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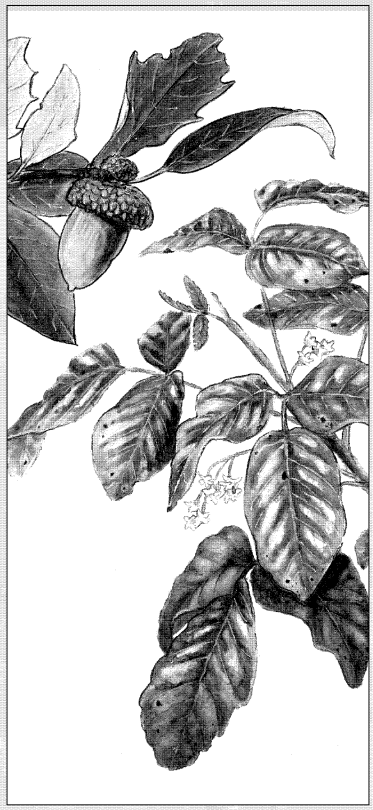

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Proceedings of the

Sudden Oak Death Second Science Symposium: The State of Our Knowledge

January 18-21, 2005 **Monterey, California**

Susan J. Frankel, Patrick J. Shea, and Michael I. Haverty
Technical Coordinators

Conference Sponsors

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Abstract

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The Sudden Oak Death Second Science Symposium provided a forum for current research on sudden oak death, caused by the exotic, quarantine pathogen, *Phytophthora ramorum*. Ninety papers and forty-six posters on the following sudden oak death/*P. ramorum* topics are included: biology, genetics, nursery and wildland management, monitoring, ecology, and diagnostics. Several papers on *P. kernoviae* and other forest *Phytophthora* species are also presented.

Key words: sudden oak death, *Phytophthora ramorum*, invasive species, tanoak, coast live oak

Contents

1 Keynote Address and Overviews

3 Keynote Address Sudden Oak Death Second Science Symposium, Monterey, California, January 19, 2005
Representative Lynn Woolsey, California's 6th Congressional District

7 USDA Forest Service, Pacific Southwest Research Station Sudden Oak Death Program: 2001-2005
Patrick J. Shea

11 Understanding Sudden Oak Death: A Model of Partnership and Collaboration
Jimmy L. Reaves

17 History and Background of the California Oak Mortality Task Force (COMTF)
Mark R. Stanley

21 Research on the Epidemiology, Ecology and Management of *Phytophthora ramorum* in California forests
David M. Rizzo

27 Epidemiology of *Phytophthora ramorum* in Oregon
E.M. Hansen, A. Kanaskie, E.M. Goheen, N. Osterbauer, and W. Sutton

31 Sudden Oak Death: Recent Developments on Trees in Europe
Clive Brasier, Sandra Denman, Joan Webber, and Anna Brown

35 *Phytophthora ramorum* in Nurseries

37 Review of *Phytophthora ramorum* in Europe and North American Nurseries
Janice Alexander

41 *Phytophthora ramorum*—Economic Impacts and Challenges for the Nursery Industry
Karen Suslow

45 APHIS *Phytophthora ramorum* Regulatory Strategy for Nurseries
Jonathan M. Jones

47 Update on Diagnostics for *Phytophthora ramorum*
Philip Berger

51 Update on European Union and United Kingdom Legislation for *Phytophthora ramorum*
Stephen Hunter

55 Biology I

57 *Phytophthora* Species Associated With Forest Soils in Central and Eastern U.S. Oak Ecosytems
Y. Balci, S. Balci, J. Eggers, W.L. MacDonald, K.W. Gottschalk, J. Juzwik, and R. Long

61 The Effects for Girdling on the Ectomycorrhizal Fungal Community Associated With Tanoak (*Lithocarpus densiflorus*)
Sarah Bergemann, Nicholas Kordesch, Matteo Garbelotto, and Timothy Metz

63 *Phytophthora* Species From Oak and Tanoak Forests in California and Oregon
Everett Hansen, David Rizzo, and Matteo Garbelotto

67 Isolation and Characterization of Phytotoxins Secreted by *Phytophthora ramorum*
Daniel K. Manter, Rick G. Kelsey, and Joseph J. Karchesy

69 Incidence of *Phytophthora ramorum, P. nemorosa* and *P. pseudosyringae* in Three Coastal California Forest Communities
Shannon K. Murphy and David M. Rizzo

73 Ecology of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in Mixed-Evergreen Forests
Allison C.Wickland and David M. Rizzo

77 Susceptibility of Conifer Shoots to Infection by *Phytophthora ramorum*
G.A. Chastagner, E.M. Hansen, K.L. Riley, and W. Sutton

79 Susceptibility Levels of *Rhododendron* Species and Hybrids to *Phytophthora ramorum*
Isabelle De Dobbelaere, Kurt Heungens, and Martine Maes

83 Variation in Phenotype for Resistance to *Phytophthora ramorum* in a Range of Species and Cultivars of the Genus *Viburnum*
Niklaus J. Grunwald, E. Anne Davis, and Robert G. Linderman

87 Variation in Susceptibility of *Umbellularia californica* (Bay Laurel) to *Phytophthora ramorum*
Matthew Meshriy, Daniel Hüberli, Tamar Harnik, Lori Miles, Keefe Reuther, and Matteo Garbelotto

91 Magnetic Resonance Imaging (MRI) of Oak Trees Infected With *Phytophthora ramorum* to Determine Potential Avenues of Infection in Bark
Edwin R. Florance

101 Adaptive Differences Between *Phytophthora ramorum* Isolates From Europe and North America: Evidence for Separate Subspecies?
Clive Brasier, Susan Kirk, and Joan Rose

105 Distribution and Etiology of Aerial Stem Infections of *Phytophthora ramorum* and *Phytophthora kernoviae* at Three Woodland Sites in the U.K.
Anna Brown, Clive Brasier, Sandra Denman, Joan Rose, Susan Kirk, and Joan Webber

109 Comparative Host Range and Aggressiveness of *Phytophthora ramorum* and *Phytophthora kernoviae* sp. nov. on North American and European Trees
Clive Brasier, Joan Rose, Susan Kirk, Sandra Denman, and Joan Webber

113 The Search for the Origin of *Phytophthora ramorum*: A First Look in Yunnan Province, People's Republic of China
Ellen Michaels Goheen, Thomas L. Kubisiak, and Wenxia Zhao

117 Genetics

119 Does Stand Density Affect Mating System and Population Genetic Structure in Coast Live Oak?
Kathryn Beals and Richard S. Dodd

123 Genetic Hitch-Hiking Extends the Range of Coast Live Oak
Richard S. Dodd, Zara Afzal-Rafii, and Wasima Mayer

125 Seasonal Trends in Response to Inoculation of Coast Live Oak With *Phytophthora ramorum*
Richard S. Dodd, Daniel Hüberli, Tamar Y. Harnik, Brenda O'Dell, and Matteo Garbelotto

129 Potential for Sexual Reproduction of *Phytophthora ramorum* in Washington State Nurseries
Matteo Garbelotto, Kelly Ivors, Daniel Hüberli, Peter Bonants, and Art Wagner

131 Phenotypic Variation Among *Phytophthora ramorum* Isolates From California and Oregon
Daniel Hüberli, Tamar Harnik, Mathew Meshriy, Lori Miles, and Matteo Garbelotto

135 Molecular Identification and Detection of *Phytophthora ramorum*
Peter Bonants, Els Verstappen, Ineke de Vries, Katarzyna Wiejacha, and Kelly Ivors

139 Molecular Detection of *Phytophthora ramorum* by Real-Time PCR Using Taqman, SYBR® Green and Molecular Beacons With Three Genes
G.J. Bilodeau, C.A. Lévesque, A.W.A.M. de Cock, C. Duchaine, G. Kristjansson, and R.C. Hamelin

141 Using Single Strand Conformational Polymorphisms (SSCP) to Identify *Phytophthora* Species in Oregon Forests Affected by Sudden Oak Death
E. Hansen, C. Hesse, P. Reeser, W. Sutton, and L. Winton

143 Application of Rapid Onsite PCR (Taq Man®) for *Phytophthora ramorum* Under U.S. Conditions
Kelvin Hughes, Jenny Tomlinson, Neil Boonham, Kelly Ivors, Matteo Garbelotto, and Ian Barker

145 Use of Microsatellite Markers Derived From Whole Genome Sequence Data for Identifying Polymorphism in *Phytophthora ramorum*
Kelly Ivors, Matteo Garbelotto, Ineke de Vries, and Peter Bonants

147 Single-Strand Conformational Polymorphism Analysis of Ribosomal DNA for Detection of *Phytophthora ramorum* Directly From Plant Tissues
Ping Kong, Patricia A. Richardson, Chuanxue Hong, and Thomas L. Kubisiak

149 AFLP Analysis of *Phytophthora nemorosa* and *P. pseudosyringae* Genetic Structure in North America
Rachel Linzer, David Rizzo, and Matteo Garbelotto

153 Molecular Markers for Identification of *P. ramorum* and Other *Phytophthora* Species From Diseased Tissue
Frank N. Martin and Paul W. Tooley

157 Genome Sequence of *Phytophthora ramorum*: Implications for Management
Brett Tyler, Sucheta Tripathy, Nik Grunwald, Kurt Lamour, Kelly Ivors, Matteo Garbelotto, Daniel Rokhsar, Nik Putnam, Igor Grigoriev, and Jeffrey Boore

159 Biology II

161 The Importance of Humans in the Dispersal and Spread of *Phytophthora ramorum* at Local, Landscape, and Regional Scales
J. Hall Cushman and Ross Meentemeyer

165 Summer Survival of *Phytophthora ramorum* in Forest Soils
Elizabeth J. Fichtner, Shannon C. Lynch, and David M. Rizzo

167 Monitoring of Natural Outbreaks of *Phytophthora ramorum* in the United Kingdom
Judith Turner, Alex Appiah, Philip Jennings, Gilli Humphries, Debbie Liddell, Sam McDonough, Jackie Stonehouse, David Lockley, and Stephen Eales

169 Examining the Relationship Between Fire History and Sudden Oak Death Patterns: A Case Study in Sonoma County
Max A. Moritz and Dennis C. Odion

179 Forecasting the Future of Coast Live Oak Forests in the Face of Sudden Oak Death
Letty B. Brown and Barbara Allen-Diaz

181 Etiology and Evidence of Systemic Acidification in SOD-Affected Forests of California
Lee Klinger and Ralph Zingaro

183 Progress Report on the Evaluation of the Susceptibility of the Holm Oak (*Quercus ilex*) Forest Ecosystem to *Phytophthora ramorum*
Eduardo Moralejo and Enrique Descals

185 A Spatial Analysis of *Phytophthora ramorum* Symptom Spread Using Second-Order Point Pattern and GIS-Based Analyses
Mark Spencer and Kevin O'Hara

187 Residual Tree Response to Tanoak Decline in California
Kristen M. Waring and Kevin L. O'Hara

191 SOD-Induced Changes in Foraging and Nesting Behavior of Insectivorous, Cavity-Nesting Birds
Kyle Apigian and Barbara Allen-Diaz

193 Patterns of Tanoak Acorn Production in *Phytophthora ramorum* Infested and Uninfested Stands in Big Sur, California, With Insights From Girdled Tanoaks
Leila Hadj-Chikh, Kerri Frangioso, Keyt Fischer, Sarah Bergemann, and Ebba Peterson

195 Potential Effects of Sudden Oak Death on the Oak Woodland Bird Community of Coastal California
William B. Monahan and Walter D. Koenig

211 Vegetation and Small Vertebrates of Oak Woodlands at Low and High Risk for Sudden Oak Death in San Luis Obispo County, California
Douglas J. Tempel, William D. Tietje, and Donald E. Winslow

233 Potenital Effects of Sudden Oak Death on Small Mammals and Herpetofauna in Coast Live Oak (*Quercus agrifolia*) Woodlands
Douglas J. Tempel and William D. Tietje

237 Management

239 Effectiveness of Fungicides in Protecting Douglas-fir Shoots From Infection by *Phytophthora ramorum*
G.A. Chastagner, E.M. Hansen, K.L. Riley, and W. Sutton

241 Fungicide Control of *Phytophthora ramorum* on Rhododendron
Kurt Heungens, Isabelle De Dobbelaere, and Martine Maes

259 Effects of Phosphonate Treatments on the Growth of *Phytophthora ramorum* in Tanoak Stems
Alan Kanaskie, Everett Hansen, and Wendy Sutton

263 Effects of Phosphonate Treatments on Sudden Oak Death in Tanoak and Shreve's Oak
Doug Schmidt, Matteo Garbelotto, Dave Chambers, and Steve Tjosvold

265 Evaluation of Chemical and Biological Agents for Control of *Phytophthor*a Species on Intact Plants or Detached Leaves of Rhododendron and Lilac
R.G. Linderman and E.A. Davis

269 Evaluation of Fungicides for the Control of *Phytophthora ramorum* Infecting *Rhododendron*, *Camellia*, *Viburnum*, and *Pieris*
S.A. Tjosvold, D.L. Chambers, and S. Koike

273 Chemical Control of *Phytophthora ramorum* Causing Foliar Disease in Hardy Nursery Stock in the United Kingdom
Judith Turner, Philip Jennings, Sam McDonough, Debbie Liddell, and Jackie Stonehouse

275 *Phytophthora ramorum* Infection in Coast Live Oaks and Shreve's Oaks Treated With Insecticide to Prevent Beetle Colonization
Brice A. McPherson, David L. Wood, David M. Rizzo, Pavel Svihra, Steve Tjosvold, Andrew J. Storer, and Richard B. Standiford

279 Effects of Sanitary Measures on the Survival of *Phytophthora ramorum* in Soil
M.M. Aveskamp, P.J.M. van Baal, and J. de Gruyter

285 Thwarting *Phytophthora ramorum*: A Proposed Disease Cycle With Mitigation Measures
Betsy Randall-Schadel and Scott Redlin

287 Introduction of Filtration Systems in Container Nurseries for Nonchemical Elimination of *Phytophthora* spp. From Irrigation Water
Thorsten Ufer, Heinrich Beltz, Thomas Brand, Katrin Kaminski, Ralf Lüttmann, Martin Posner, Stefan Wagner, Sabine Werres, and Hans-Peter Wessels

291 *Phytophthora ramorum* Disease Transmission From Artificially Infested Potting Media
Jennifer L. Parke, Melody L. Roth, Carrie Lewis, and Caroline J. Choquette

295 Pathways of Infection for *Phytophthora ramorum* in Rhododendron
Carrie D. Lewis and Jennifer L. Parke

299 Survival of *Phytophthora* Species and Other Pathogens in Soilless Media Components or Soil and Their Eradication With Aerated Steam
R.G. Linderman and E.A. Davis

303 Ecology and Monitoring

305 Potential Effects of Sudden Oak Death on Birds in Coastal Oak Woodlands
Donald E. Winslow and William D. Tietje

329 Climate-Host Mapping of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death
Glenn Fowler, Roger Magarey, and Manuel Colunga

333 Modeling Risk for SOD Nationwide: What Are the Effects of Model Choice on Risk Prediction
M. Kelly, D. Shaari, Q. Guo, and D. Liu

345 Aerial and Ground Surveys for Mapping the Distribution of *Phytophthora ramorum* in California
Jeffrey A. Mai, Walter Mark, Lisa Fischer, and Amy Jirka

361 Early Detection Monitoring of *Phytophthora ramorum* in High-Risk Forests of California
Ross Meentemeyer, Elizabeth Lotz, David M. Rizzo, Kelly Buja, and Walter Mark

365 *Phytophthora ramorum* Detection Surveys for Forests in the United States
S.W. Oak, W.D. Smith, and B.M. Tkacz

367 *Phytophthora ramorum* Experience and Approach in the Netherlands
M.H.C.G. Steeghs and J. de Gruyter

371 Pre-epidemic Mortality Rates for Common *Phytophthora ramorum* Host Tree Species in California
T.M. Barrett

379 Sudden Oak Death Disease Progression in Oaks and Tanoaks
Brice A. McPherson, Sylvia R. Mori, David L. Wood, Andrew J. Storer, Pavel Svihra, N. Maggi Kelly, and Richard B. Standiford

383 Disease Risk Factors and Disease Progress in Coast Live Oak and Tanoak Affected by *Phytophthora ramorum* Canker (Sudden Oak Death)
Tedmund J. Swiecki and Elizabeth Bernhardt

413 Surveying and Monitoring Sudden Oak Death in Southwest Oregon Forests
Ellen Michaels Goheen, Alan Kanaskie, Mike McWilliams, Everett Hansen, Wendy Sutton, and Nancy Osterbauer

417 Management II

419 The Response of Saprotrophic Beetles to Coast Live Oaks Infected With *Phytophthora ramorum*
Brice A. McPherson, Nadir Erbilgin, David L. Wood, Pavel Svihra, Andrew J. Storer, and Richard B. Standiford

423 Chemical Ecology of Sudden Oak Death/Ambrosia Beetle Interactions
Frances S. Ockels, Pierluigi Bonello, Brice McPherson, and David L. Wood

427 Relationships Between *Phytophthora ramorum* Canker (Sudden Oak Death) and Failure Potential in Coast Live Oak
Tedmund J. Swiecki, Elizabeth Bernhardt, Christiana Drake, and Laurence R. Costello

455 The Current Situation With *Phytophthora ramorum* in England and Wales
David Slawson, Lynne Bennett, Nicola Parry, and Charles Lane

459 Epidemiology of *Phytophthora ramorum* Infecting Rhododendrons Under Simulated Nursery Conditions
S.A. Tjosvold, D.L. Chambers, S. Koike, and E. Fichtner

463 Converting Biology Into Regulations: U.S. *Phytophthora ramorum* Quarantine as a Case Study
Susan J . Frankel and Steven W. Oak

465 Who Pays for Sudden Oak Death? An Economic Investigation of the Impact of an Emerging Pathogen on California Nurseries
J.K. Gilless, J. Tack, and A. Peterson Zwane

467 Understanding the Disposal and Utilization Options for *Phytophthora ramorum* Infested Wood
John Shelly, Ramnik Singh, Christine Langford, and Tad Mason

483 Extended Abstract on the Potential for *Phytophthora ramorum* to Infest Finished Compost
Steven Swain and Matteo Garbelotto

487 Ecological Evidence of Intensive Cultivation of Oaks by California Indians: Implications for the Treatment of Sudden Oak Death
Lee Klinger

489 Eradication of *Phytophthora ramorum* in Oregon Forests—Status After 3 Years
Alan Kanaskie, Nancy Osterbauer, Michael McWilliams, Ellen Goheen, Everett Hansen, and Wendy Sutton

491 Posters

493 Trace Forward, Perimeter, and National Nursery Surveys for *Phytophthora ramorum* in Texas
D.N. Appel, T. Kurdyla, R.F. Billings, K.S. Camilli, and A. Purdy

494 Small Mammal and Herpetofaunal Abundancy and Diversity Along a Gradient of Sudden Oak Death Infection
Kyle Apigian, Letty Brown, Jenny Loda, Sarah Toas, and Barbara Allen-Diaz

495 Pacific Yew as a Host for *Phytophthora ramorum* in California Forests
J.C. Bienapfl, J.W. Zanzot, and D.M. Rizzo

496 First Report of *Pyracantha koidzumii* as a Host for *Phytophthora ramorum*
Stephan C. Briere, Sarah Llewellyn, and Gary Kristjansson

497 Predicting the Potential for Establishment of *Phytophthora ramorum* in the Oak Forests of the North Central States, USA
S.D. Cohen and R.C. Venette

500 Mechanisms Underlying Differences in Inoculum Production by *Phytophthora ramorum* in Mixed-Evergreen Versus Tanoak-Redwood Forest in California
Jennifer M. Davidson, Elizabeth J. Fichtner, Heather A. Patterson, Kristin R. Falk, and David M. Rizzo

501 Detection and Distribution of *Phytophthora ramorum* in Soils
Elizabeth J. Fichtner, Shannon C. Lynch, and David M. Rizzo

502 Progress Toward the Development of a Model to Quantify the Efficacy of Detection Strategies for *Phytophthora ramorum*
Yashika Forrester, Betsy Randall-Schadel, Allan Hogue, David Oryang, and Robert McDowell

503 A Case Study to Evaluate Ground-Based, Wildland Survey Methods for *Phytophthora ramorum* in Coast Live Oak Stands in California
Susan J. Frankel, Sylvia Mori, Janice Alexander, Don Owen, Jennifer Davidson, and David M. Rizzo

509 Addressing *Phytophthora ramorum* in California: Programs of the USDA Forest Service and California Department of Forestry and Fire Protection
Susan J. Frankel and Mark Stanley

512 Anatomical Examination of *Phytophthora ramorum* Infection in *Camellia*
Elizabeth Geltz, Jessica McHugh, Lisa Baird, Sibdas Ghosh, Peter Thut, and Mietek Kolipinski

513 Application of Fungicides to Protect Four Hosts From Foliar Infection by *Phytophthora ramorum* in Curry County, Oregon
Ellen Michaels Goheen, Alan Kanaskie, Jennifer Parke, Melody Roth, Nancy Osterbauer, and Aaron Trippe

515 Amplified Fragment Length Polymorphism Assessment of Population Diversity in California Bay Laurel (*Umbellularia californica*)
Sarah Gordon, Richard Whitkus, Ross Meentemeyer, and Brian Anacker

516 Persistence of *Phytophthora ramorum* After Eradication Efforts in Oregon Tanoak Forests
E.M. Hansen and W. Sutton

517 Effect of Chemicals on Hyphal Growth, Sporangia Production and Zoospore Germination of *Phytophthora ramorum*
Tamar Y. Harnik and Matteo Garbelotto

518 Quantitative Resistance to *Phytophthora ramorum* in Tanoak
Katherine Hayden and Matteo Garbelotto

519 Distribution and Pathogencity of *Phytophthora nemoros*a and *P. pseudosyringae* in Coastal Forests of California
Camille E. Jensen, Allison C. Wickland, and David M. Rizzo

520 Inoculation Trials With *Phytophthora ramorum* on Moorland Species
Stefan Wagner, Katrin Kaminski, and Sabine Werres

523 Infectivity and Survival of *Phytophthora ramorum* in Recirculation Water
Heinrich Beltz, Thomas Brand, Katrin Kaminski, Dankwart Seipp, Stefan Wagner, and Sabine Werres

526 Ectomycorrhizal Diversity Associated With Tanoaks (*Lithocarpus densiflorus*)
Nicholas Kordesch, Sarah Bergemann, and Matteo Garbelotto

527 Epidemiological Aspects of *Phytophthora ramorum* in Redwood Forests of the Coast Range of California: A 3-Year Look
Patricia E. Maloney, Shannon C. Lynch, and David M. Rizzo

528 Sensitivity and Specificity of Inspection and Testing Procedures for *Phytophthora ramorum* on Nursery Stock
Robert McDowell, Betsy Randall-Schadel, and Shawn Robertson

529 Survival of *Phytophthora ramorum* in Tanoak and Rhododendron Leaves
I. McLaughlin, W. Sutton, and E. Hansen

530 Multihyphal Structures Formed by *Phytophthora ramorum* on Inoculated Leaves of Mediterranean Shrubs
Eduardo Moralejo and Enrique Descals

531 Monitoring *Phytophthora ramorum* Distribution in Streams Within Coastal California Watersheds
Shannon K. Murphy, Chris Lee, John Bienapfl, Yana Valachovic, and David M. Rizzo

532 Monitoring of *Phytophthora ramorum* in the North Central United States
Frances S. Ockels, Pierluigi Bonello, and Manfred Mielke

533 Genotyping *Phytophthora ramorum* Isolates From Nurseries in the United States Using PCR-RFLP and Microsatellite Analyses
Rodrigo Olarte, Matteo Garbelotto, and Kelly Ivors

534 Surveying for *Phytophthora ramorum* in Urban and Wild Forests Throughout Western Oregon
N. Osterbauer, A. Trippe, S. Suttle, M Boschee, A. Kanaskie, and L. Bauer

535 Detached-Leaf Assays With *Phytophthora ramorum*: Are They Valid?
Jennifer L. Parke, Melody L. Roth, and Caroline J. Choquette

536 Low Genotypic Diversity of *Phytophthora ramorum* in North America Revealed by Microsatellite Markers
S. Prospero, E.M. Hansen, and L.M. Winton

538 Nursery Cultural Practices and Physiological State of Nursery Stock on Susceptibility of *Phytophthora* Species, Including *P. ramorum*
Steven J. Scheuerell, Robert G. Linderman, Niklaus J. Grunwald, and E. Anne Davis

539 Environmental Limits to Infection by *Phytophthora ramorum* in Tanoak and California Bay Laurel
Lilly Schinsing, Katherine Hayden, and Matteo Garbelotto

540 Evaluating the Survival of *Phytophthora ramorum* in Firewood
John Shelly, Ramnik Singh, Christine Langford, and Tad Mason

541 Development of Detection Methods for *Phytophthora ramorum* in Georgia
Pauline Spaine, William Otrosina, and Stephen Fraedrich

542 Sudden Oak Death Bike Tire Scrubber
Peter Thut, Rocky Chavez, Desaree Williams, Sibdas Ghosh, Lisa Baird, Mietek Kolipinski, and Bruce Badzik

543 *Phytophthora ramorum* Research and Control in North Coastal California
Yana Valachovic, Chris Lee, Dave Rizzo, Jack Marshall, John Bienapfl, and Shannon Murphy

545 Characterization of European and American *Phytophthora ramorum* Isolates Due to Their Morphology and Mating Behavior *in vitro* With Other Heterothallic *Phytophthora* Species
Sabine Werres and Katrin Kaminski

548 Surveying for *Phytophthora ramorum* in Ornamental Nurseries, Home Landscapes, and Forests in Georgia, USA
Jean L. Williams-Woodward

549 Plotless Evaluation of *Phytophthora ramorum* Incidence in Oaks and Tanoaks in Two Different Forest Types in California
Brice A. McPherson, David L. Wood, N. Maggi Kelly, Andrew J. Storer, and Richard B. Standiford

552 Thwarting *Phytophthora ramorum*: An Interactive Poster on Breaking the Disease Cycle
Betsy Randall-Schadel and Scott Redlin

553 *Phytophthora ramorum* on *Calluna vulgaris*, *Photinia fraseri,* and *Pieris japonica* in Poland
Leszek B. Orlikowski, Aleksandra Trzewik, and Grazyna Szkuta

554 Comparative Evaluation of Real-Time PCR (TaqMan®) With Isolation for Diagnosis of *Phytophthora ramorum*
Kelvin Hughes, Ruth Griffin, Jenny Tomlinson, Neil Boonham, Victoria Barton, Patricia Giltrap, Ellie Hobden, Lynn Walker, Gilli Humphries, Ann Barnes, Paul Beales, Alan Inman, and Charles Lane

556 Development of Molecular Diagnostics for *Phytophthora kernoviae* a New *Phytophthora* Threatening U.K. Trees, Woodlands, and Ornamental Plants
Kelvin Hughes, Ruth Griffin, Neil Boonham, and Alan Inman

558 Isozyme Genotyping of *Phytophthora ramorum* Reveals Fixed Heterozygosity at Three Loci Suggesting a Hybrid Origin
Willem A. Man in't Veld

559 The Effects of Sudden Oak Death on Wildlife–Can Anything Be Learned From the American Chestnut Blight?
William D. Tietje, Donald E. Winslow, and Douglas J. Tempel

560 Elimination of *Phytophthora ramorum* in the Tunnel Composting Process
Maikel Aveskamp and Johanneke Wingelaar

565 Index

Keynote Address and Overviews

Keynote Address Second Sudden Oak Death Science Symposium Monterey California, January 19, 2005

Representative Lynn Woolsey, California's 6th Congressional District

It is my great pleasure to be with you today at this impressive gathering of women and men of science. As a member of the House Science Committee, I am privileged to regularly hear from people from many disciplines in the scientific community. I so enjoy those hearings with not only brilliant, rigorous thinkers, but also people with a passion for the truth and compassion for all living things. I am certain that would describe the people in this room. How could it not be when your mission is, in the simplest terms, to save this state's, this country's, and perhaps a great part of the world's woodlands?

Sudden oak death (SOD), as you know, is somewhat of a misnomer. It does not come on suddenly and it doesn't just affect oaks. However, sudden oak death is a lot easier to pronounce than its cause, *Phytophthora ramorum*, so I am going to stick to calling it that or SOD.

As you may know, SOD was first identified in Marin County, which is my Congressional District, and its first victims were tanoak trees. Those of you who are not from central or northern California may not be familiar with tanoaks. They're not particularly attractive or commercially useful, but they are fast growing and hardy. If you were to cut down a tanoak, a thick bush of sprouts soon grows from the stump. You cannot generally use them for lumber because they split when dried, although when carefully kiln-dried tanoaks have been used for flooring. But they are not the kind of awe-inspiring tree like California's signature redwoods or black oaks. That is probably why when University of California's, Cooperative Extension, Horticultural Advisor Dr. Pavel Svihra first discovered dead and dying tanoaks in the rolling hills of Marin in 1995, he had a hard time convincing authorities that his findings were consequential.

It took years, and probably only when the growing fuel load of dead tanoaks threatened million dollar homes in the hills of Marin County, that the alarm was finally heard. In fact it was not until the year 2000 that major efforts got underway to combat this disease. That was the year that local governments concerned with the spread of this disease formed the Sudden Oak Death Coastal Council and that an interagency and interdisciplinary California Oak Mortality Task Force was formed. It was also the year that Marin County, led by Supervisor Cynthia Murray, convened a Sudden Oak Death Summit that brought together local, state and federal officials. The news was out, our trees were dying, and something needed to be done about it before our woodlands were devastated and this scourge spread beyond a few coast counties.

Four bills were introduced in the California legislature in 2000, and each year since, the State has contributed to the effort. The USDA Forest Service was aware of the problem, and people like the USDA Forest Service's Susan Frankel, who headed the Task Force, took key roles in coordinating local, state, and federal activities. But we needed to get a concerted research effort off the ground and

the funding was just not there. In 2000, I wrote several letters to the USDA Forest Service and the U.S. Department of Agriculture requesting increased money and resources for research. I remember that I scrawled the exclamation "**HELP"** at the bottom of one letter that I wrote to the Secretary of Agriculture Dan Glickman. Boy, did we need help! In 2000, the total amount of federal funding directed toward this effort was a measly $85,000. Soon after the SOD Summit, the USDA began shifting funds from one account to another to help out, but still there were inadequate resources to mount a serious effort.

Clearly, the situation called for widening support. Ironically, the spread of the disease was of great help, because SOD became a problem beyond Northern California. Researchers, forestry and agricultural officials – and elected officials – soon found out that the disease was not limited to a few California counties. In fact, by 2002 the disease had worked its way up from Monterey Country to Curry County, Oregon. It was also discovered that SOD not only infected tanoaks, but a wide variety of trees, including several oak species, big leaf maple, Douglas-fir, and even the venerable California redwood. In addition, the disease did not only affect woodlands. Last year it was found at one of the State's largest commercial nurseries, infecting plants like rhododendrons and camellias.

In 2000, in collaboration with Senators Boxer and Feinstein, I worked to rally the California delegation, and then the larger Congress with the help of Congresspersons Mike Thompson, Sam Farr and Lois Capps. We doubled funding in 2001 and since than it's been going upward. Last year, was the most successful yet in terms of funding. Over $13 million was appropriated by Congress to fight sudden oak death, bringing the cumulative total to $22 million. Perhaps just as important, Congress passed a bill by Congressman Max Burns, which I joined with 19 others in the House in co-sponsoring, directing the USDA to write a national plan to deal with SOD. The plan will compile research, surveys and data on sudden oak death; assess the risks of the disease; evaluate current control and management efforts; and recommend what actions need to be taken. The plan is not to be put on a shelf somewhere because the law specifies that it is to be implemented as soon as practicable after funds have been appropriated.

It has been ten years since sudden oak death was first found on a hillside in Mill Valley, California, and we are only at the beginning stages of learning how to deal with it. There have been promising developments, including success with small-scale treatment, and an indication that controlled burning could be used as a preventative measure. I am confident that this conference will herald other important findings.

We have also learned a few things on the policy level. First, how important it is to work from the bottom up. Only because Marin and other California Counties, and their elected officials, became engaged players did the issue rise to State and national importance. Imagine if discussion of sudden oak death was mainly confined to the experts at the USDA Forest Service or the California Department of Forestry and Fire Protection. Despite their good intentions, it would still be considered one problem along with a dozen other blights affecting our woodlands. That is not to take anything away from the experts—but have you ever met the people from my district? Hand in hand with local involvement is the role of public education. Hundreds of stories about sudden oak death have appeared in newspapers and magazines, hundreds of hours of news reporting on TV and radio. Because the news got out, sudden oak death got on the public policy radar screen.

So what's ahead? Obviously more research and the development of a national plan is critical. But as we learn more, some difficult decisions are going to have to be made, both nationally and locally. Here are some tough ones to chew on:

- How much are we willing to spend to control and manage this disease? In prevention? In eradication? In dealing with the disease's aftermath?
- We also have some tough public policy questions. Should we limit public access to forests to stop the spread of the disease? How will that affect outdoor recreation and the economy that depends on it?
- Are quarantines effective and what impact will they have on commercial nurseries?
- Do we need to increase controlled burns and if we do, what will be the effect on air and water quality?

As a legislator, I know how tough it is to correctly identify a problem, find a solution, and then convince others that it is in their best interest to support that solution. I am counting on you to provide the solid scientific rationale to do what must be done.

You are not only fighting SOD, you are also pointing the way for sustaining our forests. Sudden oak death is one of the first major environmental challenges that we will face in a natural world made vulnerable to disease and other invasive species by increased international trade and travel. Your efforts will lead the way to earlier detection of plant pathogens, rapid response protocols, and new discoveries to cure disease and control their spread. So I eagerly look forward to any new information that will come out of this conference. I thank you and congratulate you on all the work you do, and look forward to continuing to be your partner in this effort.

USDA Forest Service, Pacific Southwest Research Station Sudden Oak Death Research Program: 2001-2005[1]

Patrick J. Shea[2]

Key words: sudden oak death, USDA Forest Service, request for proposals, *Phytophthora ramorum*

The Pacific Southwest Research Station (PSW), U.S. Department of Agriculture (USDA) Forest Service initiated the Sudden Oak Death Research (SOD) Program in late 2000. The program was prompted by late fiscal year funding dedicated directly to begin research on this newly discovered disease. The history of discovery of *Phytophthora ramorum*, the causative agent of SOD, is now well known and will not be repeated here. During the initial start-up of the SOD Research Program, funding came from a variety of sources. Over the course of fiscal years (FY) 2001 and 2002, research funding was received from the USDA Commodity Credit Corporation, USDA Forest Service Research and Development and through a supplemental appropriation from the United States Congress. Starting in FY 2003 the SOD Research Program was funded through the regular appropriation process and was included in the PSW budget. In addition, during FY's 2002 and 2003 the California Department of Forestry and Fire Protection provided funds to be used for funding certain research projects administered by the SOD Research Program.

At the onset of the PSW SOD Research Program research on this disease and the causative agent was already in progress at several university laboratories, such as David Rizzo's laboratory at University of California, Davis, and Matteo Garbelotto's laboratory at the University of California, Berkeley. In addition, several plant pathologists associated with the University of California Cooperative Extension program were actively pursuing research on SOD in various locations in California. It is because of this ongoing non-USDA Forest Service research and the fact that PSW was not engaged in an active forest pathology research program that the SOD Research Program was, and continues to be, a largely extramural research program.

The PSW SOD Research Program functions much like any competitive grant program: (1) research needs are identified; (2) a Request for Proposals (RFP) is issued; (3) research proposals are evaluated for relevance to program needs and sent out for peer review; and (4) a selection committee makes the final decisions on which proposal are funded. During each funding year,

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California.

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and in preparing to issue a RFP, the Program Manger convened a meeting to identify research needs/data gaps. Participants in those sessions represented a wide range of disciplines that included plant pathologists, foresters, fire ecologists, state and federal regulators, private, state, and federal land managers, wildlife biologists, entomologists, modelers, and social scientists. A RFP that highlighted high priority research needs was prepared based on the results of the research needs assessment. Upon receipt of research proposals the Program Manager acquired two peer reviews for each proposal. A selection committee composed of individuals from the USDA Forest Service-Washington Office, Research and Development; USDA Forest Service – PSW; USDA Forest Service-Pacific Southwest Region, Forest Health Protection; California Department of Forestry and Fire Protection; and the University of California, Berkeley evaluated the reviews and proposals.

Four emphasis areas have been identified for purposes of organizing the PSW SOD Research Program: (1) Pathogen biology, hosts, and epidemiology; (2) Disease management, detection, and resource utilization; (3) Disease impacts on ecosystem components; and (4) Economic and social impacts (i.e. Native American issues). The first area of emphasis includes research in such areas as growth habits and attributes of the pathogen; mechanisms of survival and infection; identification of developmental characteristics; and identification of hosts, both native to California and potential hosts. The second area of emphasis is characterized by development of treatment strategies; discovery of improved diagnostics; modeling of risks; and feasibility studies for the disposal and/or utilization of biomass killed by the disease. The third area of emphasis includes research on the potential effects on wildlife, such as small mammals, birds and insects; effects on species composition of affected forests, site productivity as a result of various levels of tree mortality; and potential effects on biodiversity. Finally, the fourth emphasis area focuses on the potential effects of SOD on local, regional, national and international economies associated with forestry, horticulture and urban land values. Most importantly this fourth area also includes social impacts, especially those that could potentially impact Native American culture.

Over the course of the last five years, funding of research proposals within the various emphasis areas has been uneven. This is to be expected when conducting a research program on an entirely new organism that only recently (2000) was given a scientific name. Pathogen biology, identification of susceptible hosts and epidemiology of *P. ramorum* received about 49 percent of the available research funds between 2001 and 2005. Research that was centered on disease management, detection and resource utilization was awarded 18 percent of the available funds and potential impacts on ecosystem components received about 19 percent during the same time period. The remaining percentage was devoted to begin determining the economic and social impacts. However, it needs to be pointed out that there has been a steady shift during FY 2004 and 2005 toward funding of research that emphasized disease management, detection, diagnostics, and the social and economic impacts. In addition, whereas much of the research activity has centered on the Pacific Coast (i.e. California and Oregon), events in the last few years has seen an increase in funding to other areas of the United States and Europe.

Funded research proposals highlight the broad array of institutions that the PSW SOD Research Program utilized to forward its objectives. Twelve academic institutions are being funded and represent both public and private universities. Geographically they range from the University of California, Davis and Berkeley, to the University of West Virginia. Two private research institutions were also recipients of SOD research funds. Federal government research agencies included USDA-Agricultural Research Service, five USDA Forest Service Research Stations and two Department of Energy agencies, Joint Genome Institute and Oak Ridge National Laboratory. Finally, because of the international importance of this organism, several European research facilities and one Mexican research institute received SOD program funding.

Our knowledge and understanding of both *P. ramorum* and the disease known as SOD has advanced greatly since the inception of the PSW SOD Research Program in late 2000. Research results, funded by the SOD Program, have assisted in the formulation of important science-based regulatory programs that aim to impede the spread of this disease. Treatment strategies have been developed that can prevent infection of individual trees that impact landscape values, especially in the urban/wildland interface. Important environmental parameters have been identified that play critical roles in the spread and infection of trees in wildland settings. New diagnostics are being developed to more rapidly identify infected plant material and aid in the delineation of infected nursery plants or wildland areas. The PSW SOD Research Program should continue to serve as the key entity in future *P. ramorum* and sudden oak death research to ensure a full understanding of this organism.

Understanding Sudden Oak Death: A Model of Partnership and Collaboration[1]

Jimmy L. Reaves[2]

Key words: tanoak, nurseries, disease spread, sudden oak death

Good morning. I want to thank the planning committee for allowing me to share some of my thoughts at this Symposium as we try to better understand sudden oak death (SOD) and *Phytophthora ramorum*. I bring you regards from Dr. Ann Bartuska, the Deputy Chief for Forest Service Research and Development. Ann is a big supporter of the work that has already been done and encourages you to continue your valiant efforts on SOD.

I would also like to thank the Pacific Southwest Research Station and State and Private Forestry for leading and coordinating the research, survey, and management efforts on SOD in the Forest Service—they have done an excellent job; the Honorable Lynn Woolsey, Barbara Boxer, Samuel Farr, and others for their support and their efforts in securing Congressional funding for SOD research and technology transfer; USDA Animal and Plant Health Inspection Service (APHIS), the Agricultural Research Service (ARS), and Cooperative, State, Research, Education and Extension Service (CSREES), as well as California, Oregon and several other states for their commitment and collaboration; and all the scientists and technology transfer specialists who are a part of our quest to enhance our knowledge and understanding of SOD and its current and potential impacts on our forest ecosystems, nurseries, and the Nation's social fabric and economy. I urge you to continue the excellent work you are doing on SOD.

In a way, I feel like I am preaching to the choir. You know what the problem is and you have a pretty good idea what needs to be done. I hope you will indulge me for just a few minutes as I share a few words about some of the accomplishments and some of the challenges we face as we continue to address SOD.

Just picture the exhilaration on the faces of your and my forefathers as they walked through the forests of the Eastern United States a hundred years ago. They likely marveled at the magnificent American chestnut trees that were abundant in those forests. Chestnut trees were used for railroad ties, telegraph and telephone poles, lumber, and firewood, and they provided products that were used to support 19 industries. In addition, American chestnuts provided food for deer and other animals. For our forefathers and some of us, chestnuts contributed to memorable Christmas joys, as some people likely roasted chestnuts over an open fire and didn't just sing this verse in a carol.

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Along with having economic value and contributing to ecosystem integrity, I am convinced that the connection made to these magnificent wonders of nature left an indelible mark on the lives of our forefathers.

Our forefathers and others living at the turn of the 20th century believed that American chestnuts and other trees would always be plentiful—the phrase "the land of plenty" was real to them. They could not know that within the next century there would not be any real chestnut trees left to support industries or to stir our spiritual awareness. Root rot caused by the pathogen *Phytophthora cinnamomi*, and, to a larger extent, chestnut blight caused by the pathogen *Cryphonectria parasitica*, did what loggers, windstorms, hurricanes, tornados, floods, and fire could not do—wipe out the beloved American chestnut trees in this country.

We are now faced with what our ancestors were faced with at the turn of the 20th century. *Phytophthora ramorum*, the pathogen that causes SOD, has appeared on the scene and has the potential to wipe out not just one species of oak, but several species, and not just in the western part of the country but in other parts of the country and elsewhere in the world. In addition, SOD has caused major losses already to a number of California nurseries, and has the potential to adversely impact our Nation's entire nursery industry, resulting in millions of dollars in losses each year. Just imagine the impact that would have on wood and paper industries in this country, wildlife habitat and forest ecosystems, hunting, recreation, and the spiritual values of oak trees that are near and dear to us.

At the turn of the 20th century, scientists were able to isolate and identify fairly early the fungus that was responsible for chestnut blight, but they did not have the scientific knowledge or the technology to develop and institute effective control measures to combat the spread of that disease. Today we are fortunate because we do have the scientific capability to develop and institute management and control measures to combat the spread and destruction caused by *P. ramorum*. We know something about the biology of this organism and have some idea how it spreads. As I speak, we are developing better ways to manage it.

We have to be vigilant in our efforts to keep SOD and *P. ramorum* from spreading beyond current boundaries. Our collective knowledge, coupled with collaborative efforts across Federal, State and international governmental agencies, is strengthened by university partnerships, the efforts of private industry, and community involvement. Our collective efforts position us well to conduct innovative research and institute management practices and appropriate policy measures to combat the spread of SOD. Since we don't fully understand the biology of this organism or the dynamics of the various diseases that it causes, we still have much to learn.

Let's take a look at what we currently know about the organism.

- *P. ramorum* was discovered in 2000.
- This virulent pathogen of unknown origin has spread to forests in 14 coastal California counties, as well Oregon's Curry County.
- It has also been found in numerous European nurseries and gardens, and most recently in nurseries in Washington, Oregon, and British Columbia, Canada.

- This past year, shipments from the West Coast sent plants from infested nurseries to several more States, triggering extensive survey and management efforts, as well as new regulations.
- The pathogen has a broad host range, including:
    - hardwood trees, such as coast live oak
    - landscape plants, such as rhododendron
    - herbaceous plants, such as western starflower
    - softwood trees, such as coast redwood and Douglas-fir
- *P. ramorum* has already killed tens of thousands of coast live oak, tanoak, and California black oak trees. The pathogen causes branch and twig dieback in conifers and several shrubs, as well as leaf blight in mountain laurel, camelia, and other species.
- In California, *P. ramorum* negatively affects ecosystem functions, increases fire and safety hazards, and reduces property values in developed areas.

I realize that this is not an exhaustive list. Research results to be presented at this Symposium will fill in a few more pieces of the SOD puzzle. As you can see, we have a formidable foe on our hands. It is going to take substantial funding, well-coordinated research and technology transfer, and sound policy decisions to effectively combat the spread of this pathogen and markedly reduce its impacts.

I have painted a rather dismal picture of the potential results that could occur from the spread of SOD. I do not want the picture I have painted to become a self-filling prophecy. However, if we don't generate the science to understand the disease and deliver this science in a usable form to our public and private land managers, what happened to our beloved American chestnuts could easily be repeated. Remember, it took more than 80 years to start to bring back the American chestnut. If we are not vigilant in our efforts against SOD, we may be faced with a similar scenario that was caused by chestnut blight and other invasive species.

Now having laid out a bleak picture on the potential adverse impacts that could result from SOD, what are some of the positives that we have in our favor? Unlike our forefathers, we do know the pathogen responsible for SOD, at what I believe is an early stage. We have some idea as to how it spreads; we know a number of its hosts; and we know how to kill it on some substrates. We have some funding thanks to the Honorable Barbara Boxer, Lynn Woolsey, and others, and I would like to thank them again for their strong support. And we have Federal, State, and local political support to study the disease, with the end goal of being able to eventually control it. We have a capable cadre of scientists to provide the necessary knowledge about the disease. We have technology transfer agents in place, and the expertise to effectively deliver information to needy users.

Our efforts to understand SOD are being refined through a SOD program review led by Forest Service Research and Development. I believe that the Pacific Southwest Research Station has done an excellent job of spearheading our SOD research program to date. Determining if there are ways we might improve on this effort will be the focus of a review team. The team first met face-to-face yesterday at the Symposium. The results of this review will be shared with everyone when completed by Forest Service Research and Development, through collaboration with Forest Service

State and Private Forestry, ARS, CSREES, and APHIS. Also, a SOD national strategic plan that was called for by Congress has been completed through collaboration among the Forest Service, ARS, CSREES, and APHIS.

Even though we have marshaled our resources in an effective and timely manner, we still face considerable challenges as we address current and emerging SOD issues. Allow me to outline what I believe are some of these challenges and possible solutions.

1) **Maintaining collaboration across organizational lines to address the science and management issues surrounding SOD**
   Proposed solution (s): We need to: (a) organize strategic alliances across organizational boundaries; (b) cooperate as a multi-faceted team as we develop and implement research programs to study SOD; (c) focus our efforts on the highest priority needs; and (d) keep all communication doors open.
2) **Maintaining a long-term research effort on SOD**
   Proposed solution(s): We need to continue to maximize the efforts of scientists in all countries and agencies involved. Include research problems in appropriate planning documents such as research work unit descriptions or problem analyses. Such efforts will then allow us to: (a) develop a better understanding of the pathogen and its dynamics; (b) get a better handle on the host range of the pathogen; (c) explore the dynamics of the disease and the importance of climatic conditions to disease development; (d) better understand potential cascading ecological effects, as well as social and economic impacts; and (e) hopefully confirm the origins of the pathogen. By knowing the pathogen's origin, we will have a better chance of identifying its natural biological enemies that could possibly be used in management practices. I believe there are some efforts underway to do just that.
3) **Securing consistent and long-term funding for SOD** ***Proposed solution(s):*** Continue to seek funding from Congress through the budget process and find ways to leverage the dollars we are able to secure. Seek funding through granting agencies outside of the Federal budget process. We need to maximize our resources to improve outcomes and address priorities.
4) **Delivering usable science to public and private landowners**
   Proposed solution(s): The public can play an integral part in our success in combating the spread of SOD. In order for that to occur, however, we must provide the public with the necessary information in ways and in a format that they can understand, not in "science-speak." There are some excellent examples out there where this has occurred. In one instance, the Fish and Wildlife Service is using trained volunteers to help with early detection, targeting invasive species at six refuges where they conduct approximately 20 percent of all work on the refuges. We could benefit from similar programs.
5) **Developing sound policies that take into consideration the economic aspects of SOD**

Proposed solution(s): We must work across organizational boundaries and provide the necessary scientific knowledge and leadership to help agencies like APHIS develop sound regulatory policies for SOD.

Please understand that what I have outlined is in no way meant to be construed as an exhaustive list, but rather as an inward look at some the challenges and possible solutions. As this is a scientific forum, I am sure that at least some of you may not agree with what I have presented. That's fine—it's healthy for the necessary debate. I believe that healthy debates lead to sound solutions. I do know that we must continue to be vigilant in our efforts against SOD.

In closing, I would like to take a phrase from one of my favorite philosophers, the sometimes gregarious Yogi Berra, the Hall of Fame catcher for the New York Yankees. Yogi once said, "You've got to be careful if you don't know where you are going, 'cause you might not get there." I feel strongly that we know where we are going. With your leadership, I am confident that we will get there.

History and Background of the California Oak Mortality Task Force (COMTF)[1]

Mark R. Stanley[2]

Abstract

The California Oak Mortality Task Force was formed in August 2000 as a consensus group to address the impacts caused by *Phytophthora ramorum*. It is over 1000 strong with over 80 agencies, universities, public, and private groups currently involved. The Task Force has been a tremendous success and may serve as model for other similar efforts.

Key words: California Oak Mortality Task Force, sudden oak death, *Phytophthora ramorum*

History and Background of the California Oak Mortality Task Force (COMTF)

I was asked to give everyone a little background on the California Oak Mortality Task Force: what it is, a little of the history of its formation, and how it functions.

The Task Force was formed in August of 2000 in response to a request from the California Board of Forestry and Fire Protection. The Task Force was originally formed as a part of the California Forest Pest Council which is a committee of the Board of Forestry and Fire Protection. At the time of its formation, *Phytophthora ramorum* had not been named and in fact was only known to be in eight counties in California and only known to affect six hosts. It was, at that time, only a wildland issue not having been found on nursery stock.

What seems to make this group a little different than most other government groups is that it is open to anyone who wants to work towards a common goal of addressing sudden oak death for California and preventing pathogen spread to new areas. We knew this had to be done quickly and that meant we had to work together towards a common goal. Although this sounds logical and straight forward to most folks it is not a simple thing to do at first. There are challenges: old history between agencies that are in competition with each other for funding and turf; personal baggage that we all bring to the table; or perhaps no history at all

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and therefore no established level of trust. You also have to have a leader that is as much a facilitator as a leader. Someone who can organize, lead, and motivate, without people feeling that they are losing their or their agencies or organization's power and identity. We were fortunate to have Susan Frankel be that leader.

We were also fortunate to have people that saw that working together was more important than building their egos or their organization. We would have a common voice for funding that would benefit the State not necessarily any one agency or group. We wanted to, and did, utilize the strengths of each organization and individual to achieve our common goals. We worked entirely on consensus. We worked on our goals and a plan of action until we reached agreement by all members of the Task Force.

We had some basic agreements and those were that information and training would be science based. We agreed that we had common goals of education, outreach, coordination and sharing information and resources. We would have a common voice for information that we communicated to the public and other professionals both inside and outside of the Task Force. To this end we had a public information officer for the task force that either spoke for, or worked with the various information officers for different agencies. This can be a very sensitive area but it can and did bring tremendous credibility to the group and to the individual agencies. We also agreed to support each other's efforts as it related to *P ramorum*.

Having a small staff is critical for coordination and communications inside and outside of the group. Everyone on the Task Force already had a full time job and the Task Force needed a few individuals that could help not only coordinate the group, but provide quick response to the needs of the public, partners, and communities that were impacted by the disease.

As a task force we wanted to lead the effort, not follow the parade. This required everyone to communicate with everyone else and so we developed a website to help with that communication. We continue to provide education and training on the pathogen, its biology and possible management, and provide publications in several languages and for scores of special user groups from arborists, to foresters, nurseries to tribal groups, recreation users to mushroom collectors. We have done scores of training sessions at very low or no cost to the participants to help get the information into the hands of the businesses, organizations, and individuals impacted by this pathogen. We have not only provided training and delivered presentations throughout the State and the nation, but we have also conducted training on how to form and operate a group like the Task Force that has proven to be successful and beneficial.

One of the reasons for our success with our various activities and success at obtaining funding to help these efforts is the press and other media folks. Contrary to popular beliefs the press

can be your friend. This takes care and nurturing to develop a good and honest working relationship as well as a trust level that you will actually work with the media.

Last and certainly not least I have to thank and commend the members of the Task Force. Everyone was fully employed and busy before this started. It is only through all the extra work, effort and commitment to work together to try and address this problem have we had success. There have been many long nights, endless conference calls and meetings, and people stepping up to do extra work. The Task Force has been fortunate to hire a few staff people to do all the coordination, website development and maintenance, and to work with the press. Without the professional work and personal commitment of these people we would not be here today. It is because of the people involved in the Task Force that we have had success in working toward our common goals.

And as always for the latest information on *Phytophthora ramorum* or sudden oak death go to www.suddenoakdeath.org.

Research on the Epidemiology, Ecology and Management of *Phytophthora ramorum* in California Forests[1]

David M. Rizzo[2]

Key words: disease management, landscape ecology, invasive species

Introduction

The ultimate goal of *Phytophthora ramorum* research is to develop disease management strategies. To date, studies have been focused at three management levels: the individual tree, the landscape (or forest stand), and the regional to international scale (Garbelotto and others 2003, Rizzo and Garbelotto 2003, Rizzo and others 2005). I will focus my brief remarks on the forest landscape, possibly the most difficult level to implement disease control strategies (for a more comprehensive discussion see Rizzo and others 2005).

From a research perspective, there are four non-exclusive areas that we must continue to focus on in order to facilitate *P. ramorum* management in forest stands:

Put the impacts of sudden oak death in context with expected successional patterns and ecosystem processes within coastal forests

For any *P. ramorum* management proposal to be successful in coastal California forests it must also be put into context with other management goals (e.g. timber extraction, wildlife) and threats (e.g., high fuel loads due to fire suppression, other invasive species) (Rizzo and others 2005). This requires continued study on the ecology of the invaded forests in addition to examining the biology of *P. ramorum*. This pathogen has invaded three broad forest types in California: mixed-evergreen forests dominated by coast live oak (*Quercus agrifolia*), mixed-evergreen forests dominated by tanoak (*Lithocarpus densiflorus*) and Douglas-fir (*Pseudotsuga menziesii*), and coast redwood *(Sequoia sempervirens*) forests. With the possible exception of the coast redwood forest type, the ecology of many coastal mixed-evergreen forests has not been well characterized (Barbour and Minnich 2000). Knowledge of the dynamics of coastal forests will allow for

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setting management objectives beyond simply disease management (e.g., Moritz and Odion 2004).

Have a good understanding of host and pathogen biology

For managing any plant disease, the importance of understanding the various components of the disease triangle is obvious, but is well worth reiterating. The establishment and spread of *P. ramorum* across a forest landscape will be dependent on pathogen biology, host susceptibility and environmental conditions. When the connection of *P. ramorum* to sudden oak death was first made in 2000, there was essentially nothing known about the biology of this organism. The finding that *P. ramorum* has a very broad host range allowed us to begin to piece together the life history of the pathogen in the forests. A key discovery was that sporulation of *P. ramorum* primarily occurs on bay laurel (*Umbellularia californica*) in California and it is this host that appears to be the main driver of the epidemic (Davidson and others 2005). After five years there is still much we do not know; numerous research projects presented at this symposium are examining many other aspects of pathogen biology such as survival in soil, infection biology, host range, and mating studies.

Coast live oak and tanoak, the two hosts impacted the most by *P. ramorum*, are not commercial timber trees. Therefore, relatively little is known about the biology of the hosts, in particular the population genetics and how this may fit into disease resistance (Dodd and others 2005). In addition, research has shown variation in susceptibility of bay laurel (Hüberli and others, 2005). At what scale resistance occurs (see below) and how to utilize such information in disease management and forest restoration are key questions that still need answers.

Consider multiple spatial scales

Research on the biology of hosts and pathogens must be taken to larger scales in order to be effectively translated in landscape management protocols. There must also be constant feedback between research at different scales. For example, Swiecki and Bernhardt (2002) were the first to document an association between sudden oak death and the presence of bay laurel at the landscape level; this was several months before bay laurel was determined to be a host based on isolation and inoculation studies. Movement between scales offers the potential to understand mechanisms and explain large scale patterns (e.g., Cushman and others 2005).

Numerous research plots have been established in California coastal forests since 2000 to understand disease progression and ecological impacts at various spatial scales

ranging from individual trees (e.g., McPherson and others, 2005) to the landscape (e.g., Swiecki and Bernhardt 2002, 2005; Maloney and others 2005). GIS has also been utilized from the earliest days of sudden oak death research (e.g., Kelly and Meentemeyer 2002). Using such information, risk models have been developed for California to target areas that are currently without the pathogen, but may be at high risk for invasion (Guo and others 2005, Meentemeyer and others 2004). These models demonstrate the integration of data on host range, transmission biology and plant community structure to develop models of *P. ramorum* establishment and the risk of spread in plant communities. Models must eventually incorporate the effects of spatial and temporal variability of multiple environmental variables on pathogen persistence.

Utilize long term observations and datasets

Many forest disease epidemics have been expressed over long periods of time (e.g., chestnut blight, white pine blister rust) (Smith 1996.). Focusing on short periods of time can give a false sense of the biology of an organism and its impacts in forest ecosystems. For example, we have been examining sporulation patterns of *P. ramorum* in coastal California forests for nearly five years (Davidson and others 2002, 2005; P. Maloney, unpublished data). Yearly variation in rainfall patterns and temperature influences sporulation patterns of *P. ramorum* (Davidson and others 2005). Late rains in spring 2003 resulted in a twenty fold increase in sporangial production, as collected in rainwater, over the previous two springs in a mixed- evergreen forest (Davidson and others 2005). Similar findings occurred in spring of 2005 (Davidson, unpublished data). Such data point to the potential importance of longer term climatic events, such as El Niño, in influencing the establishment and spread of *P. ramorum*.

There are many threats to California coastal forests (Barbour and others 1993); the damage caused by *P. ramorum* is just one of them. Through the development of collaborations between researchers (including plant pathologists, ecologists, entomologists, wildlife biologists, etc.) and managers, we can begin to integrate disease management into a broader landscape view. Much has been accomplished in *P. ramorum* research over the past five years, but there is still a ways to go.

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Epidemiology of *Phytophthora ramorum* in Oregon[1]

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Key words: sudden oak death, sporangia, aerial dispersal, rain-splash dispersal

Introduction

We are studying how *P. ramorum* survives and spreads in Oregon tanoak forests. The Oregon outbreak is similar to the epidemic in redwood-tanoak forests of California, with several important differences, however. The disease is confined to scattered stands within a 12 m^2 area, and it is subject to an ongoing eradication effort. While eradication has not yet eliminated the disease, inoculum levels are low. As a consequence, myrtlewood is seldom infected and is not important in the epidemic.

Results

Based on 1996, 1998, 2000, and 2001 color aerial photographs of the current outbreak area, the disease is of recent origin in Oregon. The first dead tanoak is visible on a 1998 photograph at one site. At the four other sites examined, the first dead trees are visible on the 2000 images. Dead trees increased rapidly through 2001, when *P. ramorum* was first identified and eradication began. The number of dead trees at the 1998 site increased from one to seven to 110 on the three sets of photographs.

We have been monitoring five eradication sites from 2001. Rainwater and streams were sampled for *P. ramorum*, and persistence of the pathogen in soil and on plants growing around the stumps of infected tanoak trees was periodically measured. Susceptible Douglas-fir and redwood seedlings were planted around stumps. In addition, other sprouting host plants were examined for infection. *P. ramorum* was recovered only once from a rain trap, and is no longer sampled. The pathogen is still recovered from

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streams 36 months after site treatment, although no new plant infections have been associated with infested streams. Eighty-eight percent of the 43 stumps sampled tested positive for the occurrence of *P. ramorum* in the associated sprouts. Infection appeared to result both from cankers already present on the stumps, and from inoculum splashed from below. Sprouts were destroyed with herbicides, and although tanoak, wild rhododendron, and evergreen huckleberry have resprouted on many plots, there were no new sprout infections detected in 2004. Douglas-fir and redwood seedlings were not infected. *P. ramorum* was recovered from soil around 18 percent of the stumps in 2004. Survival of *P. ramorum* on eradication sites was tested with inoculated tanoak and rhododendron leaves. Recovery from leaves on the soil surface dropped to near zero in 3 months, but stayed near 80 percent from buried leaves (McLaughlin, Poster in this Proceedings).

Tanoak trees with bole cankers but with green foliage, and adjacent green trees without bole cankers were felled. *P. ramorum* was isolated from leaf and twig lesions from the upper crowns of 11 of 15 tanoak trees with bole cankers, and from three of the 12 nearby canker-free trees. Infection of rhododendron and huckleberry in the understory was found only in the immediate vicinity of cankered trees. The results suggest downward spread of *P. ramorum* in a tree, from stem flow and rain splash.

New infections are mapped annually and confirmed by isolation and PCR. The distance of 137 newly detected trees (2002, 2003, and 2004) from the nearest tree detected in a previous year was calculated and plotted. The result resembles a classic aerial dispersal gradient of an infectious disease. Most new infections occur close to previously infected trees, but there is a long tail to the distribution, suggesting occasional dispersal in storm winds. About 50 percent of new infections occur within 100m of older infections. There is no “background” of infection, and there is no evidence to suggest disease spread along roads or in streams. Infected tanoaks are clustered on the landscape and are not uniformly distributed. This reflects the local intensification of infections, with occasional “spotting” via long distance dispersal. These jumps are initially detected from the air as single red-crowned trees. Follow up ground checking may reveal bole cankers on a few neighboring green trees. Eradication is imposed at this stage, but newly dead trees are often detected on the perimeter of the treatment area in the next year’s survey.

Discussion

These results are fully consistent with a recently introduced exotic pathogen, spreading by passive aerial dispersal across the landscape, with local intensification by drip and splash. Sporangia are the likely dispersal propagules. New microsatellite markers

developed by Prospero (Poster, this Proceedings) may provide an independent test of these epidemiological hypotheses for *P. ramorum*.

Sudden Oak Death: Recent Developments on Trees in Europe[1]

Clive Brasier[2], Sandra Denman[2], Joan Webber[2], and Anna Brown[2]

Key words: beech trees, Cornwall, distribution, Netherlands, oak, *Phytophthora kernoviae, Phytophthora ramorum*

Abstract

In November 2000 a new *Phytophthora* originally found on rhododendron stock in Germany and the Netherlands in 1993 and the new *Phytophthora* believed to be the cause of sudden oak death in California were shown to be the same organism. This development led to a summary Pest Risk Analysis (PRA) for Europe being prepared by Forest Research (FR), United Kingdom. Subsequently, in 2002, the new fungus was formally named *P. ramorum.* The fact that *P. ramorum* was known to be present in Germany and the Netherlands led to surveys for the pathogen in nursery material, initially in the United Kingdom and the Netherlands in 2002 and later in many European countries. By 2003 it was clear that *P. ramorum* was still spreading on ornamental nursery stock in Europe, in particular on rhododendrons and viburnums. It is now known to be present on nursery stock in at least 12 European countries.

Currently there are two countries in Europe where, outside nurseries, the spread of *P. ramorum* from infected rhododendrons onto trees is known to have occurred. These are the U.K. and the Netherlands. In the Netherlands, extensive areas of 'public green' or public recreational parks occur, many of which are wooded and have large old understorey rhododendrons. In a survey in 2003, about 30 of these sites where found to have *P. ramorum* – infected rhododendrons. At one of these sites, a single infected *Quercus rubra* (northern red oak) with bleeding stem lesions was found. In 2004, another site was found to have nine *Q. rubra* with bleeding stem lesions. Containment measures for *P. ramorum* are in progress (see M. Steeghs, this volume).

In the U.K. between 2002-4, rhododendrons and other ornamental shrubs at approximately 900 parks and wooded gardens were surveyed for *P. ramorum* by the Plant Health and Seeds Inspectorate (PHSI) and the Central Science Laboratory. Sixty such sites were found to have outbreaks of *P. ramorum*, mainly on rhododendrons. About 25 percent of these outbreaks have since been eradicated (see D. Slawson, this volume). Investigations by Forest Research, U.K. showed that *P. ramorum* was also spreading onto tree stems at two main locations. In October 2003, a single *Q. falcata* (southern red oak) with girdling bleeding stem lesions from ground level to 2m was found at a site in Sussex. This

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observation was consistent with evidence from laboratory tests by FR that bark of *Q. rubra* and, by analogy, *Q. falcata* is potentially very susceptible to *P. ramorum* (Brasier and others 2004a).

From November 2003 onwards more infected trees, notably European beech, *Fagus sylvatica*, were found in woodlands and wooded gardens in Cornwall, south-west England. Some of these sites have extensive, and locally sometimes heavily diseased, understorey *Rhododendron ponticum*. Cornwall falls within a 'higher-risk' category when a climate match is made to the southern Oregon *P. ramorum* outbreak area (R. Baker, personal communication).

At these Cornish sites new tree hosts for *P. ramorum* continue to be found. So far bleeding stem lesions caused by *P. ramorum* have been found mainly on European beech. However, stems of mature *Q. cerris* (Turkey oak), *Q. petraea* (sessile oak), *Nothofagus obliqua* (Southern beech), *Aesculus hippocastanum* (Horse chestnut) and *Acer pseudoplatanus* (European sycamore) are also affected. Diseased *R. ponticum* is thought to fuel most of these stem infections. In addition to *R. ponticum*, *P. ramorum* has been found to cause foliage and shoot infections of *Q. ilex* (Mediterranean holm oak), *Castanea sativa* (sweet chestnut), *Fraxinus excelsior* (ash), *Pieris* spp. and other ornamental rhododendron varieties.

During surveys for *P. ramorum* in Cornwall another previously unknown *Phytophthora* was isolated concurrently from a large (> 1 m^2) aerial bleeding lesion on a mature *F. sylvatica* and from *R. ponticum* in the same area. Like *P. ramorum* this new *Phytophthora* had caducous (deciduous) sporangia and was also probably aerially or splash dispersed. It was subsequently found to occur at several adjacent sites, and was apparently causing a locally more aggressive defoliation and dieback of *R. ponticum* than *P. ramorum*, leading to mortality, and locally serious damage to stems of beech. This new *Phytophthora* was informally designated *P.* taxon C and is now formally named *P. kernoviae* sp. nov. (after Kernow, the noun for Cornwall in the Cornish language, Brasier and others 2005).

Like *P. ramorum*, *P. kernoviae* is considered to be a new and invasive pathogen on trees in the U.K., most probably introduced by the plant trade, perhaps in the past 10–15 years. Possible origins for both species include the Himalayas, Yunnan and Taiwan (Brasier and others, 2004b). To date, *P. kernoviae* has been found mainly in woodlands in one area of Cornwall. Judging by its distribution, it may have arrived there before *P. ramorum*. In surveys by FR, it has been found causing bleeding stem lesions mainly on *F. sylvatica* (about 50 affected trees), but has also been isolated from bleeding stem lesions on *Q. robur* (English oak) and *Liriodendron tulipifera*. Diseased *R. ponticum* is again believed to fuel most of these stem infections. *P. kernoviae* is also causing foliar and shoot necrosis of *Gevuina*, *Magnolia*, *Michelia* and *Pieris* (in addition to *R. ponticum* and other rhododendron varieties). Interestingly, *Q. robur*, *Liriodendron* and *Michelia* are not presently known as natural hosts of *P. ramorum* in the U.K. Since *Liriodendron*, *Michelia* and *Magnolia* all belong to the Magnoliaceae, *P. kernoviae* might be a magnolia 'specialist' in its natural habitat.

Unlike *P. ramorum*, *P. kernoviae* is not widely present in the U.K. nursery trade, although it has been found at outlier locations in a garden/nursery in Swansea, south Wales, and in Cheshire, England (D. Slawson, this volume). These locations may reflect the further spread of *P. kernoviae* from the Cornish sites via the plant trade. A disease management zone has recently been set up in Cornwall by the U.K.

Forestry Commission and PHSI. Attempted eradication of infected rhododendrons is a major objective (D. Slawson, this volume).

P. kernoviae has a slightly different potential host range (see C. M. Brasier and colleagues, this volume) and a lower optimum temperature for growth than *P. ramorum.* Also, unlike *P. ramorum,* it is self-fertile i.e. probably inbreeding. Its reproductive strategy and its ecological strategy therefore may also be somewhat different. The threat that *P. kernoviae* poses to forests and natural ecosystems in other parts of the world is unknown (Brasier and others 2004b, 2005). In addition to *P. ramorum* and *P. kernoviae*, another recent 'aerial *Phytophthora*' invasive in the U.K., *P. ilicis*, plus the established species *P. cambivora*, *P. citricola* and *P. gonapodyides*, are also present on trees at the Cornish sites. Sometimes they occur together on the same tree (see A. Brown and colleagues, this volume). Consequently, another possible forest health risk is that, in the longer term, *P. kernoviae* or *P. ramorum* might adapt further to their new environment, either intrinsically or via horizontal transfer of genes with other *Phytophthora* species.

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Phytophthora ramorum in Nurseries

Review of *Phytophthora ramorum* in European and North American Nurseries[1]

Janice Alexander[2]

Key words: Phytophthora ramorum, nursery, regulations, plant pathogen quarantines.

Abstract

In 2000, a previously unidentified *Phytophthora* isolated from rhododendrons in European nurseries and gardens was determined to be the same species – but a different population and mating type – as the pathogen causing sudden oak death in California. Upon recognizing the threats that *P. ramorum* posed to both wildlands and the nursery trade, countries around the world created quarantine programs to limit the introduction and spread of the pathogen. There are currently seven countries, plus the European Union, with quarantine regulations regarding *P. ramorum*. The E.U. quarantine required member states to survey for *P. ramorum*, and through November 2004, 13 European countries have detected *P. ramorum* in nurseries. Despite quarantine efforts 381 positive sites have been identified in the United Kingdom alone.

There were also quarantines put in place in the United States, at both the state and federal level. State quarantines cover shipments within a state and are designed to prevent pest introductions to the state. The first *P. ramorum* quarantine in the U.S. was issued by the Oregon Department of Agriculture (ODA) in January 2001, and was applicable to the entire state of California. In August 2001, ODA extended the quarantine after aerial surveys detected *P. ramorum* in 40 acres of Curry County, Oregon. Since then, there has been an aggressive eradication effort in this area. In May 2001, the California Department of Food and Agriculture (CDFA) issued emergency regulations that required permits in order to move host plants or materials within or from seven infested counties.

The U.S. Department of Agriculture's (USDA) federal quarantine covers shipments between states, and went into effect in February 2002. Similar to the California regulations, it included a quarantine area – currently the 14 California counties with wildland infestations and the approximately 11 square miles of Curry County, Oregon. Since *P. ramorum* has a large host list, the federal order covers a wide range of plants and materials. Once a plant with a natural infection is detected and Koch's postulates are completed, the species or variety is added to the federal quarantine. Plants with natural detections but incomplete Koch's postulates are added to an "associated plants" list. These lists are updated frequently as new hosts are found. All unprocessed commodities made from host plants are subject to quarantine including nursery and forest stock, logs and firewood, bark, soil, wreaths, greenery, compost, and other specialty goods. Internationally, USDA Animal and Plant Health Inspection Service (APHIS) has also

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informed the European Commission of restrictions on their member country exports of *P. ramorum* host plants and propagative material.

Through 2002, it was widely believed that *P. ramorum* was an issue in European nurseries but not in their wildlands, while the opposite was believed true in North America (i.e., a forestry, not a nursery, issue). There had been only one *P. ramorum* detection from a single nursery in the U.S. in 2001, which occurred on rhododendrons in a California nursery surrounded by a heavy forest infestation in the quarantine area. In 2002, the CDFA surveyed an additional 99 nurseries in 17 counties and more than 8500 surrounding acres and did not detect the pathogen. Even through 2003 the pathogen had been detected in only seven California nurseries within the infested (quarantine) area. Outside the infested (quarantine) area, there were 10 *P. ramorum* nursery detections: in Stanislaus County, California, and in Oregon, Washington and British Columbia. Several of the Pacific Northwest detections were found to be the European (A1) strain, and were not associated with North American infestations. As part of the federal quarantine program, a U.S. National Nursery Survey was also implemented in 2002 – all results were negative in the U.S. for the National Survey. Twenty-five states contributed survey data in 2003 – one nursery in Oregon was found positive as part of the National Survey.

Just as *P. ramorum* was being detected in some North American nurseries, European forest trees were being found infected by the pathogen. In December 2003, the U.K. and the Netherlands reported that *P. ramorum* was infecting the trunks of beech, southern red oak, northern red oak, and horse chestnut. In all cases, the infected trees are located near *P. ramorum*-infected rhododendrons. In the midst of all of this, another newly discovered *Phytophthora, P. kernovii*, (initially called *Phytophthora taxon C*) has also been found causing tree deaths in the U.K.

Back in the U.S., the national survey of nurseries continued. In February 2004, as a result of the national survey and a simultaneous trace-back survey from Washington State, *P. ramorum* was detected on plants at a large nursery in Southern California. The nursery is over 400 miles from the nearest known infested forest, the climate in the vicinity of the nursery is usually hot and dry, and disease symptoms became apparent only after a period of heavy rain. After positive finds in this and other west coast nurseries, additional trace-forward surveys tracked plants to other states. At a cost of more than $15 million, over 1.6 million plants were investigated by states and USDA APHIS to check for infections and destroy them. In one nursery in Southern California alone, over 1 million camellias, worth $9 million, were destroyed because of a *P. ramorum* infestation.

In response to the finds in west coast nurseries, USDA APHIS issued an emergency order in March 2004. After the announcement, 17 states enacted emergency measures to limit plant imports from California. Reacting to these state restrictions, the National Plant Board and National Association of State Departments of Agriculture both got involved to discuss regulations on the federal and state levels. Soon after, in April 2004, USDA APHIS re-issued an order restricting all California commercial nurseries from shipping any host or associated plants out of state, and changed the diagnostic rules for *P. ramorum* to PCR-only. Not long after, the California Association of Nurserymen and Garden Centers filed a federal suit against the state of Kentucky, a state limiting imports from California beyond that allowed in the federal regulations. The state of Kentucky has since come into compliance with the federal regulations.

The final tally for 2004, including trace-forwards, trace-backs, and the national survey, was *P. ramorum* detections at 176 sites in 21 States. In cooperation with the USDA Forest Service, a national wildland survey was also carried out, with one positive site detected in Golden Gate Park, San Francisco, California. In response to these numerous finds in 2004, the USDA issued a new emergency order for nurseries in December that further restricted shipping of host material across state lines.

Phytophthora ramorum – Economic Impacts and Challenges for the Nursery Industry[1]

Karen Suslow[2]

Abstract

In March 2004, a large wholesale nursery in southern California, which ships to interstate receivers, was found to have *P. ramorum* infected nursery stock. As a result, several of the southeastern states placed a ban on all nursery stock shipping from California. Federal regulatory agencies were not able to provide background information and current research data to the states in a timely manner. As a consequence, many states instituted specific bans on host and associated host plants (HAP) of *P. ramorum* or on HAP to the genus level.

Complicating this situation, many southeastern states had a lack of confidence in California regulatory programs intended to prevent the transmission of the pathogen via nursery stock. Much of this mistrust had a foundation in the lack of communication of existing epidemiology, the gaps in basic biological information on *P. ramorum* and the lack of harmonization of regulatory approaches between California, Oregon, Washington and the Federal government.

Many state regulatory agencies were not aware of the USDA Sudden Oak Death (SOD) Compliance Agreements that formed the basis of operation for nurseries within the regulated counties of California since 2003. Further, they were unaware of the recommended Best Management Practices and how these are directly linked to current research findings that validate them. Equally, communication regarding the purpose and value of the USDA – Forest Service and California Department of Forestry and Fire Protection monitoring programs, which began in early 2000, was lacking.

When the state regulatory agencies, the federal regulatory agencies and the industry are not extensively communicating, the most severe economic consequences always fall to the industry.

