the scientific community from throughout the world working on *Phytophthora ramorum* and the phenomena known as "Sudden Oak Death"

Conference Sponsors

USDA Forest Service, Pacific Southwest Research Station
Albany, California

University of California, Integrated Hardwood and Range Management Program, Center for Forestry, Division of Agriculture and Natural Resources, Berkeley, California

Co-Chairs

Dr. Patrick J. Shea, Pacific Southwest Research Station
Davis, California

Dr. Richard B. Standiford, University of California
Berkeley, California

Table of Contents

Schedule of Events

December 15	1:00 pm – 5:00 pm	Field Trip to Big Sur to View Sudden Oak Death
December 16	2:30 pm – 5:30 pm	Early Registration for Sudden Oak Death Science Symposium
	5:30 pm – 8:00 pm	Icebreaker reception, no host bar, snacks
		Affiliated Meeting
	10:00 am – 5:00 pm	California Oak Mortality Task Force (COMTF) Meeting COMTF Business, Committee meetings, Presentations
December 17	7:00 am – 8:30 am	Continental Breakfast and Registration
	8:30 am – 9:00 am	Opening Presentation **Congressman Sam Farr, Monterey, CA**
	9:00 am – 10:00 am	Panel - Overview of Where We Are **Dr. Garland Mason, Panel Chair** **USDA Forest Service, Pacific Southwest Research Station**
		Biology, epidemiology, etc. – **Dr. Dave Rizzo, University of California, Davis** Management – **Dr. Matteo Garbelotto, University of California, Berkeley** Public Safety – **Mr. Don Gasser, PG&E**
	10:00 am – 10:20 am	Break
	10:20 am – 12:05 pm	Transmission and Epidemiology Session
	10:20 am	Transmission of *Phytophthora ramorum* in Coast Live Oak Woodlands **Jennifer M. Davidson, USDA Forest Service, Pacific Southwest Research Station**
	10:35 am	Transmission of *Phytophthora ramorum* via *Umbellularia californica* (California bay) Leaves in California Oak Woodlands, **Jennifer M. Davidson, USDA Forest Service, Pacific Southwest Research Station**
	10:50 am	Epidemiology of *Phytophthora ramorum* in Oregon Forests **Ellen Goheen, USDA Forest Service – Region 6**
	11:05 am	Epidemiology and Ecology of *Phytophthora ramorum* in Redwood/ Tanoak Forest Ecosystems of the California Coast Range, **P. E. Maloney, University of California, Davis**
	11:20 am	Progression of Sudden Oak Death Over Two Years at Sites in Marin County, California **Brice A. McPherson, University of California, Berkeley**
	11:35 am	The Spatial Pattern of Sudden Oak Death Symptoms in Coastal Redwood / Tanoak Forests **Mark Spencer, University of California, Berkeley**
	11:50 am	Factors Related to *Phytophthora ramorum* Canker (Sudden Oak Death) Disease Risk in Coast Live Oak and Tanoak **Ted Swiecki, Phytosphere Research**
	12:05 pm – 1:30 pm	Lunch (on own)

1:30 pm – 2:45 pm Molecular and Microscopic Analysis Session

1:30 pm PCR-Based Detection of *Phytophthora ramorum* in Plant Tissue
K.Hayden, University of California, Berkeley

1:45 pm Molecular Identification and Detection of *Phytophthora* Species and Populations of *P. ramorum*
Peter Bonants, Plant Research International, The Netherlands

2:00 pm Molecular Population Analysis of *Phytophthora ramorum*
Kelly Ivors, University of California, Berkeley

2:15 pm The Use of Mitochondrial Molecular Markers for Identification of *P. ramorum*
F. N. Martin, USDA Agricultural Research Service

2:30 pm Plant Structures Through Which *Phytophthora ramorum* Establishes Infections
Edwin R. Florance, Lewis and Clark College

2:45 pm – 3:10 pm Break

3:10 pm – 4:00 pm Wildlife Impacts Session

3:10 pm Effects of Sudden Oak Death-Induced Habitat Change on Insectivorus, Cavity-Nesting Birds
Kyle Apigian, University of California, Berkeley

3:25 pm Potential Effects of Sudden Oak Death on California Oak Woodland Birds
Bill Monahan, University of California, Berkeley

3:40 pm Sudden Oak Death Wildlife Impacts Study - San Luis Obispo County,
William Tietje, University of California, IHRMP

4:00 pm – 5:30 pm Survey/Monitoring Session
Dr. Wally Mark, Cal Poly SLO, Panel Chair

4:00 pm Sudden Oak Death Surveys in Oregon - 2001 & 2002,
Ellen Goheen, USDA Forest Service – Region 6

4:15 pm Development of a National Survey Protocol for Detection of *Phytophthora ramorum*
W.D. Smith

4:30 pm Spatial Modeling of Sudden Oak Death Risk and Oak Mortality Clustering, China Camp State Park
Ross Meentemeyer, Sonoma State University

4:45 pm Remote Sensing of Host Species Distribution in Hardwood Forests with Sudden Oak Death in Marin and Sonoma Counties,
Maggi Kelly, University of California, Berkeley

5:00 pm Statewide Aerial and Ground Surveys for Mapping and Monitoring the Distribution of Sudden Oak Death
Lisa Levien, USDA Forest Service – Region 5

6:00 pm – 8:30 pm Poster session, no host bar and snacks

December 18

7:00 am – 8:30 am — Continental breakfast

8:30 am – 10:00 am — Panel - Status of Quarantine (Canada, Europe, U.S., California)
Ms. Susan Frankel, Panel Chair
USDA Forest Service – Region 5

Nursery Regulations for *Phytophthora ramorum* - Jonathan Jones, USDA-APHIS, Riverdale, MD

Forestry and Special Forest Products Regulations - Shane Sela, Canada Food Inspection Agency, Victoria, B.C.

The Science Base of USDA- APHIS's *Phytophthora ramorum* Regulation - David Kaplan, USDA-APHIS, Raleigh, NC

European Regulations and Risk Assessment for *Phytophtora ramorum* - Stephen Hunter, Department of Environment, Food and Rural Affairs, York, UK

A Sideways Look at Plant Health: a Biological Perspective - Clive Brasier, Pathology Branch, Forest Research Agency, Farnham, Surrey UK

10:00 am – 10:20 am — Break

10:20 am – 12:05 pm — Pathogenicity and Resistance Session

10:20 am — Comparisons of Pathogenicity of *Phytophthora ramorum* Isolates from Europe and California
Hans de Gruyter, Plant Protection Service, The Netherlands

10:35 am — Pathogenicity of *Phytophthora ramorum* Isolates from North America and Europe to Bark of European Fagaceae, American *Quercus rubra* and Other Forest Trees
Clive Brasier, Pathology Branch, Forest Research Agency, Farnham, Surrey UK

10:50 am — Innoculation Trials of *Phytophthora ramorum* on Detached Mediterranean Sclerophyll Leaves
Eduardo Moralejo, Instituto Mediterraneo de Estudio Avanzados, IMEDEA, Spain

11:05 am — Sporulation Potential of *Phytophthora ramorum* on Leaf Disks from Selected Hosts
J. Parke, Oregon State University

11:20 am — Potential Susceptibility of Native Southwest Oregon Plant Species to *Phytophthora ramorum*
J.W. Zanzot, Oregon State University

11:35 am — Towards a Model of the Genetic Architecture of *Phytophthora ramorum* Susceptibility in Coast Live Oak
Richard Dodd, University of California, Berkeley

11:50 am — Resistance of *Umbellularia californica*(Bay Laurel) to *Phytophthora ramorum*
D. Huberli, University of California, Berkeley

12:05 pm – 1:30 pm — Lunch

1:30 pm – 3:15 pm	Management and Impacts Session
1:30 pm	*Phytophthora ramorum* in The Netherlands **Maarten Steeghs, Plant Protection Service, The Netherlands**
1:45 pm	Monitoring *Phytophthora ramorum* Within and Around Disease Patches Targeted for Eradication in Oregon **Alan Kanaskie, Oregon Department of Forestry**
2:00 pm	Fire Behavior and Fire Effects in SODS-Affected Oak Woodlands in Marin County, California **Michael Swezy, Marin Municipal Water District**
2:15 pm	Potential Impact of *Phytophthora ramorum* on Nursery Crops in the Pacific Northwest **R. Linderman, USDA Agricultural Research Service**
2:30 pm	Chemical Treatment Strategies for Control of Sudden Oak Death in Oaks and Tanoaks **D. Schmidt, University of California, Berkeley**
2:45 pm	Understanding the Disposal and Utilization Options for Wood Infected by the Sudden Oak Death Syndrome **John Shelly, University of California, Forest Product Laboratory**
3:00 pm	Survivability of *Phytophthora ramorum* in the Composing Process, **Steven Swain, University of California, Berkeley**
3:15 pm – 4:00 pm	Summary of meeting **Dr. Terry Shaw, USDA Forest Service, Washington Office**

Formal Presentations

TUESDAY DECEMBER 17, 2002

Transmission and Epidemiology Session 10:20 am - 12:05 pm

Jennifer M. Davidson Transmission of *Phytophthora ramorum* in Coast Live Oak Woodlands

Jennifer M. Davidson Transmission of *Phytophthora ramorum* via *Umbellularia californica* (California bay) Leaves in California Oak Woodlands

Ellen M. Goheen Epidemiology of *Phytophthora ramorum* in Oregon Forests

P. E. Maloney Epidemiology and Ecology of *Phytophthora ramorum* in Redwood/Tanoak Forest Ecosystems of the California Coast Range

Brice A. McPherson Progression of Sudden Oak Death Over Two Years at Sites in Marin County, California

Mark Spencer The Spatial Pattern of Sudden Oak Death Symptoms in Coastal Redwood Tanoak Forests

Ted J. Swiecki Factors Related to *Phytophthora ramorum* Canker (Sudden Oak Death) Disease Risk in Coast Live Oak and Tanoak

Molecular and Microscopic Analysis Session 1:30 pm - 2:45 pm

K. Hayden PCR-Based Detection of *Phytophthora ramorum* in Plant Tissue

Marjanne de Weerdt Molecular Identification and Detection of *Phytophthora* Species and Populations of *P. ramorum*

Kelly Ivors Molecular Population Analyses of *Phytophthora ramorum*

Frank N. Martin The use of Mitochondrial Molecular Markers for Identification of *P. ramorum*

Edwin R. Florance Plant Structures through which *Phytophthora ramorum* Establishes Infections

Wildlife Impacts Session 3:10 pm - 4:00 pm

Kyle Apigian Effects of Sudden Oak Death-Induced Habitat Change on Insectivorous, Cavity-Nesting Birds

Bill Monahan Potential Effects of Sudden Oak Death on California Oak Woodland Birds

William Tietje Sudden Oak Death Wildlife Impacts Study - San Luis Obispo County

Survey/Monitoring Session 4:00 pm - 5:30 pm

Ellen Goheen Sudden Oak Death Surveys in Oregon - 2001 & 2002

W.D. Smith Development of a National Survey Protocol for Detection of *Phytophthora ramorum*

Ross Meentemeyer Spatial Modeling of Sudden Oak Death Risk and Oak Mortality Clustering, China Camp State Park

Maggi Kelly Remote Sensing of Host Species Distribution in Hardwood Forests with Sudden Oak Death in Marin and Sonoma Counties

Lisa Levien Statewide Aerial and Ground Surveys for Mapping and Monitoring the Distribution of Sudden Oak Death

Formal Presentations

WEDNESDAY DECEMBER 18, 2002

Pathogenicity and Resistance Session 10:20 am - 12:05 pm

Hans de Gruyter Comparison of Pathogenicity of *Phytophthora ramorum* Isolates from Europe and California

Clive M. Brasier Pathogenicity of *Phytophthora ramorum* isolates from the USA and Europe to Bark of European Fagaceae, American *Quercus rubra* and Other Forest Trees

Eduardo Moralejo Inoculation Trials of *Phytophthora ramorum* on Detached Mediterranean Sclerophyll Leaves

J. L. Parke Sporulation Potential of *Phytophthora ramorum* on Leaf Disks from Selected Hosts

J.W. Zanzot Potential Susceptibility of Native Southwest Oregon Plant Species to *Phytophthora ramorum*

Richard S. Dodd Towards a Model of the Genetic Architecture of *Phytophthora ramorum* Susceptibility in Coast Live Oak

Daniel Hüberli Resistance of *Umbellularia californica* (Bay Laurel) to *Phytophthora ramorum*

Management and Impacts Session 1:30 pm - 3:15 pm

Maarten Steeghs *Phytophthora ramorum* in The Netherlands

A. Kanaskie Monitoring *Phytophthora ramorum* Within and Around Disease Patches Targeted for Eradication in Oregon

Kent Julin Fire Behavior and Fire Effects in SODS-Affected Oak Woodlands in Marin County, California

R. Linderman Potential Impact of *Phytophthora ramorum* on Nursery Crops in the Pacific Northwest

D. Schmidt Chemical Treatment Strategies for Control of Sudden Oak Death in Oaks and Tanoaks

John Shelly Understanding the Disposal and Utilization Options for Wood Infected by the Sudden Oak Death Syndrome

S. Swain Survivability of *Phytophthora ramorum* in the Composting Process

Abstract

Transmission and Epidemiology Session

Transmission of *Phytophthora ramorum* in Coast Live Oak Woodlands

Jennifer M. Davidson[1], Allison C. Wickland[2], Andrea C. Morse[2], Steven A. Tjosvold[3], David L. Chambers[3], Camille E. Jensen[2], Garey Slaughter[2], Matteo Garbelotto[4], and David M. Rizzo[2]

Controlling the spread of *Phytophthora ramorum* requires understanding conditions for spore production and movement. For the past two years (2001-2002), we have sampled throughout the seasons for the presence of *P. ramorum* in rainwater, soil, and leaf litter at a study site in a coast live oak woodland in Sonoma Co., California. Number of colony forming units per liter rainwater were assessed approximately three times per month during the rainy season, and for each summer rain, by collecting rainwater in traps placed at twelve infected oak trees surrounded by infected bay trees. In both 2001 and 2002, there was a lag of several months between the start of the rains and the presence of detectable inoculum in rain samples. In addition, the greatest number of spores per liter rainwater occurred at the end of the rainy season. Build-up of infection on bay leaves as well as rising temperatures may contribute to the increase in spore production at the end of the rainy season. This phenomenon may be accentuated in El Niño years, during which rains occur late in spring, possibly resulting in high levels of spore production and corresponding waves of infection on oak trees. The predicted 2003 El Niño may provide an opportunity to test this hypothesis through continued rainwater sampling as well as a re-census of infected coast live oak trees on a one hectare plot at the same study site.

Soil and litter samples were collected monthly throughout the year at the same locale from around fifteen infected oak trees surrounded by infected bay trees. Soil was flooded with water and baited with pears to assess presence of *P. ramorum*. Positive soil and litter samples generally occurred in the same months as did positive rain samples. Samples spanning the dry season from June - December, 2001 were negative for *P. ramorum*. Results from the 2002 dry season are pending, but appear to follow the same pattern. Our corresponding laboratory experiments indicate that infested soil can have epidemiological significance: spores in soil spread to above ground leaves of bay seedlings via infection of green leaf litter. Knowledge of time periods in which *P. ramorum* spores are present and viable in rainwater or soil may help direct management decisions for containment.

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Abstract

Transmission and Epidemiology Session

Transmission of *Phytophthora ramorum* via *Umbellularia californica* (California bay) Leaves in California Oak Woodlands

Jennifer M. Davidson[1], Patricia E. Maloney[2], Allison C. Wickland[2], Andrea C. Morse[2], Camille EJensen[2], and David M. Rizzo[2]

Quercus agrifolia (coast live oak) and *Umbellularia californica* (bay laurel) are dominant components of coast live oak woodlands in California and may comprise over 80% of the individuals (> 1 cm dbh) at a site. Both of these tree species are hosts for *Phytophthora ramorum*, the causal agent of Sudden Oak Death. However, experiments indicate that only infections on bay leaves may be important for production of spores and transmission of disease.

To better understand transmission of *P. ramorum* in leaves of bay, we marked 1000 asymptomatic bay leaves at the end of January 2002, in each of ten sites for a total of 10,000 leaves, to monitor infection and leaf fate (abscission) during the rainy season and subsequent dry season. Of the 10 sites, 4 were coast live oak-bay forests, 5 were tanoak-redwood forests, and 1 site was a mixture of the two forest types. The bay leaves were censused in April and July 2002 for *Phytophthora* symptoms and abscission. (A final census will be performed in October 2002). A small piece (approximately 9 mm2) of symptomatic leaves was removed for verification of *P. ramorum* using selective medium (PARP).

During the period of January through April, 360 of the 10,000 (3.60%) leaves became infected with *P. ramorum*. From April through July, another 114 (1.14%) were infected. Leaves that were infected by the first census date were 15 times more likely to be shed by the second census date than were non-infected leaves. Furthermore, an infected bay leaf in coast live oak forest was significantly more likely to be dropped than an infected bay leaf in tanoak-redwood forest. In two additional, separate experiments in a coast live oak-bay forest in Sonoma Co., we looked at survival of *P. ramorum* throughout the dry season in bay leaves that were attached or had abscised. Survival was assessed by plating of leaves on PARP. Survival in attached bay leaves declined, but persisted throughout the summer from approximately 90% in June to 50% in August. Survival in abscised leaves collected from leaf litter in June, July, and August was nearly zero. Given that survival of *P. ramorum* occurs in attached rather than abscised leaves, differences in abscission rates of *P. ramorum* infected bay leaves in coast live oak versus tanoak-redwood forests may be one factor contributing to differences in the onset of detectable inoculum production in these two forest types during the winter rainy season.

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Abstract

Transmission and Epidemiology Session

Epidemiology of *Phytophthora ramorum* in Oregon Forests

E.M. Goheen[1], E.M. Hansen[2], W. Sutton[3], A. Kanaskie[4], M.G. McWilliams[4], and N. Osterbauer[5]

Phytophthora ramorum was found in July 2001 near Brookings, Oregon, killing tanoak (*Lithocarpus densiflorus*) and causing dieback of closely associated wild rhododendron and evergreen huckleberry. Recent introduction and an ongoing eradication effort have contributed to the limited disease distribution (about 9 square miles) and forest impact in Oregon. The disease appears to be not only at an earlier stage of epidemic development, but perhaps also following a different trajectory in Oregon than in California. Although nearly all of the dominant vegetation in SW Oregon forests is known to be susceptible, disease remains very limited, with some common hosts in California virtually unaffected in Oregon. In this presentation, we integrate field survey and monitoring observations and isolation results, host range testing, spore trapping data, and emerging information on the population structure of *P. ramorum* in Oregon to provide a hopeful prognosis for the disease in this state.

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Abstract

Transmission and Epidemiology Session

Epidemiology and Ecology of *Phytophthora ramorum* in Redwood/Tanoak Forest Ecosystems of the California Coast Range

P. E. Maloney[1], S. F. Kane[2], C. E. Jensen[2] and D. M. Rizzo[2]

A total of 120 plots have been established in the redwood/tanoak forest type along the California coast range to study the epidemiology and ecology of *Phytophthora ramorum* in this forest ecosystem. Our plots represent a north-south gradient of *Phytophthora ramorum* populations in this forest community from Sonoma to Monterey Counties. At each site, we have collected disease, environmental, forest structure and composition data. From this data, relationships will be made between disease incidence and a number of key biological and environmental parameters. Hosts present in this forest type include redwood (*Sequoia sempervirens*), tanoak (*Lithocarpus densiflora*), bay laurel (*Umbellularia californica*), madrone (*Arbutus menziesii*), huckleberry (*Vaccinium ovatum*), toyon (*Heteromeles arbutifolia*), bigleaf maple (*Acer macrophyllum*), and Douglas-fir (*Pseudotsuga menziesii*). Because rain is a key factor in pathogen dispersal, raintraps were placed in 5 plots at Jack London State Park to determine levels of inoculum and dispersal in these forests during the winter of 2001-2002. We recovered *P. ramorum* from rainwater throughout the rainy season with a large increase in inoculum during a May rain event. Soil/litter collections were made somewhat in concert with rainwater collections. Recovery frequency of inoculum from soil during the rainy season was approximately 20% from Sonoma and Marin County sites. Summer soil collections show a dramatic decline in inoculum recovery, to 0%, of *P. ramorum*. In the spring 2002, lines with mesh bags containing rhododendron leaves, as a *P. ramorum* bait, were placed across 2 streams in three locations at Jack London SP and adjacent property. *P. ramorum* was recovered throughout the spring and summer months in these streams, regardless of any rain event. During 2002-2003, weather monitoring stations will be used to determine specific environmental conditions (i.e., relative humidity, temperature, rainfall amount and duration) on these sites that favor inoculum production. Raintraps will be stratified in the canopy to determine local dispersal (within the canopy) and long distance dispersal (inoculum leaving the canopy).

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Progression of Sudden Oak Death Over Two Years at Sites in Marin County, California

Brice A. McPherson[1], David L. Wood[2], Andrew J. Storer[3], and Richard B. Standiford[4]

Disease progression plots were established in two sites in Marin County, CA, in March 2000 to follow changes in symptoms of Sudden Oak Death, caused by *Phytophthora ramorum*, in oaks and tanoaks. Twenty plots were equally divided between China Camp State Park, along San Pablo Bay, and Marin Municipal Water District (MMWD) land, near Mt. Tamalpais. These plots included 507 coast live oaks, *Quercus agrifolia*, 45 California black oaks, *Q. kelloggii*, and 157 tanoaks, *Lithocarpus densiflorus* were assessed every 3 months. Each tree was evaluated every 3 months. Variables included bleeding status, presence of ambrosia and bark beetles, reproductive structures of the fungus *Hypoxylon thouarsianum*, and foliage condition. Bleeding cankers are the most reliable and consistent symptom of *P. ramorum* infection in *Q. agrifolia* and *Q. kelloggii*, and to a lesser extent, *L. densiflorus*. In both *Q. agrifolia* and *L. densiflorus*, the numbers of both symptomatic and dead trees increased during the monitoring period. Summed across all plots, symptomatic (bleeding) *Q. agrifolia* in the 10 China Camp plots accounted for 35% (range 11% to 95%) in March 2000, and 38% (range 19% to 100%) of living trees in March 2002. In the 8 MMWD plots, these values for *Q. agrifolia* were 14% (range 0% to 42%) in 2000, and 20% (range 0 % to 65%) in 2002. For the 6 plots with *L. densiflorus* in MMWD, symptomatic trees constituted 46% (range 20% to 64%) in 2000 and 61% (range 47% to 89%) in 2002. In both sites, combining symptomatic trees and those that died with bleeding symptoms, the total impact of the disease for *Q. agrifolia* was 32% (2000), rising to 37% (2002). Total disease impact for *L. densiflorus* was 48% (2000), rising to 67% (2002). Because the sample size for *Q. kelloggii* is small in this study, comparable values are less reliable, but the response of this species appears to be similar to that of *Q. agrifolia*.