Key words: Phytophthora ramorum, confirmed nursery, economic impacts

Economic impacts associated with instituting prevention programs

Economic burdens inherited by the industry may take the form of a one-time cost, such as soil management, or a recurring cost, such as prophylactic crop treatments. The extent of this burden will differ depending on the physical and geographical location of the nursery, environmental factors/influences and disease pressures. In addition to the costs associated

[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California

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with prevention programs, such as switching the type of soil-less media component, nurseries may opt to alter their product mix and grow less susceptible varieties. In a ripple effect of economic impact, this strategy has clear potential repercussions in the long-distance market area if specific crops cannot be grown locally and are not available in prime shipping slots, such as springtime.

Economic burdens are also associated with implementing tracking programs for inventory management and audit trails, water disinfection programs, including choice of treatment and associated water testing, and costs associated with labor practices.

Labor practices may include the redesign and implementation of the nursery field layout, wherein non-host material is interspersed as two meter or wider swaths between HAP. It is widely accepted that economics of scale and efficiency are gained when managing large, uniform blocks of crops; however, large blocks need to be broken up, should a find occur, in order to minimize the impact of a mandated or voluntary destruction protocol.

Another management practice that may be desirable to a nursery is to implement ELISA prescreening tests during the time of year when the pathogen is most prevalent. This has clear labor consequences and costs. If an ELISA screen is positive for general *Phytophthora* spp. (a *P. ramorum*-specific ELISA screen is not available at this time), then the nursery may opt to apply fungicides. The concern is that treatment may be unnecessary for two reasons; the first being that there is no established threshold directly correlated with the presence of *P. ramorum* or the likelihood of the pathogen on any host. Treatment would most likely be triggered based on background population numbers alone. Secondly, current ELISA kits are not specific for *Phytophthora* but also react to Pythiaceous fungi, so treatment may be for the wrong pathogen and unnecessary. Lastly, the treatment may actually mask incipient *P. ramorum* infections.

2004 Challenges Associated with the Implementation of the Confirmed Nursery Protocol (CNP) and the Trace-Forward Protocol

Nurseries may be found to be infected when sampled during the annual nursery inspection or as a result of a trace-back from a nursery that received infected product. Once a nursery has been confirmed to be infected via ELISA prescreen and nested PCR, the CNP is implemented. The main challenge with this protocol in 2004 was the delay in completion of the delimitation survey due to either a backlog at the state level if concurrent finds occur at multiple nurseries in the same state or the fact that the extracted DNA for the nested PCR test must be funneled through one USDA lab in Beltsville, MD. Due to the backlog in testing, salable plants are destroyed.

Additionally, a confirmed nursery has two options under the CNP. They may either hold product within a 10-meter hold area for a 90-day period and resample twice within that period, or the nursery may destroy all HAP to the genus level within the production lot plus two

meters and the 10-meter hold area. As a result of the backlog in sampling and testing, it is impossible to resample twice during a 90-day period and nurseries generally opt to destroy the plants in the 10-meter hold areas. Once again, healthy, salable plants are needlessly destroyed.

State Regulatory agencies that are attempting to track down the potentially infected material in their state are frustrated because they receive a full listing of all the HAP that were shipped into their state over the previous 12 months instead of a prioritized list of what product was found to be infected at the confirmed nursery. Unfortunately the information is provided to them prior to the completion of the confirmed nursery delimitation survey so the state agency has to determine which nurseries may be at highest risk of receiving infected product while not knowing what product was actually infected.

Another challenge the industry faces is having HAP product from one vendor commingled with product from another vendor. Should a trace forward identify that a nursery may have received infected product, all vendor HAP are pulled together and samples are pooled together. This makes the assumption that there is only one source of infected material and all vendors now have their product held pending test results. Frequently the retail yard requests that the product be disposed of properly because they cannot afford to wait the 4-6 weeks for the test results from the state or the federal government. In each case, the confirmed nursery pays for the disposal of all vendors product and for all product replacement costs.

One state, in particular, had applied the CNP at the retail level, which resulted in non-host material being held, all vendor's material being held, and the closing of many retail yards until the error in their interpretation was discovered and corrected.

Another challenge the industry faced was the remediation of trace-forward product. Finding an acceptable method of destruction (either incineration or deep burial) was, at times, very time consuming. In some locations, conflicting jurisdictions and regional or county regulations added to the cost and frustration. This resulted in retail yards holding the product for two or more months while waiting for an acceptable site to be located. This created a high level of concern for the retailer's reputation due to name-association with SOD. Reputations are difficult to rebuild after such events as well as being costly to repair.

Improvements for the Future

Improvements for the future need to include the following:

-The transfer of information in a timely manner;

-The potential utilization of UPC codes to determine origin of product (nationally and internationally);

-The trace-forward protocols to include prioritized list of infected plants from the confirmed nursery;

-Validation of accurate, timely tests;

-More accredited lab facilities;

-The avoidance of multi-vendor pooling of samples.

Due to the high financial consequences of *P. ramorum* introductions/occurrence, the industry needs all regulatory components to be functional, timely, and seamlessly coordinated. The following regulatory programs play a critical role in achieving this:

-The annual nursery inspection;

-USDA SOD Compliance Agreements;

-County Agriculture agencies linkage with State agencies;

-Restrictions on buy-ins from outside of the country;

-USDA SOD Order.

APHIS *Phytophthora ramorum* Regulatory Strategy for Nurseries[1]

Jonathan M. Jones[2]

Abstract

A review of the U. S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) regulatory response to *Phytophthora ramorum* is presented as it impacts nurseries and the nursery industry. The Agency responded in 2004 with three Federal Orders, each more restrictive than the previous one because the appropriate response called for a more robust program. The most recent response was a result of over two million host plants from infested nurseries being shipped over a one-year period to more than 11,000 locations. Consequently, APHIS is working with stakeholders to design and implement the most effective program possible for the future. Some of the initiatives described in detail are the Trace Forward Protocol used at those nurseries which have received nursery stock from another location and the Confirmed Nursery Protocol used at nurseries found with infected plants. Looking forward, APHIS is working with the Canadian Food Inspection Agency (CFIA) to harmonize approaches to the disease. Among the strategies under consideration for harmonization and implementation is a nursery "clean stock program" with a module inserted to assure freedom from *P. ramorum*.

Key words: Phytophthora ramorum, APHIS, CFIA, nursery regulation, Federal Order, Confirmed Nursery Protocol

APHIS responds to *Phytophthora ramorum* with broad regulations, recently focused on the movement of nursery stock

APHIS is charged with responding to certain plant health threats. The APHIS response to *Phytophthora ramorum* has resulted in a broad regulatory response recently focused on the movement of nursery stock. The following information details the response, what prompted the response, and some details on the regulatory program and some of the related policies and procedures. Included are details on the Federal Orders, the Trace-Forward Protocol, the Confirmed Nursery Protocol, Strategic Plans, harmonization with Canada, and a program for "clean nursery stock."

Federal Orders - April and December 2004

The first Federal Orders were issued in April 2004 in response to March detections *of P. ramorum* in California nurseries. These orders had the effect of an interim rule, but given the urgency of the situation, the Orders were implemented as measures pending the issuance of an interim rule. As Canada and some individual states took unilateral action against the movement of nursery stock from California

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and, in some instances, from Oregon and Washington as well, and as further surveys and investigations supported a wider ranging concern than just California nurseries, a new Federal Order was needed. Working closely with the nursery industry, National Plant Board, and Canada, the Federal Order signed 21 December was drafted. This Order covers most nurseries from California, Oregon, and Washington that ship interstate. The Order requires at least an annual inspection of nurseries and sampling and testing if they ship hosts or associated plants. The Order has an intended duration of three years.

Stimulus for response, Trace-Forward Protocol, and Confirmed Nursery Protocol

During the 2003-2004 shipping seasons, more than two million host and associated plants were shipped from production or wholesale nurseries found with infected plants in stock. These plants were shipped to over 5000 unique establishments nationwide. We worked with State Departments of Agriculture to respond to these shipments and do follow-up inspections at destinations using the Trace-Forward Protocol. Under these protocols plants at risk are identified, inspected, and tested for *P. ramorum*. Should the presence of *P. ramorum* be confirmed, the Confirmed Nursery Protocol (CNP) is invoked. The CNP, versions used in both the United States and Canada were designed by scientists, state and Federal officials, and the CFIA with review and input from nurserymen to put in motion steps to safeguard and eradicate *P. ramorum* from confirmed nurseries.

Strategic Plans, Harmonization with Canada, and Clean Stock Programs

As directed by Congress, the USDA Forest Service, with APHIS input, produced "…A National Strategic Plan" to demonstrate "how the Forest Service would coordinate with other(s)…to address the threat posed by *P. ramorum.*" APHIS is working on an operationally oriented Strategic Plan with Canada, which will include certain scenarios of occurrences of *P. ramorum* and the appropriate regulatory response. In December, a bill was passed directing APHIS to develop a national plan for the control and management of *P. ramorum.* Since the fall of 2004, APHIS and CFIA have been working to harmonize their programs. This includes scenario responses, the CNP, National Survey, and development of a policy toward and handling of, off-continent host imports. A significant part of this effort is the development and phase-in of a nursery "clean stock" program with a module focused on addressing, and as effectively as possible, eliminating the risk of *P. ramorum* moving in nursery stock.

Update on Diagnostics for *Phytophthora ramorum*[1]

Philip Berger[2]

Key words: Phytophthora ramorum, detection, diagnostics, PCR, nested PCR, culture

Abstract

Diagnostics used by the U.S. Department of Agriculture (USDA), to determine absence or presence of *Phytophthora ramorum* in plant samples, are based on a combination of tests and depend on collaboration with external laboratories. The system currently in place attempts to maximize limited resources and at the same time provide the most scientifically defensible, accurate, and rapid diagnostics available. Described here is an overview of the current system, followed by a discussion of future plans.

Plant tissue samples originating from trace-forward or trace-back surveys, from national surveys, or from certification surveys of nurseries are processed by state Department of Agriculture laboratories or by National Plant Diagnostic Network laboratories. This processing usually includes ELISA pre-screening (which we strongly recommend) to determine the presence of *Phytophthora* spp. Samples that are positive on the basis of ELISA are then subjected to further testing. Laboratories have the option, either before or after ELISA testing, to try to culture the organism. Presumptive positive cultures are then to be sent to Dr. Mary Palm (National Mycologist, Beltsville, MD) for confirmation. Samples where *P. ramorum* was not isolated, or if they are ELISA positive and no culture was attempted, are to have DNA extracted, and the DNA sent to the National Plant Germplasm and Biotechnology Laboratory (NPGBL) (Beltsville, MD), for PCR analysis. This system is summarized in *table 1*.

The current system was designed with a number of factors in mind. First, there are limits on the number of samples that can be processed by individual laboratories or on a national basis. Steps need to be taken to try to limit the number of samples, such as triage by ELISA. Secondly, one of the major bottlenecks in the diagnostic system is likely PCR, and particularly preparation of DNA from samples. Thus, anything that can be done to minimize the number of samples that must have DNA prepared and subjected to PCR would assist in reducing laboratory diagnosis turn around time. Thirdly, while successful isolation of *P. ramorum* is diagnostic for the presence of the pathogen, failure to isolate it does not allow one to conclude that it is not present. Furthermore, it can be difficult to isolate the pathogen from certain hosts because there are potential but unknown effects of fungicides and

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environmental effects can impact isolation efficiency. Therefore, a sensitive molecular test should provide a more reliable indication or absence of presence of *P. ramorum*. It is recognized that PCR also has limitations, but we believe that it represents the best compromise possible, when considering the entire program.

Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS PPQ) utilizes two PCR tests. The first is the multiplex PCR developed by Oregon State University. Although we have validated this method, because it is relatively insensitive compared to the nested PCR test (developed at University of California, Berkeley), it is used as a quality assurance procedure to ensure that DNA received by the NPGBL is of sufficient quantity and quality to be amplifiable in the nested PCR test. Any DNA samples that do not meet these criteria are not tested, and we request a new sample. DNA that passes this test is subjected to validated nested PCR test. Appropriate positive and negative controls are included in all tests performed. The nested PCR, while very sensitive, is technically more demanding than typical PCR assays, is more susceptible to sample-to-sample contamination, and has relatively low throughput. Nonetheless, the only validated test available to USDA APHIS PPQ at present is this method. There may also be cross-reactivity with a limited number of other *Phytophthora* spp. (and perhaps only one). We have noted that it (*P. hibernalis*) produces an amplicon in nested PCR about 20 bp larger than that produced by *P. ramorum*, and this difference can be detected by trained eyes and good laboratory technique. While any test has some rate of false positive or false negative results, the diagnostic tests currently used are such that they will more likely provide a false positive rather than a false negative, allowing the opportunity to obtain additional samples in the case of important potential detections.

At the end of June/beginning of July 2004, a Science Panel was convened, and among several topics substantial time was spent considering *P. ramorum* diagnostics. The recommendations or conclusions of the panel included: 1) Improve communications within the regulatory community as well as to stakeholders regarding diagnostics and associated issues; 2) It would be highly desirable to have an additional diagnostic test(s) to obtain confirmatory results, particularly a test that is based on a different genetic locus than that currently used. Such a test would also need to have acceptable throughput; 3) Obtain better or more complete information on the accuracy of diagnostic tests, and determine the confidence limit, rate of false positive and false negative results; 4) Culturing of the organism, while definitive when successful, is inefficient and can lead to a high rate of false negatives under certain circumstances; 5) A potentially major source of error is related to sampling methods, not necessarily the diagnostic tests used.

Much remains to be done and the program has room for improvement and/or modification. APHIS is in the process of finalizing the validation of a Real-time PCR assay for *P. ramorum*. This test is based on the test currently used in the United Kingdom and developed by the Central Science Laboratory (York, U.K.). It is robust and sensitive, and has higher throughput than current APHIS methods. However, it is still based on an ITS region. Work is underway in a highly collaborative effort involving several United States, Canadian and United Kingdom laboratories to identify the next test that will enter the validation process. Work will also be initiated to determine the effects of fungicide treatments on *P. ramorum* detectibility and to determine the correlation between ELISA, culture, and PCR detection. Finally, efforts are underway to approve laboratories external to APHIS on a provisional basis to

perform the validated diagnostic tests. The process involves inspection of laboratory facilities, determining that the laboratories seeking approval have appropriate instrumentation and training, and ensuring the proficiency of these laboratories using blind test samples. (APHIS must also pass the proficiency panel.) The system that has been developed is very similar to analogous programs used by the National Animal Health Laboratory Network. It is hoped that this program will increase national capacity for *P. ramorum* testing and decrease the time needed to process samples. The goal is to have as many as 10-15 laboratories approved by March 2005.

Table 1. *Summary of the Federal Phytophthora ramorum diagnostic system.*

Culture		Nested PCR	Action
+	Or	+	Yes
-	And	-	No
Not required	n/a	-	No
Not required	n/a	+	Yes
-	And	+	Yes
+	n/a	(optional)	Yes

Update on European Union and United Kingdom Legislation for *Phytophthora ramorum*[1]

Stephen Hunter[2]

Abstract

Legislative action at both the European Union (E.U.) and United Kingdom (U.K.) national level has been taken in response to outbreaks of *Phytophthora ramorum* and, more recently, *Phytophthora kernoviae*. Measures are aimed at identifying and preventing the movement of infected nursery stock and the containment and eradication of outbreaks at nurseries or in established plantings such as historic gardens and public parks. In addition, legislation relating to a *Phytophthora kernoviae* Management Zone has been introduced in the U.K.

Key words: Phytopthora ramorum, kernoviae, E.U. Plant Health Directive

Legislative background

Most European Union quarantine plant health controls flow from the E.U. Plant Health Directive (2000/29) which is primarily aimed at facilitating the free flow of plants and plant products within the E.U. without spreading harmful organisms (the 'Single Market'). This legislation allows E.U. Member States to introduce emergency measures but these then have to be reviewed by the Plant Health Standing Committee (PHSC).

In the U.K. implementation of these measures is through the Plant Health Act 1967. Statutory Instruments are approved by the U.K. Parliament for England and by the devolved Government arrangements in Scotland, Wales and Northern Ireland.

Chronology of legislative actions

Following the first confirmation of *Phytophthora ramorum* on a nursery in April 2002 the U.K. introduced emergency legislation in order to control imports of susceptible material from the United States and to require notification of susceptible material being moved within the U.K. Action to destroy or hold material found to be infected was taken under existing Plant Health Act powers. After discussion in the PHSC, E.U. legislation was introduced through

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Commission Decision 2002/757 of 19 September 2002. This extended controls throughout the E.U. on susceptible material imported from the U.S. and introduced a plant passport regime for movement of *Rhododendron* and *Viburnum* spp. within the E.U. This regime includes requirements (relating to inspections and eradication/quarantine procedures at the place of production) that have to be fulfilled before material can be moved. There also was a request for Member States to undertake official surveys.

What has happened since?

In December 2003 the PHSC reviewed the 2002 Decision and decided that controls needed to remain in place. The Decision was amended with *Camellia* spp. being brought within the passporting regime. Also foliage was dropped from the regime. In April 2004 ten new Member States joined the E.U. and formally came within the *Phytophthora ramorum* controls. At the December 2004 PHSC it was agreed to retain the existing E.U. controls without change.

What has this meant in practice in the UK?

The introduction of the plant passporting regime has been bolstered by increased levels of inspections at production nurseries and ports of entry into the U.K. This has been aimed at ensuring more rapid detection of any outbreaks on nurseries and reducing the chances of infected material being brought into the country. A substantial survey of woodland and other established plantings (for example historic gardens and public parks) has been undertaken to try and locate *Phytophthora ramorum* infections in the wider environment. Infected material at all sites is destroyed. This includes both places of production as required under E.U. legislation but also at other sites under national legislation. At some historic garden sites, particularly in Cornwall this has involved clearance of substantial areas of wild *Rhododendron* aided by grants under existing Forestry Commission schemes for woodland management.

The U.K. Government has also offered £200,000 towards a hardship fund to be operated by the industry. This is subject to matching funding by the industry and is aimed at those most seriously affected by the *Phytophthora ramorum* controls.

The overall thrust of the U.K. actions, which is supported by a significant scientific programme, is to contain outbreaks and to attempt to eradicate the disease. The Government is currently considering what the nature of the containment and eradication programme should be over the next two years.

Phytophthora kernoviae

The U.K. Government has also instigated containment and eradication action in relation to outbreaks of a new species, *Phytophthora kernoviae,* which is threatening native woodlands.

So far, there has only been one outbreak in a nursery (now eradicated) with the remaining outbreaks at sites in Cornwall and the Swansea area.

In response to the threat of this pathogen a *Phytophthora kernoviae* Management Zone has been created to cover the main outbreak area near Redruth in Cornwall. Access to carry out surveys, destroy infected plants, particularly wild *Rhododendron*, and the imposition of hygiene measures are covered by existing Plant Health Act powers. However, a new Statutory Instrument, The Plant Health (*Phytophthora kernoviae* Management Zone) (England) Order 2004 came into force on 21 December 2004. This provides the Plant Health authorities with powers to prohibit the removal of host plants from the Management Zone, to gain access to check compliance and to close 'rights of way' through infested areas. A Schedule to the Order sets out the geographic limits of the Management Zone.

Biology I

Phytophthora Species Associated With Forest Soils in Central and Eastern U.S. Oak Ecosystems[1]

Y. Balci[2], S. Balci[2], J. Eggers[2], W.L. MacDonald[2], K.W. Gottschalk[3], J. Juzwik[4], and R. Long[5]

Abstract

The existence of native and exotic species of *Phytophthora* in soils of eastern and central oak ecosystems is largely unknown. This informational void and the potential threat of *P. ramorum* to eastern oak species provided the impetus for a multiple state survey of soils associated with oak cover types. The initial survey was conducted from April to June 2004 in oak forests in Illinois, Indiana, Maryland, Michigan, Minnesota, Ohio, Pennsylvania, Wisconsin and West Virgina. Sampling sites were chosen to avoid areas impacted by oak wilt, recent storm damage or major defoliation. Although most stands contained a diverse community of tree species, oaks generally predominated. Stands were greater than 40 years of age and located on moist sites. Eight sites were usually sampled in each state. At each site, four soil samples were taken 1.5 m from the base of an oak tree in four cardinal directions. Sub-samples from each tree were bulked; five trees were sampled per site. As of January 2005, a total of 96 sites were surveyed and soils from 499 oak trees sampled. An oak leaf baiting procedure was used whereby soils from each tree were placed in a container, mixed and flooded with distilled water. Three- to seven-day-old *Quercus robur* leaflets were floated on the water surface to bait *Phytophthora*. Leaf samples that trapped *Phytophthora* were plated on PARPNH-medium. When initial isolations attempts failed, soils were dried at room temperature and the isolation procedure repeated. Twenty-three percent of the samples from individual trees yielded *Phytophthora*; *P. cinnamomi* was the most frequently recovered species (77 percent). Other species recovered included *P. europaea, P. cambivora, P. citricola* and yet undescribed species. A comparable survey was conducted during fall 2004 to establish a more complete assemblage of *Phytophthora* species so that studies of their role in forest health could be initiated.

Key words: oak decline, *Phytophthora cinnamomi, Phytophthora europaea, Phytophthora ramorum, Phytophthora* spp.

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Incidence of *Phytophthora* species in eastern oak forests

In total, 96 sites were surveyed and 499 oak trees sampled during spring 2004. *Phytophthora* species were isolated from 50 percent of the sites and recovered from 23 percent of the soil samples (*table 1*). During the spring sampling period at least seven species of *Phytophthora* were recovered with *P. cinnamomi* being the most frequently isolated species (76.5 percent). The second most common species was *P. citricola* (8.4 percent). Other species were less common and sporadically recovered. These include *P. europaea*, *P. cambivora* and two yet undescribed *Phytophthora* species. In prior oak forest surveys in Europe, 13 *Phytophthora* species were isolated, including five new species (Balci and Halmschlager 2003a,b; Delatour, 2003). Likewise, results of our spring survey suggest that various *Phytophthora* species exist in central and eastern oak forests, including several unknown species. Soil samples collected from Illinois, Minnesota, Michigan and Wisconsin gave fewer positive results. One reason for this discrepancy in isolation frequency could be due to the sandy soils associated with Lake State soils.

Isolation frequencies of *Phytophthora* spp. in relation to associated oak species

Soils yielding *Phytophthora* species were taken from the base of 11 oak species. Only soils from *Quercus falcata and Q. stellata* failed to yield any species of *Phytophthora*. To our knowledge *Q. coccinea*, *Q. muehlenbergii*, *Q. ellipsoidalis*, *Q. macrocarpa* and *Q. platanoides* are new host associations for *P. cinnamomi*. *Q. alba*, *Q. rubra* and *Q. velutina* are new associations for *P. europaea*.

The frequent recovery and widespread distribution of *P. cinnamomi* in hardwood forest soils within the survey areas has not been previously reported. The absence of *P. cinnamomi* in Illinois, Minnesota, Michigan and Wisconsin might suggest a climatic limitation to its survival in soils in colder regions or nonconducive soil types.

Table 1—The isolation frequencies of *Phytophthora* spp. in oak forest sites and soils from base of oak trees during spring 2004.

State	Sites	Positive sites	Total soil samples	Positive soil samples	*Phytophthora* spp.
Illinois	10	1	50	1	*P. citricola*
Indiana	8	6	40	20	*P. cinnamomi*
Maryland	12	6	62	14	*P. cinnamomi*, *P.* sp2
Michigan	6	0	30	0	-
Minnesota	8	2	40	3	*P. quercina 'like'*
Ohio	8	6	40	16	*P. cinnamomi*, *P. citricola*, *P. cambivora*, *P.* sp2
Pennsylvania	8	2	40	3	*P. cinnamomi*, *P. cambivora*, *P.* sp2
Wisconsin	8	3	40	3	*P. europaea*, *P. quercina'like'*, *P.* sp2
West Virginia	28	22	157	56	*P. cinnamomi*, *P. citricola*, *P. Europaea*, *P.* sp1, *P.* sp2
Total	**96**	**48 (50%)**	**499**	**116 (23%)**	

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The Effects of Girdling on the Ectomycorrhizal Fungal Community Associated With Tanoak (*Lithocarpus densiflorus*)[1]

Sarah Bergemann[2], Nicholas Kordesch [2], Matteo Garbelotto [2], and Timothy Metz[3]

Key words: ectomycorrhizae, *Phytophthora ramorum*, tanoak

Abstract

Phytophthora ramorum was identified as the lethal agent of tanoak (*Lithocarpus densiflorus*), black oak (*Quercus kelloggii*) and coast live oak (*Quercus agrifolia*) in central areas of California. Although the geographic origin remains unknown, its severe impact on tanoaks and *Quercus* species, its limited geographic range and the existence of a single clonal population in North America are indicative of a recently introduced pathogen. Pathogenic invasions of this magnitude are often recognized as serious threats to the maintenance of biodiversity affecting both structure and function of ecosystems. Although several studies aim to understand the epidemiology, pathogenicity and treatment of *P. ramorum*, the effects on the microbial community remain unknown. The objectives of this study were to simulate the formation of cankers formed by *P. ramorum* through physical girdling of trees and to measure the impacts on the ectomycorrhizal biomass in both roots and soil. A randomized block design was implemented with five blocks (60 x 60-m) divided into three (20 x 20-m) plots. Baseline sampling was performed to examine ectomycorrhizal composition and biomass prior to girdling. Trees were treated in one of the following manners: (1) gouged for the entire circumference to prevent translocation in phloem (fully girdled), (2) physically girdled half of the tree to reduce carbon flow (half girdled), or (3) left untreated as a control. Root tips were sampled 5, 9 and 13 months after treatment. Quantification of extracted DNA from tanoak roots and collected soil was completed using TaqMan chemistry. Primers and probes were designed to target particular guilds (*Cenococcum, Sebacina* and *Tricholoma*) that were encountered in community assays and exhibited a broad spectrum of reproductive and ecological characteristics. We found no evidence to support the loss of ectomycorrhizal biomass of specific guilds on roots; however, there was strong evidence to support significant reduction in biomass of *Cenococcum* and *Tricholoma* from soil. The ratio of pg of DNA to dry biomass was higher during drier sampling periods for *Cenococcum geophilum*, likely from imposed water stress. Given that microbial populations mediate decomposition and mineralization rates, it is not improbable to expect that the negative impacts on the ectomycorrhizal populations may influence nutrient balance in an ecosystem after invasion by *P. ramorum*.

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Phytophthora Species From Oak and Tanoak Forests in California and Oregon[1]

Everett Hansen[2], David Rizzo[3], and Matteo Garbelotto[4]

Key words: mixed-evergreen forests, *Phytophthora* spp.

Introduction

The current sudden oak death (SOD) epidemics in Europe and western North America triggered a search of North American oak forests for other *Phytophthora* species, and the results from the western United States have been surprising. *Phytophthora ramorum* has been the main quarry, and as an aerial pathogen, it is a surprise in itself. It isn't alone, however.

Results

Fifteen distinct taxa have been at least partially characterized from trees, soil or streams in oak forests in Oregon and California. Eleven are named species; four of these were described within the last five years. Four taxa await formal nomenclature, including the widespread and abundant aquatic species called "*P.g. chlamydo*." The number of *Phytophthora* species is mushrooming. Streams where SOD occurs have particularly rich *Phytophthora* flora. Seven of the 15 taxa, including *P. ramorum* and *P. nemorosa*, as well as the ubiquitous *P. gonapodyides* and *P. g. chlamydo*, have been recovered from streams. *P. cambivora* and the closely related *P. europaea* are occasionally recovered by baiting, and there are numerous unnamed isolates, including one group related to, but morphologically distinct from, *P. tropicalis*, and another closer to *P. capsici*. "Early detection" stream monitoring networks are in place for *P. ramorum* in both California and Oregon. The number of *Phytophthora* isolates of other species that these networks are yielding is overwhelming. We have developed an automated "single-strand-conformational-polymorphism" (SSCP) system, combining ITS and COX spacer primers to allow us to screen large numbers

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of isolates quickly against a suite of reference species (see Poster by Hansen et al., this Proceedings). Although we have only begun to work through our backlog of isolates, it is already clear that more species will be added to the list.

The most notorious forest *Phytophthora* species, *P. cinnamomi*, causes significant losses in forests around the world. It is present and destructive in ornamental plantings or orchards, especially in California, but is only associated with significant damage in forest plant communities in two locations. In Southern California, Matteo Garbelotto has reported it killing woodland coast live oak near infested avocado orchards, and killing manzanitas, including a rare species, adjacent to an infested Christmas tree plantation. In both cases DNA markers showed a close relationship between the forest isolates and the agricultural strains. *P. citricola, P. cambivora, P. syringae* and *P. cactorum* are also occasionally encountered in forest situations. These and other species dominate the *Phytophthora* flora on oaks in urban settings and in nurseries, yet remain rare in western forests despite repeated introductions. Clearly these common "agricultural" species of *Phytophthora* have requirements for aggressive pathogenicity that are not met in forest environments.

P. nemorosa was recently described from stem cankers on coast live oak and tanoak and leaves of various hosts. It resembles, and is closely related to, *P. ilicis* (present on holly within the SOD range). Symptoms and host range are similar to those of *P. ramorum*, although *P. nemorosa* appears to be a native forest pathogen. A third *Phytophthora* species is also present as a foliar and bark pathogen in western oak forests. *P. pseudosyringae* was first described from European oak forest soils, but is present and causes disease in western tanoak and coast live oak forests. It was first reported and is most abundant in California, but is present in Oregon as well. Other *Phytophthora* species have been isolated less frequently from leaves and stems of various SOD hosts, including *P. gonapodyides, P. syringae, P. europaea* and something akin to *P. capsici*. *P. ramorum* and *P. lateralis*, the invasive pathogen of Port-Orford-cedar, are closely related, and their ranges overlap in Oregon. *P. lateralis* also has the potential for aerial dispersal. The coincidence of two closely related species appearing as invasive exotics in the same area remains unexplained.

Discussion

The diverse array of Phytophthora species present in western forests raises a number of challenging questions. Most practically, it highlights the need for careful and complete diagnostics when working with symptomatic plant tissues. Can we distinguish indigenous from exotic species? What are these pathogens doing ecologically? What should be the regulatory consequences of these species? Why are

species known as soil inhabitants in Europe found as aerial pathogens here? Are western mixed-evergreen forest species unusually susceptible to species of Phytophthora in general? Does the moderate, Mediterranean climate favor foliar infection? These and many other questions remain.

Isolation and Characterization of Phytotoxins Secreted by *Phytophthora ramorum*[1]

Daniel K. Manter[2], Rick G. Kelsey[2], and Joseph J. Karchesy[3]

Abstract

Most *Phythophthora* species secrete a variety of small, hydrophilic proteins that induce a hypersensitive-like response to varying degrees in host and non-host plant species. Our research focuses on the potential role of these proteins in the biology and susceptibility of host species to sudden oak death (SOD). In this paper we reported on the purification and characterization of several proteins from the culture filtrates of *P. ramorum* media. Exposure of leaf discs from several host species (*Umbellularia californica* and *Rhododendron* spp.) to semi-purified protein fractions resulted in altered membrane integrity and alkalinization of a bathing solution. Such physiological changes are consistent with the hypersensitive-like response shown in other *Phytophthora* species, and appear to develop prior to any visual symptoms (e.g., wilting and/or necrosis). We have also conducted preliminary studies exploring leaf physiological responses distal to *P. ramorum* infection zones (i.e., stem inoculations). Similar to the protein assays, changes in membrane integrity and function may be observed in uninfected, non-symptomatic leaf tissues. The potential role of these phytotoxins in the development and impact of SOD was discussed.

Key words: Phytopthora ramorum, phytotoxins

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Incidence of *Phytophthora ramorum*, *P. nemorosa* and *P. pseudosyringae* in Three Coastal California Forest Communities[1]

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Key words: ecology, forest community, Generalized Additive Models, plant pathogen, *Phytophthora*, sudden oak death

Abstract

Phytophthora ramorum is well established over approximately 450 km of forests along the California coast. In the course of research on this emerging pathogen, two other aerial species of *Phytophthora*, *P. nemorosa* and *P. pseudosyringae*, were discovered. Little is known about the ecology and biology of these other species and how they interact with *P. ramorum*. Preliminary research has found that *P. nemorosa* and *P. pseudosyringae* have similar host and geographic ranges and cause similar disease symptoms as *P. ramorum*. However, *P. nemorosa* and *P. pseudosyringae* do not appear to cause landscape level mortality of oaks (*Quercus* spp.) or tanoak (*Lithocarpus densiflorus*), as does *P. ramorum*. These three species are virtually indistinguishable in the field and can only be identified microscopically.

A plot study was established to determine the distribution and incidence of *P. ramorum*, *P. nemorosa* and *P. pseudosyringae* in coastal forest communities, and to relate pathogen presence to community, structural and environmental variables. A total of 120 circular, 500-m^2 plots were established at ten sites within the known range of *P. ramorum* in Alameda, Contra Costa, Marin, Mendocino, Napa, Santa Cruz, and Sonoma counties. Field plots were installed during the summer months of 2001 and 2002 and re-sampled in the spring and summer of 2004. Six of the sites were in California state parks, three were in county or regional parks, and one was on private property. Three native forest communities were surveyed: coast redwood (redwood), and two associations of the mixed-evergreen forest, oak-bay-madrone (oak) and tanoak-madrone-live oak-Douglas-fir (tanoak) forests. Each of these forest communities contain numerous hosts of *P. ramorum* and at least two hosts of *P. nemorosa* and *P. pseudosyringae*. California bay laurel (*Umbellularia californica*) was the only plant species that occurred in all of the forest communities surveyed. Plots were located along elevational transects proceeding perpendicularly from river valleys toward ridge tops, with three independent plots along four transects at each site, placed in a stratified random design. Transects were selected to represent a variety of aspects and native plant community types, areas with minimal human disturbance, and randomly sampled for *Phytophthora* species. Each plot was evaluated for plant species composition,

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forest structure and environmental variables, and incidence of aerial *Phytophthora* species. A total of 5105 trees and shrubs were mapped and examined for *Phytophthora* species.

Based on the two sampling periods, *P. ramorum* was recovered from 29 percent of plots at seven sites, *P. nemorosa* from 9 percent of plots at four sites and *P. pseudosyringae* from 9 percent of plots at three sites (*table 1*). *P. ramorum* and *P. nemorosa* were found together at three sites, on eight plots, and were both isolated from the same California bay tree 16 times and from coast redwood (*Sequoia sempervirens*) one time. *P. ramorum* coexisted with *P. pseudosyringae* at two sites, on three plots, and with one co-occurrence on a California bay tree at Austin Creek State Park. *P. nemorosa* and *P. pseudosyringae* were not recovered together on any plots. All three *Phytophthora* species were detected at one site, Redwood East Bay Regional Park. At Big Basin State Park, no *Phytophthora* species were recovered in either sampling year. Interestingly, this is the only site where there was no California bay laurel present, from which we can postulate that bay is a key component to explain presence of the these *Phytophthora* species.

We found that the three *Phytophthora* species occupied similar ecological niches; they overlap in geographical range, forest community presence, and host range. A Pearson chi-square test analyzed associations of the pathogens with the forest communities. Each of the pathogens was positively associated with a specific community type: *P. ramorum* with the tanoak community, *P. nemorosa* with the redwood community, and *P. pseudosyringae* with the oak community. Although each *Phytophthora* species occurred more often with a particular forest community than would be expected if they were independent, it is important to recognize that both *P. ramorum* and *P. nemorosa* were found in all three forest types. These findings are consistent with observed sampling patterns by other studies (Davidson, J.M. unpublished data; Jensen, C.E. unpublished data; Wickland and Rizzo 2005; Maloney and others 2005).

Generalized Additive Modeling (GAM) was used to explore how ecological variables may contribute to pathogen presence in this study. As with previous studies, increased cover of bay was found to increase the probability of *P. ramorum* occurrence; a similar relationship with California bay laurel was noted for *P. nemorosa* and *P. pseudosyringae*. GAM substantiated the association analysis that predicted a high probability of *P. nemorosa* with the redwood forest type and *P. pseudosyringae* with the oak community. The model additionally predicts *P. ramorum* presence to be greatest at locations close to the coast, close to a river or creek, and in forests with high total basal area. There was limited recovery of *P. nemorosa* and *P. pseudosyringae* ($n<30$) so results for these pathogens are exploratory rather than statistically rigorous. However, the results are biologically plausible and consistent with observations made by other researchers.

Results from this study provide additional information about the distribution of *P. ramorum*, including examination of location and intensity of sudden oak death within state and regional parks, and within East Bay counties where *P. ramorum* plots had not previously been established. This is the first study to examine the ecological associations between these three *Phytophthora* species, across a wide geographic distribution and within several forest communities. Future research will continue to monitor spread of the pathogens within these plots, as well as further examine ecological relationships with these three pathogens using a larger data set.

Table 1- *Phytophthora species detection at all sites.*

Site	County	*Phytophthora* Species[1]		
		Pr	**Pn**	**Pps**
Boonville Property	Mendocino	+	-	-
Salt Point SP	Sonoma	-	+	-
Austin Creek SP	Sonoma	+	-	+
Armstrong Redwoods SP	Sonoma	+	-	-
Skyline Regional Park	Napa	+	-	-
Mt. Tamalpais SP	Marin	+	+	-
Briones EBRP	Contra Costa	+	-	+
Redwood EBRP	Alameda	+	+	+
Big Basin SP	Santa Cruz	-	-	-
Henry Cowell SP	Santa Cruz	+	+	-

[1]Pr= *Phytophthora ramorum*; Pn= *P. nemorosa*; Pps= *P. pseudosyringae*

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Ecology of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in Mixed-Evergreen Forests[1]

Allison C. Wickland[2] and David M. Rizzo[2]

Key words: mixed-evergreen forest, *Phytophthora nemorosa*, *Phytophthora pseudosyringae*, *Phytophthora ramorum*

Abstract

Recent research has shown that *Phytophthora ramorum* is a major threat to California's coast live oak (*Quercus agrifolia*) dominated, mixed-evergreen forests (Rizzo and Garbelotto 2003). However, the role that diseases caused by other *Phytophthora* species play in the ecology of these forest communities is less evident. Surveys in mixed-evergreen forests have revealed two additional *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*. In these forests, *P. nemorosa*, *P. pseudosyringae* and *P. ramorum* are all aerial, canker-causing pathogens with overlapping host and geographic ranges. Although all three pathogens seem to share a similar ecological niche, *P. nemorosa* and *P. pseudosyringae* are not associated with landscape level tree mortality. Additionally, the geographic distribution of *P. nemorosa* and *P. pseudosyringae* extends beyond the known distribution of *P. ramorum*. This study was initiated to learn more about the role of *P. nemorosa* and *P. pseudosyringae* in the ecology of mixed-evergreen forests and to gain an understanding of the dynamics of these forests prior to *P. ramorum* invasion. In this paper we address the incidence and mortality levels associated with *P. nemorosa* and *P. pseudosyringae* and describe biotic and abiotic variables associated with pathogen presence.

One hundred 500-m^2 circular plots were established in coast live oak and bay laurel (*Umbellularia californica*) dominated, mixed-evergreen forests outside of the known geographic distribution of *P. ramorum*. Plots were randomly distributed at each of eight sites including: Big Creek Reserve (BCR), Santa Lucia Preserve (SLP), and Hastings Reserve (HR) in Monterey Co.; Las Trampas Regional Wilderness (LTRW) and Briones Regional Park (BRP) in Contra Costa Co.; Redwood Regional Park (RRP) in Contra Costa and Alameda Cos.; and Angel Island State Park (AISP) and Point Reyes National Seashore (PRNS) in Marin Co. At each plot, data were collected on tree species, diameter, crown position, health and pests. Cover class of all woody and herbaceous species was recorded, as well as slope, aspect, elevation, distance to trail and waterway, and number of seedlings. Calculated and derived variables included basal area, importance value, diversity and various climate descriptors. Symptoms (e.g., leaf lesions, stem cankers) of *Phytophthora* infection were recorded. Samples of symptomatic tissues were returned to the laboratory for isolation and identification of the pathogen(s).

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A total of 3463 woody plants were surveyed, including 1219 coast live oak and 1408 bay laurel. *P. pseudosyringae* was a commonly isolated pathogen in these forests; it was recovered at six of the eight sites (BCR, SLP, LTRW, BRP, RRP and AISP). The pathogen was isolated from 118 bay laurel trees in 40 of the 100 plots; disease incidence on bay laurel in these plots averaged 26.9 percent. Cankers caused by *P. pseudosyringae* were found on eight coast live oaks in seven plots (a 1.7 percent infection level in plots with *P. pseudosyringae* present). Three of the plots with *P. pseudosyringae* infected coast live oaks did not have infected bay laurel trees. *P. pseudosyringae* was never found infecting tanoak (*Lithocarpus densiflorus)*. *P. nemorosa* was recovered at four of the eight sites (BCR, RRP, AISP and PRNS) and found on bay laurel in 22 plots; disease incidence on bay laurel in these plots averaged 59.1 percent. *P. nemorosa* was isolated twice from tanoak twigs and never from coast live oak. *P. ramorum* was recovered in nine of the 100 plots. These appeared to be new infection sites; there was an average cumulative oak mortality of 7.7 percent in these plots, while the other 91 plots had an average of 6.7 percent. Of the 100 plots, 11 plots had more than one *Phytophthora* species present and eight of these plots had more than one *Phytophthora* on the same tree.

The mean cumulative mortality of coast live oak in all 100 plots was 6.8 percent. To assess whether *P. pseudosyringae* was causing high levels of unidentified mortality in the plots, the mean cumulative mortality in the 43 plots with *P. pseudosyringae* infection on any host (8.9 percent) was compared to the mean cumulative mortality in the 57 plots with no infection (5.1 percent). These two means were not significantly different (Wilcoxon rank sum test, n=100, P=0.06). The low incidence and mortality of coast live oak and tanoak attributed to both *P. pseudosyringae* and *P. nemorosa* suggests that although these are common foliar pathogens of mixed-evergreen forests, at this time they are causing low levels of change and are essentially background mortality agents.

Multiple logistic regression was used to determine the environmental, compositional and structural variables that contribute to the likelihood of disease presence in a plot. *P. pseudosyringae* presence in a plot was found to be positively correlated with increased slope (P=0.003) and increased bay laurel basal area per ha (P=0.013) and negatively correlated with woody species diversity of the plot (P<0.0001) and average annual precipitation (P=0.0019). *P. nemorosa* presence in a plot was positively correlated with bay laurel basal area per ha (P=0.006) and annual average precipitation (P=0.003) and negatively correlated with elevation of the plot (P<0.0001).

The observed positive density dependence between both *P. nemorosa* and *P. pseudosyringae* and bay laurel is presumably a result of the increasing target for airborne inoculum with increasing bay laurel basal area. A similar positive association with bay laurel has been observed with *P. ramorum* (Kelly and Meentemeyer 2002, Swiecki and Bernhardt 2002, Murphy and Rizzo, this volume). Although numerous plant species were sampled in this study, bay laurel was overwhelmingly found to be the dominant foliar host of both *P. nemorosa* and *P. pseudosyringae.* As is the case with *P. ramorum* (Davidson and others., this volume), bay laurel could be the driver of *P. nemorosa* and *P. pseudosyringae* spread and survival in coastal forests by acting as the major reservoir and supplier of inoculum.

P. pseudosyringae occurrence in a plot was also strongly associated with lower diversity forests. The plots in which *P. pseudosyringae* was most commonly found were a somewhat reduced phase of the

mixed-evergreen forest in which coast live oak and bay laurel were dominant and other species, such as madrone (*Arbutus menziesii*), toyon (*Heteromeles arbutifolia*), big leaf maple (*Acer macrophyllum*) and tanoak, were uncommon. These other species, on which *P. pseudosyringae* infection was never found, may act as a barrier to the movement of spores. If this is the case, then the less bay laurel's presence is diluted by other species in the forest, or the less diverse the forest is, then the higher the likelihood of disease presence. Because of its wide host range the opposite may be true for *P. ramorum*; more diverse forests may increase the likelihood of disease occurrence (Rizzo and Garbelotto 2003).

The logistic models indicated that *P. nemorosa* was associated with higher annual average precipitation and lower elevation plots, while *P. pseudosyringae* was associated with lower annual average precipitation plots. The current broad-scale distribution of *P. nemorosa* and *P. pseudosyringae* support these results; *P. nemorosa* is more commonly found in the more mesic near-coast forests while *P. pseudosyringae* is predominantly recovered in more inland areas that receive less rain (Jensen and others., this volume).

These plots are part of a larger network established to evaluate the effects of disease on various coastal California forest types. Further research and analysis based on the combined information from all plots will help to strengthen our overall understanding of the role of native and exotic pathogens in forest communities.

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Susceptibility of Conifer Shoots to Infection by *Phytophthora ramorum*[1]

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Key words: conifers, *Phytophthora ramorum*

Abstract

Phytophthora ramorum is the pathogen that causes sudden oak death, which was first detected on tanoak in Marin County, California in 1995. The identification of several conifers as hosts of *P. ramorum* and the increased spread of this pathogen via shipment of ornamental nursery stock has the potential to severely impact the Christmas tree, conifer nursery and forestry industries if this pathogen spreads into major production areas. Four conifers (Douglas-fir, grand fir, yew and coast redwood) are among the naturally infected hosts that have been reported for this pathogen. There are large numbers of different types of conifers grown as Christmas trees and in conifer nurseries in the Pacific Northwest. To better understand the potential impact that this pathogen might have on the Christmas tree, conifer nursery and specialty forest products industries, a series of inoculation studies was conducted to determine the susceptibility of foliage and shoots from 25 conifers to *P. ramorum*. Growth stage and inoculum concentration had a significant effect on the susceptibility Douglas-fir. Needles on emerging shoots from dormant seedlings were only susceptible to infection when inoculated just after bud break. However, needles on actively growing terminals and side shoots of container-grown nursery seedlings were susceptible throughout their growing season. No disease developed when Douglas-fir and noble fir seedlings were inoculated with A1 or A2 mating types at inoculum concentrations below 10^4 zoospores per ml. Twenty of the conifers tested, including many of the important species that are used as Christmas trees, were susceptible to A2 mating types of *P. ramorum*. Symptoms included needle blight, a shoot blight resulting from needle infections, and stem dieback resulting from the growth of the pathogen from infected shoots into the stem. Significant shoot blight occurred on some *Abies* species. Our research indicates that a number of conifers are potentially susceptible to *P. ramorum*. In addition, host phenology and inoculum concentration are important factors affecting infection of conifers by *P. ramorum.*

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Susceptibility Levels of *Rhododendron* Species and Hybrids to *Phytophthora ramorum*[1]

Isabelle De Dobbelaere[2], Kurt Heungens[2,3], and Martine Maes[2]

Abstract

Until now there has been little scientific information available about the susceptibility of different *Rhododendron* species and cultivars to *Phytophthora ramorum*. However, growers could use this knowledge as part of their control strategy. In our susceptibility screening we first optimized different inoculation methods on stem and leaf material. Four methods were chosen for the actual screening: two on detached leaves and two on detached stems, each time involving either wounded or non-wounded tissues. Finally, 63 *Rhododendron* species and hybrids were screened and the correlation between the different inoculation methods was investigated. Inoculation methods involving wounding revealed limited differences in susceptibility between the species and hybrids. However, inoculation methods on non-wounded tissues showed a larger degree of variation in susceptibility. This suggests that when some form of resistance is present, it probably takes place at the level of tissue penetration.

Key words: host resistance, inoculation method, penetration

Introduction

The most important hosts of *Phytophthora ramorum* in Europe are cultivated *Rhododendron* species and hybrids. Since November 2002, EU emergency phytosanitary measures have been taken to prevent the introduction and spread of *P. ramorum* in Europe. Surveys at all commercial *Rhododendron*-growing premises are part of these measures and have led to *P. ramorum* findings at 2 to 5 percent of the inspected sites in several European countries. An eradication and quarantine program was initiated at these premises in case of positive findings, resulting in serious commercial losses for the grower. Belgium is one of the larger *Rhododendron*-growing areas in Europe, and growers are taking all possible preventive measures to

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avoid *P. ramorum* infestations at their site. These measures mainly consist of sanitation, limiting the leaf wetness period, avoiding plant injuries, and preventive fungicide treatments. Using host resistance would be another possible tool, for which there is high interest among growers. The objective of this study was to provide scientific knowledge about the relative susceptibility of several commercially important *Rhododendron* cultivars, as well as several botanical species.

Materials and methods

A total of 21 botanical *Rhododendron* species and 42 *Rhododendron* hybrids were screened for their susceptibility to *P. ramorum*. Species were selected to represent the main subdivisions within the genus *Rhododendron*. Hybrids were selected based on their economic importance. Two *P. ramorum* isolates from *Rhododendron* and two from *Viburnum* were tested on a few *Rhododendron* cultivars to check for strain effects. Different inoculation methods were tested on detached leaves and stems and evaluated based on consistency and expected relevance. Some methods were based on those described by Tooley and others. (2004). Timing and evaluation system were optimized for each inoculation method.

In the main screening experiments, four inoculation methods were used on either wounded or non-wounded leaves or stems. The methods involving non-wounded tissue were used to estimate the ability of the hosts to resist tissue penetration. The methods involving wounded tissue were geared at evaluating the resistance to pathogen growth inside leaf and stem tissue. Leaf and stem materials were as uniform as possible and less than 1 year old. Assays were performed in nine batches from June to September, each time including a standard cultivar as the internal control.

Results and Discussion

Preliminary tests using four different *P. ramorum* isolates did not indicate significant pathological variation between the four *P. ramorum* isolates. Therefore, the main tests were performed using one isolate. All cultivars and species were susceptible to some extent when wounded leaf or wounded stem tissue was inoculated. Limited differences were observed in lesion size between most cultivars and species. In contrast, inoculation of non-wounded leaves and stems did not result in symptoms on some cultivars and species. These data suggest that if resistance is present, it is most likely taking place at the level of tissue penetration. Time of year and age of plant material did not affect the results with most assays except for the one involving non-wounded leaves. There was a statistically

significant, but low, correlation between the inoculation methods. We concluded that multiple inoculation methods can be useful in these susceptibility screens as they can reveal different aspects of resistance.

This research has differentiated *Rhododendron* species and hybrids in disease susceptibility classes and can contribute to further nursery management and control strategies. Long-term application of the data may involve breeding for disease resistance.

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Variation in Phenotype for Resistance to *Phytophthora ramorum* in a Range of Species and Cultivars of the Genus *Viburnum*[1]

Niklaus J. Grunwald[2], E. Anne Davis[2], and Robert G. Linderman[2]

Key words: Phytophthora ramorum, resistance, sudden oak death, *Viburnum*

Introduction

Phytophthora ramorum is a recently introduced plant pathogen causing a range of diseases including sudden oak death, Ramorum shoot dieback and Ramorum blight (Rizzo and others 2002, 2004; Werres and others 2001). *P. ramorum* also attacks several nursery crops including viburnum and rhododendron (Werres and others 2001). Since its discovery *P. ramorum* has been shown to have a remarkably wide host range including many nursery crops (Parke and others 2002; Linderman and others 2002). It is well established that the ornamental plant *Viburnum plicatum* var. *tomentosum* cv. Mariesii and other *Viburnum* species or cultivars are highly susceptible to *P. ramorum*. What is not known is whether all cultivars in the genus *Viburnum* are equally susceptible, whether they are field- or container-grown. The objective of our research was to evaluate nine species of field-grown *Viburnum* for resistance to *P. ramorum*.

Materials and Methods

We evaluated nine species of field-grown *Viburnum* including *V. burkwoodii*, *V. dentatum*, *V. lantana*, *V. opulus*, *V. plicatum*, *V. lentago*, *V. nudum*, *V. sargentii*, and *V. trilobum* for a total of 23 cultivars for resistance to *P. ramorum* in detached leaf tests. Foliage of *Viburnum* was obtained from the Carlton Plants Nursery on September 20, 2004. Detached leaves were wound-inoculated with 6-mm agar plugs of 1-week-old colonies of *P. ramorum* using strain 4123 (isolated from *Rhododendron macrophyllum*, the predominant U.S. genotype of mating type A2) and strain 03-74-D12-A (isolated from *V. plicatum* var. *tomentosum* Mariesii the European genotype of mating type A1) grown on dilute V-8 agar. Four mycelial agar plugs were used to inoculate each of three leaves in each cultivar. Another leaf was inoculated as a control with four plain agar plugs. Leaves were incubated in moist chambers at 20 ºC for 8 days before measurements were taken. Lesion area was determined as the percentage of the total leaf area using the Assess program (APS, St. Paul, MN).

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[1]A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California.
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Results and Discussion

We obtained significant differences for levels of resistance based on percentages of leaf areas affected ($P < 0.001$) and no significant differences for isolates and interactions between isolates and cultivars. The percentages of lesion areas affected ranged from 95 percent (cvs. *V. burkwoodii* cv. unkown, *V. plicatum* var. *tomentosum* cv. Mariesii, and *V. trilobum* cvs. Alfredo and Bailey), to intermediate responses between 25 to 90 percent (cvs. *V. burkwoodii* cv. Mohawk, *V. lantana* cv. Mohican, *V. opulus* cvs. Compacta and Hanum, *V. lentago* cv. unkown, *V. sargentii* cv. Onandaga, *V. trilobum* cv. Redwing), to less than 15 percent infection (*V. dentatum* cvs. Autumn Jazz, Blue Muffin, Chicago Lustre, and Burgundy; *V. opulus* cv. Sterile, *V. plicatum* cv. Newport, Popcorn, Shasta, and Shoshon; *V. nudum* cv. Winterthur, *V. trilobum* cv. Wentworth). Our data indicate that there is a considerable range of resistance among phenotypes in the genus *Viburnum* from high susceptibility to resistance.

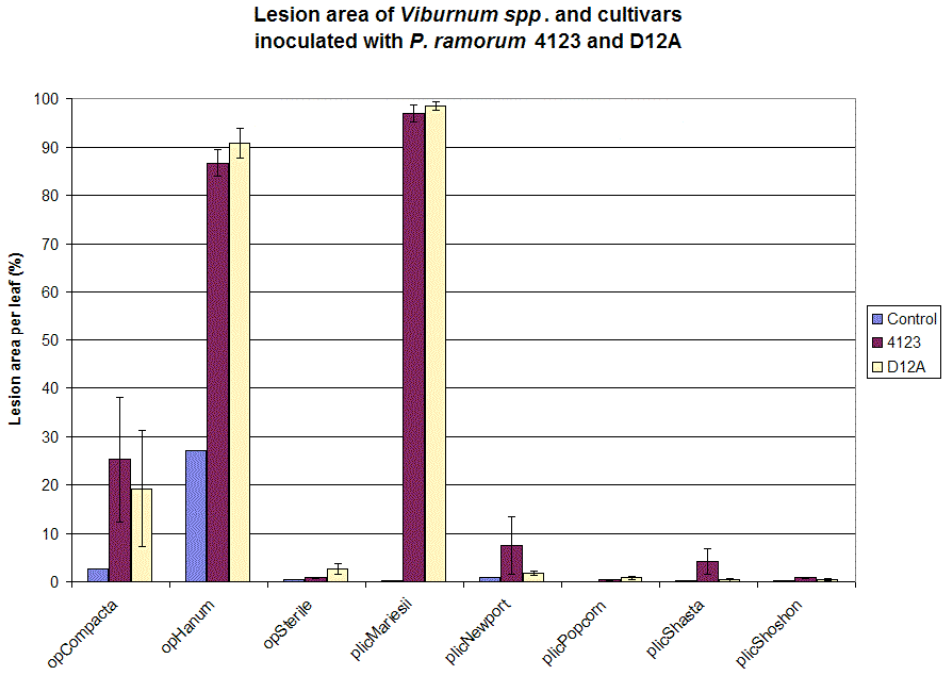

Figure 1 — Disease severity for 8 of the 23 cultivars of *Viburnum* tested for resistance to *Phytophthora ramorum* in detached leaf test assays.

Acknowledgements

We thank Carlton Plants LLC for the *Viburnum* plant material.

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Variation in Susceptibility of *Umbellularia californica* (Bay Laurel) to *Phytophthora ramorum*[1]

Matthew Meshriy[2], Daniel Hüberli[2], Tamar Harnik[2], Lori Miles[2], Keefe Reuther[2], and Matteo Garbelotto[2]

Abstract

Bay laurel (*Umbellularia californica*) is an important foliar host in terms of spore production and transmission of disease. We designed a bioassay to screen for variation in susceptibility to *Phytophthora ramorum* among populations of bay laurel collected along the coast of California to southern Oregon and also from Yosemite. Mature leaves of bay laurel were inoculated with zoospores and nine days later lesions and infection percentage were measured. Results indicated that each population comprises trees of different susceptibility. Data from three separate trials indicated that trees from Oregon were less susceptible than those from California. There was little variation among populations from California. Seasonal variation in susceptibility of bay laurel to *P. ramorum* was observed among populations. It is hypothesized that the interaction of a number of factors are responsible for the observed variation in susceptibility including host resistance, genetic structure of host species in the forests, pathogen variation, and environmental conditions.

Key words: foliar lesion, infection, inoculation, *Phytophthora ramorum*, resistance, susceptibility

Introduction

The coexistence of a number of different hosts in California forests allows *Phytophthora ramorum* to become established. In particular, severe stem cankers on coast live oak (*Quercus agrifolia*) are strongly associated with heavily infested leaves of adjacent bay laurel (*Umbellularia californica*) (Kelly and Meentemeyer 2002, Swiecki and Bernhardt 2002) and, in fact, foliar infections of bay laurel generally precede the infection of oaks (Rizzo and Garbelotto 2003).

Bay laurel is one of the most important epidemiological hosts for *P. ramorum* in California by serving as a reservoir of inoculum (Davidson and others 2002). In Oregon, on the other hand, only one infected bay laurel tree has been found (Hansen and others 2005). Why is there this difference in susceptibility between bay laurel from California and Oregon? Preliminary field and laboratory experiments have indicated that bay laurel plants have varying levels of

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susceptibility to *P. ramorum* (Rizzo and others 2002, Garbelotto and others 2003). In this paper, we investigated the susceptibility of various populations of bay laurel along the coast of California to southern Oregon to *P. ramorum*. We also investigated the seasonal variation in susceptibility within a California population of bay laurel. This knowledge is extremely important in order to develop a full understanding of the epidemiology of sudden oak death in California and Oregon.

Methods

Mature leaves from 15 bay laurel trees along transects of 18 mixed forest populations in Oregon and in California were sampled. Three to six populations were sampled in each of seven separate trials conducted from November 2003 to September 2004. In the laboratory, detached leaves were inoculated with zoospores of *P. ramorum* (isolate Pr-52) similar to the protocol outlined in Hüberli and others (2003) with the modification that detached leaves were placed into 50-ml tubes, tip-side down, and 300µl of zoospores ($2x10^4$ zoospores/ml) added to the tubes. A total of 20 leaves per tree were inoculated. After incubation at 20 ºC overnight, leaves were placed into moist chambers and incubated for 9 days at 20 ºC. Lesion area was determined using Assess (Version 1.01, APS Press, St. Paul, MN). Sections of the lesions were plated onto P_{10}ARP, a *Phytophthora*-selective agar medium modified with 25 mg of pentachloronitrobenzene (PCNB) to confirm the presence of *P. ramorum* (Rizzo and others 2002).

Results and Discussion

Bay laurel populations were significantly different ($P < 0.05$) in their susceptibility to *P. ramorum*. Within all populations sampled in California and Oregon, consistently less susceptible individuals were found. Bay laurel populations from Oregon had significantly ($P < 0.05$) smaller lesions than those from California (*fig. 1*). Seasonal variation in the susceptibility of bay laurel trees within populations was observed over a period of roughly one year, with maximum susceptibility occurring during the months of March and April. We will be conducting common garden studies of seedlings from Oregon and California, as well as molecular tests, to determine the genetic variation of bay laurel populations.

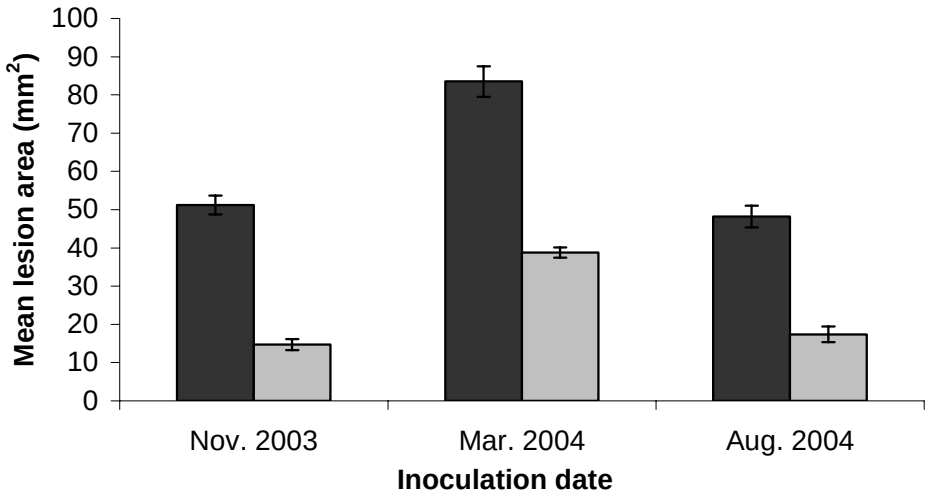

Figure 1—Mean leaf lesion area (± standard error) of *Umbellularia californica* (bay laurel) trees from Marin County, California (■) and Curry County, Oregon (□) after inoculation with *Phytophthora ramorum*. (Error bars represent standard error)

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Magnetic Resonance Imaging (MRI) of Oak Trees Infected With *Phytophthora ramorum* to Determine Potential Avenues of Infection in Bark[1]

Edwin R. Florance[2]

Abstract

Non-destructive magnetic resonance imaging (MRI) revealed pathological anatomical features of coast live oak trees (*Quercus agrifolia*) that were naturally infected with *Phytophthora ramorum*. Fresh excised whole slices showing typical macroscopic cankers and bleeding were examined. Infected areas (i.e. cankers) were compared to presumed healthy sections. Various infected tissues were revealed and the depth of infection into the xylem could be estimated. Discontinuous distribution of water in the outer layer of sapwood was observed and high water concentrations appeared in the cankers. MRI also revealed channels in the periderm (bark) with high water concentration. Microscopic examination revealed the channels to be rays continuous with the rays extending into the xylem. The rays function in the radial conduction of water, and it is suggested that they may serve as an avenue of infection for *P. ramorum*.

Key words: magnetic resonance imaging, microscopy, periderm, *Phytophthora ramorum,*

Introduction

A major question regarding the biology of *Phytophthora ramorum* is what plant structures does it utilize to establish infection? Recently hyphae of *P. ramorum* have been identified growing in lenticels of naturally field-infected material and in stomata of both field- and experimentally infected plant species (Florance 2002) (*figs. 1 & 2*). Lenticels are present in young growth, i.e. 1- to 5-year-old twigs. In deciduous plant species stomata are only present during certain parts of the growing season. However, it is known that infections occur in older stems and in very old tree trunks where *P. ramorum* causes cankers and bleeding. As a result, it is not known whether other avenues that can be utilized by *P. ramorum* to establish infections. The following questions need to be answered: 1) What structures are available as avenues for infection during the periods of the year when stomata and/or lenticels are not available? and 2) What structures could be utilized

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to establish infections in older trees, e.g., trees more than 10 years old or older? Earlier attempts to investigate the bark (periderm) of *Lithocarpus densiflorus* and *Quercus spp.* infected with *P. ramorum* using the techniques of histopathology combined with scanning electron microscopy (SEM) provided limited data (Florance 2002). Therefore, other techniques were evaluated. It was determined that high resolution Magnetic Resonance Imaging (MRI) would be a valuable technology that could expand understanding of diseased oak trees (MacFall and others 1994). This recently developed technology is utilized mostly in areas related to human medicine. It has not been generally applied in areas of plant pathology (Kramer and others 1990). However, it is highly effective, and lately has allowed for the analysis of internal wood and bark structure (Hall and others 1986; Wang and Chang 1986; Hailey and Swanson 1987; Chang and others 1989; Olson and others 1990; Chang and others 1991; Coates 1998; Contreras and others 2002). The major advantage of using MRI is that it is non-destructive. It can also provide both anatomical and functional data about the infected host (MacFall and others 1994). However, a limitation is that resolutions are in the range of approximately 100μm to 200μm. Since the anatomical structures produced by *P. ramorum* are smaller (e.g., hyphae are 5μm to 8μm in diameter), individual structures cannot be resolved. Therefore, SEM, which achieves resolutions of approximately 200 to 300Å, combined with MRI, can provide a more complete understanding of *P. ramorum* in infected oak trees.

MRI is an interaction between an external magnetic field, radio waves, and hydrogen nuclei in the tissues being examined. Because it detects hydrogen nuclei, and water is the most abundant molecule in biological material, image contrast is achieved by comparing areas high in water concentration to areas of lower concentration. The brightest areas in an MRI are areas of greatest water concentration. Preliminary data indicate: (1) high water concentration in the areas where cankers occur, (2) disruption of the outer most water conducting xylem, and (3) channels in the bark through which fungi and other organisms may infect the tree.

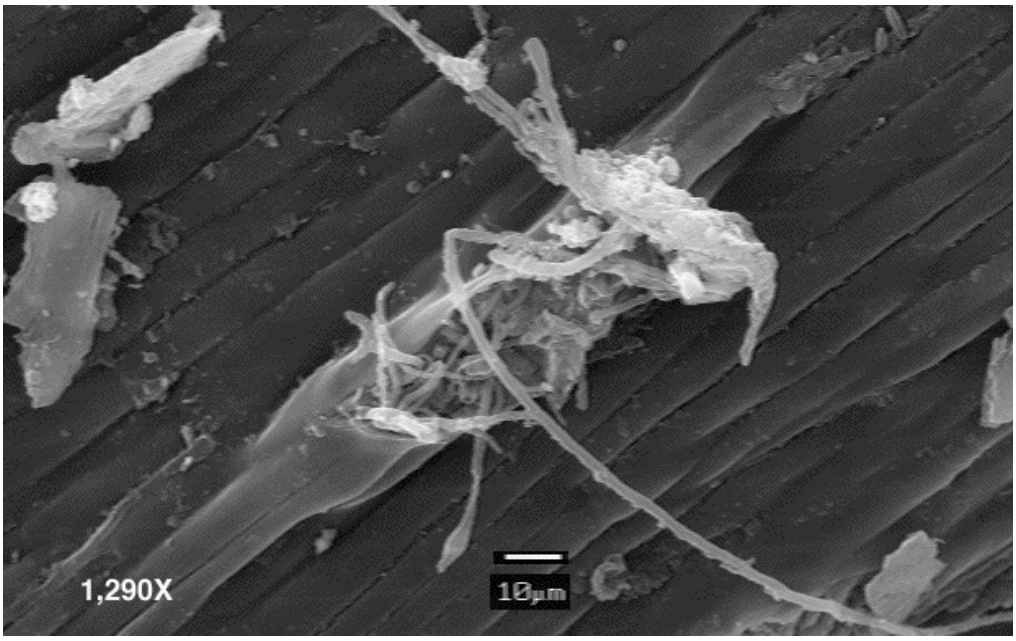

Figure 1– Hyphae of *Phytophthora ramorum* growing in a lenticel of tanoak, *Lithocarpus densiflorus*. Infection was positively identified via PCR.

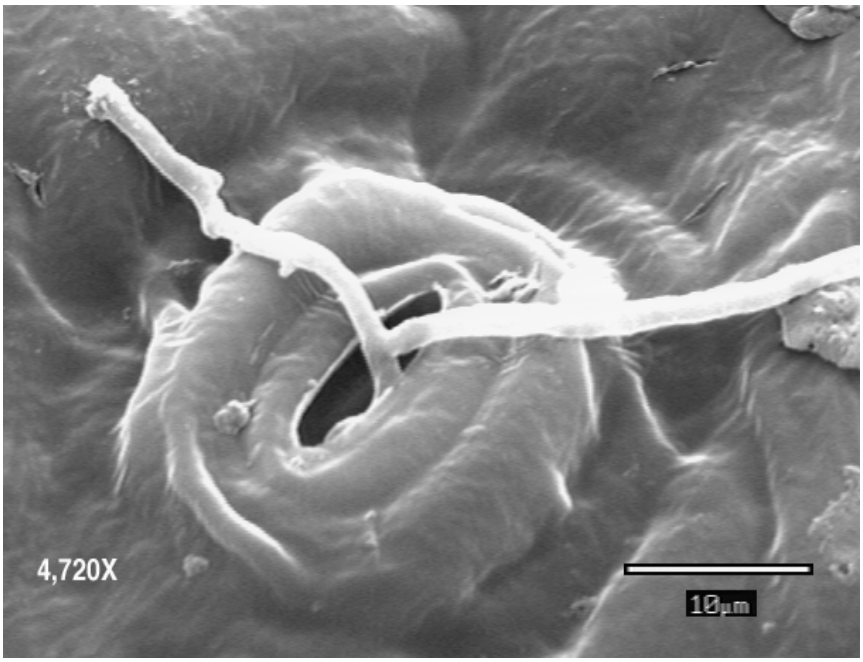

Figure 2– Hyphae growing in a stoma of infected *Umbellularia californica* (California bay laurel). The tree was naturally infected but demonstrated to be positive for *P. ramorum* via PCR.

Materials and Methods

Slices from *P. ramorum*-infected *Quercus agrifolia* were provided by Jennifer Davidson (Pacific Southwest Research Station, USDA Forest Service and the University of California, Davis) for cytological and MRI data acquisition. *Figure 3* shows an example of an infected tree cross section used as one of the study samples.

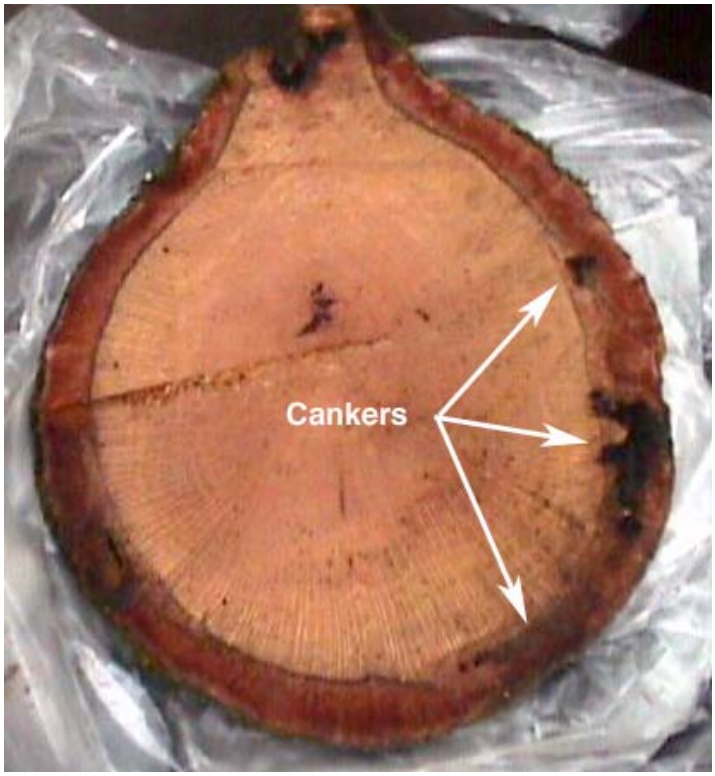

Figure 3– Slice from a *P. ramorum*-infected coast live oak tree (*Quercus agrifolia*). Note the infected areas in the bark (cankers).

The dark colored areas in the bark are the infected sites referred to as cankers. Collected material was prepared immediately for cytological examination. Bark cubes, 5 mm per side, were removed and fixed directly in 4 percent phosphate buffered glutaraldehyde (pH 7.0 at 4°C) for 2 hours. After fixation, all samples were washed thoroughly with the buffer and post-fixed in 1 percent osmium tetroxide. Dehydration was achieved through an ascending series of ethanol solutions (EtOH/Distilled H_20), 30 percent, 50 percent, 70 percent, 90 percent, and 100 percent ethanol. Samples were soaked three times for 10 minutes in each solution After dehydration the samples were prepared for critical point drying by passing them through an ascending series of ethanol/amyl acetate solutions, 30 percent, 50 percent, 70 percent, 90 percent, and 100 percent

amyl acetate. Again, the samples were given three 10-minute soaks in each solution. Samples were transferred immediately to a critical point drier using liquid CO_2 as the transition fluid. Analysis was done using either an AMRAY 1810T scanning electron microscope or a Zeiss Axiostar light microscope. Data were captured digitally and measurements were made using NIH image software. Because MRI does not require fixation procedures, cookies were maintained in a fresh condition, placed in the requisite magnetic coil, and inserted into the MRI chamber for data acquisition.

Results

MRI Data

The outer layer of xylem cells (i.e., the youngest cells, bright band) are filled with water (*fig. 4*). The water concentration in the xylem is disrupted around the upper left circumference of the tree where the cankers exist (*fig. 4*). At the site of a canker, water concentration increases significantly. Water channels in the bark that are continuous with the water conducting xylem are revealed (*figs. 4 & 5*). The channels correspond to rays observed with scanning electron microscopy (*fig. 7*).

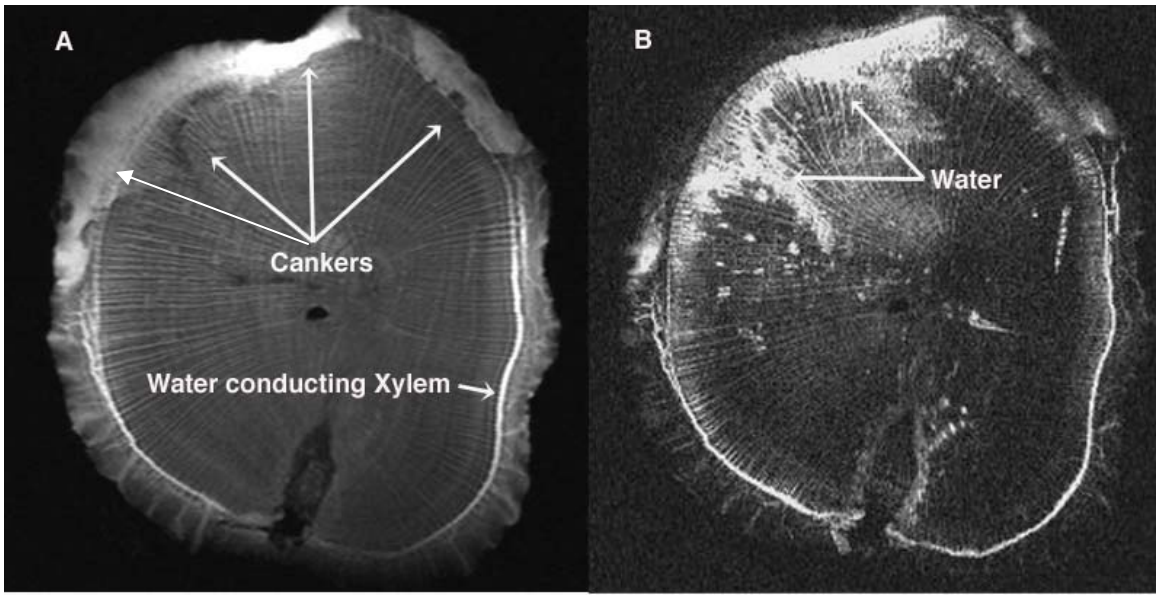

Figure 4 - MRIs of infected *Quercus agrifolia* showing the cankers (i.e. infected) areas. Also note the outer water conducting xylem and the white bands in the bark containing water. The images were captured at two different strengths of the magnetic field.

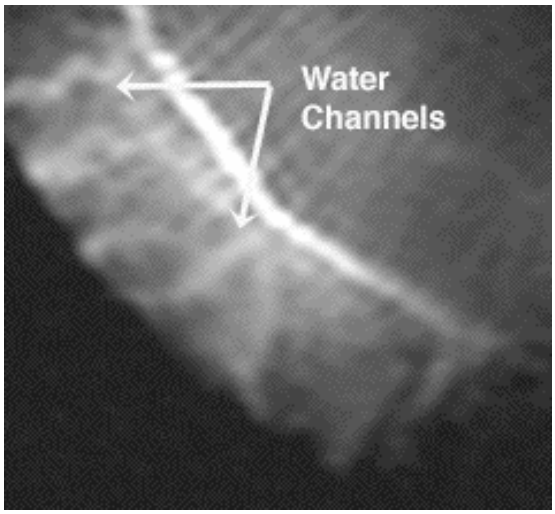

Figure 5 - Small section of the image in *fig. 4* to further demonstrate the water-filled channels in the bark.

Microscopy Data

Since MRI does not have the capability of resolving individual cell structure, the water channels were further investigated using light and scanning electron microscopy. At low magnifications, using a dissecting microscope, bands that traverse radially across the periderm can be identified (*fig. 6*).

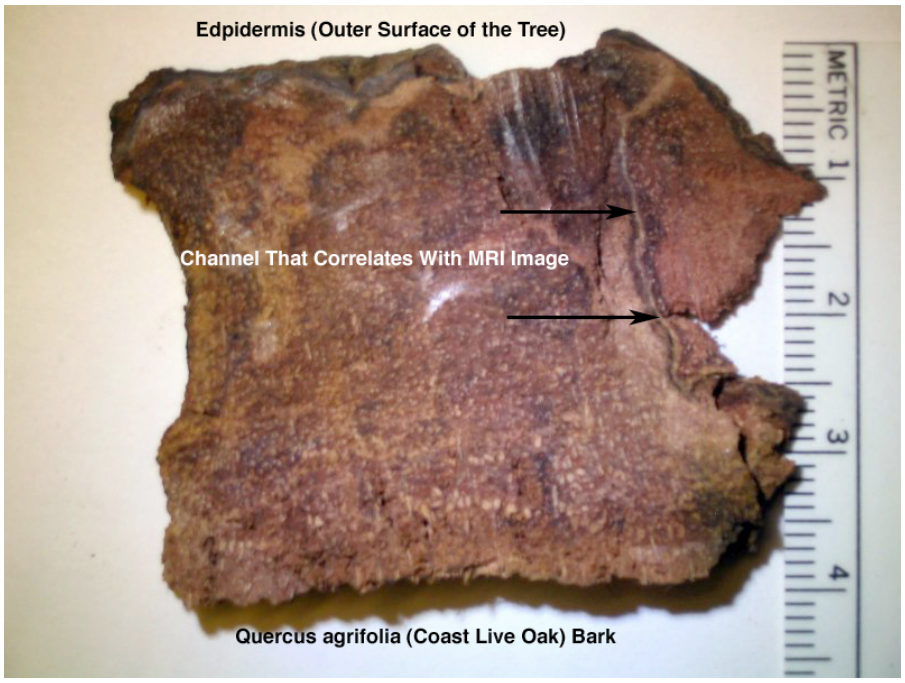

Figure 6– Low magnification photo showing a line of cells that traverse the bark. This line of cells correlates with the channels indicated in *fig. 4*.

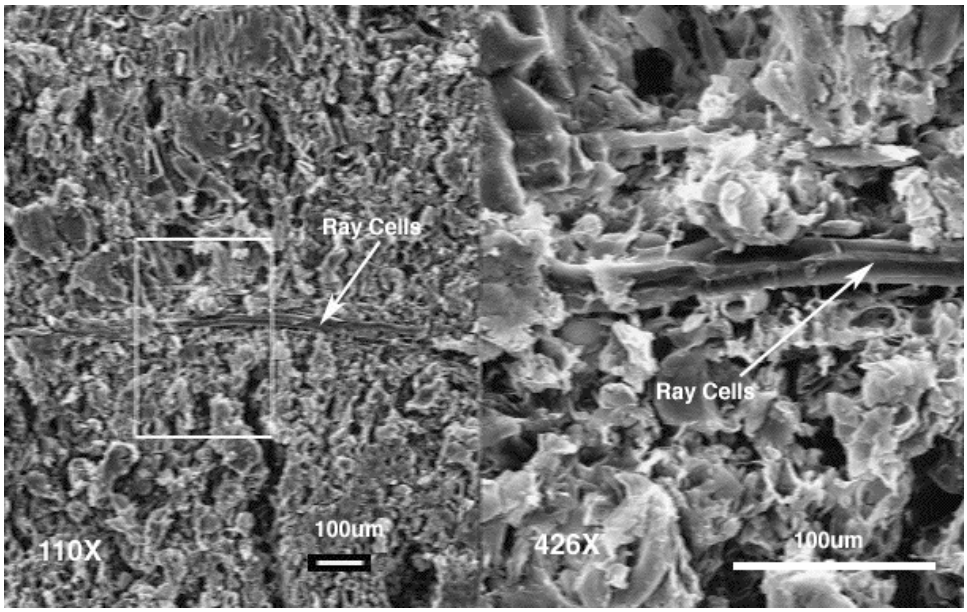

Figure 7– Scanning electron micrograph of ray cells. They are the cells that form the white, water-filled bands in the bark revealed by MRI.

Scanning electron microscopy revealed the bands to be ray cells elongated radially toward the outside of the tree (*fig. 7*). These cells correlate with the water-filled bands in the periderm shown in *fig. 5*.

Discussion and Conclusions

Technically, the outer most tissues of secondary origin in an oak tree should be referred to as the periderm. The word bark, on the other hand, is a non-technical term that refers to everything outside the vascular cambium. Each new growing season the periderm is composed of many living cells, e.g. cork cambiums, cortical cells, etc. An abundant supply of water must be radially translocated into these tissues to maintain cell division and growth. As demonstrated by MRI, the ray system functions in that capacity. And by correlating the MRI data with the structural data obtained with SEM, it has been demonstrated that the water-filled rays traverse from the xylem through the phloem and into the periderm. Regarding the various portals that may serve as infection entry points for *P. ramorum*, the bark, or periderm, appears to be a likely point of access. Bark is generally considered to be protective and more or less impenetrable by many pathogens. However, data obtained by MRI suggests otherwise. These water filled channels, or rays, could serve as natural avenues for the hyphae of *P. ramorum* to penetrate into the deeper tissues of and older oak tree.

It is important to point out that the samples studied were naturally infected in the field. Even though the presence of *P. ramorum* was demonstrated by culture and PCR, other microorganisms may have contributed to canker size and tissue disruption as demonstrated by MRI.

What has been presented above are preliminary data obtained from MRI observations correlated with considerable cytological work. MRI is a powerful technology, and when combined with scanning electron microscopy answers to the previously stated questions can be obtained. In addition, significant functional data can be acquired. For example, answers to questions about the depth and breadth of an infection may be determined, and specific tissues that have been disrupted can be identified.

Acknowledgements

The reported research has been supported, in part, by the Pacific Southwest Research Station, USDA Forest Service, through agreement No. 01-CR-11272138-174. Dr. Jennifer Davidson graciously collected and provided “cookies” of *Quercus agrifolia*. Dr. Matteo Garbelotto, University of California, Berkeley provided infected plant material. Mr. Paul Trusty, Lewis & Clark College, assisted in my laboratory.

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Adaptive Differences Between *Phytophthora ramorum* Isolates From Europe and North America: Evidence for Separate Subspecies?[1]

Clive Brasier[2,3], Susan Kirk[2], and Joan Rose[2]

Key words: genetic variation, *Phytophthora ramorum*, population structure

Abstract

The comparative risk to trees posed by European (E.U.) versus North American (U.S.) isolates of *Phytophthora ramorum*, the possible risk posed by genetic recombination between them, and whether European versus North American isolates represented discrete sub-populations was investigated. Population samples of E.U. and U.S. isolates were compared for growth rate across different environments (gene x environment tests), phenotypic stability, host range, pathogenic aggressiveness and sexual behaviour.

In linear growth tests on carrot agar at 20°C, samples of E.U. isolates grew significantly faster on average than U.S. isolates (35 to 39 isolates per sample). In several tests a total separation of the E.U. and U.S. isolates occurred. E.U. samples grew faster on average even when the U.S. samples were freshly obtained from the field (courtesy Dave Rizzo) and the E.U. isolates had been in culture for two or more years. In similar tests at 12.5°C, 15°C and 27°C the E.U./U.S. growth rate difference was maintained, but the mean growth curves for the E.U. and U.S. groups across the different temperatures were similar, indicating conspecificity. When tested at 30°C, only 37 percent of E.U. isolates grew, whereas 80 percent of U.S. isolates did so. This indicates E.U. isolates have a slightly lower maximum temperature limit for growth than U.S. isolates.

In the above growth-rate tests the variance of the E.U. samples was often very small, suggesting clonality, whereas that of the U.S. samples was much larger. Furthermore, E.U. isolates were consistently of a fairly uniform and characteristic 'wild type' colony morphology. U.S. isolates, however, were either wild type or fell into a range of morphologically variable, often slow growing 'non wild type' colonies. Routine subcultures and single hyphal tip subcultures taken from selected E.U. and U.S. isolates confirmed that U.S. isolates were intrinsically developmentally unstable, and that they could change from wild type to unstable non wild type. The basis of this instability is unknown. In a preliminary screening no viral dsRNA was detected in selected 'non wild type' isolates. In another test, a single *P. ramorum* isolate from Belgium, shown by S. Werres (personal communication) to be of A2 sexual and not A1 sexual compatibility type (see below), was shown to be a typical E.U. isolate on the basis of its growth rate and colony type.

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In repeat tests for pathogenicity to bark on the susceptible host *Quercus rubra* (via wound inoculation and measurement of lesion area), samples of E.U. isolates were, on average, significantly more aggressive than U.S. isolates, though the ranges of the two groups always overlapped. An E.U. sample was more aggressive, on average, even when the U.S. isolates were freshly obtained from the field and the E.U. isolates had been in culture two or more years. The potential host ranges of E.U. and U.S. isolates were compared by wound inoculation of mature stems of about 30 European and North American trees. Comparisons were based on lesion sizes caused on different tree species. The host ranges of the E.U. and U.S. isolates were shown to be very similar, again indicating conspecificity.

In standard sexual compatibility type tests on carrot agar, using A1- and A2-type testers of other *Phytophthora* species, samples of E.U. isolates were all of A1-type. Samples of U.S. isolates (excluding European-type nursery isolates from the Pacific Northwest) were all of A2-type (consistent with results obtained by other researchers). However, production of oogonia in these interspecific pairings was unusually slow, sparse and unpredictable. Also, using the same protocol, no oogonia could be obtained in pairings between the putative *P. ramorum* A1- and A2-types. Subsequently, a method was developed for producing oogonia of paired E.U. A1 x U.S. A2 *P. ramorum* isolates on carrot agar (Brasier and Kirk, 2004). Nonetheless, gametangial formation is still unusually sparse. Overall, it remains unclear whether *P. ramorum* is truly A1/A2 outcrossing or whether its sexual breeding system is functional.

Conclusions: (1) E.U. and U.S. isolates exhibit many similarities in continuous variables indicating conspecificity. Equally, there are important differences between them for continuous variables, such as growth rate and aggressiveness. These are likely to reflect differences in fitness, in other words adaptive differences. They are further supported by differences in predominant sexual compatibility type, and by differences in molecular polymorphisms observed by other workers. (2) For the present, the E.U. and U.S. types should be considered distinct phenotypic populations. (3) Since the E.U. population exhibits both A1 and A2 sexual compatibility types i.e. it could potentially ‘breed true’. (4) The U.S. population exhibits greater phenotypic variation and unusual developmental instability. (5) If recombination (sexual or somatic) were to occur between the E.U. and U.S. populations, further additive allelic variation is likely to be generated, in other words there is a risk that recombination could lead to the generation of further adaptive variation.

It is unclear at present how these phenotypic differences between the E.U. and U.S. populations came about. Three possible explanations are suggested: (A) That the E.U. and U.S. types are part of an original, far more variable gene pool in *P. ramorum*’s center of origin. Their differences reflect a combination of genetic bottlenecks during their introduction and the different selection pressures the two populations have experienced since their introduction into the E.U. and the United States. For example, growth rate variation in the E.U. population is narrow because this population has been subject to intense directional or stabilising selection within the European nursery environment. Growth rate variation in the U.S. population is broader because it has been exposed to a more heterogeneous set of environments or selection pressures in U.S. forests. (B) That the differences reflect their prior adaptation, before their introduction into the E.U. and United States. In other words, there may be two different ‘source’ populations of *P. ramorum*, for example in China or wherever *P. ramorum* originated (Brasier and others 2004). (C) A combination of (A) and (B) (A and B are not mutually exclusive).

Our current view is that process A is, by far, the most likely explanation. If process B were to be confirmed, however, we might need to reconceptualise the E.U. and U.S. population types as two (partially reproductively isolated?) subspecies of *P. ramorum*, for example as *P. ramorum* subspecies *europaea* and subspecies *americana*.

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Distribution and Etiology of Aerial Stem Infections of *Phytophthora ramorum* and *Phytophthora kernoviae* at Three Woodland Sites in the U.K.[1]

Anna Brown[2,3], Clive Brasier[2], Sandra Denman[2], Joan Rose[2], Susan Kirk[2] and Joan Webber[2]

Key words: etiology, *Phytophthora kernoviae, Phytophthora ramorum, Phytophthoras*

Abstract

Phytophthora ramorum and *P. kernoviae* sp. nov are recently introduced, invasive pathogens in woodlands in southern Britain. *P. kernoviae* is a newly discovered taxon, previously referred to as *Phytophthora* taxon C. Both species aggressively infect foliage and shoots of understory rhododendrons, especially *Rhododendron ponticum*. From there they can spread aerially to attack the inner bark of tree stems, especially European beech (*Fagus sylvatica*) and some *Quercus* species, causing bleeding lesions (Brasier and others 2004). Both species of *Phytophthora* are under investigation as scheduled risk organisms on trees in the U.K.

Three woodlands in southwest England, with naturalized understory *R. ponticum* infected with *P. ramorum* and *P. kernoviae* for several years, were investigated for possible spread of *P. ramorum* or *P. kernoviae*. Principal components of the woodlands are European beech (*F. sylvatica*), European sycamore (*Acer pseudoplatanus*) and Turkey oak (*Quercus cerris*). Minor species include holm oak (*Q. ilex*), English oak (*Q. robur*), European chestnut (*Castanea sativa*), European holly (*Ilex aquifolium*) and ornamentals, such as magnolia and rhododendron.

One hundred twenty-seven trees with 'suspicious' external stem symptoms (bleeding lesions) were investigated for *Phytophthora* by isolation. Overall, 39 percent of the trees investigated yielded *Phytophthora* species, with 34 percent yielding *Phytophthora* at site one; 35 percent at site two, and 66 percent at site three. A total of six different *Phytophthora* species were isolated from aerial bleeding lesions, and in one instance a single tree yielded three different *Phytophthora* species. *P. ramorum* was isolated from aerial stem lesions on beech, Turkey oak, horse chestnut (*Aesculus hippocastanum*) and southern beech (*Nothofagus obliqua*). *P. kernoviae* was isolated frequently from beech and from an American tulip tree (*Liriodendron tulipifera). P. citricola*, an established endemic, was frequently isolated from European sycamore. As with *P. ramorum* and *P. kernoviae*, *P. citricola* may also have spread from the adjacent diseased rhododendron. *P ilicis*, another recently invasive aerial *Phytophthora*,

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was obtained from stems of two Chinese *Ilex* species. This, in itself, is unusual as *P. ilicis* generally causes lesions on leaves and small twigs, not on the main stem of holly trees. Surprisingly, *P. gonapodyides*, a weak parasite and common soil inhabitant, and *P. cambivora*¸ an established endemic collar pathogen, were also obtained from aerial bark lesions on several beech trees up to 6m (see *table 1*).

Because *F. sylvatica* is the main host infected by both *P. kernoviae* and *P. ramorum* at these sites thus far, 25 mature (50 to 140 cm diameter) affected *F. sylvatica* were investigated for bark infection processes. These included proximity to rhododendron, pattern and mode of spread within a tree, and tree response to infection. Three trees were infected with *P. ramorum*, 21 with *P. kernoviae*, and one with both species of *Phytophthora*. Aerial bark lesions, indicated by stem bleeding, ranged in size from about 3-cm diameter fresh infections to lesions of greater than 1-m diameter, and occurred from near ground level to 11 m. In addition, a log experiment was established to assess whether *P. ramorum* zoospores could penetrate the bark of a range of tree species.

A study of the processes of local spread and infection indicated the following:

(i) Intact beech bark can be penetrated and infected by *P. ramorum* zoospores, and probably by *P. kernoviae* zoospores.

(ii) Close proximity to rhododendron is a major, but not universal, factor in infection with 22 of the 25 trees being within 2 m, frequently in direct contact.

(iii) Rainwater accumulation, run-off at branch forks, and sporangia carried in rain and mist may account for the remaining infections.

(iv) Simultaneous, multiple infection of stems is occurring.

(v) Vertical spread may occur within a tree as lesions develop.

(vi) *P. ramorum* and *P. kernoviae* can penetrate, and be recovered from, the underlying cambium and xylem.

(vii) The tree may respond to a bark lesion by attempting to wall it off with callus.

(viii) *P. kernoviae* (at least) may pump water out of the xylem into the inner bark.

(ix) Stem lesions sometimes extend 10 to 20 cm below ground level.

(x) One of the trees was infected by *P. kernoviae*, *P. ramorum* and *P. gonapodyides*.

(xi) Developing lesions are frequently and rapidly invaded by unknown basidiomycetes and/or ascomycetes.

Some other issues arising from these observations:

(1) Do the multiple infection points observed on stems of individual beech trees (also seen on *Quercus* and *Acer*) represent multiple infections occurring during a single temporal

event, different periods of infection, or both? Do they interact to overwhelm the trees defenses?

(2) How often does the observed callusing of stem lesions lead to arrest and walling off of the lesion? Is the phenomenon seasonally related, or host genotype related? Can it be utilized as part of a local management protocol?

(3) Does lesion invasion by basidiomycetes mask *P. ramorum*/*P. kernoviae* symptoms for diagnosis? Can it even act as a natural biocontrol? Does *Armillaria* invade and mask primary *P. ramorum* or *P. kernoviae* lesions on lower stems, especially in more resistant hosts?

(4) Have the three putatively invasive species of *Phytophthora* at these sites (*P. ramorum, P. kernoviae* and *P. ilicis*) been recently introduced, for example from Yunnan, Himalayas or Taiwan by plant collectors and/or the nursery trade?

(5) As these invasive aerial species of *Phytophthora*, plus several endemic species of *Phytophthora*, are present at these sites, sometimes in close physical contact within the same niche, is there an enhanced risk of interspecific hybridization between them (for example the new hybrid pathogen *P. alni*)?

(6) Is there a potential risk to other forest ecosystems? The host range of *P. kernoviae* and of *P. ramorum* at these sites suggests other Eurasian, North American and southern hemisphere Fagaceae and Magnoliaceae may also be at risk.

Table 1—*The number of trees yielding Phytophthora species at three sites in southwest England.*

	P. cambivora	*P. citricola*	*P. gonapodyides*	*P. ilicis*	*P. ramorum*	*P. kernoviae*
Acer pseudoplatanus	-	*11*	-	-	-	-
Aesculus hippocastanum	-	-	-	-	*1*	-
Fagus sylvatica	*1*	-	*3*	-	*4*	*22*
Ilex dypirina	-	-	-	*2*	-	-
Ilex kingiana	-	-	-	*1*	-	-
L. tulipifera	-	-	-	-	-	-
Nothofagus obliqua	-	-	-	-	*2*	*1*
***Querqus* sp.**	-	-	-	-	*1*	-
Querqus cerris	-	-		-	*2*	-
TOTAL	***1***	***11***	***2***	***3***	***9***	***23***

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Brasier, C.M.; Denman, S.; Brown, A.V.; and Webber, J.F. 2004. **Sudden Oak Death (*Phytophthora ramorum*) discovered on trees in Europe.** Mycological Research 108: 1107-1110.

Comparative Host Range and Aggressiveness of *Phytophthora ramorum* and *Phytophthora kernoviae* sp. nov. on North American and European Trees[1]

Clive Brasier[2,3], Joan Rose[2], Susan Kirk[2], Sandra Denman[2], and Joan Webber[2]

Key words: host range, *Phytophthora kernoviae, Phytophthora ramorum,* susceptibility

Abstract

Phytophthora ramorum and *P. kernoviae* are recently introduced, invasive pathogens in woodlands in southern Britain. *P. kernoviae,* previously known as *Phytophthora* taxon C, is a newly discovered taxon, (Brasier and others 2005), found during surveys for *P. ramorum* in Cornwall, southwest England in November 2003. Both species are aggressive pathogens on foliage and shoots of understory rhododendron, especially *R. ponticum*. From this infection base they can spread aerially to attack the inner bark of tree stems, especially European beech (*Fagus sylvatica*) and some *Quercus* species, causing bleeding lesions. (Brasier and others 2004). To date *P. kernoviae* has been found causing bleeding lesions on about 50 mature *F. sylvatica* trees, two common European oaks, *Quercus robur,* and a *Liriodendron* at several adjacent woodland sites. The wider international risk to trees that *P. kernoviae* poses is unknown. The comparative host range and aggressiveness of *P. kernoviae* and *P. ramorum* to bark and foliage of North American and European tree species is therefore being investigated.

The potential host range of *P. ramorum* on bark of native and plantation trees in the U.K. has been assessed previously (Brasier and others 2003; http://rapra.csl.gov.uk). The test method comprises wound inoculating fresh cut 1.1-m long by 16 to 20-cm diameter stems at 20°C, and assessing the comparative susceptibility of the inner bark to *P. ramorum.* Thirty hosts have been tested and assigned to 'more susceptible', 'less susceptible' and 'resistant' categories. Tests are carried out under licence in quarantine chambers. Comparative tests of *P. ramorum* versus *P. kernoviae*, begun in 2004, are scheduled to continue in 2005. Present results are therefore provisional. Twenty-eight hosts have been inoculated to date, using three isolates each of *P. kernoviae* and *P. ramorum*. *F. sylvatica* is being used as a control host for most tests.

F. sylvatica fell into the 'more susceptible' category with regard to both *P. kernoviae* and *P. ramorum*. In four tests, *P. kernoviae* was, on average, slightly more aggressive on *F. sylvatica* than *P. ramorum*

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(total lesion areas of 282 cm^2 and 242 cm^2, respectively). However, there was also evidence of marked seasonal or genetic influences on susceptibility, leading to critical threshold effects (Brasier and Kirk 2001). European chestnut, *Castanea sativa*, was moderately susceptible to both species. Some tree species, including *Liriodendron tulipifera*, were resistant to both pathogens. Most of the other tree species tested, whether assigned to 'more susceptible' or 'less susceptible' categories, tended to be more susceptible to *P. ramorum* than to *P. kernoviae*. Among these the oak species *Quercus robur, Q. cerris, Q. ilex, Q. rubra* and *Q. palustris* were all susceptible to *P. ramorum* to some degree, but all these hosts fell into the 'resistant' category with *P. kernoviae*. These tests will be repeated.

The North American tree species *Q. palustris, Abies grandis* and *Tsuga heterophylla* and the European species *Taxus baccata* and *Acer pseudoplatanus* fell into the 'more susceptible' category in these tests with regard to *P. ramorum.* In previous tests in the U.K. with *P. ramorum* these species were either assigned to the 'less susceptible' category, or were not included in the tests (Brasier and others 2003).

The comparative foliage susceptibility tests involve dipping unwounded leaves of about 30 hosts in zoospore suspensions of three isolates each of *P. kernoviae* and *P. ramorum* at 20°C.In tests to date, *P. kernoviae* has been more aggressive than *P. ramorum* on foliage of *L. tulipifera* and *Aesculus hippocastanum*. *P. kernoviae* and *P. ramorum* were equally aggressive on rhododendron and *Magnolia* species. *P. kernoviae* was less aggressive than *P. ramorum* on all other susceptible hosts. These tests will be repeated.

To date, field data on stem susceptibility of U.K. trees to *P. ramorum* and *P. kernoviae* in Cornwall are fairly consistent with the above laboratory data. For example, stems of *F. sylvatica* have proved very susceptible to both *P. ramorum* and *P. kernoviae* in the field. Notable exceptions at present are *Q. robur* and *L. tulipifera*. Bark of these species were 'resistant' to *P. kernoviae* in these preliminary laboratory tests, yet mature *Q. robur* and *L. tulipifera* have been found with bleeding stem lesions caused by *P. kernoviae* in the field. These discrepancies may reflect: (1) environmental influences, such as episodes of very high *P. kernoviae* inoculum levels, or varying degrees of individual tree stress, in the field, or (2) variation in genetic resistance of individual *Q. robur* trees to *P. kernoviae*. In previous laboratory tests, *Q. robur* has been shown to vary considerably in its susceptibility to *P. ramorum*. Most individuals fall in the 'less susceptible' category but some fall in the 'more susceptible' category.

Also European sycamore, *Acer pseudoplatanus*, falls in the *P. kernoviae* 'less susceptible' category in the laboratory tests, yet no infected individuals have been found in the field, despite heavy exposure to the pathogen at the same Cornish sites as the affected beech. This may reflect an inability of *P. kernoviae* zoospores to penetrate unwounded bark of *A. pseudoplatanus*.

Clarification of these discrepancies is needed to more confidently assess the wider risk that it poses to trees in the U.K., in Europe or to trees in global ecosystems, should it escape beyond the present disease management zones in Cornwall.

One suggested approach, in addition to conducting further laboratory tests, is to back up these tests with well replicated field inoculation of trees of *F. sylvatica*, *Q. robur* and *A. pseudoplatanus* (under regulated conditions) within part of the present *P. kernoviae* management zone. This could help to validate the results of the laboratory tests, give insight into the environmental and seasonal influences on infection processes and disease expression in the field, and provide much needed insight into the

extent of genetic variation in resistance within each species. Infected tree bark is not presently believed to be a significant source of *P. kernoviae* inoculum. Furthermore, small lesions, such as those initiated via artificial inoculations over a few weeks, can usually be excised from trees (A. V. Brown and C. M. Brasier, unpublished studies).

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The Search for the Origin of *Phytophthora ramorum*: A First Look in Yunnan Province, People's Republic of China[1]

Ellen Michaels Goheen[2], Thomas L. Kubisiak[3], and Wenxia Zhao[4]

Key words: Phytophthora ramorum, Yunnan Province

Abstract

Phytophthora ramorum, a previously unknown pathogen, was first seen in Europe on ornamental nursery stock in the early 1990s (Werres and others 2001). It has since been implicated in the death of thousands of oaks (*Quercus agrifolia* and *Q. kelloggii*) and tanoaks (*Lithocarpus densiflorus*) in the western United States (Rizzo and others 2002). Although populations of *P. ramorum* in Europe and the United States are largely distinct from one another, the organism is considered invasive in both locations with an operating hypothesis that it was introduced on both continents (Brasier and others 2004). It is possible that *P. ramorum* may have originated somewhere in Asia and was unknowingly transported on commercial or privately collected ornamental plants. Asia is a center of diversity for many genera of plants that are *P. ramorum* hosts. More specifically, northern Yunnan Province in the People's Republic of China has an abundance of oaks and tanoaks in a variety of forest ecosystems. Rhododendrons and azaleas, among others, have been widely collected in this region for at least 150 years by plant enthusiasts from across the globe. Furthermore, the climatic conditions in this part of the Province correlate well with those predicted to be favorable for *P. ramorum.*

The International Forestry, Forest Health Protection, and Vegetation Management and Protection Research staffs of the Forest Service, U.S. Department of Agriculture, have been working cooperatively with forestry officials in China over the past several years on a variety of issues related to forest insects and pathogens. In August 2004 a small team of USDA Forest Service scientists traveled to Yunnan Province in southwestern China to begin collaboration with scientists from the Chinese Academy of Forestry (CAF) on projects investigating forest *Phytophthora* species in China with the more immediate goal of initiating a survey specifically for the pathogen *P. ramorum*. International Forestry, using its network of contacts in China, arranged for field personnel in China to assist with site identification, visits and sampling.

We visited four forest sites where a variety of species of *P. ramorum* host genera occur. Three sites were in Diqing Prefecture in the vicinity of the city of Zhongdian (Shangri-la) in northwestern Yunnan Province. Plant associations on these sites included evergreen oak (*Quercus*), *Larix*, and *Picea* stands

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with *Rhododendron* understories, evergreen oak and pine (*Pinus*) stands, and pine forests with *Lithocarpus* species and other oak species in the understory. Sites ranged in elevation from approximately 2000 m to 3500 m. Genera of other *P. ramorum* hosts were also present including *Lonicera* and *Rosa*. We also visited a site outside of the city of Kunming in central Yunnan province. Forests there were composed of several *Quercus* species with *Lithocarpus* species and *Rhododendron* species in the understory.

We observed foliar and dieback symptoms similar to those associated with *P. ramorum* in the United States and Europe on a variety of plants. Symptoms included inner bark necrosis, shepherd's crooking of small diameter stems and new growth, leaf tip dieback, leaf margin necrosis, and leaf spotting. Symptomatic tissues were collected in the field, plated on CARP media in the evenings after collection, and then transported to and processed in the CAF pathology laboratory in Beijing for sub-culturing and DNA extraction. In Beijing, mycelium and plant tissues contained within a single plate were pooled together and DNA was extracted using a standard CTAB-based extraction protocol. We also extracted DNA from an additional 30 symptomatic plant tissue samples. The DNA assay performed while in Beijing was inconclusive and DNA samples were brought back to the United States. All DNA samples were further purified and amplified using the ITS 6 and 7 rDNA primers developed specifically for use on Oomycetes. Out of 92 total samples, 29 samples amplified at least a single visible band based on ethidium bromide-stained gels. Due to the manner in which samples were taken and the redundancy of band profiles across some samples, only 19 bands from 18 samples were subject to further analysis. *P. ramorum* was not immediately identified from these samples. We did find DNA sequence BLAST search matches with *Cryptococcus flavescens* and *Mortierella alpina* for some samples. The possibility that other *Phytophthora* species are involved in symptom development is currently under investigation.

The forests we visited in Yunnan Province were remarkably similar in genera, yet profoundly different in terms of species diversity and composition relative to forests on the west coast of the United States where *P. ramorum* is currently found. Symptoms similar to those caused by *P. ramorum* on wildland and nursery stock target species (*Quercus*, *Lithocarpus*, *Rhododendron*) in the western United States, as well as minor *P. ramorum* hosts (such as *Lonicera*, *Smilacina*), were abundant. While our laboratory results were not definitive, the team sees promise for additional investigations and received an enthusiastic response from our Chinese colleagues for continued collaboration. Further field work in Yunnan Province may include sampling additional hosts and forest types, as well as sampling forest soil and water. Understanding the origin of a presumably introduced pathogen, such as *P. ramorum*, will help us better understand its biology, ecology, pathology, and genetics, and may lead to management actions that minimize its impact.

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Genetics

Does Stand Density Affect Mating System and Population Genetic Structure in Coast Live Oak?[1]

Kathryn Beals[2] and Richard S. Dodd

Key words: mating system, pollen flow, *Quercus agrifolia*, stand structure

Introduction

Coast live oak *(Quercus agrifolia)* is a major species at risk in the current *Phytophthora ramorum* epidemic in California's oak woodlands. To search effectively for resistant genotypes, it is imperative to have an understanding of the existing host population genetic structure in these forests, and how its reproductive capacity may be affected by major losses. The purpose of our study is to assess and quantify the distribution of genetic variation of coast live oak in central California. To understand the factors that may limit the ability of these populations to recover, we will compare the current mating system in relatively dense coastal populations to open woodland populations in the interior.

To inventory the existing genetic structure, we sampled adult trees from five large, contiguous coastal and interior populations, which provided an appropriate sample of the species range in central California. Spatial locations of these trees were mapped, and leaf tissue was collected for DNA extraction. The multilocus genotypes of these individuals were identified using 13 neutral, nuclear, microsatellite markers.

Preliminary Results

Analyses using Wright's F-statistics and Hardy-Weinberg tests revealed a global Fst of 0.037 for the entire sampling area, suggesting high outcrossing that is typical of oaks, and low population differentiation. When a stepwise mutational model for microsatellite alleles is assumed, we detected heterozygote deficiency in all populations, suggesting that, rather than evidence of a genetic bottleneck, these

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populations have been expanding in recent generations. We detected up to 23 alleles per locus. These findings indicate that 96 percent of the molecular variance occurs within populations, and though genetically diverse, the population structure of coast live oak is reasonably uniform throughout this range.

Wright's Fis estimates of single-population inbreeding revealed small, but statistically significant, differences between the coastal and interior regions that were sampled. Coastal populations, where stand structure is denser and contains a mixture of species, had higher rates of inbreeding than the interior populations. The interior populations, which exhibit higher outcrossing, are close to the limit of the species range and may be benefiting from more open pollen flow.

Current Research

The second part of our study addresses the most recent mating between the adult trees in these populations. We have collected acorns from the adult study trees in the fall of 2004 and are using the same 13 markers to determine their paternity. From these data we will estimate the proportion of effective pollen donors in each population. Incorporating the spatial locations of the parent trees, we will estimate the average distance of successful pollen dispersal within a stand. This information will indicate the success of the current mating, and the ability of these stands to recover their reproductive capacity after a reduction in their effective population size.

To follow the movement of genes via seed, we are genotyping these adult trees using five maternally inherited chloroplast primers. This will allow us to gauge the component of gene flow that has resulted from the distribution of acorns, in addition to what we know about gene flow via pollen.

Conclusions

From the preliminary results of this study, we conclude that coast live oak has low differentiation in population genetic structure throughout central California. A vast majority of the genetic variation in this species is contained at the population level, so searches for resistant genotypes could be limited to smaller areas of the species distribution.

Coastal populations exhibit more inbreeding than interior populations, which may be a result of their dense, mixed species stand structure. Pollen flow may be less restricted in open woodland interior populations, resulting in greater outcrossing.

Current research will provide more insight into pollen movement in these two stand types, and the contribution that maternal seed distribution makes towards gene flow in this species. Evaluating the current mating system of coast live oak during the *Phytophthora* outbreak will help to predict the impact of adult tree mortality on the future genetic diversity of these populations, and allow better allocation of conservation resources.

Genetic Hitch-Hiking Extends the Range of Coast Live Oak [1]

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Key words: chloroplast DNA, introgression, nuclear DNA, *Quercus agrifolia*, *Quercus wislizeni*, red oaks

Abstract

The northernmost range of coast live oak (*Quercus agrifolia*) is reported from the Ukiah Valley (Mendocino County, California). Here, field observations suggest that hybridization with interior live oak (*Q. wislizeni*) is important. Elsewhere in northern California, morphology of coast live oak can be highly variable (particularly foliar form) and this has commonly been attributed to "noise" from a polymorphic species belonging to a genus renowned for its variability. This variability can be worrisome to scientists working on sudden oak death, since it may result in confounding species in ecological and pathogenicity studies.

To better understand the causes of this variability, within and among the major red oak species of the coastal oak woodlands of California, we have been studying population structure and differentiation by comparing nuclear and chloroplast DNA. The nuclear genome is bi-parentally inherited and provides important information on population demographic processes, such as gene flow and historical fluctuations in population size. The chloroplast genome in oaks is maternally inherited and is therefore passed on clonally through seed from mother tree to progeny. The heavy seeds of oaks are not likely to be widely dispersed resulting in strong population structure in chloroplast DNA that provides historical signals of population lineages in time and space.

Our nuclear DNA studies have shown important interspecific gene flow among the three evergreen red oaks of California (*Q. agrifolia*, *Q. wislizeni* and *Q. parvula*) and minor gene flow between these species and the deciduous red oak, *Q. kelloggii* (Dodd and Afzal-Rafii 2004). In populations morphologically assigned to *Q. wislizeni*, genetic admixture from up to three of the other red oak species can be detected in an individual's genotype. We have shown that admixture proportions at a population level are well correlated with environmental conditions at that site suggesting that introgression among species can produce genotypes that may be better adapted to the local habitat than are pure parental genotypes. Since introgression among red oaks is important in northern California, in the region of heavy infection from *Phytophthora ramorum*, we compared chloroplast DNA to investigate possible cyto-nuclear disequilibrium that would suggest differences in the relative success of one species as maternal or paternal parent.

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Chloroplast DNA diversity based on six microsatellite loci was documented for 28 populations of *Q. agrifolia*, including 12 from northern California (north of San Francisco Bay), eight from central California and eight from southern California and Baja California, Mexico. As the chloroplast genome is non-recombining, the six loci are inherited together as a single locus that we refer to as a haplotype. A total of 27 different haplotypes were detected, including six haplotypes in northern California, seven in central California and 14 in southern California. This higher haplotype diversity in the south, suggests this to be a region in which coast live oaks have a longer history than in the north and may be a refugium during the last glacial advance. We found that chloroplast haplotypes may be shared among species, where populations occupy the same geographic range. This is particularly true for coast live oak in northern California. The northernmost populations of coast live oak, including hybrid populations at Hopland and Yorkville, were characterized by a single haplotype. Interestingly, this haplotype is the only one found in interior live oak over a broad zone north from Ukiah. We conclude that these northernmost populations of coast live oak must have an interior live oak maternal lineage. This suggests that coast live oak has colonized this region through pollen hybridizing with interior live oak, followed by generations of backcrossing to "regenerate" the coast live oak morphotype. Further south, including the well-studied populations of Marin County, two or three chloroplast haplotypes were detected including the northern interior live oak haplotype and two coast live oak haplotypes. This indicates that these populations are of mixed species lineages through seed and pollen dispersal. We hypothesize that coast live oak has been advancing northwards through pollen "hitchhiking" on interior live oak and that, once established, occasional seed dispersal from more southerly populations of coast live oak produces a population of mixed maternal lines.

Our findings on population structure of California red oaks indicate that these are not well-defined species genetically. Ecological studies and studies of pathogenicity may need to take account of this and that "pure" species' habitat preferences are not necessarily appropriate in these mixed populations.

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Seasonal Trends in Response to Inoculation of Coast Live Oak With *Phytophthora ramorum* [1]

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Key words: inoculation, lesion size, *Phytophthora ramorum, Quercus agrifolia,* sudden oak death

Abstract

We developed a branch cutting inoculation method to provide a controlled system for studying variation in response to inoculation of coast live oak (*Quercus agrifolia*) with *Phytophthora ramorum*. This method has advantages over inoculations of trees in the field, in containing the inoculum and in allowing high levels of replication and the possibility of time series of responses. We previously reported significant tree-to-tree variation, with little population variation in lesion size using this method (Dodd and others 2005). Here we report on a time series in which branch cuttings were collected from the same trees at eight dates through a full year cycle. Branch cuttings were sampled from 33 trees from two sites at China Camp in Marin County, California, including 18 trees from one site that had suffered heavy mortality from this disease (Miwok Meadows) and 15 trees from a second site that has had little infection (Chicken Coop Island).

Our inoculation experiments showed a very marked seasonal cycle in lesion size (*fig. 1*). The largest lesions were produced in the spring and declined through the summer, reaching a minimum in the August inoculation. There was an increase in response in the October inoculation to levels that were not significantly different to those in the late spring and early summer. The large lesions produced in the spring are at a time when cambial activity and sugar mobilization are likely to be at a maximum in this Mediterranean climate species and could provide a very suitable environment for the pathogen. The minimum lesion size in August is likely to coincide with very low activity of the cambium during summer drought. The increase in lesion size in October suggests that sugars may be more important than the size of the cambial zone in determining activity of the pathogen. At this time of year the cambium has generally completed cell divisions and cell differentiation and is likely to be composed of only a few cells in thickness. However, sugars may be actively transported to storage sites and possibly for root growth in the late autumn. We propose to test whether cambial activity or sugar contents in the phloem are correlated with seasonal activity of the pathogen in future studies.

Our inoculations confirm significant tree-to-tree variation in lesion size after inoculation that we have reported previously. At the time of maximum response to inoculation (greatest lesion size), we detected a five to six fold difference between the largest and smallest lesions produced. This difference fell to

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only two fold at times during the season when the lesions produced were small. Trees were ranked according to their lesion size at each of the harvest dates and rankings were compared across harvests. No trees ranked equally across all harvest dates. However, in comparing rankings for the two dates in which lesion sizes were maximum and differences among individuals were five to six fold, three individuals ranked in the five smallest lesion size class at both dates and three trees ranked in the five smallest lesion size class at each date. Interestingly, the three individuals with smallest lesions were all from Miwok Meadows, the site that had suffered heavy mortality before our sampling. The three individuals with largest lesion sizes were all from Chicken Coop Island, the site where prior infection had been light.

The seasonal cycle in response to inoculation indicates that for comparative purposes, susceptibility tests should be carried out at the same time during the season to avoid confounding seasonal cycles in response. Late spring to late summer inoculations are likely to show the least seasonal variation in response, but because of relatively small lesions, are likely to provide poor levels of detection of differences among individuals. Lesion size varied significantly among individuals within a population, providing support to our earlier report of probable variation among individuals of coast live oak in response to infection with *P. ramorum*.

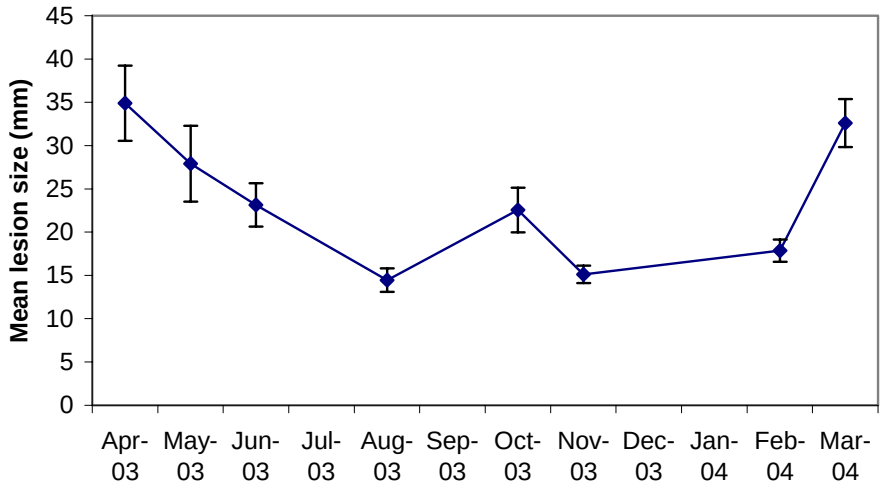

Figure 1— Mean length of lesion on coast live oak cuttings after two weeks incubation following inoculation with *Phytophthora ramorum*. Vertical bars are 95% confidence intervals about the mean.

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Potential for Sexual Reproduction of *Phytophthora ramorum* in Washington State Nurseries[1]

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Key words: mating types, microsatellites, *Phytophthora ramorum*, RFLP

Abstract

In 2003, isolates belonging to the Al mating type were reported from commercial nurseries in Oregon. Soon thereafter, we reported the presence of both mating types of *P. ramorum* in nurseries in Washington. AFLP, microsatellite, and RFLP of the Cox I region indicated the Al isolates belonged to the European (E.U.) lineage of *P. ramorum*. In 2003, 33 percent of the isolates analyzed belonged to European lineage, while the rest belonged to the North American (N.A.) lineage. Isolates belonging to the two lineages were found in the same block of plants, and even on adjacent plants. By using tester strains (A2 from California and Al from Oregon nurseries) we confirmed that all North American isolates belonged to the A2 mating type, and all of the European isolates belonged to the Al mating type. The coexistence of both mating types on adjacent plants increases the chances for sexual recombination between these two genetically divergent lineages. Crosses with tester strains were 100 percent successful, but a very high proportion of oospores appeared to be aborted, suggesting the mating system of *P. ramorum* is not perfectly functional, potentially due to the long isolation of the two mating types. Nonetheless, a significant number of oospores appeared to be viable. In 2004, the survey of isolates from nurseries in Washington continued. PCR-RFLP of the Cox I region and morphology of the colony (appressed for N.A. and fluffy for E.U. isolates) indicated that 84 appressed colonies had the E.U. RFLP pattern, while only one isolate had fluffy colony morphology and had a RFLP pattern matching that of the E.U. lineage. Despite the sharp decrease of Al isolates in 2004, we encountered one isolate that had fluffy colony morphology and RFLP pattern of the N.A. isolates. Out of 12 microsatellite loci analyzed for this isolate, two had alleles exclusively found in North America, two had alleles exclusively found in Europe, one had one allele that is shared by both lineages, and seven loci had novel alleles. AFLP analysis showed this isolate has bands typical of the N.A. lineage, bands typical of the E.U. lineage, and a significant number of new bands. This isolate is likely to be either a recombinant between Al and A2 or an isolate representative of a third yet unknown lineage. Mating and phenotype of this isolate were discussed, including implications for the evolution of *P. ramorum*.

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Phenotypic Variation Among *Phytophthora ramorum* Isolates From California and Oregon[1]

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Key words: phenotypic variation, *Phytophthora ramorum*, sudden oak death

Introduction

To manage and control *Phytophthora ramorum* successfully, it is important to know the amount of phenotypic variation within a given pathogen population. Because the pathogen has only recently been described, there are few studies on morphological and pathological variation of isolates from the United States. One study has compared growth rate on agar, aggressiveness to *Quercus rubra* (Eastern American red oak), and developmental stability of European and U.S. isolates. In this study, the U.S. isolates were significantly slower growing, less aggressive, morphologically more unstable, and had a larger range in growth rates than the European isolates (Brasier and others unpublished data; Brasier 2003; Brasier and others, this Proceedings). However, there is no information on fungicide tolerance, spore production or aggressiveness to hosts from the western United States to U.S. isolates. This study examines these phenotypic characters among 12 isolates from a broad range of hosts, locations within California and Oregon, and AFLP genotypes. We examine variation in aggressiveness of isolate populations from California. Additionally, we determined whether measurement of lesions on inoculated coast live oak stems is a good measure of pathogenicity.

Methods

Twelve *P. ramorum* isolates were used in all experiments, unless specified otherwise. These isolates were from a broad range of hosts and locations within California and Oregon, and AFLP genotypes (see Ivors and others 2004). For fungicide tolerance experiments, isolates were grown on vegetable-8 juice agar (V8A) modified with 53 and 2134 _g/mL phosphite (Agrifos 400; Agrichem, Loganholme, Qld, Australia) at 22 °C. Colony size was calculated as a percentage of the control after 10-days growth on agar. Sporangia were produced in Multiwell 24 well plates (Falcon, Franklin Lakes, NJ) containing 350 _L sterile water. All sporangia were counted within the well. Oospores were produced in two separate experiments by pairing the cultures with an A1 and A2 tester isolate on separate carrot agar plates (Brasier and others 2004). A 10-mm square was removed from each mashed paired culture and examined under the microscope. For pathogenicity experiments, bay laurel leaves were

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inoculated with zoospores (Hüberli and others 2003) and coast live oak seedlings were inoculated with agar discs (Dodd and others 2004). Each inoculation was repeated once. Forty-five isolates from three different counties in California were used to inoculate coast live oak seedlings. Stems from four coast live oak trees were inoculated with three isolates as described in Dodd and others(2004). Small sections (0, 0.5, 1.5, 2 and 3 cm from beyond both sides of the longitudinal lesion) were plated onto P_{10}ARP.

Results and Discussion

There was large and significant ($P < 0.001$) isolate variation in all morphological and pathological phenotypes examined (*table 1*). A significant positive correlation ($r = 0.89$, $P < 0.001$) between lesions on bay laurel and coast live oak was found. Two low and three highly aggressive isolates were identified for both hosts. No other linear correlations were found between different phenotypes measured. However, the two low aggressive isolates produced low concentrations of sporangia, and vice versa for the highly aggressive isolates. All three highly aggressive isolates were able to grow on the highest concentration of phosphite. One highly aggressive isolate was identified as having high fungicide tolerance, and produced abundant sporangia and oospores. It is these types of isolates that need to be used in control studies, host resistance screening and recombination analysis.

Inoculation of coast live oak seedlings with 45 isolates from three populations (Santa Cruz, Sonoma and Monterey Counties) in California revealed that Santa Cruz isolates were more pathogenic than those from the other two counties. In these inoculations, Pr-102 and Pr-52 routinely used in coast live oak (Dodd and others 2004) and bay laurel (Meshriy and others, this Proceedings) inoculations were low to moderately highly aggressive. Hosts identified as being less susceptible with these two isolates need to be screened against a range of highly aggressive isolates to confirm if they are robustly resistant. Population variation in a range of other phenotypes is currently being investigated.

We observed that after removal of the bark from coast live oak stems, recovery beyond macroscopically visible lesions is minimal for *P. ramorum* (*fig. 1*). The recovery of the pathogen from apparently beyond the lesion (2 cm for 2 percent of stems) is explained by some stems producing lesions that are diffuse with non-defined lesion margins. This is in contrast to what has been found for *P. cinnamomi* where the pathogen has been recovered from beyond well-defined lesions of between 3 to 6 cm or more (Hüberli and others 2002a, b).

Table 1—*Mean isolate range and* P*-values for different morphological and pathological phenotypes of 12 isolates of Phytophthora ramorum from California and Oregon.*

Phenotype	Isolate range	*P* value
Growth on phosphorus acid (% of control) at:		
53 _g/mL V8A [a]	50-92	< 0.001
2134 _g/mL V8A	0, 10-22	< 0.001
Sporangia production (no./mL)	205-8863	< 0.001
Oospore production	0, + - +++[b]	NA
Bay laurel lesion area (mm^2)	0, 1-31	< 0.001
Coast live oak lesion length (mm)	19-52	< 0.001

[a] V8A, vegetable-8 juice agar
[b] Numbers of oospores observed within a 10-mm square of the carrot agar directly beneath the mashed paired isolates; + = few oospores, +++ = many oospores

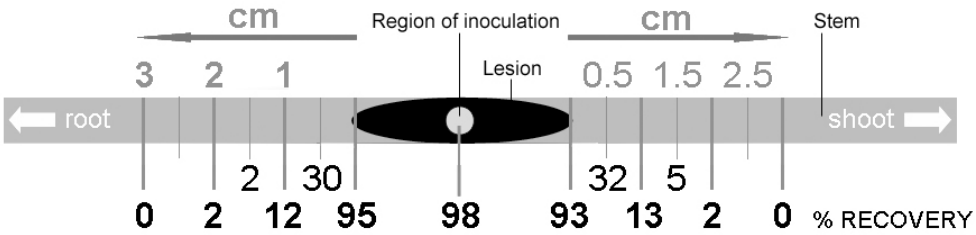

Figure 1—Recovery (percent of 60 stems) of *Phytophthora ramorum* on *Phytophthora*-selective agar medium (P_{10}ARP) from beyond a well-defined, visible lesion of a wound inoculated *Quercus agrifolia* (coast live oak) stem.

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Molecular Identification and Detection of *Phytophthora ramorum*[1]

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Key words: AFLP, identification detection methods, ISSR, ITS-PCR, PCR-RFLP, *Phytophthora ramorum*, SSR, TaqMan®

Introduction

The genus *Phytophthora* comprises over 70 described species, however many new species have been reported recently as a result of the discovery of previously undetected species or by the hybridization of known species. *Phytophthora ramorum*, one of the new *Phytophthora* species, is considered a high phytosanitary risk because it is currently responsible for large-scale oak mortality in coastal California forests. In Europe, the disease occurs mainly in nurseries on *Rhododendron*, *Viburnum* and *Camellia*. However, in landscapes, infected trees of *Quercus rubra, Q falcata, Q. ilex, Aesculus hippocastanum* and *Fagus sylvatica* were reported recently on sites previously identified with infected *Rhododendron* plants. In addition, numerous nurseries in the U. S. have recently been diagnosed with infected *Rhododendron* and *Camellia* spp.

P. ramorum is a heterothallic species and is known to exist as two distinct populations in California/Oregon (U.S. type) and Europe (E.U. type), respectively. In Europe, almost exclusively A1 mating type isolates have been found, while in the United States A2 type isolates are most often identified. A single A2 isolate was found in Europe and recently several A1 isolates were identified in nurseries in the United States.

Measures are in force to prevent spread of this pathogen, as well as to prevent co-existence of both types. Therefore, adequate detection and identification methods are urgently needed.

Several molecular techniques have been developed for detection and characterization of this new species of *Phytophthora*. Molecular differences between U.S.- and E.U.-isolates of *P. ramorum* exist and can be demonstrated with the techniques at hand. Data thus far obtained of isolates from throughout Europe and from the United States will be discussed.

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Results

For identification of *P. ramorum* several methods have been used:

AFLP: Amplified Fragment Length Polymorphism

This method consists of digesting genomic DNA with two restriction enzymes (a rare and a frequent cutter), ligation of adapters to the restriction fragments and two rounds of amplification: the first round using primers of the adapter sequence, which amplify all restriction fragments, and a second round using a specific amplification of a subset of restriction fragments using extra selective bases in the primers. One of the second round primers is fluorescently labeled; therefore, running the amplicons on a sequencing system will generate a DNA fingerprint pattern. Relatedness of isolates or species can be seen by scoring the number of identical and unique bands; these data can be presented in a dendogram. Large numbers of *P. ramorum* isolates have been analyzed with AFLP (Ivors and others 2004). Results show two clusters, with E.U. isolates and U.S. isolates grouping separately. A1 mating type isolates from the U.S. and the E.U. A2 mating type isolate cluster within the E.U. clade.

ISSR: Inter Simple Sequence Repeats

ISSR is a technique that uses primers to amplify regions between two microsatellites utilizing PCR. Microsatellites are Simple Sequence Repeats (SSR) occurring in the genome of many organisms (e.g. $(AG)_{12}$ or $(CTG)_7$). Several primers were tested on two E.U. and two U.S. isolates. Many E.U. and U.S. isolates of *P. ramorum* were then screened with polymorphic primers Although differences were identified between E.U. and U.S. populations, polymorphisms were not identified within each population with this technique.

Microsatellites (SSR: Simple Sequence Repeats)

Using the whole genome sequence of *P. ramorum*, SSR techniques were used for fingerprinting large numbers of *P. ramorum* isolates originating from different host species within Europe and the United States. Using a computer program developed at Plant Research International, 1334 potential microsatellite loci were identified. Primers were selected from over 110 flanking regions of SSRs and tested in PCR reactions to amplify repeats. Thirty-one polymorphic loci were identified and 14 primer sets were optimized for isolate genotyping. Many loci showed variation between the E.U. and U.S. populations. Minor variation was found within the E.U. populations and even less variation within the U.S. population. This information provided insight regarding the amounts of genetic variation within populations, identified new genotypes, and separated isolates into two distinct lineages correlated with continental provenance. Comprehensive data will be published soon (Ivors and others 2006).

Sequence analysis of several genes

In a recent study several genes of numerous *Phytophthora* species were sequenced (Kroon and others 2004). ß-tubulin and elongation factor 1_ as nuclear genes and NADH dehydrogenase I and Cox1 as mitochondrial genes were sequenced and phylogenetic trees were generated. Results show a close relationship between *P. ramorum* and *P. lateralis*.

For detection of *P. ramorum*, several other methods can be used:

ITS-PCR

ITS regions of the rDNA have been sequenced for many *Phytophthora* isolates. PCR-based detection methods using primers derived from those sequences have been developed for many *Phytophthora* species. PCR primers for *P. ramorum* have been developed and validated in this way. Detection of the pathogen in plant, water and soil samples has been successful.

TaqMan® PCR (also known as real-time PCR)

A TaqMan® probe has been developed based upon the ITS sequence (Ivors and Garbelotto 2002). A TaqMan probe is a probe with two fluorescent labels that can be used in PCR for real time analysis. In this way, quantification of the pathogen in infected material can be estimated.

PCR-RFLP

A single nucleotide difference in the Cox 1 gene has been observed between E.U. and U.S. isolates of *P. ramorum*. This single nucleotide polymorphism (SNP) exists at an *Apo*I restriction site. Based on this SNP, a PCR-RFLP method was developed to distinguish between E.U. and U.S. isolates of *P. ramorum*. This test can also be used on infected material (*in planta*) without a pure culture isolate.

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Molecular Detection of *Phytophthora ramorum* by Real-Time PCR Using Taqman, SYBR®Green and Molecular Beacons with Three Genes[1]

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Key words: β-tubulin, CBEL, diagnosis, elicitin, genotyping, sudden oak death

Abstract

Sudden oak death, caused by *Phytophthora ramorum*, is a severe disease that can affect numerous species of trees and shrubs. This pathogen has been spread via nursery stock, and quarantine measures are currently in place to prevent further spread. Molecular assays have been developed to rapidly detect and identify *P. ramorum*, but one difficulty encountered with some of these assays is the inability to reliably distinguish between *P. ramorum* and closely related species. To increase redundancy and therefore confidence, ß-tubulin and elicitin regions from a collection of *Phytophthora* species were sequenced and searched for single nucleotide polymorphisms (SNPs). New assays were designed based on internal transcribed spacer (ITS) sequences as well as new ß-tubulin and elicitin sequences. Primers specific to *P. ramorum* were designed to amplify a 171-bp fragment of ß-tubulin and these were used in a real-time PCR assay in conjunction with molecular beacons, Taqman probes and SYBR®Green, to compare the three reporter systems. The best performing system (Taqman probes) was also used to compare the three DNA regions. The real-time PCR assays differentiated *P. ramorum* from the 65 *Phytophthora* species tested, including *P. lateralis*. The assays were also used with DNA extracted from plants infected with *P. ramorum*. Overall, ITS and elicitin Taqman assays had the best combination of sensitivity and specificity. Intra-specific polymorphisms were also discovered in ß-tubulin and CBEL (cellulose binding elicitor lectin). Allele specific oligonucleotide (ASO) assays were developed for real-time PCR with SYBR®Green to distinguish between North American and European *P. ramorum*. These markers, used with Belgium samples possessing the two mating types, occurred in isolates with different DNA backgrounds. With the availability of the *P. ramorum* genome, more genes were analysed for polymorphisms. Multiple intraspecific and interspecific polymorphisms were found in most genes assayed by sequencing. We have sequenced 12 genes with polymorphisms and discovered 61 new SNP markers. The combination of SNP polymorphisms of an organism can provide

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a fingerprint or a biological barcode that uniquely identifies an individual. Using these 61 SNPs we found that isolates from Holland and Belgium had identical DNA profiles to samples from British Columbia (B.C.) isolated in 2003, but that profile was distinct from those found in the California isolates at all 61 SNPs. However, isolates from B.C. discovered in 2004 had a DNA profile that matched 100% with DNA profiles of the California isolates. Genotyping assays are currently being developed to allow simultaneous diagnosis at the species level, as well as for multilocus fingerprinting of individuals. This should be a useful tool in understanding the origin and migration of *P. ramorum.*

Using Single Strand Conformational Polymorphisms (SSCP) to Identify *Phytophthora* Species in Oregon Forests Affected by Sudden Oak Death[1]

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Key words: Phytophthora ramorum, Phytophthora species identification, sudden oak death

Introduction

Phytophthora species are abundant in streams, widespread in soils and occasionally found in diseased plants in the tanoak forests of southwestern Oregon. It is time-consuming and expensive to identify hundreds of isolates to species using morphology or internal transribed spacer (ITS) sequencing. We modified a published *Phytophthora* single strand conformation polymorphism (SSCP) protocol (Kong and others 2003) to use fluorescent-labeling chemistry and an additional marker locus to allow quantitative matching of unknown isolates.

Methods

The ITS1 region of rDNA was amplified with primers ITS6 and ITS7 (Cooke and others 2000) labeled with fluorescent HEX and FAM, respectively, yielding approximately a 300-bp product. The mitochondrial COX gene spacer region (Martin and Tooley 2003) was amplified with primers FMPh8 and FMPh10 labeled with fluorescent HEX and FAM, respectively, yielding approximately a 500-bp product. Amplified products were mixed with formamide and ROX 500 marker, heated to 95 ºC for 3 minutes and cooled in ice for 5 minutes. Samples were run on an ABI 3100 Capillary Sequencer with a 36-cm array and 4 percent GeneScan polymer, with 10 percent glycerol, and 1X TBE, at 25ºC. Fluorescence was analyzed with GeneScan 3.7 and electrophoretic mobility reported as scan number.

Results

Electropherograms generated by GeneScan typically revealed one peak for the forward strand and one peak for the reverse strand in both ITS1 and COX gene spacer regions. All reference

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isolates were well separated, except when comparing the closely related *P. cambivora* and *P. europaea*. Repeat analysis of references showed good repeatability.

We drew a blind sample of 54 unknowns from a larger collection of isolates from streams, soil and plants, and ran a preliminary test with 16 reference *Phytophthora* species, chosen for their perceived relevance to the sampling region. The sixteen references consisted of *P. cambivora*, *P. citricola*, *P. citrophthora*, *P. europaea*, *P. fragariae*, *P. gonapodyides*, *P. hibernalis*, *P. ilicis*, *P. lateralis*, *P. megasperma*, *P. nemorosa*, *P. pseudosyringae*, *P. psychrophila*, *P. ramorum*, *P. syringae* and *P.* taxon 'Pg chlamydo.' SSCP separated the 54 unknowns into 11 distinct groups. Five isolates matched taxon 'Pg chlamydo,' six isolates matched *P. nemorosa*, 22 isolates matched *P. gonapodyides*, and one isolate matched *P. ramorum*. The remaining seven unmatched groups suggest a large diversity of *Phytophthora* species in natural environments. Four isolates from one unique group have been characterized by ITS sequence and culture morphology, and appear to be a new species of *Phytophthora*. Several of the unknown isolates from stream samples were identified as *P. nemorosa*, previously known only from tanoak cankers in our area.

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Application of Rapid Onsite PCR (TaqMan®) for *Phytophthora ramorum* Under U.S. Conditions[1]

Kelvin Hughes[2], Jenny Tomlinson[2], Neil Boonham[2], Kelly Ivors[3], Matteo Garbelotto[3], and Ian Barker[2]

Key words: on-site PCR, *Phytophthora ramorum*, SmartCycler®, sudden oak death, TaqMan®

Abstract

Currently, diagnosis of *Phytophthora ramorum* involves sending samples to a laboratory for traditional isolation and morphological characterisation, and/or PCR analysis. This can take as long as 2 weeks from sampling to final diagnosis. However, the Plant Health Group, Central Science Laboratory, has produced on-site DNA extraction and real-time PCR (TaqMan®) methods, which use field-stable reagents for diagnosis of symptomatic infections of *P. ramorum*, in under 2 hours (Tomlinson and others, 2005). In March 2004 this method was evaluated, with the help of the University of California, Berkeley and the USDA Forest Service, on leaf and stem samples from 21 plant species collected from four sites in the vicinity of San Francisco, California. DNA was extracted from each sample using a Bio-Nobile QuickPick™ plant kit and added to a TaqMan® reaction mix developed for on-site use. This contained reagents for specific amplification of *P. ramorum* DNA and a universal plant gene (cytochrome oxidase (COX), used as an internal reaction control). Each sample was subjected to thermal cycling in a Cepheid SmartCycler® and the real-time data from these reactions interpreted. The majority of testing was performed at the University of California, Berkeley, for logistical reasons. However, on one day, samples were tested in the field at China Camp State Park using the complete on-site protocol in less than 2 hours, confirming this protocol can be performed independent of all laboratory support. At the University of California, Berkeley, plant material was plated out onto a *Phytophthora*-specific media (PARP) for comparative purposes to determine the presence of *P. ramorum* in each sample. COX amplification was achieved for all 21 host species with *P. ramorum* being detected by TaqMan® and isolation from leaf samples of tanoak, Douglas-fir, honeysuckle and bay laurel. *P. ramorum* was also identified by TaqMan® from stem samples of madrone, Shreve's oak, tanoak, redwood and Douglas-fir, but only isolated from madrone and tanoak. This study showed that real-time PCR diagnosis of *P. ramorum* can be performed on-site in under 2 hours on symptomatic stem and leaf material. This protocol will be further developed in the E.U. Portcheck project that aims to develop on-site methods for diagnosis of E.U. plant pathogens at national inspection points. Its use for on-site asymptomatic detection for *P. ramorum* is also underway.

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Use of Microsatellite Markers Derived From Whole Genome Sequence Data for Identifying Polymorphism in *Phytophthora ramorum*[1]

Kelly Ivors[2], Matteo Garbelotto[3], Ineke de Vries[4], and Peter Bonants[4]

Abstract

Investigating the population genetics of Phytophthora ramorum, the causal agent of sudden oak death (SOD), is critical to understanding the biology and epidemiology of this important phytopathogen. Raw sequence data (445,000 reads) of P. ramorum was provided by the Joint Genome Institute. Our objective was to develop and utilize Simple Sequence Repeat (SSR) techniques for fingerprinting large numbers of P. ramorum isolates originating from different host species within Europe and the United States. Using a computer program developed at Plant Research International, 1334 potential microsatellite loci were identified. Primers were selected from over 110 flanking regions of SSRs and tested in PCR reactions to amplify repeats. Thirty-one polymorphic loci were identified and 14 primer sets were optimized for isolate genotyping. Three loci showed variation among nursery isolates from Europe and the United States, although no variation was identified among isolates from forests in the United States. This information provided insight regarding genetic variation within populations, identified new genotypes, and separated isolates into two distinct lineages correlated with continental provenances.

Key words: microsatellites, *Phytophthora ramorum*, polymorphism

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Single-Strand Conformation Polymorphism Analysis of Ribosomal DNA for Detection of *Phytophthora ramorum* Directly From Plant Tissues[1]

Ping Kong[2], Patricia A. Richardson[2], Chuanxue Hong[2], and Thomas L. Kubisiak[3]

Abstract

At the first Sudden Oak Death Science Symposium, we reported on the use of a single strand conformation polymorphism (SSCP) analysis for rapid identification of *Phytophthora ramorum* in culture. We have since assessed and improved the fingerprinting technique for detecting this pathogen directly from plant tissues. The improved SSCP protocol uses a single run PCR reaction with the same primer pair (ITS6/7) and consistently detects *P. ramorum* at 10 fg per reaction or above. It provides reliable diagnoses of *P. ramorum*, whether it is a single infection or dual infection (a second *Phytophthora* species involved). This technique also can provide accurate diagnoses of diseases caused by 12 other species of Phytophthora without additional work. These species (*P. cactorum, P. cambivora, P. cinnamomi, P. cryptogea, P. citricola, P. citrophthora, P. gonapodyides, P. lateralis, P. megasperma, P. nemorosa, P. nicotianae and P. pseudosyringae*) are common in ornamental plant and forest tree nurseries, as well as in natural forested environments. The through-put capacity of this technique can be greatly improved by use of fluorescence-based technologies, such as those common to most commercially available DNA sequencers. This study provides an alternative protocol with increased detection scope and accuracy, at a reduced cost, for future surveys of nurseries, parks and forests for *Phytophthora* species.

Key words: Phytophthora ramorum, single strand conformation polymorphism, sudden oak death

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AFLP Analysis of *Phytophthora nemorosa* and *P. pseudosyringae* Genetic Structure in North America[1]

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Key words: California, endemic, forest *Phytophthora* species, genetic diversity, homothallic, Oregon

Introduction

In California and Oregon, *Phytophthora ramorum* has an overlapping host and geographic range with two newly described homothallic *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*. *P. nemorosa* alone causes symptoms similar to those of *P. ramorum*, including lethal tanoak cankers, and *P. pseudosyringae* is associated with oak decline in Europe. However, epidemiological observations, namely broader geographic distribution and reduced virulence, suggest *P. nemorosa* and *P. pseudosyringae* are endemic in this region, while *P. ramorum* is hypothesized to have been introduced. Though molecular evidence suggests that *P. nemorosa* and *P. pseudosyringae* are each other's closest known relatives, both are rather distantly related to *P. ramorum*. Little is known about the characteristics of these newly described forest pathogens; however, the two putative endemic *Phytophthora* species can apparently share the same niche as *P. ramorum* and may affect *P. ramorum* disease epidemiology. Understanding their genetic structure, then, may contribute to understanding the range of potential interactions and outcomes during infection by these *Phytophthora* species. Our aim is a preliminary assessment of the genetic structure of *P. nemorosa* and *P. pseudosyringae* in western North America using Amplified Fragment Length Polymorphism (AFLP) genetic markers.

Materials and Methods

We analyzed 38 *P. nemorosa* and 29 *P. pseudosyringae* isolates selected from throughout most of their known host and geographic ranges in the western United States and included one *P. ilicis* isolate for use as an outgroup. Distribution of the isolates was rather continuous

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through the west coast of California and Oregon, excepting one *P. nemorosa* and two *P. pseudosyringae* isolates from Mariposa County, CA. For each isolate, total genomic DNA was extracted from 10 to 20 mg freeze-dried mycelium using the Puregene DNA extraction kit (Gentra, Minneapolis, MN). AFLP reactions were performed as in Ivors and others. (2004). We screened a total of 18 primer pairs for each species, selecting from among them six informative primer pairs for *P. nemorosa* and four for *P. pseudosyringae.* As there was only partial overlap between the informative primer pairs for each species, we included two isolates of *P. nemorosa* in the analysis of the full *P. pseudosyringae* dataset, along with *P. ilicis*, to function as outgroups, and *vice versa* for the full *P. nemorosa* analysis. The AFLP procedure was replicated for 10 percent of isolates in this preliminary work. Data analysis was performed as in Ivors and others (2004). To assess the degree of genetic similarity between each isolate and all of the others, we calculated a distance matrix for each species using the Jaccard coefficient of similarity, which measures the proportion of shared AFLP markers in a pairwise fashion, while correcting for the dominant nature of the data. We then constructed a neighbor-joining dendrogram for *P. nemorosa* and one for *P. pseudosyringae;* outgroups used in the dendrograms were as above.

Results

We scored 214 AFLP bands for *P. nemorosa,* 20 of which (10.7 percent) were polymorphic, and identified a total of 12 distinct AFLP genotypes among the 38 *P. nemorosa* isolates. Our measure of degree of genetic similarity, Jaccard similarity coefficients (S_J), fell between 0.941 and 1.000 (0.990 ± 0.011; mean ± SD) for *P. nemorosa* isolates – a score of 1.0 is equivalent to 100 percent identity. Over 50 percent of the pairwise comparisons showed 100 percent genetic similarity between isolates. There was no evidence of partitioning by host or by area of origin of the isolates based upon the branching pattern of the neighbor-joining dendrogram (tree not shown). We scored 198 AFLP bands for *P. pseudosyringae,* 20 of which (9.9 percent) were polymorphic, and identified 13 distinct AFLP genotypes among the 29 *P. pseudosyringae* isolates. The range of S_J values was 0.953 to 1.000 (0.987 ± 0.011; mean ± SD), with only about 20 percent of the pairwise comparisons yielding 100 percent similarity values within *P. pseudosyringae* isolates. As with *P. nemorosa,* the neighbor-joining dendrogram yielded no evidence of partitioning of the genetic variation by host or area of isolate origin (tree not shown).

Discussion

In our preliminary assessment of the genetic structure and diversity of both *P. nemorosa* and *P. pseudosyringae* from the west coast of the United States, we found extremely high levels of within-species genetic similarity and no evidence of partitioning of genetic diversity based upon the host or site from which study isolates were collected, including those from the isolated site in Mariposa county. The degree of genetic similarity is comparable to that found

in a similar study carried out for *P. ramorum*, in which the North American population is dominated by a single clone genotype with a few rare, but highly genetically similar, variants (Ivors and others 2004). While this lack of genetic diversity is unsurprising for the putatively non-native *P. ramorum*, as exotic species often undergo a population genetic bottleneck upon introduction to a new area, it is somewhat unexpected for a native organism. Therefore, our results for *P. nemorosa* and *P. pseudosyringae* are consistent with the hypothesis of recent, or relatively recent in the context of evolutionary time, introductions. However, there is a scarcity of studies on the genetic structure and diversity of homothallic, endemic *Phytophthora* species outside of the intense, artificial selection regimen of agriculture. These *Phytophthora* species may be endemic, and the low amount of genetic variation and lack of structure in the variation present may be due to self-compatible sexual reproduction. Also, the AFLP primers chosen may have had insufficient resolution to detect existing genetic variation. To assist us in distinguishing between these hypotheses, we will repeat and fully replicate this preliminary study using more isolates, expanding the host and geographic ranges covered, and including multiple European *P. pseudosyringae* isolates.

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Molecular Markers for Identification of *P. ramorum* and Other *Phytophthora* species From Diseased Tissue[1]

Frank N. Martin[2] and Paul W. Tooley[3]

Abstract

Molecular techniques have been developed for detection and identification of *P. ramorum* and other *Phytophthora* species that are based on the mitochondrially encoded sequences. One technique uses a *Phytophthora* genus specific primer to determine if a *Phytophthora* species is present, followed by a nested amplification with species-specific primers to determine if the pathogen is *P. ramorum* or one of several other species (such as *P. nemorosa* or *P. pseudosyringe*). This procedure also uses a plant primer pair to serve as a positive control ensuring the ability of the template DNA to amplify. These species-specific and plant markers have been modified for use with real-time PCR. A second technique relies on RFLP analysis of the Cox I and II gene cluster to identify isolates of *Phytophthora* to a species level.

Key words: detection, identification, mitochondrial DNA, *Phytophthora ramorum*

Introduction

The use of molecular methods for detection of plant pathogens can reduce the time required to process samples, as well as improve the accuracy of pathogen identification. Rather than focus on identifying a single species, methods that focus first on identification at the genus level, followed by a nested species-specific detection, could facilitate identification of a broader number of pathogens. For example, while *P. ramorum* is the causal agent of sudden oak death, other species can be recovered from forest samples exhibiting similar symptoms. The diversity of *Phytophthora* species recovered from nursery samples that cause symptoms similar to *P. ramorum* would be even greater. Having a marker system that would first accurately determine if a *Phytophthora* species was present, and then allow identification to a species level, would simplify sample processing, as well as provide additional data on the diversity of *Phytophthora* species present on the hosts under investigation.

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Species-specific PCR Detection

A PCR detection system has been developed that is based on amplification of the spacer region between the mitochondrially encoded Cox I and II genes of *Phytophthora* using genus-specific primers from the conserved regions of the flanking genes (Martin and others 2004). The first round, multiplex amplification contained two primer pairs, one for amplification of plant sequences to serve as an internal, positive control to ensure that extracted DNA was of sufficient quality to allow for PCR amplification, and a second primer pair specific for amplification of sequences from *Phytophthora* species. The products of the first round amplification were amplified with a nested primer pair that was specific for either *P. ramorum*, *P. nemorosa* or *P. pseudosyringae*. Using purified pathogen DNA, the limit of detection for *P. ramorum* using this marker system was approximately 2.0 fg of total DNA. However, when this DNA was spiked with DNA from healthy plant tissue extracted with a commercial miniprep procedure, the sensitivity of detection was reduced by 100- to 1000-fold. This was dependent on the plant species and suggests that the techniques used for DNA extraction did not remove all PCR inhibitors. Using a dilution series of purified DNA from *P. ramorum* the nested mitochondrial marker system was found to have a level of sensitivity comparable to the nested ITS marker system (Garbelotto and others 2002). This marker system was validated with DNA extracted from naturally infected plant samples collected in the field by comparing the sequence of the *Phytophthora* genus-specific amplicon, morphological identification of cultures recovered from the same lesions, and for *P. ramorum*, amplification with a previously published rDNA ITS species-specific primer pair. Results were compared and validated with three different brands of thermalcyclers in two different laboratories to provide information about how the described PCR assay performs under different laboratory conditions. The specificity of the *Phytophthora* genus-specific primers suggests they will have utility for pathogen detection in other *Phytophthora* pathosystems, and the variability encountered in the spacer regions should be useful for constructing additional species-specific primers (markers have been developed for five additional species). More recent work has demonstrated that RFLP analysis of the genus-specific amplicons can be used for identification of unknown isolates (Martin, unpublished). The plant primers and species-specific primers for *P. ramorum* and *P. pseudosyringae* from this marker system also have been adopted for use with TaqMan real-time PCR (Tooley and others 2004 and unpublished). Conditions for multiplexed amplification with the plant, *P. ramorum*, and *P. pseudosyringae* primers have been identified and additional work to add *P. nemorosa* to this multiplexed amplification is in progress.

Identification of *Phytophthora* spp. to Species Level by RFLP Analysis

A RFLP method has been developed for identification of *Phytophthora* species based on PCR amplification of a region spanning the mitochondrially encoded Cox I and II genes (Martin and Tooley 2004). Of the 24 species where multiple isolates were examined, intraspecific

polymorphisms were not observed for 16 species, while five species exhibited limited intraspecific polymorphism that could be explained by the addition/loss of a single restriction site. Intraspecific polymorphisms were observed for three species; however, these differences may be a reflection of the variation that has been reported in the literature. The use of the computer program BioNumerics simplified data analysis and identification of isolates. Successful template amplification was obtained with DNA recovered from hyphae using a boiling miniprep procedure, thereby reducing the time and materials needed for conducting this analysis. While the primers were capable of amplifying target DNA from 300 isolates of 31 species in the genus *Phytophthora* tested at the time of publication, additional testing has identified several species that do not amplify. Work is currently in progress to develop primers to amplify all *Phytophthora* species, but not plant DNA, to enable their use for amplification directly from diseased tissue (Martin, unpublished). Additional details on these marker systems can be found at http://www.ars.usda.gov/Research/docs.htm?docid=8728.