Even though *P. ramorum* was already well established in these areas of Marin County before plots were initiated, considerable change is evident after 2 years. The number of symptomatic trees has continued to increase, as has the number of trees that have died with symptoms of *P. ramorum* infection. There is a consistent association of bark and ambrosia beetles with the bleeding cankers of infected *Q. agrifolia*. The significance of these insects in disease progression is unknown, but every symptomatic tree that died during the study period (n=37) had been colonized by these beetles while foliage was green. A number of trees that were bleeding and had been colonized by March 2000 were still producing apparently healthy foliage in June 2002, indicating that the duration of infected tree survival is variable, and can be much greater than 2 years. This conclusion is supported by the fact that no trees have progressed from asymptomatic to infected, and then to dead during the first two years of this study.

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Abstract

Transmission and Epidemiology Session

The Spatial Pattern of Sudden Oak Death Symptoms in Coastal Redwood/Tanoak Forests

Mark Spencer[1] and Kevin O'Hara[2]

This paper presents the results of a spatial analysis of the point pattern of SOD symptoms and tanoak (*Lithocarpus densiflorus*) mortality in coastal redwood (*Sequoia sempervirens*) forests in Marin and Mendocino counties. The study incorporates five plots on Marin Watershed lands and three plots on Jackson State Forest in Mendocino. These plots average 450 stems per plot and include data on location, species, disease status, diameter, height, and crown position for each stem. Mortality for tanoak stems ranges from 18-50% on Marin plots and from 10-18% on Mendocino plots free of SOD symptoms. Spatial point pattern analysis does not provide direct causal understanding of the processes driving the generation of mortality and disease patterns. However comparison of Marin stand patterns against SOD-free Mendocino stand patterns and patterns generated under the Poisson distribution or other models enhances our understanding of the scale at which the underlying processes function. This paper emphasizes the implications of these results for silvicultural treatment of diseased stands and indicates the ways in which spatial pattern analysis can enhance our understanding of *Phytophthora ramorum* and *Hypoxylon thouarsianum* epidemiology.

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Abstract

Transmission and Epidemiology Session

Factors Related to *Phytophthora ramorum* Canker (Sudden Oak Death) Disease Risk in Coast Live Oak and Tanoak

T. J. Swiecki[1] and E. A. Bernhardt[2]

This paper reports on the first three years of data from a case-control study to examine the role of water stress and various other factors on the development of *Phytophthora ramorum* stem canker (Sudden Oak Death) in coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). The study compares subject trees that exhibited symptoms of *Phytophthora* infection (case trees) with symptomless (control) trees. In September 2000, 2001, and 2002, we collected data in 150 circular plots (8 m radius) in areas where disease caused by *Phytophthora ramorum* was prevalent. Each plot was centered around a case or control subject tree. Plots were established at 10 locations in Marin County, and 1 location each in Sonoma and Napa Counties.

A number of plot and tree factors were associated with disease in the subject tree in logistic regression models for coast live oak. Vegetation-related plot variables that were positively correlated with disease in coast live oak included the count of California bay (*Umbellularia californica*) trees in the plot, the number of plot trees with *Phytophthora* canker symptoms, and the presence of poison oak (*Toxicodendron diversilobum*) in the plot. Tree-related factors that were associated with disease included multiple stems, large stem cross-sectional area, high levels of canopy exposure, and high stem water potential (SWP). In addition to these factors, logistic models based on plot trees other than the subject trees showed a negative association between *Phytophthora* canker symptoms and decline symptoms associated with agents other than *Phytophthora*.

The direction of the effects of a number of variables in several different analyses suggests the possibility that *Phytophthora* canker in coast live oak is more likely to occur in trees that are vigorous and/or fast-growing (larger, more dominant, less waterstressed, not in decline due to other agents) than in trees that are suppressed and/or slow-growing. Significant positive correlations between canopy dieback and *Phytophthora* canker may indicate that diffuse dieback in the canopy is an early indicator of *Phytophthora* canker for both coast live oak and tanoak.

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Abstract

Molecular and Microscopic Analysis Session

PCR-Based Detection of *Phytophthora ramorum* in Plant Tissue

Katherine Hayden[1], Justin Tse[2], Kelly Ivors[2], Matteo Garbelotto[2], and Cheryl Blomquist[3]

We report on the use of Polymerase Chain Reaction (PCR) as a diagnostic test for *Phytophthora ramorum*, the causal agent for Sudden Oak Death. Our protocol allows the detection of *P. ramorum* in DNA extracted directly from plant samples using equipment available in most molecular biology laboratories. It is, therefore, an easily accessible method that bypasses the difficulties inherent in culturing *Phytophthora* species. Ten of the 16 known hosts for *P. ramorum* were discovered with this method; this is the first time a new forest disease has been studied from the time of its discovery using DNA technology. This protocol has been used to diagnose *P. ramorum* infection in multiple hosts across plant families; these data are reported here. We use a nested approach, first selectively amplifying a 687bp fragment in the Internal Transcribed Sequence (ITS) region of the ribosomal DNA; from those fragments an internal 291bp fragment is amplified. This approach allows us to maximize sensitivity while maintaining specificity.

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Abstract

Molecular and Microscopic Analysis Session

Molecular Identification and Detection of *Phytophthora* Species and Populations of *P. ramorum*

Peter Bonants[1], Marjanne de Weerdt[2], Robert Baayen[3], Hans de Gruyter[3], Willem Man in 't Veld[3] and Laurens Kroon[2]

The genus *Phytophthora*, one of the most devastating plant pathogens, currently consists of more than 50 recognized species, which can be divided into six main groups based on morphological features of the sexual structures and sporangia. Other criteria widely used to distinguish species are cardinal growth temperature and mating behavior. Identification of *Phytophthora* species based on morphology is difficult due to intraspecific variation and overlap of morphological characters between species. Specialists capable of identifying *Phytophthora* to the species level are less common and available. Since the last decade a number of biochemical techniques have been developed to aid in identification: Isozyme-, RAPD- and mtDNA analysis. For *Phytophthora*, internal transcribed spacer (ITS) sequence analysis of rDNA has been shown to be very useful for species identification. Although some species have exactly the same ITS sequence (e.g. *P. infestans* and *P. mirabilis*), most species have a unique sequence. In this way new species have been identified: e.g. *P. multivesiculata, P. ipomoeae, P. pistaciae, P. brassicae, P. psychrophila, P. europaea, P. uliginosa,* and *P. ramorum*. These sequence differences can be used to develop very sensitive diagnostic methods using PCR. In this way we developed several tools for diagnosis and detection of *P. fragariae*. Another important technology is AFLP (amplified fragment length polymorphism) in which a DNA fingerprint is generated to study genetic differences within a species. Although designed for construction of genetic maps, we used AFLP to study more than 400 isolates comprising many *Phytophthora* species. Clear differences between species of *Phytophthora* have been observed. In this way an AFLP database has been constructed. Also gene sequencing can be used for identification of species. Sequence analysis of house-keeping genes with structural and metabolic functions was used for phylogenetic analysis, and unknown isolates could be placed in the right species, based on sequence homology. Since the discovery of *Phytophthora ramorum* on *Rhododendron* in The Netherlands and on oak in the USA, several of the techniques mentioned above have been used for identification and detection of this new species of *Phytophthora*. Minor differences between USA* and EU# isolates of *P. ramorum* exist and can be shown with the techniques at hand. Isozyme profiles, gene sequences and minor variation in AFLP fingerprint patterns support that USA and EU populations belong to the same species (*P. ramorum*), despite the difference in mating type (A2 in the USA, A1 in Europe) and unsuccessful attempts to cross European and American isolates.

* USA isolates used in this study were obtained from Dave Rizzo, Matteo Garbelotto and Kelly Ivors University of California, USA).

#EU isolates from Germany used in this study were obtained from Sabine Werres (BBA, Braunschweig, Germany).
Their collaboration is greatly appreciated.

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Abstract

Molecular and Microscopic Analysis Session

Molecular Population Analyses of *Phytophthora ramorum*

Kelly Ivors[1], Katy Hayden[2], Matteo Garbelotto[2], and David Rizzo[3]

Phytophthora ramorum, the causal agent of Sudden Oak Death, is presumed to be an introduced aggressive pathogen in the United States and is responsible for the mortality of at least 3 species of native California oaks (*Quercus* spp.), and tanoak (*Lithocarpus densiflora*). Amplified fragment length polymorphism (AFLP) analyses were conducted to determine the genetic variability and sub-structuring of this pathogen isolated from different hosts and locations in North America and Europe. Among the North American isolates tested, we found 12 different AFLP genotypes. A single genotype accounted for 82% of all isolates; no other genotype had more than 2 isolates represented. In contrast, each of the isolates tested in a recent AFLP study of *P. ramorum* in Europe represented a different genotype, as also observed with the seven European isolates used in our study. The main genotype of *P. ramorum* was recovered throughout the known geographic range of the pathogen, including the northernmost (Oregon) and southernmost locations (Big Sur), a distance of approximately 650 km. These results indicate a largely clonal population in North America with no subdivisions based on geography or host. Such data strongly suggest that the pathogen may move from one host to another with no evidence of host specificity between different isolates. The apparent limited gene pool of *P. ramorum* in North America is suggestive of an introduced or emergent organism, but the actual origin and global genetic structure of the pathogen remains unknown. Although sample size from the European population was limited, AFLP results place the European and North American populations in two separate clades. The results and implications of these population analyses will be further discussed.

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Abstract

Molecular and Microscopic Analysis Session

The Use of Mitochondrial Molecular Markers for Identification of *P. ramorum*

Frank N. Martin[1], P.W. Tooley[2], and R. Frederick[2]

The genus *Phytophthora* contains approximately 67 described species, many of which cause economically important plant diseases. Accurate diagnosis of pathogens to a species level has traditionally required isolation from infected tissue and classification based on morphological criteria. This can be a time intensive endeavor (for isolation and waiting for cultures to grow enough for morphological assessments to be made) that can be further complicated by morphological variation among isolates of a species, thereby requiring a certain level of taxonomic expertise with the genus by the diagnostician. The use of molecular markers can simplify and improve the accuracy of the diagnostic assay.

PCR markers based on the internal spacer sequences of the ribosomal DNA for identification of *P. ramorum* have been previously described. An advantage to using this region for primer construction is that it is present in high copy number (thereby increasing the sensitivity of detection). However, a challenge in using this region is that the rate of evolutionary change may be low, making it difficult to discriminate among more closely related species. Another genomic region that holds promise for construction of molecular markers for *Phytophthora* spp. is the mitochondrial DNA. Previous research has shown that specific regions are highly conserved within a species, yet divergent among even closely related species. This genome also is present in high copy number, which increases the sensitivity of the marker system.

A molecular marker system has been designed that can be used in multiplex amplifications to determine if the sample is infected by *P. ramorum*, other *Phytophthora* spp., or to determine if the extracted DNA contains PCR inhibitors. Several sets of PCR primers have been constructed that are highly specific for *P. ramorum*. Nested amplification can be done with these primers, thereby enabling amplification of target sequences present in low concentrations. A high degree of species specificity has been observed. When tested against 26 other *Phytophthora* spp., one primer pair amplified a smaller band from one other species, *P. heveae* (the design of this primer pair is being reevaluated) while a primer internal to these amplified only *P. ramorum* without background amplification from any other species. The primers amplify the fragment diagnostic for *P. ramorum* from all *P. ramorum* isolates evaluated, including those collected from California, Germany, and the Netherlands. *Phytophthora* genus-specific PCR primers that are compatible with the *P. ramorum* specific primers in multiplex PCR reactions are being tested. These will provide an additional diagnostic tool for determining if any other *Phytophthora* spp. are present in the plant material when the *P. ramorum* specific band is not amplified. Plant specific PCR primers have been developed that are compatible with the *P. ramorum* specific primers in multiplex PCR reactions. These will be used as internal controls to verify that the DNA extraction did not contain PCR inhibitors and reduce the risk of false negatives. The development of a real-time PCR diagnostic system is in progress. Mitochondrial gene sequences have been used to estimate the phylogenetic relationship of *P. ramorum* with other species in the genus; based on cox II sequences *P. ramorum* was most closely related to *P. hibernalis*.

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Abstract

Molecular and Microscopic Analysis Session

Plant Structures through which *Phytophthora ramorum* Establishes Infections

Edwin R. Florance[1]

A major question regarding the biology of *Phytophthora ramorum* is the pathway through which the fungus enters the plant in order to establish an infection. Two possible points of entry are stem lenticels and leaf stomata. Stem and leaf tissue samples of *Umbellularia californica*, *Quercus* spp. and *Rhododendron macrophyllum* infected with *P. ramorum* were collected in both California and Oregon. Samples were fixed immediately in buffered 4% glutaraldehyde, followed by 1% osmium tetroxide, and prepared for both scanning electron and light microscopic analysis. Major tissues of the plants were observed. Data indicate that both lenticels and stomata can serve as points of entry for the hyphae of *P. ramorum*. Hyphae were also observed growing in the vascular tissues of the stem.

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Effects of Sudden Oak Death-Induced Habitat Change on Insectivorous, Cavity-Nesting Birds

Kyle Apigian[1] and Donald L. Dahlsten[1]

Sudden Oak Death (SOD) has killed thousands of trees in oak woodland in 12 coastal California counties. Such disease-induced habitat changes can have severe impacts on wildlife populations by altering their resource base. For example, forest dieback can result in declines or changes in insect populations, which can subsequently impact insectivorous birds by forcing them to switch prey items, change foraging substrates, or increase foraging time. Ultimately, this can impact the nesting success of these bird species. The goal of this study is to examine the effects of SOD-induced stand structure changes in oak woodlands on insectivorous cavity nesting birds, particularly oak titmice (*Baeolophus inornatus*) and chestnut backed chickadees (*Poecile rufescens*). We are examining the impacts of SOD on these species on two levels: the population ecology level by studying nesting success and productivity and the behavioral ecology level by studying diet composition, foraging behavior, and habitat use. This study is part of an integrated, collaborative study (Allen-Diaz and O'Hara labs, UC Berkeley, ESPM) on the ecological effects of SOD that utilizes common, 1-ha study plots established in SOD infected (treatment) and uninfected (control) areas. We will present background for our component of the study, our methods, and preliminary results from the spring of 2002.

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Abstract

Wildlife Impacts Session

Potential Effects of Sudden Oak Death on California Oak Woodland Birds

Bill Monahan[1] and Walt Koenig[2]

SOD threatens to seriously reduce or eliminate several common California oaks, including tanoak, coast live oak, and California black oak. We investigated the potential effect that the elimination of these species would have on a series of resident California oak woodland birds by combining data from the Audubon Christmas Bird Counts with GIS layers of oak distributions from the California GAP analysis. Two estimates were used: first, controlling for other factors, we statistically compared the expected relative density and population variability of birds in areas with and without a particular oak species. Using this method, bird species most strongly affected by the elimination of coast live oaks include California towhees, wrentits, and California thrashers. Elimination of tanoaks and California black oaks had little or no negative effect on relative bird densities, probably because the bird species considered were mostly characteristic of open oak woodland rather than the mixed evergreen forests where these two oak species are most common. The second method we used was to capitalize on the significantly positive relationship between oak species diversity and relative density of many oak woodland birds, including Nuttall's woodpecker, acorn woodpecker, California scrub jay, California towhee, Hutton's vireo, plain titmouse, and western bluebird. By comparing the current range of areas containing different numbers of oak species with the range upon the elimination of SOD-sensitive species, we estimated the overall population reductions that these oak-affiliated avian species are likely to suffer due to the elimination of one or more oak species due to SOD. Our approach yields quantitative estimates of the potential ecological effects of SOD and should help focus attention on the conservation of particular oak species whose loss will apparently have relatively greater effects on wildlife.

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Abstract

Wildlife Impacts Session

Sudden Oak Death Wildlife Impacts Study—San Luis Obispo County

William Tietje[1] and Donald Dahlsten[1]

Sudden Oak Death (SOD) has been identified in coast live oak and tan oak woodlands in 12 California coastal counties. Woodlands in several other counties are at risk. This study is part of a collaborative effort in which UC Berkeley researchers are investigating the effects of SOD on vertebrates by comparing data from infected and uninfected counties. This presentation will focus on the objectives, study design, first-year results, and future work in coastal oak woodlands of San Luis Obispo County, an uninfected county with thousands of ha of woodlands at risk. A risk-prediction map was used to place study plots in coast live oak woodlands of high and low levels of risk for SOD. Amphibians and reptiles are sampled with coverboards, while small mammals are live-trapped. Nest boxes and foraging observations are used to study the diet composition and behavioral ecology of insectivorous cavity-nesting birds. Sampling for vertebrate species is conducted on 1-ha plots where vegetation is also sampled. California Wildlife Habitat Relationships System-identified at-risk wildlife species are of primary interest. Data from San Luis Obispo County will be used to generate habitat models and to evaluate the effects of SOD on wildlife.

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Abstract

Survey/Monitoring Session

Sudden Oak Death Surveys in Oregon—2001 and 2002

Ellen Goheen[1], Mike McWilliams[2], Alan Kanaskie[2], Nancy Osterbauer[3], Everett Hansen[4], and Wendy Sutton[4]

2001: Two aerial surveys in southwest Oregon, one in July and one in October, 2001, detected 33 sites with recently killed tanoak trees, a possible indicator of Sudden Oak Death (SOD). Field crews during ground-verification identified eight additional sites with recently killed tanoak trees. Of these 41 sites, 9 were confirmed positive for the SOD pathogen, *Phytophthora ramorum*, based on cultural morphology and PCR analysis. The infested sites all were within a 9-square mile area near Brookings, Curry County, Oregon, and ranged in size from 0.5 to approximately 12 acres. They occurred on industrial forestland, non-industrial forestland, and Federal land (Bureau of Land Management). The primary plant species killed by *P. ramorum* was tanoak (*Lithocarpus densiflorus*), with Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*) also infected and occasionally killed. Other causes of tanoak mortality included *Armillari*a sp. (9 sites), intentional herbicide or mechanical damage (7 sites), an unidentified *Phytophthora* species (5 sites), and unknown agents (11 sites). *Armillaria* sp. was the most frequently encountered pathogen associated with single trees or small clumps of 2 or 3 dead tanoaks, while larger groups of dead tanoak were more likely to be SOD. Ground surveys and roadside surveys not associated with the aerial surveys failed to detect Sudden Oak Death.

2002: From January through August, 2002, ten new sites with SOD were detected during the course of eradication and monitoring activities. All of these sites were quite small (0.2 to 1 acre) and in close proximity to previously identified sites. Most of these new sites have already been cut and burned; the remainder will be treated as soon as safely possible. In July 2002, an aerial survey of the tanoak forest types in southwest Oregon (2.1 million acres) detected 89 sites with recently killed tanoak trees. As of August 26, 2002, 80 sites had been checked, and no *P. ramorum* has been found. To date, *P. ramorum* has only been detected within the 9 mi2 regulated area that was established by the Oregon Department of Agriculture in 2001.

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Abstract

Survey/Monitoring Session

Development of a National Survey Protocol for Detection of *Phytophthora ramorum*

W.D. Smith[1], E.M. Goheen[2], F. Sapio[3], K.W. Gottschalk[4], S. Frankel[5], and P. Dunn[6]

Sudden Oak Death, a newly identified forest disease caused by the pathogen *Phytophthora ramorum*, has been killing thousands of tanoak and oaks and infecting shrub species such as rhododendron and evergreen huckleberry in coastal areas of central California. Small infestations were recently found in southern Oregon and eradication efforts have begun. Laboratory investigations indicate that other oak species, including northern red and pin oak, are susceptible to the pathogen. Concerns regarding the risk of this disease to the Nation's oak forests have led to development of a National Sudden Oak Death Detection Survey of forests through the Forest Health Monitoring program.

A risk map was developed for detecting the possible presence of *P. ramorum* based on three factors: (1) distribution of known and suspected host species, (2) likely pathways of introduction to new areas, and (3) climatic conditions that favor or limit the development of the pathogen.

Host susceptibility studies have only recently begun; preliminary results suggest that members of the white oak group are not susceptible to *P. ramorum*; therefore, in addition to those species confirmed to be susceptible, the red oak, live oak, and willow oak groups and evergreen Ericarious shrubs such as are assumed to be at risk.

The evergreen hosts such as rhododendron and huckleberry are currently considered to be very important inoculum sources. Introduction into the East Coast is likely through *Rhododendron* nurseries. Recent reports on the susceptibility of Douglas-fir suggest that Christmas tree farms in the east are an additional pathway of introduction.

Climatic factors were the maximum and minimum temperature that *Phytophthora ramorum* spores are unlikely to survive and the number of the months of adequate rainfall (5 inches or 3 inches and one day of dense fog) during optimum temperature (60 and 80 degrees F) for infection.

An ordinal scale risk map was developed by the spatial intersection of the three factors identifying regions of high (X), moderate (1/3X), and low (1/64X) risk. This approach will allow the participating agencies to focus the initial detection surveys in high-risk areas in order to maximize the efficiency of the limited resources available. Locations of known infected areas are not used in the mapping process. Comparison of infected locations with expected risk provides an opportunity to evaluate the veracity of the procedure.

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Abstract

Survey/Monitoring Session

Spatial Modeling of Sudden Oak Death Risk and Oak Mortality Clustering, China Camp State Park

Ross Meentemeyer[1] and Maggi Kelly[2]

Spatial pattern is a fundamental property of disease dynamics because it reflects the environmental forces acting on the dispersal and life cycles of a pathogen. For this reason research in plant disease epidemics is beginning to focus on spatially explicit approaches to quantify and model landscape patterns of disease spread. Despite the strengths of a spatial approach, relatively few studies have developed spatial models of plant disease spread in natural systems due to the challenge of integrating numerous, spatially-referenced samples of disease incidence with digital maps describing spatial variations in environmental factors.

We present a landscape-scale study on the distribution of *Phytophthora ramorum*-related oak mortality and underlying environmental factors. Second order spatial point pattern analysis techniques (Ripley's K) are applied to the distribution of dead tree crowns (derived from high-resolution imagery) in Marin County, CA to determine the scale and extent to which oak mortality is spatially clustered in 2 years (2000 and 2001). Both years show clustering patterns between 100 and 300 m. A statistical model is developed to predict spatial patterns of risk for oak mortality based on several landscape-scale variables. Proximity to forest edge is the most important explanatory factor, followed by potential topographic moisture, abundance of Umbellularia californica, and potential solar radiation. This preliminary research demonstrates the utility of integrating remote sensing with GIS and spatial modeling for understanding the spread of exotic species invasions. Future work will use these mapping technologies in combination with fieldwork and DNA analysis to more effectively characterize spatial patterns of disease factors such as the genetic background of host species, plant community structure, and weather patterns.

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Abstract

Survey/Monitoring Session

Remote Sensing of Host Species Distribution in Hardwood Forests with Sudden Oak Death in Marin and Sonoma Counties

Maggi Kelly[1] and Ross Meentemeyer[2]

Marin and Sonoma counties have pockets of extensive oak mortality throughout forest stands, and several of these areas are recognized hot-spots for the disease Sudden Oak Death. The disease type common in these areas causes overstory crown color change and mortality which is visible from above. Airborne Digital Acquisition and Registration (ADAR) imagery was gathered in Marin county in spring 2000, 2001 and 2002, and in Sonoma county in summer 2001. The 4-band (red, green, blue and near infrared) imagery has a ground resolution of 1-meter, sufficient to map individual tree crowns. The imagery was analyzed for hardwood species discrimination and for individual tree mortality in the study areas using a combination of supervised and unsupervised classification routines. Maps of hardwood species distribution were created. In addition, the spatial pattern of the resulting array of dead tree crowns by year was quantified to demonstrate clustering of the mortality across multiple scales. These data were used in further research that models the underlying environmental factors influencing mortality. This paper presents results from the remote sensing and spatial analyses and discusses the advantages and challenges of remote approaches to mapping Sudden Oak Death.

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Abstract

Survey/Monitoring Session

Statewide Aerial and Ground Surveys for Mapping and Monitoring the Distribution of Sudden Oak Death

Lisa Levien[1], Jeff Mai[1] and Walter Mark[2]

California's oaks are an essential part of our heritage and natural landscape. Since 1995, SOD has been confirmed in California from Humboldt County to Monterey County, as well as in Curry County, Oregon, and is particularly severe in Marin, Santa Cruz, and Monterey Counties in California. The actual current geographic range of the fungus that causes SOD, *Phytophthora ramorum*, is unknown. A team of USDA Forest Service, California State University (CSU), and University of California (UC) researchers was assembled to conduct a statewide survey to map and identify the fronts of infection and overall distribution of SOD on overstory hosts, including coast live oak (*Quercus agrifolia*), tanoak (*Lithocarpus densiflorus*), Shreve oak (*Quercus parvula* var. *shrevii*), and California black oak (*Quercus kelloggii*). The 12 currently infested counties plus those counties south of San Francisco (San Benito, Fresno, San Luis Obispo, Kern, Santa Barbara, Ventura, and Los Angeles), and the Sierra Nevada foothill band of black oak were jointly surveyed. Additional counties bordering infested counties including Solano, Yolo, Lake, Glenn, Tehama, Trinity and Siskiyou were also surveyed. Aerial surveys were conducted from May 29, 2002 through July 3, 2002 over 20 million acres of species susceptible to SOD. Approximately 14,000 miles of ground were flown and 450 polygons exhibiting oak mortality mapped. The area included in the polygons was approximately 157,000 acres, a nearly four-fold increase in the area mapped in the 2001 aerial survey. Ground surveys of a prioritized sample of the aerially surveyed polygons were checked primarily for new infestations and for infestations that expand the existing range of SOD. Field crews were provided with GPS coordinates to center points of a polygon, navigated to the center and determined if SOD symptoms were present in any one of the susceptible species. Established sampling protocols were followed and all samples were shipped to the laboratory for confirmation of SOD. Results from the entire aerial and ground survey were compiled and evaluated.

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Abstract

Pathogenicity and Resistance Session

Comparison of Pathogenicity of *Phytophthora ramorum* Isolates from Europe and California

Hans de Gruyter[1], Robert Baayen[2], Johan Meffert[2], Peter Bonants[3], and Fons van Kuik[4]

The first findings of *Phytophthora ramorum* in Europe date from 1993 (The Netherlands) and Germany (1994). Sudden Oak Death, also caused by *P. ramorum*, was observed in California from 1995. Most probably, the fungus was separately introduced both in Europe and in the USA. In California, *P. ramorum* has since killed large numbers of coast live oaks and tan oaks, and a number of new host plants representing several plant genera has been found during the last 2 years. However, in Europe, *P. ramorum* has only inflicted damage on *Rhododendron* spp. and *Viburnum* spp. plants so far.

It is not clear why *P. ramorum* has not been associated with oak death in Europe. Climatic differences would not seem the most likely explanation, because the fungus does spread on *Rhododendron* and *Viburnum* species. European white oak species, in northwestern Europe, mainly *Quercus robur* and *Q. petraea*, might be resistant to *P. ramorum*, in contrast to native red oaks in California. Alternatively, the pathogenicity of the American population of *P. ramorum* might differ from its European counterpart. The latter hypothesis is supported by apparent differences in mating type (A1 versus A2), and minor molecular differences between both *P. ramorum* populations. However, such small differences could also be due to a founder effect, with two narrow and slightly distinct lineages having been introduced independently in Europe and California.

We tested whether European white oaks are susceptible and also whether European *P. ramorum* isolates differ in pathogenicity from American ones. In a glasshouse experiment under strict quarantine conditions, the type isolate (CBS 101553) and isolate O4 from *Q. agrifolia*, California were inoculated on young plants of *Q. robur, Q. rubra, Fagus sylvatica, Lonicera periclymenum, Vaccinium myrtillus, Viburnum* (x) *bodnantens*e 'Dawn', and *Rhododendron ponticum*. Isolate O4 had been generously made available by Matteo Garbelotto (Berkeley) and Dave Rizzo (Davis). Two treatments were applied, viz. dipping the foliage into a suspension of sporangia and mycelial fragments of the fungus, and placing discs of agar-bearing mycelium on wounded stems. As a control, foliage of plants was dipped into water, or pure agar discs were placed on wounded stems, respectively. Plants were maintained at 18&Mac176;C and 100% humidity (after 2 weeks 80% humidity) for three months. As expected, *Rhododendron ponticum* plants were highly affected, developing severe leafspots and stem lesions after 1 week. Plants of the European wild blueberry, *V. myrtillus*, died within 4 weeks. Plants of *F. sylvatica* and *Q. rubra*, infected on wounded stems, showed a severe dieback of twigs after 4 weeks. Plants of *Q. robur* seemed free of damage, as was the case with *V.* x *bodnantense*. Plants of *L. periclymenum* also remained unaffected. All control plants remained healthy. The European *P. ramorum* isolate was equally pathogenic as the American one.

Our results suggest that European *Q. robur* is not susceptible to *P. ramorum*; however, European beech and *Q. rubra*, also common indigenous respectively exotic species in European forests, proved to be susceptible. The importance of stem wounding for establishing infections suggests that the latter two species may have some field resistance. The lack of damage on *V.* (x) *bodnantense* may indicate that infection occurs by root infection, in line with the formation of basal cankers on this host and the absence of leaf infection in practice. More research is required to test whether or not native European oak species and other trees found not susceptible in this experiment can be infected through the roots or stem base. Further experiments on European forest tree species, lane trees and hardy ornamentals will be conducted in the coming year.

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Pathogenicity of *Phytophthora ramorum* Isolates from North America and Europe to Bark of European Fagaceae, American *Quercus rubra,* and Other Forest Trees

Clive M. Brasier[1], Joan Rose[2], Susan A. Kirk[2], and Joan F. Webber[2]

P. ramorum has not been found on trees in Europe. In 2001, a series of pathogenicity tests was initiated with the aim of assessing: (i) the risk posed by *P. ramorum* to native UK/European *Quercus*, Fagaceae and other European trees; (ii) the comparative risk posed by the North American (NA) and European (EU) *P. ramorum* populations; (iii) the comparative susceptibility of *Quercus rubra*. The experiments (in quarantine chambers) employ the 'log inoculation method' later adapted for assessing susceptibility of *Quercus*, *Alnus* and other tree species to *Phytophthora* bark pathogens. As this method involves wound inoculation of freshly cut logs, it tests the pathogen's potential to attack the phloem and cambium after bark entry. It provides consistent linear measurements for assessing genetic differences in aggressiveness between wild isolates or progenies. After a 5-6 week test at 20°C, unattacked bark is usually still 'green.' About 8 replicate inoculations per isolate, randomized overall, are used. In UK, tests are usually best conducted during summer.

Three experiments were conducted in 2001 and another five in 2002, on a total of 23 hosts. Common European deciduous oak (*Quercus robur*) was included in seven experiments and eastern American red oak (*Q. rubra*) in three. Most tests have involved at least two EU and two NA isolates of *P. ramorum*. Control isolates of *P. cinnamomi*, *P. cambivora* and alder *Phytophthora* have behaved as predicted. Results so far are summarized here. (i). Bark of the following species appears more susceptible to *P. ramorum*: *Q. ilex*, *Q. cerris*, *Q. rubra*, *Fagus sylvatica*, *Picea sitchensis*, *Pseudotsuga menzeisii*, *Chamaecyparis lawsoniana* and *Rhododendron ponticum*. (ii). The following appear less susceptible: *Q. robur*, *Q. suber*, *Castanea sativa*, *Betula pubescens*, *Aesculus hippocastanum*, *Prunus laurocerasus*, *Acer pseodoplatanus*, *Alnus glutinosa*, *Sequoia sempervirens* and *Taxus baccata*. (iii). The following appear resistant: *Tilia cordata*, *Carpinus betula*, *Populus* sp., *Ulmus procera* and *Fraxinus excelsoir*. *Q. robur* was in the 'less susceptible' category in all seven experiments. *Q. cerris* was 'more susceptible' to two EU isolates but 'less susceptible' to two NA isolates. Further tree species will be tested in future. 'More susceptible' hosts will be investigated under different test conditions.

In culture North American *P. ramorum* isolates were, on average, slower growing at 20°C than European isolates. NA isolates were also more phenotypically variable, comprising both a wild-type form and a range of non wild-type forms; whereas EU isolates were very uniform and wild-type. In a

direct comparison 15 EU isolates were, on average, more aggressive than 15 wild-type NA isolates in bark of both *Q. rubra* (mean lesion areas 31.3 and 22.3 cm^2) and *Q. robur*. However there was a large range overlap, some NA isolates being about as aggressive as the most aggressive EU isolates. Further NA/EU comparisons are in progress.

These studies complement work by UK Central Science Laboratory on foliage susceptibility of woody ornamental and hedgerow plants.

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Abstract

Pathogenicity and Resistance Session

Inoculation Trials of *Phytophthora ramorum* on Detached Mediterranean Sclerophyll Leaves

Eduardo Moralejo[1] and Luz Hernández[1]

Phytophthora ramorum, the causal agent of Sudden Oak Death syndrome in California, has been recently isolated from ornamental Rhododendron sp. in Mallorca (Balearic Islands, Spain). This is the first record of this polyphagous pathogen from a Mediterranean climate and vegetation area outside California. To date, it seems that the Sudden Oak Death epiphytotic is limited to the fog belt area along the Pacific Coast where special climatic conditions within the Mediterranean climate pattern are produced. It is unclear whether *P. ramorum* could thrive in drier areas.

In order to assess the risk of spread of *P. ramorum* from Mallorca nurseries to natural ecosystems, as it is believed has occurred before in California, we have tested the susceptibility of species from an assortment of local flora. Inoculation trials were carried out on plants of Mediterranean evergreen oak forest and maquis-type vegetation. The abaxial side of detached leaves of *Quercus ilex, Arbutus unedo, Lonicera implexa, Rhamnus alaternus, Pistacia lentiscus, Ceratonia siliqua, Smilax aspera, Olea europea, Cistus salvifolius, Rubus ulmifolius, Rosa sempervirens* and *Clematis flammula* were inoculated with 20 μl drops containing 100 to 200 zoospores, which were placed on the laminar base, margins, center and tip of six leaves of each species. These were incubated in humid chambers at 17 º C under 12 h daily fluorescent white light. Lesion development and the formation of sporangia and other vegetative structures were checked daily after 72 h, as well as sporangia produced per unit leaf area.

All plants except *Clematis flammula* and *Rubus ulmifolius* were susceptible to the pathogen. However, there were some differences of lesion expansion, sporangial production and chlamydospore formation among the species tested. *Quercus ilex* developed limited necrotic lesions after 9 days' inoculation but the pathogen could be reisolated. Conspicuous necrotic lesions were produced 72 h after inoculation in the other plants tested and extensive blight after 9 days occurred on leaves of *A. unedo, O. europea, C. siliqua, R. alaternus, P. lentiscus and L. implexa*. On several hosts, abundant chlamydospores were readily formed in condensation drops on leaf lesions and also sporadically and subepidermally on the adaxial side. Sporangial density ranged from 1 to 11 sporangia mm2. Structures produced by *P. ramorum* similar to coelomycete acervuli, which we believe have not been previously reported for oomycetes, have been consistently observed after 2 weeks' inoculation on the adaxial side of leaves of *O. europea, C. siliqua* and *P. lentiscus*. Sporangiogenous layers were formed within the hosts and dehiscence was by rupture of the overlying tissue; slimy sporangial masses were liberated. The term "sporangiomata" is proposed to describe these structures. These preliminary studies indicate a significant susceptibility of some widespread Mediterranean plants to *P. ramorum*. Newly morphological characters, herein reported (aerial chlamydospores and sporangiomata) give new insights on how this pathogen might become established in drier environments.

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Abstract

Pathogenicity and Resistance Session

Sporulation Potential of *Phytophthora ramorum* on Leaf Disks from Selected Hosts

J. L. Parke[1], E. M. Hansen[2], and R. G. Linderman[3]

Although *Phytophthora ramorum* can infect a broad range of plant species, some hosts appear to contribute to the disease epidemic more than others. One reason for this is that hosts could differ in their capacity to support inoculum production by the pathogen. We assessed the sporulation potential of *P. ramorum* on various host species under controlled conditions by quantifying zoospores and chlamydospores produced from inoculated leaf disks during a 7-day period. Host species included in the assay were California bay laurel (*Umbellularia californica*), tanoak (*Lithocarpus densiflorus*), madrone (*Arbutus menziesii*), Pacific rhododendron (*Rhododendron macrophyllum*), and evergreen huckleberry (*Vaccinium ovatum*). Additional non-host plant species included in the assays were vine maple (*Acer circinatum*), salal (*Gaultheria shallon*), red alder (*Alnus rubra*) and Oregon white oak (*Quercus garryanna*).

Leaf disks (6 mm diam) from each plant species were dipped in a zoospore suspension (6 x 104 zoospores mL-1) and incubated on moist filter paper in a petri dish for 24 hr. Each disk was then floated on the surface of sterile water (1 mL) placed in a well of a 24- well microtitre plate. Control treatments consisted of leaf disks dipped in sterile water rather than in the zoospore suspension. In each assay, there were 5 replicate leaf disks per plant species and inoculation treatment, and treatments were completely randomized within the microtitre plates.

Leaf disks and the well contents were examined daily for sporangia, zoospores, and chlamydospores. If sporangia were observed, zoospore release was induced by chilling (at 4°C) the microtitre plates containing the leaf disks, then returning the plates to room temperature. After removal of the disks, the contents of each well were mixed, and 25- 100 _L of the suspension was plated onto modified PARP medium to enumerate colonies arising from zoospores. Chlamydospores surrounding the leaf disks were counted 7 days after inoculation. Leaf disks were then cleared in 10% KOH, rinsed in water, acidified in dilute HCl, and stained in 0.05% trypan blue in lactoglycerin to reveal additional chlamydospores in or on the plant tissue.

The capacity for *P. ramorum* to produce sporangia, zoospores, and chlamydospores on leaf disks was greatest and most rapid on California bay laurel. Abundant sporangia and active zoospores were released within 24 hr following placement of leaf disks in the wells, and zoospores continued to be released for several days. Tanoak also supported numerous sporangia and zoospores soon after infection. On madrone leaf disks, only chlamydospores were produced. On other hosts, a few to many sporangia were produced over the course of several days, with chlamydospore production following. Of the species not reported as hosts, inoculum production was most abundant on vine maple and salal.

Despite the artificial nature of this assay, results indicate that certain foliar hosts enable rapid and prolific reproduction of the pathogen, whereas other hosts do not. The possible epidemiological ramifications of this finding will be discussed.

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Abstract

Pathogenicity and Resistance Session

Potential Susceptibility of Native Southwest Oregon Plant Species to *Phytophthora ramorum*

J. W. Zanzot[1], J. L. Parke[2], and E. M. Hansen[2]

In contrast to the situation in California where disease is widespread, Sudden Oak Death (SOD) is restricted to a small geographic area in Oregon. As eradication efforts progress, the potential effect on surrounding plant communities remains a contingency to be addressed.

The southwestern corner of Oregon, where SOD has been found, is adjacent to the Siskiyou Mountains, which harbor some of the most diverse plant communities in North America. Tanoak, the principal host for SOD in Oregon, is found throughout many of these communities, on the western side of the range. To better predict the disease risk to plant communities in this region, we are employing an USDA Forest Service classification scheme in which 17 tanoak associations are defined by species presence and correlated with temperature and precipitation. We are using a detached leaf assay to identify potential foliar hosts among tree, shrub, and herb species in each of these tanoak associations. Leaves are dipped in a suspension of *P. ramorum* zoospores and incubated in a moist chamber for seven days. The area of necrotic leaf tissue is determined, and compared to symptoms on leaves of known hosts. Previous work supports the validity of the detached leaf assay as an indicator of whole plant susceptibility.

Many plant species associated with tanoak not yet exposed to the pathogen in nature appear susceptible to foliar necrosis by *P. ramorum*. This includes the understory species *Chimaphila umbellata* (common prince's pine), *Vaccinium parvifolium* (red huckleberry) and *Linnaea borealis* (twinflower). Understanding the potential susceptibility of tanoak associates to *P. ramorum* is a first step in predicting which plant communities in southwestern Oregon could be at greatest risk should the disease spread, and may help to inform the decision-making process.

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Abstract

Pathogenicity and Resistance Session

Towards a Model of the Genetic Architecture of *Phytophthora ramorum* Susceptibility in Coast Live Oak

Richard S. Dodd[1], Daniel Hüberli[2], Vladimir Douhovnikoff[2], Tamar Harnik[1], Zara Afzal-Rafii[3], and Matteo Garbelotto[2]

The appearance of SOD symptoms on coast live oak trees is spatially uneven within stands and is unrecorded over a large part of the natural range of this species. Among the many factors that could play a role in this uneven distribution of disease is genetic-based variation in levels of host susceptibility both at the local and at the population-wide levels. We have begun a series of studies to investigate whether variations in field observations of disease symptoms may be associated with genetic structure of coast live oak.

1. To investigate variation in susceptibility, we designed an *in vitro* system to assay response of excised shoots to inoculation of *Phytophthora ramorum*. Inoculum was placed under the bark and the excised shoots were grown on the greenhouse bench for about 3 weeks. Response to inoculation was assessed by measuring the size of the resulting lesion. We tested populations from southern California, from an east-west transect in central California and from northern populations in Marin County. All shoots responded to the inoculation by producing lesions, but those from southern California produced only small lesions that tended to be surrounded by a sharply demarcated boundary layer, suggesting a reaction by the plant to the pathogen. There was significant variation among individuals in all populations and the repeatability of this differential response is being tested.

2. An AFLP molecular study of range-wide populations of coast live oak is underway. Our preliminary data indicate a marked differentiation of southern California populations from those of central and northern California. Further inoculation studies are needed to test whether the north-south segregation of populations observed from the molecular data corresponds with a similar geographical division in responsiveness to inoculation with *P. ramorum*.

3. DNA fingerprints, using a large number of AFLP primers, are being analyzed for all inoculated individuals and also for a large number of field site individuals ranked as showing serious signs of infection, moderate infection and no signs of infection, to look for possible associations between the AFLP banding patterns and degree of infection.

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Abstract

Pathogenicity and Resistance Session

Resistance of *Umbellularia californica* (Bay Laurel) to *Phytophthora ramorum*

Daniel Hüberli[1], Will Van Sant[1], Steven Swain[3], Jennifer Davidson[3], and Matteo Garbelotto[2]

Recent research suggests California bay laurel trees, Umbellularia californica, act as a critical vector of *Phytophthora ramorum*, the causal agent of Sudden Oak Death. Severe stem cankers on Quercus agrifolia (coast live oak) are strongly associated with heavily infested adjacent bay trees. This discovery has inspired us to conduct research on both genetic and environmental resistance factors to *P. ramorum* among bays. By understanding the conditions most conducive for *P. ramorum* infecting bays, we can determine which oak woodlands contain the highest risk of potential infection by the pathogen.

The methodology used includes collection of healthy bay samples throughout the known geographic range of the pathogen and then inoculating the leaves with zoospores of *P. ramorum* in a controlled environment. Leaf lesions are then measured to determine susceptibility of trees. Preliminary results suggest there is a range of resistance among infested geographic regions. Comparisons between trees with high infection levels and those which show little or no symptoms were also completed. It is hypothesized that a number of factors contribute to these epidemics including host resistance, genetic structure of host species in the forests, pathogen variation, and environmental conditions. These issues are currently being assessed.

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Abstract

Management and Impacts Session

Phytophthora ramorum in The Netherlands

Maarten Steeghs[1] and Alwin de Haas[1]

Since 1993, a hitherto unidentified *Phytophthora* species has been found associated with twig blight disease in *Rhododendron* and, sporadically, *Viburnum*. The fungus was described in 2001 as *P. ramorum*. Between 1993 and 2000, a total of 18 samples of infected *Rhododendron* plants had been collected in The Netherlands from 15 locations of public green and nurseries. A single finding had been made in 1998 on *Viburnum x bodnantense* 'Dawn'. Unexpectedly, this fungus proved to be conspecific with a new *Phytophthora* species identified in 2000 as the cause of large scale oak mortality in California. The awareness of the potential destructiveness of *P. ramorum* for our ecosystem, landscape, recreation, and, secondarily, trade caused great concern.

In order to make a first assessment of the phytosanitary situation, a general and a targeted survey were carried out in 2001. For the general survey, 214 locations with *Rhododendron* or *Viburnum* were checked. Infestations were found in 11 out of 78 nurseries and garden centers, and in 7 out of 136 locations in public and private green. In the targeted survey, 15 locations where *P. ramorum* was previously found were visited. At 5 locations inspections were not possible due to the absence of host plants. One out of 2 nurseries infected in the previous years still was infected, and 3 out of 8 locations in public or private green. In both surveys, potential host plants including *Quercus*, *Fagus*, and *Castanea* approximately 100 m around infected *Rhododendron* or *Viburnum* plants were checked for symptoms of *P. ramorum*. In none of the cases bleeding cankers or other symptoms suggesting Sudden Oak Death were encountered. Our experience so far suggests that in The Netherlands the disease is restricted to *Rhododendron* (particularly *R. ponticum* and *R. x catawbiense*) and *Viburnum* (particularly *V. x bodnantense* 'Dawn'). In nurseries, *P. ramorum* was restricted to infested lots of these two genera; no spread was found in adjacent lots of (potential) host plants. In public and private green, only *Rhododendron* was infected. No spread from *Rhododendron* to other plants was observed. Control measures taken in nurseries were aimed at securing that plant material for trade is free from *P. ramorum*. Infected plants and neighboring plants within 1 meter were destroyed; remaining plants had been treated chemically beforehand as a preventive measure. Re-inspection took place during the quarantine period of three months. Lots found to be infected at re-inspection were destroyed. On the plots where plants had to be removed because of infestation, a prohibition (3 years) on growing host plants was imposed. In 2001, in public and private green, infested plant parts were pruned and destroyed, fallen leaves were removed and wound treatment was applied.