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Genome Sequence of *Phytophthora ramorum*: Implications for Management[1]

Brett Tyler[2], Sucheta Tripathy[2], Nik Grunwald[3], Kurt Lamour[4], Kelly Ivors[5], Matteo Garbelotto[6], Daniel Rokhsar[7,8], Nik Putnam[7], Igor Grigoriev[7], and Jeffrey Boore[7,8]

Key words: DNA sequence, *Phytophthora ramorum*

Abstract

A draft genome sequence has been determined for *Phytophthora ramorum*, together with a draft sequence of the soybean pathogen *Phytophthora sojae*. The *P. ramorum* genome was sequenced to a depth of 7-fold coverage, while the *P. sojae* genome was sequenced to a depth of 9-fold coverage. The genome size of *P. ramorum* was estimated to be significantly smaller than that of *P. sojae*, 65 Mb compared to 95 Mb, with the difference lying primarily in the amount of repetitive sequences in the *P. sojae* genome. Computer predictions estimate the number of genes in *P. ramorum* to be 15,743, while 19,027 are predicted for *P. sojae*. Most of the differences in gene number result from larger multigene families in *P. sojae*. Six hundred twenty four genes were predicted to be unique to *P. ramorum*, while 1755 were predicted to be unique to *P. sojae*. The generally high level of similarity of most *P. ramorum* and *P. sojae* genes predicts that, in general, chemical treatments developed for other *Phytophthora* species should also be effective against *P. ramorum*. The small size of the *P. ramorum* genome and lack of extensive numbers of duplicated chromosomal segments effectively eliminates the hypothesis that *P. ramorum* is a recent hybrid between two other *Phytophthora* species. The two *Phytophthora* genome sequences are available at http://genome.jgi-psf.org/ and http://phytophthora.vbi.vt.edu.

A critical need in understanding the epidemiology of *P. ramorum* is the need to be able to distinguish different genetic individuals of *P. ramorum* so that patterns of spread can be traced. However, very little genetic variation can be detected in *P. ramorum* isolates from the United States, using conventional techniques such as AFLPs (Ivors and others 2004), presumably because most of the population has derived clonally from a single introduction or a small number of introductions of closely related strains. The *P. ramorum* genome sequence now offers the possibility of examining the genome directly for regions that may be useful in genetically distinguishing closely related strains. Simple Sequence Repeats (SSRs) or microsatellites have been used for genetic typing of an extensive variety of eukaryotic organisms. A total of 1,000 microsatellite loci were observed in the genome of *P. ramorum*.

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Dinucleotide repeats were the most abundant microsatellite repeats making up 56 percent of all repeats followed by trinucleotide repeats at 29 percent. Single Nucleotide Polymorphisms (SNPs) offer another resource for identifying recent variation, such as gene conversion or mitotic crossing over (Chamnanpunt and others 2001). Sequencing of the *P. ramorum* genome identified approximately 200,000 sites at which the genome sequence of this diploid organism is polymorphic. Screening of these SSR and SNP sites is underway to determine whether any of these sites are variable enough to detect recent genetic divergence in the *P. ramorum* population that could be used to track patterns of spread.

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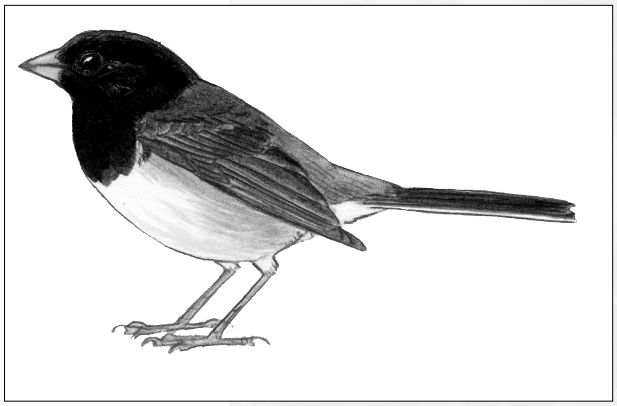
Biology II



The Importance of Humans in the Dispersal and Spread of *Phytophthora ramorum* at Local, Landscape, and Regional Scales[1]

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Key words: plant disease spread, human dispersal, effects of recreation

Abstract

Determining how *Phytophthora ramorum* is dispersed across the landscape is critical for understanding the ecology and epidemiology of this influential pathogen. To date, researchers have shown that abiotic factors – such as rain-splash, wind-blown rain and down-stream transport of inoculum – are critical mechanisms for the dispersal of this pathogen. In contrast, little research has focused on the potential for human dispersal of *P. ramorum*, although work on other pathogens – such as *P. lateralis* attacking Port Orford cedar – suggests that humans can play an influential role in disease spread. Here, we summarize results from a study conducted in Sonoma County that addresses three research questions examining the importance of human dispersal at the local, landscape and regional scales: 1) Is *P. ramorum* more frequently found in soil along hiking trails than in soil off trail? 2) Do areas visited intensively by humans have a greater proportion of hosts showing symptoms of infection by *P. ramorum* than areas visited less frequently? and 3) Do wildlands surrounded by dense human populations have greater levels of infection by *P. ramorum* than areas surrounded by less dense populations?

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

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dispersal agents of *P. ramorum* and are able to transport the pathogen into areas that lack a local source of inoculum.

To determine whether disease symptoms were greater in areas with high human activity, we used 202 15x15-m plots established in a 275-km^2 area in eastern Sonoma County. Approximately half of these plots occurred in areas that experienced high visitation rates by humans (primarily county and state parks), whereas the other half occurred in areas that had low visitation rates (primarily rural private land). During the spring and early summer of 2004, we sampled all foliar and terminal hosts in these plots for symptoms of infection by *P. ramorum*. After taking into account the influence of elevation, precipitation, solar radiation (February-April), topography, and cumulative DBH of bays (a host that is thought to play an important role in disease establishment), we found that the proportion of symptomatic bay trees was significantly greater in plots experiencing high levels of human activity than those with low activity levels. We also performed the same analysis on terminal hosts – coast live oak, black oak (*Q. kellogii*), and tanoak (*Lithocarpus densiflorus*) with trunk cankers and/or canopy dieback – but did not detect a significant effect of human activity level. We hypothesize that effects similar to those found for bays will eventually appear, but that there are lag effects because foliar hosts at a site get infected by *P. ramorum* first and only later do terminal hosts become infected.

To evaluate the relationship between human density surrounding wildlands and disease incidence, we first delineated a 'zone of infestation' in the 14 Bay Area counties of northern California where *P. ramorum* and sudden oak death are known to occur. This was achieved by inscribing a 20-km buffer around the 275 sites that have been confirmed by the California Department of Food and Agriculture (CDFA) to contain host plants infected with *P. ramorum* (see the California Oak Mortality Task Force's webpage: www.suddenoakdeath.org). Within this zone, we examined the infection status of all foliar hosts present at 166 randomly located sites, based on data collected by Meentemeyer et al. (this volume). Two 50 x 10-m belt transects were established at each site, foliar hosts contained within these transects were assessed for evidence of infection by *P. ramorum*, and tissue from all symptomatic hosts was evaluated by CDFA to test for the presence of *P. ramorum*, using culturing and PCR techniques. Results from this assessment revealed that *P. ramorum* was detected on foliar hosts at 33 sites, whereas hosts at 133 sites showed no evidence of disease. We then evaluated the degree to which human density predicted the presence of *P. ramorum* at these 166 sites, after taking into account the influence of six potentially influential variables. Human population density within a 50-km radius of each site was quantified in a GIS using data from the U.S. Census Bureau; minimum daily temperature (December-February), maximum daily temperatures (December-May), annual precipitation, and relative humidity (December-May) were estimated using the PRISM model; fire history since 1950 was estimated from records compiled by the California Department of Forestry and Fire Protection; and the presence or absence of bay and tanoak was quantified in the field at each of the 166 sites by Meentemeyer and others (this volume). Our analysis indicated that *P. ramorum* was more likely to be present in wildlands that were surrounded by high human population density. In addition, *P. ramorum* was more likely to be found in plots containing bay and receiving greater amounts of precipitation.

In summary, we have presented three lines of evidence – at the local, landscape and regional scales – which each suggest that intensive human activity is associated with – and we hypothesize leads to – increased levels of disease in bays, a foliar host that is thought to play a key role in the establishment

and spread of sudden oak death. Our data indicate that there may be conflicts between recreation and disease spread, and that efforts to address this epidemic may require active management of human activity.

Summer Survival of *Phytophthora ramorum* in Forest Soils[1]

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Key words: chlamydospore, *Phytophthora ramorum*, soil, sudden oak death, summer survival

Abstract

Recovery of *Phytophthora ramorum* from soils throughout Sudden Oak Death-affected regions of California illustrates that soil serves as an inoculum reservoir for the pathogen, but the potential for survival in soils throughout the summer is largely unknown. In this study we assess pathogen survival in infected leaf tissue in the upper soil profile in a redwood-tanoak forest ecosystem in Sonoma County, California.

Over 4,000 rhododendron leaf disks were inoculated with an isolate of *P. ramorum* that was recovered from soil at Jack London State Park. Inoculated leaf disks were incubated for one week in a moist chamber to allow for colonization of leaf tissue. Ten leaf disks were placed in each of 360 mesh sachets and transferred to the field in April 2004. The sachets were dispersed under 10 trees each of tanoak (*Lithocarpus densiflorus*), California bay laurel (*Umbellularia californica*), and coast redwood (*Sequoia sempervirens*), and at three vertical locations: i) leaf litter surface, ii) litter/soil interface, and iii) below the soil surface. Sachets were arranged vertically within partially submerged cylindrical barriers to prevent lateral movement of *P. ramorum* and were retrieved 1, 2, 8 and 24 weeks after introduction.

At each time point, sachets were retrieved from the field, and soil samples were taken from within the cylindrical barriers and from the bulk soil. In the laboratory, the wet weight of leaf disks was recorded as a measure of water retention; soil moisture content was also determined. *P. ramorum* was baited from soil samples, both inside the cylinders and from the bulk soil under each tree. Pathogen recovery was determined by submerging the leaf disks in selective medium and incubating at room temperature for 14 days. If a leaf disk was considered *P. ramorum*-negative, that disk was subsequently incubated in water for three weeks before being returned to selective medium. After determination of pathogen recovery, leaf disk samples retrieved after 24 weeks were cleared with 1 M KOH and chlamydospore populations were assessed within the tissue.

After one week in the field, the pathogen was recovered from 1 percent of the disks at the leaf litter surface. Recovery from the two subsurface treatments remained over 80 percent over the first 2 weeks. After 8 weeks 80 percent and 65 percent pathogen recovery were observed in the soil and at the

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litter/soil interface, respectively. Over 60 percent pathogen recovery was observed in leaf disks remaining in the soil for 24 weeks. After incubation of P. ramorum-negative disks in water, recovery at 8 weeks was enhanced by 3 percent and 10 percent in soil and interface disks, respectively. Significantly more chlamydospores were observed in leaf disks ranked P. ramorum-positive after incubation in water than those ranked P. ramorum-negative, and chlamydospore germination was occasionally observed within *P. ramorum*-positive leaf disks. Furthermore, leaf disks incubated in soil contained significantly more chlamydospores than those incubated at the surface or litter/soil interface.

Soil moisture content varied under the different tree species, with the highest moisture content persisting under redwoods, as well as tree species associated with organic soils. Soil moisture content was positively correlated with wet weight of leaf disks ($P \leq 0.01$), and leaf disk wet weight was significantly correlated with recovery ($P \leq 0.01$) over the first 8 weeks; however, no direct correlation between soil moisture content and pathogen recovery was observed. *P. ramorum* was baited from soil within 33 percent of the cylinders 1 week after introduction of inoculum, and from 16 percent of the cylinders at every subsequent sample time. Conversely, *P. ramorum* was rarely recovered from soil sampled outside the cylinders.

Heightened recovery of *P. ramorum* from soils after a period of oversummering suggests that infested soil may serve as a source of primary inoculum for fall disease development. The ability to bait *P. ramorum* from soils within cylinders demonstrates the transmission of the pathogen from infected leaf tissue to the bulk soil. Recovery of *P. ramorum* after incubation in water coupled with high populations of chlamydospores in water-soaked P. ramorum-positive tissue suggests the potential role of hydration in breaking chlamydospore dormancy. Further studies are needed to address the relationship between pathogen survival and soil moisture content and soil matric potential; however, the results of this study suggest that natural pedoturbation processes resulting in burial of inoculum may enhance pathogen survival.

Monitoring of Natural Outbreaks of *Phytophthora ramorum* in the United Kingdom[1]

Judith Turner[2], Alex Appiah[2], Philip Jennings[2], Gilli Humphries[2], Debbie Liddell[2], Sam McDonough[2], Jackie Stonehouse[2], David Lockley[3], and Stephen Eales[4]

Key words: Phytophthora ramorum, sudden oak death, management, monitoring

Abstract

Over 40 outbreaks of *Phytophthora ramorum* have occurred in managed gardens in the United Kingdom. Three of these sites, one in the southeast of England and two in the southwest, have been closely monitored since October 2003. These sites represented differing disease scenarios at the start of monitoring, as eradication action had already taken place at the site in the southeast, whereas no action had taken place in the southwest. The progress of existing infections and the development of new infections were monitored at all sites. A number of current or previously infected areas within the gardens was selected for experimentation and marked out in 1-m^2 grids to cover at least 3 m in all directions from the original infected plant. Samples of soil and leaf litter, taken each month, were analyzed for presence of *P. ramorum* by isolation and microscopic examination of the cultures; positives were confirmed by TaqMan® PCR. In areas of high infection, sequential sampling was carried out through the soil profile to examine the depth of contamination. Baits were deployed along all watercourses on a 3-month basis to investigate extent of contamination in ponds and streams within the gardens, and to quantify the effect of seasonal variation on inoculum levels. The influence of environmental factors on timing and quantity of spore release was investigated using volumetric spore samplers, rainwater traps and logging of meteorological data by weather stations. Sampling strategies have successfully established the level of contamination at each of the sites and have shown that the pathogen can survive winter conditions in the south of England within plant material and in the wider environment. Monitoring of the selected sites revealed different levels of infection in terms of the number of plants affected and the degree of soil contamination. Higher levels of infection and contamination occurred in the southwest sites. No direct evidence of significant vector involvement in pathogen dispersal was found, but a number of pathways used by members of the public were found to be infested in the southeast site. The seasonal baiting of watercourses at this site has indicated widespread contamination of streams, ponds and lakes. However, significantly lower levels of *P. ramorum* contamination were detected in the summer when compared to the spring. This may reflect a general reduction in inoculum as a result of the earlier eradication strategies adopted but may also

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indicate less favorable conditions for sporulation and spread during the summer. There have been no new plant infections at the southeast site (other than on regrowth) and levels of soil and leaf litter contamination are low. This also suggests that the strategy of early removal of infected plants and surface leaf litter has been successful in reducing disease inoculum and spread.

Examining the Relationship between Fire History and Sudden Oak Death Patterns: A Case Study in Sonoma County[1]

Max A. Moritz[2] and Dennis C. Odion[3]

Abstract

Fire is often integral to forest ecology and can affect forest disease dynamics. Sudden oak death has spread across a large, fire-prone portion of California, killing large numbers of oaks and tanoaks and infecting most associated woody plants. Building on our earlier study of fire-disease dynamics, we examined spatial patterns of confirmed infections in relation to past fire history for a single county using a geographic information system (GIS). The region analyzed here is Sonoma County, where infections have been documented in past burn areas and exposure to the disease is thought to be extensive. We found that the disease is extremely rare inside recently burned areas (since 1950), although the relationship in this region is somewhat weaker than previous findings across a much larger area. This highlights how factors, such as variation in vegetation, climate, and patterns of severity, within burn areas are important to consider in the landscape pathology of sudden oak death. Despite confirmation of earlier findings at a different scale of analysis, it remains to be seen whether the observed negative relationship between current infection locations and past fires is due to a fire-related mechanism, a surrogate of fire, and/or an artifact of the datasets used. Regardless, it is likely that interactions between the disease and fire will vary with local differences in host species composition, the stage of disease progression, and the scales of space and time used in analyses.

Key words: spatial pattern analysis, landscape pathology, fire management, forest disease epidemiology

Introduction

Sudden oak death (SOD), a disease caused by the pathogen *Phytophthora ramorum*, has reached epidemic proportions over the last decade across landscapes of northern coastal California. Based on climatic factors and host distributions, there are large portions of the state at risk for the disease, including ecosystems of the Sierra Nevada foothills (Meentemeyer

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and others 2004, Guo and others 2005). Suitable habitat for the disease may exist over extensive areas in the western U.S. and beyond, and there is great concern over the cascading ecological effects that will occur in affected areas (Rizzo and others 2002, Rizzo and Garbelotto 2003). A variety of modeling tools exist for predicting the spatial-temporal dynamics of plant disease epidemics (van Maanen and Xu 2003), although presently not enough is known about SOD to parameterize such models.

Forest diseases involve interactions among pathogens and hosts in heterogeneous, dynamic environments (Lundquist and Klopfenstein 2001), all of which are components of the plant "disease triangle." This conceptual model illustrates a fundamental paradigm in plant pathology: that is, the existence of a disease caused by a biotic agent requires the interaction of a pathogen, susceptible hosts, and an environment favorable to disease development (Stevens 1960). Recently there has been increasing awareness of landscape-scale factors in forest diseases, exemplified by the emergence of "landscape pathology" as a field of study (Holdenrieder and others 2004). This approach is an extension of the traditional disease triangle that explicitly includes coarse-scale landscape patterns and processes as part of disease dynamics. Examples include landscape fragmentation effects on pathogen dispersal and how vegetation diversity patterns might limit the availability of susceptible hosts (Holdenrieder and others 2004).

Fire is one of the dominant pattern-forming forces on terrestrial landscapes (Bond and others 2005), and it is an important natural disturbance in ecosystems currently impacted by SOD. Our goal has, therefore, been to understand the strength and possible causes of a relationship between past fires and current disease patterns (Moritz and Odion 2005). Given how little is known about the landscape pathology of SOD and the possible role of fire, here we reanalyze fire-disease patterns in a specific area, to see if our earlier findings hold at a finer spatial scale. We also discuss how geographic variation in fire and processes it affects at different scales of space and time may influence the landscape pathology of the disease. While there are many ways fire can influence the landscape pathology of the disease, specific mechanisms must be identified to confirm that the observed spatial relationship between current *P. ramorum* infections and long unburned areas is caused by fire.

Case Study: Sonoma County

The analysis presented in this paper is an extension of previous analyses (*fig. 1a*), which are described elsewhere in detail (Moritz and Odion 2005). In our earlier work we found a strong and consistent negative relationship between *P. ramorum* infections across northern California and the locations of fires since 1950, and this relationship was robust under different scenarios of susceptibility in host abundance and elimination of spatially dependent sample observations. Notably, we also found that the reduced probability of infections within areas burned in the past was not due to undersampling in previously burned areas, supporting the hypothesis that past fire occurrence is somehow important in the spread of the disease. Sonoma County contained the portion of the study area in which the relationship appeared to

be weakest, because it was the only region in which infections were found in past burned areas. Sonoma County is also thought to be an area that has been completely exposed to *P. ramorum*, which may not be the case for all susceptible landscapes examined in our earlier study (D. Rizzo, personal communication). In this paper we examine Sonoma County in particular (*fig. 1b*) to address possible concerns about variation in pathogen exposure across a study area, and to test the fire-disease relationship in a region where it appears to be least robust. This case study will also allow for a finer spatial scale of analysis; however, the temporal scale will remain the same as in Moritz and Odion (2005).

Methods and Data

The infection database used for this study contains confirmed *P. ramorum* infection locations as of October 2002, reported through the California Oak Mortality Task Force (COMTF) (Kelly and others 2004). Only those samples testing positive and not originating from a nursery were used in our analyses, which were restricted to Sonoma County (*fig. 1b*). We used the mapped fire history in a GIS format from the California Department of Forestry and Fire Protection (CDF)[4] and merged this with a supplemental CDF coverage of management burns. We then used a GIS to identify portions of the landscape that had burned in the relatively recent past (i.e., since 1950) and those we considered "long unburned" for further analysis. It is worth noting that these mapped fire databases omit many of the smaller fires that have occurred in the past (in other words < ~120 ha, based on official documentation); however, these fires cumulatively account for a very small proportion of the landscape burned over time. In addition, many areas burned in past smaller fires have been subsequently reburned in larger, mapped events, so omission of small fires in the database should not bias analyses.

[4] See http://frap.cdf.ca.gov/data/frapgisdata/select.asp for additional details and metadata documentation.

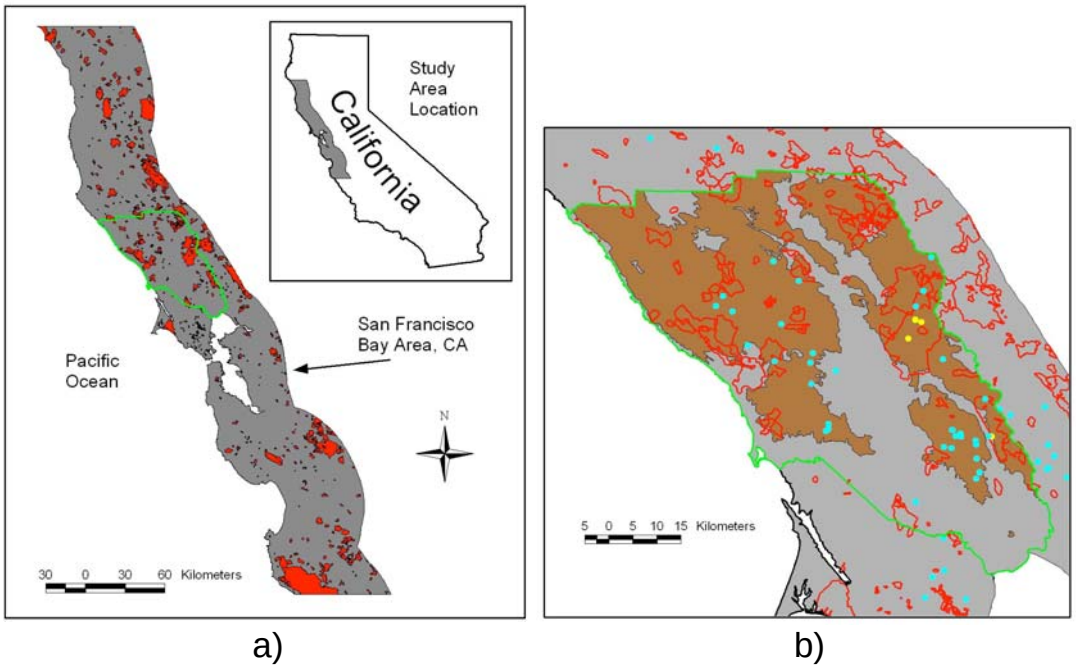

Figure 1—Map of study area. Panel a) shows the portion of California analyzed in Moritz and Odion (2005), which extended approximately 61 km inland from the Pacific coastline; mapped fire history (1950-2000) is shown in red, and Sonoma County is highlighted with a green border. Panel b) shows the study area of Sonoma County analyzed here, with landscape susceptibility scenario L3 in brown, past fire perimeters in red, and positive *P. ramorum* infection locations shown as points; infections points located in past burns are shown in yellow, while others are in light blue. See text for details about infection sampling and vegetation scenarios used in analyses.

As in Moritz and Odion (2005), we examined the strength and consistency of the fire-disease relationship in Sonoma County using different sampling and landscape susceptibility scenarios. We did this because the original COMTF database had been populated by sampling symptomatic plants on an *ad hoc* basis, so it was possible that an observed relationship between infections and past fires could be due to how one defined the study area for analysis, or artifacts of sampling. We found that the original sampling effort was actually biased somewhat toward sampling in past burned areas (Moritz and Odion 2005), which makes the consistent negative relationship in fire-disease patterns even more striking. Although we discuss the issue of possible sampling bias later in this paper (see Assumptions and Limitations section), we do not reanalyze the sampling effort itself (in other words including samples testing negative) further.

For the Sonoma study area, we employed three landscape susceptibility scenarios. These were based on the abundance of susceptible host species in vegetation units mapped for the California Gap Analysis Project (Davis and others 1998), which contain floristic detail on the extent of vegetation types (in other words primary, secondary, and tertiary coverages) within

mapped polygons. Details of these scenarios are given in Moritz and Odion (2005), although the following provides an overview of the screening categories resulting from characteristics of the dominant mapped vegetation types: L1 specifies that susceptible vegetation dominates the primary, secondary, or tertiary types; L2 requires that either the primary or secondary vegetation types are of susceptible species; and L3 means that the primary cover type must be mapped as susceptible vegetation[5]. This approach produced a gradient of increasingly restrictive screens, progressively excluding areas that might fall near urban and/or agricultural landscapes.

Plant diseases can result in clustered symptoms and infections, and many samples from the COMTF database were collected in close proximity. For these reasons, it is likely that many of these samples do not constitute spatially independent observations. To create an alternative subsampling scenario to address possible spatial dependence issues in sampling, we aggregated closely spaced sample points into "sites." Based on published information on clustering of *P. ramorum* symptoms at 100 to 300 m (Kelly and Meentemeyer 2002), we chose a conservative buffer distance of 400 m. For the COMTF positive infections in Sonoma County, this aggregation process resulted in n=89 sample points and n=34 sample sites.

To investigate the spatial relationship between positive infection locations and fire history, given the two sampling scenarios and three landscape susceptibility scenarios, we tested the null hypothesis that the proportion of infections in previously burned areas is not lower than would be expected by chance. The expected value was based on the proportion of each landscape susceptibility scenario (L1-L3) that had burned since 1950 within the boundaries of Sonoma County. We used a test statistic based on the normal approximation to the binomial distribution, representing the portion of a normal distribution lying beyond a given normal deviate (Zar 1999).

Results and Discussion for Sonoma

Similar to our earlier findings statewide, in Sonoma County we found fewer positive *P. ramorum* infections in areas burned in the relatively recent past (in other words since 1950). When original COMTF sampling points were compared to mapped fire history patterns, there were consistently far fewer infections in burns than expected, based on the proportion of Sonoma County that had burned in the past (top half of Table 1). After aggregating closely spaced sample points into sites, the negative relationship between infection locations and past fires is not as statistically strong; however, the relationship is still weakly significant for landscape susceptibility scenario L3, the most spatially restrictive vegetation screen (last row of Table 1). One caveat worth mentioning is that the relatively low number of observations for sites (n≈30) raises questions about the statistical power of this test (in other words normal approximation to the binomial).

[5] Primary, secondary, and tertiary refer to the 3 vegetation communities that dominate, in order of their spatial extent, each mapped unit on the landscape.

Table 1—*Results for spatial analyses of whether infection locations occur less frequently in previously burned areas than expected; study area is limited to Sonoma County.*

POINTS	Landscape area (ha)	Landscape burned (ha)	Proportion (expected)	Infections in scenario	Infections burned	Proportion (observed)	P-value
Scenario L1:	294751	54343	0.1844	85	7	0.0824	0.0067*
Scenario L2:	290666	53933	0.1856	83	7	0.0843	0.0088*
Scenario L3:	245716	50344	0.2049	82	6	0.0732	0.0016*
SITES							
Scenario L1:	294751	54343	0.1844	31	4	0.1290	0.2134
Scenario L2:	290666	53933	0.1856	30	4	0.1333	0.2310
Scenario L3:	245716	50344	0.2049	29	3	0.1034	0.0880**

* Significantly lower than expected, _ =.05 ** Weakly significant, _ =.10

In comparison to our earlier analyses across a larger area (*fig 1a*), evidence for a negative relationship between SOD and fire history is not as strong in Sonoma County. The weaker statistical results for Sonoma County were to be expected, given that this is the only county in which any COMTF positive infections were found in past burned areas. In a statistical sense, this is at least partially offset by the fact that more of Sonoma County has burned since 1950 (18 to 20 percent; Table 1) than in our earlier study area (13 to 15 percent; Moritz and Odion 2005). Regardless, several of the scenarios analyzed for Sonoma County did still show a significant apparent avoidance of the disease in places that have burned in the last 50 years.

Assumptions and Limitations

We have already described several possible fire-related mechanisms for the observed negative relationship between past fire locations and current *P. ramorum* infections (Moritz and Odion 2005). In short, there are many fire-related factors that can affect the three sides of the plant disease triangle, such as changes in host densities, plant stress levels due to mineral nutrition, or effects of the chemical environment on the pathogen. The observed negative spatial relationship may be caused by fire-related mechanisms, and we have ongoing research in this area. However, it may also be due to something closely correlated with fire patterns and/or an artifact of the datasets themselves. Here we focus on several important caveats about the data used in this study and other possibly confounding factors.

As mentioned above, one concern about the observed relationship between past fires and current infection locations is that it could be caused by a sampling bias with respect to

previously burned areas. We addressed this issue in Moritz and Odion (2005) by examining the overall sampling effort, using a subset of the COMTF sample points from the California Department of Food and Agriculture (CDFA). The CDFA subset contained sampling locations taken for symptomatic plants, regardless of infection status (in other words both positive and negative for *P. ramorum*), and we tested these to see if there had been undersampling with respect to fire history. In our analysis we found that symptomatic samples were being taken roughly as often in past burned areas as one would expect by chance, and even significantly more often in some scenarios. This is based on the assumption that the CDFA data were representative of the overall sampling effort. While this might be additional evidence to support a hypothesis that burned areas are less susceptible to *P. ramorum*, there are additional considerations. If a substantial number of samples found to be negative for *P. ramorum* were erroneous, which may be the case (M. Garbelotto personal communication), then *P. ramorum* could be much more common in areas burned since 1950 than the confirmed positive infection data suggest. Without good estimates of how often one might expect false positives in different parts of the study area, among different host species, and in burned versus unburned areas, it is difficult to asses how strongly this issue may influence findings (in other words by raising the number of additional positive infections in previously burned areas).

Regardless of uncertainty due to inconsistent identification of *P. ramorum* in samples, a CDFA sample testing negative was about twice as likely to have been taken in a past burned area as a positive (data not shown). It is interesting that more sampling may have been done in burned areas (Moritz and Odion 2005), and, for whatever reason, it appears there are many plants that are symptomatic for *P. ramorum* in places that have burned relatively recently. Perhaps the most likely reason for concentrated sampling for SOD in burns and failure to document *P. ramorum* is that much similar mortality may have occurred there as a result of bark beetles, which can increase after fire. In fact, McPherson and others (these proceedings) reported more rapid mortality in bark beetle infested hosts, and the trees they monitored were in or near very recent burn areas. In addition, many symptomatic samples may test positive for other plant diseases (for example, *Phytophthora* spp.), which could be more likely to occur in areas burned since 1950.

A further complication is that human activities that may affect both fire and the disease are not randomly dispersed on the landscape: 1) Fire patterns, in particular the locations of ignitions, can often be associated with the road network; 2) Fire suppression efforts typically utilize the road network and are often strongest near areas of human habitation; and 3) Fire size and frequency can be affected by fragmentation of natural vegetation patterns due to both urban and rural development. Despite our findings of sampling patterns being unbiased with respect to fire history, one might still question whether fire-disease dynamics are somehow strongly driven by human population patterns, road locations, and/or access constraints. Our landscape susceptibility scenarios L1-L3 indirectly address this concern, as they progressively exclude portions of the landscape that may be affected by human activities. The negative relationship between SOD and fire history was consistently statistically significant across these analyses (Moritz and Odion 2005), as were several of the scenarios examined here for Sonoma County.

Conclusions

Just as there is spatial variation in the risk of *P. ramorum* infections based on patterns of host distribution and climatic factors (Meentemeyer and others 2004, Guo and others 2005), there will be natural spatial variation in many different fire regime parameters (in other words fire intensity, size, season, frequency) within and between different ecosystems. The spatial variability of fire at microscales can even be considerable. For example, continuous temperature measurements by sensors less than 0.5 m apart can vary as greatly as among sensors several meters apart, with overall levels of heating at the soil surface varying by over an order of magnitude (Odion and Davis 2000). Factors such as the hydrophobicity of soil, organic matter consumption, and thermal alteration of soil chemistry may, therefore, show considerable fire-related variation at relatively fine spatial scales. Such variation may interface with the life history of *P. ramorum* dispersed to new areas, for example by human activities (Cushman and Meentemeyer, these proceedings). It is also likely that the factors that drive ongoing disease progression in a given location are different from those that may control the initial invasibility of an ecosystem, and fire-related effects on the disease will probably vary by the host species in question. We may therefore expect substantial variation in how a fire-disease relationship manifests itself, depending on the stage of disease progression and the scales of space and time used in examining the landscape pathology of SOD.

Spatial analysis of Sonoma County's fire history and locations of confirmed *P. ramorum* infections from the COMTF database supports our earlier findings of an apparently lower probability of *P. ramorum* in places that have burned in the relatively recent past. Nonetheless, further research is needed to determine whether this relationship is due to a fire-related mechanism, a surrogate of fire, and/or an artifact of available datasets. The differences between prescribed burns and past wildfires must also be understood before advocating fire as a tool in managing *P. ramorum* infections (Moritz and Odion 2004). Little is known about fire-disease dynamics in the ecosystems affected by SOD and the landscape pathology of this disease is likely to be complex.

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Examining the Relationship between Fire History and Sudden Oak Death Patterns: A Case Study in Sonoma County[1]

Max A. Moritz[2] and Dennis C. Odion[3]

Abstract

Fire is often integral to forest ecology and can affect forest disease dynamics. Sudden oak death has spread across a large, fire-prone portion of California, killing large numbers of oaks and tanoaks and infecting most associated woody plants. Building on our earlier study of fire-disease dynamics, we examined spatial patterns of confirmed infections in relation to past fire history for a single county using a geographic information system (GIS). The region analyzed here is Sonoma County, where infections have been documented in past burn areas and exposure to the disease is thought to be extensive. We found that the disease is extremely rare inside recently burned areas (since 1950), although the relationship in this region is somewhat weaker than previous findings across a much larger area. This highlights how factors, such as variation in vegetation, climate, and patterns of severity, within burn areas are important to consider in the landscape pathology of sudden oak death. Despite confirmation of earlier findings at a different scale of analysis, it remains to be seen whether the observed negative relationship between current infection locations and past fires is due to a fire-related mechanism, a surrogate of fire, and/or an artifact of the datasets used. Regardless, it is likely that interactions between the disease and fire will vary with local differences in host species composition, the stage of disease progression, and the scales of space and time used in analyses.

Key words: spatial pattern analysis, landscape pathology, fire management, forest disease epidemiology

Introduction

Sudden oak death (SOD), a disease caused by the pathogen *Phytophthora ramorum*, has reached epidemic proportions over the last decade across landscapes of northern coastal California. Based on climatic factors and host distributions, there are large portions of the state at risk for the disease, including ecosystems of the Sierra Nevada foothills (Meentemeyer

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California. The work described here is an extension of previous work that is in press at the journal *Oecologia*.

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and others 2004, Guo and others 2005). Suitable habitat for the disease may exist over extensive areas in the western U.S. and beyond, and there is great concern over the cascading ecological effects that will occur in affected areas (Rizzo and others 2002, Rizzo and Garbelotto 2003). A variety of modeling tools exist for predicting the spatial-temporal dynamics of plant disease epidemics (van Maanen and Xu 2003), although presently not enough is known about SOD to parameterize such models.

Forest diseases involve interactions among pathogens and hosts in heterogeneous, dynamic environments (Lundquist and Klopfenstein 2001), all of which are components of the plant "disease triangle." This conceptual model illustrates a fundamental paradigm in plant pathology: that is, the existence of a disease caused by a biotic agent requires the interaction of a pathogen, susceptible hosts, and an environment favorable to disease development (Stevens 1960). Recently there has been increasing awareness of landscape-scale factors in forest diseases, exemplified by the emergence of "landscape pathology" as a field of study (Holdenrieder and others 2004). This approach is an extension of the traditional disease triangle that explicitly includes coarse-scale landscape patterns and processes as part of disease dynamics. Examples include landscape fragmentation effects on pathogen dispersal and how vegetation diversity patterns might limit the availability of susceptible hosts (Holdenrieder and others 2004).

Fire is one of the dominant pattern-forming forces on terrestrial landscapes (Bond and others 2005), and it is an important natural disturbance in ecosystems currently impacted by SOD. Our goal has, therefore, been to understand the strength and possible causes of a relationship between past fires and current disease patterns (Moritz and Odion 2005). Given how little is known about the landscape pathology of SOD and the possible role of fire, here we reanalyze fire-disease patterns in a specific area, to see if our earlier findings hold at a finer spatial scale. We also discuss how geographic variation in fire and processes it affects at different scales of space and time may influence the landscape pathology of the disease. While there are many ways fire can influence the landscape pathology of the disease, specific mechanisms must be identified to confirm that the observed spatial relationship between current *P. ramorum* infections and long unburned areas is caused by fire.

Case Study: Sonoma County

The analysis presented in this paper is an extension of previous analyses (*fig. 1a*), which are described elsewhere in detail (Moritz and Odion 2005). In our earlier work we found a strong and consistent negative relationship between *P. ramorum* infections across northern California and the locations of fires since 1950, and this relationship was robust under different scenarios of susceptibility in host abundance and elimination of spatially dependent sample observations. Notably, we also found that the reduced probability of infections within areas burned in the past was not due to undersampling in previously burned areas, supporting the hypothesis that past fire occurrence is somehow important in the spread of the disease. Sonoma County contained the portion of the study area in which the relationship appeared to

be weakest, because it was the only region in which infections were found in past burned areas. Sonoma County is also thought to be an area that has been completely exposed to *P. ramorum*, which may not be the case for all susceptible landscapes examined in our earlier study (D. Rizzo, personal communication). In this paper we examine Sonoma County in particular (*fig. 1b*) to address possible concerns about variation in pathogen exposure across a study area, and to test the fire-disease relationship in a region where it appears to be least robust. This case study will also allow for a finer spatial scale of analysis; however, the temporal scale will remain the same as in Moritz and Odion (2005).

Methods and Data

The infection database used for this study contains confirmed *P. ramorum* infection locations as of October 2002, reported through the California Oak Mortality Task Force (COMTF) (Kelly and others 2004). Only those samples testing positive and not originating from a nursery were used in our analyses, which were restricted to Sonoma County (*fig. 1b*). We used the mapped fire history in a GIS format from the California Department of Forestry and Fire Protection (CDF)[4] and merged this with a supplemental CDF coverage of management burns. We then used a GIS to identify portions of the landscape that had burned in the relatively recent past (i.e., since 1950) and those we considered "long unburned" for further analysis. It is worth noting that these mapped fire databases omit many of the smaller fires that have occurred in the past (in other words < ~120 ha, based on official documentation); however, these fires cumulatively account for a very small proportion of the landscape burned over time. In addition, many areas burned in past smaller fires have been subsequently reburned in larger, mapped events, so omission of small fires in the database should not bias analyses.

[4] See http://frap.cdf.ca.gov/data/frapgisdata/select.asp for additional details and metadata documentation.

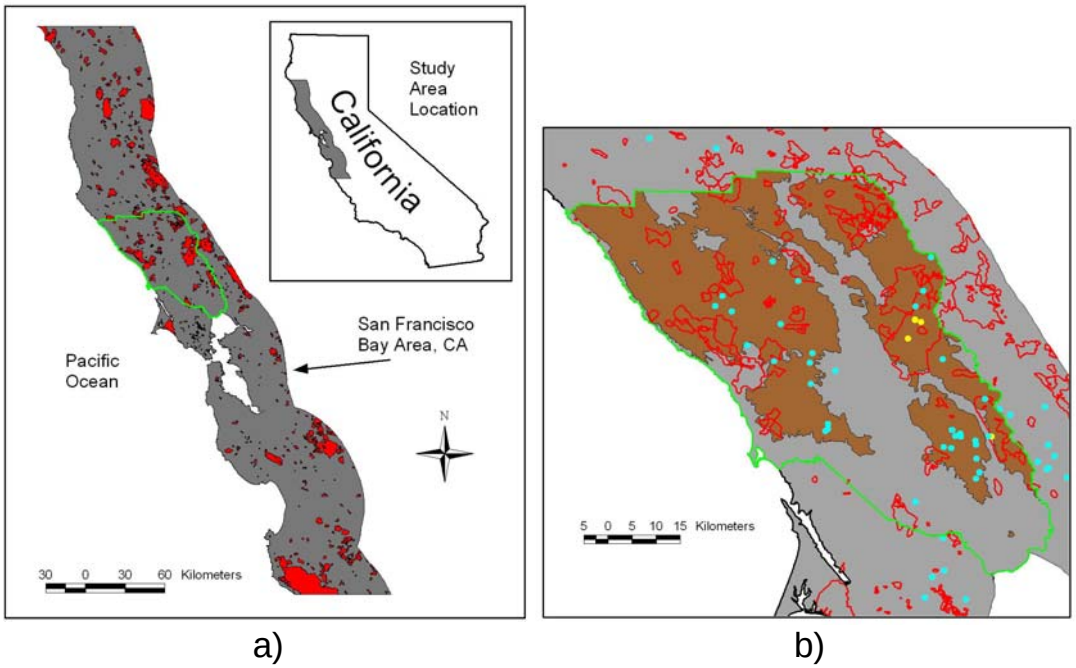

Figure 1—Map of study area. Panel a) shows the portion of California analyzed in Moritz and Odion (2005), which extended approximately 61 km inland from the Pacific coastline; mapped fire history (1950-2000) is shown in red, and Sonoma County is highlighted with a green border. Panel b) shows the study area of Sonoma County analyzed here, with landscape susceptibility scenario L3 in brown, past fire perimeters in red, and positive *P. ramorum* infection locations shown as points; infections points located in past burns are shown in yellow, while others are in light blue. See text for details about infection sampling and vegetation scenarios used in analyses.

As in Moritz and Odion (2005), we examined the strength and consistency of the fire-disease relationship in Sonoma County using different sampling and landscape susceptibility scenarios. We did this because the original COMTF database had been populated by sampling symptomatic plants on an *ad hoc* basis, so it was possible that an observed relationship between infections and past fires could be due to how one defined the study area for analysis, or artifacts of sampling. We found that the original sampling effort was actually biased somewhat toward sampling in past burned areas (Moritz and Odion 2005), which makes the consistent negative relationship in fire-disease patterns even more striking. Although we discuss the issue of possible sampling bias later in this paper (see Assumptions and Limitations section), we do not reanalyze the sampling effort itself (in other words including samples testing negative) further.

For the Sonoma study area, we employed three landscape susceptibility scenarios. These were based on the abundance of susceptible host species in vegetation units mapped for the California Gap Analysis Project (Davis and others 1998), which contain floristic detail on the extent of vegetation types (in other words primary, secondary, and tertiary coverages) within mapped polygons. Details of these scenarios are given in Moritz and Odion (2005), although

the following provides an overview of the screening categories resulting from characteristics of the dominant mapped vegetation types: L1 specifies that susceptible vegetation dominates the primary, secondary, or tertiary types; L2 requires that either the primary or secondary vegetation types are of susceptible species; and L3 means that the primary cover type must be mapped as susceptible vegetation[5]. This approach produced a gradient of increasingly restrictive screens, progressively excluding areas that might fall near urban and/or agricultural landscapes.

Plant diseases can result in clustered symptoms and infections, and many samples from the COMTF database were collected in close proximity. For these reasons, it is likely that many of these samples do not constitute spatially independent observations. To create an alternative subsampling scenario to address possible spatial dependence issues in sampling, we aggregated closely spaced sample points into "sites." Based on published information on clustering of *P. ramorum* symptoms at 100 to 300 m (Kelly and Meentemeyer 2002), we chose a conservative buffer distance of 400 m. For the COMTF positive infections in Sonoma County, this aggregation process resulted in n=89 sample points and n=34 sample sites.

To investigate the spatial relationship between positive infection locations and fire history, given the two sampling scenarios and three landscape susceptibility scenarios, we tested the null hypothesis that the proportion of infections in previously burned areas is not lower than would be expected by chance. The expected value was based on the proportion of each landscape susceptibility scenario (L1-L3) that had burned since 1950 within the boundaries of Sonoma County. We used a test statistic based on the normal approximation to the binomial distribution, representing the portion of a normal distribution lying beyond a given normal deviate (Zar 1999).

Results and Discussion for Sonoma

Similar to our earlier findings statewide, in Sonoma County we found fewer positive *P. ramorum* infections in areas burned in the relatively recent past (in other words since 1950). When original COMTF sampling points were compared to mapped fire history patterns, there were consistently far fewer infections in burns than expected, based on the proportion of Sonoma County that had burned in the past (top half of Table 1). After aggregating closely spaced sample points into sites, the negative relationship between infection locations and past fires is not as statistically strong; however, the relationship is still weakly significant for landscape susceptibility scenario L3, the most spatially restrictive vegetation screen (last row of Table 1). One caveat worth mentioning is that the relatively low number of observations for sites (n≈30) raises questions about the statistical power of this test (in other words normal approximation to the binomial).

[5] Primary, secondary, and tertiary refer to the 3 vegetation communities that dominate, in order of their spatial extent, each mapped unit on the landscape.

Table 1—*Results for spatial analyses of whether infection locations occur less frequently in previously burned areas than expected; study area is limited to Sonoma County.*

POINTS	Landscape area (ha)	Landscape burned (ha)	Proportion (expected)	Infections in scenario	Infections burned	Proportion (observed)	P-value
Scenario L1:	294751	54343	0.1844	85	7	0.0824	0.0067*
Scenario L2:	290666	53933	0.1856	83	7	0.0843	0.0088*
Scenario L3:	245716	50344	0.2049	82	6	0.0732	0.0016*
SITES							
Scenario L1:	294751	54343	0.1844	31	4	0.1290	0.2134
Scenario L2:	290666	53933	0.1856	30	4	0.1333	0.2310
Scenario L3:	245716	50344	0.2049	29	3	0.1034	0.0880**

* Significantly lower than expected, α =.05 ** Weakly significant, α =.10

In comparison to our earlier analyses across a larger area (*fig 1a*), evidence for a negative relationship between SOD and fire history is not as strong in Sonoma County. The weaker statistical results for Sonoma County were to be expected, given that this is the only county in which any COMTF positive infections were found in past burned areas. In a statistical sense, this is at least partially offset by the fact that more of Sonoma County has burned since 1950 (18 to 20 percent; Table 1) than in our earlier study area (13 to 15 percent; Moritz and Odion 2005). Regardless, several of the scenarios analyzed for Sonoma County did still show a significant apparent avoidance of the disease in places that have burned in the last 50 years.

Assumptions and Limitations

We have already described several possible fire-related mechanisms for the observed negative relationship between past fire locations and current *P. ramorum* infections (Moritz and Odion 2005). In short, there are many fire-related factors that can affect the three sides of the plant disease triangle, such as changes in host densities, plant stress levels due to mineral nutrition, or effects of the chemical environment on the pathogen. The observed negative spatial relationship may be caused by fire-related mechanisms, and we have ongoing research in this area. However, it may also be due to something closely correlated with fire patterns and/or an artifact of the datasets themselves. Here we focus on several important caveats about the data used in this study and other possibly confounding factors.

As mentioned above, one concern about the observed relationship between past fires and current infection locations is that it could be caused by a sampling bias with respect to previously burned areas. We addressed this issue in Moritz and Odion (2005) by examining the overall sampling effort, using a subset of the COMTF sample points from the California Department of Food and Agriculture (CDFA). The CDFA subset contained sampling locations taken for symptomatic plants, regardless of infection status (in other words both positive and negative for *P. ramorum*), and we tested these to see if there had been undersampling with respect to fire history. In our analysis we found that symptomatic samples were being taken roughly as often in past burned areas as one would expect by chance, and even significantly more often in some scenarios. This is based on the assumption that the CDFA data were representative of the overall sampling effort. While this might be additional evidence to support a hypothesis that burned areas are less susceptible to *P. ramorum*, there are additional considerations. If a substantial number of samples found to be negative for *P. ramorum* were erroneous, which may be the case (M. Garbelotto personal communication), then *P. ramorum* could be much more common in areas burned since 1950 than the confirmed positive infection data suggest. Without good estimates of how often one might expect false positives in different parts of the study area, among different host species, and in burned versus unburned areas, it is difficult to asses how strongly this issue may influence findings (in other words by raising the number of additional positive infections in previously burned areas).

Regardless of uncertainty due to inconsistent identification of *P. ramorum* in samples, a CDFA sample testing negative was about twice as likely to have been taken in a past burned area as a positive (data not shown). It is interesting that more sampling may have been done in burned areas (Moritz and Odion 2005), and, for whatever reason, it appears there are many plants that are symptomatic for *P. ramorum* in places that have burned relatively recently. Perhaps the most likely reason for concentrated sampling for SOD in burns and failure to document *P. ramorum* is that much similar mortality may have occurred there as a result of bark beetles, which can increase after fire. In fact, McPherson and others (these proceedings) reported more rapid mortality in bark beetle infested hosts, and the trees they monitored were in or near very recent burn areas. In addition, many symptomatic samples may test positive for other plant diseases (for example, *Phytophthora* spp.), which could be more likely to occur in areas burned since 1950.

A further complication is that human activities that may affect both fire and the disease are not randomly dispersed on the landscape: 1) Fire patterns, in particular the locations of ignitions, can often be associated with the road network; 2) Fire suppression efforts typically utilize the road network and are often strongest near areas of human habitation; and 3) Fire size and frequency can be affected by fragmentation of natural vegetation patterns due to both urban and rural development. Despite our findings of sampling patterns being unbiased with respect to fire history, one might still question whether fire-disease dynamics are somehow strongly driven by human population patterns, road locations, and/or access constraints. Our landscape susceptibility scenarios L1-L3 indirectly address this concern, as they progressively exclude portions of the landscape that may be affected by human activities. The negative relationship

between SOD and fire history was consistently statistically significant across these analyses (Moritz and Odion 2005), as were several of the scenarios examined here for Sonoma County.

Conclusions

Just as there is spatial variation in the risk of *P. ramorum* infections based on patterns of host distribution and climatic factors (Meentemeyer and others 2004, Guo and others 2005), there will be natural spatial variation in many different fire regime parameters (in other words fire intensity, size, season, frequency) within and between different ecosystems. The spatial variability of fire at microscales can even be considerable. For example, continuous temperature measurements by sensors less than 0.5 m apart can vary as greatly as among sensors several meters apart, with overall levels of heating at the soil surface varying by over an order of magnitude (Odion and Davis 2000). Factors such as the hydrophobicity of soil, organic matter consumption, and thermal alteration of soil chemistry may, therefore, show considerable fire-related variation at relatively fine spatial scales. Such variation may interface with the life history of *P. ramorum* dispersed to new areas, for example by human activities (Cushman and Meentemeyer, these proceedings). It is also likely that the factors that drive ongoing disease progression in a given location are different from those that may control the initial invasibility of an ecosystem, and fire-related effects on the disease will probably vary by the host species in question. We may therefore expect substantial variation in how a fire-disease relationship manifests itself, depending on the stage of disease progression and the scales of space and time used in examining the landscape pathology of SOD.

Spatial analysis of Sonoma County's fire history and locations of confirmed *P. ramorum* infections from the COMTF database supports our earlier findings of an apparently lower probability of *P. ramorum* in places that have burned in the relatively recent past. Nonetheless, further research is needed to determine whether this relationship is due to a fire-related mechanism, a surrogate of fire, and/or an artifact of available datasets. The differences between prescribed burns and past wildfires must also be understood before advocating fire as a tool in managing *P. ramorum* infections (Moritz and Odion 2004). Little is known about fire-disease dynamics in the ecosystems affected by SOD and the landscape pathology of this disease is likely to be complex.

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Forecasting the Future of Coast Live Oak Forests in the Face of Sudden Oak Death[1]

Letty B. Brown[2] and Barbara Allen-Diaz[2]

Key words: sudden oak death, coast live oak, California bay laurel, stand reconstruction, ecological impact

Abstract

Little is known about the potential short- and long-term impacts of sudden oak death (SOD) on forest structure and composition. This study began in 2002 to evaluate the effects of SOD on coast live oak (*Quercus agrifolia*) - California bay laurel (*Umbellularia californica*) forests over a gradient of *Phytophthora ramorum* infection. In order to evaluate stand level impacts of the disease, we present mortality data from three time periods: recent past (five to 10 years before 2002), present (2002-2004), and future (next five years). Because large numbers of recently downed stems (mainly *Q. agrifolia*) were found in 2002, in areas of high known infection levels of *Phythophthora ramorum*, and because most SOD monitoring efforts began in 2001 or later, stand reconstruction methods were employed to accurately capture the impact of the disease on forest stands.

Our eight study sites (1 hectare in size) are located throughout the San Francisco Bay Area. These were chosen based on dominance by *Q. agrifolia* and *U. californica*, and to span a wide variability of *P. ramorum* infection, in terms of visually manifested signs of the disease. Five .08–hectare, fixed-radius plots were placed using a stratified random design within each of the eight study sites, for a total of 40 plots. Results are based on measurement of diameter at breast height (dbh), as well as mortality and disease characteristics of all stems (standing and downed) within these 40 plots. Stems are defined as forking below dbh, and are 10 cm dbh or greater.

To estimate current and past stand parameters, we used tree mortality data collected in the spring of 2002, 2003, and 2004, and stand reconstruction methods to establish (presumed) pre-SOD reference stand structure. Stand reconstruction involved inclusion and measurement of all recently downed stems. Based on a decay index, recently downed stems were defined as stems with fine twigs and leaves still attached or with solid, intact bark. Tissue samples taken from *U. californica* foliage confirmed the presence of *P. ramorum* at six sites at the high and medium ends of the gradient. Based on the decay index and similar studies completed in hardwood forests, we estimate that these stems had senesced within the last five to 10 years. These estimates include background mortality as well as *P. ramorum* - influenced mortality.

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[1]A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California
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To forecast mortality in the near future, we assumed that all currently bleeding *Q. agrifolia* stems will senesce. Based on our observations and the SOD literature to date, we estimate that these stems will senesce in the next five years. These estimates assume that seeps are due *to P. ramorum*, that current rates of infection will continue, and do not account for potential host resistance.

A total of 1029 *Q. agrifolia* stems were sampled, out of 2306 stems of all species in all plots. Mortality losses are reported in terms of stem basal area (m^2/ha). Results indicate that between 2002 and 2004, from 2 percent (at a low infection site) to 27 percent (at a high infection site) of *Q. agrifolia* basal area (m^2/ha) senesced. From the recent past through 2004, stand reconstruction results indicate that sites have lost from 4 percent (at a low infection site) to 55 percent (at a high infection site) of the *Q. agrifolia* basal area (m^2/ha). Future forecasting results indicate that from the recent past to the future, from 15 percent (at a low infection site) to 69 percent (at a high infection site) loss of *Q.agrifolia* basal area (m^2/ha) will occur. In terms of impacts to the total stand (looking at all species), because of the senescence of *Q. agrifolia* (the dominant or co-dominant tree), up to 42 percent (in a high infection site) of the total stand basal area (m^2/ha) is predicted to be lost within the next five years. The importance of other non-terminal host tree species is expected to increase, in particular *U. californica*, which is the co-dominant in these stands. These estimates of *Q. agrifolia* mortality are greater than previously shown in the literature.

Further analysis will investigate the impacts of SOD on understory light levels, understory shrub and herbaceous layer plant dynamics, and woody seedling and sapling recruitment, over the same gradient of *P. ramorum* infection.

Etiology and Evidence of Systemic Acidification in SOD-Affected Forests of California[1]

Lee Klinger[2] and Ralph Zingaro[3]

Key words: acidification, soil, mosses

Abstract

Pathologists investigating the widespread death of oak trees in the forest ecosystems of northern California concluded, in 2000, that the problem was due to a new plant disease, dubbed sudden oak death (SOD), which is caused by the fungal pathogen *Phytophthora ramorum*. Since then this one organism has been the focal point of notable efforts to understand, monitor, and control SOD. While not disputing that *P. ramorum* is involved in the final demise of many oaks, there is a growing number of scientists and arborists who do not agree that this pathogen is the fundamental cause of the overall decline. These experts point out that most of the dying oaks in SOD-affected forests show no expression of *P. ramorum*. They further note that the etiology of SOD closely resembles that seen in other aging forests where the decline of the trees has been attributed to an increase in acidity and mineral deficiency of the water and soils. In these places, acidic conditions create mineral imbalances and deficiencies in trees, especially calcium, which greatly weaken the trees, raising their susceptibility to secondary pests and pathogens. Here we present evidence that suggests systemic acidification of forests can explain, quite well, the entire SOD phenomenon.

The etiology of SOD in California coincides closely with the symptoms of systemic acidification in aging forest ecosystems. Dieback starts with the upper and outer branches in the crown, showing a pattern of wilting and browning of leaves, along with dead, small branches and progressively spreading to the lower parts of the crown over several years. The decline affects nearly all kinds of oaks, as well as bays, buckeyes, and pines, hitting mainly the larger trees in mixed-oak savannas and forests, most of which have been under strict fire control for more than 50 years. Areas near the coast and those experiencing frequent seasonal fog are especially hard hit by SOD. Affected trees tend to occur in mature forests (greater than 100 years old) and are always found in association with a heavy cover of mosses and lichens. Moss mats have been shown in both laboratory and field studies to create conditions acidic enough to kill the underlying fine roots and mycorrhizae, which leads to water and nutrient stress and reduced radial growth in nearby trees. Mosses and lichens are also observed to degrade the protective bark layer, allowing for pests/pathogens to more easily infest/infect the tree. In general, the etiology of SOD in California is much like that seen elsewhere in dying oaks (e.g., Texas, Missouri, Pennsylvania, Manitoba, and Europe), except that *P. ramorum* is not found to be involved in these other declines.

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California
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Data on pH from 34,700 soil samples taken from a wide range of agricultural and forest soils in California indicate that between 14 and 21 percent of the soils are acidic (pH < 6.0) and 3 to 4 percent are strongly acidic (pH < 5.0). However, a subset of samples taken from SOD-affected sites indicates that 72 percent of these soils are acidic and 4 percent are strongly acidic (median pH = 5.7; n = 132). The soils from these sites were also found to be consistently low in calcium and very high in soluble aluminum and iron. Spatial analysis reveals a strong coastal gradient in soil pH with the lowest pH values found near the coast. Strong coastal gradients are also apparent in soil calcium, which is lowest near the coast, and in soil aluminum, which is highest near the coast. Precipitation chemistry data from this region also reveal a coastal pH gradient much like that found in the soils. Similar coastal gradients in precipitation pH have been reported from the Olympic Peninsula, from southeast Alaska, and from Scandinavia.

These results lend further support to the hypothesis that systemic acidification is adversely affecting the health of the trees and soils in SOD-affected forests. The situation described here in California is not unlike that in other regions of the world where aging forests are experiencing decline. From this and other work (e.g., studies at Hubbard Brook), we strongly believe that the cause (and the definition) of SOD is still an open question, and that the scope of SOD research should be expanded to include studies of acidification by cryptogams in the context of forest and soil ecology.

Etiology and Evidence of Systemic Acidification in SOD-Affected Forests of California[1]

Lee Klinger[2] and Ralph Zingaro[3]

Key words: acidification, soil, mosses

Abstract

Pathologists investigating the widespread death of oak trees in the forest ecosystems of northern California concluded, in 2000, that the problem was due to a new plant disease, dubbed sudden oak death (SOD), which is caused by the fungal pathogen *Phytophthora ramorum*. Since then this one organism has been the focal point of notable efforts to understand, monitor, and control SOD. While not disputing that *P. ramorum* is involved in the final demise of many oaks, there is a growing number of scientists and arborists who do not agree that this pathogen is the fundamental cause of the overall decline. These experts point out that most of the dying oaks in SOD-affected forests show no expression of *P. ramorum*. They further note that the etiology of SOD closely resembles that seen in other aging forests where the decline of the trees has been attributed to an increase in acidity and mineral deficiency of the water and soils. In these places, acidic conditions create mineral imbalances and deficiencies in trees, especially calcium, which greatly weaken the trees, raising their susceptibility to secondary pests and pathogens. Here we present evidence that suggests systemic acidification of forests can explain, quite well, the entire SOD phenomenon.

The etiology of SOD in California coincides closely with the symptoms of systemic acidification in aging forest ecosystems. Dieback starts with the upper and outer branches in the crown, showing a pattern of wilting and browning of leaves, along with dead, small branches and progressively spreading to the lower parts of the crown over several years. The decline affects nearly all kinds of oaks, as well as bays, buckeyes, and pines, hitting mainly the larger trees in mixed-oak savannas and forests, most of which have been under strict fire control for more than 50 years. Areas near the coast and those experiencing frequent seasonal fog are especially hard hit by SOD. Affected trees tend to occur in mature forests (greater than 100 years old) and are always found in association with a heavy cover of mosses and lichens. Moss mats have been shown in both laboratory and field studies to create conditions acidic enough to kill the underlying fine roots and mycorrhizae, which leads to water and nutrient stress and reduced radial growth in nearby trees. Mosses and lichens are also observed to degrade the protective bark layer, allowing for pests/pathogens to more easily infest/infect the tree. In general, the etiology of SOD in California is much like that seen elsewhere in dying oaks (e.g., Texas, Missouri, Pennsylvania, Manitoba, and Europe), except that *P. ramorum* is not found to be involved in these other declines.

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These results lend further support to the hypothesis that systemic acidification is adversely affecting the health of the trees and soils in SOD-affected forests. The situation described here in California is not unlike that in other regions of the world where aging forests are experiencing decline. From this and other work (e.g., studies at Hubbard Brook), we strongly believe that the cause (and the definition) of SOD is still an open question, and that the scope of SOD research should be expanded to include studies of acidification by cryptogams in the context of forest and soil ecology.

Progress Report on the Evaluation of the Susceptibility of the Holm Oak (*Quercus ilex*) Forest Ecosystem to *Phytophthora ramorum*[1]

Eduardo Moralejo[2] and Enrique Descals[2]

Key words: Phytophthora ramorum, susceptibility tests, Mediterranean vegetation and sporulation

Abstract

In preliminary studies on the susceptibility of plant members of the holm oak (*Quercus ile*x) forest, detached leaves of several woody species were highly susceptible when inoculated with zoospore suspensions of local isolates of *Phytophthora ramorum* (Moralejo and Hernández 2002). Since then, there have been reports of natural infections by *P. ramorum* on leaves and shoots of *Q. ilex* in the UK, and new findings on *Viburnum tinus* in nurseries across Europe. Taken together, this indicates that at least some areas of Mediterranean vegetation with a prevalence of the *Arbutus unedo-Viburnum tinus-Quercus ilex* association should be closely monitored. Yet, there are, so far, no outdoor infections in the Mediterranean basin.

For having a more complete picture of what might occur at the plant community level, we are further *in vitro* testing the susceptibility of fruits, leaves and twigs of shrubs and vines of the understory, as well as logs of *Q. ilex* to different isolates of *P. ramorum*. The sporangial production on leaf lesions is being assessed to identify potential major sources of inoculum. Available protocols of the European Project RAPRA, either for testing the susceptibility of tree and non-tree species or for determining sporulation capacity, are being used. Leaf lesion areas developed 4 and 7 days after inoculating are measured in cm^2, and the sporangial production is rated as number of sporangia/leaf lesion unit (cm^2). The length of the twig lesions is measured 10 days after wound inoculating. For the log trials, the necrotic area is calculated in cm^2 ca. 40 to 50 days after wound inoculating.

Results of the ongoing research on susceptibility of fruits (nine species), leaves and twigs (seven species), and logs (*Q. ilex and Pinus halepensis*) to five *P. ramorum* isolates will be presented. Collected data are being analyzed to determine (i) whether isolates of *P. ramorum* differ in pathogenicity, (ii) to rank the susceptibility of different organs of each potential host, (iii) to identify which hosts might act as major sources of inoculum, (iv) to calculate the latent period, i.e. time from infection to the first appearance of sporangia, and (v) to determine the capacity of *P. ramorum* for invading the bark of *Q. ilex* and *P. halepensis*.

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California

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A Spatial Analysis of *Phytophthora ramorum* Symptom Spread Using Second-Order Point Pattern and GIS-Based Analyses[1]

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

Abstract

Phytophthora ramorum is a major source of tanoak (*Lithocarpus densiflorus*) mortality in the tanoak/redwood (*Sequoia sempervirens*) forests of central California. This study presents a spatial analysis of the spread of the disease using second-order point pattern and GIS analyses. Our data set includes four plots in tanoak/redwood (*Sequoia sempervirens*) forests in Marin County, California. Each plot contains roughly 400 individual stems. We transformed our point pattern maps into continuous density surfaces using a gaussian kernel function for the 2001 and 2003 disease symptom surveys. Subtracting one year from the other with a cut and fill function we in turn developed a triangulated irregular network (TIN). The TIN provides a means to estimate the spread of symptoms, directionality of spread and the average increase of symptomatic stem density. Our analysis indicates an average median spread of 2.1 meters and an average density increase that ranged from 32 to 100 symptomatic stems per hectare between 2001 and 2003. We detected no signs of anisotrophy. Our localization of the scale of spread using Ripley's L(t) analysis supports the GIS analysis results. Together these results reflect a spread of disease from existing foci without the development of new foci within the stands.

Key words: sudden oak death, *Phytophthora ramorum*, spatial spread analysis

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Residual Tree Response to Tanoak Decline in California[1]

Kristen M. Waring[2] and Kevin L. O'Hara[3]

Key words: sudden oak death, redwood, tanoak, stand development, tree response

Abstract

Coast redwood (*Sequoia sempervirens*) frequently grows in association with tanoak (*Lithocarpus densiflorus*) along the central and northern California coast. High rates of tanoak mortality beginning in the mid 1990's have been attributed to *Phytophthora ramorum*, a water mold believed to be non-native. Of the many species that host *P. ramorum*, tanoak is one of the most susceptible and thousands of tanoak trees have died in the past ten years. In a two species forest, the implications of losing one species may be severe, especially when one species is a mast producer such as tanoak. These trees provide numerous ecological benefits, many of which have yet to be quantified. Tanoak has long been classified as a weed by the timber industry because of its ability to colonize and re-sprout following disturbance. For this reason, tanoak is frequently the target of vegetation control treatments using herbicides to reduce competition with the commercially valuable redwood trees. Tanoak does have considerable ecological value as the only mast-producing species in redwood/tanoak forests. Development patterns in redwood/tanoak forest stands have not been quantified, nor has the response pattern of residual trees following invasion of a stand by *P. ramorum*. The objectives of this study were to quantify stand development patterns in redwood/tanoak stands, assess residual tree response to tanoak decline and mortality, and develop guidelines for restoration and management of affected stands.

Two study areas were utilized: one currently exhibiting high rates of tanoak mortality from *P. ramorum* and a second site outside the current range of *P. ramorum* infection and exhibiting background rates of tanoak mortality. The infected site was located on the Marin Municipal Water District, Marin County where four stands were selected for sampling. Three stands were selected on Jackson Demonstration State Forest, Mendocino County, which is currently uninfected. All stands were similar in age and species composition. Three circular, fixed plots

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between .01 and .10 hectare in size were installed in each stand. For each tree, species, diameter and location were recorded. Between six and 10 “sample” trees were then selected for more intensive data collection, including total tree height, live crown length, crown radii, sapwood area, age and growth rates. Trees were either cored or felled for stem analysis to obtain data for height/age development patterns. Leaf area was estimated for each redwood sample tree using an equation developed by the silviculture laboratory at the University of California, Berkeley. Calculations of crown surface area for redwood used a paraboloid shape and a hemispherical shape for tanoak. Redwood basal area growth was calculated in a subset of the data for two time periods and compared: pre- and post- *P. ramorum*. A second set of comparisons was made assuming a 2-year lag time between tanoak mortality and redwood response.

Stand development patterns at both sites were similar: even-aged stands with redwood stratifying above the tanoak. This pattern appears early in stand development and tanoak continues to hold a sub-canopy position as the stand ages. Approximately 50 years into stand development, some of the redwood trees begin falling behind in height growth and join the tanoak in the lower canopy layer as the lagging redwood slow in growth and the tanoak maintains a more steady growth rate. Redwood sample trees at each site showed a similar relationship between leaf area (m2) and diameter at breast height (1.37m). However, sample trees from Marin County had higher crown surface area (m2) than trees from Mendocino County. While this difference may be due to stand density, neither trees per hectare nor basal area per hectare were significantly different between sites ($p>0.05$). Basal area growth trends showed typical age-related decline at both sites for the post-*P. ramorum* time period and rates were similar between sites. However, when basal area growth was compared using a time lag between tanoak mortality and redwood response, only the Mendocino non-infected site showed the expected age-related decline. However, these differences were small and not significant ($p>0.05$). Overall, Marin County trees showed a trend toward greater basal area growth in addition to the greater crown surface area. The reasons behind greater growth could be attributed to several factors, including the beginning signs of individual tree response to tanoak decline. While the sites are similar, they are removed from each other by approximately 130 miles. It is possible that the differences observed thus far in the analysis are due to a difference in site such as soil moisture. Additionally, redwood has unusual tree ring patterns that may result in underestimation of growth rates and age estimates. As tanoak continues to decline in abundance in these stands, a shift in species composition will trend towards single-species redwood forests. In these situations, trees of the lesser canopy classes in addition to codominant trees, are the most likely to respond to increased growing space. Where large gaps are created, less susceptible tree species may regenerate or redwood sprouts may respond through rapid increases in height and diameter growth. Further analysis will

investigate the affect of variable redwood rings on tree age and growth rate measurements, whether crown surface area differs by canopy class and a more in-depth view of tree growth pre- and post- *P. ramorum*.

SOD-Induced Changes in Foraging and Nesting Behavior of Insectivorous, Cavity-Nesting Birds[1]

Kyle Apigian[2] and Barbara Allen-Diaz[3]

Key words: chestnut-backed chickadee, oak titmouse, foraging, sudden oak death, *Phytophthora ramorum*

Abstract

Sudden oak death (SOD) is a tree disease caused by a recently described pathogen, *Phytophthora ramorum*. The disease affects dozens of plant species, but its effects are particularly pronounced in stands of coast live oak (*Quercus agrifolia*), often resulting in large stands with dead canopies and many downed trees. Such disease-induced habitat changes may impact bird populations both directly and indirectly. For example, forest dieback can result in changes to insect populations, which subsequently impact insectivorous birds by forcing them to switch prey items, change foraging substrates, or increase foraging time. This can ultimately impact the nesting success of these birds. The goal of this study is to examine the effects of SOD-induced changes in oak woodlands on insectivorous cavity-nesting birds, particularly oak titmice (*Baeolophus inornatus*) and chestnut-backed chickadees (*Poecile rufescens*).

We have been examining the impacts of SOD on these birds by investigating the population status of their common prey items, their foraging ecology, and their nesting behavior. We established study plots in the San Francisco Bay Area in several stands that represent a gradient from high SOD impact (many downed trees, high infection) to low impact from the disease. We used branch clipping to compare arthropod populations on coast live oaks and California bay-laurel (*Umbellularia californica*) trees, as well as symptomatic versus asymptomatic coast live oaks. Direct foraging observations of birds on our plots allowed us to determine preferred tree species for foraging, as well as the changes to foraging that result in areas of high SOD impact. We used artificial nest boxes and video cameras to examine nestling diet, foraging effort (length of foraging trips), box occupancy, and nestling survivorship. This scheme allowed us to measure the impacts of SOD at two trophic levels (prey and predator) and through several aspects of cavity-nesting bird natural history (foraging, diet, and nesting).

Our diet studies indicate that foliage-dwelling arthropods, particularly Lepidoptera larvae, are important to chickadees and titmice during the breeding season. There were significantly greater numbers of arthropods on coast live oak trees than California bay laurel trees throughout the spring. One group of arthropods ("leaf-feeders", including Lepidoptera) was greater on asymptomatic oaks than on

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symptomatic oaks early in the spring. Our foraging observations show that coast live oak is the primary foraging substrate for oak titmice, while chestnut-backed chickadees prefer to forage on a diverse range of tree species. Both species avoid bay trees for foraging, most likely as a result of the low arthropod abundance on bays. In heavily affected stands, birds tend to switch to a variety of other trees and shrubs (but *not* bays) as foraging substrates, rather than feed more heavily on remaining oaks. As a result, the loss of oaks may be particularly detrimental to oak titmice, as they are forced to switch to sub-optimal foraging locations. Chickadees may be better able to adjust to the loss of some oaks, due to their more flexible foraging niche. Foraging effort, measured as time away from the nest on a foraging trip, was greater on heavily affected sites, while diet composition remained relatively stable. This indicates that the birds may spend more time foraging in heavily affected sites to find the same types of food. Low sample sizes prevented us from making conclusive results about box occupancy and survivorship, but we have limited evidence that nestling survivorship was lower on heavily affected sites.

These results indicate that sudden oak death may affect insectivorous birds through a number of different pathways, including prey reduction and loss of foraging substrates. Changes to these environmental factors affect where these bird species forage, the amount of time spent foraging, and survivorship of nestlings. We suggest that any management plans related to SOD recognize the importance of protecting healthy coast live oaks while promoting diverse stands in affected areas.

Patterns of Tanoak Acorn Production in *Phytophthora ramorum* Infested and Uninfested Stands in Big Sur, California, With Insights From Girdled Tanoaks[1]

Leila Hadj-Chikh[2], Kerri Frangioso[2], Keyt Fischer[2], Sarah Bergemann[3], and Ebba Peterson[4]

Key words: Phytophthora ramorum, tanoak acorn production

Abstract

Although tanoak (*Lithocarpus densiflorus*) acorns are a valuable source of food for many species of wildlife few data on tanoak acorn production exist. We conducted studies of acorn production in tanoaks to understand the potential impact of *Phytophthora ramorum* on acorn availability to wildlife. In 2002 we established 3-ha seed-trap grids in redwood-tanoak habitat to estimate acorn production at the stand level. We established one grid in Pfeiffer Big Sur State Park (PBS), where *P. ramorum* has caused high levels of tanoak morbidity and mortality, and another in the Landels-Hill Big Creek Reserve (BCR), where *P. ramorum* has not yet been found. In 2004, we established an additional 3-ha grid in Julia Pfeiffer Burns State Park (JPB), where *P. ramorum* is present, but most tanoaks are still asymptomatic. Each grid consisted of an array of 0.5-m^2 seed traps placed at 25-m intervals, and was checked weekly during the period of acorn fall (Sept-Dec). To estimate acorn production by individual trees, we also identified 25 focal trees at our study sites in PBS and BCR, and 40 focal trees at our site in JPB. For each focal tree, we used a spotting scope to sample 5 m^2 of crown for acorns in late summer, and monitored two 0.5-m^2 seed traps placed beneath the crown. Focal trees at the infested site in PBS significantly out-produced those at BCR in both 2003 and 2004 (Wilcoxon Rank Sum; P's < 0.01). Because several of the more productive trees in PBS were clearly dying from Sudden Oak Death (SOD) in this period, we hypothesized that their high levels of acorn production may have been at least partly a reproductive response to infection with *P. ramorum*. To examine whether girdling by *P. ramorum* infections may lead to increased reproductive effort in tanoaks, we studied acorn production in tanoaks that had been mechanically girdled in Whitethorn, Humboldt County, CA, in January 2003. Because tanoak acorns require a full year to develop, acorns produced in 2004 were from the first crop of flowers fertilized after girdling. On 31 August 2004, we conducted visual surveys of acorn production in 20 girdled and 20 ungirdled trees at the study site. For each tree, two observers using binoculars conducted simultaneous 15-sec counts of acorns in the crown. Both counts were averaged to

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provide an estimate of acorn production. Acorn counts of girdled trees (mean ± SE = 21.1 ± 3.0 acorns/15 sec) were significantly higher than acorn counts of ungirdled trees (mean ± SE = 3.8 ± 2.2 acorns/15 sec; Wilcoxon Rank Sum, $P < 0.0001$). These results suggest that infection by *P. ramorum* could theoretically trigger increased acorn production in trees girdled by the pathogen. To better understand the relationship between infections and acorn production in tanoaks, we are monitoring symptom progression in our focal trees at our three study sites in Big Sur, as we continue to measure acorn production in these trees. We are also analyzing data from a 2004 survey of acorn production in 300+ tanoaks in infested and uninfested stands in Big Sur, to understand patterns of spatial synchrony in tanoak acorn production. Collectively, these data will provide us with a clearer picture of natural patterns of acorn production in tanoaks, and changes in these patterns that may result from SOD.