All member states of the EU are concerned about the disease. In July 2002, the European Commission decided to take precautionary measures for *P. ramorum*. The member states have to carry out national surveys in addition to the implementation of the official measures. The precautionary measures of the EU are partly based on the assumption that *P. ramorum* strains from the USA are different from those in Europe. This assumption is based on genetic differences and differences in epidemic development between California and Europe. The assumed differences are subject of international research.

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In The Netherlands, in 2002 and 2003, the Plant Protection Service is performing inspections on all 700 nurseries cultivating *Rhododendron* and *Viburnum*. Potential host plants including *Quercus* receive special attention during inspection for symptoms of *P. ramorum*. Measures in nurseries are according to the new EU legislation. Furthermore, 2000 locations where *Rhododendron* is present in public and private green will be inspected. Inspections are aimed at determining the spread of the disease and at checking for the presence of additional host species in our ecosystem. Following findings in public or private green, measures are imposed aiming at preventing fungal spread and reducing the inoculum pressure; the infested plants are to be removed.

The results of this survey will be used by the government of The Netherlands to determine the approach for 2003 and thereafter regarding the eradication or management of *P. ramorum*. The Plant Protection Service also initiates research in areas where basic information for this policy is lacking, such as pathogenicity testing, population genetics of the pathogen, and development of detection methods.

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Abstract

Management and Impacts Session

Monitoring *Phytophthora ramorum* Within and Around Disease Patches Targeted for Eradication in Oregon

Alan Kanaskie[1], Nancy Osterbauer[2], Ellen Goheen[5], Everett Hansen[3], Michael McWilliams[4], and Wendy Sutton[3]

Sudden Oak Death, caused by *Phytophthora ramorum*, was found in late July 2001 near Brookings, Oregon, killing tanoak (*Lithocarpus densiflorus*) and infecting Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*). Because of the relative isolation and small size of the infestation, eradication is being attempted. In 2001, nine disease centers totaling 40 acres were treated by cutting and burning all host plants within the disease patch and within at least 50 feet of its edge. In post-treatment monitoring of these sites, we analyzed 786 plant samples and 714 soil samples for presence of *P. ramorum*. *P. ramorum* was detected in five of the 786 plant samples and in three soil samples. All positive plant isolations were from tanoak. The infested soil samples all were from within the same site.

In December 2001 and January 2002, we sampled for *P. ramorum* in the forest within 100 feet of the edge of the nine sites that were cut and burned. We installed 82 plots, each 0.05 acre, and collected symptomatic and non-symptomatic plant material from the following plant species: tanoak, evergreen huckleberry, Pacific rhododendron, Oregon myrtle (*Umbellularia californica*), manzanita (*Manzanita* sp.), Pacific madrone (*Arbutus menziessi*), Cascara (*Rhamnus pursiana*), honeysuckle (*Lonicera* sp.), salal (*Gaultheria shallon*), wax-myrtle (*Myrica californica*), and Sword-fern (*Polystichum* sp.). All plant samples were plated onto a *Phytophthora*-selective medium and tested using PCR analysis and ELISA. Of the approximately 400 samples analyzed, only one, a tanoak, tested positive for *P. ramorum*. This finding resulted in an expansion of the treatment area to include the additional infected tree and neighboring host plants.

In June and July of 2002, the perimeter survey was repeated at the nine original sites plus one new site, and 58 samples were analyzed for presence of *P. ramorum,* which was recovered from tanoak within the 100-foot perimeter zone at three sites. At one of these sites, *P. ramorum* also was recovered from pacific rhododendron. Treatment areas have been enlarged to include the infected plants and neighboring host plants. We observed an increased frequency of symptomatic plants and recovery of *P. ramorum* in June and July 2002 that could be related to warm wet weather during May.

As of November 5, 2002, 11 new disease patches (in addition to the original nine patches identified in 2001) totaling 9 acres have been identified. All occur within the nine square-mile regulated area and are near known disease patches, but beyond the 100- foot wide survey zone. Treatment and monitoring are underway on these sites.

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Abstract

Management and Impacts Session

Fire Behavior and Fire Effects in SODS-Affected Oak Woodlands in Marin County, California

Kent Julin[1], David Sapsis[2], Janet Klein[3], Michael Swezy[4]

Information regarding the impacts of SODS mortality on wildland fire behavior is needed to guide public policy decisions regarding mitigation of potential hazards. This study monitored fire behavior and fire effects on a 110 acre prescribed fire in oak woodlands in 2001 on watershed lands managed by the Marin Municipal Water District. Pre-fire stand characteristics, fuel loading, and incidence of SOD were assessed. A prescribed fire was conducted in August, 2001 under relatively mild summer conditions. Rate of spread, flame length, and tree torching activity were documented. Post fire tree mortality, fuel loading, scorch height, and other variables were recorded post-fire. Results will be summarized.

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Abstract

Management and Impacts Session

Potential Impact of *Phytophthora ramorum* on Nursery Crops in the Pacific Northwest

R. Linderman[1], J. Parke[2], and E. Hansen[2]

The discovery of *Phytophthora ramorum* infecting plants such as *Rhododendro*n and *Viburnum* that are grown extensively in nurseries raised the question of what its potential impact would be if introduced into production areas of the nursery industry in the Pacific Northwest. We predict very high risk because of the wide range of tree and shrub plant species and cultivars grown; because the climatic conditions in Oregon's Willamette Valley would be optimum for the pathogen; because irrigation and fertilization practices might favor infections in the nursery; because plant material at all stages of growth is moved within and between nurseries; and because symptoms caused by *P. ramorum* and other *P.* species on any one host, such as rhododendrons, might be similar and therefore preclude its detection. Furthermore, symptoms caused by *P. ramorum* might not be the same on different hosts. As a result, infected symptomatic or symptomless plants might be shipped to other nurseries.

Our studies sought to determine (1) if there would be any distinguishing symptoms caused by *P. ramorum* on a range of ornamental plants compared to those caused by other *P.* species known to occur in Oregon (*P. cactorum, P. syringae, P. citricola, P. hevea, P. parasitica*, *P. citrophthora*, and *P. cinnamomi*); (2) how many plants would be potential hosts for *P. ramorum* compared to other *P.* species, (3) if *P. ramorum* is more virulent than other *P.* species on any given host plant, and (4) if the biological traits for *P. ramorum*, especially sporulation capacity, would increase its potential to have a major disease impact on nursery production.

Inoculation of detached leaves of all hosts with mycelial plugs of each *P.* species resulted in varied susceptibility and severity of symptoms based on visual ratings of lesion size. On any given host, lesions were essentially identical in appearance, but on hosts like laurels, "shot-hole" lesions developed compared to general necrosis seen on most hosts. Species of *Rhododendron*, *Pieris, Vaccinium, Syringa, Prunus* (Laurel), *Cotoneaster*, and *Arctostaphylos* were the most susceptible to the most *P.* species. Some plants were susceptible to *P. ramorum* but not other *P.* species (*Viburnum plicatum* 'Tomentosum' and *V. davidii*), while others were susceptible to other *P.* species but not *P. ramorum*. *P. ramorum, P. citricola*, and *P. citrophthora* were the most aggressive/virulent pathogens, often spreading throughout the entire leaf. Of the many plant species and cultivars tested by inoculation with mycelial plugs or by dipping leaves in a zoospore suspension, some were essentially resistant, others varied from low to moderate to highly susceptible. Sporulation (sporangia/zoospores and chlamydospores) by *P. ramorum* also varied on different hosts when leaf discs were inoculated by floating them on a zoospore suspension, and it was more profuse on infected tissues and on agar media than any other *P.* species. These results indicate (1) that a wider range of nursery and landscape plants should be surveyed for early detection of *P. ramorum* and other aggressive species in the nurseries; (2) that *P. ramorum* is as virulent as *P. citricola* and *P. citrophthora* on some hosts, uniquely virulent on some hosts, and less virulent on others; and (3) that the sporulation potential of *P. ramorum* exceeds that of most *P.* species, leading to the conclusion that *P. ramorum* would have very significant disease-causing potential in nurseries in the Pacific Northwest.

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Chemical Treatment Strategies for Control of Sudden Oak Death in Oaks and Tanoaks

D. Schmidt[1], T. Harnik[1], D. Rizzo[3], and M. Garbelotto[2]

Field experiments were conducted in Marin, Alameda, and Santa Cruz counties to evaluate the effectiveness of chemical treatments for controlling Sudden Oak Death. Nursery-grown saplings as well as native populations of mature coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflora*) were tested. Trees were infected by placing a small amount of mycelium of *Phytophthora ramorum* under the bark. A variety of commercially available chemicals that have been used on other *Phytophthora* species were evaluated. Application methods included trunk injections, soil drenches, topical applications, and foliar sprays.

Treatments with phosphonate compounds significantly reduced lesion size in both oaks and tanoaks. Injecting the chemicals into the trunk of the tree was found to be the most effect method. Treatment of the tree prior to infection was found to be significantly more effective at controlling the disease than treatment after infection. Phosphonate treated trees remained resistant to new *P. ramorum* infections for at least 3 months. However, mature trees may require a longer period following chemical treatment to become resistant to infection.

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Abstract

Management and Impacts Session

Understanding the Disposal and Utilization Options for Wood Infected by the Sudden Oak Death Syndrome

John Shelly[1], Ramnik Singh[2], Katalin Jackovics[3], and Tad Mason[4]

Removal of trees infected with the Sudden Oak Death Syndrome (SOD) might be desirable because of hazard issues or utilization potential. This project was initiated to analyze the potential value of using the infected wood for value-added products and to further understand the risk of spreading the disease through disposal and utilization channels. Of the hardwood host trees known to be infected, only *Lithocarpus densiflorus* (tanoak) and *Quercus keloggii* (California black oak) have any previously demonstrated value as commercial lumber. However, if other infected species can be effectively used to produce a value-added product they can help defer some of the costs of removal and disposal. In addition, if it is confirmed that other commercial lumber species are viable hosts for the disease and likely to aid in the spread of the disease then SOD has the potential to be a major threat to the forest products industry. The primary focus of this study is to evaluate the effects this disease has on the raw material properties of infected material and to evaluate disposal and utilization options. The objectives are to: (1) survey the existing utilization markets and processing infrastructure for the host tree species, (2) analyze the basic wood properties of the most severely infected tree species, and (3) determine the utilization potential and best uses for infected wood.

This paper reports on the preliminary wood quality results of *Quercus agrifolia* (coast live oak). Physical and chemical properties of healthy (control specimens) trees were compared with early symptomatic, advanced symptomatic, and dead trees. Two different sites in Marin County were sampled. Properties measured include wood density, extractive content, and sugar analysis (total polysaccharides and degradation of cellulose and hemicellulose). Density, a benchmark property for utilization, decreased with increasing levels of disease, as much as a 20% reduction was measured in infected, dead trees but the results are confounded by the large variations of density within the species tested – coast live oak. Sapwood extractive content also followed the progression of the disease and decreased from about 10% (by mass) to 2% in the dead specimens, suggesting metabolic activity of biodeteriogens. A slight decrease in NaOH solubility with advancing levels of disease also supports the observation of sapwood degradation. At this time, the role of secondary pathogens (hypoxylon, other decay fungi, ambrosia beetles, and bacteria) is not clear. Lignin and sugar contents, tree form and the large density variation found in infected trees preclude the use of coast live oak for lumber. Based on the preliminary chemical analysis, this material has potential for uses as firewood, compost, hog fuel, pulp, and fuel ethanol. Further study of each of these suggested uses is needed to determine the effects of processing on the viability of the disease and the risk of spread in processed material.

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Abstract

Management and Impacts Session

Survivability of *Phytophthora ramorum* in the Composting Process

S. Swain[1], T. Harnik[1], M. Mejia-Chang[1], J. Creque[2], M. Garbelotto[1]

It is estimated that millions of cubic tons of green-waste may be infected by *Phytophthora ramorum* in California. Disposal of this material is a major regulatory and management issue within the state. Here we show that composting effectively eliminates *P. ramorum* from green-waste. In laboratory heat treatment tests, wood chips and cankered stems from coast live oak, and infected bay leaves were all non-infectious after a 2 week exposure at 55 degrees centigrade. These same types of infectious plant materials were also used in field composting trials, utilizing both window and forced-air methods. All plant material extracted from compost piles was free from *P. ramorum* after 2 weeks. Pathogen viability of was tested both with direct plating on PARP selective medium and with pear-baiting, both before and after treatment. We will show whether compost can be re-infected after treatment, and draw conclusions on the overall potential of composting as a viable method of eliminating *P. ramorum* from green-waste.

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Poster Sessions

Poster Session A -Transmission and Epidemiology

- A01 Cheryl Blomquist Can Vertebrates Transmit Viable *Phytophthora ramorum* Spores - Data from the Lab
- A02 Jennifer M. Davidson Spatial Distribution of Disease Caused by *Phytophthora ramorum* in Coast Live Oak Woodlands
- A03 Ross Meentemeyer Modeling the Spread of *Phytophthora ramorum*: Integrating Spatial Data on Abiotic Conditions, Plant Community Structure, and Population Genetics of Host Species
- A04 Shannon K. Murphy A Survey of Sudden Oak Death in Native California Forest and Woodland Communities: Relating Incidence and Intensity of *Phytophthora ramorum* to Plant Community and Site Variables
- A05 T. J. Swiecki Disease Progress and Changes in Stem Water Potentials of Coast Live oak and tanoak affected by *Phytophthora ramorum* Canker (sudden oak death)
- A06 S.A. Tjosvold Incidence of *Phytophthora ramorum* Inoculum Found in Streams Running through Areas of High Incidence of Sudden Oak Death in Santa Cruz County
- A07 S.A. Tjosvold Incidence of *Phytophthora ramorum* Inoculum Found in Soil Collected from a Hiking Trail and Hikers' Shoes in a California Park

Poster Session B - Molecular and Microscopic Analysis

- B01 E. R. Florance Ultrastructural Features of *Phytophthora ramorum* and Infection Morphology on Leaves
- B02 Jeffrey Boore Genomic Approaches Soon to be Available for Understanding Sudden Oak Death
- B03 Christopher Fung AFLP Analysis of Single-Zoospore Isolates of *Phytophthora ramorum*
- B04 Kelly Ivors TaqMan PCR for Detection of *Phytophthora* DNA in Environmental Plant Samples
- B05 Ping Kong Rapid Identification of *Phytophthora ramorum* Using Single Strand Conformation Polymorphism of Ribosomal DNA
- B06 Linda Kox Validation of a PCR method for detection and identification of *Phytophthora ramorum*
- B07 Willem A. Man in 't Veld Isozyme Genotyping of *Phytophthora ramorum* reveals a remarkable genetic homogeneity
- B08 Britta Zielke Taxonomic Investigations of European and American Isolates of *Phytophthora ramorum*
- B09 G. Bilodeau Detection and Identification of *Phytophthora ramorum* Causal Agent of Sudden Oak Death by Molecular Beacon

Poster Sessions

Poster Session C - Impacts of Sudden Oak Death

C01 Kyle Apigian Effects of Sudden Oak Death on Vertebrate Communities in Coast Live Oak and Tanoak/Redwood Ecosystems: a Collaborative Study

C02 Kristen Baker Investigating the Relationship of Stand Structure and Development to Spread and Incidence of Sudden Oak Death in Redwood / Tanoak Forests

C03 Letty Brown Effects of Sudden Oak Death on Coast Live Oak Woodland Vegetation Structure

C04 Brandon M. Collins Stand Structure and Fuel Loads in Sudden Oak Death Syndrome Infected Forests in Marin County

C05 Danny L. Fry Sudden Oak Death Infection Levels and Associated Forest Characteristics

C06 Lee Klinger The Ecology of Mosses in Forest Decline

C07 Brice A. McPherson Interactions among Bark and Ambrosia Beetles (*Coleoptera*: *Scolytidae*) and a Novel Disease of Oaks in California

Poster Session D - Management and Disposal

D01 S. Bergemann Detection and Identification of Decay and Pathogenic Fungi from Tanoak and *Quercus* ssp. Directly from Wood

D02 Frank Betlejewski Lessons from *Lateralis*: Experiences with Managing Another Forest *Phytophthora*

D03 Tamar Y. Harnik Effect of Chemical and Biological Control Agents on *Phytophthora ramorum* Growth in *in vitro* Trials

D04 John Shelly Wood Volume Estimation of SOD Infected Coast live oak in Marin County

D05 Andrew J. Storer Michigan - An Uninfested State Responding to the Threat of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death

D06 Pavel Svihra Study of a Prophylactic Insecticidal Treatment for Preventing Emergence of Oak Bark Beetle and Oak Ambrosia Beetle Broods

D07 Andy Trent Use of Power Washer for Sudden Oak Death Mitigation

Poster Session E - Pathogenicity and Resistance

E01 Richard S. Dodd The California Red Oaks: a Pandora's Box

E02 E. Goheen Plant Species Naturally Infected by *Phytophthora ramorum* in Oregon Forests

E03 Kurt W. Gottschalk Potential Susceptibility of Eastern United States Forests to *Phytophthora ramorum*

E04 E. Hansen *Phytophthora ramorum* and Oregon Forest Trees - One Pathogen, Three Diseases

E05 Daniel Hüberli Worldwide Plant Hosts of *Phytophthora ramorum*

Poster Sessions

E06 A.J. Inman Comparative Pathogenicity of European and American Isolates of *Phytophthora ramorum* to Leaves of Ornamental, Hedgerow and Woodland Understory Plants in the UK

E07 Alan Kanaskie Monitoring Insect Activity Near Sites Infested with *Phytophthora ramorum* in Southwest Oregon

E08 R. G. Linderman Relative Virulence of *Phytophthora* Species, Including the Sudden Oak Death Pathogen, *P. ramorum*, on Leaves of Several Ornamentals

E09 P. E. Maloney Studies on the Effect of *Phytophthora ramorum* on Madrone

E10 J. L. Parke Differential Susceptibility to *Phytophthora ramorum* Among Vaccinium Species and Cultivars

E11 J. L. Parke Assessing the Susceptibility of Pacific Northwest Nursery Plants to *Phytophthora ramorum* Using a Detached Leaf Assay

E12 Friederike Pogoda Pathogenicity of European and American *P. ramorum* Isolates to *Rhododendron*

E13 S.A. Tjosvold Susceptibility of Azalea (*Rhododendron*) to *Phytophthora ramorum*

E14 Claude Delatour Discovery of *Phytophthora ramorum* on *Rhododendron* sp. in France and Experimental Symptoms on *Quercus robur*

Poster Session F - Monitoring

F01 Desheng Liu Evaluation of ADAR Imagery for Mapping Moisture Stress in Oaks with Advanced Symptoms of Sudden Oak Death

F02 Ross Meentemeyer Mapping the Risk of Sudden Oak Death for California

F03 N.K. Osterbauer Survey of Oregon Nurseries and Other Commodities for *Phytophthora ramorum*

F04 R. Pu Spectroscopic Determination of Health Levels of Coast Live Oak (*Quercus agrifolia*) Leaves

F05 S.A. Tjosvold Evaluation of a Novel Diagnostic Procedure to Detect the Presence of *Phytophthora ramorum* by Sampling Ooze from Infected Cankers

F06 Karin Tuxen OakMapper webGIS for monitoring Sudden Oak Death

F07 Sharon von Broembsen Pilot Survey of Oklahoma Ornamental Nurseries for *Phytophthora ramorum*

F08 R. Ioos Results of French Survey of *Phytophthora ramorum* in 2002 (abstract not available at time of printing)

Abstract

Poster Session A
Transmission and Epidemiology

Poster A01
Can Vertebrates Transmit Viable *Phytophthora ramorum* Spores - Data From the Lab?

Cheryl Blomquist[1], Keyt Fischer[2], and Michael Fry[3]

Vertebrates have been identified as potential vectors of *Phytophthora* canker disease (a.k.a. Sudden Oak Death or SOD). This experimental study examines two aspects of potential transmission by vertebrates. First, we determined whether chlamydospores of *Phytophthora ramorum* survive gut transit in birds and small mammals. Pigeons (*Columba livia*) and rodents (*Mus musculus*) were fed known concentrations of chlamydospores and hyphae in cornmeal agar. Feces were collected and plated on PARP medium at regular intervals during the course of the experiment. Second, we examined the ability of birds and small mammals to externally transport *Phytophthora ramorum* zoospores, sporangia and chlamydospores. Pigeons and rodents were misted with zoospores and sporangia suspended in distilled water. Samples were collected by swabbing and rinsing the animals at timed intervals. Rinse water and feces were assayed by baiting and plating onto PARP medium. In a separate experiment, chlamydospores suspended in cornmeal agar were mixed with damp soil and the mixture was placed on cage surfaces. Animals were allowed full access to the cage surfaces and after exposures of varying intervals samples were collected and plated on PARP medium.

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Poster A02
Spatial Distribution of Disease Caused by *Phytophthora ramorum* in Coast Live Oak Woodlands

Jennifer M. Davidson[1], Allison C. Wickland[2], Andrea C. Morse[2], Camille E. Jensen[2], Rebecca G. Albright[2], Shannon C. Lynch[2], Justin Tse[3], Garey Slaughter[2], Matteo Garbelotto[3], and David M. Rizzo[2]

We established three one hectare plots in coast live oak woodlands infested with *Phytophthora ramorum* to study the spatial distribution and spread of disease among all hosts of *P. ramorum*. On each plot, all individuals greater than 1 cm dbh were tagged, mapped, and visually assessed for disease symptoms due to *P. ramorum*. For all hosts except madrone, tissue samples were taken from symptomatic plants for isolation of *P. ramorum* on selective medium (PARP). Because madrone is a difficult host from which to culture *P. ramorum*, molecular diagnostics were used to test for

the presence of *P. ramorum*. In addition, failed culturing for coast live oak, big leaf maple, toyon, coffeeberry, honeysuckle, and hazelnut were also subjected to molecular testing. In total, 1533 plants were censused on the three plots. Two of the three plots (Fairfield Osborn Preserve (FOP), Sonoma Co. and Napa Skyline Wilderness County Park, Napa) were dominated by coast live oak and bay. Coast live oak and bay accounted for 38.7% (164/424) and 45.0% (191/424) of the tagged species at FOP, and 45% (241/535) and 40.6% (217/535) of the tagged species at Skyline, respectively. In the third plot in Pacheco Hills, Marin Co., coast live oak and madrone were the dominant tree species, comprising 58.0% (333/574) and 29.6% (170/574) of the individuals, respectively. Identification of infected individuals on these plots via molecular analysis is still in progress. Final data will be analyzed for spatial distribution of disease on coast live oak versus other hosts. Future work will monitor how infection spreads on each of the plots, especially in years of high versus low rainfall.