Potential Effects of Sudden Oak Death on the Oak Woodland Bird Community of Coastal California[1]

William B. Monahan[2] and Walter D. Koenig[3]

Abstract

Oak-dependent birds are expected to suffer severe population declines as a result of sudden oak death (SOD). We investigated how the disappearance of two highly SOD-sensitive tree species, tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*), may in turn affect levels of bird species richness, diversity, and equitability in coastal oak habitats of California. Combining avian census data from Audubon Christmas Bird Counts and North American Breeding Bird Surveys with oak distributional data from the California Gap Analysis, we use the statistical relationships between bird community indices and oak species diversity and areal extent to quantify expected bird responses to SOD while assuming complete loss of *Q. agrifolia* or *L. densiflorus* and complete, partial, or no loss of oak habitat. Additionally, we combine existing *Phytophthora ramorum* locality data with estimates of temperature, precipitation, and seasonality to model the potential distribution of SOD. Results suggest that species richness, diversity, and equitability of oak-dependent birds will decline by 5 to 15 percent in areas where SOD eliminates either tanoak or coast live oak. According to the spatial disease model, these declines are expected over 10,130 km^2, or 24 percent of the combined California ranges of *Q. agrifolia* and *L. densiflorus*. Although the overall influence of SOD on the statewide avian community is likely to be modest, bird losses are predicted to be relatively great for central regions already harboring the disease and, following a slight southward expansion of *P. ramorum* into San Luis Obispo and Santa Barbara counties, within neighboring oak habitats that will potentially suffer SOD mortality in the near future.

Key words: California birds, *Phytophthora ramorum,* sudden oak death, *Quercus agrifolia, Lithocarpus densiflorus,* anthropogenic climate change

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Introduction

Sudden oak death (SOD) threatens to reduce or eliminate several species of oaks (family Fagaceae) endemic to the west coast of North America. Since its detection in 1995, SOD has caused significant mortality of coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*) within primarily coastal areas of central California (Garbelotto and others 2001, Rizzo and others 2002, Swiecki and Bernhardt 2002) (*fig. 1*). The disease is caused by the fungus-like pathogen *Phytophthora ramorum* and promotes the rapid development of bleeding bark cankers and foliar pigment degeneration in mature trees, often resulting in tree death (McPherson and others 2002, Rizzo and Garbelotto 2003). Known mortality from SOD is recent and thus the wildlife impacts remain largely unknown (CalPIF 2002).

Here we develop regression models for estimating the effects of SOD on the California oak woodland bird community. Birds are particularly useful indicators since excellent survey databases are readily available for both wintering and breeding populations. The most extensive of these are the Audubon Christmas Bird Count (CBC) and North American Breeding Bird Survey (BBS), both of which have been used extensively and highly successfully to study avian distribution and abundance (Bock and Root 1981, Root 1988, Price and others 1995, Koenig 1998), as well as model the population-level effects of SOD on certain California birds (Monahan and Koenig [In press]). Our approach here estimates SOD impacts using these two independent datasets whose seasonal timing and methodologies are quite different and thus to some degree complementary.

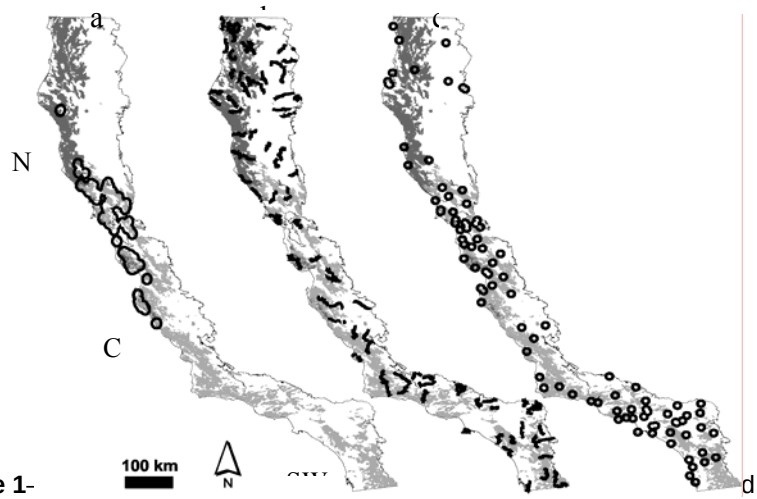

Figure 1- d locations of BBS routes (b) and CBC circles (c) used in the present study. Maps of a, b, and c as well as the California distributions of *Q. agrifolia* (light gray) and *L. densiflorus* (dark gray) are all bounded by three Jepson ecological regions (Northwestern-NW, Central Western-CW, and Southwestern-SW).

In this study we 1) use the statistical relationships between major avian community indices (species richness, diversity, and equitability) and oak species diversity and areal extent to quantify expected bird responses to SOD while assuming complete loss of *L. densiflorus* or *Q. agrifolia* and complete, partial, or no loss of associated oak habitat, 2) map the predicted bird

responses in geographic space, and 3) consider their spatial associations to climatic SOD models developed using existing *P. ramorum* point occurrence data.

Methods

Data overview

In order to focus on birds most likely to be affected by the loss of oaks and avoid problems associated with habitat diversity within sites, analyses were restricted to 16 oak-affiliated year-round resident bird species: acorn woodpecker (*Melanerpes formicivorus*), Nuttall's woodpecker (*Picoides nuttallii*), black phoebe (*Sayornis nigricans*), Hutton's vireo (*Vireo huttoni*), Steller's jay (*Cyanocitta stelleri*), western scrub-jay (*Aphelocoma californica*), chestnut-backed chickadee (*Poecile rufescens*), oak titmouse (*Baeolophus inornatus*), common bushtit (*Psaltriparus minimus*), brown creeper (*Certhia americana*), western bluebird (*Sialia mexicana*), wrentit (*Chamaea fasciata*), California thrasher (*Toxostoma redivivum*), rufous-sided towhee (*Pipilo maculatus*), California towhee (*Pipilo crissalis*), and dark-eyed junco (*Junco hyemalis*). These species were chosen for their high dependence on oak habitats for survival and reproduction (Grinnell and Miller 1944, Pitelka 1951, American Ornithologists' Union 1957, Verner 1979, American Ornithologists' Union 1998, Koenig and Haydock 1999, Cicero 2000).

Three common indices were used to characterize diversity of this oak-dependent avian community. These included richness (number of species counted during the survey; maximum = 16); Shannon's diversity index $H = -\sum p_i \ln p_i$, where p_i = the proportion of the ith species; and Shannon's equitability index $E_H = H / \ln S$, where S is the total number of species found (species richness). The minimum value for H is 0 but it is otherwise unbounded; E ranges from 0 to 1 and is a measure of how evenly the numbers of the different species observed are distributed, with 0 being the most skewed (almost all individuals of one species) and 1 being most even (the same number of all species observed) (Magurran 1988, Rosenzweig 1995).

Bird distribution and abundance data were obtained from Audubon Christmas Bird Counts (CBC) (Butcher 1990) and the North American Breeding Bird Survey (BBS) (Sauer and others 2003). A single CBC involves between a few and over 100 people counting all the birds they can during a single day within the last two weeks of December within a 24 km diameter circle (total area approximately 450 km^2). Sites are usually centered on some geographic landmark and are in many cases surveyed year after year. CBC analyses utilized data from the 38 years, 1960 to 1997, inclusive. Breeding Bird Surveys consist of 3-minute censuses at a series of 50 stops, 0.8 km apart, conducted once during the breeding season along a road transect. BBS data used spanned 37 years from 1966 to 2002; we included recent survey data since only a handful of sites were associated with areas where SOD symptoms continue to develop. For each BBS or CBC site surveyed, bird community indices were calculated using only the 16 oak-associated species. For the CBC data, community measures were correlated with effort (party hours). The closest linear relationship between these measures and effort was achieved when dividing indices by the log-transformed number of

party hours, thus all CBC estimates were divided by this value. For both BBS and CBC data, average values per site were then calculated by determining mean indices across all years during which a particular circle or route was surveyed.

In order to control for major habitat differences between census sites, we only considered CBC circles and BBS routes occurring within the Jepson ecological regions (Hickman 1993) bounding the complete distributions of *Q. agrifolia* or *L. densiflorus* (*fig. 1*). This effectively excluded large portions of the state devoid of either oak species. Analyses thus involved 60 CBC circles and 56 BBS routes intersected with the Northwestern (NW), Central Western (CW), and Southwestern (SW) California Jepson regions.

Distributional data for California oaks were determined from the California Gap Analysis Project (GAP) (Davis and others 1998). These data are 80 percent accurate (with 95 percent confidence) to a spatial resolution of 100 ha (in other words, 1/450 of the area encompassed by a CBC circle or 1/40 of the length of a BBS route). Target species included *Q. agrifolia*, *L. densiflorus*, and seven other major tree oaks: California black oak (*Q. kelloggii*), canyon live oak (*Q. chrysolepis*), blue oak (*Q. douglasii*), Engelmann oak (*Q. engelmannii*), Oregon white oak (*Q. garryana*), valley oak (*Q. lobata*), and interior live oak (*Q. wislizenii*). Analyses thus considered all tree species of oaks in mainland California; shrub oaks were not included.

Geographic distributions were generated separately for *Q. agrifolia* and *L. densiflorus* occurring in the absence of all other focal oaks and for all possible combinations of 1, 2, and ≥ 3 additional oak species. Geographic overlap between *Q. agrifolia* and *L. densiflorus* is relatively small (approximately 900 km^2), and thus the effect of simultaneous loss of both species in the same areas was not explored. The maximum number of target oak species found within a particular CBC circle or BBS route was used to estimate oak species diversity. The proportion of the total CBC area or length of BBS route that included any of the species of oaks as determined by the GAP distributions was used as an estimate of oak areal extent. Spearman rank correlations considered the influence of oak species diversity and areal extent on the avian community indices.

Point occurrence data for *P. ramorum* were obtained from the California Oak Mortality Task Force archive (Kelly and Tuxen 2003). Climate data used in the analyses originated from WorldClim (Hijmans and others [In press]).

Estimating the effects of SOD on birds

In all cases, we assumed a reduction in oak diversity due to SOD and equivalence of *Q. agrifolia* or *L. densiflorus* with the other tree oak species. We then used three methods characterized by slightly different assumptions to estimate how SOD might affect California oak woodland birds through elimination of *Q. agrifolia* or *L. densiflorus*, differing according to the post-SOD projections of how much oak area would be retained after the disease sweep. Since the ultimate effects of SOD on oak habitats remain unknown, selected habitat loss scenarios were designed to bracket the range of expected outcomes.

In method one (M1), oak area was assumed to decrease by the amount initially occupied by either *Q. agrifolia* or *L. densiflorus*; that is, M1 assumed that there was no small-scale habitat overlap between the target species and any other oak species. In method two (M2), oak area was assumed to decrease by the average amount of area lost when oak diversity dropped by one focal tree species, where values were determined by averaging across all CBC sites or BBS routes minus one tree oak species (including *Q. agrifolia* or *L. densiflorus*, when initial oak species diversity > 1). Finally, in method three (M3), we assumed that there would be no change in oak area, except when oak diversity was limited to a single oak species, in which case the comparison was between sites containing only *Q. agrifolia* or *L. densiflorus* and sites containing no species of oaks, but still within the appropriate Jepson regions. This last method thus assumed complete habitat overlap between *Q. agrifolia* or *L. densiflorus* and other oak species.

We regressed oak woodland bird species richness, diversity, and equitability on oak species diversity and areal extent and calculated new values for oak areal extent under post-SOD conditions, that is, when oak diversity declined by one through loss of *Q. agrifolia* or *L. densiflorus*. We used these post-SOD values for oak species diversity (in other words, one less oak species in all cases) and oak habitat in conjunction with the regression coefficients to calculate post-SOD estimates for each bird index. We then weighted these predicted changes by the areas within California that would be affected by the loss of *Q. agrifolia* or *L. densiflorus* to estimate the total changes in bird species richness, diversity, and equitability expected following a disease sweep. Thus, values presented are estimates of the overall change in avian diversity that is expected to occur within the statewide tanoak and coast live oak geographic ranges.

Predicting the geographical extent of SOD

In all cases, we assume complete elimination of either *Q. agrifolia* or *L. densiflorus*. To determine the extent to which this is realistic over large spatial scales, we developed climatic models aimed at predicting the potential geographic range of *P. ramorum* and, by extension, SOD. Optimal infection of the California bay (*Umbellularia californica*) by *P. ramorum* requires 9 to 12 hr of leaf moisture and temperatures of 18 to 22 °C (Garbelotto and others 2003). Associated climate variables used in our models consisted of 19 measures of temperature, precipitation, and seasonality: 1) annual mean temperature, 2) mean diurnal range, 3) isothermality, 4) temperature seasonality, 5) maximum temperature of the warmest month, 6) minimum temperature of the coldest month, 7) temperature annual range, 8) mean temperature of the wettest quarter, 9) mean temperature of the driest quarter, 10) mean temperature of the warmest quarter, 11) mean temperature of the coldest quarter, 12) annual precipitation, 13) precipitation of the wettest month, 14) precipitation of the driest month, 15) precipitation seasonality, 16) precipitation of the wettest quarter, 17) precipitation of the driest quarter, 18) precipitation of the warmest quarter, and 19) precipitation of the coldest quarter. Climate data were obtained at 30 s resolution (approximately 1 km^2) for 32° 30' to 42° N and 124° 30' to 114° W.

We used a simple profile-matching algorithm (BIOCLIM) (Nix 1986, Busby 1991) to project into geographic space the association between existing pathogen localities and present-day climate. Analyses used the 19 climatic attributes of 183 confirmed *P. ramorum* localities rendered spatially unique at 30 s resolution. Localities were randomly assigned to training (n = 92) and testing (n = 91) subsets for purposes of selecting a single optimal model using approximate Bayesian sampling techniques (see Monahan and Koenig [In press]); pseudo-absence testing data (n = 91) were randomly drawn from areas throughout California where *P. ramorum* is currently absent. The Bayesian analysis provided a framework for comparing among candidate models developed using prior knowledge of typical distributional attributes of BIOCLIM parameters. We decided *a priori* to maximize model sensitivity, specificity, and improvement over chance expectations (Kappa) (Fielding and Bell 1997). Uniform priors were selected to define all minimum (0-0.75 quantiles) and maximum (0.925-1.0 quantiles) model parameters (38 total). Analyses used the priors to randomly determine parameter values from the training data. Combining each new candidate BIOCLIM model with both sets of testing data, sensitivity, specificity, and Kappa were calculated along with their deviations from unity. We allowed for 20,000 iterations and used 10 percent of the ranked output data to define posterior parameter distributions for use in subsequent analyses. The posteriors effectively identify particular combinations of BIOCLIM quantiles that return "best-fit" models; posteriors may also be used to draw inferences about which climate variables most greatly influence overall accuracy.

Results

Oak variables

CBC and BBS datasets yielded comparable estimates of the effects of oak species diversity and oak areal extent on oak woodland bird species richness, diversity, and equitability (*table 1*). All oak woodland bird indices were positively correlated (P < 0.01) with either oak diversity or the amount of oak habitat. These results suggest that oak species diversity and areal extent are key predictors of oak woodland bird species richness, diversity, and equitability and therefore useful variables for purposes of estimating the effects of SOD.

Table 1—*Spearman rank correlations assessing the influence of oak species diversity (*N*) and oak areal extent (*A*) on oak woodland bird species richness, diversity, and equitability. Results reported for two independent bird census datasets (BBS and CBC). Sample sizes (*n*) refer to the number of BBS routes or CBC circles included in the analyses.*

	BBS[1]			CBC[1]		
Bird Index	*N*	*A*	*n*	*N*	*A*	*n*
Richness	0.620**	0.388*	56	0.659**	0.447**	60
Diversity	0.577**	0.364*	56	0.590**	0.567**	60
Equitability	0.365*	0.056	56	0.405**	0.531**	60

[1] * $P < 0.01$, ** $P < 0.001$

Effects of SOD on birds

The three regression models (M1 through M3) used to simulate the effects of SOD yielded predictably different oak woodland bird community responses. M1, which assumed the greatest loss of oak habitat due to loss of *Q. agrifolia* or *L. densiflorus,* generally predicted declines of the greatest magnitude, while M3, which assumed no loss of oak habitat except for sites containing only *Q. agrifolia* or *L. densiflorus*, yielded the smallest estimated declines (*fig. 2a*). Although M1 and M3 paint unrealistically severe (M1) and unrealistically mild (M3) pictures of the likely effects of SOD on oak distributions, the probable effects of SOD on oak areal extent are unknown, and we consider results averaged across M1 through M3 as providing the best current estimates of the effects of the disease on birds. Similarly, given *a priori* knowledge of the seasonal and methodological biases of the survey data, SOD effects are also likely best approximated by averaging across BBS and CBC data.

Using these values, results suggest that bird species richness will decline by 15.2 percent with the loss of *Q. agrifolia* and by 8.7 percent with the elimination of *L. densiflorus*. Similarly, bird species diversity is expected to decrease by 13.4 percent given complete removal of *Q. agrifolia* and 8.9 percent with the loss of *L. densiflorus*. However, the associated estimated declines in relative abundance vary greatly depending on choice of census data. Equitability as determined from BBS data essentially remained unchanged, but based on the CBC was predicted to decline by 11 percent (*Q. agrifolia*) and 10 percent (*L. densiflorus*). Hence, according to the CBC results, but not the BBS, SOD is expected to skew the oak woodland bird community towards fewer, more dominant species.

Partitioning results by the number of oak species in the sites and averaging across M1 through M3, most of the estimated declines in bird species richness, diversity, and equitability were predicted for areas where initial oak species diversity was low (*fig. 2b*). Following the loss of *Q. agrifolia*, 90 to 93 percent of the estimated weighted declines were predicted to occur in woodlands where oak diversity was limited to ≤ 2 species, and 72 to 78 percent in areas with just coast live oak. Similarly, following the loss of *L. densiflorus*, 74 to 82 percent of the estimated weighted declines were expected for areas with ≤ 2 oak species, and 48 to 72 percent in areas where initial oak species diversity was limited to tanoak.

Current geographical extent of SOD

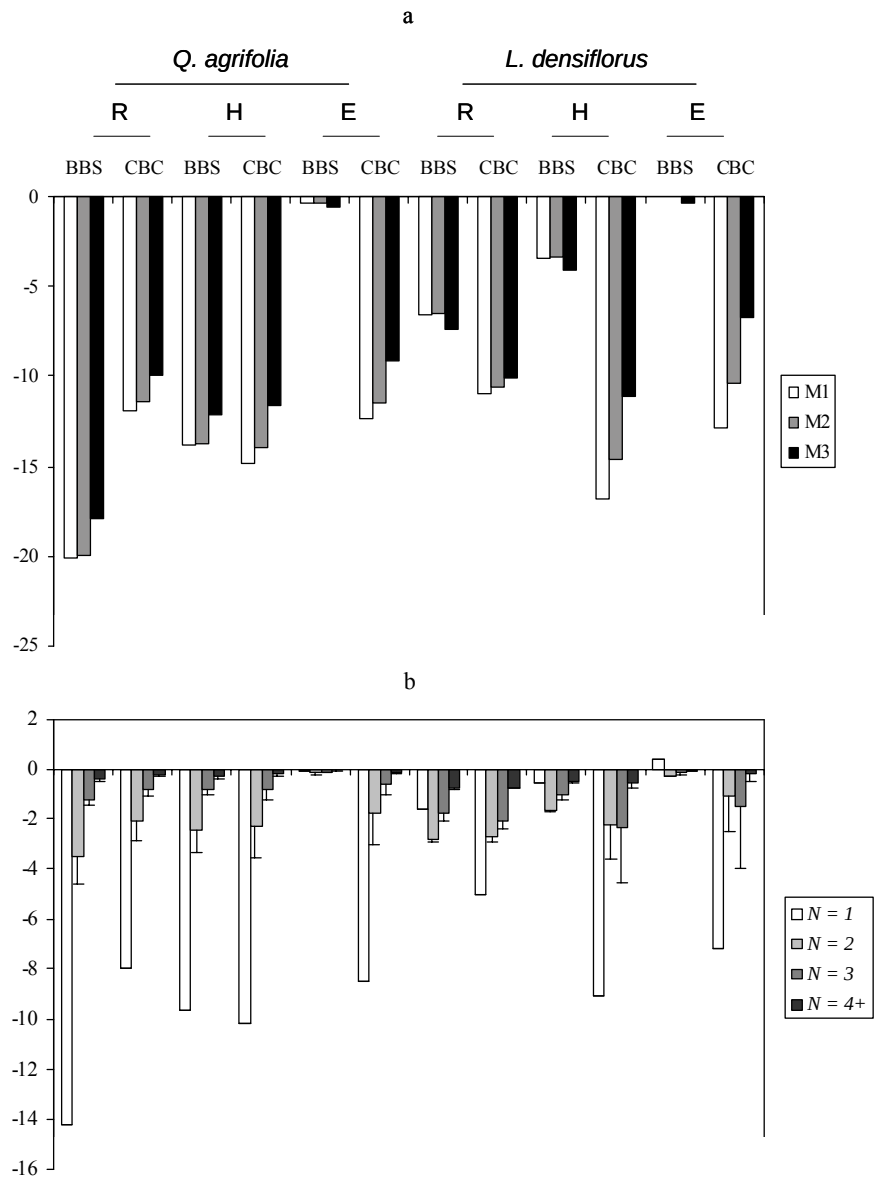

Figure 2—Estimated effects of SOD on bird species richness (R), diversity (H), and equitability (E). Results reported for separate BBS and CBC analyses involving the statewide elimination of either *Q. agrifolia* or *L. densiflorus*; results partitioned according to regression model (a) and initial oak species diversity (b, mean M1 through M3 ± SD).

The optimal SOD model was obtained using a 100 percent bounding envelope with 3-variable relaxation. Hence, minimum and maximum values for each of the 19 starting climate variables were best defined using 0 and 1 quantiles, respectively, and the final model encompassed intersections of 17 or more of these univariate climate distributions projected in geographic space. The final model was extremely accurate at predicting where *P. ramorum* currently does and does not occur (sensitivity = 0.97, specificity = 0.99). It also provided significant

improvement over chance expectations (Kappa = 0.96; according to Landis and Koch [1977], models yielding Kappa values above 0.75 should be considered excellent). Qualitatively the model showed close spatial agreement with other risk models developed using different techniques (Meentemeyer and others 2004, Fowler and Magarey 2005, Guo and others 2005, Kelly and others 2005).

The optimal model identified 17,570 km^2 of coastal California as climatically suited to infection by *P. ramorum*, of which 10,130 km^2 (58 percent) intersect the statewide geographic ranges of *Q. agrifolia* and *L. densiflorus* and 5,000 km^2 (28 percent) intersect areas where *N* is limited to either *Q. agrifolia* or *L. densiflorus* (*fig. 3*). Results suggest that the disease may eventually encompass 6,175 km^2 (24 percent) of the *Q. agrifolia* distribution and 4,693 km^2 (27 percent) of *L. densiflorus*. SOD-suitable areas of special future concern include 750 km^2 of the southern CW Jepson ecological region (San Luis Obispo and Santa Barbara counties). Thus far, SOD has primarily been found in the NW and northern CW Jepson regions, while the geographically disjunct areas predicted for the southern CW region remain disease free. Re-weighting bird results obtained from M1 through M3 by the proportion of the *L. densiflorus* range encompassed by the optimal SOD model, oak woodland bird species richness is expected to decline by 8.5 percent, diversity by 9.6 percent, and equitability by 5.8 percent. Similarly, loss of *Q. agrifolia* within SOD-suitable habitats is expected to reduce bird richness by 13 percent, diversity by 11.3 percent, and equitability by 4.7 percent.

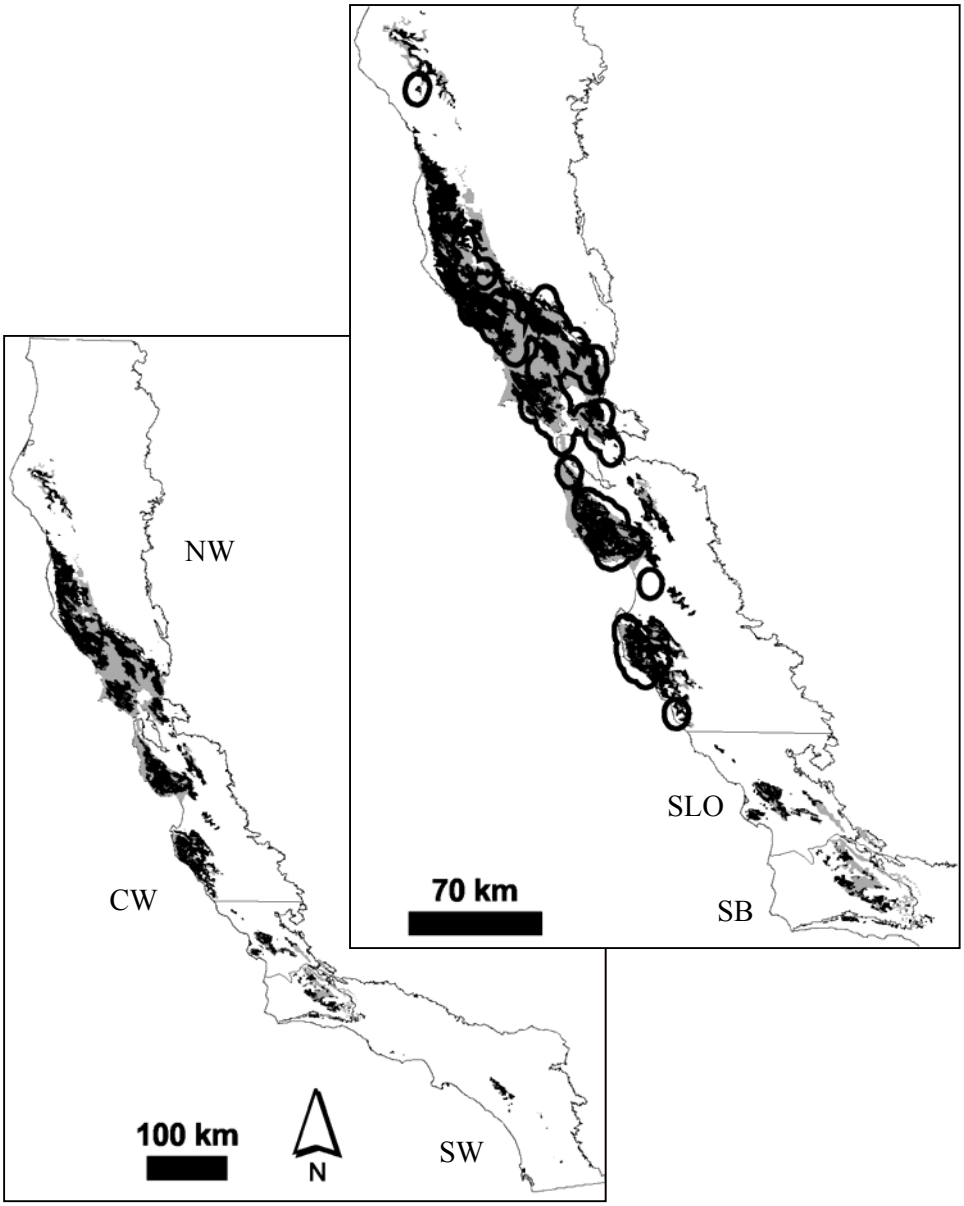

Figure 3—Current climatic SOD model (gray) and intersections with either *Q. agrifolia* or *L. densiflorus* (black). *P. ramorum* localities as of December 2004 are given by the bold polygon. Both San Luis Obispo (SLO) and Santa Barbara (SB) counties possess SOD-suitable climates.

Discussion

We used observed relationships between bird species richness, diversity, and equitability and oak species diversity and areal extent to quantify how the loss of two major tree oak species from California may impact the coastal oak woodland bird community. Three different assumptions were made concerning overlap between the SOD-sensitive oak species (*Q. agrifolia* or *L. densiflorus*) and other tree oaks, ranging from no overlap (and thus complete

loss of *Q. agrifolia* or *L. densiflorus* with loss of oak area [M1]) to complete overlap (and thus no loss of oak habitat with loss of *Q. agrifolia* or *L. densiflorus* [M3]). Since the probable effects of SOD on oak areal extent are unknown, we consider results averaged across M1 through M3 as providing the best current estimates of how the disease stands to impact the avian community. Similarly, the effects of SOD are also likely best approximated by averaging results obtained from the two independent census datasets. Using these values, the statewide elimination of *Q. agrifolia* is predicted to result in 15.2 percent decline in oak woodland bird species richness, 13.4 percent decline in diversity, and 5.7 percent decline in equitability within the current range (25,282 km^2, or 6.2 percent of California's land area) of this species. Complete loss of *L. densiflorus* is expected to reduce oak woodland bird species richness by 8.7 percent, diversity by 8.9 percent, and equitability by 5 percent within its current range (17,173 km^2, or 4.2 percent of California's land area). Weighted estimates obtained for areas encompassed by the optimal SOD model were comparable.

Our post-SOD bird projections are not without precedent, for avian declines of this magnitude have been previously documented in other habitats following forest conversion. For example, the rapid loss of Appalachian Fraser fir (*Abies fraseri*) from an introduced insect (*Adelges piceae*) resulted in >50 percent population declines for six of 11 common bird species in the breeding community (Rabenold and others 1998). The establishment of chestnut blight fungus (*Cryphonectria parasitica*), which virtually exterminated the American chestnut (*Castanea dentata*) throughout much of its original range within 50 years (Anagnostakis 1987, McKeen 1995), was contemporaneous with declines in rank abundance for certain cavity nesting bird species (Haney and others 2001). Rapid loss of the American elm (*Ulmus americana*) following Dutch elm disease (*Ophiostoma ulmi*) (Karnosky 1979) was associated with pronounced population declines in mature forest bird species (Canterbury and Blockstein 1997). Furthermore, sizeable population declines are expected among at least five California bird species, including cavity nesters and mature oak woodland specialists such as acorn and Nuttall's woodpeckers, Hutton's vireo, and oak titmouse, following the SOD-induced loss of *Q. agrifolia* (Monahan and Koenig [In press]).

There are, however, several potentially mitigating factors that may lessen the ultimate impact of SOD as predicted by our models. Oak habitats rich in other tree species might allow certain insectivorous bird species to switch foraging substrates following loss of *Q. agrifolia* (Apigian and Allen-Diaz 2005). Even without this potential buffering capacity, stand-level tree mortality resulting from SOD has thus far not usually been complete (McPherson and others 2002, Rizzo and others 2002), approaching only 50 percent for *Q. agrifolia* in areas of high infection (Brown and Allen-Diaz 2005). Furthermore, if the disease locally eliminates coast live oak or tanoak, recolonization remains a possibility (Weste and others 2002). In the absence of complete elimination of *Q. agrifolia* or *L. densiflorus*, understanding changes in stand structure may be key to accurately estimating bird responses at smaller spatial scales (Winslow and Tietje 2005). Thus, the present post-SOD estimates likely represent the most extreme scenarios for oak woodland birds.

Despite uncertainty surrounding future spatiotemporal dynamics of the disease, most field and laboratory studies indicate that the ultimate influence of SOD will be to significantly reduce numbers of *Q. agrifolia* and *L. densiflorus* in California (Rizzo and Garbelotto 2003). These findings are supported by our climatic SOD model, which predicts that *P. ramorum* could expand to occupy 10,130 km^2 or 24 percent of the total statewide *Q. agrifolia* and *L. densiflorus* distributions (5,000 km^2 or 23 percent of areas where oak species diversity is limited to either *Q. agrifolia* or *L. densiflorus*). Coast live oak woodlands in San Luis Obispo and Santa Barbara counties are of special concern with respect to establishment of the disease, and future SOD projections further suggest that long-term disease sweeps may occur in Sonoma, Monterey, and San Luis Obispo counties (Monahan and Koenig [In press]). Based on our findings, current SOD management decisions aimed at lessening the ultimate impact of the disease on both oaks and birds should target these California counties and direct preventative treatments primarily towards coast live oak woodlands that are low in initial oak species diversity.

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Vegetation and Small Vertebrates of Oak Woodlands at Low and High Risk for Sudden Oak Death in San Luis Obispo County, California[1]

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Abstract

San Luis Obispo County contains oak woodlands at varying levels of risk of sudden oak death (SOD), caused by a fungal pathogen (*Phytophthora ramorum*) that in the past decade has killed thousands of oak (*Quercus* spp.) and tanoak (*Lithocarpus densiflorus*) trees in California. SOD was most recently detected 16 km north of the San Luis Obispo County line. Low-risk woodlands occupy 57 percent of the county's land area, whereas high-risk woodlands occupy only 1 percent. During 2002-2004, we collected data on vegetative structure and small vertebrate (birds, small mammals, amphibians, reptiles) abundance in both types of woodland within the county. One study site was located in low-risk habitat and two in high-risk habitat. The two high-risk sites were similar in terms of vegetative structure and wildlife, while being much different from the low-risk site. Of the 11 vegetation attributes measured, tree density, basal area, canopy cover, coarse woody debris, and litter depth were greater at both high-risk sites ($P < 0.05$). Small mammals and amphibians were more abundant at the high-risk sites ($P < 0.05$), while birds and reptiles were more abundant at the low-risk site ($P < 0.05$). Species that were notably abundant in high-risk compared to low-risk habitat included the chestnut-backed chickadee (*Poecile rufescens*), Steller's jay (*Cyanocitta stelleri*), orange-crowned warbler (*Vermivora celata*), dusky-footed woodrat (*Neotoma fuscipes*), brush mouse (*Peromyscus boylii*), Monterey salamander (*Ensatina eschscholtzii eschscholtzii*), and slender salamander (*Batrachoseps* spp.). Due to the scarcity and fragmented distribution of high-risk woodlands within San Luis Obispo County, these wildlife species may be severely impacted if the pathogen reaches the county.

Key words: California, oak woodlands, *Phytophthora ramorum, Quercus agrifolia,* small vertebrates, sudden oak death, wildlife habitat

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Introduction

Many wildlife species utilize California's oak woodlands (*Quercus* spp.) for food, shelter, and reproduction (Pavlik and others 1991, California Department of Fish and Game 2002). Acorns are such an important food resource that deer and rodent populations can fluctuate in synchrony with the annual mast crop (Barrett 1980). Food resources are not limited to acorns, however. The dusky-footed woodrat (*Neotoma fuscipes*) feeds heavily upon coast live oak (*Quercus agrifolia*) shoots and leaves in some locations (Linsdale and Tevis 1951), and several bird species preferentially forage on oak trees (Block and Morrison 1987, Apigian and Allen-Diaz this volume). Some species, such as the acorn woodpecker (*Melanerpes formicivorus*), may even be dependent upon oaks for their continued survival (CalPIF 2002).

California oak woodlands are threatened, however, by natural and anthropogenic disturbances, including poor oak regeneration, firewood cutting, housing and vineyard development, and severe wildfires (Merenlender 2000, Fire and Resource Assessment Program 2003). Within the last 10 years, perhaps the most serious threat to emerge has been sudden oak death (SOD), a disease caused by the fungal pathogen *Phytophthora ramorum*. This pathogen primarily kills coast live oak, tan oak (*Lithocarpus densiflorus*), and California black oak (*Q. kelloggii*) (Rizzo and Garbelotto 2003). Although we currently have little knowledge of the disease's impacts upon wildlife communities, previous forest pathogen outbreaks suggest that significant impacts to wildlife may occur (Liebhold and others 1995).

In San Luis Obispo County, the Agriculture and Open Space Element of the General Plan makes mention of the scenic beauty and wildlife habitat provided by the county's coastal oak woodlands (San Luis Obispo County 1998), and a recent revision (San Luis Obispo County 2004) remarked: "… special standards need to be applied so that the woodlands are protected". Although SOD has not yet been identified in San Luis Obispo County, the disease is currently known in southern Monterey County, 16 km north of the San Luis Obispo County line. Meentemeyer and others (2004) identified 102 km^2 of San Luis Obispo County's coastal oak woodland (1.2 percent of the total county area) to be at high risk for the disease compared to 4,891 km^2 at low risk (56.9 percent of the total county area). Nearly all high-risk areas occur within 20 km of the coast in the northern third of the county and in a mosaic that is closely associated with medium- and low-risk habitats. If the disease becomes established in San Luis Obispo County, planners and natural resource managers will require information on the habitat and faunal characteristics of the high-risk coastal oak woodlands on which to base protective measures.

We sampled the habitat structure and wildlife communities in San Luis Obispo County oak woodlands at one site at low risk of SOD infection and two sites at high risk. We chose to study four wildlife taxa (birds, small mammals, amphibians, and reptiles), which we believed would be sensitive to habitat alterations caused by SOD should the disease become established in the county. Our study objectives were to: 1) compare vegetation attributes and small vertebrate occurrences and relative abundances in oak woodlands at low risk and high risk for SOD, and 2) provide baseline data on the habitat and small vertebrate wildlife potentially useful to natural resource planners and managers striving to protect the wildlife within the county's oak woodlands.

Study Areas

Low-Risk Habitat

Our low-risk (hereafter "LR") study site was within the "high country" of Camp Roberts, a training facility of the Army National Guard in north-central San Luis Obispo County (*fig. 1*), at which we have conducted wildlife research since 1993. The northern portion of Camp Roberts is in Monterey County. Within the 17,800-ha facility, 7,200 ha are classified oak woodland (Camp Roberts 1989). The woodland is within the area classified by Meentemeyer and others (2004) at low risk of SOD infection. Blue oak (*Quercus douglasii*), a non-host of *P. ramorum*, is the predominant tree species in the overstory with a variable contribution of coast live oak. When present, the understory is composed of poison oak (*Toxicodendron diversilobum*), toyon (*Heteromeles arbutifolia*), redberry (*Rhamnus crocea*), bigberry manzanita (*Arctostaphylos glauca*), and ceanothus (*Ceanothus* spp.). On the woodland floor, wild oats (*Avena* spp.), bromes (*Bromus* spp.), and fescues (*Festuca* spp.) dominate. Common forbs include deerweed (*Lotus scoparius*), fiddleneck (*Amsinckia* spp.), filaree (*Erodium* spp.), hummingbird sage (*Salvia spathacea*), and the exotic yellow star thistle (*Centaurea solstitialis*). Most of the "high country" on Camp Roberts receives minimal use by military personnel and is not managed specifically for livestock, woodcutting, or other land-use activities. Sheep grazing is conducted on Camp Roberts each spring, but our study sites receive minimal or no grazing due to the steep terrain and dense shrub and tree cover.

High-Risk Habitat

In 2002, within areas of high risk (hereafter "HR") habitat in San Luis Obispo County, we established two study sites (hereafter HR1 and HR2) on private cattle ranches (*fig. 1*). Sites were chosen based on accessibility and the presence of coast

live oak woodlands having large oak trees (>50 cm dbh), a dense canopy layer, and California bay (*Umbellularia californica*) as a co-dominant tree species. The presence of California bay is a strong predictor of woodlands at high risk for SOD (Meentemeyer and others 2004). Lesser amounts of Pacific madrone (*Arbutus menziesii*) and tanoak are present. The understory is composed of shrubs, including California blackberry (*Rubus ursinus*), creeping snowberry (*Gaultheria hispidula*), poison oak, and toyon. Ground vegetation comprises a mix of herbaceous plants such as Western bracken fern (*Pteridium aquilinum*), California polypody (*Polypodium californicum*), fiesta flower (*Pholistoma* spp.), and miner's lettuce (*Claytonia perfoliata*). Our study sites receive only minimal grazing by cattle due to the steep terrain and limited production of forage.

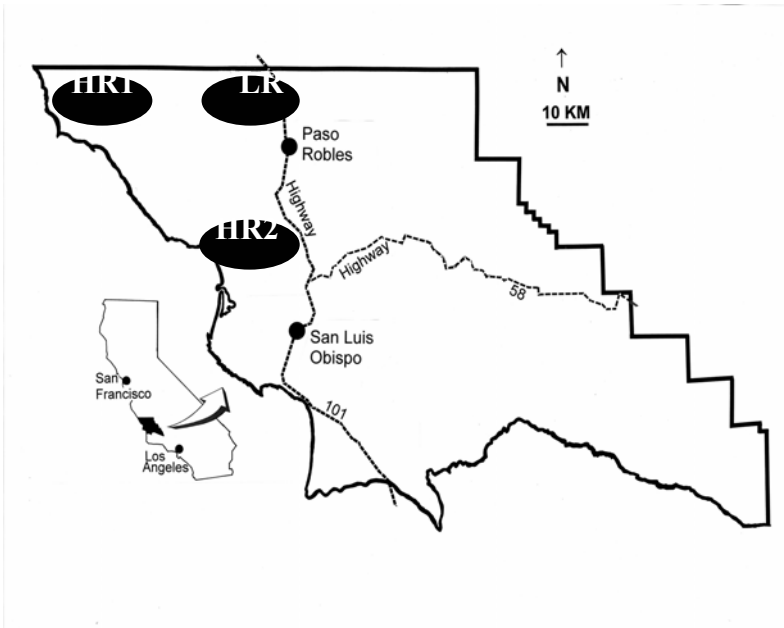

Figure 1—Location of study sites containing oak woodlands at low risk and high risk of SOD infection, San Luis Obispo County, California, 2002-2004. LR = low-risk site, HR1 = high-risk site 1, HR2 = high-risk site 2.

Methods

Field Methods

Vegetation

We gathered data on the following vegetation attributes: tree (≥10.0 cm dbh) density and diameter, snag (≥10.0 cm dbh) density and diameter, shrub cover, canopy cover, coarse woody debris (CWD) volume, litter depth, and number of woodrat dwellings (hereafter "houses"). At site LR, we primarily used vegetation data from prior studies. The sampling protocol for snags, canopy, shrubs, litter, and woodrat houses

is described in Vreeland and Tietje (2002). The sampling protocol for trees is described in Tietje and others (1997). In February 2005, we collected more detailed CWD data at site LR on 11 randomly selected small-mammal trapping grids (see below). Within each grid, we located four sampling plots at random grid intersections (44 plots total). From the plot center, we established 10-m transects in each cardinal direction and recorded the length and diameter of each CWD piece (≥7.6 cm in diameter, ≥1 m in length) crossing a transect (Waddell 2002). In 2004, we collected data on all of the above vegetation attributes on 10 small-mammal trapping grids (see below) on the HR sites (three at site HR1, seven at site HR2). Within each grid, we located eight plots at random grid intersections (80 plots total). We used the same plot design (10-m-radius circle) and sampling techniques used at site LR with the exception of tree density. Whereas we had used a point-center quarter method at site LR (Cottam and Curtis 1956), we opted to count all trees within each plot at sites HR1 and HR2.

Breeding Birds

At sites LR and HR2 during March-June, 2002-2003, we conducted point-counts of breeding birds at point-count stations that were at least 150 m apart. In 2002, we visited 86 point-count locations at site LR and 30 point-count locations at site HR2. In 2003, we visited a subset of 12 randomly selected point-count locations at each site. Each year, we made five visits to a sampled point-count location. Each visit lasted 10 minutes and occurred between official sunrise and 1100 hours. We recorded all birds detected within 50 m of a point per standard breeding-bird survey protocols (Bibby and others 1992).

Small Mammals

We sampled small mammals on 1.1-ha, 8 X 8 trapping grids (64 intersections, 15-m spacing). We trapped on 22 grids at site LR, three grids at site HR1, and five grids at site HR2 (adding two more grids at HR2 for the final trapping session and for sampling vegetation [see above]). We conducted trapping sessions during April-May 2003, October-November 2003, and April-May 2004. We trapped for three consecutive nights. We placed a single Sherman live trap (7.6 X 9.5 X 30.5 cm) baited with COB (rolled corn, oats, and barley laced with molasses) at each grid intersection. We tagged captured animals with individually numbered ear tags and recorded the tag number, species, capture location, sex, mass (g), and age class (juvenile or adult). If an individual's pelage was >25 percent gray, we recorded the animal as a juvenile. We handled the animals in accordance with the University of

California, Berkeley, Animal Use Protocol #R166-0199, and released them at the site of capture.

Amphibians and Reptiles

During 2002-2004, we counted amphibians and reptiles under plywood coverboards as described in Tietje and Vreeland (1997). At sites HR1 and HR2, we placed coverboards along the A, C, F, and H rows of 8 X 8 small mammal trapping grids, for a total of 32 coverboards per grid. (We did not place coverboards on the grids added to HR2 in 2004 for trapping small mammals and sampling vegetation [see above].) At site LR, we sampled coverboards along alternate rows of 17 X 17 trapping grids (17 X 8; 136 coverboards per grid) that we have used for wildlife study since 1993 (Tietje and others 1997). From January to mid-May, we checked each coverboard every 2-3 weeks. We recorded the location, species, and number of individuals for all detections. In 2002, we checked coverboards at site LR (eight grids) and site HR2 (five grids). In 2004, we checked coverboards at site HR1 (three grids) and site HR2 (five grids). We made eight sampling visits to each grid in 2002 and six visits to each grid in 2004. Three species of slender salamander (*Batrachoseps nigriventris, B. incognitas,* and *B. minor*) potentially occur at each study site (Jockusch and Wake 2002). These species cannot be distinguished in the field, so we grouped all *Batrachoseps* species into one category (hereafter "slender salamanders").

Data Analysis

Climate

We downloaded climate data for San Luis Obispo County weather stations from an online database (University of California 2004). We used data from stations operated by the U.S. Department of Commerce/National Oceanic and Atmospheric Administration. We obtained daily maximum temperature, minimum temperature, and precipitation data from 1994-2004 for three weather stations near our study sites. We chose station #NCDC 6730 (Paso Robles) for its proximity to site LR, and stations #NCDC 5866 (Morro Bay) and #NCDC 7851 (San Luis Obispo) for their proximity to sites HR1 and HR2. We then calculated average daily values over the 10-year period for the "low-risk" weather station data and for the combined data of the "high-risk" stations.

Vegetation

We compared 11 vegetation attributes among study sites using a 1-way ANOVA (α = 0.05; Quinn and Keough 2002). We treated each trapping grid as a sampling unit. Thus, each vegetation plot within a grid was a subsample. We calculated CWD volume according to Waddell (2002). We transformed CWD volume (natural log) and canopy cover (arcsine) to achieve normal distributions.

Breeding Birds

We used the California Wildlife Habitat Relationships computer program (California Fish and Game 2002) to identify breeding bird species in each habitat type. For each breeding bird species, we calculated its annual relative abundance at each point-count location by using the maximum number of individuals recorded during a single visit. We then computed an annual Shannon-Wiener diversity index at each point-count location using the species abundance data (Ricklefs 1979). We determined the annual species richness at each point-count location by counting the total number of breeding bird species recorded from all five visits. We compared attributes of the bird community (*see table 3*) between the two study sites using a 3-way ANOVA (α = 0.05; Quinn and Keough 2002) with site, time period (year), and their interaction as the independent variables. We were mainly interested in study site as the independent variable, but included time and a time*site interaction term to control for temporal variation.

Small Mammals

For all small mammal species, we calculated seasonal relative abundance on each grid by determining the minimum number of animals (MNA) captured during each trapping session. We then computed the Shannon-Wiener diversity index for each trapping session on a grid using the species abundance data (Ricklefs 1979). We determined seasonal species richness on each grid by counting the total number of species recorded during each trapping session. Because small mammals frequently lost weight with successive captures during a trapping session, we used the maximum weight recorded for each individual during a trapping session. We compared attributes of the small mammal community (*see tables 4 and 5*) among study sites using a 3-way ANOVA (α = 0.05; Quinn and Keough 2002) with site, time period (trapping session), and their interaction as the independent variables. We were mainly interested in study site as the independent variable, but included time and a time*site interaction term to control for temporal variation. Because sample sizes varied among study sites, we used Type III sum of squares to determine statistically significant

effects (Quinn and Keough 2002). When ANOVA indicated that study site was a significant factor for a given variable, we performed pairwise comparisons among study sites using Ryan's *Q* test (Quinn and Keough 2002).

Amphibians and Reptiles

We calculated the annual relative abundance of each amphibian and reptile species on each grid by using the maximum number of individuals recorded during a single visit. Because grids at site LR contained more coverboards than grids at sites HR1 and HR2 (136 vs. 32), we divided the species abundance on each LR grid by 4.25. For both the amphibian and reptile communities, we then computed an annual Shannon-Wiener diversity index on each grid using the species abundance data (Ricklefs 1979). We determined annual species richness on each grid by counting the total number of species recorded during all visits. We compared attributes of the amphibian and reptile communities between sites LR and HR2 (2002 data, see *table 6*) and between sites HR1 and HR2 (2004 data, *see table 7*). We were unable to transform either the amphibian or reptile abundance data to achieve a normal distribution, so we performed non-parametric Mann-Whitney *U* tests for each comparison ($\alpha = 0.05$; Quinn and Keough 2002).

Results

Climate

Temperature extremes at the Paso Robles weather station (in effect, "low-risk" habitat) were greater than those at the Morro Bay and San Luis Obispo weather stations (in effect, "high-risk" habitat, table 1). Although all weather stations experienced arid conditions during the warmer months (May-October), precipitation during the cooler months (November-April) averaged 20 cm more at the "high-risk" stations (table 1).

Table 1—*Average daily maximum and minimum temperatures and seasonal precipitation at three weather stations near study sites containing oak woodlands at low risk (LR) and high risk (HR) of SOD infection, San Luis Obispo County, California, 1994-2004. Climatic data was obtained from weather stations operated by the U.S. Department of Commerce/National Oceanic and Atmospheric Administration. The LR weather station was at Paso Robles, California, and the HR weather stations were at Morro Bay and San Luis Obispo, California.*

	LR	HR
Average daily temperatures (°C)		
Maximum (May-October)	30	22
Minimum (May-October)	9	11
Maximum (November-April)	18	18
Minimum (November-April)	3	7
Average seasonal rainfall (cm)		
May-October	3	5
November-April	32	52

Vegetation

At site LR, all trees were either blue oak (81 percent) or coast live oak (19 percent). In contrast, sites HR1 and HR2 contained greater amounts of coast live oak and a more diverse tree community. At these two sites, observed tree species included coast live oak (58 percent), California bay (24 percent), and madrone (12 percent). The remaining 6 percent of the trees were predominantly tanoak, valley oak (*Quercus lobata*), and bigleaf maple (*Acer macrophyllum*).

Both HR sites (HR1, HR2) had well-structured overstories, compared to site LR. Tree density was over twice as great and tree basal area over three times greater on both HR sites (*table 2,* $P < 0.05$). In addition, tree canopy covered approximately 90 percent of both HR sites, compared to 74 percent of site LR (*table 2,* $P < 0.05$). Presumably due to their greater tree densities, both HR sites also had more CWD and a deeper litter layer than site LR (*table 2,* $P < 0.05$).

Site LR did have higher observed values than either site HR1 or HR2 for some vegetation attributes. Due to a more open canopy, shrub cover was greater at site LR (*table 2,* $P < 0.05$). In addition, we observed a greater density of woodrat houses ($P < 0.10$) and snags ($P < 0.10$) at site LR, although the differences were not significant at our specified α level of 0.05.

Table 2— *Mean values (and standard errors) of vegetation attributes on study sites containing oak woodlands at high risk (HR1, HR2) and low risk (LR) of SOD infection, San Luis Obispo County, California.*

	HR1 (n = 3)	HR2 (n = 7)	LR (n = 11)*
Tree density (stems/ha)	[A] 493.5 (201.3)	[A] 506.0 (26.7)	[B] 238.6 (22.9)
Tree diameter (cm)	[A] 30.9 (5.4)	[AB] 27.6 (0.7)	[B] 22.6 (0.8)
Tree basal area (m^2/ha)	[A] 29.5 (1.0)	[A] 30.1 (1.4)	[B] 9.3 (0.7)
Snag density (stems/ha)	[A] 17.3 (11.3)	[A] 21.6 (3.1)	[A] 39.4 (7.9)
Snag diameter (cm)	[A] 26.7 (6.2)	[A] 18.9 (1.3)	[A] 21.4 (2.0)
Snag basal area (m^2/ha)	[A] 0.8 (0.4)	[A] 0.6 (0.1)	[A] 1.3 (0.2)
Canopy cover (%)	[A] 91.0 (1.3)	[A] 89.1 (1.9)	[B] 73.5 (2.9)
Shrub cover (%)	[A] 50.3 (14.9)	[A] 50.6 (5.6)	[A] 71.2 (4.4)
CWD (natural log [m^3/ha])	[A] 1.15 (0.03)	[A] 1.11 (0.04)	[B] 0.84 (0.04)
Litter depth (cm)	[A] 6.1 (1.6)	[A] 6.0 (0.3)	[B] 2.7 (0.2)
Woodrat houses (no./ha)	[A] 22.5 (8.7)	[A] 18.8 (4.2)	[A] 45.7 (8.5)

* n = 9 for tree density, tree diameter, and tree basal area.

[A, B] *Sites sharing a common letter are not significantly different for a given attribute (Ryan's* Q *test, α = 0.05).*

Birds

At site LR, the most abundant bird species were the oak titmouse (*Baeolophus inornatus*), bushtit (*Psaltriparus minimus*), house finch (*Carpodacus mexicanus*), lesser goldfinch (*Carduelis psaltria*), western scrub-jay (*Aphelocoma californica*), and dark-eyed junco (*Junco hyemalis*) *(table 3)*. At site HR2, the most abundant species were the chestnut-backed chickadee (*Parus rufescens*), Steller's jay (*Cyanocitta stelleri*), dark-eyed junco, and orange-crowned warbler (*Vermivora celata*) *(table 3)*. Total abundance of all species, species richness, and Shannon-Wiener diversity index were all significantly higher at site LR (*table 3, P* < 0.05). Of the eight focal conservation species identified in the Oak Woodland Bird Conservation Plan (CalPIF 2002), seven were significantly more abundant at site LR (*table 3, P* < 0.05).

Table 3—*Mean values (and standard errors) of bird species abundance and species diversity on 50-m-radius plots in oak woodlands at high risk (HR1, HR2) and low risk (LR) of SOD infection, San Luis Obispo County, California. Five annual visits were made to each plot during March-June, 2002 and 2003. The following species are listed below: a) the 10 most abundant species at each site, and b) focal conservation species identified in CalPIF (2002).*

	HR2 (n = 42)	LR (n = 98)
Species abundance (maximum no. individuals/single visit)		
All species	24.1 (0.8)	34.6 (0.8) **
#Acorn woodpecker (*Melanerpes formicivorus*)	0.1 (0.1)	0.8 (0.1) **
Ash-throated flycatcher (*Myiarchus cinerascens*)	0.0 (0.0)	1.4 (0.1) **
# Blue-gray gnatcatcher (*Polioptila caerulea*)	0.0 (0.0)	0.7 (0.1) **
Brown creeper (*Certhia americana*)	1.1 (0.1) **	0.0 (0.0)
Bushtit (*Psaltriparus minimus*)	0.8 (0.2)	2.5 (0.3) **
Chestnut-backed chickadee (*Poecile rufescens*)	2.8 (0.4) **	0.3 (0.1)
Dark-eyed junco (*Junco hyemalis*)	2.2 (0.2)	2.3 (0.2)
House finch (*Carpodacus mexicanus*)	0.1 (0.0)	2.5 (0.2) **
Hutton's vireo (*Vireo huttoni*)	1.3 (0.1)	1.3 (0.1)
# Lark sparrow (*Chondestes grammacus*)	0.1 (0.0)	0.5 (0.1) **
Lesser goldfinch (*Carduelis psaltria*)	0.3 (0.1)	3.3 (0.2) **
# Nuttall's woodpecker (*Picoides nuttallii*)	0.4 (0.1)	0.8 (0.1) **
# Oak titmouse (*Baeolophus inornatus*)	0.9 (0.1)	3.6 (0.1) **
Orange-crowned warbler (*Vermivora celata*)	2.1 (0.2) **	0.1 (0.0)
Pacific-slope flycatcher (*Empidonax difficilis*)	1.3 (0.1) **	0.1 (0.0)
Spotted towhee (*Pipilo maculatus*)	1.6 (0.2) *	1.2 (0.1)
Steller's jay (*Cyanocitta stelleri*)	2.6 (0.2) **	0.0 (0.0)
Violet-green swallow (*Tachycineta thalassina*)	0.2 (0.1)	1.8 (0.1) **
Warbling vireo (*Vireo gilvis*)	1.3 (0.2) **	0.0 (0.0)
# Western bluebird (*Sialia mexicana*)	0.0 (0.0)	1.1 (0.1) **
# Western scrub-jay (*Aphelocoma californica*)	0.3 (0.1)	2.5 (0.1) **
# Yellow-billed magpie (*Pica nuttalli*)	0.0 (0.0)	0.1 (0.0)
Species richness (no. of species per point-count location)	14.3 (0.5)	17.2 (0.3) **
Shannon-Wiener diversity index	2.50 (0.04)	2.64 (0.02) **

Identified as focal species in Oak Woodland Bird Conservation Plan (CalPIF 2002).
* *Difference between sites statistically significant at* P < *0.05 (Mann-Whitney* U *test).*
** *Difference between sites statistically significant at* P < *0.01 (Mann-Whitney* U *test).*

Small Mammals

Total small mammal abundance was much greater on both HR sites than on site LR (*table 4*). Among the commonly captured species, the dusky-footed woodrat, brush mouse (*Peromyscus boylii*), and California mouse (*P. californicus*) were all more abundant at sites HR1 and HR2 than at site LR (*table 4, P* < 0.05). Brush mice were particularly abundant at site HR1, being three times more abundant than at site HR2 and 10 times more abundant than at site LR (*table 4*). Only the piñon mouse (*P. truei*) was more abundant at site LR than either HR site (*table 4, P* < 0.05). Species diversity was similar at all three sites, both in terms of species richness and the Shannon-Wiener diversity index (*table 4*).

Table 4—*Mean values (and standard errors) of small mammal abundance (minimum number of animals) and species diversity on 1.1-ha trapping grids in oak woodlands at high risk (HR1, HR2) and low risk (LR) of SOD infection, San Luis Obispo County, California. Three trapping sessions were conducted on each grid (spring 2003, fall 2003, spring 2004). Each trapping session lasted for 3 consecutive nights.*

	HR1 (n = 9)	HR2 (n = 17)	LR (n = 66)
Species abundance (minimum no. animals per grid)			
All species	[A] 86.2 (6.0)	[B] 47.6 (4.2)	[C] 24.1 (1.4)
Brush mouse (*Peromyscus boylii*)	[A] 46.0 (10.0)	[B] 14.1 (1.9)	[C] 4.3 (0.5)
California mouse (*P. californicus*)	[A] 12.9 (1.3)	[A] 15.3 (2.3)	[B] 1.5 (0.3)
Dusky-footed woodrat (*Neotoma fuscipes*)	[A] 20.8 (4.8)	[A] 16.8 (1.4)	[B] 9.0 (0.7)
Piñon mouse (*P. truei*)	[B] 1.0 (0.7)	[B] 0.0 (0.0)	[A] 7.0 (0.6)
Species richness (no. of species per grid)	[A] 4.22 (0.14)	[A] 3.88 (0.21)	[A] 3.79 (0.15)
Shannon-Wiener diversity index	[A] 1.05 (0.10)	[A] 1.12 (0.03)	[A] 1.08 (0.03)

[A, B, C] *Sites sharing a common letter are not significantly different for a given attribute (Ryan's* Q *test, α = 0.05).*

Both male and female dusky-footed woodrats were significantly heavier on sites HR1 and HR2 than site LR (*table 5, P* < 0.05). Male and female California mice body masses were also higher at sites HR1 and HR2 than site LR, although the difference was significant only for site HR1 (*table 5, P* < 0.05). We detected no significant differences in body mass among study sites for male or female brush mice (*table 5*).

Table 5— *Mean body masses (and standard errors) in grams of 3 small mammal species trapped on 1.1-ha trapping grids in oak woodlands at high risk (HR1, HR2) and low risk (LR) of SOD infection, San Luis Obispo County, California. Trapping was conducted during spring 2003, fall 2003, and spring 2004.*

	Body mass (g)		
	HR1*	HR2*	LR*
Dusky-footed woodrat (*Neotoma fuscipes*)			
Male (*n* = 419)	[A] 243.5 (4.3)	[A] 239.2 (3.8)	[B] 218.7 (3.2)
Female (*n* = 521)	[A] 214.0 (3.4)	[A] 213.2 (3.6)	[B] 196.7 (1.9)
California mouse (*Peromyscus californicus*)			
Male (*n* = 203)	[A] 37.2 (0.5)	[A B] 36.4 (0.4)	[B] 35.1 (0.6)
Female (*n* = 197)	[A] 40.5 (0.8)	[B] 38.3 (0.5)	[B] 36.7 (0.9)
Brush mouse (*P. boylii*)			
Male (*n* = 408)	[A] 21.9 (0.2)	[A] 22.2 (0.2)	[A] 22.0 (0.2)
Female (*n* = 423)	[A] 22.5 (0.3)	[A] 22.1 (0.3)	[A] 21.9 (0.3)

[A, B] *Sites sharing a common letter are not significantly different for a given attribute (Ryan's* Q *test, α = 0.05).*

Amphibians and Reptiles

During 2002, amphibian abundance was nearly eight times greater at site HR2 than site LR (*table 6, P* < 0.05), mainly due to the Monterey salamander (*Ensatina eschscholtzii eschscholtzii*). Monterey salamanders were abundant on site HR2, but absent from site LR. Slender salamanders (*Batrochoseps* spp.) were more abundant on site HR2 (*table 6, P* < 0.10), although the difference was not significant at our specified α level of 0.05. In addition, species richness and the Shannon-Wiener diversity index were significantly higher at site HR1 (*table 6, P* < 0.05). In contrast, reptiles were observed almost exclusively on the LR site (*table 6*). While we recorded 10 reptile species at site LR (three lizard, one skink, and six snake spp.), we observed only one reptile species at site HR1 (western fence lizard [*Sceloporus occidental*]).

Table 6—*Mean values (and standard errors) of amphibian and reptile species abundance and diversity on study sites containing oak woodlands at high risk (HR2) and low risk (LR) of SOD infection, San Luis Obispo County, California. Sampling was conducted during January-May 2002. The maximum numbers of animals observed per grid on the LR site were divided by 4.25 to account for a larger grid size at site LR (136 coverboards per grid at site LR, 32 coverboards per grid at site HR2).*

	HR2 (n = 5)	LR (n = 8)
Amphibians		
Species abundance (maximum no. individuals/single visit)		
All species	17.8 (4.7) **	2.3 (0.8)
Monterey salamander (*Ensatina e. eschscholtzii*)	12.6 (3.8) **	0.0 (0.0)
Slender salamander (*Batrochoseps* spp.)	4.6 (2.1)	2.2 (0.8)
Species richness (no. of species per grid)	2.4 (0.2) *	1.4 (0.2)
Shannon-Wiener diversity index	0.62 (0.12) *	0.13 (0.07)
Reptiles		
Species abundance (maximum no. individuals/single visit)		
All species	0.2 (0.2)	6.9 (1.0) **
California legless lizard (*Anniella pulchra*)	0.0 (0.0)	1.2 (0.6) *
Southern alligator lizard (*Gerrhonotus multicarinatus*)	0.0 (0.0)	0.4 (0.1) *
Western fence lizard (*Sceloporus occidentalis*)	0.2 (0.2)	1.7 (0.3) **
Western skink (*Eumeces skiltonianus*)	0.0 (0.0)	3.1 (0.5)**
Snakes (all snake species)	0.0 (0.0)	0.6 (0.1) *
Species richness (no. of species per grid)	0.2 (0.2)	5.8 (0.6) **
Shannon-Wiener diversity index	0.00 (0.00)	1.20 (0.09) **

** Difference between sites statistically significant at* P < *0.05 (Mann-Whitney* U *test).*
*** Difference between sites statistically significant at* P < *0.01 (Mann-Whitney* U *test).*

During 2004, the amphibian and reptile communities were similar on sites HR1 and HR2. No comparison between sites HR1 and HR2 for any species or group of amphibians or reptiles was significant (*table 7*). Species richness and the Shannon-Wiener diversity index were also similar for both amphibians and reptiles at each site (*table 7*). As in 2002 at site HR1, amphibians were observed frequently, but few reptiles were found (*tables 6 and 7*).

Table 7— *Mean values (and standard errors) of amphibian and reptile species abundance and diversity on study sites containing oak woodlands at high risk (HR1, HR2) of SOD infection, San Luis Obispo County, California. Sampling was conducted during January-May 2004. No attributes were significantly different between study sites (*P *< 0.05, Mann-Whitney* U *test).*

	HR1 (n = 5)	HR2 (n = 3)
Amphibians		
Species abundance (maximum no. individuals/single visit)		
All species	26.4 (8.2)	20.7 (0.7)
Monterey salamander (*Ensatina eschscholtzii*)	17.4 (6.3)	18.3 (1.5)
Slender salamander (*Batrochoseps* spp.)	8.8 (3.1)	2.3 (0.9)
Species richness (no. of species per grid)	2.2 (0.2)	2.0 (0.0)
Shannon-Wiener diversity index	0.60 (0.05)	0.34 (0.09)
Reptiles		
Species abundance (maximum no. individuals/single visit)		
All species	0.6 (0.4)	0.3 (0.3)
California legless lizard (*Anniella pulchra*)	0.0 (0.0)	0.0 (0.0)
Southern alligator lizard (*Gerrhonotus multicarinatus*)	0.2 (0.2)	0.3 (0.3)
Western fence lizard (*Sceloporus occidentalis*)	0.4 (0.2)	0.0 (0.0)
Western skink (*Eumeces skiltonianus*)	0.0 (0.0)	0.0 (0.0)
Snakes (all snake species)	0.0 (0.0)	0.0 (0.0)
Species richness (no. of species per grid)	0.6 (0.4)	0.3 (0.3)
Shannon-Wiener diversity index	0.14 (0.14)	0.00 (0.00)

Discussion

Differences Between Low-Risk and High-Risk Sites

Compared to the low-risk site, more moderate annual temperatures and over 1.6 times the rainfall characterized our San Luis Obispo sites at high risk of SOD infection (*table 1*). Warm, wet winters typify coastal oak woodlands in California at high risk of SOD (Major 1988, Rizzo and Garbelotto 2003). These climatic conditions are largely responsible for the habitat differences that we observed between our low-risk and high-risk study sites. Our low-risk study site contained more open stands of smaller trees (predominately blue oak), somewhat more shrub cover, but much less downed wood and litter. Our high-risk study sites were

characterized by stands of larger trees (predominately coast live oak and California bay) with a dense canopy, somewhat more open understory, large amounts of downed wood, and a well-developed litter layer. We surmise that these structural differences in vegetation between the low-risk and high-risk study sites were primarily responsible for the observed differences in the small vertebrate communities.

Birds were more abundant and diverse in low-risk habitat than high-risk habitat. This result surprised us because more complex overstory at the surveyed high-risk site should have provided additional niches for bird species, as proposed by MacArthur and MacArthur (1961). Horizontal habitat patchiness, however, was greater on the low-risk site, which contained a more heterogeneous mosaic of woodland, grassland, and chaparral (personal observation). In addition, the understory was more developed at the low-risk site, as indicated by greater shrub cover. Both of these factors may have contributed to a more diverse avian community at the low-risk site (Roth 1976). Each habitat type had distinct avian communities with some species being much more abundant in one habitat type than the other. The surveyed high-risk site had low numbers of the eight focal conservation species identified by CalPIF (2002), relative to the low-risk site. The chestnut-backed chickadee, Steller's jay, orange-crowned warbler, Pacific-slope flycatcher (*Empidonax difficilis*), warbling vireo (*Vireo gilvis*), Wilson's warbler (*Wilsonia pusilla*), and Cassin's vireo (*Vireo cassinii*), however, were found only or predominantly at the high-risk site.

Small mammals were much more abundant in the high-risk habitat, and some species (particularly the dusky-footed woodrat) were in better physical condition, as measured by body mass. High-risk habitat characteristics that likely contributed to these differences include greater amounts of water, food (larger and more numerous oak trees), vertical habitat structure, and downed wood. For example, in a prior study on our low-risk study site (Tietje and others 1997), the abundance of woodrats, piñon mice, brush mice, and California mice all were positively and strongly correlated with tree canopy, and cover of shrubs and downed wood. Because some small mammal populations are characterized by cycles lasting 3-4 years (Boonstra and others 1998), we investigated the possibility that population cycles at the low-risk and high-risk sites were asynchronous during our study (in effect, low-risk site at low point in cycle, high-risk sites at peak in cycle) by examining our unpublished data from Camp Roberts. We found that total small mammal population densities were never as great at Camp Roberts during 1993-2002 as they were at the high-risk sites during this study.

The greater abundance of amphibians (primarily salamanders) in high-risk habitat and of reptiles (primarily lizards) in low-risk habitat can be attributed to a combination of climatic and habitat conditions. In terms of climate, the wetter winters and cooler summers of the high-risk sites are more favorable for amphibians than reptiles. Block and Morrison (1998) also found that mesic sites supported more amphibians and fewer reptiles than xeric, blue-oak-dominated sites in the Sierra Nevada and Tehachapi Mountains. In terms of habitat, Monterey salamander and slender salamander abundances in the Pacific Northwest were positively correlated with litter depth and downed wood volume (Corn and Bury 1991, Aubry and Hall 1991). These two habitat elements protect salamanders from desiccation and predators and provide them with foraging habitat (California Department of Fish and Game 2002, Wisely and Golightly 2003). Although litter depth and downed wood are also important for reptile species such as the western fence lizard and western skink (*Eumeces skiltonianus*), they prefer more open areas and generally avoid densely forested areas (California Department of Fish and Game 2002).

Potential bias in our results may have been introduced by differences in species detectability between the low-risk and high-risk sites. Detectability may have been particularly important with respect to birds where between-site differences in the bird community and habitat structure may have influenced detectability (Boulinier and others 1998). As a result, our observations of avian abundance and species richness may have been biased if detection probabilities were higher at the low-risk site. With respect to small mammals, we believe that by marking individuals we controlled potential bias to a large degree. We generally observed that most captures on the final night of a trapping session were recaptures, and thus, the minimum number of animals captured was a robust index for the true population density. With respect to amphibians and reptiles, the observed differences in abundance were so large between the high-risk and low-risk sites that we believe the differences to be genuine.

Although low-risk habitat is widespread in San Luis Obispo County (Meentemeyer and others 2004), we sampled only one location. Camp Roberts, our low-risk site, contains well-structured, relatively undisturbed blue oak-coast live oak woodlands (Tietje and others 1997). Combined with our casual observations of low-risk habitat throughout the county, we believe that Camp Roberts contains above-average wildlife habitat for low-risk portions of the county. Thus, we believe that small mammals and amphibians are more abundant in high-risk habitat than low-risk habitat throughout the county. On the other hand, birds and reptiles may not be as abundant at other low-risk habitat sites as they were at Camp Roberts.

Implications of SOD for San Luis Obispo County Wildlife

If SOD infects coastal oak woodlands within the county, impacts to the structure and composition of these woodlands will vary according to the time scale under consideration. In the short term (perhaps 5-30 years after infection), we would expect the generation of large amounts of CWD from stem failure of dying coast live oak trees (Swiecki and Bernhardt this volume), a reduction in the density of tree-sized stems, and the creation of canopy gaps leading to patches of increased shrub growth. The impacts of such major habitat changes on wildlife will be species-specific and are difficult to predict. Some small mammal species might benefit from an increase in downed wood and shrub growth, but the loss of vertical habitat structure could outweigh any benefits. Similarly, salamanders might be adversely affected by canopy gaps that result in warmer, drier microclimates and reduced amounts of leaf litter (deMaynadier and Hunter 1995), but the increase in downed wood might mitigate these impacts.

In the long term, we would expect California bay trees to replace many of the dead coast live oak trees in the forest canopy (Brown and Allen-Diaz this volume). We found few reports on wildlife use of California bay trees in the ecological literature. Dusky-footed woodrats, California mice, and western gray squirrels (*Sciurus griseus*) consume the fruit of California bay trees (Linsdale and Tevis 1951, Stienecker and Browning 1970, Merritt 1974), and woodrats use California bay cuttings and leaves for house construction and nest lining (Linsdale and Tevis 1951, Hemmes and others 2002). On the other hand, California bay trees have fewer cavities for nesting birds compared to coast live oaks (Winslow and Tietje this volume), are used less frequently than coast live oaks by foraging birds (Apigian and Allen-Diaz this volume), and contain monoterpenoid compounds that may deter the consumption of bay leaves by deer and other herbivores (Goralka and others 1996). Given the well-documented use of oak trees by many wildlife species for food, cover, and reproduction (Barrett 1980, Block and Morrison 1987, Pavlik and others 1991, CalPIF 2002), we would expect long-term negative impacts on most wildlife species if California bay largely replaced coast live oak in the forest canopy.

High-risk habitat in San Luis Obispo County is relatively scarce and fragmented in distribution. A reduction in habitat quality, therefore, could threaten populations of species that are particularly abundant in or restricted to high-risk woodlands. The dusky-footed woodrat is perhaps the most ecologically important species in high-risk woodlands, and a decline in its population could impact a wide range of species that prey upon woodrats or utilize woodrat houses for shelter and foraging (Linsdale and Tevis 1951). In addition, dusky-footed woodrats are the major food item for the California spotted owl (*Strix occidentalis occidentalis*) in coastal oak woodlands

(Verner and others 1992), which provide the preponderance of suitable owl habitat west of the Sierra Nevada. Several other bird species may be largely restricted to high-risk habitat within the county (see above), and we would expect at least one of these species (the chestnut-backed chickadee) to be adversely affected by the loss of coast live oaks (Apigian and Allen-Diaz this volume). Finally, Monterey and slender salamanders may be particularly susceptible to SOD impacts because they would be less capable of dispersal to nearby uninfected stands. The Monterey salamander was abundant on our high-risk study sites and coastal oak woodlands likely constitute its primary habitat within the county. Several slender salamander species (*Batrachoseps* spp.) occur within the county, at times within a few km of each other (Jockusch and Wake 2002). Even localized occurrences of SOD may lead to the isolation and loss of *Batrachoseps* species populations with highly localized distributions (see for example, Bradford and others 1993).

Should SOD become established in San Luis Obispo County, natural resource managers and land-use planners will be challenged to protect the county's coastal oak woodlands. This study provides a better understanding of the distinctive habitat and wildlife communities found in these woodlands. Our results could potentially serve as baseline data should SOD spread to the coastal oak woodlands of San Luis Obispo County.

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Potential Effects of Sudden Oak Death on Small Mammals and Herpetofauna in Coast Live Oak (*Quercus agrifolia*) Woodlands[1]

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Key words: oak woodlands, sudden oak death, wildlife-habitat relationships, small mammals, forest salamanders, *Quercus agrifolia*, *Phytophora ramorum*, forest pathogen impacts

Abstract

Within San Luis Obispo County, California, coast live oak (*Quercus agrifolia*) woodlands provide important habitat for many wildlife species (see Tietje and others, this volume). Unfortunately, many of these woodlands are at high risk of sudden oak death (SOD) infection should the pathogen (*Phytophthora ramorum*) become established in the County (Meentemeyer and others 2004). To assess the potential impacts of SOD on the small mammal and herpetofaunal communities in this high-risk habitat, we surveyed for these taxa at three separate high-risk study sites within the County. We considered the co-occurrence of California bay laurel (*Umbellularia californica*) to be an indicator of high-risk woodlands. We then developed *a priori* regression models relating the abundance of the most common species to habitat structure and compared competing models using adjusted Akaike's Information Criterion (AIC_c) values (Burnham and Anderson 2002). Finally, we projected the potential effects of SOD on these species in San Luis Obispo County by comparing vegetation data that we collected at infected and uninfected sites in the San Francisco Bay Area.

At the three high-risk sites in San Luis Obispo County, we established 13, 1.1-ha trapping grids (8 _ 8, 15-m spacing between traps). From 2002-2004, we live-trapped small mammals for three consecutive nights each spring and fall; captured animals were eartagged and released. We later conducted mark-recapture analyses to estimate the abundances of common species on each grid. During January-April of 2003 and 2004, we surveyed for reptiles and amphibians using plywood coverboards (61 _ 61 _ 1.6 cm) placed along every other row of each grid; coverboards were checked every 2-3 weeks. We treated the total number of observations for each species on a grid as an index of species abundance.

During June and July 2004, we collected data on habitat elements of potential importance to small mammals and herpetofauna. On each grid, we randomly selected eight grid intersections at which we

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located 10-m-radius, circular vegetation plots (2 plots per grid quadrat, all plots separated by at least 20 m). Within each 10-m plot, we recorded: 1) the size, species, condition (live or dead), and number of cavities for all trees ≥ 10 cm dbh; 2) canopy cover; 3) vertical obstruction from 0-5 m above ground level (a surrogate for shrub cover; see Griffith and Youtie 1988); 4) amount of fine (< 7.6 cm diameter) and coarse woody debris (≥ 7.6 cm; CWD); 5) depth of the duff and litter layers; and 6) number of dusky-footed woodrat (*Neotoma fuscipes*) dwellings. During October 2004, we used the same sampling protocol to collect habitat data on 10, 10-m plots on each of two additional sites—a site that was heavily infected with SOD in Marin County (China Camp State Park) and an uninfected site in nearby Sonoma County (Helen Putnam Regional Park). At China Camp, each plot was centered three m from a randomly selected, SOD-killed coast live oak tree located on research plots established by Phytosphere Research (see Swiecki and Bernhardt, this volume). At Helen Putnam, the plots were centered three m from healthy coast live oak trees of similar size to the selected trees on the infected site.