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Poster A03

Modeling the Spread of *Phytophthora ramorum*: Integrating Spatial Data on Abiotic Conditions, Plant Community Structure, and Population Genetics of Host Species

Ross Meentemeyer[1], Hall Cushman[2], James Christmann[2], Nathan Rank[2], Rich Whitkus[2], and Dave Rizzo[3]

We are researching the ecological and genetic factors that influence the spread of the SOD pathogen, *Phytophthora ramorum*, in the Sonoma Valley of northern California. The multidisciplinary work uses computer mapping technologies in combination with fieldwork and DNA analysis to integrate spatial data on host genetics, community structure, and environmental variation with investigations of mechanisms underlying spread of *P. ramorum*. We aim to accomplish the following: 1) Develop a statistically based model that predicts spatial patterns of SOD risk on the landscape, based on relationships among critical environmental factors. These include proximity to infected locations, genetic background of foliar host species, plant community structure, landscape structure, abiotic conditions, and anthropogenic activities. 2) Develop a mechanistic model that predicts spatial patterns of pathogen activity and host susceptibility based on empirical studies of pathogen dispersal between hosts and environmental conditions conducive to pathogen reproduction and dispersal. 3) Develop a integrated model of SOD spread based on the most predictive components of the statistical and mechanistic models. To accomplish these goals, numerous field plots are being established throughout Sonoma Valley. In each plot, we are quantifying plant community structure, recording weather conditions, sampling for *P. ramorum* infection status, and collecting samples for genetic analysis. These data will be linked with high-resolution aircraft imagery and integrated into a GIS database for spatial modeling. This work is funded by the National Science Foundation for four years.

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Poster A04

A Survey of Sudden Oak Death in Native California Forest and Woodland Communities: Relating Incidence and Intensity of *Phytophthora ramorum* to Plant Community and Site Variables

Shannon K. Murphy[1] and David M. Rizzo[2]

Ten sites were surveyed for incidence of *Phytophthora ramorum* on all woody plant species in native forest and woodland plant communities. Sites were sampled in Alameda, Contra Costa, Marin, Mendocino, Napa, Santa Cruz, and Sonoma counties. Six of the sites were in California state parks, three were in county or regional parks, and one was on a private property protected with a conservation easement. Sampling occurred during the summer months of 2001 and 2002. At each site, twelve tenth hectare circular plots were surveyed. Plots were located along elevational gradients proceeding perpendicularly from river valleys toward ridge tops, with three plots along four transects at each site. Plots were selected based on aspect, plant community type, and areas with minimal human disturbance, rather than presence of *P. ramorum*. Each plot was evaluated for plant species composition, shrub and tree density, tree basal area, site variables, and presence of *P. ramorum*. Within each tenth hectare plot, a nested hundredth hectare circular plot was evaluated for tree density by plant species, soil/litter depth, and intensively sampled for percent incidence of *P. ramorum* in each woody plant species. Symptomatic leaf, stem, and bark tissue were assessed for presence of *P. ramorum* by culturing onto PARP media. Additional samples, as well as those with negative isolation results, were examined using PCR molecular detection protocols. Preliminary isolation results confirm *P. ramorum* presence at four of ten sites and within 14 of 120 plots. Symptomatic oaks with bleeding cankers characteristic of *P. ramorum* were observed at five additional sites but were not successfully isolated. *P. ramorum* was positively isolated from the following host species: *Corylus cornuta, Heteromeles arbutifolia, Lithocarpus densiflorus, Quercus agrifolia, Quercus chrysolepis, Quercus parvula, Umbellularia californica*, and *Vaccinium ovatum*. PCR detection will be completed in the fall and winter of 2002. Results from this study may provide additional information about the distribution of *P. ramorum*, including examination of location and intensity of *P. ramorum* within state and regional parks, and within east bay counties like Alameda and Contra Costa where Sudden Oak Death (SOD) plot studies have not previously been established. Additionally, this project may reveal new host species of *P. ramorum*, as well as provide information about whether or not infection in other host species can precede infection in *Quercus* spp. and *Lithocarpus densiflorus*. Furthermore, this study may identify influential plant community characteristics, as well as both biotic and abiotic site variables that contribute to the presence, spread, and intensity of the SOD disease. Information garnered in this study will provide additional basic information for ecological understanding and management of SOD.

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Poster A05

Disease Progress and Changes in Stem Water Potentials of Coast Live Oak and Tanoak affected by *Phytophthora ramorum* Canker (Sudden Oak Death)

T. J. Swiecki[1], and E. A. Bernhardt[2]

In September of 2000, 2001, and 2002, we recorded detailed symptom information and measured late summer midday stem water potentials (SWP) of coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*) with and without symptoms of *Phytophthora ramorum* stem canker (Sudden Oak Death).

Disease progress in trees with symptoms of *Phytophthora* canker was more rapid for tanoak than for coast live oak. *Phytophthora*-related mortality between 2000 and 2001 was greater for tanoak (19% of symptomatic plot trees) than for coast live oak (6% of symptomatic plot trees). Apparently new *Phytophthora* canker symptoms were also more common in tanoak than in coast live oak (8% and 1.6% of previously asymptomatic plot trees, respectively). Somewhat less than half of the coast live oak and tanoak cases showed no obvious advancement of disease symptoms between 2000 and 2001. Preliminary disease progress models for coast live oak indicate that most of the factors associated with disease occurrence are not associated with disease progress in trees that are already symptomatic. This pattern would be consistent with a disease model in which infection events occur infrequently and disease progress is due primarily to canker expansion rather than the initiation of additional cankers.

Tree SWP readings for 2001 were 0.54 MPa lower on average than 2000 SWP readings across both species. Overall average SWP readings in 2002 were intermediate between 2000 and 2001 readings. SWP readings for individual trees in all three years were highly correlated. SWP readings made on multiple trees within plots were also correlated, suggesting that plot soil moisture levels account for much of the variation in SWP between plots. Trees with

Phytophthora canker symptoms did not show a significant overall reduction in SWP between 2000 and 2001 relative to asymptomatic trees, and trees that died between 2000 and 2001 had higher than average SWP readings in 2000. Disease progress from 2000 to 2001 was not correlated with changes in SWP. From these results and other observations, we conclude that trees with *Phytophthora* canker develop substantial water stress in the canopy within the final year before the top is killed. Hence, canopy water stress appears to be a relatively late symptom of disease.

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Poster A06

Incidence of *Phytophthora ramorum* Inoculum Found in Streams Running through Areas of High Incidence of Sudden Oak Death in Santa Cruz County

S.A. Tjosvold[1], D.L. Chambers[2], J.M. Davidson[3], and D.M. Rizzo[3]

Phytophthora ramorum, the causal agent of the disease commonly known as Sudden Oak Death is a prevalent pathogen in California with its effects evident in 12 counties and found on 14 different oak, tanoak and non-oak hosts. In Santa Cruz County, several streams run through affected woodland. It is important to know if and when *P. ramorum* is found in these streams. Potentially the pathogen could be dispersed from affected drainages downstream for long distances. In some cases, stream water is used for irrigation by some nurseries and landowners situated along these streams. Water contaminated with inoculum might be inadvertently dispersed to nursery hosts or other landscape hosts. In addition, if *P. ramorum* could be reliably detected from streams draining through diseased woodland, this detection technique might be used as a novel technique to identify and delineate potentially affected woodland areas that are not easily accessible.

Sampling of stream water for the presence of *Phytophthora ramorum* inoculum occurred approximately weekly, from April 2001 to April 2002, in 7 rivers and creeks in Santa Cruz County. All streams were perennial except one that dried in late spring. All northern streams drained through woodland known to contain Sudden Oak Death. The two most southern streams drained through woodland that contained hosts of Sudden Oak Death but the pathogen has not been confirmed in those regions. The samples were taken downstream from the suspected diseased watersheds. Approximately 8 liters of water were sampled at each site. In the laboratory, the water samples were baited with pears, inspected for characteristic *P. ramorum* lesions, and pear tissue from suspicious lesions were transferred to PARP selective media for pathogen identification.

P. ramorum was detected in all streams except the two most southern streams, Corralitos Creek and Aptos Creek. The pathogen was found almost entirely during the period following the beginning of winter rainfall and through early spring (January 2002 through March 2002). There was no *P. ramorum* detected in streams during the dry months except on one occasion;

P. ramorum was detected in September 2001 in the water collected from the San Lorenzo River following a short rain in the river's drainage.

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PosterA07

Incidence of *Phytophthora ramorum* Inoculum Found in Soil Collected from a Hiking Trail and Hikers' Shoes in a California Park

S.A. Tjosvold[1], D.L. Chambers[2], J.M. Davidson[3], and D.M. Rizzo[3]

Phytophthora ramorum, the causal agent of the disease commonly known as Sudden Oak Death is a prevalent pathogen in California with its effects evident in 12 counties and found on 14 different oak, tanoak and non-oak hosts. The disease has been commonly found in areas of high recreational use such as state parks and forest service lands. In some of the most heavily diseased areas, recreational hikers frequent the trail systems. It is important to understand if the pathogen inoculum is found in soil along the hiking trails and, if inoculum is there, whether it could be picked up on shoes of recreational hikers. The spread of inoculum could then be a real threat as the hiker visits new areas.

Sampling occurred following rainy periods in March and May and then monthly through the dry summer and fall period 2002 in a popular California State Park. At each sampling date, soil was sampled from five locations along a 1.3 km "nature trail" loop and from the bottom of shoes of hikers who had hiked the trail. In the laboratory, soil samples were baited with pears, inspected for characteristic *P. ramorum* lesions, and pear tissue from suspicious lesions were transferred to PARP media for pathogen identification.

In the spring rainy periods, the incidence of successful pear baiting for *P. ramorum* varied from 40 - 60 % success rate for trail soil and 40 - 95% success rate for soil removed from hiker's shoes. In the dry summer period, no baiting was successful from trail soil or shoe soil.

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Abstract

Poster Session B

Molecular and Microscopic Analysis

Poster B01

Ultrastructural Features of *Phytophthora ramorum* and Infection Morphology on Leaves

E. R. Florance[1] and J. L. Parke[2]

The anatomy and infection morphology of *Phytophthora ramorum* on leaves of various species were analyzed using light and scanning electron microscopy. Leaves of *Umbellularia californica*, *Rhododendron macrophyllum*, *Vaccinium ovatum* were inoculated under controlled conditions using zoospore suspensions. Horticultural cultivars of *Rhododendron* and *Vaccinium* were also included in the assay. Leaf tissue was harvested at various time intervals to study the sequence of infection events. Samples were fixed in 4% glutaraldehyde followed by 1% osmium tetroxide and prepared for microscopic analysis. Hyphal growth appeared more abundant on the ventral than on the dorsal surface of leaves, and preliminary analysis indicates that stomata are one portal of entry to the interior tissues. Hyphae, sporangia, and chlamydospores were more abundant on *Umbellularia californica* than on other hosts in this assay.

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Poster B02

Genomic Approaches Soon to be Available for Understanding Sudden Oak Death

Jeffrey Boore[1], Susan Lucas[2], Paul Richardson[2], Daniel Rokhsar[2], Eddy Rubin[2], and Brett Tyler[3]

The Joint Genome Institute (JGI) in Walnut Creek, CA, (http://www.jgi.doe.gov/) is a high throughput center for genome sequencing and analysis, with a capacity for producing nearly two billion nucleotides of DNA sequence per month and an extensive history of sequencing whole genomes. The JGI now intends to generate high depth draft sequence of the complete genomes of *Phytophthora sojae* and *P. ramorum*. Sequencing of *P. sojae* is already well underway and DNA is being isolated from *P. ramorum*. Both genomes are expected to be completed not later than June 2003. The JGI, along with collaborators at Virginia Bioinformatics Institute, will also perform deep annotation to identify all genes of *P. ramorum* as well as comparative sequence analyses of the two organisms. These sequences, along with gene annotations, etc. will be made available to all interested researchers via the web. We are in the process of setting up a genomic infrastructure for *P. ramorum* that will include both bioinformatics tools and laboratory reagents. The goal is to provide resources to enable JGI and other genome scientists to interact with the community of *P. ramorum* researchers to do such things as (1) Develop genomic markers with higher resolution for tracking the pattern and mode of infection; (2) Build a strategy for screening for early infection where treatment is most likely to be effective; (3) Select genes whose products are most likely to be effective targets for prevention or treatment of infection; (4) Screen compounds *in vitro* that might be effective for prevention or treatment. Further, the JGI hopes to offer opportunities for training of scientists interested in using genome technology to combat *Phytophthora* infections through our visiting scientists program and to provide a center for interaction of *Phytophthora* researchers, including those doing work in the field and at the whole organism level. We are describing this project here in its early stages in order to get researchers' input on how we might best meet the needs of the community and to inform scientists of the resources soon to be available.

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Poster B03

AFLP Analysis of Single-Zoospore Isolates of *Phytophthora ramorum*

Christopher Fung[1], Kelly Ivors[2], Matteo Garbelotto[2]

Phytophthora ramorum, the oomycete pathogen responsible for Sudden Oak Death, is thought to reproduce asexually through production of zoospores, chlamydospores and sporangia. AFLP (Amplified Fragment Length Polymorphism) analyses were conducted on ten sets of ten single-zoospore isolates. These samples were generated from cultures of *P. ramorum* collected from five different host species (*Quercus agrifolia*, *Quercus kelloggii*, *Umbellularia californica*, *Rhododendron* spp., and *Lithocarpus densiflora*) located in different Northern California counties (Marin, Sonoma, Solano, Santa Cruz and San Mateo). Genomic DNA was extracted from pure cultures of each single zoospore isolate and digested with the restriction enzymes EcoRI and MseI. Fragments of the genomic DNA were ligated to standard blunt end adaptors and amplified by two successive rounds of PCR (Polymerase Chain Reaction). This resulted in the creation of a unique genetic "fingerprint". Fingerprint patterns were analyzed using an ABI 3100 Genetic Analyzer. Genetic similarity or variation between single-zoospore isolates determined from these analyses will be presented.

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Abstract

Poster Session B
Molecular and Microscopic Analysis

Poster B04
TaqMan PCR for Detection of *Phytophthora* DNA in Environmental Plant Samples

Kelly Ivors[1] and Matteo Garbelotto[2]

Diagnosis of *P. ramorum* was initially attempted via direct isolation from symptomatic plant tissue. However, *Phytophthora* species are often difficult to culture from plants, which can lead to false-negative isolations and misdiagnosis of infected plant material. The detection and extent of *P. ramorum* colonization in various host plant tissues was estimated with real-time quantitative polymerase chain reaction (PCR) using TaqMan chemistry. Pathogen DNA was amplified by species-specific primers and a fluorogenic TaqMan probe based on a 74 base pair sequence of the internal transcribed spacer (ITS2) of nuclear ribosomal DNA (rDNA). To check for specificity, the primers (Pram5 and Pram6) and probe (Pram7) were applied to DNA dilutions of 10 *P. ramorum* isolates and 14 other *Phytophthora* species. This assay was positive for all dilutions of *P. ramorum* and *P. lateralis*, and negative for all other species tested with quantification of *P. ramorum* cells linear over a range of 4 orders of magnitude.

Recent observations indicate that another new and undescribed *Phytophthora* species (temporarily referred to as *P. ilicis*-like) is often co-isolated with *P. ramorum* from infected plant material. To help discern the distribution of this new species and its relationship with *P. ramorum* within host plants, another set of species-specific TaqMan primers and probe were developed for *P. ilicis*-like. This protocol amplifies a 64 base pair sequence of the ITS2 region of the rDNA. To check for specificity, the primers (Pi-like1 and Pi-like2) and probe (Pi-like3) were applied to DNA dilutions of 10 *P. ilicis*-like isolates and 14 other *Phytophthora* species.

Using this technique with a multiplex approach, both *P. ramorum* and *P. ilicis*-like DNA can be simultaneously amplified in the same reaction tube, but individually detected by species-specific primers and TaqMan probes labeled with different fluorescent dyes. The total assay can be completed in less than 3 hours without the need to run additional rounds of PCR, agarose gels, or sequencing reactions. The TaqMan probes and primer sets designed in this study can be used as a rapid screening tool for the detection and quantification of *P. ramorum* and *P. ilicis*-like DNA in pure-culture and environmental plant extracts without prior isolation and characterization of the organisms by traditional microbiological methods.

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Abstract

Poster Session B

Molecular and Microscopic Analysis

Poster B05

Rapid Identification of *Phytophthora ramorum* Using Single Strand Conformation Polymorphism of Ribosomal DNA

Ping Kong[1] and Chuanxue Hong[1]

Phytophthora ramorum is an important quarantine pathogen for most parts of the United States and many other countries worldwide. Current approach to identification of this pathogen is primarily based on morphology, which can be difficult and may lead to misidentification due to interspecific overlap and intraspecific variations of diagnostic characters. This study provided an alternative technique to distinguish *P. ramorum* from its genetically close relatives, and those that are morphologically similar or that have an overlap of host range. The technique consists of three major steps: (i) simplified mycelium boiling DNA extraction, (ii) DNA amplification with ITS primers, and (iii) single strand conformation polymorphism (SSCP) analysis. It takes no more than 6 hr to run through the entire procedure, which can determine identities of 13 to 44 isolates of *Phytophthora*, depending on the capacity of thermocycler and electrophoresis unit used. Use of the technique described in this work provides rapid and reliable identification of *P. ramorum* and its close relatives, while requiring minimal training.

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Poster B06

Validation of a PCR Method for Detection and Identification of *Phytophthora ramorum*

Linda Kox[1], Hans de Gruyter[1], Matteo Garbelotto[2], Ilse van Brouwershaven[1], Joke Admiraal[1], and Robert Baayen[1]

Identification of *Phytophthora ramorum* Werres, De Cock & Man in 't Veld, the causal agent of Sudden Oak Death in California and associated in Europe with disease symptoms on *Rhododendron* spp. and *Viburnum* spp., is commonly based on microscopical examination of the morphological structures. Routinely this takes 5-10 days. Recently Matteo Garbelotto, University of Berkeley, California, developed a PCR method which is less time-consuming. We implemented this PCR method in our laboratory. The PCR method is based on sequences in the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA gene repeat. This region is broadly used in diagnostic and evolutionary studies because it is conserved within species and generally variable between species, and is present in multiple copies in each cell.

For the validation of the PCR method, we investigated 129 samples possibly infected with *P. ramorum*. These samples were received as part of a survey held to obtain information concerning the distribution of *P. ramorum* in The Netherlands. The samples consisted of mainly *Rhododendron* spp. (93) as well as *Viburnum* spp. (23), *Quercus* spp. (6), *Aesculus* sp. (2), *Buxus* sp. (1), *Castanea sativa* (1), *Larix* sp. (1), *Sambucus* sp. (1), and *Vaccinium* sp. (1). The samples from *Aesculus*, *Buxus*, *Castanea*, *Quercus*, *Larix*, and *Sambucus* were taken from shrubs or trees in the vicinity of diseased *Rhododendron* plants. Plant parts were cut in 32 small pieces and divided randomly for culturing and PCR. DNA for PCR was isolated using the DNAeasy Plant Kit (Qiagen) and further purified using polyvinyl polypyrrolidone (PVPP) columns. Fifty-three samples were positive both after culturing and with PCR, and 51 samples (including those from *Aesculus*, *Buxus*, *Castanea*, *Larix*, *Sambucus*, *Quercus*, and *Vaccinium*) were negative with both methods. This results in a concordance of 80% between both methods (104/129). Five samples were positive by culturing only, and 20 samples were positive by PCR only. When the total of positives is used as the golden standard, the sensitivity of PCR and culturing is 94 % and 72 %, respectively. Samples of healthy control plants of the aforementioned trees and shrubs were all negative. In several cases where PCR testing was positive but culturing was not successful, sporangia of *P. ramorum* were observed with the microscope but the plant material was apparently too dry. Further investigations should be directed at explaining the remaining differences between culturing and PCR.

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Poster B07

Isozyme Genotyping of *Phytophthora ramorum* Reveals a Remarkable Genetic Homogeneity

Willem A. Man in 't Veld[1], Hans de Gruyter[2], Peter J.M. Bonants[3], and Robert P. Baayen[2]

Three enzyme stains, malate dehydrogenase (MDH), malic enzyme (MDHP) and lactate dehydrogenase (LDH), altogether comprising five putative loci (*Mdh*-2, *Mdhp*, *Ldh*-1, *Ldh*- 2 and *Ldh*-3) were used to characterize twenty strains of *Phytophthora ramorum*. All enzymes used are known to be powerful diagnostic enzymes in the genus *Phytophthora*. The tested strains were isolated from *Rhododendron* spp. and *Viburnum* spp. from six European countries, and from various hosts in the USA (*Arbutus menziesii*, *Lithocarpus densiflorus*, *Quercus agrifolia*, *Rhododendron* spp., *Umbellularia californica*, *Vaccinium* spp. and *Viburnum* spp.). Strikingly, so far all European strains were of the A1 mating type whereas all American strains belonged to the A2 mating type.

All strains contained identical isozyme alleles at all putative loci. At the *Ldh*-2 locus, which is known to encode for dimeric enzymes, two different alleles were present in all strains, indicating the heterozygous state. Similarly, at *Ldh*-3, which is known to encode for monomeric enzymes, two different alleles were present also indicating heterozygosity.

Interestingly, *P. lateralis*, which is phylogenetically the closest neighbour of *P. ramorum*, was homozygous at all isozyme loci except *Ldh*-3, where it had one of the two alleles in common with *P. ramorum*; *P. lateralis* had also one allele in common with *P. ramorum* at *Ldh*-2.

Although a limited number of *P. ramorum* strains have been subjected to isozyme analysis so far, and a limited number of enzymes have been used, the first results indicate that *P.ramorum* is a very homogeneous species, in spite of different mating types, geographic origins and hosts. The apparent genetic homogeneity, as demonstrated by isozyme analysis, provides no indication of differences in pathogenicity between strains originating from America and Europe.

The heterozygosity at *Ldh*-2 and *Ldh*-3 loci of *P. ramorum* is intriguing because first attempts to cross the European A1 and the American A2 mating types have not been successful. It is tempting to speculate that the heterozygosity at two isozyme loci of *P. ramorum*, having one allele at both loci in common with *P. lateralis*, may be the result of reticulation events in its evolutionary past, possibly involving *P. lateralis*. Alternatively, the lineages of *P. ramorum* that have been introduced into Europe and California originate from a sexually outcrossing ancestral population elsewhere in the world. In that case, fine scale differences between geographic populations as recently found with AFLP need not have any meaning in terms of natural species boundaries or pathogenic potential.

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Poster B08

Taxonomic Investigations of European and American Isolates of *Phytophthora ramorum*

Britta Zielke[1] and Sabine Werres[2]

Fourteen German isolates, eight isolates from other European countries and ten American isolates were examined. The study mainly focused on comparing vegetative growth at different temperatures, comparing size and shape of sporangia and chlamydospores, and investigating mating reactions by crossing experiments. The German isolates chosen for this study originated from different *Rhododendron* and *Viburnum* species. The other European isolates originated mainly from *Rhododendron* spp. The American isolates were from hosts other than *Viburnum*.