After an extensive literature review, we developed a set of linear regression models for each frequently captured species relating its abundance to our measured habitat variables. For the small mammal habitat models, we considered each trapping session on a grid to be a repeated measurement when fitting the regression models (see Littell and others 1996) and included study site as a covariate in all models. For the salamander habitat models, we calculated a single abundance (i.e., no repeated measures) on each grid and included an interaction term(s) between study site and the habitat variable(s) in each model. We then calculated AIC_c values and Akaike weights (w_i) for the suite of candidate models for each species. To predict changes in animal abundance should SOD reach San Luis Obispo County, we adjusted the habitat variables for each trapping grid based on the observed differences between the Bay Area sites. For each species, we then used model-averaging based on Akaike weights to calculate a change in abundance due to SOD.

Three species of cricetid rodents—dusky-footed woodrat, brush mouse (*Peromyscus boylii*), and California mouse (*P. californicus*)— dominated the small mammal community in San Luis Obispo County high-risk habitat, comprising 95 percent of all captures. Two forest salamanders—Monterey salamander (*Ensatina eschscholtzii eschscholtzii*) and slender salamander (*Batrachoseps* spp.)—dominated the herpetofaunal community, comprising 98% of all detections. For each of these five species, one candidate model was much more likely than the competing models. The best models suggested that each species responded differently to the measured habitat elements (*table 1*).

On the sampling plots in the Bay Area, we observed differences in several of the habitat variables that were important predictors of species abundance in San Luis Obispo County. For example, CWD volume was nearly 70 times greater in plots at the infected site relative to those at the uninfected site, while canopy cover was lower on the infected plots (79 percent vs. 84 percent). Vertical obstruction was higher on the infected plots (57 percent vs. 31 percent), but this difference was largely due to fallen trunks and branches, rather than increased shrub growth. The abundances of all three small mammal species showed a significant increase ($P < 0.05$), while the two salamander species showed no significant change.

Table 1—*Most supported abundance vs. habitat linear regression models for common small mammal and salamander species in San Luis Obispo County coast live oak woodlands at high risk of* Phytophora ramorum *infection. Abundance surveys were conducted during 2002-2004 for small mammals and during 2003-2004 for salamanders.*

Species	Habitat variables [1]	Akaike weight [2]
Dusky-footed woodrat	Shrub cover (+), coarse woody debris (–)	0.91
Brush mouse	Tree density (–)	0.88
California mouse	Shrub cover (+), coarse woody debris (+)	0.74
Monterey salamander	Elevation (+)	0.60
Slender salamander	Litter depth (+)	0.78

[1] For each habitat variable, (+) indicates a positive correlation with abundance, while (–) indicates a negative correlation.
[2] Akaike weight (w_i) represents the likelihood of a specific model, given the set of candidate models for each species. The sum of w_i for each species' set of candidate models is 1.00.

For each small mammal and salamander species, the relationship between abundance and the habitat variable(s) in the best habitat model was in general agreement with the literature (see a review for each species in CDFG [2002]). One notable exception was the negative correlation between woodrat abundance and CWD. This relationship was much weaker than the positive correlation with shrub cover, however, as evidenced by the projected increase in woodrat abundance after SOD.

The abundances of the three mammal species were correlated with habitat features (shrub cover, CWD, tree density) that were substantially altered at the infected site, in a manner that would be beneficial for each species. We project that woodrats would benefit from an increase in "shrub cover," California mice would respond favorably to the large increase in CWD, and brush mice would benefit from lower tree densities. In contrast, the two salamander species may be relatively unaffected by the disease because their abundances were correlated with habitat features (litter depth, elevation) that were relatively or completely unchanged by SOD.

On a cautionary note, should SOD become established in San Luis Obispo County high-risk woodlands, the levels of some habitat variables (e.g., amount of CWD) would lie well outside the current range of values. As a result, our habitat models may be less applicable under future conditions. In addition, we note that the disease has been present at the infected site in the Bay Area for less than 10 years, so our results represent short-term wildlife responses. Long-term responses (e.g., over the next 50 years) are uncertain. Nonetheless, our results provide a basis for hypothesis development for future wildlife studies conducted in areas where SOD is present.

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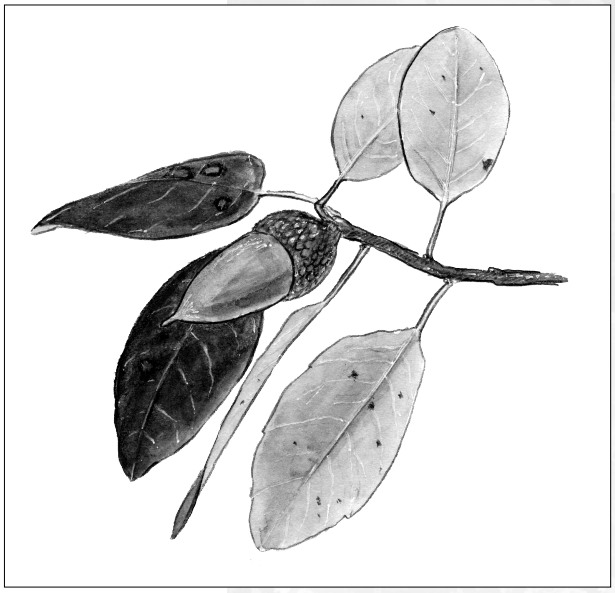
Management



Effectiveness of Fungicides in Protecting Douglas-fir Shoots From Infection by *Phytophthora ramorum*[1]

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Key words: Phytophthora ramorum, sudden oak death, fungicide effectiveness, Douglas-fir seedlings

Abstract

The effectiveness of 20 systemic and contact fungicides in protecting Douglas-fir seedlings from infection by *Phytophthora ramorum* was determined. Some systemic products were applied about a week prior to bud break, while most treatments were applied just after bud break. In addition to the fungicides, two surfactants were included in the post-bud break application tests. One day after the post-bud break treatment applications, all the seedlings were inoculated by spraying seedlings with zoospore suspensions obtained from isolates of A1 and A2 mating types of *P. ramorum*. Inoculated seedlings were then incubated under optimal conditions for disease development. The only pre-bud break treatment that completely prevented infection was the drench application of Subdue MAXX. Pre-bud break drench applications of Stature, Insignia, and Terrazole had no affect on the number of infected seedlings. The reduction in infection by the pre-bud break applications of Heritage and Chipco Signature was variable and applications of Phostrol reduced infections by 71 to 75 percent. Post-bud break applications of the contact fungicides Dithane, Gavel, Maneb, and Polyram provided 100 percent control. Although not as consistently effective, applications of Champ Formula 2F, Reason, Daconil Ultrex, Stature, and IKF – 916 reduced the number of infected seedlings by 70 to 100 percent. Most of the other fungicides included in these tests provided more limited or variable reductions in the number of infected seedlings. Post-bud break applications of the organosilicone surfactant, Silwet L-77 had no effect on infection of seedlings. However, the post-bud break application of Latron CS-7 reduced infection by 67 to 100 percent. Our results indicate that several fungicides and possibly some surfactants have the potential to provide excellent control of *P. ramorum*. One common concern associated with the potential use of fungicides to control this disease is the possibility that fungicides might suppress symptom development on infected plants. While systemic fungicides might have the potential to suppress symptom development, it is unlikely that this would be an issue with any of the contact types of fungicides that were found to be effective in protecting seedlings from *P. ramorum.*

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Fungicide Control of *Phytophthora ramorum* on Rhododendron[1]

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Key words: chemical control, fungicide efficacy, *in vitro*, *in planta*, resistance management, protective, curative, *Phytophthora ramorum*

Abstract

Commercial rhododendron plants have been the most important hosts of *Phytophthora ramorum* in Europe. As part of the European Union (EU) emergency phytosanitary measures 2002/757/EU and 2004/426/EU all commercial rhododendron-growing premises are surveyed for *P. ramorum*. Detection of *P. ramorum* leads to quarantine measures, including destruction of plants, and could lead to considerable financial damage for the companies involved. Growers are taking all possible measures to avoid *P. ramorum*, including preventive fungicide treatments. However, because little information is available on the effect of the different oomycete fungicides on *P. ramorum*, there is a danger of conducting sub-optimal protective fungicide treatments. The objective of this research was to evaluate the efficacy of different oomycete fungicides against *P. ramorum*. Active ingredients from most of the oomycete fungicides on the Belgian market were screened for their *in vitro* effect on the mycelial growth of four *P. ramorum* isolates. Based on the results from the *in vitro* experiments, a selection of these fungicides was tested against *P. ramorum* on rhododendron plants. Application time and application method were included as variables. A wide range of *in vitro* fungicide activity was observed. Metalaxyl, dimethomorph, and benthiavalicarb-isopropyl showed complete inhibition of mycelial growth at 1 µg ml^{-1} or less. Cymoxanil, etridiazole, and mancozeb caused complete growth inhibition at 1 to 100 µg ml^{-1}. Chlorothalonil, Cu-oxychloride, famoxadone, fluazinam, and cyazofamid did not completely inhibit growth at 100 µg ml^{-1}. Fosetyl-Al and propamocarb did not cause growth inhibition at 100 µg ml^{-1}. Fungicide effects were independent of the strain of *P. ramorum* used, except for one strain, which showed a decreased sensitivity to metalaxyl. A subset of the fungicides was tested on plants. Overall, fungicides that performed best on plants were metalaxyl, cyazofamid, and benthiavalicarb-isopropyl. Dimethomorph and fosetyl-Al had intermediate effects. Cymoxanil and mancozeb were the least effective of the products tested. Protective effects were best

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when the lower surface of the leaf was covered with the fungicide, consistent with the observation that infection of non-wounded leaves inoculated with zoospores takes mostly place through the lower surface of the leaves. Curative fungicide treatments 2 days after zoospore inoculation were much less effective than protective treatments (1 day before zoospore inoculation). This research shows that protective applications of specific fungicides can contribute to effective control strategies of *P. ramorum* on rhododendron. Considering that we identified a strain with decreased activity against metalaxyl, it seems advisable to limit the number of consecutive uses of products with the same mode of action. However, growers may face few options in alternating fungicides due to the limited number of products with use permits on rhododendron.

Introduction

In 1993, a new species of *Phytophthora* was found responsible for an aggressive twig and leaf blight of rhododendron and a stem blight of viburnum in German and Dutch nursery settings (Werres and Marwitz 1997, Werres and others 2001). Starting in 1995, extensive mortality of native oak species such as tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) occurred in California and in one part of Oregon, caused by a disease called sudden oak death (SOD) (Goheen and others 2002, Rizzo and others 2002). This same new *Phytophthora* species that was responsible for the diseases on the European ornamentals was found to be responsible for SOD. It has since been designated *P. ramorum* (Werres and others 2001). In the United States, an increasing number of over 30 natural hosts have been identified, mostly in natural settings (APHIS 2004). Depending on the host, symptoms vary from leaf spots, leaf blight, twig dieback, to cankers, and ultimately plant mortality. In Europe, the pathogen has been found in Belgium, Denmark, France, Germany, Ireland, Italy, Netherlands, Norway, Poland, Slovenia, Spain (Galicia, Islas Baleares: Mallorca), Switzerland, Sweden and the United Kingdom (EPPO 2005), but almost exclusively in nursery settings. A limited number of findings took place on trees in England and in the Netherlands, but each time it was found in the vicinity of infected rhododendron plants (DEFRA 2003a, DEFRA 2003b, PD Nederland 2003). There are genetic differences between the United States and the European populations of *P. ramorum* (Ivors and others 2004, Kroon and others 2004) but the pathogenicity is not substantially different. The detection of the pathogen in an increasing number of European countries and the potential danger for spread from nurseries to natural oak and beech populations has prompted the EU to install emergency phytosanitary measures (directives 2002/757/EU and 2004/426/EU). These measures include inspections on all nurseries where rhododendron, viburnum or camellia are grown. The most important host of *P. ramorum* in Europe is rhododendron. Although the

percentage of positive sites is comparable to that of its neighboring countries, the impact of the EU measures on the Flemish rhododendron industry is considerable because Flanders (Northern part of Belgium) is one of largest rhododendron-producing areas in Europe. Therefore, rhododendron growers are taking all possible measures to avoid *P. ramorum*, including use of preventive fungicide treatments. The objective of this research was to do an initial evaluation of the efficacy of the different oomycete fungicides on the Belgian market against *P. ramorum* on rhododendron.

Materials and Methods

In vitro experiments

Four *Phytophthora ramorum* isolates from four different Flemish nurseries were used in this study. Isolates 02-880 and 02-1658 were from rhododendron; isolates 02-2084 and 02-2086 were from viburnum. The isolates were maintained on V8 agar and CMA at 4ºC and transferred periodically. *In vitro* evaluation of fungicide activity was based on inhibition of mycelial growth only. Fungicides used were benthiavalicarb-isopropyl (non-commercial; 15 percent a.i.), chlorothalonil (Clortosip; 50 percent a.i.), Cu-oxychloride (Cupravit Forte; 50 percent a.i.), cyazofamid (non-commercial; 40 percent a.i.), cymoxanil (non-commercial; 96 percent a.i.), dimethomorph (non-commercial; 50 percent a.i.), etridiazole (Aaterra; 35 percent a.i.), famoxadone (non-commercial; 96 percenta.i.), fluazinam (Shirlan; 50 percenta.i.), fosethyl-Al (Aliette; 80 percenta.i.), mancozeb (Dithane WG; 75 percent a.i.), metalaxyl (Ridomil 5G, 5 percent a.i.), and propamocarb (Previcur N; 72.2 percent a.i.). Except for benthiavalicarb-isopropyl, the active ingredients listed were available in at least one commercial product on the Belgian market at the time this research was initiated. However, inclusion in this study does not imply they are registered for use against *P. ramorum* at this time. Stock solutions of the fungicides were made in DMSO or sterile water and added to V8 agar to a final concentration of either 0, 0.001, 0.01, 0.1, 1, 10, or 100 µg active ingredient ml^{-1}. Rifampicin was also added at a final concentration of 10 µg ml^{-1} to suppress potential development of bacteria originating from the fungicide formulations. For each *P. ramorum* isolate used, separate plates were inoculated with a mycelial plug from an actively growing culture. Plates were incubated at 20°C in the dark until the colony margin of the cultures in the control treatment almost reached the edge of the plate. Growth inhibition in the fungicide treatments was calculated based on the proportional decrease in radial growth to the control plates. A minimum of two replicate plates were used for every combination of fungicide concentration and fungal isolate. All experiments were conducted twice.

In planta experiments

A subset of the fungicides from the *in vitro* experiments was selected based on the extent of their *in vitro* effect. These selected fungicides were tested in three separate *in planta* experiments. Fungicides used were benthiavalicarb-isopropyl (non-commercial formulation, 15 percent a.i.), cyazofamid (Ranman A+B; 40 percent a.i.), cymoxanil (Cymopur; 35 percent a.i.), dimethomorph (Paraat; 50 percent a.i.), mancozeb (Dithane WG; 75 percent a.i.), and mefenoxam (non-commercial formulation, 48 percent a.i.). Although results with fosethyl-Al (Aliette; 80 percent) were poor in the *in vitro* experiments, it was included in the *in planta* experiments based on its expected activity in plants. Fungicide solutions were prepared at the following rates and applied to run-off: benthiavalicarb-isopropyl (75 ppm), cyazofamid (80 ppm), cymoxanil (119 ppm), dimethomorph (150 ppm), fosethyl-Al (2000 ppm), mancozeb (1575 ppm), and mefenoxam (192 ppm). Rates are expressed as active ingredient and were based on label (or company) recommendations. Cyazofamid was used in trials two and three only.

Potted rhododendron plants were obtained from a commercial nursery. Plants within each trial originated from the same batch. In trial one and two, cultivar "Percy Wiseman" was used. In trial three, cultivar "Germania" was used. To avoid residual effects from preventive fungicide applications at the rhododendron nursery, plants from trials two and three were maintained in our greenhouse for at least 6 weeks before use in the experiments. All experiments took place in a climatized greenhouse during the months October through April with a day temperature setting of 20°C and a night temperature setting of 15°C. Pots were placed in individual saucers and watered from below. Plants received natural light only.

The pathogen inoculum consisted of an equal mixture of zoospores of *P. ramorum* isolates 02-1658, 02-2084, and 02-2086. A $5x10^4$ ml^{-1} zoospore suspension was applied at 20 ml per plant. The zoospores were sprayed onto the lower side of all leaves with a modified syringe. The syringe was modified in such a way that a sufficiently fine droplet size was obtained to get good distribution but without causing the zoospores to encyst by the spraying process. Post inoculation, each plant was individually covered with a large plastic bag to ensure high humidity conditions. Bags were removed one week after inoculation. To produce zoospores, sporangia from each isolate were separately harvested from 10-day-old cultures on diluted (10 percent clarified juice) V8 agar with a total of 20 ml sterile MilliQ water per plate. Sporangia solutions were chilled in a refrigerator and zoospores allowed to emerge over a period of 2 to 3 hours. Zoospores were separated from sporangia by filtering through a nylon mesh (10 µm) and their concentrations were determined with a

Bürker chamber. Equal numbers of zoospores from the three strains were mixed and diluted to the desired concentration with chilled MilliQ water. Zoospore solutions were kept on ice until application. At the end of the experiment, the mobility of the remainder of the zoospores was verified microscopically. No decrease in zoospore mobility was observed.

In the first *in planta* experiment, fungicides were applied in a preventive manner only, but with different application methods: (1) to the upper side of the leaves (1 day before pathogen inoculation), (2) to the lower side of the leaves (1 day before pathogen inoculation), or (3) systemically, to the potting medium (7 days before pathogen inoculation). Systemic application was not made with mancozeb and cymoxanil, as those are contact fungicides only. Applications via the potting medium (and roots) consisted of adding 100 ml of fungicide solution to the saucers in which the pots were placed. The liquid was absorbed within 8 hours for all pots. An equal amount of water was applied in the same manner to the other treatments. In the second and third experiment, fungicides were applied to the upper side of the leaves only, but either preventive (1 day before pathogen inoculation) or curative (2 days post pathogen inoculation). There were five replicate plants per treatment in trial one, and six replicates in trials two and three. The experimental setup was a randomized complete block, with blocks consisting of location in the greenhouse and pathogen inoculation order. There was no contact between individual plants. Non-inoculated controls and non-fungicide treated controls were included.

Plants were scored for symptoms on a weekly basis until no new symptoms developed. The number of leaves with lesions and number of stems with lesions were counted. No distinction was made between the size of the lesions. Stem lesions were evaluated in experiments one and two. Leaf lesions were evaluated in experiments two and three. In the first experiment, a part of each lesion was plated on selective medium (PARP) to confirm the cause of the lesion. In later experiments only lesions that were not typical were plated. Analysis of variance or Kruskal-Wallis tests were performed on the lesion data where appropriate, using Minitab 12.1. To summarize the effects of the fungicides in the different experiments, the results of each fungicide were put in one of four different categories within each experiment. Based on cut-off levels of the number of lesions, the following categories were established: 1 = excellent control, 2 = good control, 3 = some control, 4 = inadequate control.

The plants from experiment two were maintained in the greenhouse for 6 months after the end of the experiment (no new lesions), under stressful conditions in terms of temperature (high) and water availability (low). At the end of this 6-month period, mortality was assessed and correlated with previous infection level.

Results

In vitro experiments

A wide range of *in vitro* fungicide activity was observed. The effects were separated in four groups. Fungicides in group one (metalaxyl, dimethomorph, and benthiavalicarb-isopropyl) showed 100 percent growth inhibition at 1 µg ml^{-1} or less (*Fig*. 1A). Fungicides in group two (cymoxanil, etridiazole, and mancozeb) showed 100 percent growth inhibition at 1 to 100 µg ml^{-1} (*Fig.* 1B). Fungicides in group three (chlorothalonil, Cu-oxychloride, cyazofamid, famoxadone, and fluazinam) did not reach 100 percent growth inhibition at 100 µg ml^{-1} (*Fig*. 1C). Lastly, fungicides in group four (fosetyl-Al and propamocarb) did not show any *in vitro* activity (*Fig*. 1D). The fungicide effects were independent of the strain of *P. ramorum* used, except for strain 02-880, which showed a decreased sensitivity to metalaxyl. The smallest concentration at which inhibitory activity was observed was 0.001 µg ml^{-1} with cyazofamid (23 percent growth inhibition). Interestingly, growth inhibition with this compound leveled off at approximately 80 percent starting at 1 µg ml^{-1}.

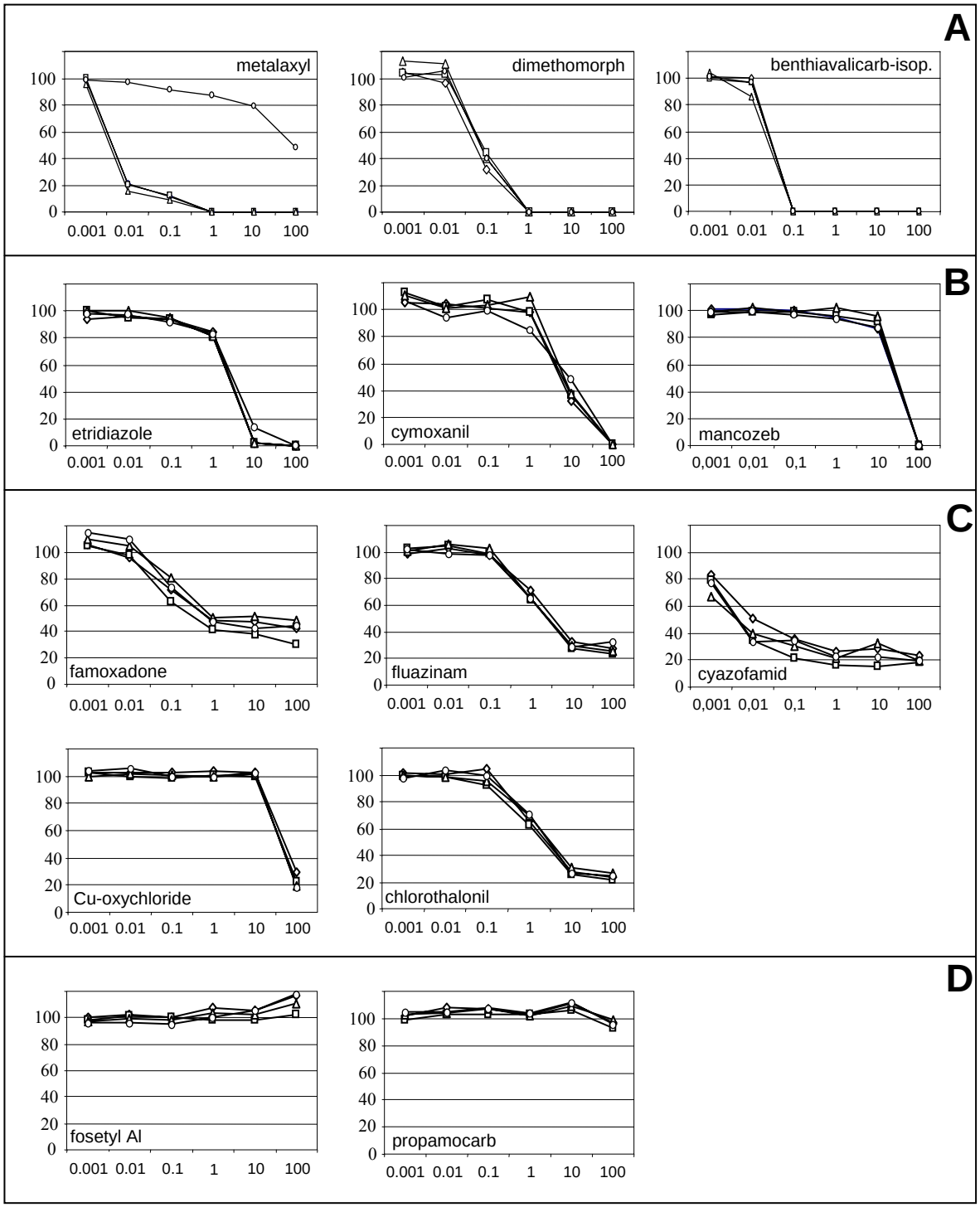

Figure 1— Mycelial growth of *P. ramorum* in agar medium amended with fungicides, expressed as a percentage (Y-axes) of the growth in the control plates (=100 percent). Each graph is labeled with the corresponding fungicide and fungicide concentrations are given in ppm (X-axes). Each graph contains the results of four *P. ramorum* isolates: 02-880 (circles), 02-1658 (squares), 02-2084 (diamonds), 02-2886 (triangles). The four subparts of the graph (A through D) correspond with the four efficacy classes (groups 1 through 4), as indicated in the results section of the text.

In planta experiments

No new primary leaf lesions developed after 14 days. Secondary leaf lesions, which only occurred if the pathogen spread via the stem, were not counted. Stem lesions took longer to develop, mostly between 4 and 10 weeks post inoculation.

Results from the first *in planta* experiment are presented in *table 1*. The overall level of infection was lower than expected based on preliminary experiments. Therefore, only the total number of lesions in the five replicates are presented for this first experiment. Residual effects from fungicides applied at the nursery where the plants originated were probably responsible for the limited number of lesions. To prevent this effect, plants from nurseries were maintained in the greenhouse for at least 6 weeks in all later experiments. Even though the level of infection was low in trial one, some important trends were clearly visible. The application method played a major role in the level of infection: application of the fungicides to the lower side of the leaves, where the infection with zoospores takes place, gave the best protection. Even the contact fungicides (mancozeb and cymoxanil) were very effective with this application method. Applying the fungicides via the potting medium was least effective. Overall, mefenoxam, benthiavalicarb-isopropyl, and dimethomorph (except when applied systemically) gave the best results.

Table 1—*Total number of stems with infections due to* Phytophthora ramorum *in the first* in planta *experiment. Fungicides were applied either systemically, onto the upper side of the leaves, or onto the lower side of the leaves.*

Fungicide treatment	Fungicide application mode		
	via roots	upper leaf side	lower leaf side
no pathogen control[2]	0	0	0
no fungicide control[2]	9	9	9
mefenoxam	5	1	0
fosetyl-Al	5	3	1
benthiavalicarb-isopropyl	5	0	0
dimethomorph	12	0	0
mancozeb	NA[1]	5	2
cymoxanil	NA	8	0
mean±stdev[3]	6.8±3.5	2.8±3.2	0.5±0.8

[1] NA = Not Applicable (contact fungicides)
[2] Considering controls did not involve a fungicide treatment, they were the same for each application mode

[3] Mean ± standard deviation of application method, excluding the control treatments

Results from the second *in planta* experiment are presented in *table 2*. The overall level of infection was much higher in this experiment than in the first one, hence the evaluation of leaf infections as well as stem infections. As expected, the average level of infection was significantly higher in the curative treatment than in the preventive

treatment (p<0.0001). This was clear in the average number of infected leaves per plant (4.95 versus 0.67 infected leaves on 5.86 versus 2.00 plants out of 6) and the average number of stem lesions per plant (1.26 versus 0.12 infected stems on 3.43 versus 0.29 plants out of 6). There was no significant block effect when evaluating leaf (p=0.35) or stem (p=0.52) lesions. Effects on stems and leaves were similar for most fungicides, except for dimethomorph and cymoxanil, which were considerably better on stems than on leaves in preventive treatments, and cyazofamid, which was considerably better on stems than on leaves in the curative treatment.

Table 2—*Mean+/-stdev number of leaves and stems with lesions per plant in the second* in planta *experiment. Fungicides were either applied 1 day before (preventive) or 2 days after the pathogen (curative).*

Fungicide	preventive				curative			
	leaves		stems		leaves		stems	
	lesions	pl.[1]	lesions	pl.[1]	lesions	pl.[1]	lesions	pl.[1]
no pathogen control	0.00±0.00	0	0.00±0.00	0	0.00±0.00	0	0.00±0.00	0
no fungicide control	8.50±3.94	6	2.83±1.60	6	8.00±3.03	6	1.50±1.52	4
fosetyl-Al	0.67±0.61	3	0.00±0.00	0	5.67±4.03	6	1.83±2.56	3
mefenoxam	0.50±0.55	3	0.17±0.41	1	3.17±2.56	6	1.00±1.55	3
benthiavalicarb-isopropyl	0.00±0.00	0	0.00±0.00	0	4.83±3.97	5	0.83±2.56	4
cyazofamid	0.00±0.00	0	0.00±0.00	0	4.50±2.74	6	0.00±0.00	0
dimethomorph	1.67±3.61	2	0.00±0.00	0	5.33±2.34	6	1.17±0.75	5
cymoxanil	1.33±1.51	3	0.00±0.00	0	4.67±2.25	6	1.33±1.97	3
mancozeb	0.50±0.55	3	0.67±1.63	1	6.50±2.51	6	2.67±1.03	6
mean±stdev[2]	0.67±0.63	2.00	0.12±0.25	0.29	4.95±1.04	5.86	1.26±0.83	3.43

[1] number of replicate plants (out of 6) with at least one lesion
[2] mean (± standard deviation) within each column, excluding the control treatments

The effects of the fungicides could be divided in different groups. When averaged over effects on leaves and stems, excellent control was obtained with cyazofamid and benthiavalicarb-isopropyl for the preventive treatments. For the curative treatments, cyazofamid, mefenoxam, and benthiavalicarb-isopropyl resulted in the smallest number of lesions. These three fungicides were the only ones that were significantly different from the positive control according to the Kruskal-Wallis test and comparison of the average ranks with the Dunns test (leaf data, averaged over preventive and curative treatments). Using the same test and when averaged over leaf and stem data, these same three fungicides were significantly different from the positive control in the preventive treatments. In the curative treatments, no fungicide

was significantly different from the control (averaged over stem and leaf lesions). Fungicides with intermediate effects were fosetyl-Al and dimethomorph. Fungicides with the smallest effects were cymoxanil and mancozeb, although mancozeb still showed reasonable effect in the preventive treatment.At the end of the second *in planta* experiment, which contained 108 plants total, 12 plants were heavily infected (three or more stem lesions) and 23 were moderately infected (one or two stem lesions). After 6 extra months, during which the plants were stored under stressful conditions, 50 percent of the heavily infected plants and 35 percent of the moderately infected plants had died. Mortality was 10 percent among the plants without stem lesions. Both ratios are significantly different from the control ratio ($p=0.006$ for heavy infection and $p=0.017$ for moderate infection) based on χ^2 tests. Within the infection classes, no obvious relationship was present between mortality and previous fungicide treatment.

Results from the third *in planta* experiment are presented in *table* 3. In this last experiment a different rhododendron cultivar was used. A higher level of leaf infections was observed with this cultivar: an average of 2.71 versus 0.67 infected leaves per plant in the preventive treatments and 10.31 versus 4.95 infected leaves in the curative treatment. The number of plants on which leaf lesions were observed was also higher in the preventive treatment: 4.86 versus 2.00. In contrast to the large number of leaf infections, hardly any stem infections were observed over the entire third experiment and therefore not reported. As in the previous experiments, a major difference was observed between the preventive and the curative treatments ($p<0.001$): the average number of infected leaves per plant was 2.71 versus 10.31 in the respective groups. In the curative treatment, no fungicide gave good control and no significant difference was obtained between the fungicide treatments (except for the negative control) using the Kruskal-Wallis and the Dunn tests. In the preventive treatments, benthiavalicarb-isopropyl, mefenoxam and cyazofamid gave the best control, but only benthiavalicarb-isopropyl was significantly different from the positive control (Kruskal-Wallis and the Dunn tests). Mancozeb, fosetyl-Al, and dimethomorph gave intermediate effects, and cymoxanil did not give acceptable control.

An interpreted summary of the results from the three experiments is presented in *table* 4. It consists of the effects on stem lesions in experiments one and two and leaf lesions in experiments two and three. Benthiavalicarb-isopropyl, cyazofamid, and mefenoxam were the best products for the preventive treatments (summarized over stem and leaf). Those same products also were the best for the curative treatments, be it in a different order and at an overall less favorable score. In the curative treatments, a few products ranked worse than the positive control.

Table 3—*Mean +/-stdev number of leaves with lesions per plant in the third* in planta *experiment. Fungicides were either applied 1 day before (preventive) or 2 days after the pathogen (curative).*

Fungicide	preventive		curative	
	lesions	plants[1]	lesions	plants[1]
no pathogen control	0.00±0.00	0	0.00±0.00	0
no fungicide control	9.33±4.80	6	6.33±1.86	6
fosetyl-Al	3.83±4.17	5	5.83±5.31	6
mefenoxam	1.33±0.82	5	7.00±5.93	5
benthiavalicarb-isop.	0.83±0.98	3	12.50±6.28	6
cyazofamid	1.33±1.21	4	10.00±8.92	6
dimethomorph	3.50±2.81	5	8.33±3.88	6
cymoxanil	5.50±3.08	6	13.33±5.09	6
mancozeb	2.67±1.86	6	15.17±9.77	6
mean±stdev[2]	2.71±1.68	4.86	10.31±3.48	5.86

[1] number of replicate plants (out of 6) with at least one leaf lesion
[2] mean (± standard deviation) within each column, excluding the control treatments

Table 4—*Average category of fungicide effects in the three* in planta *experiments, based on the following categories: 1 = excellent control, 2 = good control, 3 = some control, 4 = inadequate control. Within each group, fungicides with the same score were sorted based on the actual number of lesions observed.*

timing of fungicide application	leaf		stem		leaf + stem	
	rank	fungicide	rank	fungicide	rank	fungicide
preventive	1.0	no pathogen	1.0	no pathogen	1.0	no pathogen
	1.5	benthiavalicarb	1.0	benthiavalicarb	1.3	benthiavalicarb
	1.5	cyazofamid	1.0	cyazofamid	1.3	cyazofamid
	2.0	mefenoxam	1.0	dimethomorph	1.8	mefenoxam
	2.5	fosetyl-Al	1.5	mefenoxam	2.0	fosetyl-Al
	2.5	mancozeb	1.5	fosetyl-Al	2.0	dimethomorph
	3.0	dimethomorph	2.0	cymoxanil	2.5	mancozeb
	3.5	cymoxanil	2.5	mancozeb	2.8	cymoxanil
	4.0	no fungicide	4.0	no fungicide	4.0	no fungicide
curative	1.0	no pathogen	1.0	no pathogen	1.0	no pathogen
	2.0	mefenoxam	1.0	cyazofamid	2.0	cyazofamid
	2.5	fosetyl-Al	2.0	benthiavalicarb	2.5	mefenoxam
	3.0	cyazofamid	3.0	mefenoxam	2.8	benthiavalicarb
	3.0	dimethomorph	3.0	dimethomorph	3.0	dimethomorph
	3.0	no fungicide	3.0	cymoxanil	3.0	no fungicide
	3.5	benthiavalicarb	3.0	no fungicide	3.3	fosetyl-Al
	3.5	cymoxanil	4.0	fosetyl-Al	3.3	cymoxanil
	4.0	mancozeb	4.0	mancozeb	4.0	mancozeb

Discussion

Prevention of the introduction of *Phytophthora ramorum* and its spread within the EU is the objective of the current European phytosanitary measures. Prevention of the spread via infected plants is in part accomplished through a plant passporting system involving surveys, eradication of infected plants within a 2 m radius, and quarantine measures of the remaining plants within a 10 m radius. The potential threat from the pathogen and the impact of the quarantine measures has caused growers to also take preventive initiatives. They try to prevent the introduction and spread within their nursery through sanitation, reduction of disease conducive conditions such as wounding and high moisture conditions, avoidance or protection of susceptible cultivars, and preventive fungicide applications. Curative fungicide applications, or applications in the 10-m quarantine zone, are not allowed under the EU phytosanitary measures. Our data tend to support that eradication through curative application of fungicides is extremely difficult under optimal conditions for pathogen development. However, preventive use of fungicides was very effective with selected fungicides, even under the conditions of high inoculum dose used, and may be encouraged as a protective measure. Therefore, our data can contribute to the more effective use of fungicides.

Our approach was to pre-select fungicides based on their *in vitro* activity. In most cases this approach was effective as the fungicides with excellent *in vitro* effect such as metalaxyl (and mefenoxam) and benthiavalicarb-isopropyl were among the fungicides with the best *in planta* effects. Cyazofamid, which also had excellent *in planta* effects, did not show complete growth inhibition *in vitro*. When using *Phytophthora infestans* as the test organism, 100 percent growth inhibition was obtained at 0.1 ppm cyazofamid (data not shown). Although cyazofamid did not give 100 percent growth inhibition *in vitro* against *P. ramorum*, it was the fungicide with activity at the lowest concentration. This concentration effect may have been sufficient to give superior results on plants. Alternatively, the *in planta* effect of cyazofamid may have been the result of an effect on zoospores instead of on mycelial growth. Effects on zoospores were not tested in the *in vitro* experiments. This could also explain why the curative effect of cyazofamid on leaves was less extensive, as that kind of effect mostly involved activity on mycelial growth. Dimethomorph, which gave excellent effects *in vitro*, only gave intermediate overall results *in planta*. However, this was mostly due to its inferior results on leaf lesions. Results on stem lesions with dimethomorph were comparable to those of mefenoxam. Differences in results on leaves and stems could indicate that the formulation and/or (semi)-systemic characteristics of the fungicide may also play a role in the effect on plants.

The *in vitro* fungicide effects were independent of the strain of *P. ramorum* used, except for strain 02-880, which showed a decreased sensitivity to metalaxyl. The risk of rapid development of resistance against metalaxyl is well known (Gisi 1992). We can only speculate that this strain had been exposed to metalaxyl in the field. The three most effective fungicides in our experiments (mefenoxam, cyazofamid, benthiavalicarb-isopropyl) are single target fungicides, indicating a higher risk for the development of resistance. Even though most of them are commercialized in a mixture with a broad spectrum fungicide like mancozeb, alternating their use seems highly advisable.

Although there were differences in the details of each of the three *in planta* experiments, no major differences were observed in the relative efficacy of the fungicides throughout the three experiments. An apparent difference was the smaller effect of dimethomorph in experiment two as compared to experiment one, but this was due to the smaller protective effect of this compound on leaves in experiment two. In experiment one, only stem lesions were taken into account. In the curative treatment of experiment three, a somewhat different result was obtained with fosetyl-Al (better) and with benthiavalicarb-isopropyl (worse) as compared to the other experiments. However, under the intense disease pressure of the curative part of experiment three, none of the treatments were performing particularly well. Several fungicide treatments even performed worse than the control. This is in part because the score for the positive control for the curative treatment of experiment three was better than expected. However, this negative effect of some fungicides in the curative treatment was also observed in experiment two (stem lesions). It is possible that lesion development was enhanced if the plants were stressed by the fungicide applications. Overall, when averaged over the three experiments, mefenoxam, cyazofamid and benthiavalicarb-isopropyl performed the best. Fosetyl-Al and dimethomorph gave intermediate results, and mancozeb and cymoxanil gave poorer protection. Some fungicides such as mefenoxam also had a considerable effect on lesion size. Those data are not presented in this paper, as only presence or absence of lesions was recorded, independent of lesion size. Lesion size is relevant in terms of level of pathogen growth and probably in terms of the amount of secondary inoculum that can be produced. However, when the Plant Health Inspection Service takes samples, lesion size does not affect the level of consequences when samples are found positive.

The difference in the results with the two rhododendron cultivars was rather striking: on cv. Percy Wiseman, a moderate number of leaf lesions and moderate number of stem lesions where observed, while on cv. Germania, a large number of leaf lesions was observed, but hardly any stem lesions. Differences in the number and extent of

leaf and stem lesions was in correspondence with results from a study comparing the susceptibility of many rhododendron cultivars (De Dobbelaere and others 2005). One reason for the smaller number of stem infections in cv. Germania could be that a large proportion of its infected leaves dropped from the plant before the lesion spread extensively within the leaf. This prevents the spread of the pathogen from infected leaves into the stem.

The application method of the fungicides played a major role in the effect, with application to the lower part of the leaves as the most effective. This was not surprising, considering the zoospores were also applied to that surface. Preliminary experiments showed repeatedly that infection of non-wounded leaves inoculated with zoospores almost exclusively takes place through the lower side of the leaves (Heungens and others 2003). So even if the zoospores had been applied to both sides of the leaves, application of the fungicides to the lower leaf surface would still be expected to be significantly better than the other application modes. The excellent control effect of the preventive fungicide applications to the lower leaves also shows that the application of the zoospores in itself did not substantially wash the fungicide from the leaves, which could have happened with the contact fungicides. At nurseries, fungicides are usually applied through overhead irrigation systems. Application methods which help reach the lower side of the leaf, such as higher pressure application or application with air support could potentially increase the protective effect. Application via the roots gave a smaller protective effect than expected, even with compounds that have proven systemic activity such as mefenoxam. This could be due to the time of year (reduced evapotranspiration or reduced translocation) or to a suboptimal concentration of the fungicide.

Progression of *P. ramorum* twig infections often seems to halt when conducive conditions (high moisture) stop. Although anecdotal and not the primary goal of this research, we considered it interesting to verify this assumption and observe the mortality data of plants with different levels of infection, several months after inoculation. If there is progression of disease then mortality is expected to be larger in infected plants than in control plants. Our results did indeed indicate that mortality of plants was correlated with *P. ramorum* infection level under the stressful conditions used. However, we can not exclude that the increased level of mortality is in part due to the already weakened status of the plant instead of the continued activity of the fungus.

One of the concerns with the use of fungicides is that symptoms may be suppressed without completely killing the fungus, thus facilitating its latent spread. In our experiments, no problems were experienced with the isolation of *P. ramorum* on

PARP medium from any of the lesions. Based on our results, the risk for missing the detection of the pathogen through false negative plating results of suspected material thus seems limited. Of course, finding suspected material can become problematic if symptoms are considerably fewer or smaller.

Until recently, reports on the activity of fungicides against *P. ramorum* were limited and mostly involved trees. Phosphonates were one class of compounds able to reduce *P. ramorum* lesions and canker size on trees (Garbelotto and others 2002, Garbelotto and others 2003, Schmidt and others 2002). One such compound (fosetyl-Al) was included in our studies. Although this fungicide has interesting systemic properties and has a good reputation for its activity against *Phytophthora* species, it only gave intermediate effects in our experiments. This could in part be due to the limited physiological activity of the plants during the period of the experiment. Other compounds tested on trees and woodland shrubs are metalaxyl, copper sulfate pentahydrate and dimethomorph+mancozeb (Garbelotto and others 2002, Goheen and others 2005). Especially metalaxyl and dimethomorph+mancozeb had an effect, but rarely the complete suppression of the pathogen. At the SOD Science Symposium II, several new reports were presented on fungicide activity against *P. ramorum* in horticultural settings (Chastagner and others 2005, Linderman and Davis 2005, Tjösvold and Chambers 2005, Turner and others 2005). In general, similar results were reported as to the effect of the different fungicides. Overall, metalaxyl and cyazofamid were among the better products. However, cyazofamid did not have a consistent effect on all strains tested in one report (Linderman and Davis 2005). Compounds with good results on plants that were tested in other studies but not in ours were azoxystrobulin (Turner and others 2005), fenamidone (Tjösvold and Chambers 2005, Turner and others 2005), and pyraclostrobin (Tjösvold and Chambers 2005). Future experiments are needed to evaluate their activity under our test conditions.

This research shows that protective applications of specific fungicides can contribute to effective control strategies of *P. ramorum* on rhododendron. Considering that we identified a strain with decreased activity against metalaxyl, it seems advisable to limit the number of consecutive uses of products with a single target site. However, depending on the specifics of each country, growers may face few options in alternating fungicides due to the limited number of products with use permits on rhododendron.

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Effects of Phosphonate Treatments on the Growth of *Phytophthora ramorum* in Tanoak Stems[1]

Alan Kanaskie[2], Everett Hansen[3], and Wendy Sutton[3]

Introduction

In Oregon, tanoak (*Lithocarpus densiflorus*) is the tree species most susceptible to *Phytophthora ramorum*, and Sudden Oak Death (SOD) occurs only in forests where tanoak is present. Oregon is attempting to eradicate the pathogen by complete destruction of host plants in and near infested sites. Although the eradication effort has been successful to a degree, it is clear that we need other tools to limit spread of the pathogen in forests. The purpose of this study is to begin evaluating phosphonate (Agrifos-400®) treatments as tools for managing SOD in Oregon Forests. Phosphonate (Agrifos-400®) treatments have been shown to reduce *P. ramorum* lesion development in coast live oak and tanoak in California, but the results for tanoak suggest that bark application on tanoak may not be effective (Garbelotto and others 2003). There is little or no information as to whether or not application of phosphonate to the lower bole by any method can reduce infection on the upper stem or foliage, or reduce sporulation on these same parts. The objectives of our study were to: 1) compare the effectiveness of lower bole bark application and trunk injection in terms of lesion development in the lower bole, upper bole, and leaves of tanoak; 2) evaluate the relative effectiveness of a single application of Agrifos® to two applications separated by 3 months; 3) evaluate the relative effectiveness of maximum label rate compared to two times the maximum label rate, and; 4) describe the duration of treatment effect.

Key words: sudden oak death, phosphonate, tanoak, Agrifos®, tree injection, *Phytophthora ramorum*

Methods

Two study sites were located in young-growth tanoak stands on industrial forestland near Brookings, Curry County, Oregon. We tested two methods of application (injection and bark spray), two application rates (maximum label rate (1X) and double the maximum label rate

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(2X)), and two treatment schedules (a single treatment on February 10, 2004 and treatment on February 10 and May 5, 2004. Twelve trees at each site were untreated controls. We injected trees with a Sidewinder® tree injector, one injection per 15 cm of trunk circumference at or below 1.5 m. above ground. Holes were drilled approximately 2 cm into the sapwood. The injection fluid was 1 part Agrifos-400® and 2 parts water. For the 1X treatment each injection delivered 10 ml of solution. The 2X treatment delivered 20 ml of solution per injection. The 5 mm injection holes were sealed with threaded plastic plugs. We applied Agrifos® to the lower 2.5 meters of the trunk with a back-pack sprayer. The 1X spray solution contained 21 parts Agrfos-400®, 21 parts water, and 1 part pentra-bark® surfactant. The 2X treatment was 42 parts Agrifos-400®, 21 parts water, and 1 part pentra-bark surfactant. Bark was sprayed to runoff. We harvested trees from the February 10-only treatments on April 9 (8 weeks) and on June 23 2004 (18 weeks). We harvested trees from the February 10-May 5 treatments on June 23 and July 26 2004, 7 and 12 weeks after treatment, respectively. At each sampling time we harvested three trees from each treatment at each site plus six control trees (three bark spray controls and three injection controls). We felled each sample tree and cut a 1 meter long (10 to 20 cm diameter) section of bole immediately above the stump and another (3-8 cm diameter) from 10 meters above stump height. We randomly collected 18 leaves from the upper crown of each sample tree. Log ends were sealed with a wax-emulsion sealer to prevent water loss and delivered to Oregon State University in Corvallis, OR. We inoculated each bole section with one of each of the following agar plugs: *P. ramorum* North American genotype-A2 mating type; *P. ramorum* European genotype-A1 mating type, and; sterile V-8 juice agar. We inserted the 5mm diameter plugs into holes created in the bark with a cork borer, and covered the inoculation point with sterile cotton and tape. Logs were incubated inside clear polyethylene sleeves for 4 weeks at constant temperature (21 degrees C). At the end of the incubation period we shaved the bark, measured the longitudinal and radial extent of lesions associated with each inoculation point, and attempted re-isolation of the pathogen from the lesions. We rinsed leaves with sterile water, placed them in a moist chamber, and placed agar plugs of two *P. ramorum* isolates and sterile agar (described above) on the leaf surface. We incubated leaves for 10 to 14 days in the dark at 21 degrees C and then measured the area of necrosis associated with each inoculation point.

Results

For trees treated in February only, after 8 weeks Agrifos® applied either by injection or bark spray clearly reduced lesion size in the lower bole, but not in the upper bole. Treatment rate (1X or 2X) did not affect lesion size. The European isolate consistently caused larger lesions than the North American isolate, but the latter was losing vitality in culture and behaving erratically in other studies, and was excluded from subsequent analyses. After 18 weeks, lesion size did not differ statistically (ANOVA, $p>.05$) between treated and untreated stems from either the upper or lower bole. Injection treatments appeared to have a more lasting effect than the bark application.

For trees treated in February and May, after 7 and 12 weeks (following the May treatment) the bark treatments reduced *lower* bole lesion size compared to controls, but injection treatments did not. Injection treatments reduced lesion size in the *upper* bole compared to controls but bark treatments did not. It is possible that injected material moved upward in the xylem stream and accumulated in expanding foliage, whereas bark-applied material may have been absorbed directly into the inner bark. Bark application may be more effective than injection for protecting the bole during spring vegetative growth. Injection may be more effective than bark spray for reducing lesion size in the upper stem. Much statistical variation was due to little or no lesion development at some inoculation points regardless of treatment.

The leaf test failed because unknown micro-organisms confounded lesion measurements. We were unable to determine whether or not Agrifos® treatments affected susceptibility of leaves or petioles to infection by *P. ramorum.*

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Effect of Phosphonate Treatments on Sudden Oak Death in Tanoak and Shreve's Oak[1]

Doug Schmidt[2], Matteo Garbelotto[2], Dave Chambers[3], and Steve Tjosvold[3]

Abstract

Field experiments were conducted to evaluate the effectiveness of phosphonate chemical treatments for control of sudden oak death in tanoak (*Lithocarpus densiflorus*) and Shreve's oak (*Quercus parvula* var. *Shrevei*). Native stands of mature trees were preventatively treated with Agrifos® systemic fungicide and subsequently infected with *Pytophthora ramorum*. Both injection treatments as well as topical application of Agrifos® with Pentrabark® surfactant were evaluated. Experimental methods were designed to test both within tree and between tree variability. Leaves and wood samples were also collected from the treated trees for chemical analysis of phosphonate levels.

The injection and topical phosphonate treatments significantly reduced lesion size in tanoaks and Shreve's oaks compared to untreated control trees. However, the extent of moss covering the tree trunk affected the Shreve's oaks topical treatment. A combined treatment of injection and topical application methods was found to be most effective in Shreve's oak. The results suggest that the dosage of phosphonate that is successfully introduced into the tree may be as important as the application method. In addition, the range of resistance and susceptibility to *P. ramorum* that exists in native stands may affect the success of phosphonate treatments.

Key words: sudden oak death, *Phytophthora ramorum*, phosphonate treatment

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Evaluation of Chemical and Biological Agents for Control of *Phytophthora* Species on Intact Plants or Detached Leaves of Rhododendron and Lilac[1]

R.G. Linderman[2] and E.A. Davis[2]

Abstract

The recent incidence of Ramorum blight, caused by *Phytophthora ramorum*, on many nursery crops has focused attention on improving management strategies against *Phytophthora* diseases in nurseries. We evaluated several chemical agents that target Oomycete pathogens for their capacity to inhibit infection of rhododendron or lilac leaves by *P. ramorum* (both NA strain 2027 mating type A2 and European strain D12A mating type A1) compared to other species, including *P. cactorum*, *P. citricola*, *P. nicotianae*, and *P. citrophthora*. We inoculated needle-wounded leaves from plants previously treated with various chemicals that were (a) removed and inoculated, or (b) inoculated on intact plants maintained in high humidity. Inoculation of leaves on chemically-treated intact plants with *P. ramorum* or other *Phytophthora* species yielded similar results to those from inoculation of leaves detached from the same treated plants. Most of the chemicals tested had some efficacy on some species of *Phytophthora*, but Subdue Maxx (drench or foliar) had the greatest disease-suppressive activity against all species of *Phytophthora* except *P. citrophthora*, and was effective for at least 6 weeks after drench application. Some chemicals had varied efficacy depending on the species of *Phytophthora*. All chemicals were fungistatic, not fungicidal. Dipping leaves in the chemicals 24 hr prior to inoculation resulted in the same activity profile as applying chemicals to intact plants or detached leaves. Bacterial antagonists (*Bacillus brevis* or isolates of *Paenibacillus polymyxa*) significantly inhibited all *Phytophthora* species in *in vitro* challenges, but were ineffective when applied to leaves 24 hr prior to inoculation with *P. ramorum* or other species.

Key words: Phytophthora ramorum, sudden oak death, ramorum blight, chemical control, biological control

Introduction

Surveys in many areas of the world indicate that many species of *Phytophthora* can cause diseases in the nursery industry but are not being controlled by current management practices.

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That fact and the recent emergence of Ramorum blight in nurseries, caused by *P. ramorum*, suggest the need for improved control strategies, including application of chemical and biological agents. The purpose of these studies, therefore, was to evaluate a range of chemicals, specifically targeted to Oomycete pathogens, and some prospective bacterial antagonists that strongly inhibit *Phytophthora* spp. *in vitro*, for their ability to suppress infections by a selection of *Phytophthora* species, including *P. ramorum*, that can cause foliar blights on rhododendrons and lilacs.

Materials and methods

Inoculation and disease severity assessment. Leaf material used was from outdoor container-grown rhododendron 'Nova Zembla' and lilac 'Monge' plants. Three replicate leaves per fungal isolate were inoculated with mycelial inoculum plugs taken from agar cultures of the different species of *Phytophthora* and placed directly on needle wounds. Inoculated detached leaves were placed in zip-lock polyethylene bags and hand-misted with water as the bags were closed several times during incubation. Leaves on intact plants were similarly inoculated and the entire plant enclosed in a polyethylene bag. All inoculated plants or leaves were maintained in an incubator at 20 ^{o}C for 8 days, and then leaves were photographed to obtain digital images from which percentage of leaf area with lesions was assessed quantitatively using the lesion assay software ASSESS (Lakhdar Lamari, APS, St. Paul, Minn.).

Chemical and biological agents. Eleven chemicals plus a control were tested for efficacy in controlling six species of *Phytophthora* inoculated onto rhododendron or lilac leaves. Chemicals used were: BAS 500, Agriphos, Aliette, Biophos, Fenamidone, Fosphite, Heritage, Ranman, Stature, Subdue Maxx, and Truban, all at label rates. Chemical agents were applied either by: (a) spraying foliage or drenching roots of container plants, or (b) by dipping detached leaves removed from container-grown plants. Bacteria were isolated from organic materials and tested *in vitro* against numerous *Phytophthora* species; all showed strong antagonism. Bacterial isolates were identified by FAME analysis (Texas A & M University) as *Bacillus brevis* and *Paenabacillus polymyxa.* Leaves to be inoculated were dipped in the bacterial suspensions and held for 24 hr prior to inoculation with the *Phytophthora* pathogens. Pathogenic *Phytophthora cactorum*, *P. citricola*, *P. citrophthora*, *P. nicotianae* (= *P. parasitica*), and two isolates of *P. ramorum* were grown on dilute V8 juice agar medium for 14 days before plugs were removed as inoculum.

Results and discussion

Inoculation of leaves on chemically-treated intact plants with *P. ramorum* or other *Phytophthora* species yielded similar results to those from inoculation of leaves detached from the same treated plants. Most of the chemicals tested had some efficacy on some species of *Phytophthora*, but Subdue Maxx (drench or foliar) had the greatest disease-suppressive activity against all species of *Phytophthora* except *P. citrophthora*, and was effective for at least 6 weeks after application. Some chemicals had varied efficacy depending on the species or isolate of *Phytophthora* and the host used. Dipping leaves in the chemicals 24 hr prior to inoculation resulted in the same activity profile as applying chemicals to intact plants or detached leaves, and in some cases efficacy was increased. Bacterial antagonists (*Bacillus brevis* or isolates of *Paenibacillus polymyxa*) significantly inhibited all *Phytophthora* species in *in vitro* challenges, but were ineffective in inoculation assays of leaves dipped in a cell suspension of each antagonist 24 hr prior to inoculation with *P. ramorum* or other species. These tests indicate that inoculating detached leaves was comparable to inoculating intact plants to evaluate chemical and biological agents against *Phytophthora* species, and that several systemic or translaminar chemicals were effective in suppressing infections but without eradicating the pathogens.

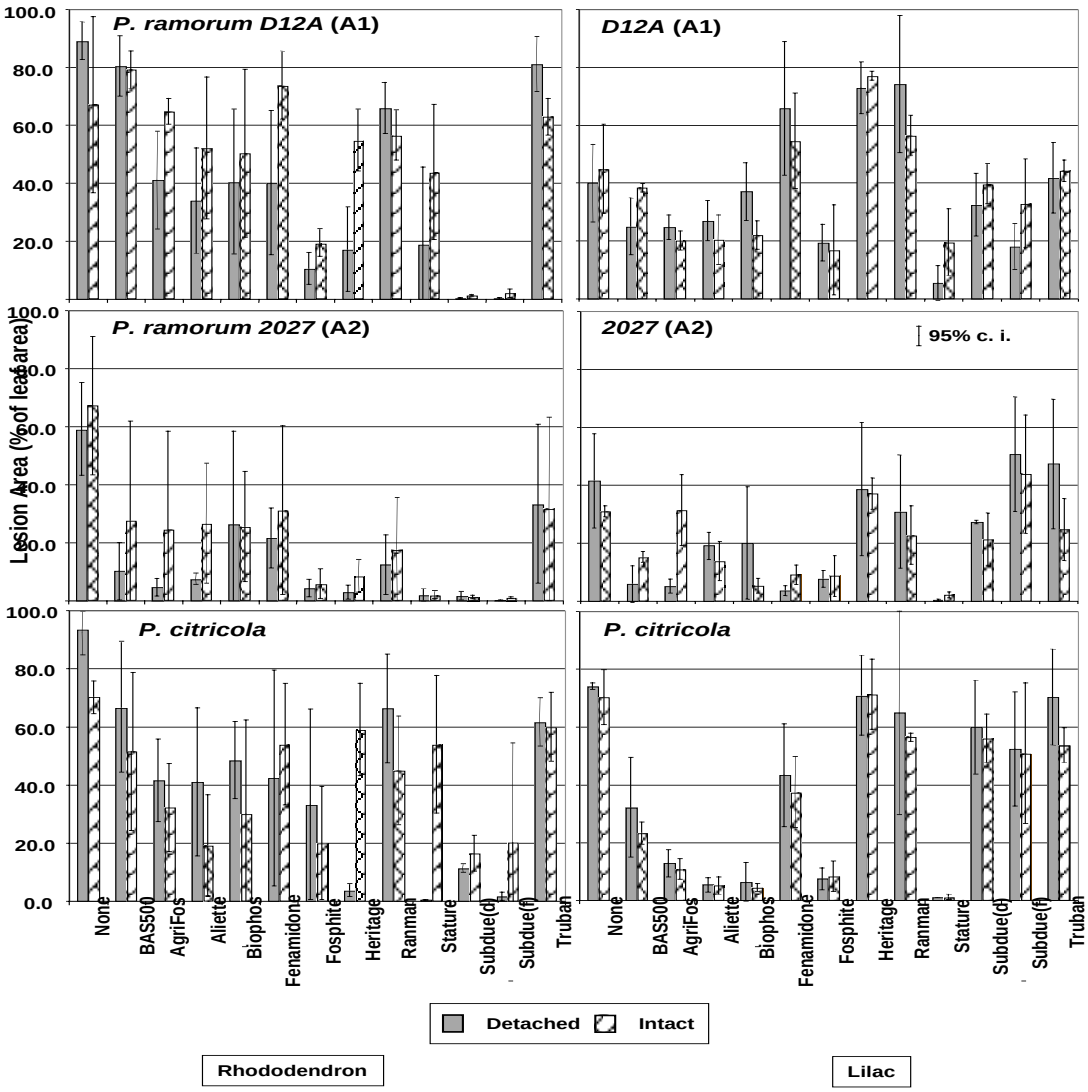

Figure 1 – Disease severity on chemically-treated rhododendron or lilac leaves inoculated with isolates of *Phytophthora ramorum* (isolates 2027 or D12A) or *P. citricola*. Bars on columns represent 95 percent confidence intervals.

Evaluation of Fungicides for the Control of *Phytophthora ramorum* Infecting *Rhododendron*, *Camellia*, *Viburnum,* and *Pieris*[1]

S.A. Tjosvold[2], D.L. Chambers[2], and S. Koike[3]

Abstract

Phytophthora ramorum has been detected infecting ornamental hosts in European nurseries and gardens beginning in 1993, and detected in North American nurseries beginning in 2000. Nursery operators need a comprehensive program to insure that nursery stock remain disease free. Fungicides could be part of an integrated pest management approach to meet that goal. It is also important to evaluate whether fungicides are masking symptoms and the detection of the pathogen.

For two years we evaluated fungicides for the prevention and eradication of *P. ramorum* on four important ornamental genera of nursery hosts: two cultivars of *Rhododendron* (R. 'Cunningham's White' and R. 'Irish Lace') in 2003, and *Camellia japonica* 'Elena Nobile', *Pieris japonica* 'Whitewater', and *Viburnum tinus* 'Compacta' in 2004. First, we screened prospective fungicides by evaluating the preventative control of a wide range of commercially-available and experimental Oomycete fungicides. Second, we selected the fungicides that provided the best efficacy in the fungicide screen and repeated these applications to determine their residual action to prevent infection. Efficacy was also judged by how well the pathogen was recovered from lesions resulting from infections. Finally, the eradicative potential of these fungicides were judged by the success of recovery of the pathogen from lesions that were treated with these fungicides.

Key words: Phytophthora ramorum, fungicide, Azalea, fosetyl-Al, phosphorous acid, copper sulfate pentahydrate, cuprous oxide, azoxystrobin zoxamide, mancozeb

Rhododendron (2003)

Screen potentially effective fungicide

In both R. 'Cunningham's White' and R. 'Irish Lace', the treatments that provided the smallest lesion development were mefenoxam (foliar), dimethomorph, fenamidone, and pyraclostrobin. Cuprous oxide was ineffective on both cultivars and was removed from subsequent experiments. Fosetyl-Al applied as a soil drench was not effective, but the foliar

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treatments were somewhat better than the water check. Fosetyl-Al as a foliar treatment was still included in subsequent experiments to see if additional time for metabolism and movement might result in better efficacy. Plants treated with copper sulphate pentahydrate developed significant phytotoxicity on both cultivars.

Determine residual fungicidal action

All tested fungicide treatments were effective in reducing lesion development if applied 1 day, 1 week or 2 weeks before inoculation, but not if applied 4 weeks before inoculation. There were no differences of lesion development that could be attributed to a specific fungicide. The pathogen was re-isolated from a high percentage of leaves from most treatments. However, recovery was consistently lower in those leaves that were treated with dimethomorph over all treatment-time regimes.

Determine eradicative action

Post-infection fungicide treatments did not impede lesion development on infected leaves remaining on the plant or leaves that abscised. When sampled up to 6 weeks after fungicide treatment, intact leaves had lesions that covered 13.0 to 32.0 percent of the leaf with a mean coverage of 23.0 percent. Fallen leaves had lesions that covered 44.0 to 88.0 percent of the leaf with a mean of 61.0 percent. There were no significant differences (ANOVA) in lesion development between treated and water-check treated lesions for intact and abscised leaves. In addition, *P. ramorum* was usually readily re-isolated. There was a general decline of isolation success with time, and isolation was less successful on fallen leaves.

Camellia, Viburnum, and Pieris (2004)

Screen potentially effective fungicides

The treatments had similar relative effects, regardless of species, even though leaf lesions of each species had different growth rates. Dimethomorph, pyraclostrobin, fenamidone, mefenoxam provided preventative control better than the other fungicides, as they did in the fungicide screen for rhododendron. Zoxamide and cyazofamid were tested on these species and provided preventative control. There was less control with mancozeb, zoxamide + mancozeb, azoxystrobin, mono and di-potassium phosphorous acid and fosetyl-Al than with the other fungicides.

Determine residual fungicidal action

For camellia, several fungicides provided preventative control for at least 2 weeks. Generally efficacy was not as strong at 4 weeks as indicated by weaker statistical significance ($p < .06$), although cyazofamid and dimethomorph were clearly efficacious at 4 weeks. For Japanese pieris and viburnum, dimethomorph, fenamidone, and cyazofamid provided preventative

control for up to 4 weeks. To a lesser extent, mefenoxam, pyraclostrobin, and zoxamide provided preventative control. There were generally lower isolation success rates with dimethomorph and cyazofamid.

Determine eradicative action

Post-infection fungicide treatments did not impede lesion development on infected leaves when lesion size was evaluated 2 weeks after fungicide treatment. There were no significant differences (ANOVA) in lesion development. Lesion diameter varied with species, with mean diameters at 2 weeks for Japanese pieris, camellia and viburnum, 33.6, 19.8 and 11.1 mm respectively. In addition, *P. ramorum* was commonly re-isolated, with no significant differences between fungicide treatments and the water-check. Success rate for isolation varied from 86 to 100 percent. Even though isolations were highly successful there was a difference in the general vigor of growth from isolation. Fungicide treated isolations were weaker, especially when treated with cyazofamid and dimethomorph.

Summary

Cyazofamid, dimethomorph, mefenoxam, pyraclostrobin and fenamidone applied as foliar sprays consistently provided preventative control as expressed by reduced lesion size compared to water controls. These fungicides provided preventative activity for at least 4 weeks, except in rhododendron in which the tested fungicides were active up to 2 weeks following their application. When lesions occurred, the pathogen was successfully recovered from the lesions by isolation on selective media. Only cyazofamid and dimethomorph significantly reduced the success of isolation recovery. Post-infection treatments of leaf lesions with foliar- and soil-applied fungicides were ineffective in reducing the size of lesions and the success of pathogen recovery from those lesions. The pathogen was recovered from lesions consistently for at least 6 weeks after fungicide application in rhododendron regardless of treatment.

Fungicides will not be effective in eradicating existing infections and lesion development is not slowed significantly by fungicide application. Infected plants will need to be destroyed in quarantine situations. Laboratory isolation success declines with time after fungicide application and infection so agricultural inspectors should locate leaves that have recently formed lesions for isolation. Lesion development can occur even with preventative fungicide application for various reasons: imperfect application uniformity, incomplete movement of fungicide, inherent fungicide efficacy, and weakened or wounded plants. If lesions occur, cyazofamid and dimethomorph could have a significant negative impact on isolation recovery.

Chemical Control of *Phytophthora ramorum* Causing Foliar Disease in Hardy Nursery Stock in the United Kingdom[1]

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Key words: Chemical control, *Phytophthora ramorum*, nursery stock

Abstract

A range of fungicides have been tested for activity against *P. ramorum* using both *in vitro* and *in vivo* tests. All fungicides had proven activity against *Phytophthora* species and either had full approval for use on hardy ornamental nursery stock in the United Kingdom, or could be used under the Revised Long Term Arrangements for Extension of Use (2002). Fungicides were initially tested for *in vitro* activity against mycelial growth and spore germination of two isolates of *P. ramorum* from the U.K. Agar plate tests showed that metalaxyl-M (SL 567A), dimethomorph/mancozeb (Invader), etridiazole (Standon Etridiazole 35) and fenamidone/mancozeb (Sonata) were the most effective fungicides against mycelial growth. Tests on activity of the fungicides against spore germination showed that metalaxyl-M, SL 567A, etridiazole, fenamidone/mancozeb, azoxystrobin (Amistar) and famoxadone/cymoxanil (Tanos) were most effective. In both tests metalaxyl-M was by far the most effective of all the fungicides tested. Seven fungicides: metalaxyl-M, dimethomorph/mancozeb, cymoxanil/mancozeb (Curzate M68), fenamidone/mancozeb, etridiazole, azoxystrobin and famoxadone/cymoxanil were tested for protectant and eradicant activity on containerized rhododendron and viburnum. All fungicides were applied as foliar sprays at the manufacturer's recommended rate. On rhododendron, metalaxyl-M, azoxystrobin and fenamidone/mancozeb completely inhibited symptom development when applied as protectant treatments either 4 or 7 days prior to inoculation. However, on viburnum only metalaxyl-M was completely effective at all protectant timings. Fenamidone/mancozeb was effective when applied 4 days prior to inoculation but efficacy was greatly reduced when the treatment was applied 3 days earlier. Fungicides were generally less effective when applied as eradicants. The most effective was metalaxyl-M, completely inhibiting disease development when applied 4 days after inoculation. None of the fungicides completely controlled disease development on viburnum when applied after the same time period. Despite the fact that metalaxyl-M was the most effective fungicide for control of *P. ramorum*, use of this fungicide has not been recommended due to the significant risk of the rapid development of fungicide resistance in the pathogen. Co-formulations and mixtures of metalaxyl-M with other active ingredients, including those shown to be effective in this study (e.g. azoxystrobin and

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fenamidone/mancozeb), need to be investigated to develop a protocol for durable fungicidal control of *P. ramorum*.

Overall, the project has demonstrated that, subject to further investigation, chemical treatments could play a major role in future control and containment strategies for *P. ramorum*. However, before growers in the U.K. could adopt the results of the work there would need to be changes in the current eradication policy for *P. ramorum* and further experimental work carried out on rates and methodologies for fungicide application and on the development of sound anti-resistance strategies.

Phytophthora ramorum Infection in Coast Live Oaks and Shreve's Oaks Treated With Insecticide to Prevent Beetle Colonization[1]

Brice A. McPherson[2], David L. Wood[3], David M. Rizzo[4], Pavel Svihra[5], Steve Tjosvold[6], Andrew J. Storer[7], and Richard B. Standiford[2]

Key words: Phytophthora ramorum, coast live oak, Shreve oak, ambrosia beetles, insecticides

Abstract

As the name implies, sudden oak death, caused by *Phytophthora ramorum*, kills many, if not most of the coast live oaks, *Quercus agrifolia*, that become infected (McPherson and others, 2005). Several genera of ambrosia and bark beetles (Coleoptera: Scolytidae) colonize bleeding (infected) trees and are suspected to hasten tree death. Insecticide treatment is being offered to property owners to prevent the death of high value trees, although controlled studies of its efficacy are lacking. In this study, we are testing the efficacy of the synthetic pyrethroid insecticide permethrin in prolonging the life of infected coast live oaks and the closely related Shreve oaks, *Q. parvula* var. *shrevei.* This study was also designed to follow the progression of *P. ramorum* infection in the absence of beetle colonization. Asymptomatic coast live oaks (80 in each of two Marin County sites) were randomly assigned to three treatments in July 2002: inoculated (80), mock-inoculated (40, wounded but not inoculated), and control (40). Half of each group was randomly selected for spraying with permethrin twice each year, in August and February, prior to beetle flight periods, following recommended application levels. The study on Shreve oaks was started in March 2003 in one site in Henry Cowell State Park, Santa Cruz County. After one year, 72 percent of the inoculated coast live oaks were symptomatic (bleeding). Considerable variation was seen in the number of discrete bleeds on trees and in the total area affected per tree. Permethrin treatment prevented colonization through March and April 2003. However, by July, the mean number of beetle tunnels per tree did not differ as a function of permethrin treatment, indicating that efficacy decreased with time (*fig.* 1). Beetles colonized approximately half the infected, bleeding trees in both the insecticide-treated and untreated groups. However, although the number of beetle-colonized trees did not differ between groups, significantly fewer beetles tunneled into the

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permethrin-treated trees than the untreated trees. This difference was maintained through October 2004. Once insecticide-treated trees were colonized, significantly more beetles were trapped than on the trees that did not have any beetle attacks (*table 1*). This response demonstrates the production of aggregation pheromones by the colonizing beetles. This phenomenon is well documented in a number of ambrosia and bark beetles. The bark cankers produced by *P. ramorum* in coast live oaks often lead to cracking of the bark, with attendant fissures that disrupt any insecticide barrier on the surface. This damage to the bark may negate the effect of the insecticides. Ambrosia beetles, *Monarthrum scutellare* in particular, may tunnel to 10 cm and deeper in the sapwood (Svihra and Kelly, 2004). The attraction of beetles to trees in which colonizing beetles have established tunnels may overwhelm any natural resistance of the tree to *P. ramorum*. Ambrosia beetles have the capability to introduce microorganisms, including decay fungi, deep into the sapwood of trees (Hijii and others, 1991). Once the protection of the bark has been breached, a tree may have little resistance to introduction of fungi and bacteria into its conductive tissues. Of the five inoculated trees that had died by August 2004, one was in the permethrin-treated group. This study is continuing.

Bleeding developed in 45 percent of the inoculated Shreve oaks in one year. Very little is known about the course of *P. ramorum* infection in this species. Bleeding in inoculated trees tends to be more limited, and the association with beetles appears to be less consistent, than in coast live oaks. Through October 2004, no inoculated trees had died.

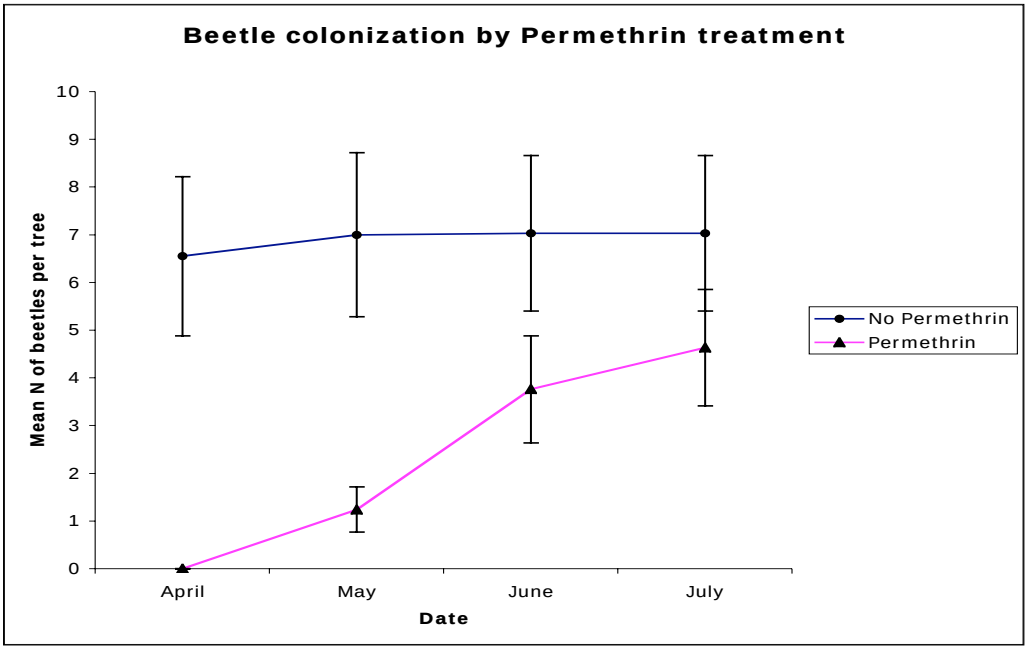

Figure 1—Numbers of beetle tunnels in inoculated bleeding coast live oaks sprayed with Dpermethrin (triangles) and unsprayed (circles), for April through July 2003 (mean ± SE)

Table 1—*Trap catch on insecticide-treated bleeding coast live oaks as a function of prior beetle colonization during the 2003 season, mean ± SE; beetle trap counts were statistically different by t-tests. May, p = 0.0016; July, p = 0.006*

Tree Status (Colonized, or not)	**May**	**July**
No Beetles	2.6 (1.2)	0.6 (0.2)
Beetles	9.0 (1.1)	14.0 (5.9)

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Effect of Sanitary Measures on the Survival of *Phytophthora ramorum* in Soil[1]

M.M. Aveskamp[2,3,4], P.J.M. van Baal[2], and J. de Gruyter[2]

Abstract

In May 2003, a *Phytophthora ramorum* infested garden in the surroundings of Nijmegen, the Netherlands, was cleared of infected Rhododendron shrubs by cutting the plants back at 30 cm. In an experiment the remaining parts of the Rhododendron plants were treated with thiophanate–methyl, glyphosate or untreated. In the same experiment, the effect of additional phytosanitary measures, removal of plant debris and humus or plant debris only, was tested. The re-growth and occurrence of new infections were monitored. Soil samples were taken monthly at 20 cm depth or at soil surface and tested for the presence of *P. ramorum* using a rhododendron-leaf baiting test, followed by a TaqMan® ITS-PCR. Two months after cutting back, development of new shoots was observed in all objects. A number of the new shoots were infected from the initial growth onwards by *P. ramorum*. These infections proved that the remaining parts of the stems were already infected. The different treatments had no influence on the percentage of infected shoots. The detection of *P. ramorum* in soil samples showed that the pathogen seems to be present more abundantly at 20 cm depth than at the soil surface. In the samples taken from the soil surface, *P. ramorum* was mainly found in samples from plots where both humus and plant debris were present. The recovery of *P. ramorum* in the samples taken from 20 cm depth was independent of the soil coverage. The results indicate that *P. ramorum* is capable of surviving in sandy soil for at least one year. Furthermore, in this experiment, cutting back *Rhododendron* shrubs at 30 cm was not sufficient for eradication.