The twenty-two European isolates were very homogenous. They showed only small variations in colony morphology, growth rate, size and shape of sporangia and chlamydospores. However, there was a high degree of variability amongst the ten American isolates. Some were identical to the European isolates but others showed quite different colony patterns and had a very slow vegetative growth rate. Furthermore, some of these American isolates produced sporangia that were different in shape to those of the European isolates. All European isolates belonged to the A1 mating type and could be successfully crossed *in vitro* with *P. cryptogea*. A small number of oogonia were also produced by most of these isolates after pairing with *P. cinnamomi*. In contrast, those American isolates which could be crossed sucessfully belonged to the A2 mating type. The most successful partners for the American isolates were *P. cryptogea* and *P. cambivora*. However, two American isolates did not produce oogonia with any of the partners. The in vitro pairing experiments of the European isolate BBA 9/95 (the type strain of *P. ramorum*) with the ten American isolates were not successful. Oogonia were not produced on either carrot piece agar or on *Rhododendron* agar after an incubation period of eight weeks. However in first studies with green *Rhododendron* twigs inoculated with the European isolate BBA 9/95 and the American isolate PR6, oogonia were produced on the outgrowing hyphae. The studies have not been finished.

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Poster B09

Detection and Identification of *Phytophthora ramorum* Causal Agent of Sudden Oak Death by Molecular Beacon

G. Bilodeau[1], C.A. Lévesque[2], A.W.A.M. De Cock[3], G. Kristjansson[4], J. McDonald[4], R.C. Hamelin[5]

Phytophthora ramorum, the causal agent of the Sudden Oak Death (SOD), is decimating oak trees in California. This disease is spreading rapidly. A quarantine currently in effect to prevent spread and eradication is being attempted in Southern Oregon where the disease was found recently. New methods are being developed to detect and identify the pathogen reliably in order to implement this quarantine. The most commonly used assay is based on Internal Transcribed Spacer (ITS) DNA analysis. However this assay cannot distinguish reliably between *Phytophthora ramorum* and *Phytophthora lateralis*. In order to overcome this problem, the Beta-tubulin gene was sequenced and more polymorphisms between these two species and between *Phytophthora ramorum* and other *Phytophthora* were found compared to the ITS region. Specific primers for Allelic Specific Polymerase Chain Reaction (AS-PCR) and molecular beacons have been developed to detect and identify this pathogen. The primers are Lock Nucleic Acids (LNA) which show a high specificity and yield. The preliminary assay with the molecular beacon and the primers differentiate *P. ramorum*

from the different species. This new assay will be validated this fall using Canadian Food Inspection Agency (CFIA) and USDA-APHIS samples from the survey.

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Abstract

Poster Session C
Impacts of Sudden Oak Death

Poster C01
Effects of Sudden Oak Death on Vertebrate Communities in Coast Live Oak and Tanoak/Redwood Ecosystems: a Collaborative Study

Kyle Apigian[1], Donald L. Dahlsten[1], and Willam Tietje[1]

The occurrence of Sudden Oak Death (SOD), a disease resulting from a newly described pathogenic organism, *Phytophthora ramorum*, provides a unique opportunity to study its effects on the oak woodland ecosystem in California. Few studies have examined the effects of a widespread forest pathogen at the community level but, due to the potentially widespread deleterious affects of SOD, it is important to begin ecological studies early so the effects of SOD can be anticipated, properly interpreted, and minimized.

Our study examines the impact of habitat change caused by SOD on the bird, small mammal, amphibian, and reptile communities in oak woodlands. We have established 1- ha study infected and control plots in the San Francisco Bay. In San Luis Obispo County, out of the current range of SOD, we established control plots for comparison to the Bay area plots and to obtain pre-infection data. We began data collection in the spring of 2002 by using point counts and foraging observations to study birds, live traps to monitor small mammals, and coverboards to monitor amphibians. This collaborative study will use vegetation data collected on the same plots by collaborating researchers (Allen-Diaz and O'Hara labs, UC Berkeley, ESPM) to establish a link between SOD-induced stand structure alterations and wildlife functional and demographic responses. This poster will present background for our project, our methods, and our preliminary results from 2002.

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Poster C02
Investigating the Relationship of Stand Structure and Development to Spread and Incidence of Sudden Oak Death in Redwood/Tanoak Forests

Kristen Baker[1], Mark Spencer[2], Kevin O'Hara[2], and Scott Stephens[2]

This poster presents the integrated design of three studies investigating relationships between stand structure and development and the spread and incidence of *Phytophthora ramorum* (causal agent of Sudden Oak Death) in tanoak (*Lithocarpus densiflorus*) / redwood (*Sequoia sempervirens*) stands. These three studies include a spatial analysis study to explore spatial patterns of stems with stem

Abstract

Poster Session C

Impacts of Sudden Oak Death

mortality and disease symptoms, a stand development study to reconstruct height and diameter development of stands under various levels of infections, and a thinning/burning study to assess the effect of fire and alterations in stand structure on disease spread. Our studies encompass eight 1 ha plots, four on Marin Municipal Watershed lands and three on Jackson Demonstration State Forest (Mendocino County). We present the research methods of the three studies, display a diagram illustrating the design of our plots and discuss the benefits of our coordinated research design.

The primary objective of the first study is to determine the relationship of spatial pattern of individual stems to patterns of disease spread. Spatial analysis plots, with a minimum of 300 trees, are installed, with all trees tagged and mapped. Species, height, crown class and diameter are recorded. The data include recently killed trees and records of SOD symptoms. Plots are being re-visited regularly to assess changes in disease incidence and spread over time.

The second study involves the reconstruction of individual tree height and diameter development in stands under various levels of infection. Smaller circular reconstruction plots, ranging in size from 0.10 to 0.25 ha., are established within or adjacent to the spatial analysis plots. A subsample of 5-10 trees on each plot are selected for more intensive sampling, which includes either coring all trees in three locations, measurements of crown radii and tree vigor, or stem analysis of selected trees to reconstruct past growth.

The third study will implement thinning and burning treatments to assess the effect of alterations in stand structure on disease spread. These plots, at least one-hectare in size, will be separate from those previously described, and will be established beginning in 2003. Smaller plots will be established within the thinning and burning treatments to evaluate the effects of changing density and species composition.

These three studies will provide valuable information on the incidence and spread of sudden oak death in redwood/tanoak forest stands. Spatial patterning can be a valuable resource for predicting and mitigating effects of disease, while information on stand development patterns provides the necessary background information for developing management tools. Finally, information on thinning and burning treatments and their effect on Sudden Oak Death may lead to the development of new management approaches for treating both infected forests and forests threatened by infection.

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Abstract

Poster Session C

Impacts of Sudden Oak Death

Poster C03

Effects of Sudden Oak Death on Coast Live Oak Woodland Vegetation Structure

Letty Brown[1] and Barbara Allen-Diaz[2]

This study seeks to identify correlates between the spread of non-native *Phytophthora ramorum* and the structure of native coast live oak (*Quercus agrifolia*) plant communities. We compare stand structure, plant species composition, and environmental characteristics in coast live oak woodlands over a gradient of Sudden Oak Death (SOD) infection. The primary objective is to establish baseline information about the types and rates of change in successional pathways, including the potential for species invasions and effects on vegetation productivity. Five 1-ha plots have been established; 3 in Marin County and 2 in Contra Costa County. Within each 1-ha plot, five 0.08 ha subplots were established to sample species composition of herbaceous, shrub, and tree layers, tree seedling density, tree diameter and height, and SOD disease characteristics. Tissue samples from all plots are being collected and tested for presence of *P. ramorum*. Environmental attributes include slope, aspect, elevation, light intensity, and soil type. Characteristics of downed woody debris are also recorded. On the 25 sub-plots established thus far, slope varies between 20% and 60%, with a 40% average. All aspects are represented, but plots tend to occur in mesic *Q. agrifolia* community types with California bay (*Umbellaria californica*), black oak (*Quercus kelloggii*), and/or buckeye (*Aesculus californica*), or madrone (*Arbutus menziesii*) represented. As an indicator of slightly drier sites, toyon (*Heteromeles arbutifolia*) may be present but with much less frequency than the other tree species. Understory herbaceous and shrub species in these stands tend to be sparse. In order to establish additional uninfected control plots, we will incorporate plots in Mendocino and San Luis Obispo counties. This research is funded by the USDA Pacific Southwest Station of the Forest Service and is part of a larger study that includes research by collaborators in tanoak (*Lithocarpus densiflorus*) communities, and estimates of reptiles and amphibians, small mammals, and birds utilizing these SOD-infected and uninfected sites.

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Abstract

Poster Session C

Impacts of Sudden Oak Death

Poster C04

Stand Structure and Fuel Loads in Sudden Oak Death Syndrome Infected Forests in Marin County

Brandon M. Collins[1] and Danny L. Fry[2]

Stand Structure and fuels were investigated in forests affected by Sudden Oak Death Syndrome (SODS) in Marin County, California. The sites chosen for this study were China Camp State Park and Marin Municipal Watershed District. Stand structural characteristics, as well as surface and ground fuel loads were compared between plots with different SODS infection levels. Only the mortality volume of SODS host species, California black oak (*Q. kelloggii*), coast live oak (*Quercus agrifolia*), tanoak (*Lithocarpus densiflorus*), California bay laurel (*Umbellaria californica*), madrone (*Arbutus menziesii*), toyon (*Heteromeles arbutifolia*), were significantly correlated with plot infection levels. Plots were grouped into low (0-25%), mid (>25-50%), and high level (>50%) infection, to explore trends between these infection levels and the plot structural characteristics and fuel loads. Although no significant differences were found, due to tremendous variability, trends did exist between infection levels. Oak and tanoak basal area, as well as total basal area, tended to be lowest in plots with high level infection. In addition, 1000 hour fuel load tended to be highest in plots with high level infection. All host oak trees found in the plots, California black oak, coast live oak, and tanoak, were compared with their site, plot, and individual characteristics, using logistic regression, to test for correlations with individual tree infection. Site aspect and individual tree diameter at breast height (d.b.h.) were found to be significantly correlated with individual tree infection. This study is a preliminary attempt to quantify structural characteristics, and fuel loads in SODS affected forests. This study suggests that some characteristics can be correlated with SODS infection.

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Abstract

Poster Session C
Impacts of Sudden Oak Death

Poster C05
Sudden Oak Death Infection Levels and Associated Forest Characteristics

Danny L. Fry[1], Brandon Collins[2], Brice A. McPherson[2], Maggi Kelly[3], Andrew J. Storer[4], David L. Wood[5], and Richard B. Standiford[2]

Accurate assessment of the Sudden Oak Death epidemic in California requires reliable information on disease impact at both local and landscape scales. Surveys of forested landscapes must be unbiased, efficient, reliable, and capable of covering relatively large areas. We are using the point-centered quarter plotless method to estimate the extent of Sudden Oak Death in two forests with different compositions of susceptible host species in California. Parallel transects were laid out through the forests, with sampling nodes placed every 100 m. The nearest host tree in each cardinal direction was assessed, for a maximum of 4 trees per node. Each tree was also assigned coordinates using global positioning system (GPS). Variables recorded were tree size and basal area, bleeding status, presence of bark and ambrosia beetles (*Scolytidae*), reproductive structures of *Hypoxylon thouarsianum*, and foliage condition. Additional information about the woody vegetation composition and density was obtained using prism sweeps around each node. We conducted surveys in 2001 in China Camp State Park (Marin Co.), where coast live oak, *Q. agrifolia*, and California black oak, *Q. kelloggii*, are the known oak hosts. Coast live oak is the most abundant oak species in China Camp, which has probably been infected since the early 1990s. In 2001-2002, we ran a similar survey in Soquel Demonstration State Forest (Santa Cruz Co.), where *Phytophthora ramorum* is suspected to have become established more recently than in China Camp. Tree species in Soquel known to be susceptible include *Q. agrifolia*, Shreve oak, *Q. parvula* var. *shrevei*, and tanoak, *Lithocarpus densiflorus*. Shreve oak is the most abundant oak species in the Soquel site, which also features extensive redwood stands. The course of the disease in Shreve oak is poorly understood. Sites were similar in dominance by one species, 85% coast live oak in China Camp and 76% tanoak in Soquel. Although much less abundant at China Camp, black oak was similar to coast live oak in the relative proportion of symptomatic trees and the presence of associated organisms. Approximately 16% of the trees were observed with at least one bleeding canker, evidence of wood boring beetles, and fruiting bodies of *H. thouarsianum*. This was not the case for Soquel where the oaks and tanoaks were symptomatic in different proportions. Tanoak had the highest proportion of symptomatic trees with beetles and Hypoxylon (approximately 1%) while the few black oaks sampled were asymptomatic.

Binary logistic regression was used to determine if any variables predicted the presence of bleeding cankers on trees. For both sites, diameter at breast height (DBH) and basal area of associated live trees were positive significant predictors, although oak density and oak basal area were not. Basal area of dead oak trees was also a significant predictor at China Camp but not at Soquel. Additionally, basal area of dead non-oak trees was found to be significant at Soquel. Although it has been suggested that associated tree species, such as bay laurel, may be important in the dispersal of

P. ramorum, bay laurel alone was not a significant predictor. The association of dead oak basal area with bleeding trees may indicate a longer presence of the pathogen in China Camp. This research was designed to evaluate the usefulness of the point centered-quarter method by comparing apparent infection and mortality levels with those obtained from disease progression plots in Marin County For China Camp, the estimates for apparent infection and mortality appear to be in good agreement with those obtained from disease progression plots, for both coast live and black oak. Estimates of the impact of the disease on the trees in Soquel indicate lower levels of infection and mortality for tanoak than in Marin County disease progression plots. Inadequate understanding of Shreve oak symptomology precludes comparable analysis.

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Poster C06

The Ecology of Mosses in Forest Decline

Lee Klinger[1]

In recent decades scientists worldwide have been puzzled by the seemingly rapid decline in the quality and health of many mature and old-growth forests, called “novel forest decline”. Interestingly, forests which are quite removed from the impact of natural and human disturbances often seem to be suffering the worst. Because so many causes have been postulated for this decline, ranging from acid rain and global warming to pathogenic fungi and viruses, most scientists now accept a multiple factor explanation for forest dieback. Indeed, it is common for forests in decline to be simultaneously affected by both environmental stresses (e.g., drought, acid rain, and soil acidification) and biological stresses (e.g., root rot, bark beetles, and budworms). Still, current approaches have yet to yield any unified set of mechanisms which can account for the similarity in the symptoms and etiology of forest decline globally.

A major breakthrough in our understanding of forest decline, including Sudden Oak Death Syndrome, has been achieved by approaching this problem from a systems’ perspective. Complexity is a new theoretical arena, perhaps even a new paradigm in science, that defines the mechanisms by which natural systems self-organize and self-regulate. Applied to forest ecosystems, complexity emphasizes ecological interactions, synergistic couplings, and feedback connections among all the organisms in a forest.

Abstract

Poster Session C
Impacts of Sudden Oak Death

Findings are summarized from more than 20 years of field and laboratory studies in biocomplexity of forest ecology and decline conducted at the University of Colorado and at the National Center for Atmospheric Research. Published research findings from Alaska, Colorado, Canada, Venezuela, Africa, China, and elsewhere show, unequivocally, that an intricate ecological subsystem, comprised of a suite of cryptogamic organisms, plays a central role in the decline and death of woody plants. This has allowed us to identify an underlying physiology of the forest, a physiology analogous to the immune system of an organism, but for an entire ecosystem. The pH balance, a critical parameter in this physiology, is found to shift as the result of successional changes in the ecosystem involving cryptogams, especially mosses. The onset of novel forest decline is observed to be closely associated with an increased cover of mosses, which are shown to kill fine "feeder" roots of plants. Symptoms of novel forest decline, globally, are observed to adhere closely to those expected with acidification and root mortality in these forests.

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Poster C07

Interactions among Bark and Ambrosia Beetles (*Coleoptera: Scolytidae*) and a Novel Disease of Oaks in California

Brice A. McPherson[1], Richard B. Standiford[2], David L. Wood[3], Andrew J. Storer[4], and Pavel Svihra[5]

Insects have many varied roles in tree diseases. They may disseminate a pathogen by scattering or broadcasting inoculum, or inoculate the host with a pathogen by placing the inoculum on the infection court. They may facilitate ingression of a pathogen into the host tissues, invasion of a pathogen through the host tissues or preservation of a pathogen under unfavorable environmental conditions. Interactions among tree diseases and bark and ambrosia beetles that involve predisposition of the host trees to insect attack have been well documented in a number of pathosystems. For example, conifers infected by the fungus *Heterobasidion annosum*, a root pathogen, are prone to mass attack by bark beetles, resulting in elevated mortality. Ambrosia beetles, *Xylotera domesticum*, frequently colonize the bark of beech trees (*Fagus* spp.) that have been infected by fungal bark pathogens, *Nectria* spp., which can lead to structural failure ("beech snap").

Coast live oaks, *Quercus agrifolia*, infected by *Phytophthora ramorum* produce a viscous red to black exudate, referred to as bleeding, that is associated with subcortical cankers, usually on the main stem. Bark and ambrosia beetles consistently colonize the bleeding areas of infected trees while these trees maintain functional green foliage. These saprotrophic insects typically attack recently killed trees and branches, but have not been reported to colonize living trees. In disease progression plots that we have monitored every three months since March 2000, up to 53% of living symptomatic (bleeding) trees were colonized by bark beetles. Through June 2002, every symptomatic tree that died (n = 37) had been colonized by beetles while the foliage was still green.

Abstract

Poster Session C
Impacts of Sudden Oak Death

A beetle-infested tree may also suffer breakage of the major stem at the point of the beetle tunneling. In an effort to better understand the factors influencing colonization of *P. ramorum*-infected trees by ambrosia and bark beetles, we monitored the association of these insects with *Q. agrifolia* in natural forests. During beetle flight seasons for 2 years, sticky traps were placed on trees classified as non-symptomatic, symptomatic (bleeding), and symptomatic/beetle-infested. The mean number of beetles trapped on symptomatic/beetle-infested trees was significantly greater than on both nonsymptomatic and symptomatic trees. This result implies pheromone-mediated colonization of diseased portions of trees, which may contribute to tree mortality. There was no significant difference in catch between symptomatic and non-symptomatic trees. Since the course of the disease in mature trees has not been observed in the absence of ambrosia and bark beetles, the ability of trees to recover from *P. ramorum* infection has not been determined. Studies designed to assess the role of these beetles in Sudden Oak Death Syndrome will be described.

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Abstract

Poster Session D
Management and Disposal

Poster D01

Detection and Identification of Decay and Pathogenic Fungi from Tanoak and *Quercus* ssp. Directly from Wood

S. Bergemann[1], K. Hayden[1], L. Costello[2], T. Swiecki[3], and M. Garbelotto[1]

The disease progression by secondary plant pathogens may be interrelated with *Phytophthora ramorum* in tanoaks and *Quercus* spp., however the synergistic effects remains unknown. Since plant pathogens and other agents of decay cannot be distinguished with certainty in trees on the basis of morphological characters and decay characteristics, the goals of the present research are to identify the pathogenic and decay agents from colonization patterns in wood and plant material. In California, 12 genera of basidiomycetes were identified that are responsible for most of the oak tree failures reported in California including *Armillaria*, *Ganoderma*, *Hericium*, *Inonotus*, *Laetiporus*, *Omphalotus*, *Oxyporus*, *Phellinus*, *Pleurotus*, *Schizophyllum*, *Stereum*, and *Trametes*. The focus of our diagnostic approach is aimed at developing primers at the generic level using the 28S region of the nuclear ribosomal DNA. Each taxon-specific primer was designed to produce a DNA molecule of different length; therefore, multi-plex PCR diagnosis may be performed to identify generic-specific taxa from the size of the PCR-amplified fragment. Primers were tested on DNA extracts from pure fungal cultures, and then on DNA extracts from wood colonized by decay agents. The development of rapid, accurate molecular approaches is pivotal for the identification and will aid in future research in understanding disease development, identifying diseased trees, spread from disease centers, and the synergism between *P. ramorum* and wood decay organisms.

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Poster D02

Lessons from *Lateralis*: Experiences with Managing Another Forest *Phytophthora*

Frank Betlejewski[1], Pete Angwin[2], and Don Goheen[3]

Phytophthora lateralis, the pathogen that causes Port-Orford-cedar (*Chamaecyperis lawsoniana*) root disease, has been present within the natural range of Port-Orford-cedar since the 1950s. Since then, a number of management strategies have been adopted to limit the spread of the disease. Designating the season of activity, scheduling of operations, road closures, and washing of equipment, vehicles and boots have all had some success in reducing the spread of *P. lateralis*. Some of these techniques may be useful in the management of Sudden Oak Death (*Phytophthora ramorum*).

Abstract

Poster Session D
Management and Disposal

The success of various management strategies for Port-Orford-cedar root disease has depended upon complexity, cost, public awareness, and public acceptance. We have learned that while there are many possible methods of management, proposed treatments must be both biologically effective and economically efficient. Monitoring of results is a critical component of disease management. It quantifies results for the scientists and demonstrates the value of applied management to the public.

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Poster D03

Effect of Chemical and Biological Control Agents on *Phytophthora ramorum* Growth in *in vitro* Trials

Tamar Y. Harnik[1], Monica Mejia-Chang[2], and Matteo Garbelotto[2]

Phytophthora ramorum is a major pathogen of native plant communities in California, and currently there is no official method available to control the disease it causes. Chemical and biological control products that are available in the market to control other diseases might be effective against *P. ramorum*. We tested chemical compounds and biological control agents.

Different methodologies were used to test the products in *in vitro* trials including agar incorporation and pathogen inhibition studies. We incorporated the chemicals or the biological control agents into the V8 agar in different concentrations. In these studies, we measured hyphal growth rates and germination rates of zoospores. A few of the biological control products were tested using pathogen inhibition assay. Preliminary results show a significant reduction in colony size as an influence of chemical/biological control agents presence. We also plan to conduct in planta studies using zoospores.

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Poster D04

Wood Volume Estimation of SOD Infected Coast Live Oak in Marin County

John Shelly[1], Katalin Jackovics[2], Ramnik Singh[3], and Tad Mason[4]

Sudden Oak Death has dramatically increased the number of dead and dying trees in areas with a high incidence of infection. These infected trees contribute greatly to the potential hazard for wildfire, physical damage, injury, and disease spread. This situation has led to an interest in developing management strategies to deal with the removal, disposal, and possible utilization of infected trees. One of the most important pieces of information needed to plan for future disposal and utilization needs is a reliable estimate of the volume of material that must be dealt with in both the short term and the long term. Aerial surveys and satellite imagery are known to be helpful in identifying areas of infected trees and even individual trees. However, the lack of information on tree volumes for many of the host species makes it difficult to use this remote sensing data to estimate the volume of infected wood that may be available for disposal or utilization. A relationship between crown spread and wood volume could be used to predict wood volume from remote data.