Key words: Phytophthora ramorum, *Rhododendron*, sanitary measures, survival, soil

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Introduction

Rhododendron proved to be the main source of infection for *Phytophthora ramorum* in the public green in the Netherlands. Results of surveys indicate that a complete eradication of *P. ramorum* is not possible. Most important is to obtain an acceptable reduction of the infection pressure especially to avoid spread of *P. ramorum* from infected Rhododendrons to trees nearby. Questions arose about the methods of eradication, and the means of handling the infected plant material. In addition, data about the infection level and the longevity of *P. ramorum* in soil at infested sites was lacking. A field experiment was conducted to test the effect of cutting back infected rhododendron plants at 30 cm compared to removing whole rhododendron plants. The effect of treatment of the stumps with an herbicide or fungicide on the occurrence of re-infections was also studied.

In addition, burying infected plant material at 1 m depth, or burning all infected plant material at the site is not always possible and may involve high costs. Therefore, in the same experiment phytosanitary measures, such as removal of plant debris and humus or plant debris only, was compared with leaving the chipped plant debris and humus on the soil surface. Soil samples were taken monthly at 20 cm depth and at the soil surface and tested for the presence of *P. ramorum*, to determine the infection levels in soil during the experiment.

Material and methods

In May 2003, a *P. ramorum* infected garden in the surroundings of Nijmegen, the Netherlands, was cleared of infected *Rhododendron* shrubs by cutting back the plants at 30 cm. In another experiment, on nine 200 m^2 plots, the remaining stumps of the approximately 70-year old *Rhododendron* plants were treated with thiophanate–methyl, glyphosate or left untreated. The different plant debris treatments within each object are given in *table 1*. The re-growth and occurrence of new infections were monitored monthly in the period from May 2003 to January 2004.

Table 1—*Treatments.*

Object
A: Application of herbicide on cut rhododendron stumps
B: Application of fungicide on cut rhododendron stumps
C. Cutting of rhododendrons only
Treatments within each object:
A1,B1,C1: chipped plant debris and humus left on the soil surface
A2,B2,C2: plant debris removed
A3,B3,C3: plant debris and humus layer removed

The date of the cutting, removal of plant debris and the various fungicide and herbicide applications was May 20, 2003. The fungicide application was carried out with Topsin M/S (thiophanate–methyl). The application of the herbicide Round-up (glyphosate) to each stump was repeated at July 23, 2003.

On each plot, a mixed soil sample of 60 sub samples, 1.5 cm diam. was taken monthly at alternating soil surface level or at 20 cm depth, in the period from May 2003 to December 2004. Presence of *P. ramorum* was tested by a baiting method using freshly picked leaves of *Rhododendron catawbiense* 'Cunningham's White' (Themann. and Werres 1998), followed by a Taqman® ITS-PCR on the bait (Ivors and Garbelotto 2002).

Results

Two months after cutting back the shrubs, development of new shoots was observed in all objects (*table 2*). The lowest numbers were in the glyphosate treatment. However, the re-growth indicates that the treatment was not completely effective. A number of the new shoots were infected from the initial growth onwards by *P. ramorum*. These infections proved that the remaining parts of the stems were already infected. The different treatments had no reliable influence on the percentage of infected plants.

Table 2— *Percent re-growth and percent infected Rhododendron plants 8 months after cutting at 30 cm and treatment with glyphosate, thiophanate-methyl or untreated.*

Object	# plants	percent re-growth	percent infected	(SD)
Glyphosate	64	16	60	(32)
Thiophanate-methyl;	75	85	48	(15)
Untreated	81	67	41	(13)

Table 3— *Percent re-growth and percent infected rhododendron plants 8 months after cutting at 30 cm, followed by removal of plant debris and humus, plant debris only or leaving the plant debris on the soil surface.*

Object	# plants	percent re-growth	percent infected	(SD)
All debris left	68	57	46	(33)
All debris removed	78	68	45	(5)
Debris and humus removed	7	49	47	(20)

The different treatments in the objects, all debris left, all debris removed and debris and humus removed, did not give reliable differences in the percentage of infected plants (table 3). Probably, infection did not take place from infected soil or plant debris.

The detection of *P. ramorum* in soil samples (*fig. 1.*) showed that the pathogen seems to be present more abundantly at 20 cm depth than at the soil surface.

Figure 1—Number of positive samples period May 2003 to December 2004, at soil surface or at 20 cm depth.

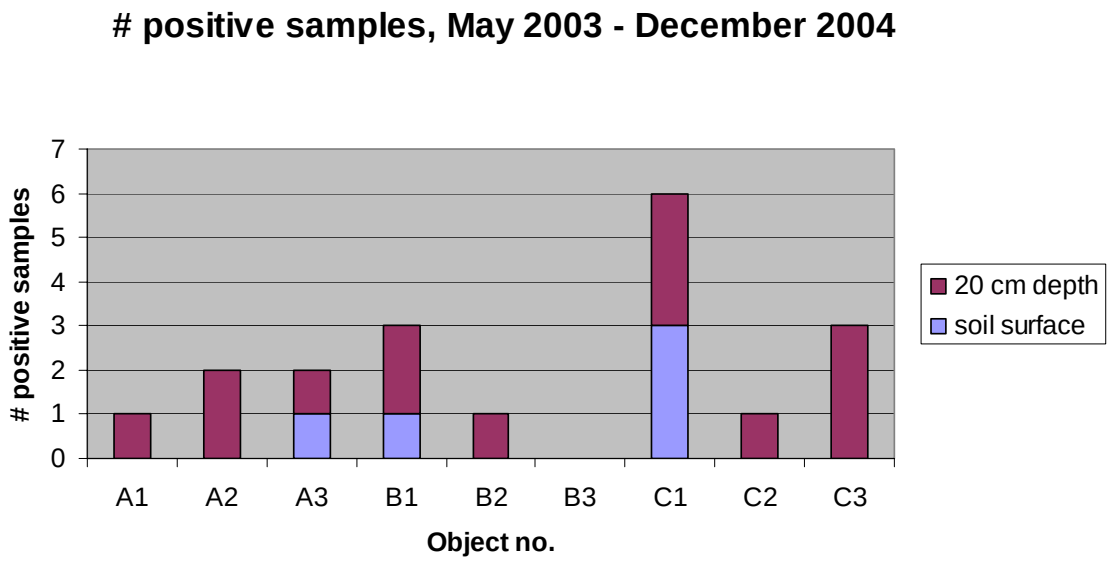
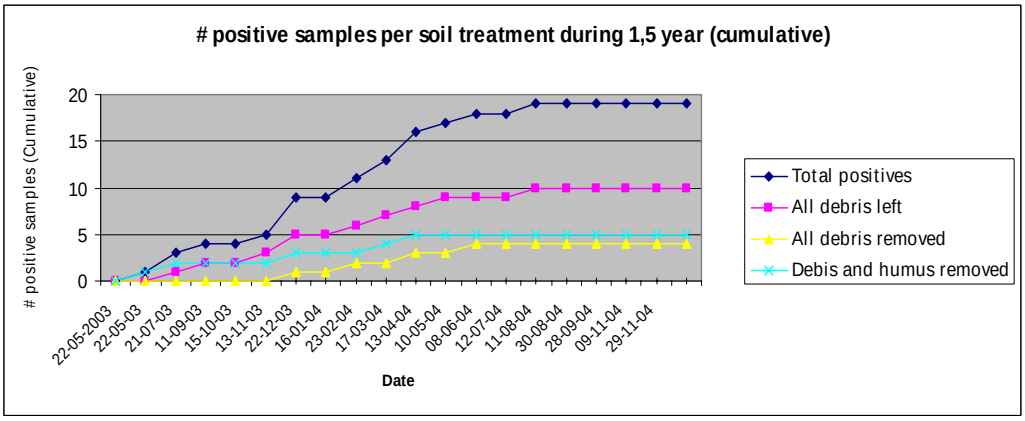


Figure 2—Number of positive samples period May 2003 to December 2004



This is probably due to a higher variation of environmental conditions in the upper soil surface. In the samples taken from the soil surface, *P. ramorum* was mainly found in samples from plots where both humus and plant debris were present. The recovery of *P. ramorum* in the samples taken from 20 cm depth was independent of the soil coverage.

Conclusions

The results indicate that in this experiment cutting back *Rhododendron* shrubs at 30 cm was not sufficient to eradicate *P. ramorum*, because new developing shoots became infected directly from the remaining stumps. The percentage re-infected plants, almost 50 percent, was high, however, the number of infected shoots per plant was relatively low. Infected shoots easily break off. Experiences in practice on 40 sites where infected Rhododendrons were cut off, showed that at 50 percent of the sites developing shoots were directly infected from the stumps, usually in low percentages.

In this experiment, *P. ramorum* proved to be capable of surviving in sandy soil for at least one year. The highest number of infections was found in the samples taken at 20 cm depth, in the plots where all debris was left. However, the number of positive samples was relatively low, and the difference was not statistically reliable.

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Thwarting *Phytophthora ramorum:* A Proposed Disease Cycle With Mitigation Measures[1]

Betsy Randall-Schadel[2] and Scott Redlin[3]

Abstract

Phytophthora ramorum has become increasingly disruptive to the movement of nursery stock since it was described in 2001. Risk and mitigation assessments for *P. ramorum* have been done or are underway by APHIS. Because of the impact of this pathogen on forests and the nursery industry, accelerated research efforts are underway. Capturing new information in a graphic format was desirable. The lack of a single mitigation measure to effectively eliminate *P. ramorum* from nursery stock suggested the value of a diagram highlighting points in the disease cycle where mitigations may be effective. For each point, potential mitigation measures are listed. The diagram was compiled from scientific sources including published papers and proceedings of science panels. The proposed disease cycle originally had four phases: foliar hosts, canker hosts, soil/potting medium and dormancy phases. The multicyclic foliar and dieback hosts were linked with the monocyclic potential of canker hosts. The recent reports of the inoculation with infested potting media and persistence of the pathogen in potting media prompted the inclusion of the soil/potting medium phase. The potential survival of chlamydospores and mycelia are included in the dormancy phase. The four phases are color coded and linked by reproduction and survival of the pathogen. The proposed disease cycle is being presented in a variety of scientific forums to elicit feedback. The disease cycle and mitigation diagram was presented at the 2004 Ornamental Workshop hosted by North Carolina State University. Additions to the diagram following that interaction included sexual reproduction and the biological requirements to transition from one phase to the next. The proposed cycle (*fig. 1*) and mitigation measures are presented in an interactive poster format to prompt discussion.

Key words: Phytophthora ramorum, risk assessment, mitigation assessment

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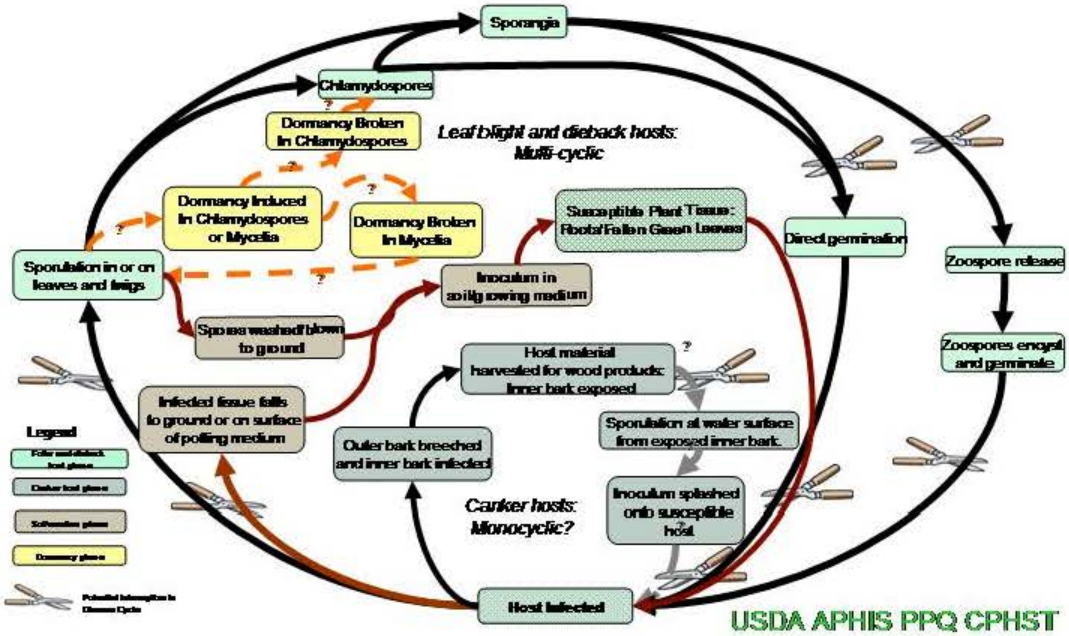
Proposed disease cycle for *Phytophthora ramorum*. Potential points for mitigation measures are indicated with pruning shears.



Introduction of Filtration Systems in Container Nurseries for Nonchemical Elimination of *Phytophthora* spp. From Irrigation Water[1]

Thorsten Ufer[2], Heinrich Beltz[3], Thomas Brand[3], Katrin Kaminski[2], Ralf Lüttmann[3], Martin Posner[4], Stefan Wagner[2], Sabine Werres[2], and Hans-Peter Wessels[5]

Abstract

In a 3-year project the elimination of *Phytophthora* spp. from the recirculation water with different kinds of filtration systems will be tested under commercial conditions in container nurseries. First results indicate that the filtration systems eliminate *Phytophthora* spp. from the water.

Key words: Phytophthora spp., recycling water, filtration systems, commercial nurseries

Introduction

The production of hardy ornamental nursery stock (HNS) outdoors in containers is an important part of the German horticultural industry. The plants are cultivated outdoors in containers in special areas (container stands). On these areas the surplus water from irrigation and natural rain is collected via drains and/or special drainage systems and stored in special reservoirs. These reservoirs usually have capacities between 400 and 25,000 m^3. The reservoir water is usually taken for irrigating the container nursery, hence the possibility exist that the entire nursery can be contaminated. If there is not enough water in the reservoirs (e.g. during summer) ground water from wells is frequently used for refilling.

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Studies in commercial container nurseries have shown that *Phytophthora* spp. can be present in the drains and in the reservoirs (but not in the wells) irrespective of the season (Themann et al. 2002). A 3-year project has been started to test different filtration systems for eliminating *Phytophthora* spp. from recycling water under commercial conditions in nurseries. Preventing spread of these pathogens reduces the risk of infections and leads to environmentally sound production, due to fewer applications of chemicals. The German Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL) and the Federal Agency for Agriculture and Food (BLE) funded the project.

Material and Methods

Filtration Systems – Three different filtration systems are tested: slow sand filtration, filtration with lava grain (SHIEER BIO FILTER®) and constructed wetlands with different plant species.

Samples and sampling – Water and/or sediment samples before and after filtration as well as samples from drains and clean water reservoirs were taken at three different dates over the course of the year.

Detection methods – Presence of *Phytophthora* propagules in the samples will be tested with the rhododendron leaf test, determination of the species with morphological studies and/or with Polymerase Chain Reaction (PCR). In addition the rhododendron leaf test should help to detect active and viable propagules of the pathogens.

Filtration systems data – Technical data of the different filtration systems like filtration capacity, necessity and frequency of cleaning of the filter surface (slow sand filtration) etc. will be recorded.

Water data – Chemical data of the water like nutrition, pH etc. will be analysed.

Costs – Cost for running the recirculation system under commercial conditions will be calculated.

Preliminary results

Preliminary results indicate that all three filtration methods eliminate *Phytophthora* spp. from the water under commercial conditions. Isolation and determination procedures have not yet been completed.

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Phytophthora ramorum Disease Transmission From Artificially Infested Potting Media[1]

Jennifer L. Parke, Melody L. Roth, Carrie Lewis, and Caroline J. Choquette[2]

Abstract

Potted rhododendrons grown in potting media amended with inoculum of *Phytophthora ramorum* became infected and showed symptoms of stem necrosis, leaf wilting, and death. *P. ramorum* was isolated from roots and stems of infected plants.

Key words: nursery, potting media, rhododendron, *Phytophthora ramorum*

Introduction

Phytophthora ramorum, a pathogen of aerial plant parts, causes Ramorum shoot dieback and leaf blight on many nursery species in Europe and North America and sudden oak death (SOD) on certain members of the Fagaceae. It has been detected in soil and potting media, but root infections have not been observed. The purpose of these experiments was to determine if potting media infested with *P. ramorum* could serve as a potential source of inoculum for potted rhododendrons, and to determine if *P. ramorum* is capable of infecting roots.

Materials and Methods

Rhododendron 'Nova Zembla' plants grown from rooted cuttings and native Pacific rhododendron *(R. macrophyllum)* plants grown from seed were transplanted into potting media artificially infested with *P. ramorum*.

Inoculum consisted of V-8-broth-vermiculite cultures of *P. ramorum* or chopped infested rhododendron leaves inoculated with Oregon nursery isolate 03-74-N11A (European genotype, A1 mating type). A zoospore dose-response experiment was

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also conducted with *Rhododendron* 'Nova Zembla' using two isolates: 03-74-N11A and 4143 (North American genotype, A2 mating type) and on *R. macrophyllum* with isolate 03-74-N11A. A suspension of zoospores (3×10^5, 3×10^4, or 3×10^3 zoospores mL^{-1}) was pipetted onto the surface of potting media in a 5-cm-diameter circle around the base of the stem. Non-inoculated controls received water only. Disease incidence based on above-ground symptoms was recorded every few days for 6 weeks. As plants wilted and died, samples of roots, belowground stems, aboveground stems and leaves were plated onto PAR selective medium for recovery of the pathogen. Roots were surface-sterilized in 10 percent bleach solution for 30 sec. before plating. Duplicate tissue samples were processed for multiplex PCR analysis. There were five or six replicate plants per inoculation treatment for each host, and each experiment was conducted twice. In all experiments, plants were watered only from the bottom to prevent splash dispersal of inoculum onto stems and foliage.

Results

Both infested amendment treatments, and inoculation of potting media with higher doses of zoospores, resulted in plant mortality. Disease incidence was greater among plants inoculated with 03-74-N11A as compared to plants inoculated with 4143, and plant response reflected zoospore inoculum dose. Leaves wilted and collapsed within 3 to 6 weeks and upwardly-expanding necrotic lesions became apparent on the stems within 4 to 6 weeks. *P. ramorum* was isolated at high frequency from fine roots, large roots, belowground stems, and aboveground stems of plants grown in artificially infested potting media. Occasionally it was also recovered from petioles of inoculated plants. Detection of the pathogen was confirmed with PCR analysis. Control plants grown in non-amended, non-inoculated potting media remained healthy and did not yield *P. ramorum.*

Discussion

Potted rhododendrons grown in potting media amended with inoculum of *P. ramorum* became infected and showed symptoms of stem necrosis, leaf wilting, and death. *P. ramorum* was isolated from roots and stems of infected plants. These results are similar to those reported for other rhododendron dieback-causing *Phytophthora* species that also were able to cause root infections (Benson and Jones 1980). Disease transmission from infested potting media to above-ground plant parts and recovery of *P. ramorum* from asymptomatic root tissue demonstrate the need to monitor potential

belowground sources of inoculum of *P. ramorum* for preventing pathogen spread on nursery stock.

References

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Pathways of Infection for *Phytophthora ramorum* in Rhododendron[1]

Carrie D. Lewis[2] and Jennifer L. Parke[2]

Key words: cyst, germination, histology, root infection, stomata, xylem, *Phytophthora ramorum*

Abstract

The lack of knowledge regarding infection biology of *Phytophthora ramorum* limits our understanding of its ecology and epidemiology. Pathways of infection in *Rhododendron* 'Nova Zembla' were investigated using tissue culture plantlets and 3-year-old container plants inoculated with zoospore suspensions (6 x 10^4 zoospores mL^{-1}) or mycelial plugs of Oregon nursery isolate 03-74-N11A. The histology of inoculated roots, stems and leaf surfaces was evaluated to identify pathways by which *P. ramorum* infects and colonizes plants. To observe infection, inoculated roots, stems, and leaves were examined by means of epi-fluorescence, scanning electron and scanning laser confocal microscopy.

To follow infections initiated from roots into above-ground plant parts, container plants were repotted in a 2:1 mixture of potting soil and V8-vermiculite inoculum of *P. ramorum*. Leaves never touched the potting media, and plants were watered from the bottom only. Leaves wilted and collapsed within 3-6 weeks and upwardly-expanding stem lesions became apparent within 4-6 weeks. *P. ramorum* was recovered through both isolation onto PAR selective medium, and multiplex PCR analysis from symptomatic roots, stems and petioles. Control plants not inoculated with *P. ramorum* remained healthy and did not yield *P. ramorum*. Samples from stems, petioles and below-ground tissues were used for microscopy and PCR analysis. PCR analysis indicated *P. ramorum* was present in roots, stems, and petioles. Tissue samples for microscopy were fixed, embedded, sectioned, stained with 0.01 percent Calcofluor White MR2 and viewed with epi-fluorescence. Preliminary observations of root tissues indicated the presence of *P. ramorum* hyphae on the root surface. Microscopy of stem tissues indicated the presence of *P. ramorum* hyphae in primary xylem at the leading edge of infection. More advanced stem infections indicated the presence of hyphae in pith cells, primary and secondary xylem (*fig. 1*), and chlamydospores in the cortex.

To evaluate root infections, rooted tissue culture plantlets were inoculated by submersing 5-cm sections of rooted stems in a zoospore suspension for 4 or 48 hr. Tissues were fixed, embedded, sectioned, stained with 0.01 percent Calcofluor White MR2 and viewed with epi-fluorescence. Hyphae from germinating cysts were observed penetrating root primordia both inter- and intra-cellularly. Zoospores

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congregated near root primordia, emerging lateral roots, and wound sites. After encystment, germination appeared to be directed toward those sites.

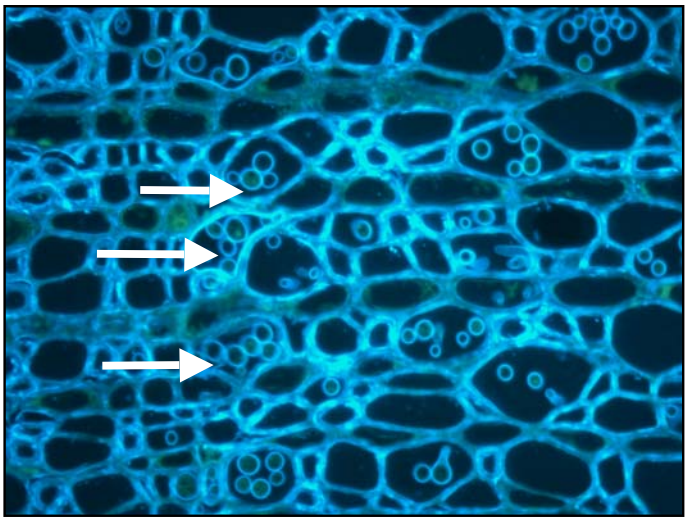

Figure 1- Secondary xylem cells from above-ground stem tissue of rhododendron grown in potting media artificially infested with *P. ramorum*. Cross-sections of hyphae (white arrows) can be seen inside individual cells one month after inoculation.

To observe infections inside root tissues, tissue culture plantlets were inoculated by submersing the rooted portion of stem in a zoospore suspension for 1 hour and then incubating for one week in moist paper towels. Roots were removed, stained with 0.01 percent Calcofluor White MR2 and viewed by scanning laser confocal microscopy. Hyphae from germinating cysts oriented towards emerging lateral roots, penetrated the root epidermis, formed hyphae inside root tissue and produced sporangia.

To observe and follow spread of infection in container plants, leaves were wounded and inoculated with mycelial plugs. Plants were grouped by two treatment methods: leaves were inoculated either on the leaf midrib, or on the leaf blade halfway between the midrib and the margin. Two leaves from each plant were inoculated: one young leaf from new growth and one mature leaf from older growth. After 1 to 2 weeks, inoculation near the midrib resulted in more rapid development of leaf necrosis as compared to inoculation of the leaf blade. Infections initiated in young leaves spread through petioles and into stems of new growth, and the necrosis appeared to spread equally up and down the stem as well as into petioles of adjacent leaves. Removal of the leaf directly above the stem lesion revealed discolored vascular bundles in the leaf scar. Infections initiated in mature leaves appeared to stay within the leaf without advancing into petioles. Lesions or necrosis were never observed on non-inoculated but wounded control plants. Stems from new growth and all leaves were plated onto PAR selective medium. *P. ramorum* was isolated slightly beyond the lesion margins. Isolations from older stems indicated that *P. ramorum* spread into stem tissue from leaf inoculation sites even when petioles were not necrotic.

A time-course study was conducted to observe the development of infection on abaxial leaf surfaces. Leaves from tissue culture plantlets were inoculated with a zoospore suspension and incubated for 1.0, 1.5, 2.0 and 2.5 hrs. Leaves were fixed and critical point dried in preparation for scanning electron microscopy. SEM images of leaf surfaces showed that germinating cysts were occasionally found associated with stomata, but stomata are not required for infection. Germinating cysts were more

abundant at wound sites than stomatal openings. Some cyst germ tubes had structures at the growing tip that resembled appresoria, but their function is unclear.

Survival of *Phytophthora* Species and Other Pathogens in Soilless Media Components or Soil and Their Eradication With Aerated Steam[1]

R.G. Linderman[2] and E.A. Davis[2]

Abstract

Phytophthora ramorum, while thought to be primarily an aerial pathogen, can be introduced into soilless potting media in the nursery industry as sporangia or chlamydospores and be disseminated widely without being detected. Inoculum of this pathogen, both North American (A2) and European (A1) isolates were used to infest potting media components or soil as sporangia or chlamydospores produced in vermiculite culture or in infected rhododendron leaves. Vermiculite chlamydospore/oospore inoculum of *P. citricola*, *P. cactorum*, and *P. citrophthora* were included for comparison. Survival was determined by monthly sampling by baiting (B) or direct plating (DP) on selective medium. Results indicated that *P. ramorum* can survive in most media components for up to 6 months, whether introduced as sporangia or chlamydospores. Experiments were also conducted to determine the lethal temperature needed to eradicate *P. ramorum* from infested potting medium or contaminated plastic containers (over a range of 45 to 70 ºC for 30 min using aerated steam mixtures). Other soilborne pathogens included for comparison were *Pythium irregulare*, *Thielaviopsis basicola*, *Cylindrocladium scoparium*, and their survival was determined by baiting methods. All pathogens were killed by temperature treatments of 50 ºC or greater. These results show that *P. ramorum* can survive in potting media if introduced as sporangia or chlamydospores, and infested media or contaminated containers can be sanitized by aerated steam treatment.

Key words: Phytophthora ramorum, sudden oak death, Ramorum blight, soil pasteurization, eradication, soilless potting media

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Introduction

Phytophthora ramorum on nursery crops, thought to be largely a foliar pathogen, could be incorporated into container soilless potting media and thus become soilborne. Also, nursery containers from which infested media or infected plants were removed could be contaminated, as is true with other soilborne fungal pathogens. Inoculum from either source may initiate infections on subsequent crops grown in infested media or contaminated containers. Pathogens could be eradicated from soilless media by heat from steam, composting, or solarization; or by chemical fumigation. Growers currently attempt to decontaminate used containers by pressure washing and/or chemical sanitization. Many simply apply fungicides during the production cycle to prevent infections or to respond to occurrence of diseases. Thus our objectives were to: (1) determine the capacity of *P. ramorum* (NA genotype isolate 2027, A2 mating type; and European genotype isolate D12A, A1 mating type), compared to *P. cactorum*, *P. citricola*, and *P. citrophthora*, to survive in potting media components, and (2) determine the efficacy of heat from aerated steam mixtures to eradicate *P. ramorum* and other pathogens (as colonized vermiculite) from potting medium in containers.

Materials and methods

For objective (1), the soilless media components used were river sand, Douglas-fir (DF) bark, coir, sphagnum peat, redwood (RW) sawdust, a bark-peat-pumice potting mix (40:30:30 by volume), a dairy compost, and a garden soil. Inocula for the survival study were sporangia (*P. ramorum* only), infected dry rhododendron leaf pieces containing chlamydospores or oospores, or chlamydospore or oospore inoculum produced in culture on vermiculite. Survival of *Phytophthora* species was determined monthly by baiting (B) or direct plating (DP) on selective agar medium. For objective (2) we used heat from aerated steam mixtures to sanitize plastic containers filled with infested potting medium. Varied temperatures were established, from 4°C to 70 °C for 30 min at °C increments, by changing the air/steam ratio (1). The pathogens used were *Phytophthora ramorum*, *Pythium irregulare*, *Thielaviopsis basicola*, and *Cylindrocladium scoparium*. Pathogen mortality was determined by DP or B.

Results and discussion

P. ramorum was detected for 6 months by B or DP from all substrates amended with sporangia or chlamydospores in vermiculite, but was not detected by either B or DF

from infected leaf inoculum. *P. ramorum* sporangia survived best in peatmoss, potting mix, coir, and DF bark, and poorest in sand or soil. By comparison, *P. cactorum* was recovered after 5 to 6 months from infected leaf inoculum in all media. *P. citricola* was recovered by B for only 3 months in coir, potting mix, sand and soil, but not at all from compost, DF bark, peatmoss, or RW sawdust. *P. citrophthora*, as with *P. ramorum*, was never recovered from leaf inoculum in any material at any time. All pathogens were killed by aerated steam treatments of infested medium at 50º C or higher.

These results indicate (a) that *P. ramorum* can survive very well in potting mix components or soil as culture-produced sporangia or chlamydospores, but was not detected from infected leaf pieces compared to other *Phytophthora* species that were, and (b) that aerated steam pasteurization is an effective means of eradicating *P. ramorum* as well as other pathogens from infested media and contaminated containers without destroying the containers.

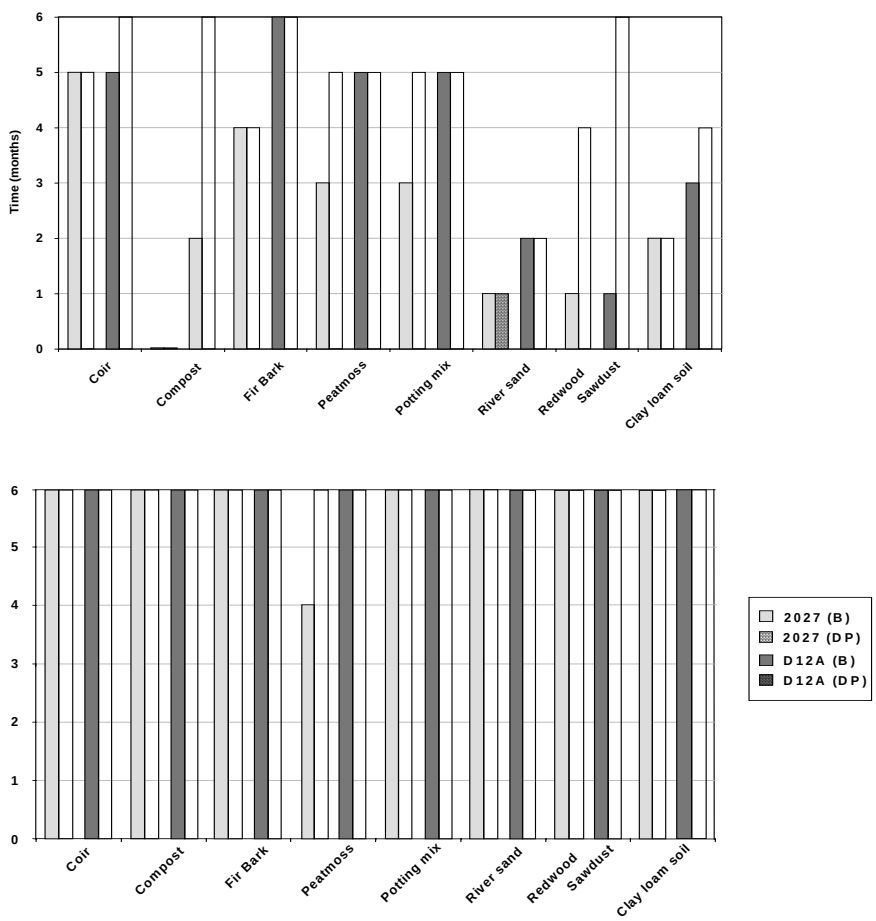

Figure 1—Survival of *Phytophthora ramorum* introduced into soilless media or soil as sporangia (top) or chlamydospores (bottom) in vermiculite.

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Ecology and Monitoring

Potential Effects of Sudden Oak Death on Birds in Coastal Oak Woodlands[1]

Donald E. Winslow[2,3] and William D. Tietje[2]

Abstract

Tree pathogens can affect community composition and structure over wide areas. *Phytophthora ramorum,* cause of sudden oak death (SOD), occurs in the wild in California from Humboldt County to southernmost Monterey County. *P. ramorum* has killed many trees at some sites and may spread to affect near and distant forests. The pathogen has not yet been detected in San Luis Obispo County outside of nurseries, but threatens coast live oak (*Quercus agrifolia*) woodlands there. SOD-induced changes in vegetation structure and tree community composition may cascade to affect vertebrate communities. From 2002-2004 we counted breeding birds and measured habitat characteristics at 78 points distributed among four sites in coastal oak woodlands at high risk from SOD in San Luis Obispo County. Each point was visited three times each year to conduct 10-minute counts of all adult birds detected within 50 m. In 2004 we surveyed trees within 10 m of each point. We found 13 tree species; 63.8 percent of the individuals recorded were coast live oak and 19.6 percent were California bay laurel (*Umbellularia californica*). We recorded 75 bird species at the census points. The most abundant species were Steller's jay (*Cyanocitta stelleri*, 8.9 percent of individuals), orange-crowned warbler (*Vermivora celata*, 8.2 percent), dark-eyed junco (*Junco hyemalis*, 7.4 percent), and spotted towhee (*Pipilo maculatus*, 6.8 percent). Avian species diversity (Shannon-Wiener index) showed no clear pattern of variation with basal area of oaks (*Quercus* spp) and tanoaks (*Lithocarpus densiflorus*) in 2004 (r_s = -0.10, $0.2 < p < 0.5$), but oak titmouse (*Baeolophus inornatus*) abundance was associated with oak/tanoak basal area (r_s = 0.40, $p < 0.001$). We used these data to evaluate six habitat association models for cavity-nesting birds. Using $QAIC_c$ as an information criterion, the most favorable model indicated that cavity-nesting birds select microsites with large-diameter trees (Akaike weight = 0.47). Changes in tree size class distribution resulting from SOD may lead to a slight decrease in cavity-nesting bird abundance over time. Effects on individual species may be more dramatic.

Key words: Quercus agrifolia, avian community structure, cavity-nesting birds, California Central Coast, disturbance, community dynamics

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Introduction

Tree pathogens can act as keystone species, altering forest community composition and structure over wide areas (Hudler 1998). These changes may in turn affect faunal communities. Dutch elm disease (*Opiostoma ulmi*), for instance, has impacted avian populations in Europe and North America (Canterbury 1997, Osborne 1985, Osborne 1983, Osborne 1982). Haney and coworkers (2001) implicated chestnut blight (*Cryphonectria parasitica*) as one factor influencing historical changes in a southern Appalachian breeding bird community.

Sudden oak death (SOD), caused by the oomycete pathogen *Phytophthora ramorum*, causes high levels of adult tree infection and mortality (Brown and Allen-Diaz, this volume; Frankel and others, this volume; McPherson and others 2002, Garbelotto and others 2001). Although infection rates are generally higher for tanoak (*Lithocarpus densiflorus*) than for coast live oak (*Quercus agrifolia*) in the same stands (Swiecki and Bernhardt, this volume; McPherson and others 2002, Kelly and McPherson 2001), coast live oak mortality exceeds 50 percent of standing basal area at some locations in China Camp State Park in Marin County (Brown and Allen-Diaz, this volume).

P. ramorum has the potential to spread to near and distant regions and may dramatically affect vegetation structure in multiple forest communities (Cushman and Meentemeyer, this volume; Kelly and others, this volume; Oak and others, this volume; Meentemeyer and others 2004). SOD has not yet been detected in the wild in San Luis Obispo County, but threatens coast live oak woodlands there (Meentemeyer and others 2004). Coast live oak forests are highly susceptible to sudden oak death, in part because of high moisture levels in coastal areas. Rain is considered an important dispersal mechanism for *P. ramorum* spores (Davidson and others 2002). Also, California bay laurel (*Umbellularia californica*), which serves as a source of *P. ramorum* inoculum (Davidson and others 2002), is often an important overstory component of woodlands dominated by coast live oak. For these reasons, coastal forests in San Luis Obispo County may be at high risk of invasion by *P. ramorum*.

Over the short term, the death of a large proportion of the trees in a stand decreases canopy cover and may thus lead to an increase in shrub cover. As regeneration progresses, we may expect future stands to exhibit greater relative abundances of less susceptible tree species. California bay laurel, for instance, develops foliar lesions but does not suffer serious effects from *P. ramorum* (Davidson and others 2003) and thus may be more highly represented in future stands that have suffered high oak mortality.

Changes in vegetation structure and tree community composition resulting from sudden oak death may cascade to affect vertebrate communities and other ecosystem attributes (CalPIF 2002). The physical structure of vegetation is an important determinant of avian community composition; i.e., the species present and their relative abundances (e.g., Bub and others 2004, Urban and Smith 1989, Collins and others 1982, Anderson 1981).

The avifauna of coast live oak forests is relatively unstudied, although many of the breeding bird species in this system have been studied in other oak forest types (CalPIF 2002, Wilson and others 1991, Block 1989, Verner 1980). California oak woodlands are characterized by a high diversity of bird species (CalPIF 2002, Tietje and Vreeland 1997), many of which use acorns and cavities provided by mature oaks and tanoaks (Koenig 1990, Tietje 1990, Wilson and others 1991, Verner 1980). We would expect these vulnerable bird species, such as Acorn Woodpecker (*Melanerpes formicivorus*) and Oak Titmouse (*Baeolophus inornatus*), to become less abundant where oaks have declined in dominance (CalPIF 2002). Changes in canopy and shrub cover resulting from *P. ramorum* infestation would also be expected to change avian community composition. Dark-eyed Juncos (*Junco hyemalis*), for instance, are more abundant at sites with higher canopy cover (Tietje and Vreeland 1997, Tietje and others 1997). We would expect this species to be less abundant in an oak woodland infected with sudden oak death than at a comparable healthy site.

The objectives of this contribution are to (1) characterize the habitat structure and avifauna of coast live oak woodland in San Luis Obispo County, (2) describe how habitat structure affects the abundance of cavity-nesting birds, and (3) predict the potential effects of sudden oak death on cavity-nesting bird communities in high-risk coast live oak forests.

Study Area

The coastal oak woodland landscape in San Luis Obispo County is a mountainous mosaic of woodland, pasture, chapparal, and ecotonal habitats, characterized by a high density of abrupt edges between habitat types (Vreeland and Tietje 2004). The overstory is dominated by coast live oak, with significant components of California bay laurel, Pacific madrone (*Arbutus menziesii*), and tanoak. Toyon (*Heteromeles arbutifolia*) is a common understory tree. The shrub layer is quite dense in places, and features such species as poison oak (*Toxicodendron diversiloba*), California blackberry (*Rubus ursinus*), and creeping snowberry (*Gaultheria hispidula*). Herbaceous ground flora include Western brackenfern (*Pteridium aquilinum*), California polypody (*Polypodium californicum*), fiesta flower (*Pholistoma* spp.), and

miner's lettuce (*Claytonia perfoliata*). Temperatures are moderated by the proximity of the ocean (Pavlik and others 1991), and do not reach the extreme highs and lows of more interior oak woodlands (Tempel and others, this volume). We examined climate data from National Oceanic and Atmospheric Administration weather stations (University of California 2005) from 1994 to 2004 and found that annual rainfall ranged from 276-939 mm at Morro Bay and 384-1052 mm at San Luis Obispo. Most of this precipitation falls during the winter months (November through April; Tempel and others, this volume). Winter rainfall is supplemented by fog interception (Pavlik and others 1991).

We surveyed breeding birds and measured habitat characteristics at four coastal oak woodland sites in San Luis Obispo County on the Central Coast of California, USA (*fig*. 1). The four sites include multiple ownerships and experience varied land uses. San Simeon Creek consists of two private residences and a portion of one private cattle ranch. Atascadero Ranches includes portions of two private cattle ranches. Camp San Luis Obispo consists of two areas within a National Guard base (Camp San Luis Obispo), used infrequently for training exercises and cattle grazing. Irish Hills consists of a rugged nature preserve owned by The Nature Conservancy, mostly inaccessible to human use.

Methods

Field methods

We conducted 10-minute variable-radius counts (Ralph and others 1995) of breeding birds at 78 points distributed among the four sites from 2002-2004. This sample size is adequate for most purposes (Ralph and others 1995). Because our analyses involved predictors that vary among points and we modeled the abundance of a nesting guild (rather than individual species), we had plenty of statistical power to detect relationships. Count stations were located in coast live oak woodland and were ≥ 250 m apart. We visited each point at least three times from March to June of each year to conduct 10-minute variable-radius counts. One to three observers conducted counts in any given year. We rotated station visitations to balance observers, time of season, and time of day. All counts took place between sunrise and 1200 PDT. We recorded the species, sex (when known), and vocal behavior of each adult bird detected within the timed counts and estimated the distance of each bird from the observer. We censused 30 points at one site in 2002, 23 points at two sites in 2003, and 78 points at four sites in 2004.

We measured habitat characteristics within 25 m of each count point in 2004. We measured the diameter at breast height of each woody stem ≥10 cm within 10 m of

each count point. We recorded the species and status (dead or alive) of each stem and the number of cavities suitable for bird nesting on each one. A cavity was judged to be suitable for bird nesting if the opening was wide enough for an adult bird to enter. The four observers examined some stems together to reduce the subjectivity of these judgments.

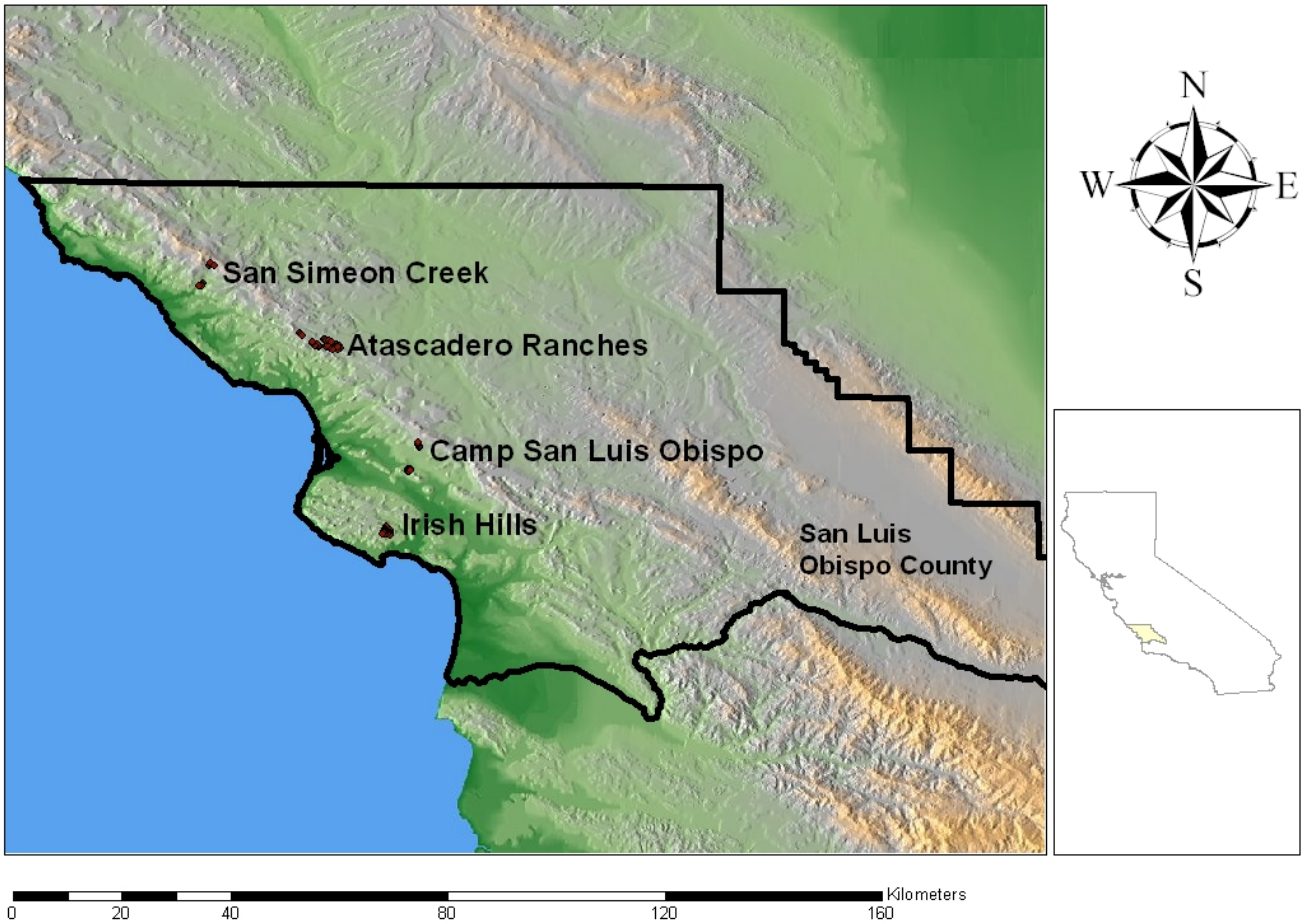

Figure 1—Clouds of bird survey points at four sites in San Luis Obispo County.

We estimated canopy cover with densiometers at count points and 25 m in each cardinal direction. We measured shrub cover with a cover pole placed 25 m in each cardinal direction from each point. The pole was divided into five 0.5-m sections to measure visual obstruction from 0 to 2.5 m above the ground. Each section was subdivided into five 10-cm bands (alternating black and white). The observer stood 10 m from the pole in the direction of the point and recorded the number of bands in each section at least 25 percent obstructed by shrubs or other objects.

Statistical analysis

For each point where we surveyed birds in 2002 and 2003, we chose three visits in each year to correspond to the three visits in 2004. We matched these visits so that the dates sampled were similar among the three years. We determined the maximum number (among three visits) of individuals of each bird species detected within 50 m of each count point during the 10-minute surveys to obtain abundance indices for each species for each year. Using only data from 2004, we tallied the number of species detected at each point as an index of species richness and used the abundance estimates to calculate species diversity using the Shannon-Wiener index (Ricklefs 1979).

The distribution of cavity abundance among stems was not close to normal. Most stems had no cavities, but some had over 20. Rather than make distributional assumptions, we used a Spearman rank correlation test (SPSS 2003) to examine the association between tree diameter and number of cavities. We compared cavity counts on coast live oaks and bay laurels using a Mann-Whitney *U* test (SPSS 2003).

Using the tree survey data we calculated six habitat measures related to stand structure for each point station: total basal area, basal area of oaks and tanoaks, density of oak and tanoak stems, mean diameter at breast height, snag density, and cavity density. Avian species richness, as a count variable, would not be expected to be normally distributed. The Shannon-Wiener index can never be positive, so an assumption of normality does not seem appropriate for this index. Rather than make distributional assumptions, we used Spearman rank correlation tests (Zar 1996) to determine if avian species richness or diversity were related to the basal area of oaks and tanoaks in 2004.

We estimated canopy cover for each count area by averaging the five cover values measured in each plot. For shrub cover we calculated the percentage of bands at least 25 percent obstructed in the top four sections of the pole, because the bottom section was often obstructed by the ground or leaf litter. We then averaged the four shrub cover values for each plot.

For this contribution, we decided to model the abundance of birds in the cavity-nesting guild. By modeling an entire guild of species, rather than individual species, we hoped to uncover relationships useful for the conservation of multiple species (Block and others 1986). We calculated abundance of cavity-nesting birds by summing abundance indices for each species at each point. We reviewed species accounts in the California Wildlife Habitat Relationships (California Department of Fish and Game 2002) database in order to select appropriate habitat measures to include in candidate models. We developed a set of *a priori* poisson regression

models with cavity-nester abundance as the dependent variable, one or more habitat measures as predictor variables, temporal measures as "nuisance" predictors, and study site as a random effect. We considered observations from separate years to be repeated measures at the same points. We fitted these models with the GLIMMIX macro in SAS Proc Mixed (Littell and others 1996). We evaluated competing models for explanatory power and parsimony using a quasi-likelihood-based version of Akaike's Information Criterion ($QAIC_c$, Burnham and Anderson 2002).

We evaluated six competing models of cavity-nester abundance, using a poisson error structure with an overdispersion factor and site as a random effect. Predictor variables included five temporal nuisance parameters (year, earliest date of three visits, latest date of three visits, earliest start time of three visits, and latest start time of three visits) and the habitat measures relevant to each hypothesis. The candidate models included a cavity-availability limitation hypothesis (with cavity density as a habitat predictor), a snag-availability limitation model (with snag density), an acorn-availability limitation model (with oak/tanoak basal area), a multiple-limiting-factor hypothesis (with cavity density, snag density, and oak/tanoak basal area), a habitat selection hypothesis assuming a preference for oaks (with oak/tanoak basal area and density of oak/tanoak stems), and a habitat selection hypothesis assuming a preference for large-diameter trees (with mean diameter and total basal area).

We fitted the global model (including all considered nuisance and habitat measures) with various covariance structures and used $QAIC_c$ to select the most appropriate one. The most favorable covariance structure was first-order autoregressive, so we used this structure with all subsequently fitted models. We then fitted a set of models that included all the habitat predictors, but varied by which nuisance parameters were included and whether site was included as a random effect. We compared all combinations in this model set and found the most favorable model to be the one including only year, latest date, and latest time (without site as a random effect).

We used available information on effects of sudden oak death on coastal oak woodland stand structure (Swiecki and Bernhardt, this volume; 2004; 2003) to simulate expected changes in an infected stand over 40 years. We used this simulation of habitat change to examine how cavity-nesting bird abundance might change under the selected habitat association model.

Results

Vegetation Attributes

We found 13 tree species within 10 m of the count points; 42.2 percent of the stems recorded were coast live oak (*table 1*). The next most abundant species was California bay laurel, comprising 19.6 percent of the stems. Cavities were most abundant on old oak trees (*table 1*, *fig. 2*). Across species, cavity count increased with increasing diameter (r_s = 0.415, p < 0.0005, N = 1226, *fig. 2*). Coast live oak stems contained nearly four times as many cavities as California bay (*table 1*, *fig.* 2, Mann-Whitney U = 4794.5, p < 0.0005). Of 454 cavities on 1127 stems, only 11 were excavated by birds. Most cavities resulted from the scars of fallen limbs or decomposition of dead wood. Descriptive statistics for habitat measures are presented in *table 2*.

Bird Community Structure

We recorded 84 bird species at census points from 2002-2004, including 18 cavity-nesters, 15 species that use acorns, and five California endemics (CalPIF 2002). The overall 2004 Shannon-Wiener index was 3.5 (71 species, 1564 individuals). Of 75 species recorded within 50 m during the three years, the four most abundant species were all open-nesters: Steller's jay (*Cyanocitta stelleri*), orange-crowned warbler (*Vermivora celata*), dark-eyed junco (*Junco hyemalis*), and spotted towhee (*Pipilo maculatus*, *table 3*). Sixteen of these 75 species nest primarily in cavities.

The basal area of oaks and tanoaks showed no clear relationship to species richness (r_s = -0.08, 0.2 < p < 0.5) or diversity (r_s = -0.10, 0.2 < p < 0.5) in 2004 (*fig. 3*). Counts of oak titmice were positively correlated with the basal area of oaks and tanoaks in 2004 (r_s = 0.40, p < 0.001, *fig. 4*).

Table 1—*Tree stems >10 cm diameter at breast height found in 78 plots of 10-m radius, centered at bird census points.*

Species	Stems	Total cavities	Mean cavities/stem	Minimum # of cavities	Maximum # of cavities
California bay laurel (*Umbellaria californica*)	82	48	0.59	0	8
Big-leafed Maple (*Acer macrophyllum*)	11	3	0.27	0	1
California black oak (*Quercus kelloggii*)	1	2	2.00	2	2
Ceanothus (*Ceanothus* spp.)[1]	18	0	0.00	0	0
Coast live oak (*Quercus agrifolia*)	177	398	2.25	0	21
Elderberry (*Sambucus nigra* ssp. *caerulea)*	1	0	0.00	0	0
Gray pine (*Pinus sabiniana*)	1	0	0.00	0	0
Pacific madrone (*Arbutus menziesii*)	57	58	1.02	0	6
Tanoak (*Lithocarpus densiflorus*)	31	0	0.00	0	0
Toyon (*Heteromeles arbutifolia*)	26	2	0.08	0	2
Valley oak (*Quercus lobata*)	8	10	1.25	0	6
Willow (*Salix* spp.)	6	0	0.00	0	0
Total	419	521	1.24	0	21

[1]We found at least two species in this genus, but not all individuals were identified to species.

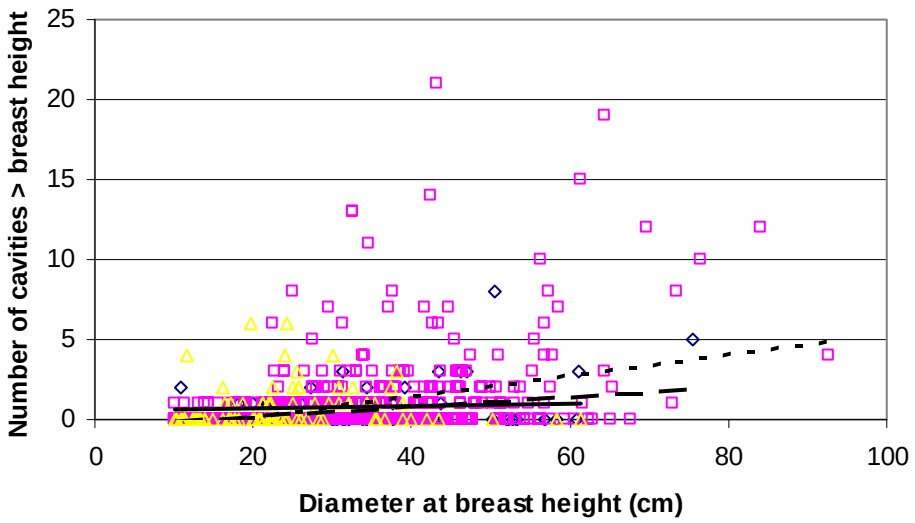

Figure 2—Relationship between diameter and the number of cavities for three most common tree species: coast live oak (squares), California bay laurel (diamonds), and Pacific madrone (triangles). Linear regressions of cavity count on diameter are indicated for oak (dotted line), bay (dashed line), and madrone (solid line).

Table 2—*Descriptive statistics for habitat measures at 78 plots of 10 m radius, centered at bird census points.*

Habitat Measure	Mean	Standard Error	Minimum[1]	Maximum
Mean diameter at breast height (cm)	26.42	0.89	0[2]	55.50
Cavities per ha	2652.58	497.53	0	24191.55
Oak/tanoak stems per ha	332.59	28.12	0	1209.58
Oak/tanoak basal area (m^2/ha)	24.55	1.79	0	66.45
Total basal area (m^2/ha)	32.22	1.90	0	84.08
Snags per ha	37.14	5.52	0	190.99
% Canopy cover	81.63	1.68	23	98
% Shrub cover	54.63	2.32	7.50	96.25

[1]One plot contained no stems > 10 cm; thus minimum values for tree-stem variables are equal to zero.
[2]The value for the plot with no stems > 10 cm would technically be undefined, but we set this datum equal to zero to reflect that this plot contained only small trees.

Habitat relationships of cavity-nesting birds and potential effects of SOD

We fitted one model for each of the competing hypotheses of cavity-nester abundance (see Methods), only including the selected nuisance variables. The most favorable model was the large-diameter tree hypothesis (Akaike weight = 0.47, *table 4*). The large-diameter tree model fit the data adequately with the estimated overdispersion parameter (scaled deviance = 118.5, $df = 130$, $0.5 < p < 0.75$), but the range of observed values was substantially larger than the range of predicted values (*fig. 5*).

The habitat variables included in the large-diameter tree model include total basal area and mean diameter of all species. Sudden oak death would be expected to cause values of both of these habitat measures to decline (for information on the effect of diameter on susceptibility to sudden oak death see Swiecki and Bernhardt 2003, 2002a, 2002b). The parameter estimate for the effect of mean diameter on cavity-nester abundance was significantly greater than 0 (0.021, SE = 0.0082, $t = 2.54$, $df = 75$, $p = 0.0132$), but the parameter estimate for the effect of basal area was not significantly different from 0 (-0.0011, SE = 0.00040, $t = -0.29$, $p = 0.7746$, *table 5*).

Table 3—*Bird species detected within 50 m of 78 survey points during timed counts in 2002-2004. Only the 40 most abundant species are listed.*

Species	Nesting Guild[1]	Birds per point[2]	Birds per ha (SE)[3]
Steller's Jay (*Cyanocitta stelleri*)	canopy	1.69	2.16 (0.15)
Orange-crowned Warbler (*Vermivora celata*)	ground	1.56	1.99 (0.13)
Spotted Towhee (*Pipilo maculatus*)	ground	1.31	1.66 (0.11)
Dark-eyed Junco (*Junco hyemalis*)	ground	1.42	1.81 (0.11)
Chestnut-backed Chickadee (*Poecile rufescens*)	cavity	1.22	1.56 (0.18)
Pacific-slope Flycatcher (*Empidonax difficilis*)	ground	0.98	1.25 (0.09)
Hutton's Vireo (*Vireo huttoni*)	canopy	0.89	1.13 (0.07)
Warbling Vireo (*Vireo gilvus*)	canopy	0.80	1.02 (0.12)
Oak Titmouse (*Baeolophus inornatus*)	cavity	0.56	0.72 (0.10)
Bushtit (*Psaltriparus minimus*)	canopy	0.50	0.64 (0.12)
House Wren (*Troglodytes aedon*)	cavity	0.54	0.69 (0.09)
Black-headed Grosbeak (*Pheucticus melanocephalus*)	canopy	0.50	0.63 (0.09)
Anna's Hummingbird (*Calypte anna*)	canopy	0.37	0.48 (0.08)
Hairy Woodpecker (*Picoides villosus*)	cavity	0.33	0.42 (0.06)
Northern Flicker (*Colaptes auratus*)	cavity	0.29	0.37 (0.06)
Western Scrub-jay (*Aphelocoma californica*)	canopy	0.33	0.42 (0.07)
Brown Creeper (*Certhia americana*)	under bark	0.44	0.56 (0.06)
Bewick's Wren (*Thryomanes bewickii*)	cavity	0.37	0.48 (0.07)
Wrentit (*Chamaea fasciata*)	shrub	0.37	0.48 (0.07)
Purple Finch (*Carpodacus purpureus*)	canopy	0.42	0.53 (0.06)
Lesser Goldfinch (*Carduelis psaltria*)	canopy	0.38	0.49 (0.07)
Turkey Vulture (*Cathartes aura*)	cliff	0.11	0.15 (0.04)
California Quail (*Callipepla californica*)	ground	0.15	0.18 (0.05)
Mourning Dove (*Zenaida macroura*)	canopy	0.19	0.24 (0.05)
Band-tailed Pigeon (*Columba fasciata*)	canopy	0.11	0.15 (0.04)
Acorn Woodpecker (*Melanerpes formicivorus*)	cavity	0.12	0.16 (0.04)
Nuttall's Woodpecker (*Picoides nuttallii*)	cavity	0.25	0.32 (0.05)
Cassin's Vireo (*Vireo cassinii*)	canopy	0.14	0.17 (0.05)
American Crow (*Corvus brachyrhynchos*)	canopy	0.15	0.18 (0.05)
Violet-green Swallow (*Tachycineta thalassina*)	cavity	0.15	0.19 (0.05)
Ruby-crowned Kinglet (*Regulus calendula*)	canopy	0.14	0.17 (0.04)
American Robin (*Turdus migratorius*)	canopy	0.27	0.35 (0.06)
Townsend's Warbler (*Dendroica townsendi*)	canopy	0.24	0.30 (0.06)
Wilson's Warbler (*Wilsonia pusilla*)	ground	0.26	0.33 (0.05)
Western Tanager (*Piranga ludoviciana*)	canopy	0.11	0.15 (0.04)
California Towhee (*Pipilo crissalis*)	shrub	0.17	0.21 (0.04)
Brewer's Blackbird (*Euphagus cyanocephalus*)	canopy	0.11	0.14 (0.09)
American Goldfinch (*Carduelis tristis*)	shrub	0.10	0.13 (0.04)
Ash-throated Flycatcher (*Myiarchus cinerascens*)	cavity	0.08	0.11 (0.03)
Swainson's Thrush (*Catharus ustulatus*)	shrub	0.08	0.11 (0.04)

[1] Most frequent nest location for each species (Ehrlich and others 1988, Small 1994, personal observation).
[2] Sample size = 131 (30 points censused in 2002, 23 in 2003, and 78 in 2004).
[3] Estimated by dividing birds/plot by area within 50 m of plot center. These must be considered minimum estimates of density, because some individuals remain undetected (Buckland and others 1993).

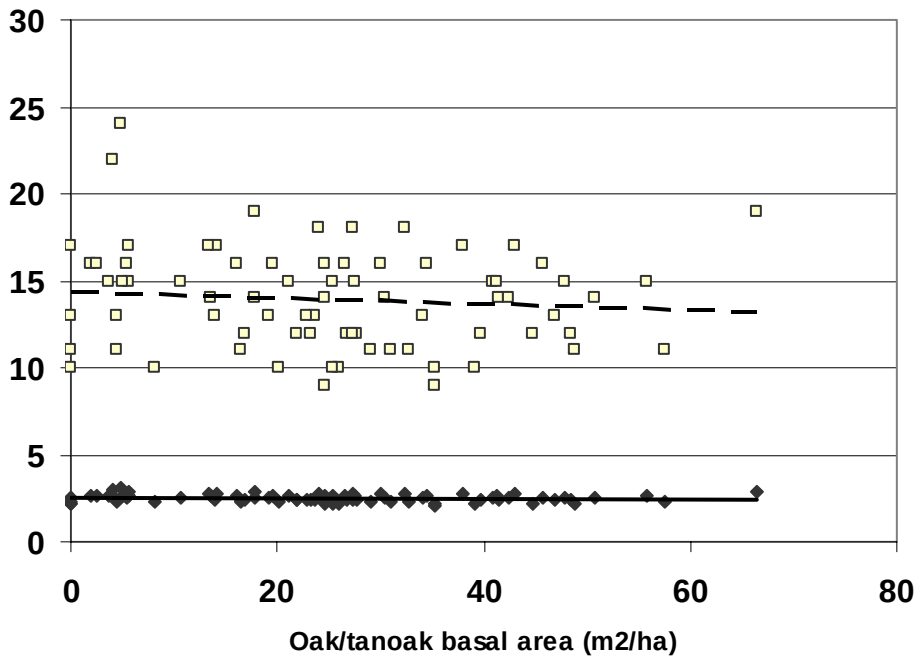

Figure 3—Avian species richness (clear squares) and species diversity (Shannon-Wiener index, solid diamonds) in 2004 at 78 bird survey points varying in the basal area of oaks and tanoaks. Linear regressions of richness (dashed line) and diversity (solid line) on basal area are indicated.

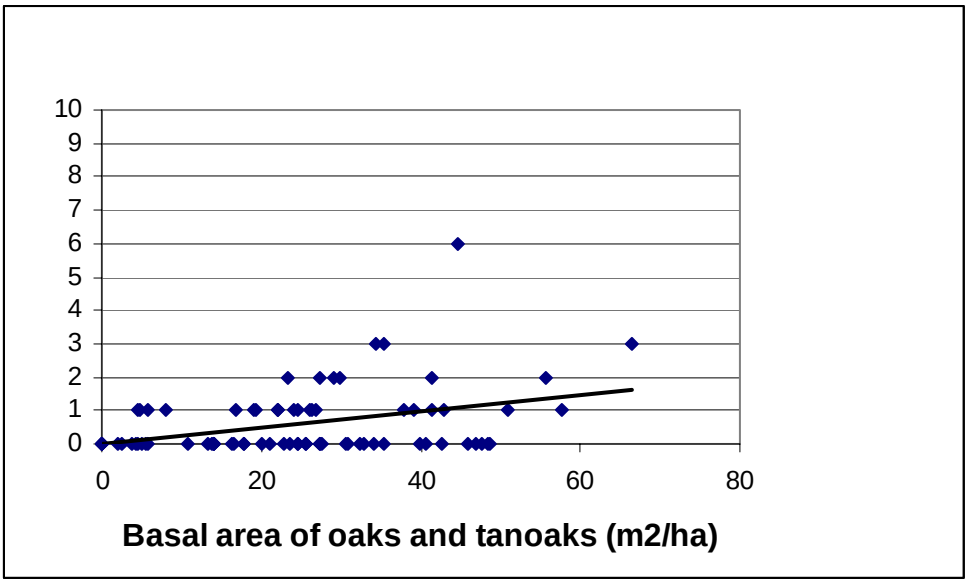

Figure 4—Abundance of Oak Titmice (individuals per survey point) in 2004 at 78 bird survey points varying in the basal area of oaks and tanoaks. Linear regression of titmouse abundance on basal area is indicated.

Table 4—*Model selection statistics for cavity-nesting bird abundance. The most favorable model (boldface font) indicates a habitat preference for stands with large-diameter trees.*

Model	Scaled Deviance[1]	*df*	*p*	Over-dispersion	-2log*L*	Parameters	$QAIC_c$[2]	Δ*i*	w_i
Cavity limitation	116.7	125	0.5<*p*<0.75	1.5739	244.8	8	184.2	3.0	0.105
Snag limitation	117.0	125	0.5<*p*<0.75	1.5980	245.1	8	184.4	3.2	0.095
Acorn limitation	116.7	125	0.5<*p*<0.75	1.5547	243.1	8	183.1	1.8	0.188
Multiple limiting factors	116.1	123	0.5<*p*<0.75	1.5526	243.4	10	187.9	6.7	0.017
Large tree selection	**118.5**	**124**	**0.5<*p*<0.75**	**1.4759**	**237.1**	**9**	**181.2**	**0.0**	**0.471**
Oak selection	116.9	124	0.5<*p*<0.75	1.5239	241.0	9	183.9	2.7	0.121

[1] Deviance (scaled by overdispersion factor) is used as a lack-of-fit statistic with df degrees of freedom; p is the probability of a fit as bad or worse as the observed deviance if the model is correct (Littell and others 1996).

[2] *QAICc* is calculated from -2*log-likelihood, the number of parameters in the model, and the sample size (131). The model with the lowest value of *QAICc* is considered the most favorable model (Burnham and Anderson 2002). The *QAICc* difference (Δi) for the most favorable model is by definition 0, and that model has the highest Akaike weight (wi).

We carried out a crude simulation of change in stand structure and cavity-nester abundance over 39 years in a SOD-affected coast live oak woodland, using vegetation parameters estimated from information published by Phytosphere Research (Swiecki and Bernhardt, this volume; 2004; 2003) and from our own habitat measurements (*table 6*).

Because the trees studied by Swiecki and Bernhardt (this volume, 2004, 2003) were greater in diameter than the mean diameter in our study plots, we estimated initial stem density at the Phytosphere plots by applying a regression equation of stem density on mean diameter from 77 of our study plots (stem density = 807.065 stems/ha – 11.217*diameter in cm, t = -2.95, p = 0.004, adjusted r^2 = 0.092, SPSS 2003) to the mean diameter reported by Swiecki and Bernhardt (2003). Every three years of the simulation we reduced the stem density based on the infection and failure parameters in *table* 6. We assumed the mean diameter of failed stems to be 48.5 cm, as reported by Swiecki and Bernhardt (2003), and used this value to calculate the mean diameter of remaining stems after each three-year increment. We assumed that recruitment exactly compensated for non-SOD mortality. We estimated basal area using the simplifying assumption that all trees were equal in diameter. After 39 years, 55.7 percent of the stems had failed, the basal area had declined from 47.2 to 14.8 m^2/ha, and the mean diameter was 36.2 cm. Using these values of basal area and

diameter in the large-diameter model of cavity-nester abundance, we estimated a decline in cavity-nester abundance from 7.1 to 6.4 adults per ha over 39 years (*fig. 6*).

Table 5—*Model statistics for large-diameter tree model of cavity-nesting bird abundance (individuals/point).*

Effect	Numerator *df*	Denominator *df*	*F*	*p*	Parameter Estimate	SE	*t*
Intercept		75		0.0065	1.247	0.445	2.8
Year	2	49	6.49	0.0032			
2002		49		0.0701	-0.377	0.203	-1.85
2003		49		0.0048	0.370	0.125	2.95
2004					0.000		
Latedate[1]	1	49	3.40	0.0712	-0.011	0.006	-1.84
Latetime[2]	1	49	2.06	0.1571	0.085	0.059	1.44
Meandbh[3]	1	75	6.44	0.0132	0.021	0.008	2.54
Totalba[4]	1	75	0.08	0.7746	-0.001	0.004	-0.29

[1]Latedate is the latest date of three visits in days after Vernal Equinox.
[2]Latetime is the latest start time of three visits in hours after sunrise.
[3]Meandbh is the mean diameter at breast height in cm of trees > 10 cm within 10 m of each survey point.
[4]Totalba is the total basal area in m^2/ha of trees of all species > 10 cm diameter within 10 m of each survey point.

Table 6—*Parameter values used in simulation of change in stand structure and cavity-nester abundance in response to sudden oak death.*

Parameter	Symbol	Value	Units	Source
Initial proportion infected	ι	0.224	Individual^{-1}	Swiecki and Bernhardt (2004)
Rate of new infections	ν	0.015	$\text{Individual}^{-1}*3\ \text{years}^{-1}$	Swiecki and Bernhardt (2004)
Probability of failure with infection	φ	0.318	Individual^{-1}	Swiecki and Bernhardt (2004)
Proportion of failures that are bole failures	β	0.58	Individual^{-1}	Swiecki and Bernhardt (2004)
Bole failure rate	ρ	0.0028	$\text{Individual}^{-1}*3\ \text{years}^{-1}$	$\nu*\varphi*\beta$
Mean diameter of failed stems	μ	48.5	cm	Swiecki and Bernhardt (2003)
Mean diameter of non-failed stems	o	42.8	cm	Swiecki and Bernhardt (2003)
Initial mean diameter	δ	43.1	cm	$\rho*\mu + (1 - \rho)*o$
Initial stem density	σ	324.1	stems/ha	See text.

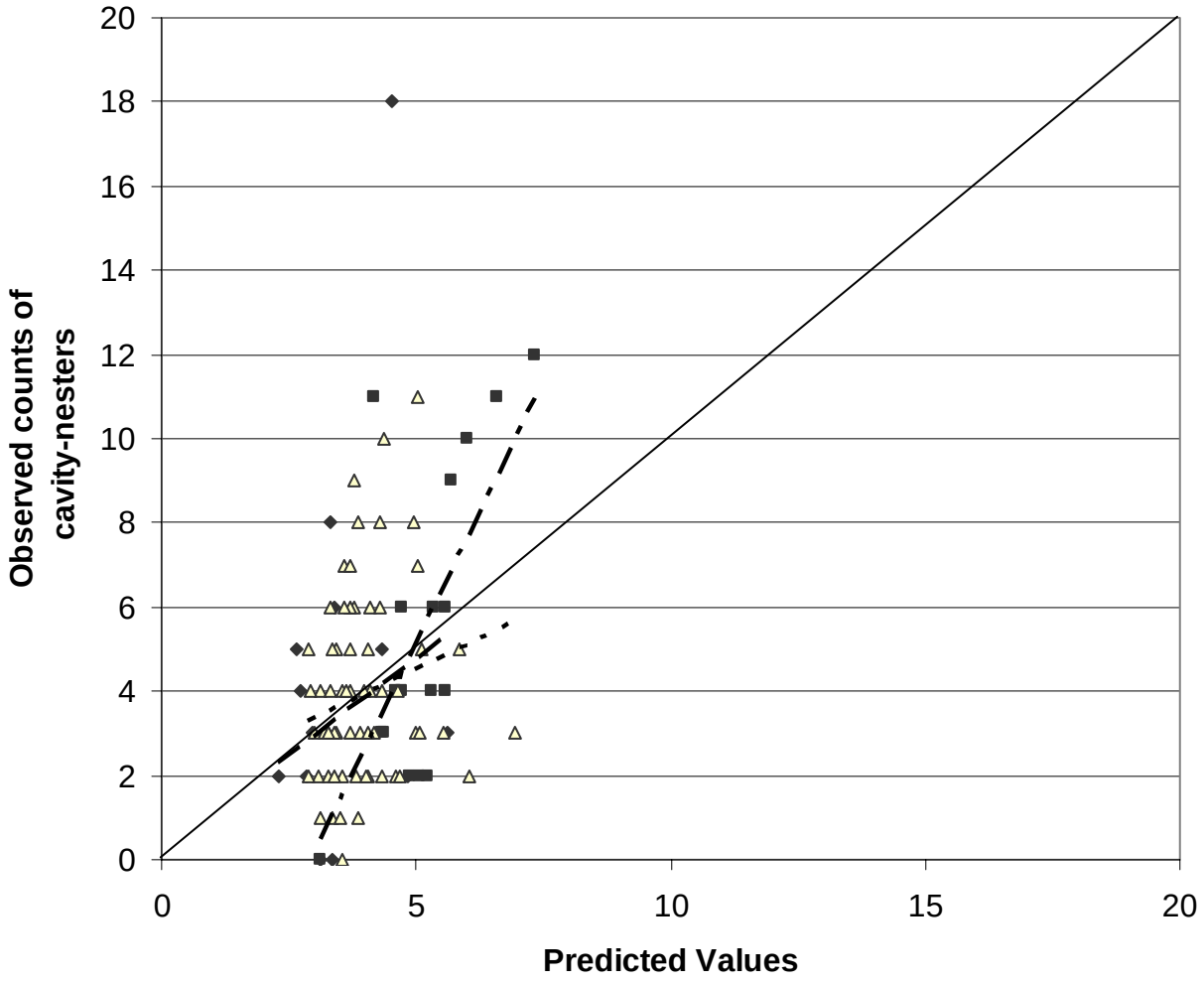

Figure 5—Observed abundance of cavity-nesting birds from 2002-2004 at 78 bird survey points plotted versus values predicted from the large-diameter tree habitat preference model. Points from 2002 are indicated by diamonds, from 2003 by squares, and from 2004 by triangles. Solid line indicates hypothetical perfect prediction of observed values. Linear regressions of observed on predicted values are indicated (dashed line, 2002; alternating dots and dashes, 2003; dotted line, 2004).

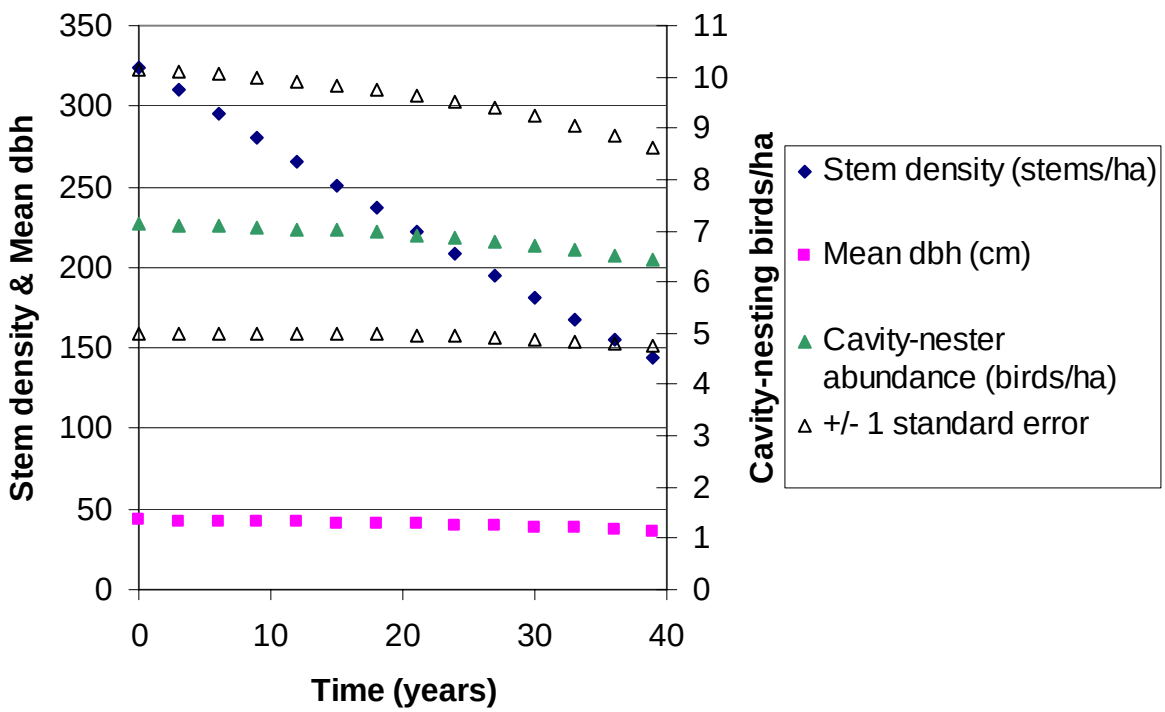

Figure 6—Simulation of change in stand structure and cavity-nester abundance over 39 years in coast live oak woodland infected with sudden oak death. Minimal standard errors of cavity-nester abundance were estimated by substituting values of mean-dbh coefficient adjusted by +/- 1 SE (*table 5*). Error estimates would be greater if SE's of other parameter estimates were considered.