The purpose of this study is to estimate the amount of SOD infected biomass available for utilization and/or disposal in Marin County. The goal is to develop a volume model based on aerial surveys that can expedite the inventory process throughout all infected counties. Timber cruise data, including the area of the canopy cover, has been collected on 87 *Quercus agrifolia* (coast live oak) trees from a one acre plot in China Camp State Park, San Rafael, CA. Data measured includes canopy size, DBH, total tree height, merchantable height for lumber (up to 8-inch top), and main stem height to a 4-inch top. This data was used to calculate average merchantable wood volume and total biomass volume of the main stem. Canopy size of each tree was estimated by measuring the distance in the four cardinal directions from the center of the tree to the edge of the canopy. The data was analyzed by regression techniques to develop a correlation between canopy size and both merchantable volume and biomass volume. Although preliminary crown spread data resulted in less than ideal correlation coefficients, the method shows promise. Recent refinements in measuring technique and model description are expected to improve the correlation. If successful, the technique will be applied to other host species. These relationships will then be used to estimate total volumes of infected wood from the crown spread areas measured in aerial and satellite surveys.

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Poster D05

Michigan - An Uninfested State Responding to the Threat of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death

Andrew J. Storer[1], Gerard C. Adams[2], John Hill[3], Frank J. Sapio[4], and Robert Heyd[5]

The pathogen that causes Sudden Oak Death, *Phytophthora ramorum*, is not known to occur in the state of Michigan. This pathogen causes a canker disease on oaks and tanoaks and other diseases on host plants in other plant families. The current knowledge

base about this pathogen suggests that the risk of introduction into Michigan is through the movement of diseased or otherwise contaminated nursery stock. Other risks for entry include recreational activities that involve the transport of fire wood or contaminated soil. The potential impact in Michigan is great due to the presence of known susceptible hosts, including red oak (*Quercus rubra*), and climatic conditions that have not yet been demonstrated to limit the survival of the pathogen. This impact will be increased if other susceptible hosts are identified from the native plants of Michigan, especially understory plants that may serve as reservoirs of inoculum in forest environments.

Given the potential impact of this pathogen, all available measures should be taken to preclude the possibility of this pathogen being introduced into Michigan. The current federal regulation does not completely protect Michigan from contaminated material from States not listed as infested by this pathogen, and the screening methods for shipments of plants may be inadequate. In addition, early detection of the pathogen in Michigan will be essential to any plan for eradication. Towards this end an initial survey of nurseries carrying rhododendron stock has been completed. In this survey, rhododendron leaves with leaf spots were collected from nurseries around the state and screened for the presence of *P. ramorum*. Screening techniques included isolation onto PARP medium and the use of molecular diagnostic tools developed in California. While nationwide detection efforts include a focus on evergreen understory species, deciduous hosts are also known to harbor the pathogen in the understory in infested areas.

Specific information needs for Michigan include:

- Environmental constraints on the establishment and development of the pathogen
- Susceptibility of shrubby plants associated with Michigan hardwood forests
- Further information on the biology and epidemiology of *P. ramorum*
- Amount and location of host material imported from California and other states
- Optimal survey protocols for nurseries and the field that include timing of survey, evaluation of observed and reported oak and other host mortality, training resource managers in Sudden Oak Death identification and sampling procedures, and an effective reporting system
- Planned responses to detection of the pathogen

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Abstract

Poster Session D
Management and Disposal

Poster D06

Study of a Prophylactic Insecticidal Treatment for Preventing Emergence of Oak Bark Beetle and Oak Ambrosia Beetle Broods

Pavel Svihra[1], Deborah Fenner Crosby[2], and Becky Duckles[3]

Phytophthora ramorum infection of some oaks and tanbark oaks have been accompanied by increased incidence of oak bark beetle and oak ambrosia beetle outbreaks. The effects of these beetles preventing emergence from the infested oaks and averting their destructive activity in the adjacent trees is the objective of this study.

In June 2000, three 40 cm long bolts were removed from eight heavily infested coast live oaks *Quercus agrifolia*, and one black oak *Q. kelloggii* in the following sequence: the first bolt 1 meter above the ground, the second one under the main limbs' crotch, and the third one from a 3-inch diameter branch. Each bolt was then cut vertically into two halves. One half was selected at random and its bark surface sprayed with Permethrin, while the corresponding other half (control) was not sprayed. Bolt halves were placed into separate air-conditioned rearing cans to collect daily emerging beetles.

The results showed that Permethrin significantly reduced emergence of both the oak ambrosia beetle, *Monothrum scutellare*, and the oak bark beetle, *Pseudopityphthorus pubipennis* from the treated logs (a = 0.05).

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Poster D07

Use of Power Washer for Sudden Oak Death Mitigation

Andy Trent[1], and Dick Karsky[2]

The USDA Forest Service, Missoula Technology and Development Center (MTDC) has designed, fabricated and tested a prototype portable vehicle washer to help prevent the spread of noxious weed seeds and spores from vehicles entering and leaving infected areas. The concept may have application in the prevention of Sudden Oak Death disease. The MTDC vehicle washer is a closed loop system where the water used to wash the vehicles is recycled. The system uses two high pressure wands to wash the sides, wheels, and wheel wells and an oscillating, high pressure, underbody washer to wash the vehicle's undercarriage. An industrial rubber mat with foam filled barriers is used to contain the wash water. The contained wash water is pumped from the mat

Abstract

Poster Session D

Management and Disposal

to two 175 gallon settling tanks where large particulate will sink to the bottom of the tanks. The effluent from the settling tanks is then pumped through two filters. The filters use felt bags designed to remove particulate down to 3 microns if desired. After the water is passed through the filters, it is dumped into a 350 gallon holding tank where a high pressure (approximately 800 to 1000 psi), high volume (about 20 gallons per minute) pump pushes the water through the wands and underbody washer. The whole system is mounted on a double axle, 8 by 16 foot trailer that can be towed behind a _ ton pickup. The pumps, generator, and tanks are permanently mounted on the trailer. Space is provided on the trailer to transport the wash mat, hoses, and miscellaneous equipment. The vehicle does not use hot water nor does it use any chemicals, soaps, or detergents to wash the vehicles.

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Abstract

Poster Session E
Pathogenicity and Resistance

Poster E01
The California Red Oaks: a Pandora's Box

Richard S. Dodd[1], Zara Afzal-Rafii[2], and Vladimir Douhovnikoff[3]

Although in most cases oak species are distinct and readily identifiable in the field, species boundaries may break down locally due to a combination of recent speciation followed by slow rates of morphological divergence and of hybridization among closely related taxa. These two processes appear to have occurred in the coastal woodlands of California, leading to confusion in the identification of some trees that may be showing variable symptoms of infection by *Phytophthora ramorum*. Recognition of *Quercus parvula* var. *shrevei* (Shreve oak) as a distinct taxon from *Q. wislizeni* var. *wislizeni* (interior live oak) has not received full acceptance by the scientific community because of variable and overlapping morphological traits. Our morphological, biochemical and molecular data indicate that these two taxa are differentiated. However, the molecular data suggest a recent divergence that has not yet been accompanied by full reproductive isolation. The habitat preferences of Shreve oak and interior live oak are distinct and are reflected in the very marked divergence in biochemical characters that we have shown are indicators of adaptation to habitat stress. We suggest that Shreve oak and interior live oak have arisen from a recent common ancestor that may have diverged due to isolation in different glacial refugia. Because of the lack of full reproductive isolation, hybridization can be locally common between these two species and also with sympatric populations of *Quercus agrifolia* (coast live oak). Preliminary trials in which we have inoculated coast live oak, Shreve oak and interior live oak with *Phytophthora ramorum*, suggest that interior live oak is less susceptible than the two other species. If true, introgressed populations of Shreve oak and coast live oak with interior live oak genes may prove less susceptible than pure populations. We are testing this in in vitro inoculation studies.

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Poster E02
Plant Species Naturally Infected by *Phytophthora ramorum* in Oregon Forests

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Phytophthora ramorum was found in July 2001 near Brookings, Oregon, killing tanoak (*Lithocarpus densiflorus*) and causing dieback of closely associated wild rhododendron and evergreen huckleberry. Subsequent examinations have found a few more plant species naturally infected, but despite intensive surveys in areas of infection, and the presence of many species known to be susceptible in artificial inoculation, the list remains much shorter than in California, and in most cases, disease

incidence is very low. The Oregon list includes: tanoak, wild rhododendron (*R. macrophylum*), evergreen huckleberry (*Vaccinium ovatum*) poison oak (*Rhus diversiloba*), cascara (*Rhamnus purshiana*), and salmon berry (*Rubus spectabilis*). Symptoms from natural infection and artificial inoculation are illustrated, and field disease incidence is recorded.

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Poster E03

Potential Susceptibility of Eastern United States Forests to *Phytophthora ramorum*

Kurt W. Gottschalk[1], Randall S. Morin[2], and Andrew M. Liebholds[2]

Sudden Oak Death syndrome is caused by the organism, *Phytophthora ramorum*, and was first discovered in central coastal California in 1995. The non-native disease has killed large numbers of oaks (coast live oak, *Quercus agrifolia*, California black oak, *Q. kelloggii*, and Shreve oak, *Q. parvula* var. *shrevei*), tanoaks (*Lithocarpus densiflorus*), and Pacific madrone (*Arbutus menziesii*) and has recently been found in coastal Oregon. The organism has not affected California species in the white oak group. Greenhouse tests of eastern oak species pin oak (*Q. palustris*) and northern red oak (*Q. rubra*) have shown that these species are just as susceptible to the pathogen as their west coast relatives. Based on this knowledge, we developed a preliminary map of the potential susceptibility to *Phytophthora ramorum* for Eastern forests in order to prepare for quarantine and management actions should the pathogen be introduced or move naturally to the Eastern U.S.

The map was developed using FIA (Forest Inventory and Analysis) periodic inventory plot data from the Eastwide FIA database. While only two species have been tested, based on the California data, we assumed that all species in the red oak and live oak groups in the East would be susceptible to the pathogen. The proportion of the total plot basal area that included these oak species was mapped for all Eastern state periodic FIA points. Kriging was used to develop a surface from the point data. A forest density layer was used to mask the surface to remove non-forested areas and to reduce the proportions based on forest density. The Northeastern states conducted a shrub inventory during these periodic inventories. This shrub data was recently obtained and binary classifications were used based on presence or absence of *Phytophthora ramorum* shrub hosts, primarily evergreen ericaceous species. Indicator kriging was used to generate a probability surface of shrub host presence.

The result of the overstory tree analysis is a map that shows potential susceptibility of forests containing those two oak groups for the eastern United States. This map shows that over half of the forests in the Eastern U.S. contain some susceptible hosts. The majority of these susceptible forests contain only a small percentage of susceptible hosts (less than 20 percent), so impacts on these forests may be less critical than in the areas that contain large percentages (up to 90 percent in some areas). Areas with high proportions of susceptible hosts include the Ozark-Ouachita Highlands, pin oak sand flats in the Lake States, and live oak areas in Florida. These susceptible areas can be further defined by using understory hosts. The Northeastern states shrub data is being used to refine the overstory susceptibility for that area. Climatic factors are also an important component of vulnerability of hosts to the pathogen. Future work will add these factors into the analysis.

The Eastern oak forests of the United States appear to have plenty of susceptible hosts, both overstory and understory, that would make the establishment of *Phytophthora ramorum* in these forests a very serious threat. In a worst case scenario, it could rival or exceed the chestnut blight in its impact on the forest ecosystems of the East.

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Poster E04

Phytophthora ramorum and Oregon Forest Trees - One Pathogen, Three Diseases

E. Hansen[1], W. Sutton[2], J. Parke[2], and R. Linderman[3]

Phytophthora ramorum causes girdling cankers on tanoak (*Lithocarpus densiflorus*) and foliar and dieback symptoms on a few other hosts, including native rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*) in forests of SW Oregon. Our objective was to test the potential susceptibility of other forest trees native to Oregon.

We inoculated freshly cut logs, 10-20 cm dia. and about 1 m long, small diameter (<1cm) stems of potted plants, and leaves of native conifer and angiosperm tree species. For log inoculations, plugs of bark (5 mm dia.) were removed to the cambium, and a matching plug of colonized agar (or sterile agar) was inserted; wounds were covered by wet gauze and foil. Bark was stripped after 5-7 weeks and extent of phloem necrosis was measured. Isolations were made from canker margins. Stem inoculations were made by inserting a small piece of colonized agar into a horizontal slit cut to the cambium. Detached leaves and intact plants were also dipped in zoospore suspension without any overt wounding. Tree species differed in the extent and pattern of symptom development following the different inoculation treatments. Phloem necrosis following log inoculation developed in most tree species tested, but large, expanding lesions were regularly seen only in a few members of the Fagaceae. A broader range of species was susceptible to stem inoculations, but girdling cankers formed on only about 18 of 41 species tested, including several conifers. Leaf dip resulted in variable symptoms

on all hosts except willows and poplars, and conifers. Dipping intact shoot tips of whole plants in zoospores gave perhaps the most realistic test. Foliar symptoms were similar to the leaf dip test, but disease progressed into the twigs of only a few species of Ericaceae, Fagaceae, and several conifers. The combined inoculation results and field observations suggest that *P. ramorum* causes three distinct disease syndromes on different plant species. "Sudden Oak Death," characterized by lethal bole cankers, is presently confined to a few species of the family Fagaceae. "*ramorum* leaf blight" follows exposure of foliage of many (most?) angiosperm plant species to sufficient inoculum levels. "*ramorum* shoot dieback" results from foliar infection and/or direct infection of stems on a limited number of species, including tanoak, madrone, some *Vaccinium* and *Rhododendron* species, and several conifers.

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Poster E05

Worldwide plant hosts of *Phytophthora ramorum*

Daniel Hüberli[1], Ellen Simms[2], Chris Carmichael[3], Matteo Garbelotto[4]

Phytophthora ramorum is a phytopathogen that has large impacts on the Californian native ecosystem and has been isolated from 6 European countries in mainly nursery settings or non-native vegetation. The pathogen has the potential to be a worldwide problem.

Symptomatic leaf material from a wide range of plants growing on the university campus and the botanical garden were sampled for PCR analysis. The garden contains over 13,238 plant taxas from around the world, many of which are rare or endangered. It provides an opportunity to investigate the extent of plants that are susceptible to the pathogen.

Twenty-four plants were PCR positive for *P. ramorum* and included shrubs and trees from America (South and North), Asia, Australia, and Europe. Of the 13 different plant families identified as potential hosts, 10 plants were Ericaceous. Eight different *Rhododendron* spp. were positive for *P. ramorum*. Koch's Postulates will be fulfilled for all plants identified by PCR analysis as potential hosts.

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Abstract

Poster Session E

Pathogenicity and Resistance

Poster E06

Comparative Pathogenicity of European and American Isolates of *Phytophthora ramorum* to Leaves of Ornamental, Hedgerow and Woodland Understory Plants in the UK

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Investigations are being done on the potential foliar susceptibility of UK ornamental, hedgerow and woodland under-story plants to European and US isolates of *P. ramorum*. Whereas leaves and shoots of ornamentals are an important means of introduction and spread, those of under-story and hedgerow species represent potentially important sources of inoculum for tree epidemics. The work is complementary to the UK Forest Research Agency's studies on leaves of European tree species. Three representative European isolates (Germany/UK, ex. *Viburnum*/ *Rhododendron*) and three US isolates (ex. *Quercus*, *Rhododendron*, *Vaccinium*) were selected.

In preliminary studies on the development of a detached leaf assay, mini-inoculators (modified microcentrifuge tubes) containing mycelial plugs were used with two UK isolates to investigate the effect of leaf age, leaf surface and wounding/non-wounding on infection of *Rhododendron*. Mini-inoculators were held in place on the leaves using modified hair clips. Infection occurred only on wounded leaves, regardless of leaf age or leaf surface inoculated. A zoospore dipping method (K. Park/E. Hansen) was compared with the mini-inoculators. Two isolates (UK/USA) were tested on 15 plant species: leaves were dipped into a zoospore suspension either by their tip end (unwounded) or by their petiole end (wounded). Infection only occurred on the wounded petiole end. Mini-inoculators gave comparable, but more consistent, data to the dipping method.

The mini-inoculator test using detached, wounded leaves was selected for large scale host tests as it is easy to use and allows quantification. Infection was recorded after 7 days at 20°C and a lesion index rating assigned (cf. Linderman et al., 2002): 0 = no lesion; 1 = lesion not extending beyond the plug (<5 mm diam.); 2 = lesion extending 1–2 mm beyond plug (c. 7 - 9 mm diam.); 3 = lesion extending 3 - 7 mm (c. 11 - 19 mm diam.); 4 = lesion extending >8 mm (>21 mm diam.). There were few differences between the European and American isolates in their ornamental and non-tree host range and pathogenicity, though there were some differences in aggressiveness between individual isolates. Plant species (>51 species from >19 families) were assigned to susceptibility categories based on their mean lesion index for all isolates, as follows: virtually immune (<0.49); resistant (0.5 - 1.49); slightly susceptible (1.5 - 2.49); moderately susceptible (2.5 - 3.49); highly susceptible (>3.5). Potentially highly susceptible species included those in the following genera: *Sambucus* (Caprifoliaceae); *Rhododendron*, *Camellia*, *Pieris*, *Leucothoe* (Ericaceae); *Syringa* (Oleaceae); *Eucalyptus* (Myrtaceae). Moderately susceptible species included: *Viburnum*, *Symphoricarpus* (Caprifoliaceae); *Arctostaphylous* (Ericaceae); *Fuchsia* (Onagraceae); *Photinia* (Rosaceae);

Ulmus (Ulmaceae). Slightly susceptible species included: *Acer* (Aceraceae); *Gaultheria* and some azaleas (Ericaceae); *Malus* and *Prunus* (Rosaceae). Of the potentially susceptible species, *Acer, Fraxinus, Sambucus* and *Ulmus* are important hedgerow or under-story species in the UK. Hedgerow or under-story species considered potentially resistant or immune included: *Alnus*, *Corylus*, *Crataegus*, Ilex, *Rubus fructicosus* and *Viburnum opulus*. With the exception of *Rhododendron* and *Viburnum*, none of these laboratory-tested hosts showing physiological leaf susceptibility are recorded as natural hosts in the UK. Hosts showing susceptibility will be investigated further using attached leaves; stem infection will also be investigated. The data will contribute to UK/EU risk assessments for *P. ramorum*.

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Poster E07

Monitoring Insect Activity Near Sites Infested with *Phytophthora ramorum* in Southwest Oregon

Alan Kanaskie[1], Karen Violet[2], Everett Hansen[3], and Wendy Sutton[2], and James Labonte[4]

Sudden Oak Death, caused by *Phytophthora ramorum*, was found in late July 2001 near Brookings, Oregon, killing tanoak (*Lithocarpus densiflorus*) and infecting Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*). In 2001, nine disease patches totaling 40 acres in size were treated by cutting and burning all host plants within the disease patch and within at least 50 feet of its edge. In May 2002, we distributed 15 pairs of vane traps (3 or 4 per site) around the perimeters of four of the disease patches. Traps within a pair were baited with either slow-release ethanol or freshly-cut tanoak logs. Insects were captured at the bottom of the vane trap in a plastic container armed with a kill-strip. At two-week intervals through October 2002, we collected insects and rainwater from traps, and examined logs for evidence of beetle attacks. We focused on insects that we considered likely associates with Sudden Oak Death, mainly *Monarthrum* spp., *Xyleborus* spp., *Pityophthores* sp., *Pseudopityophthores* spp., and *Xyleborinea* sp. Insects and rainwater were periodically analyzed for presence of *P. ramorum*. The most frequently captured insects were *Xyleborinea saxesenii*, *Pseudopityophthores pubipennis*, and *Monarthrum scutellare*. *P. ramorum* occasionally was recovered from rainwater accumulated in the traps. Beetles were recovered from some of the trap logs, but *P. ramorum* was not.

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Abstract

Poster Session E
Pathogenicity and Resistance

Poster E08

Relative Virulence of *Phytophthora* Species, Including the Sudden Oak Death Pathogen, *P. ramorum*, on Leaves of Several Ornamentals

R. G. Linderman[1], J. L. Parke[2], and E. M. Hansen[2]

Several *Phytophthora* species cause leaf and shoot dieback diseases of ornamentals similar to that caused by the Sudden Oak Death pathogen, *P. ramorum*. Detached leaves of several landscape plants were inoculated with *P. ramorum*, *P. cactorum*, *P. syringae*, *P. citricola*, *P. hevea*, *P. parasitica*, *P. citrophthora*, and *P. cinnamomi*. *Rhododendron*, *Pieris*, and *Laurel* were the most susceptible to the most pathogens, based on lesion severity ratings, with variation depending on the host-pathogen combination. Few hosts were not susceptible to some *Phytophthora* species, and most pathogens infected some hosts. Necrotic lesions were initially similar, but generally with most pathogens subsequent spread was limited. In contrast, *P. ramorum* and *P. citrophthora* lesions spread throughout the entire leaf, suggesting greater virulence and thus underscoring the risk to nursery and landscape plants should the quarantined *P. ramorum* become more widespread.

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Poster E09

Studies on the Effect of *Phytophthora ramorum* on Madrone

P. E. Maloney[1], S. F. Kane[2], C. E. Jensen[2], and D. M. Rizzo[2]

Symptoms and disease progression have not been well characterized for most of the hosts of *Phytophthora ramorum*. On madrone (*Arbutus menziesii*), *P. ramorum* has been confirmed from leaf lesions and stem cankers using molecular, and to a lesser extent, cultural techniques. Difficulty in culturing *P. ramorum* from lesions, in addition to lack of unique symptomatology, complicate diagnosis of *P. ramorum* infection on madrone.

Presently, molecular PCR-based techniques are the best way to confirm presence of *P. ramorum* in madrone. To further complicate field diagnosis, madrone may also be infected with *Nattrassia mangiferae* and *Botryosphaeria dothidea*. These pathogens overlap in geographic range with *P. ramorum*. *N. mangiferae* causes distinct sunken cankers that look like open sores on the branches and trunk. *B. dothidea* causes dieback of twigs and leaves that could be confused with *P. ramorum* infection. Generally, neither pathogen causes death of madrone. Given that *P. ramorum* and

B. dothidea co-occur and cause similar symptoms on madrone, laboratory and field studies were established to characterize symptoms of *P. ramorum* infection as opposed to those of *B. dothide*a. In artificial inoculation experiments, *P. ramorum* formed very dark lesions on leaves. On stems, the lesion initially was hardly apparent and appeared as a slight water soaking or darkening of the bark that traveled very quickly throughout the stem and then killed the tissue. In growth chamber experiments, *P. ramorum* was recovered from stems and leaves 28 days after plants were inoculated and at 42 days *P. ramorum* was recovered from stems and only 1 in 30 leaf samples. Because detection of *P. ramorum* in the field is difficult, we placed madrone saplings (approximately 1 m tall) in 10 plots in a redwood/tanoak forest at Jack London State Park to observe disease development over time. When exposed to natural levels of inoculum, five of the ten madrones became infected with *P. ramorum* within 2 months and 2 more became infected 3 and 6 months later. Both stems and foliage were infected. Five of the saplings died from *P. ramorum* infection within 6 months. Further laboratory experiments will be conducted to assess the interactions between *P. ramorum* and *B. dothidea*. Field experiments will be repeated and expanded during winter 2002-2003 to determine the effects of *P. ramorum* on madrone populations in redwood/tanoak forests.

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Poster E10

Differential Susceptibility to *Phytophthora ramorum* Among *Vaccinium* Species and Cultivars

J. L. Parke[1], R. G. Linderman[2], K. Hummer[3], and E. M. Hansen[4]

Phytophthora ramorum, cause of sudden oak death in California and Oregon, causes foliar necrosis and dieback on several forest understory species including evergreen huckleberry (*Vaccinium ovatum*). We compared the potential susceptibility of other wild *Vaccinium* species and of horticulturally important *Vaccinium* crops such as blueberry, cranberry, and lingonberry in detached leaf assays.

Leaves were dipped in a suspension of *P. ramorum* zoospores and then incubated in moist chambers for seven days. The proportion of necrotic leaf tissue was compared to that resulting from leaf inoculation of known foliar hosts (bay, evergreen huckleberry, rhododendron). A wide range of disease responses, from resistant to highly susceptible, was observed among the *Vaccinium* species. Differences were also observed among cultivars.

Entire plants (selected species and cultivars) were also inoculated by dipping foliage into zoospore suspensions. The disease phenotype expressed in the detached leaf assay was a good indicator of whole plant response. We are currently using the detached leaf assay to screen the USDA-

Agricultural Research Service collection of *Vaccinium germplasm* (25 species, multiple cultivars) to identify possible sources of resistance to *Phytophthora ramorum*.

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Poster E11
Assessing the Susceptibility of Pacific Northwest Nursery Plants to *Phytophthora ramorum* Using a Detached Leaf Assay

J. L. Parke[1], R. G. Linderman[2], and E. M. Hansen[3]

Nursery and greenhouse crops together comprise Oregon's largest agricultural commodity. *P. ramorum* poses a threat to nursery production and export, but the magnitude of the threat is difficult to assess because the susceptibility of most horticultural plant species to this new pathogen is unknown. Quarantine restrictions prevent large-scale greenhouse inoculations with *P. ramorum*, so we developed a detached leaf assay to determine the potential susceptibility of nursery plants to *Phytophthora ramorum* foliar necrosis.

Freshly collected leaves are dipped for 5 sec, midway, petiole first, into a suspension of 6 x 104 zoospores mL-1. The inoculum is a combination of three Oregon isolates originating from rhododendron, tanoak, and evergreen huckleberry. Control leaves are dipped in sterile water. Leaves are placed in a moist chamber at 20°C for 7 days. Leaves are photographed digitally and the percent necrotic area quantified with image analysis software. A known susceptible host (evergreen huckleberry or rhododendron cv. 'Cunningham's White') is included in each assay as a positive control. Previous work (Parke, Linderman, and Hansen, 2002) demonstrated that symptoms that developed in the detached leaf assay reflect those resulting from inoculation of whole plants.

We have used this method to evaluate the relative susceptibility of more than 60 nursery plant taxa belonging to 24 families. Results indicate that a wide range of nursery plants is potentially susceptible to *P. ramorum* foliar necrosis. Some of the most susceptible plants tested to date include *Acer palmatum* (Aceraceae), *Viburnum tinus* (Caprifoliaceae), *Oxydendrum arboreum* (Ericaceae), *Rhododendron* 'Exbury Hybrids' (Ericaceae), *Ribes sanguineum* (Grossulariaceae), *Gleditsia triacanthos* (Fabaceae), *Buddleia davidii* (Loganiaceae), *Clematis montana* (Ranunculaceae), *Ceanothus impressus* (Rhamnaceae), and *Hamamelis vemalis* (Styracaceae). No symptoms were observed on inoculated *Cornus florida* (Cornaceae), *Hypericum* 'Hidcote' (Hypericaceae), *Chaenomeles speciosa* (Rosaceae), *Cotoneaster multiflorus* (Rosaceae), *Photinia serrulata* (Rosaceae), *Rhaphiolepis umbellata* (Rosaceae), or *Philadelphus coronarius* (Saxifragaceae).

Considerable variation was observed among different species within the same genus (*Acer*, *Rhododendron*, *Viburnum*), or even different cultivars within a single species (*Acer palmatum*). Our results indicate that many nursery and landscape plants should be surveyed for early detection of *P. ramorum* infection. The detached leaf assay appears to provide a simple, reproducible method to determine potential susceptibility of plant hosts to *P. ramorum*. Inoculum can be adjusted to a consistent, quantifiable dose. Incubation conditions can be maintained at a constant environment and containment is relatively simple. Small quantities of plant leaves are used, and many plant species may be assayed simultaneously.

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Poster E12

Pathogenicity of European and American *P. ramorum* Isolates to *Rhododendron*

Friederike Pogoda[1] and Sabine Werres[2]

In experiments under controlled conditions, *P. ramorum* isolates from different European countries and ten American isolates were tested for their pathogenicity to *Rhododendron*. For these experiments, freshly cut green twigs of the *Rhododendron* hybrid ‘Cunninghams White’ were inoculated at the cut ends with a mycelium plug. The twigs were incubated at 25°/20°C and a daylength of 16 hours. All the European isolates caused a twig dieback and disease development was identical for all isolates. Nine days after inoculation, the length of the discolouration on the twigs ranged from 5 to 6 cm for nearly all isolates. The pathogen was successfully re-isolated from all the inoculated twigs. In contrast, the virulence of the American isolates was highly variable: some caused a twig dieback identical in severity to that caused by European isolates; some caused only a moderate dieback; and others caused no discolouration on the *Rhododendron* twigs, or else the discolouration was less than 1 cm in length. Isolates causing symptoms were all successfully re-isolated from the inoculated twigs; the single isolate that did not produce symptoms could not be re-isolated from the inoculated twig. The host origin of the isolates did not seem to influence their agressiveness towards *Rhododendron*. However, agressiveness did appear to correspond to their vegetative growth on carrot piece agar. American isolates with a very slow growth rate on carrot piece agar did not cause a severe twig dieback on *Rhododendron* in these experiments; it has not yet been determined whether this is due to potential degeneration of these isolates, or whether this reflects true genetic variation within the American *P. ramorum* population.

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Abstract

Poster Session E

Pathogenicity and Resistance

Poster E13

Susceptibility of Azalea (*Rhododendron*) to *Phytophthora ramorum*

S.A. Tjosvold[1], S.T. Koike[2], J.M. Davidson[3], and D.M. Rizzo[3]

Phytophthora ramorum, the causal agent of the disease commonly known as Sudden Oak Death is a prevalent pathogen in California with its effects evident in 12 counties and found on 14 different oak, tanoak and non-oak hosts. *Rhododendron* and azalea (*Rhododendron*) are very important horticultural commodities and potentially could be important long-distance carriers of *Phytophthora ramorum* through commercial trade. Although plants of the rhododendron subgenus has been found naturally infected in commercial nurseries and landscapes, plants in the azalea subgenus, so far, have not been found naturally infected. The question arises as to whether azaleas are susceptible to *P. ramorum*, and, if so, to what extent.

Twenty commercially available cultivars or species were tested for susceptibility to *P. ramorum* in a series of experiments. Plants were selected to include a broad genetic diversity, including deciduous and evergreen selections. *Rhododendron* 'Cunningham's White' was used in all experiments as a standard susceptible rhododendron cultivar. Susceptibility was gauged using several inoculation procedures.

P. ramorum inoculations were made on leaves on potted plants in a greenhouse (18º C mean) or on detached leaves and incubated in a dark chamber (20º C) at high humidity. Inoculum included: 1) mycelial agar plugs with pin wounding or 2) mycelial agar plugs without pin wounding, or 3) zoospore suspension.

With inoculum plugs, pin wounding proved necessary to reliably infect intact plants in the greenhouse or detached leaves in the incubation chamber. With zoospore inoculum, infection was most successful when leaves were removed, inoculated, and incubated in the humid chamber rather than the leaves inoculated and incubated, intact, on the plant in the greenhouse.

Zoospore inoculation of detached leaves resulted in small lesions forming on all cultivars. *P. ramorum* was successfully isolated from these lesions in all but one cultivar. In those leaves where lesions were formed, the pathogen was isolated in 17 to 100 percent of those cases.

The deciduous azaleas 'Northern Hilights' and *R. occidentale*, and the standard susceptible rhododendron, 'Cunningham's White', proved to be highly susceptible. Deciduous azaleas generally were more susceptible than evergreen azaleas.

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Abstract

Poster Session E
Pathogenicity and Resistance

Poster E14
Discovery of *Phytophthora ramorum* on *Rhododendron* sp. in France and Experimental Symptoms on *Quercus robur*

Claude Delatour[1], Carole Saurat[2], Claude Husson[3], Renaud Ioos[4] and Nathalie Schenck[2]

The first evidence of *Phytophthora ramorum* in France was found in several garden centres on *Rhododendron* sp. in April 2002. Brown spots on leaves, buds and twigs necrosis were observed. During 2002, the unit of Mycology of the French Plant Protection Service carried on a national survey in nurseries. About 300 samples were analysed by isolation on a *Phytophthora* selective medium. Species identification was based on morphological characterisation. *P. ramorum* was detected in 68 samples (63 from *Rhododendron* and 5 from *Viburnum* spp.) originating from several geographical areas covering the entire french territory.

Concerning the experimental symptoms, saplings and seedlings of *Quercus robur* were inoculated with a French isolate. Two types of inoculation were performed: by inserting mycelium on bark wounds and by spraying sporocysts. All the inoculated cuttings produced bark necrosis with occasional dark bleeding. The length of necrosis on the stem ranged from 1.4 to 8.3 cm. These results are similar to those obtained on tanoak and coast live oaks but we observed no girdling, no wilting and no death on *Q. robur* even 40 days after inoculation. With respect to the damage on leaves, necrosis was not frequent. Therefore, *Q. robur* seems to be less susceptible than *Q. agrifolia* and *Lithocarpus densiflorus*. In addition, our results suggest that bark necrosis, dark bleeding and occasionally leaf necrosis are good candidate symptoms of *P. ramorum* for future forest surveys.

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Abstract

Poster Session F
Monitoring

Poster F01

Evaluation of Airborne Digital Acquisistion and Registration (ADAR) Imagery for Mapping Moisture Stress in Oaks with Advanced Symptoms of Sudden Oak Death

Desheng Liu[1] and Maggi Kelly[2]

We investigated the ability of high spatial-resolution 4-band imagery Airborne Digital Acquisition and Registration (ADAR) to discern moisture stress in trees affected by Sudden Oak Death. The disease progression in oaks includes a stage where the canopy fades quickly as a result of advanced cancer growth on the main stem. Immediately prior to this color change, we assume the leaves experience moisture stress. It has been well documented that moisture stress in vegetation is correlated with a decrease in reflectance in the near and middle infrared portions of the electromagnetic spectrum. We wanted to test ADAR imagery could be used to distinguish between green oak trees with advanced SOD trunk symptoms, and green oaks with no SOD trunk symptoms.

ADAR imagery of China Camp State Park in Marin County was flown in spring 2000. Training samples from the field consisting of the locations of 25 green healthy oaks and 25 green stressed oaks were used to locate individual tree crowns in the imagery, from which spectral signatures were defined for the two classes. Both hierarchical unsupervised classification (HUC) and maximum likelihood classification (MLC) were used to classify the two groups. Accuracy assessment and other spectral measurements were performed to analyze the separability of the two signatures. Poor overall accuracy 55.17% was obtained by the HUC method. A better overall accuracy 74.19% was obtained by MLC method, but the low transformed divergence (1448) indicated poor separability of the training samples. The poor accuracy results can be explained by the fact that ADAR image has relatively broad spectral bands that combine narrow stress-sensitive regions with broader stress-insensitive regions; such combination could decrease the capability of ADAR to detect moisture stress. In addition, healthy oaks in the area display a marked variability in canopy condition, making it impossible to separate healthy trees from those experiencing some stress. In conclusion, the classified results indicated the limitation of using ADAR image to map moisture stress in oaks.

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Abstract

Poster Session F
Monitoring

Poster F02
Mapping the Risk of Sudden Oak Death for California

Ross Meentemeyer[1], Liz Lotz[2], Dave Rizzo[3], Wally Mark[4], and Maggi Kelly[5]

Information on Sudden Oak Death risk is needed to target distribution surveys and predict potential impacts on California's natural resources. We present a geographical model of disease risk for California. The model is based on host species distribution data and several critical environmental factors. These factors include GIS-derived maps of proximity to infested locations, weather and climate conditions, proximity to commercial nurseries and human features, and landscape structure of host connectivity and edge effects. Two modeling approaches are evaluated. One is statistically based while the other uses human input from preliminary field and laboratory studies of environmental conditions conducive for pathogen reproduction and dispersal. In the statistical approach, observations of disease incidence (the response variable) are obtained from FIA field plots and COMTF ground surveys, and linked with the predictor variables in the GIS database. Multivariable relationships are statistically analyzed to develop a model that quantifies the degree to which predictor variables are related to disease presence. The model's equation is applied to mapped predictor variables in the GIS to calculate the risk that an unsampled location could develop infestation, or already has. Twenty-five percent of the observations are held back during model development to evaluate model performance.

We also develop a standard overlay model, which uses human input, rather than statistical inference. In collaboration with Dave Rizzo (University of California, Davis), each of the mapped predictor variables (described above) are scored and ranked based on potential infestation risk. The maps are overlayed and summed to produce a composite map of risk for the whole state. Risk values range continuously from 0 (low risk) to 100 (high risk). Proximity to infected sites gives the model a dynamic component. As additional sites are infected, the model targets new areas at risk and tracks patterns of our knowledge through time. Model performance is evaluated by comparing the resultant risk maps to field observations of disease incidence.

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Abstract

Poster Session F
Monitoring

Poster F03
Survey of Oregon Nurseries and Other Commodities for *Phytophthora ramorum*

N.K. Osterbauer[1], L. Rehms[2], and J. Hedberg[2]

Phytophthora ramorum is established in 12 coastal counties in California and has also been detected (and targeted for eradication) in a limited area in Curry County, Oregon. This pathogen has a wide host range that includes at least 17 naturally infected host species in 10 different plant families. Many of these hosts are important parts of Oregon's various agricultural industries. In response to this threat, the Oregon Department of Agriculture has conducted a detection survey for *P. ramorum* in Oregon nurseries, plantations, and "high risk" sites (e.g., botanical gardens) for the past two years. Susceptible host plants at each site were visually surveyed and samples collected from symptomatic plants. The samples were then processed in the laboratory using standard isolation techniques and analyzed for the presence of *P. ramorum*. In 2001, 69 nurseries and "high risk" sites were visually surveyed for *P. ramorum* and a total of 2,657 samples collected and analyzed. *Phytophthora ramorum* was not recovered from any of the samples submitted. Other *Phytophthora* species were detected at 30 of the sites surveyed. In 2002, 98 nurseries, plantations, and "high risk" sites were visually surveyed for *P. ramorum* with a total of 3,927 samples collected and analyzed. Once again, no *P. ramorum* was found. However, like last year, other *Phytophthora* species were detected at 20 of the sites surveyed. Based upon these results, Oregon nurseries, plantations, and "high risk" sites remain apparently free of *P. ramorum*.

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Poster F04
Spectroscopic Determination of Health Levels of Coast Live Oak (*Quercus agrifolia*) Leaves

R. Pu[1], Q. Chen[2], D. Graham-Squire[2] and P. Gong[2]

A total of 153 reflectance spectra (covering 350 - 2500 nm) from coast live oak (*Quercus agrifolia*) leaves were measured in the laboratory with a spectrometer FieldSpec®Pro FR for "Sudden Oak Death (SOD)" monitoring. It is well known that the spectroscopic determination of health status of the coast live oak leaves is very difficult due to subtle spectral differentiation between different health levels. In this study, two spectroscopic classification algorithms, cross correlogram spectral matching (CCSM) and penalized discriminant analysis (PDA), are applied to identify the two health levels of oak leaves: healthy and infected. CCSM is practiced by calculating the cross correlation at different match positions between a test spectrum and a

reference spectrum. A test spectrum with a higher cross correlation will have a perfect matching to a reference spectrum, which leads to the test spectrum classified to the reference spectrum. PDA is a penalized version of Fisher's linear discriminant analysis (LDA) and can considerably improve upon LDA when it is used in classification of hyperspectral data. In this experiment, the 153 spectra (54 for healthy and 99 for infected) was randomly divided into three nonoverlapping groups, and among them two groups were used as training samples and the remaining one as test samples (repeating the selection three times for obtaining three non-overlapping groups of test samples) for testing the two classification algorithms. Experimental results indicate that the PDA algorithm has produced a slightly higher classification accuracy (68.0%) than that (65.2%) by CCSM for identifying the two health levels.

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Poster F05

Evaluation of a Novel Diagnostic Procedure to Detect the Presence of *Phytophthora ramorum* by Sampling Ooze from Infected Cankers

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Phytophthora ramorum, the causal agent of the disease commonly known as Sudden Oak Death is a prevalent pathogen in California with its effects evident in 12 counties and found on 14 different oak, tanoak and non-oak hosts. On oak and tanoak, cankers are formed along the lower trunk and often ooze seeps from them. The traditional field diagnostic technique to confirm the presence of the pathogen in these species involves shaving away the outer bark near the perimeter of the canker, collecting pieces of inner bark at the perimeter of the canker, and placing these pieces on selective PARP media. This sampling technique is often very destructive, leaving large wounds on the lower trunk of the tree. Also, this technique is not very dependable; at best, it may require multiple sampling to get an affirmative result on known diseased trees. Since diseased trees commonly have cankers where ooze seeps to the surface of the bark- and *P. ramorum* sporangia have been observed in this ooze- it is possible that sporangia or other fungal components could be detected with the right detection technique. This study evaluates the reliability of using PCR (polymerase chain reaction) and traditional PARP selective media to confirm the pathogen's presence in canker ooze. For 9 months, ooze samples were taken weekly from naturally infected, seeping cankers of 10 coast live oaks (*Quercus agrifolia*) and 4 tanoaks (*Lithocarpus densiflora*) in several locations in north Santa Cruz County. Ooze was tested for the presence of *P. ramorum* with PCR or by plating on PARP selective media. In addition, ooze was periodically examined for fungal particles under a light microscope. At each sampling, cankers were sampled using traditional techniques, where inner bark is placed on selective media. The three detection techniques, 1) inner bark plated on PARP, 2) ooze tested with PCR, and 3) ooze plated on PARP resulted in 26.3, 17.6, and 1.8 % with positive findings of *P. ramorum* respectively. Traditional sampling of inner bark was less effective on tanoak than on live oak. No fungal structures of *Phytophthora* were observed in ooze samples. Ooze was not always present for sampling on some trees during some dry months, particularly following exceptionally warm weather.

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Poster F06

OakMapper webGIS for Monitoring Sudden Oak Death

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Research on and community interest in Sudden Oak Death is very high in the areas near affected forests. Because of this interest, many people want to be involved in the monitoring process. The overall monitoring strategy of the California Oak Mortality Task Force is multi-scale, multi-source, flexible, and geographically organized. Coordinated by the Monitoring Committee, it combines several on-going efforts at multiple scales. At the local level, we have developed an Internet-based GIS ("webGIS") application that distributes information about trees confirmed with Sudden Oak Death, and collects information from a wide community of users about trees suspected of having Sudden Oak Death. To date, the website has received over 300 submissions of symptomatic trees, from as far all regions of California. While these submissions are not laboratory confirmations of Sudden Oak Death and should not be considered official, they do give a snapshot of the distribution of public interest in and awareness of the disease. This poster displays examples of the interactive OakMapper webGIS application, showing both the data distribution component, and the latest results from public use of the site.

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Abstract

Poster Session F
Monitoring

Poster F07

Pilot Survey of Oklahoma Ornamental Nurseries for *Phytophthora ramorum*

Sharon von Broembsen[1] and Brian Olson[2]

In April 2002, when APHIS-USDA-PPQ sent out the guidelines for the Sudden Oak Death national nursery survey, it seemed obvious that it would be in Oklahoma's best interest to participate in the survey. Funding was obtained through the Western Region beginning in July 2002. Although all ornamental nursery stock meeting the survey criteria will be surveyed in May-June of 2003, a pilot survey of the two largest ornamental nurseries was conducted during late July 2002 to get the sampling and assay systems in place. PCR technology was chosen as the primary means of detecting *P. ramorum* in plant samples. Dr. Matteo Garbelotto, University of California-Berkeley, supplied us with the specific protocols, primer sequences, and enough extracted *P. ramorum* DNA to be able to run the PCR assays. Cultural methods were also used on some samples to establish what other *Phytophthora* spp. were present. In June 2002, a training session was held for all personnel that would be involved in the surveys, including the Oklahoma Department of Agriculture nursery inspectors, who are responsible by law for the actual sampling of all nursery stock in Oklahoma. However, we assisted the inspectors with sampling of the two pilot nurseries so that the sampling methods and data handling procedures could be established for the 2003 survey. During August 2002 assaying of the survey samples was initiated.

A number of challenges were encountered in this pilot study. The first set of these concerned the sampling of the nursery material. It is always difficult to survey for something that is not expected to be found, but it is especially difficult when information on symptoms, plant parts affected, and the disease cycle is preliminary. Should only symptomatic above-ground parts be sampled and tested? Several *Phytophthora* spp. cause shoot disease on rhododendrons in Oklahoma, so inspectors should be familiar with these and should be able to take appropriate samples. But what do *P. ramorum* symptoms really look like on viburnum? Only one poor photo from Europe was available as a guide. *Viburnum* is a very common nursery plant in Oklahoma nurseries so this is no small question. What tissues should be sampled? To detect *P. ramorum*, it is critical that the small amounts of tissue selected for the PCR assays be those most likely to have the fungus in them. The second set of challenges concerned the PCR assays. Unlike the simpler PCR assays that were part of the lab's repertoire, the *P. ramorum* protocol was a considerably more complicated, nested assay requiring two DNA amplifications. Work programs had to be adapted to the much larger volume of assays as compared to that encountered for diagnostic samples. It took some time to get the procedures working and to maximize efficiency. One frustration of being outside the quarantine area is not having reference cultures of *P. ramorum* to work with and not being able to include *P. ramorum* infected plant material in assays to increase confidence. No *P. ramorum* was been detected in any of the samples and no cross reactions with other *Phytophthora* spp. have been encountered, even in samples shown by culturing to have other *Phytophthora* spp. present.

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