Discussion

Although researchers have devoted considerable effort toward modeling habitat relations of birds in California oak woodlands (e.g., Wilson and others 1991, Block 1989, Dedon and others 1986), woodlands dominated by coast live oak deserve more attention. Bird species often vary geographically in habitat relations (Block and Morrison 1991), and avian communities vary in the relative abundances of species in common. For instance, we found that the most abundant bird species were open-nesting birds, while blue oak (*Quercus douglasii*) woodlands are often dominated by cavity-nesters (Wilson and others 1991). Tempel and others (this volume) found the most abundant breeding bird species in blue oak woodlands in San Luis Obispo

County to be the cavity-nesting Oak Titmouse, although open-nesters were well-represented among the most common species.

The high diversity of breeding birds we observed in coast live oak woodlands demonstrates the rich biota characteristic of this habitat. In contrast, Wilson and others (1991) found 51 breeding bird species in blue oak woodland near Hopland, California. Tempel and others (this volume), however, found higher avian species diversity (Shannon-Wiener index) and richness in blue oak woodlands at Camp Roberts than at our coastal oak woodland sites.

Of the models we examined, the most favorable in terms of fit and parsimony indicated that cavity-nesters prefer stands with large trees. The limiting-factor models were inferior to this habitat selection model when all three years of avian census data were considered. Model selection on 2004 data alone, however, indicated that limiting factors such as cavity and acorn availability may be important. Although secondary cavity-nesting birds are often assumed to be limited by nest-site availability, this is not always true in California oak woodland habitats (Waters and others 1990). Excavating species, such as woodpeckers, might be expected to be limited by the availability of snags. Snag availability was not an important factor in determining cavity-nester abundance in any of the models we examined. Because large living oaks tend to have many cavities (*table 1, fig. 2*) and dead branches suitable for excavation (Garrison and others 2002), snag availability is not likely to be limiting for cavity-nesting birds in California oak woodlands. Acorn woodpeckers along the Pacific coast may be limited by acorn abundance and diversity (Koenig and Haydock 1999), but it seems unlikely that an entire nesting guild would be limited by this food resource.

In highly mobile organisms such as birds, we might expect local abundances to be determined by habitat preference rather than by limiting resources. It is important to remember that preferred habitats are not necessarily optimal habitats in terms of individual fitness or population viability (Battin 2004, Purcell and Verner 1998). Modeling relationships between abundance of animals and habitat characteristics is only the first step in understanding how populations may change with habitat alteration. Information on breeding success, mortality, and dispersal is necessary for demographic modeling.

The simulation of change in stand characteristics with *P. ramorum* infection indicated that cavity-nester abundance might only experience slight declines even with dramatic overstory mortality. This is not a surprising result, given that cavity-nesting birds are common in open oak woodlands. There are reasons to be skeptical of these results, however. The selected model of cavity-nester abundance has not

been validated on other data sets, and thus we cannot know if it is generalizable to coastal oak woodland birds at other sites (Morrison and others 1992). It might not even be generalizable to the sites we studied, considering that we observed variation in apparent habitat relations among years. Furthermore, we have not yet conducted sensitivity analyses, and simulation results are likely to be sensitive to initial parameters. Values and variance of parameters can be refined as specific information on the effects of sudden oak death on vegetation structure becomes available (Brown and Allen-Diaz, this volume).

How can we build better models? We fitted a *post hoc* model with observer history as an additional nuisance variable. The results suggested that the marked overdispersion in 2002 was due to variation among observers, but the additional parameters made this model less favorable by $QAIC_c$ than the previously selected model. Because bird species vary in habitat relations, the total abundance of cavity-nesters averages over counts of individual species that may increase or decrease with habitat predictors. For instance, secondary cavity nesters might be influenced by cavity availability, but excavators would not be dependent on this resource. We anticipate that tighter fits will be obtained when we model the abundances of individual species, given that species vary in habitat associations. This is suggested by the observation that counts of oak titmice were positively correlated with the basal area of oaks and tanoaks, but avian species richness and diversity (community attributes) were not related to oak/tanoak basal area. The simulation of change in vegetation structure in SOD-affected woodland could be much improved with species- and size-class-specific data on infection and mortality rates of trees from affected stands.

If sudden oak death becomes established in San Luis Obispo County, it could alter much of the remaining coast live oak woodland. As trees die and fall, the canopy would become more open, woody debris would accumulate on the ground, and increased light levels may result in dense shrub cover. We may expect that sudden oak death would lead to an increase in the representation of bay laurel in affected stands. This change in tree species composition might be detrimental to birds for several reasons. We found lower numbers of cavities on bay trees than on coast live oaks, so cavity-nesting birds may find fewer nest sites in SOD-affected stands. We might also expect bird species that use acorns to decline in number as the oaks become less dominant. In addition, Apigian and Allen-Diaz (this volume) found that oak titmice preferentially forage in the canopies of coast live oaks. Titmice and chestnut-backed chickadees (*Poecile rufescens*) avoid bays, which harbor fewer insect prey items.

Because many of these bird species vary in habitat relations over space and time (Block and Morrison 1991), care should be exercised in extending these findings to other oak woodland types. Long-term studies are needed in multiple habitats. Variation in climate among years can affect food availability and predation risk of songbirds (Sillet and others 2000, Morrison and Bolger 2002). Variation in acorn productivity among years (Koenig and others 1991) may also cause temporal variation in avian abundance. It is especially important for standardized censuses to be conducted in woodlands experiencing structural changes, such as those affected by sudden oak death.

Recommendations for management

We should make every effort to insure that *P. ramorum* does not become established in San Luis Obispo County or otherwise expand beyond its current range. Current regulations for survey and control of nursery and agricultural industries should be followed with diligence and resolve. Given the possibility that human recreationists may be important vectors for the pathogen (Cushman and Meentemeyer, this volume), it would be valuable to develop infrastructure to help hikers and bikers clean equipment and vehicles before leaving infected areas (Thut and others, this volume). Inaccessible forests such as the nature preserve in the Irish Hills may prove to be valuable refugia for coast live oaks.

Forest and rangeland managers should retain large oaks with numerous cavities to provide foraging and nesting sites for cavity-nesting birds. Diseased and dead oaks should only be removed from infected stands if it can be confidently established that so doing will contain the infestation.

Conclusions

Sudden oak death is likely to change stand structure in ways important to breeding birds. Cavity-nesting birds may be affected by changes in the size class distribution of trees or the availability of cavities and acorns. Nevertheless, sudden oak death is unlikely to have dramatic effects on the abundance of cavity-nesters as a guild in the short term. Effects on individual species may be more dramatic.

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Josh Jones, Robert McKee, Dan Mummert, Will Fields, and Kim Vincent counted birds in 2002 and 2003. We appreciate the cooperation of the personnel at Camp San Luis Obispo and private landowners for granting and facilitating access to study sites.

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Climate-Host Mapping of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death[1]

Glenn Fowler[2,3], Roger Magarey[2], and Manuel Colunga[4]

Abstract

Phytophthora ramorum infection was modeled using the NAPPFAST system for the conterminous United States. Parameters used to model *P. ramorum* infection were: leaf wetness, minimum temperature, optimum temperature and maximum temperature over a specified number of accumulated days. The model was used to create maps showing the frequency of favorable years for infection and these were compared with historical *P. ramorum* observations from California. The majority of historical observations were located in the greater than 90 percent favorable zone using 10 year climate data. Climate risk areas for *P. ramorum* were then overlaid with hardwood forest density to generate a preliminary composite map that was demarcated based on the degree of climate match and hardwood forest density. These maps are designed to improve the efficacy and economy of survey and detection activities for *P. ramorum* by federal, state and local regulatory agencies. Work is in progress to create international climate risk maps using a daily data set that is temporally and spatially interpolated from the Intergovernmental Panel of Climate Change (IPCC) data set.

Key words: climate, hardwood density, historical observations, infection, *Phytophthora ramorum*, survey

Introduction

In March 2004, considerable quantities of *P. ramorum* infected nursery stock were shipped throughout the United States by retail and wholesale nurseries. This necessitated an emergency response by Plant Protection and Quarantine (PPQ). Federal, state and local regulatory personnel were activated. Their combined efforts have helped mitigate and manage the situation.

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One aspect of the emergency response was GIS mapping of areas with climates conducive to *P. ramorum* infection. These climate maps are designed to increase the efficacy of surveys, provide information to regulatory decision makers and assist decision-making that would mitigate economic and environmental damage associated with the disease.

Methods

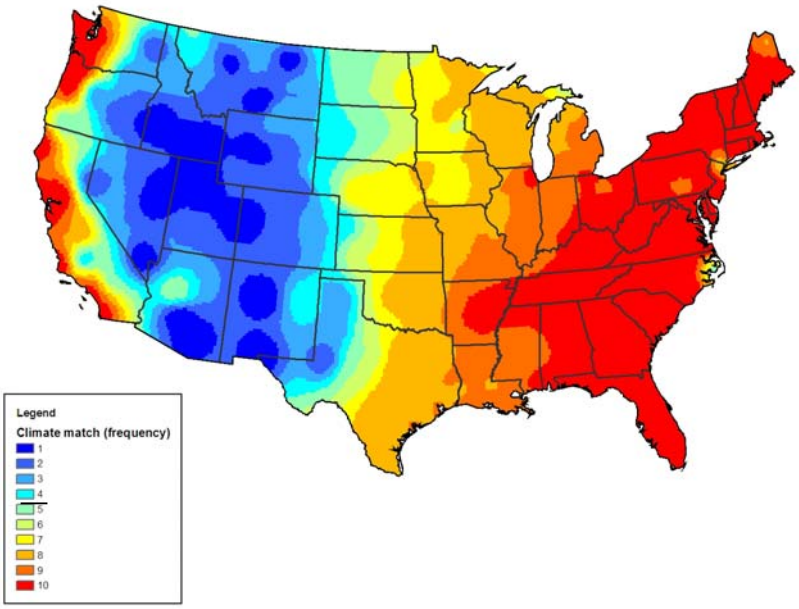
The NCSU/APHIS Plant Pest Forecasting System (NAPPFAST) was used to model *P. ramorum* infection. Historical daily climate data for the 10 year period between 1995 and 2004 was used to construct the model. Temperature was recorded at 1,882 weather stations throughout North America. Leaf wetness was derived for 200 weather stations throughout the conterminous United States. The temperature and leaf wetness data was used to map potential areas of infection with the Barnes interpolation at a 10 km resolution. The parameters used were: Tmin (3 °C); Topt (20 °C); Tmax (28 °C) and a minimum accumulated leaf wetness of 12 hours (Huberli and others, 2003; Olikowski and Szukuta, 2002; Werres and others 2001). An area was considered to be at risk if the climate met these infection conditions for 10 or more accumulated days in a month (Jones pers. comm., 2004) or more than 60 days in a year. A probability map was generated using 10 years of climate data. The geotiff images created in NAPPFAST were incorporated into GIS for further analysis.

Model validation points were obtained from locations in California where the presence of *P. ramorum* has been confirmed (UC Berkeley, 2003) and then overlaid with the annual climate match map. The number of confirmations associated with each climate match area was plotted. A distribution for the probability that a confirmation would occur in the 10 year climate match area was determined.

Forest density (1 to 100 percent) was estimated across the conterminous United States at a 1 km resolution using the 1992 National Land Cover Data (USGS, 1992). A hardwood GIS layer was obtained by combining the NLCD deciduous forest and 50 percent of mixed forest density classes. It was assumed that the mixed forest was 50 percent hardwoods. After the climate match layer was converted to a percentage it was summed with the hardwood forest density layer and divided by two to generate a preliminary relative risk map. A mask where no hardwoods occurred was then applied to eliminate areas without suitable hosts.

Results and Discussion

The climate maps demonstrated a good correlation (greater than 90 percent) with known occurrences of *P. ramorum* in California. This indicates that the model is a good predictor of disease establishment. The climate maps were used in the 2004 *P. ramorum* PPQ National Survey manual and the updated maps will be incorporated into the 2005 version. The preliminary climate match-host density maps indicate that the eastern United States has a high likelihood for *P. ramorum* infection if an inoculum source is available. More sophisticated climate match-host density models and global climate match mapping capabilities are being developed.



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Modeling Risk for SOD Nationwide: What Are the Effects of Model Choice on Risk Prediction?[1]

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Abstract

Phytophthora ramorum has the potential to infect many forest types found throughout the United States. Efforts to model the potential habitat for *P. ramorum* and sudden oak death (SOD) are important for disease regulation and management. Yet, spatial models using identical data can have differing results. In this paper we examine the results from five types of models generated from common input parameters, and investigate model agreement for distribution of risk for *P. ramorum*. We examine five models: (1) Rule-based, (2) Logistic regression, (3) Classification and Regression Trees, (4) Genetic Algorithm modeling, and (5) Support Vector Machines. The models differed in terms of parametric and non-parametric requirements, necessity for presence/absence data, and whether or not the explanatory variables were determined *a priori* or revealed during the model process. Nationwide input data included vegetation/host (hardwood diversity and hardwood density), topography, and climate (e.g. precipitation, frost days, temperature, and many other layers). We developed a risk map for the conterminous United States in which probabilities for *P. ramorum* disease establishment were based not on one model, but on agreement between multiple models. The five models were consistent in their prediction of some SOD risk in coastal CA, OR and WA. All models predicted some risk in the northern foothills of the Sierra Nevada mountains in CA. Outside of the west coast, the combined models predicted highest risk for SOD in a east-west oriented band including eastern OK, central AR, TN, KY, northern MI, AL, GA and SC, parts of central NC, and eastern VA, DL and MD. The paper also discusses issues of input data accuracy, coverage, availability of nationwide host datasets, data scale, and model computational requirements. Although theoretical in nature, the results of this paper have practical and applied value for managers and regulators of this disease.

Key words: geographic information systems, spatial modeling, sudden oak death

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Introduction

Phytophthora ramorum has the potential to infect many forest types found throughout the United States. Efforts to model the potential habitat for *P. ramorum* and sudden oak death (SOD) are important for disease regulation and management. For example, in California, researchers used an expert-knowledge driven Rule-based model to predict risk of SOD based on host, temperature and moisture data (Meentemeyer and others 2004) which has been used to guide sampling, aerial surveys, and other statewide monitoring efforts. Rule-based models such as this one use expert input rather than statistical inference, and thus the predictor ecological variables used are known *a priori*. Other spatially referenced ecological niche models require presence data to train the model, possibly revealing ecological niches. The variety of techniques used for ecological niche modeling is growing (Guisan and Zimmermann 2000), and there has been a corresponding increase in the spatial modeling literature in work that compares results from different models (Guisan and Zimmermann 2000; Manel and others 1999; Muñoz and Felicísimo 2004). These efforts show that spatially referenced models using identical data often have differing results, due in part to 1) the fact that models can be either parametric or non-parametric with varying reliance on variable distribution, 2) user-defined weightings placed on variables can differ by analyst, and 3) the different methods used for generating absence data for input to models can influence results, among other factors. In this research, we examined a collection of model types and produced a risk map for SOD nationwide based on agreement between these models. We examined five classes of models that differed in terms of parametric and non-parametric requirements, necessity for presence/absence data, and whether or not the explanatory variables were determined *a priori* or revealed during the model process. Our spatial models include the following: (1) Rule-based, (2) Logistic regression (LR), (3) Classification and Regression Trees (CART), (4) Genetic Algorithm (GA) modeling, and (5) Support Vector Machines (SVM).

Model Descriptions

Rule-based spatial models use research data and expert input, rather than statistical inference to determine the importance of predictor variables (Meentemeyer and others 2004). Predictor variables are given weights based on importance, and all weighted variables are summed in a geographical information system (GIS) overlay procedure to produce a mapped output. This method is straightforward and not computationally intensive. Logistic regression (LR) is a variation of ordinary regression which is used when the dependent (response) variable is a binary variable

which usually represents the occurrence or non-occurrence of some outcome event, usually coded as 0 or 1, and the independent (input) variables are continuous, categorical, or both. Resulting probabilities can be mapped over space for an easily understood cartographic representation of modeled distribution. LR is a powerful parametric yet computationally non-intensive method for ecological niche modeling (Felicisimo and others 2002; Kelly and others 2001; Mladenoff and others 1995). Classification trees are a non-parametric alternative to parametric techniques such as logistic regression (De'ath and Fabricius 2000) and Linear Discriminant Analysis (LDA) (Feldesman 2002). The tree models are developed by recursively partitioning the response variable into increasingly homogeneous binary subsets based on critical thresholds in predictor variables. The split chosen is the one that most reduces the average impurity in the resulting bins (Breiman and others 1984; De'ath and Fabricius 2000; Venables and Ripley 2002). The resulting “trees” are often displayed graphically, and are easy to understand as a series of if/then conditions, but they can be complex to render cartographically (Kelly and Meentemeyer 2002; Muñoz and Felicísimo 2004). Genetic Algorithm (GA) modeling is a an evolutionary computing system that has excellent capabilities for delineating ecological niches and geographical distributions of species (Raxworthy and others 2003; Stockwell 1999). The method uses genetic algorithms to predict the potential distribution of a species by generating a set of rules. Essentially, genetic algorithms apply the operational concepts similar to evolutionary biology such as mutation and crossover to evolve the solutions in order to find the best solutions. GA modeling is less susceptible to local maxima and able to handle various data formats (continuous and discrete). Finally, Support Vector Machines (SVM) are a new generation of learning algorithms that can perform binary classification (pattern recognition) and real valued function approximation (regression estimation) tasks. SVMs have been developed on a solid base of statistical learning theory and are designed especially to provide high flexibility for approximating class boundaries, while avoiding over-fitting phenomena. Functionally, SVMs seek to find an optimal separating hyperplane with the maximal margin between the training points for presence and absence data in multidimensional space (Cristianini and Scholkopf 2002; Huang and others 2002). SVMs are able to handle non-linear and categorical data, make no assumption on the probability density of the data, and are competitive with the best available machine learning algorithms in classifying high-dimensional data sets (Guo and others 2005). Model characteristics for these five models are summarized in *table 1*.

Table 1—*Characteristics of the five classes of models used.*

Model Name	Presence/absence data required?	Parametric/Non-parametric	Important Variable selection	Output	Computational requirements
Rule-based	No	Non-parametric	*a priori*	Ranked	Low
Logistic Regression	Yes	(semi-) Parametric	Through training	Probability	Low
CART[1]	Yes	Non-parametric	Through training	P/A based on # runs	Low
GA[2]	Yes	Both	Through training	P/A based on # runs	High
SVM[3]	No (1-class) Yes (2-class)	Non-parametric	Through training	P/A based on # runs	High

[1] Classification and Regression Tree; [2] Genetic Algorithm; [3] Support Vector Machines.

Methods

Predictor Ecological Variables

We developed five spatial models using common nationwide data. Physical variables included topography and climate; we used Digital Elevation Model (DEM) and DAYMET weather and climatologically modeled raster surfaces gridded at 1 km to summarize physical conditions for the pathogen. Daymet is an assortment of climate raster surfaces interpolated from ground-based meteorological stations on a daily basis over an 18 year period (1980 to 1997) (Thornton and others 1997). The primary climate surfaces utilized in this modeling project included total annual precipitation, total annual frost days, average minimum temperature, average maximum temperature and average maximum August temperature. Topography was derived from United States Geological Survey (USGS) National Elevation Dataset (USGS. 1999b).

Host/Vegetation Data

We had a considerable challenge finding a detailed vegetation map for the conterminous United States with sufficient floristic and spatial detail to allow modeling. We explored the utility of several datasets. The first among these was the National Land Cover Data (NLCD) 1992 dataset, a 21-category classification derived primarily from Landsat Thematic Mapper (TM) imagery from 1992, containing categories for several different forest types including deciduous, conifer and mixed (Vogelmann and others 1998; Vogelmann and others 2001). The NLCD classification supplies high spatial resolution (30-m) but poor floristic detail, providing only three general vegetation categories relevant to our project: deciduous forest, evergreen forest, and mixed forest. Using this information, we created a 1-km gridded vegetation dataset depicting "hardwood density" based on the percent of deciduous and mixed forest found within each 1-km cell. The second dataset we investigated was the digital tree range maps for North America created by the USGS for a vegetation climate modeling study (USGS. 1999a), which provided more floristic detail, but was coarse in spatial detail. This product was based upon a series of tree range maps assembled by Elbert L. Little, Jr. in the 1970s as the "Atlas of the United States Trees" (Little 1971; Little 1976; Little 1977; USGS. 1999a). Of the 58 digital oak species maps created by USGS, we determined that 34 were potentially susceptible to *P. ramorum*. These 34 oak species maps were then combined with digital maps of 12 other tree range maps of species found to either be directly susceptible to the pathogen or to be related (*i.e.*, in the same taxonomic genus as a susceptible species). The 46 tree range maps were then combined to form a "hardwood diversity index" map. It should be emphasized that the hardwood diversity index as calculated for this study was limited to a portion of the tree range maps made available by the USGS and contains only a minimal number of shrub or understory species. It is only intended to represent areas within the United States that potentially contain high numbers of susceptible *P. ramorum* host species (both foliar and terminal hosts) with the recognition that there are many more species not included. The final vegetation layer examined was provided by the USDA-Forest Service Northeastern Research Station (Gottschalk and others 2002). In this product, Forest Inventory and Analysis (FIA) plot data were used to calculate the percentage of forest basal area composed of the red and live oak groups and these points were kriged to create a continuous raster surface for the eastern United States. Percent basal area estimates were adjusted for forest density using the NLCD dataset.

Model Creation

We first developed our nationwide rule-based model using similar input data to those used in the California model. Climate variables for six winter months were parameterized and placed into weighted classes in accordance with the methods of Meentemeyer and others (2002). Two different coarse-resolution vegetation maps (hardwood diversity and hardwood density) were used as a surrogate for the detailed vegetation map used in the California modeling case. The remaining models (with the exception of GA) required that presence and absence data be generated for model training. For presence data we used the database of locations (n = 169) of confirmed *P. ramorum* existing in 12 counties in California and one in Oregon provided through the University of California, Berkeley (Kelly and others 2004). Absence data (or in this case 'pseudo-absence' data, as we do not have samples of *P. ramorum* absence) were generated in the following manner. We created a zone of infestation within California consisting of the 12 infested counties and six border counties. We then constrained the algorithms to search for and generate pseudo-absence data from locations in California outside this zone.

We began with CART modeling. Using Splus v. 6.2 for Windows, we generated 100 classification trees using 100 unique pseudo-absence point distributions and the 169 *P. ramorum* presence points. Each "tree" was pruned by examining a plot of deviance and tree complexity (Feldesman 2002), and the resulting tree models were implemented in ArcInfo using Arc Macro Language (AMLs). We then developed LR equations using Splus v. 6.2 for Windows and implemented the results in ArcInfo. Desktop GARP (Genetic Algorithm for Rule-set Production) (Stockwell and Peters 1999) software was used for the application of the GA model. One hundred model runs were performed using presence data only. Finally, we developed SVM models using Matlab and LIBSVM software (Chuang and Lin 2001). Cross-validation was used for each of the 100 runs to optimize parameter selection. It became clear early on that the vegetation information would not be useful in the initial modeling exercises, as detailed vegetation maps are not available for every state. The initial model inputs (with the exception of the Rule-based model) were limited to climate variables.

The different predicted *P. ramorum* risk map results of the models examined in this project displayed significant levels of variation depending on the model in question and the input climate variables used. Therefore, a combination of several different model input formulas was used to create a cumulative and weighted map result. The four predictive models (excluding Rule-based) were run 100 times each with the

following three collections of input predictor variables: 1) precipitation total and frost days, 2) precipitation total, frost days and average maximum temperature, and 3) precipitation total, average minimum temperature and average maximum temperature. Model results were added together along with proportional values from the Rule-based models to create a finalized *P. ramorum* risk grid. Finally, in an effort to eliminate areas of non-hardwood forest in the final risk map, we filtered the combined model results through the NLCD and red oak basal area vegetation maps by multiplying the map by each vegetation map rescaled from 0 to 1. We did not include the USGS vegetation map in this exercise as the spatial fidelity of the product seemed problematic. The representation of hardwood diversity in the southeast United States may not be accurate due to the relatively small number of tree ranges used.

All model results were normalized for visual display purposes using the following technique: the mean value was calculated and then arbitrary boundaries were set for plus or minus two standard deviations. Any values above or below were reclassed to the minimum or maximum of the 95th percentile. This grid was then rescaled from 0 to 100, and classed into five classes of risk: 0 to 20 percent low, 20 to 40 percent, 40 to 70 percent medium, 70 to 90 percent, and 90 to 100 percent. The exception to this approach was the Rule-based model, which was scaled and displayed according to Meentemeyer and others (2004).

Results

The results from each of five models are shown in *Figure 1*. The rule-based model shows a high risk for *P. ramorum* spread across the southeast from eastern TX to VA, with declining risk to the north. The model also shows high risk in the northwest and risk in the northern foothills of the Sierra Nevada mountains in CA. While this model is a replica of that provided by the Meentemeyer and others (2004) model, this is a different result and likely due to qualitative differences in input data and spatial resolution. The LR results show a broadly similar pattern to the Rule-based model, with less risk on the west coast and less overall risk in the southeast: the model constrains the highest risk to the deep southern states of LA, AL and MS. The LR formula determined that precipitation total, average minimum temperature and average maximum temperature were important in risk prediction. CART and GA results are similar, with a band of highest risk occurring throughout the middle southeast from OK in the west to VA and NC in the east. This is likely due to the importance both models placed on latitudinally controlled temperature variables. Both models found precipitation total, frost days and average maximum temperature to be the most important predictors. The SVM result is coarser than the others; the

algorithm required resampling of the input data to a coarser spatial resolution (~12 km) due to computational limitations. The SVM algorithm predicted risk as a result of precipitation total, frost days and average maximum temperature.

The final risk map – a combination of the results from five models – is shown in *Figure 2*. The five models were consistent in their prediction of some *P. ramorum* risk in coastal CA, OR and WA, although LR and GA show less risk than do the other models. All models predicted some risk in the northern foothills of the Sierra Nevada mountains in CA. Outside of the west coast, the combined models predicted highest risk for *P. ramorum* in an east-west oriented band including hardwood forested areas of OK, AR, TN, KY, northern portions of MI, AL, GA and SC, parts of central NC, eastern VA, DL and MD. This area includes portions of several ecoregions, including the Piedmont ecoregion of NC, SC, GA, AL and MI, a transitional area between the mostly mountainous ecoregions of the Appalachians to the northwest and the relatively flat coastal plain to the southeast. Much of this region has reverted to successional pine and hardwood woodlands, with an increasing conversion to an urban and suburban land cover (ECOMAP 1993; Omernik 1987; Omernik 1995). There is also predicted risk for *P. ramorum* in hardwood forests of the Southwestern Appalachains in TN and in the primarily oak-hickory forests of the Interior Plateau in KY and TN. Eastern OK and central AR shows high risk in the oak-hickory-pine forests of the Ouachita Mountains, and in the red oak, white oak, and hickory dominated forests of the Boston Mountains, and in the predominantly oak forests of the Southern Ozarks (ECOMAP 1993; Omernik 1987; Omernik 1995). Coastal MD and DL, part of the Middle Atlantic Coastal Plain, are climactically susceptible and have forests at risk in riparian areas.

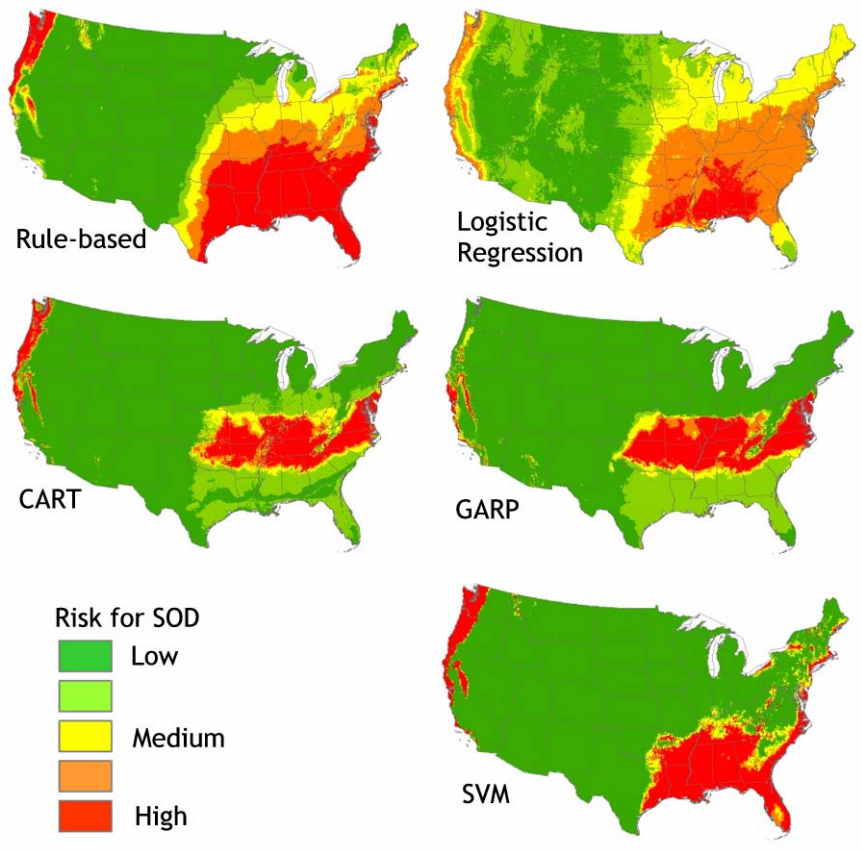

Figure 1—Risk for Sudden Oak Death in the conterminous United States: results from five spatially referenced models**.**

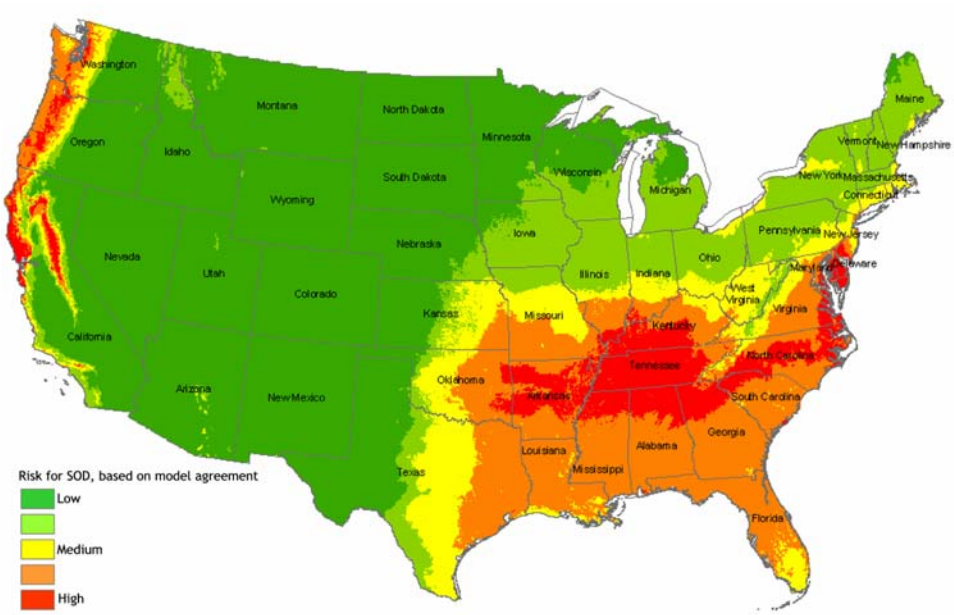

Figure 2—Risk for Sudden Oak Death in the conterminous United States based on agreement between five spatially referenced models.

Discussion and Conclusions

Because there are no wildland cases of SOD outside of California and Oregon, none of these models can be assessed for accuracy. This is an unfortunate but not uncommon situation when modeling invasive species (Muñoz and Felicísimo 2004). Several of the models allow for some form of cross-fold validation tools for assessing accuracy, but these tools can be problematic due to the small number of training samples and their concentrated distribution (Graham and others 2004). The generation of pseudo-absence data must also be examined. We do not have reliable negatives for *P. ramorum*, so we used a common method for generation of pseudo-absence data, constraining the pool of possible absence points to be taken from outside the zone of infestation in California. Experiments with pseudo-absence data generated within the zone of infestation resulted in models that over-predicted the risk of the disease. One-class SVMs are able to model species distributions without absence data (Guo and others 2005), and this method might be of use in this case. We only examined two-class SVMs in this paper, for consistency. Finally, the host data available for the entire United States was the largest limiting factor in our modeling exercise; all nationwide vegetation layers we used had significant drawbacks. For example, the NLCD data was the most spatially comprehensive layer, with complete coverage at a high spatial resolution; however, specific floristic detail was absent, and the vegetation classes were much too broad to be of use in the modeling exercise. The USGS layer had sufficient floristic detail, but was tremendously course in resolution. Finally, the FIA product only covered the east coast area, and thus could not be used in the models that required training. A similar west coast product is not available. Computationally, the Rule-based, Logistic regression and CART were the least computationally demanding of the algorithms. GARP and SVM require more CPU time. This work examined common ecological niches for *P. ramorum*, but an investigation of the human component to disease establishment and spread should also be considered. The locations of nurseries might be an additional important component to this research. Attempts at finding similar climate datasets for European locations of *P. ramorum* were not successful prior to completion of this paper but should be considered. Although theoretical in nature, the results of this paper have practical, applied value for managers and regulators of this pathogen.

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Aerial and Ground Surveys for Mapping the Distribution of *Phytopthora ramorum* in California[1]

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Key words: aerial surveys, ground surveys, *Phytophthora ramorum*, sudden oak death

Abstract

Since 2001, the USDA Forest Service and California Polytechnic State University, San Luis Obispo have been collaborating for early detection and monitoring of the occurrence of *Phytophthora ramorum*, the pathogen known to cause sudden oak death (SOD). The effort consists of annual aerial surveys to map hardwood mortality in overstory tree species including coast live oak (*Quercus agrifolia*), tanoak (*Lithocarpus densiflorus*), Shreve oak (*Quercus parvula* var. *shrevei*) and California black oak (*Quercus kelloggii*). These aerial surveys are followed by ground surveys to locate and sample both symptomatic overstory and understory host plants. Over the past few years the surveys have focused on early detection within minimally infested counties or counties with no known occurrence of *P. ramorum* but share a common border with regulated (infested) counties. The team of cooperators includes assistance from the University of California (UC), the California Department of Food and Agriculture (CDFA), county agricultural commissioners and cooperation from numerous private and public entities. Success in the program can be looked at in at least two ways, 1) by identifying new disease infestations and 2) by not finding new disease infestations. Over the past four years, the combination of aerial survey and ground confirmation efforts have identified new fronts of infection and mapped the distribution of *P. ramorum* within counties, or portions of counties, not previously known to have the disease (including recent expansion into southern Monterey County and new occurrences in Lake County). Following aerial surveys, ground surveys are targeted within select areas to check for infestations that would

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expand the current range of *P. ramorum*. All areas mapped from the air within counties adjacent to currently infested counties are checked on the ground. Within minimally infested counties, ground surveys are targeted toward those areas in the county not known to be infested. Established sampling protocols are followed to determine if *P. ramorum* symptoms are evident in any of the susceptible plant species present. All samples are shipped to the appropriate laboratory for confirmation of *P. ramorum* as well as other Phytophthoras. Results from aerial and ground surveys conducted over the last four years were compiled and evaluated for over 1,000 discrete areas mapped from the air and hundreds of sites visited on the ground showing the distribution of *P. ramorum*-caused hardwood mortality across the landscape.

Introduction

Tens of thousands of tanoak *(Lithocarpus densiflorus*), coast live oak *(Quercus agrifolia*), California black oak *(Quercus kelloggii*), Shreve oak *(Quercus parvula* var. *shreve*i), and have been killed by a non-native, invasive pathogen, *Phytophthora ramorum*. This pathogen, causing a disease referred to as sudden oak death (SOD), was first reported in Marin County in 1995 (McPherson and others, 2000). The pathogen also infects rhododendron species including *Rhododendron macrophyllum*, madrone *(Arbutus menziesi*i), huckleberry *(Vaccinium ovatu*m), California bay laurel also known as Oregon myrtle *(Umbellularia californic*a), and California buckeye *(Aesculus californic*a), but usually causes only leaf spot and twig dieback on these hosts. The host list is expected to grow as researchers continue their investigations of affected ecosystems. (See host information at http://suddenoakdeath.org/ for the most current host list).

The known distribution of *P. ramorum* is expanding. According to the California Oak Mortality Task Force (COMTF), there are, as of December 2004, fourteen counties with wildland confirmations of *P. ramorum:* Alameda, Contra Costa, Humboldt, Lake, Marin, Mendocino, Monterey, Napa, San Francisco, San Mateo, Santa Clara, Santa Cruz, Solano and Sonoma (*fig. 1)*. The farthest north wildland confirmed location of *P. ramorum* in California is near Redway, in Humboldt County. The farthest south confirmed wildland location is Plaskett Creek, in Monterey County. The wildland location farthest from the coast is 50 miles inland and in Solano County. The disease is widespread in Marin and Santa Cruz Counties in redwood forests that have tanoak as an understory, and in mixed hardwood forests of oaks, bay, madrone and other species (California Oak Mortality Task Force 2004).

Figure 1—Counties with confirmed *P. ramorum* locations.

Over the course of the last four years (2001 to 2004), this cooperative effort between California Polytechnic San Luis Obispo (Cal Poly) and USDA Forest Service (USDA-FS) Pacific Southwest Region, Forest Health Protection in the air and on the ground has generated 1,337 polygons mapped containing hardwood mortality. Survey methods and sampling techniques have remained generally consistent throughout all years. However, the areas aerially surveyed have varied from year to year based on several factors including funding, confirmed locations of the pathogen during any given year, and relative risk for establishment and spread (*fig. 2)*. Similarly, the sites selected from the aerial surveys for ground checking have varied from year to year depending primarily on results from the aerial surveys as well as funding.

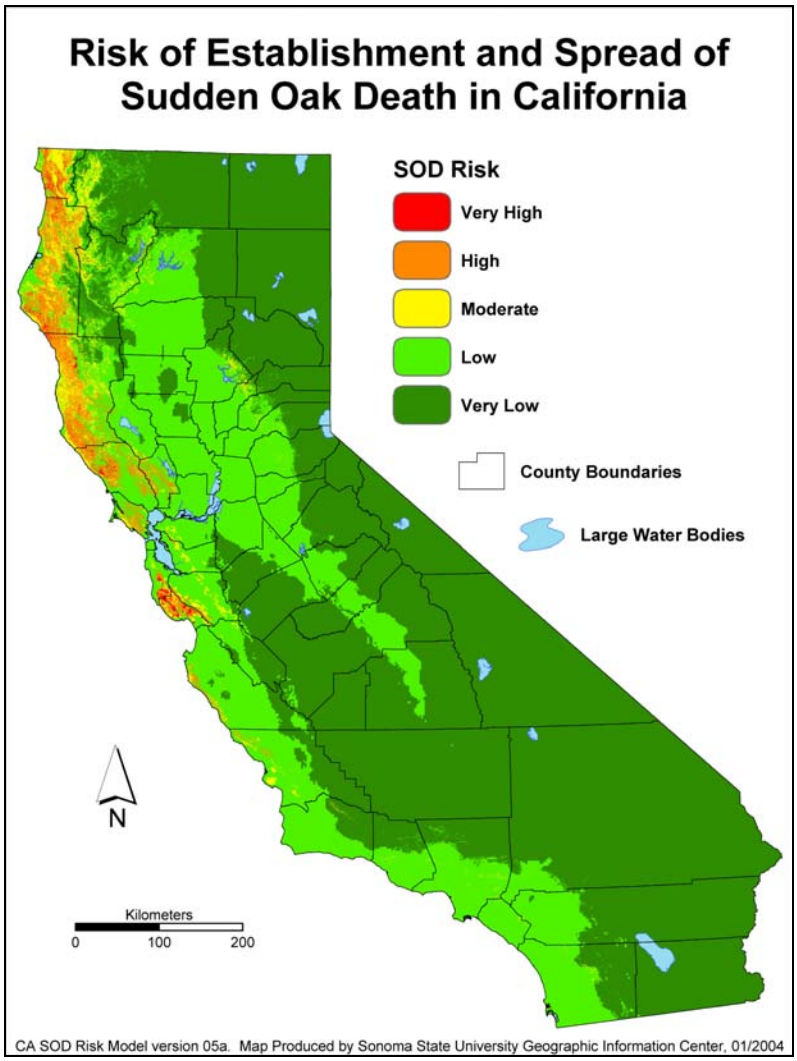

Figure 2—Statewide Risk Map (Meentemeyer, Sonoma State University) used to aid planning and implementation of aerial and ground efforts.

Aerial Survey Methods

Various fixed and rotor-wing aircraft were utilized during the aerial mapping of host hardwood mortality, these include fixed-wing Cessna models 182, 206T, 337 and rotor-wing Bell 206 B-III and 206 L-3 helicopters. During 2001, accuracy of sketch-mapping onto paper maps was aided through the use of a handheld global positioning system (GPS). Beginning 2002, a digital aerial sketch-mapping system (DASM) was used rather than the more traditional, paper mapping method. The DASM provides an outstanding navigational and data capture platform. Facilitated by GPS, laptop, and touch screen, the real-time moving map display enables relatively accurate heads-up digitizing of host hardwood mortality. Host tree mortality was mapped in California black oak, coast live oak and redwood/tanoak-dominated forest types. The intent of

aerial mapping was to capture all observed host mortality possible, recognizing inherent physical limitations of the aerial observer and technical limitations of the equipment and aircraft. Nonetheless, an attempt was made to capture mortality polygons "wall to wall" within areas surveyed. Polygon areas mapped ranged in size from 0.3 of an acre to 22,297 acres. For each area mapped, attributes captured included affected host tree species and amount of mortality. Oblique 35mm photographs were taken of the mortality and surrounding area, frame numbers were also captured as attributes during data collection. Additional attributes sometimes included secondary host species if present, aspect and any comments the observer considered relevant. Comments included details on best access route and description of any structures or landmarks nearby that would be helpful as navigational aids during subsequent field checks. Finally, polygon centroid coordinates were calculated in UTM NAD83 (either zone 10 or 11) and retained within the polygon attribute table. Mortality data captured during neighboring surveys in Oregon that happened to occur just over the California border were incorporated with the California survey data; likewise California mortality data occurring just over the Oregon border was provided to the Oregon Department of Forestry and USDA-FS in the Pacific Northwest Region.

Beginning in 2003, helicopters were used to re-fly select areas mapped during the fixed-wing flights. Helicopter flights, used only for a limited number of sites, were prioritized based on a combination of factors including proximity to existing infections, number of dead trees, high risk areas based on the Meentemeyer risk model and, to some extent, range of the aircraft operating out of airports with correct fuel type. Once high priority polygons were identified, additional polygons were included in the helicopter flight plan that happened to occur along the planned route. To direct the helicopter, the polygon centroid coordinates were entered in a handheld GPS used on board. Upon arriving at each GPS waypoint, affected host and level of mortality was confirmed or updated as appropriate, additional observations were made describing the mortality and access, and a new GPS waypoint captured while hovering. This new GPS waypoint is more accurate than the original centroid coordinates calculated for the polygon and subsequently used by field crews to more easily locate trees mapped from the air. Analysis of fixed-wing mapping accuracy comparing original polygon centroids to revised helicopter GPS waypoint is displayed in *table 1*.

Table 1—*Accuracy of original digital sketch-mapping (centroid) compared to revised coordinates captured from helicopter using GPS, 2003 aerial survey data is analyzed for 92 polygons within six counties*[6]

COMPARISON OF HELICOPTER GPS TO POLYGON CENTROID

Distance to Centroid	DN	HUM	MEN	SBAR	SLO	KERN	TOTAL
# of Points within 200 feet	1	2		1	1		5
# of Points within 400 feet	2	4	1		2	1	10
# of Points within 600 feet	3	5	6	2	3	2	21
# of Points < 1320 feet (1/4 mile)	3	9	11	3	9	3	38
# of Points > 1320 feet (1/4 mile)	1	4	3	1	5	4	18
Nearest GPS to Centroid (feet)	144	145	295	110	81	372	
Average GPS to Centroid (feet)	759	859	887	695	1083	1131	

COMPARISON OF HELICOPTER GPS TO DETERMINE "IN" OR "OUT" OF ORIGINAL POLYGON & SIZE

Distance to Centroid	**DN**	**HUM**	**MEN**	**SBAR**	**SLO**	**KERN**	**TOTAL**
# of GPS points inside polygon	1	4	4	3	5	4	21
# of GPS points outside polygon	9	20	17	4	15	6	71
Smallest Polygon (acres)	0.7	0.7	0.8	3.9	2.6	10.5	
Average Polygon (acres)	2.3	14.5	29.9	24.7	22.0	61.1	
Largest Poly (acres)	4.4	169.5	237.4	38.6	171.5	455.5	

[6] '#' Quantity of GPS locations captured during subsequent helicopter flights, Global Positioning System (GPS), Centroid is the center of the original polygon mapped from fixed-wing for which UTM coordinates are calculated and the helicopter GPS is compared, county abbreviations: DN - Del Norte, HUM – Humboldt, MEN – Mendocino, SBAR – Santa Barbara, SLO – San Luis Obispo; factors to be considered that affect interpretation of this data include: error induced during geographic transformation of map data (re-projection and coordinate conversion), location of polygon centroid within the original polygon (calculated centroids are not always positioned in polygon center, polygon shape affects calculation) and size of original polygon (many of the polygons include single or very few trees and are less than an acre in size)

Ground Survey Methods

Personnel employed over the last four years have varied to some extent but ground survey methods have remained generally consistent, including:

- Structured, pre-season training for field crews in use of map materials generated from aerial survey, host and symptom recognition and how to collect samples
- Identification of landowner and contact with landowner in advance or during visit
- Host plant examination for symptoms while navigating to the GPS waypoint to locate tree(s) mapped from the air, the search area typically extends well beyond any given polygon
- Proper documentation of the visit and handling/shipment of samples for lab diagnostics
- Emphasis on equipment and personal sanitation to prevent pathogen spread

During 2001, "in kind" support was provided by USDA-FS, California Department of Forestry and Fire Protection (CDF), and UC Davis pathologists and others. During 2002, the USDA-FS contracted field work to a consulting firm and Cal Poly utilized its employees and students. During 2003 and 2004, the USDA-FS began hiring and supervising student temporary employees (STEPs) to accomplish what had previously been contracted or completed "in-kind" in the past. The STEP program is viewed as an excellent way to give college students, interested in natural resources, an opportunity to work for the USDA-FS. Cal Poly continued their program of summer recruitment throughout all four years. Whenever possible, employees with previous experience were rehired for both USDA-FS and Cal Poly crews.

Cal Poly and USDA-FS field crews, lead by Amy Jirka and Jeff Mai respectively, trained together within infested areas (Jack London and China Camp State Parks) to recognize hosts and symptoms of the pathogen. Additionally, practical exercises in making landowner contacts and collecting samples were included as part of the training. Pathologists from CDF, UC Davis, and the USDA-FS have been a key component during each of the annual training sessions. The majority of field checks are completed during the summer months when roads are open and dry, during the period of employment for our temporary workforce and immediately following aerial survey. However, field checks typically continue beyond the summer season to the degree possible utilizing regular, full time personnel and occasionally with assistance from various county agriculture departments, UC Davis and California Department of Food and Agriculture (CDFA) pathologists.

Field materials consist of navigational equipment, including handheld GPS, data collection sheets, California's standard Pest Detection Report (PDR), 35 mm photographs, digital orthoquads (DOQs) with flightlines and polygons, and topographic map with flightlines, polygons and table of attributes collected during the aerial survey. All field materials are produced by the USDA-FS and Cal Poly. The details of every visit and status of any samples submitted are tracked by the USDA-FS. Periodic status updates are provided to the UC Berkeley-maintained Oakmapper interactive website http://kellylab.berkeley.edu, results and web link are communicated to interested parties. The website provides easy access for anyone interested in SOD or this project specifically. Public and government agencies can view all project information from the last four years including flightlines, areas mapped, and attributes of each area and the status of any completed ground checks. Additional outreach and communication is accomplished at regular intervals via email messages and accomplishment reports to COMTF cooperators, county agriculture departments, CDF and CDFA.

Aerial Survey Results and Accomplishments

The area surveyed has varied every year since 2001, see *fig. 3* and *table 2*. Factors influencing areas surveyed include 1) host habitat within un-infested counties, adjacency to regulated counties and within infested counties having relatively large unifested areas, 2) abundance of host species and modeled risk, 3) current distribution of the pathogen, and, 4) staffing and budget. During 2001 the first survey was fairly limited, focusing on infested areas and on host habitat immediately adjacent to those infested areas (generally within counties in proximity to the San Francisco Bay Area). With new discoveries of *P. ramorum* in Curry County, Oregon and elsewhere in California, 2002 saw the largest survey area to date and nearly all host habitats were flown (the exception being counties south of Los Angeles). During 2003, the survey area was reduced but more focused within areas not yet known to be infested. Further reduction in area flown occurred in 2004, with the exclusion the California black oak-type in the Sierra foothills and concentration on host habitat at risk but not yet known to be infested within minimally infested counties and un-infested counties immediately adjacent to infested areas. A list of counties surveyed and number of polygons within each county are displayed in *table 3*.

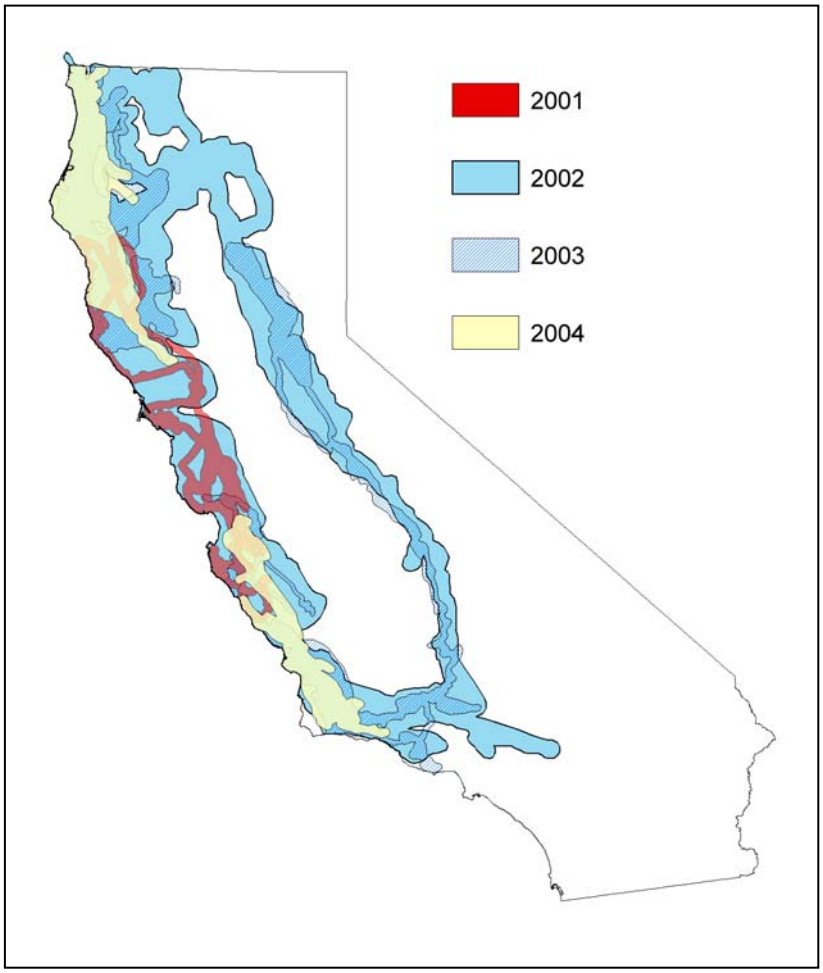

Figure 3—California aerial survey coverage by year.

Table 2—*Aerial survey accomplishments summarized by year*.

Year	Miles Flown	Surveyed Area (acres)	Mortality Mapped (acres)
2001	2,100	5,000,000	13,200
2002	14,500	20,000,000	149,000
2003	10,200	13,000,000	8,200
2004	7,100	8,000,000	3,300

Table 3—*Counties Surveyed and Polygons Mapped Each Year; Subtotal by county and total number of polygons by year displayed will differ from previous reports due to some polygons split by county boundaries and additional sites visited in route to aerial survey polygons (not included above); many counties listed had only partial coverage during the aerial survey due presence of host tree species.*

California Counties Surveyed	Surveyed 2001	Polygons Mapped 2001	Surveyed 2002	Polygons Mapped 2002	Surveyed 2003	Polygons Mapped 2003	Surveyed 2004	Polygons Mapped 2004
Alameda			X	36				
Amador			X		X	3		
Butte			X	7	X	25		
Calavaras			X	1	X			
Colusa			X					
Contra Costa			X	14				
Del Norte			X	7	X	12	X	23
Eldorado			X	6	X	10		
Fresno			X		X	1		
Glenn			X		X	1		
Humboldt			X	84	X	120	X	194
Kern			X	1	X	17		
Lake			X		X	6	X	6
Lassen			X					
Los Angeles			X		X			
Madera			X		X			
Marin	X	9	X	20				
Mariposa			X		X			
Mendocino	X	3	X	42	X	117	X	123
Monterey	X	13	X	44	X	40	X	2
Napa	X	1	X	26				
Nevada			X	4	X			
Placer			X	8	X	3		
Plumas			X					
San Benito			X	18	X	5	X	3
San Bernardino			X					
San Luis Obispo			X	10	X	33	X	45
San Mateo	X	3	X	5				
Santa Barbara			X	3	X	18	X	7
Santa Clara	X	7	X	39				
Santa Cruz	X	5	X	17				
Shasta			X		X			
Sierra			X					
Siskiyou			X	1	X	1		
Solano	X	1	X	13				
Sonoma	X	4	X	40				
Tehama			X		X	1		
Trinity			X	5	X	8	X	4
Tulare			X		X	2		
Tuolumne			X	3	X			
Ventura			X	4	X	2	X	7
Yolo			X					
Yuba			X	1	X	3		
TOTAL		**46**		**459**		**428**		**414**

An important consideration for the aerial survey is timing which has remained fairly consistent during each year of the project. Flights typically occur during June and July after spring flowering in host trees, particularly coast live oak and tanoak, and before much of the drought-deciduous California buckeye foliage turns brown. Approximately 6 to 22 days of flight have been required in each project year (excluding helicopter) with up to 8 hours per day in the plane. Flight days are scheduled for drier counties first then progress into moister habitats, thus further minimizing confusion from flowering hardwood or browning buckeye foliage. Presence of summer fog has been problematic but not insurmountable. Simply maintaining flexibility and surveying what is visible one day, then moving into areas previously not visible the next has largely been successful in minimizing fog obstruction.

During 2002, the year with the largest survey area, the aerial survey portion of this project covered approximately 20 percent of California. For all years combined, the project has accumulated approximately 34,000 miles flown which is a distance nearly equal to 1.5 times around the earth. Ignoring annual overlap, the polygons mapped cover 174,000 acres which equals an area half the size of San Mateo County.

Field Survey Results

Approximately 400 of 1,337 sites have been visited by trained field crews on the ground. Samples were collected from approximately two thirds of all sites visited (*fig. 5)*. Studies by Davidson and others (2003) have shown that timing of field visits and sample collection has a bearing on the likelihood of recovering *P. ramorum*. Climatic conditions and sporulation are most favorable for handling of samples and pathogen recovery during spring months, however, workforce and access issues limit the number of visits conducted during this time of year. As of December 2004, most of the priority sites have been checked. Weather and access permitting, the remainder will be checked in spring of 2005. The most limiting factor in completing ground checks is obtaining landowner permission to access sites since the majority of mortality mapped occurs on private ownerships (*fig. 6)*. Ground visits must be conducted when access conditions are most favorable, surface roads are open for travel and reasonably dry to avoid damage to road surface and minimize the chance of spreading *P. ramorum* and other pathogens such as *P. lateralis*.

For all years, laboratory diagnostics include culture and isolation for *P. ramorum* and a few other commonly occurring but less pathogenic *Phytophthoras*. Revisits to sites checked during the previous survey year are conducted if laboratory results are negative but host plants were described as highly symptomatic. To date, only one

such revisit has resulted in a positive isolation of *P. nemorosa*. Beginning 2004, the Rizzo Laboratory at UC Davis began incorporating ELISA screening to determine presence of the genus *Phytophthora*. In the event that ELISA was positive but culture negative for any *Phytophthora*, a sample was forwarded to the Garbelotto Laboratory at UC Berkeley for PCR diagnostics. To date, all PCR results have been negative for *P. ramorum*. In the event a PCR positive is determined, the site would be revisited to collect another sample and culture attempt. For all years combined, samples collected during the field checks have resulted in positive cultures for *P. ramorum* (27 positives), *P. nemorosa* (11 positives), *P. pseudosyringae* (one positive), and *P. gonapodyides* (one positive). California bay laurel has been the host with greatest success of recovering *P. ramorum* from, as shown in *fig. 7*. However crews examine all host species for symptoms and, due to Oregon myrtle's minimal importance as a host in Oregon, look more closely at wild rhododendron when working in the northernmost area of California.

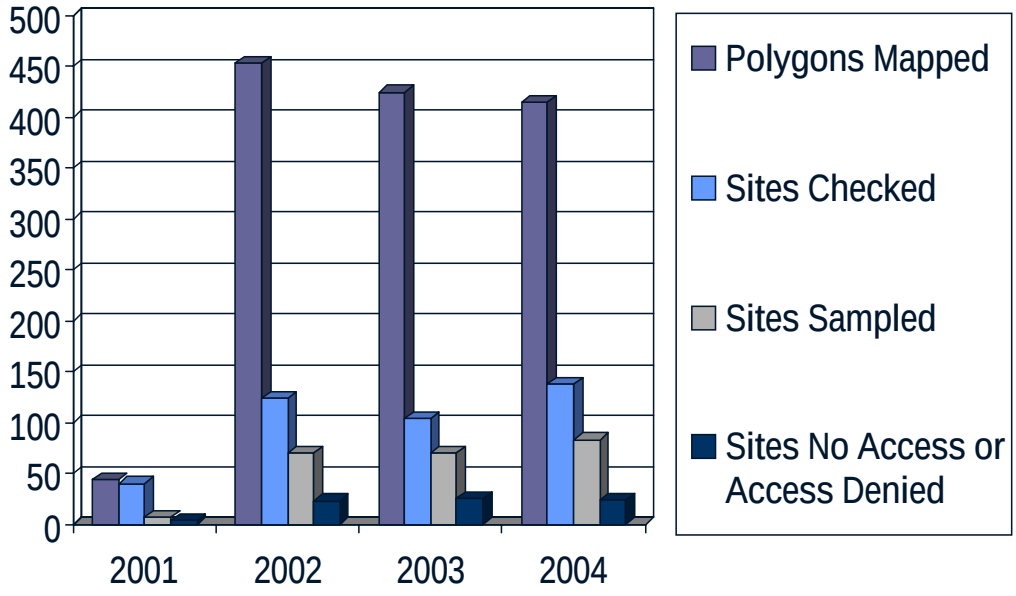

Figure 5—Number of Polygons by Survey Year; the majority of polygons mapped and not checked are located in low-priority areas within currently infested counties, the majority of sites in the no access or access denied category are situations where multiple attempts to contact landowner failed.

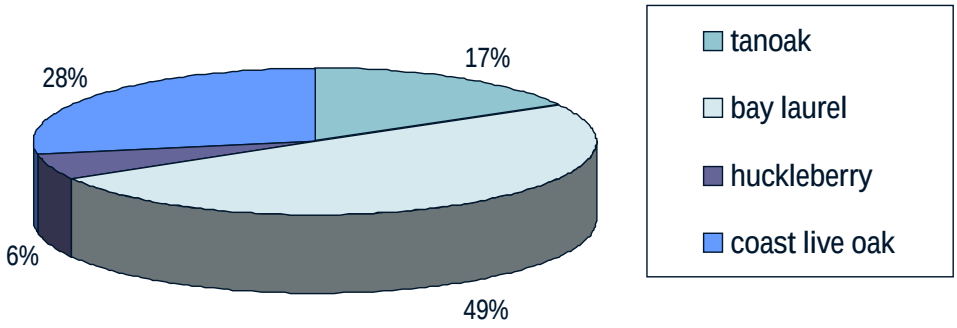

Figure 6—Species distribution with positive lab cultures; includes 18 specimens from 2004 only.

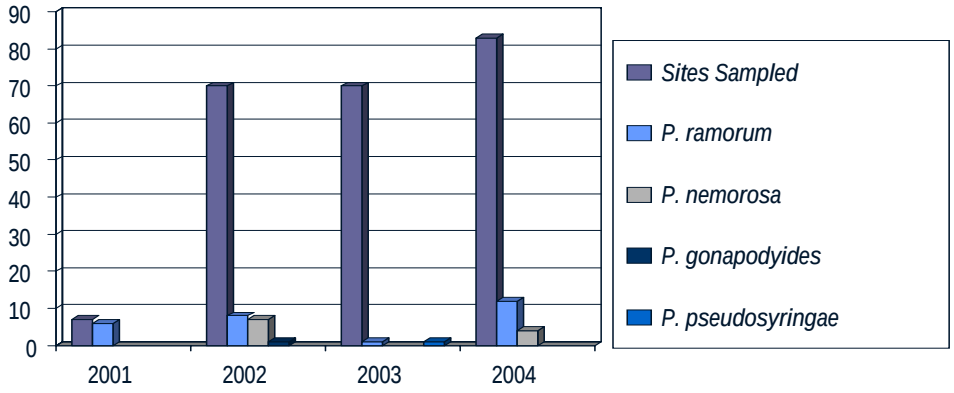

Figure 7—Summary of Lab Results by Year for Sites Sampled; results displayed are for positive cultures only and exclude PCR positives (of which there have been three to date for other *Phytophthoras*).

During the history of aerial surveys in California, there have been several important confirmations as a result of subsequent ground checks. Significant field confirmations include:

- 2001 - Solano County collection by Adams & Kliejunas shortly after first detection by others
- 2002 - Contra Costa County collection by Jirka & Piazza shortly after first detection by others
- 2003 - Monterey County collection by Corrella & Wicklander approximately 25 miles south of previous confirmations and 10 miles from uninfested San Luis Obispo County
- 2004 - Lake County collection by Mai & Jirka and the first confirmation in the county adding it to the list of regulated counties; Humboldt County collections by Harrington & Langenbeck 2 miles east and 2 miles south of previous confirmations in Redway.

Also of significance is the documentation of pathogen spread. Areas flown overlap during various years of survey and *fig. 9* documents how one infested area near Big Sur in Monterey County has grown in size.

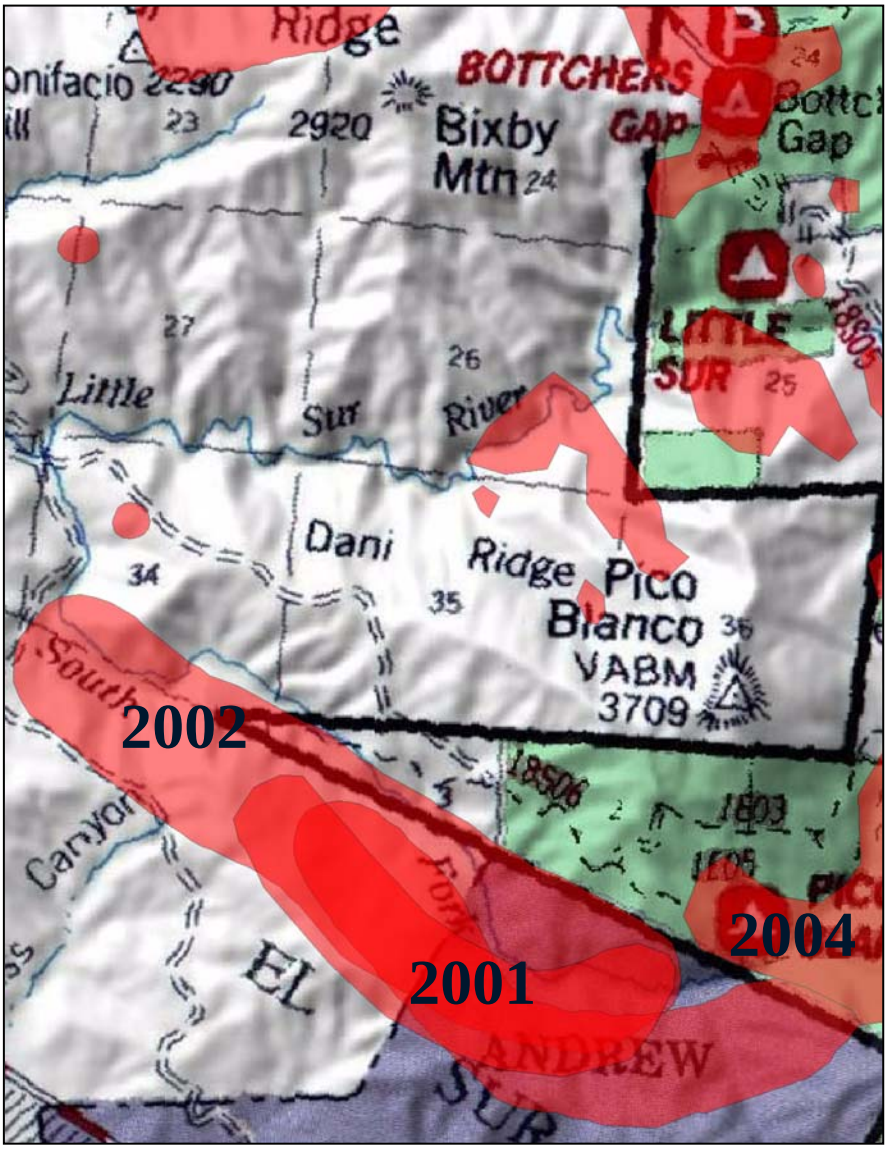

Figure 9—Spread of *P. ramorum*; the magnitude of change documented from three aerial surveys, area at north end of Andrew Molera State Park near Big Sur in Monterey County was initially mapped in 2001 at 500 acres, in 2002 grew to 1200 acres and, finally, 1800 acres in 2004.

Conclusion and Recommendations

The methods employed to conduct aerial survey and focused ground checks have effectively detected new areas of infestation and documented spread of *P. ramorum*. Timing of various phases of the project is optimized considering the logistical,

physical and climatic limitations described previously. Helicopters are beneficial for pinpointing locations that are often very difficult to find due to terrain, dense cover and minimal visibility of the target from the ground. Use of helicopters for the entire project is cost-prohibitive and not practical for the scope of survey in California. The prioritization of areas to be flown with helicopter and/or checked on the ground, a subset of all areas mapped with emphasis on host habitats at relatively higher risk in un-infested areas and in counties adjacent to infested counties, is appropriate for the scope of the problem in California and effective in finding the pathogen in the most critical locations. Communication of project progress and accomplishments at key intervals has been sufficient to provide information to important partners and cooperators during all phases via email, accomplishment reports, USDA-FS Pacific Southwest Region State and Private Forestry Forest Health Protection and Oakmapper websites. Additional public relations efforts would increase awareness of *P. ramorum*, potentially reducing the chance of spreading the pathogen and improving access to private property. Notification and coordination with other agencies to participate and/or conduct field checks independently has been moderately successful. However, increased interest and involvement by other qualified individuals with the USDA-FS, CDF, CDFA, UC Cooperative Extension and county agriculture departments to conduct additional field checks would be beneficial.

The project has had success in both finding and in not finding new disease locations. While not finding *P. ramorum* in any given location does not definitively indicate absence, there is perhaps some comfort in negative detection. Not finding the pathogen with the level of effort expended on this and other detection efforts can be viewed as a success. However, one of the most compelling unanswered questions remains, is the pathogen present in Del Norte County? To date, results from this project and other detection efforts (stream baiting and focused ground surveys based on modeled risk) have all been negative. Emphasis will continue for additional aggressive detection efforts in Del Norte County. This is particularly important to Oregon's effort to eradicate *P. ramorum* in a regulated area within Curry County, adjacent to the California border.

Future surveys are planned to be implemented similarly with improvements. During 2005, the USDA-FS will likely add staff to conduct localized outreach in communities of particular interest to facilitate more effective landowner contact and aid in tracking status of field checks. Surveys have not been conducted over the infested counties surrounding the Bay Area since 2001 and 2002. There is interest in updating the database for mortality that has occurred since that time. The area

planned for 2005 survey will be similar to 2004 with ground checks prioritized as described; however, current plans consider inclusion of infested areas in proximity to the Bay Area for the aerial portion of the project.

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Early Detection Monitoring of *Phytophthora ramorum* in High-Risk Forests of California[1]

Ross Meentemeyer[2], Elizabeth Lotz[2], David M. Rizzo[3], Kelly Buja[4], and Walter Mark[4]

Key words: early detection monitoring, GIS risk model, *Phytophthora ramorum*, sudden oak death

Abstract

Early detection monitoring is essential for successful control of invasive organisms. Detection of invasions at an early stage of establishment when a population is small and isolated makes eradication more feasible and less costly. Sudden oak death, caused by the recently described pathogen *Phytophthora ramorum*, is an emerging forest disease that has reached epidemic levels in coastal forests of central and northern California. To control the spread of the pathogen and protect susceptible habitats, California has established an extensive monitoring program focused on early detection of pathogen activity at isolated locations, where it may be possible to apply chemical treatments or attempt eradication. It is also essential to understand when and where the risk of establishment is elevated in order to effectively monitor the pathogen and manage threatened forests in a state as large (408,512 km^2) and environmentally variable as California. Funded by the USDA Forest Service and California Department of Forestry and Fire Protection, the objectives of this research are to: (1) use GIS-based risk modeling to target early detection sampling efforts in the most threatened forests across the state of California; and (2) discover emergent infections of *P. ramorum* before they become well-established. Here, we present two years (2003-04) of results from California's early detection survey of *P. ramorum*.

The risk model used to target sampling incorporates the effects of spatial and temporal variability of multiple variables on the pathogen's persistence based on current knowledge of host susceptibility, pathogen reproduction, and pathogen transmission with particular regard to host species distribution and climate suitability (Meentemeyer *et al.* 2004). Maps of host species distributions and monthly weather conditions are spatially analyzed in a GIS and parameterized to encode the magnitude and direction of each variable's effect on disease establishment and spread.

A total of 496 locations were assessed for the presence of *P. ramorum* on public land across 38 counties (n = 138 in 2003; n = 347 in 2004). One hundred nineteen of these sites were located within 10 km of a known occurrence of *P. ramorum* and 376 sites were further than 10 km. Along two transects at each

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California

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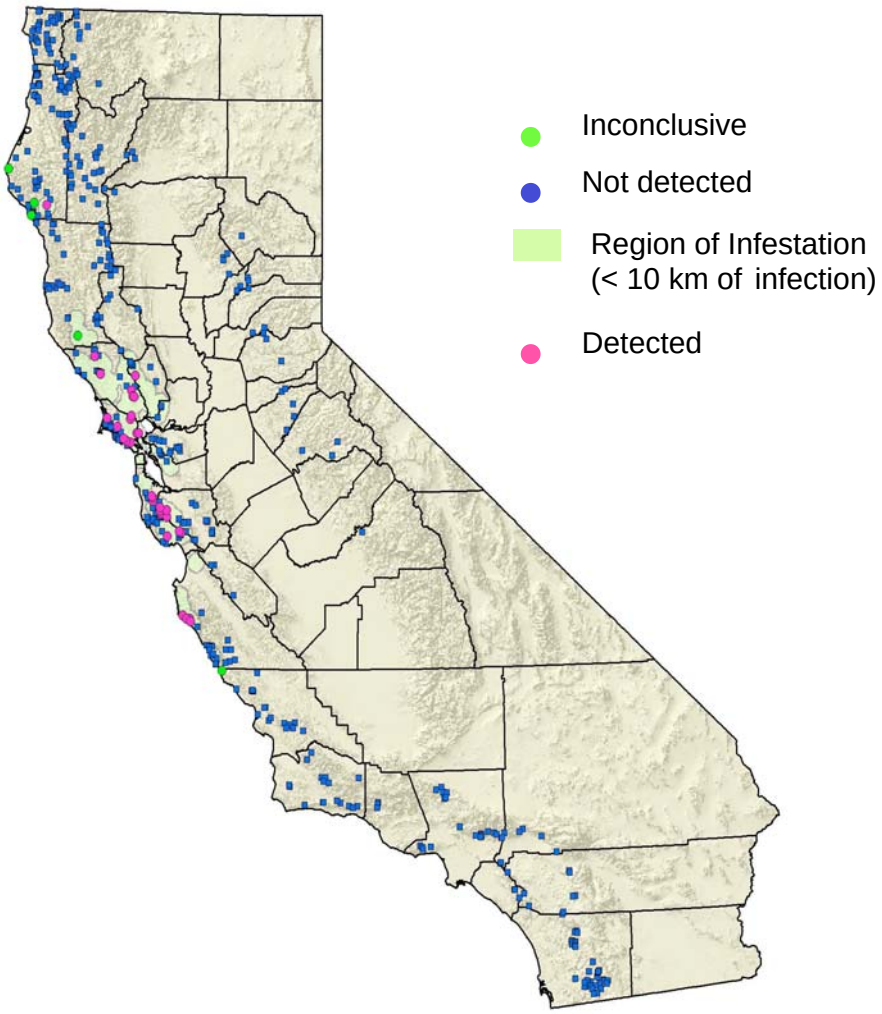
site, leaf and canker samples were collected from all host plants showing potential symptoms of *P. ramorum* and examined using culture and PCR methods. Samples from infested counties were examined by California Department of Food and Agriculture (CDFA) for pathogen identification and by University of Calfornia, Davis in un-infested counties. Of the 496 sites assessed, *P. ramorum* was found at 32 of the 119 sites occurring within 10 km of a known occurrence, but only one of the 376 sites occurring further than 10 km from an already confirmed occurrence of *P. ramorum*. The pathogen was not discovered in any un-infested counties. These results suggest that *P. ramorum* has not spread significant distances over the past two years in California's forest environments. While these results are encouraging, the extensive size of California allowed us to assess only a small fraction of the state's high-risk habitats even with targeted sampling and a relatively large sample size. Moreover, almost 80 percent of California's most threatened forests are privately owned, which presents challenging access issues for early detection and management of disease spread. Given the extensive number and diversity of threatened habitats in California, risk-based site selection is an effective tool for early detection monitoring and protection. This strategy may play an especially important role in identifying emergent infections before they have established significantly.

Figure 1—Distribution of site locations and results of the early detection across the state of California.



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Phytophthora ramorum Detection Surveys for Forests in the United States[1]

S.W. Oak[2], W.D. Smith[3], and B.M. Tkacz[4]

Key words: forest survey, *Phytophthora ramorum*, risk rating

Abstract

Diseases caused by *Phytophthora ramorum* in forest landscapes of North America are presently confined to areas of the Pacific coast in the states of CA and OR. However, the vulnerability of other ecosystems is suggested by the discovery in Europe and the U.K. of disease in hosts which are abundant in oak-dominated ecosystems of eastern North America; successful greenhouse inoculation trials of these and other potential eastern tree and shrub hosts; and brisk domestic and international trade in woody ornamental hosts. Overlay analysis was used to project low, moderate or high risk of *P. ramorum* diseases to U.S. forests using spatial estimates of overstory and understory host presence and density, climatic variables, and potential pathways of pathogen introduction (Smith and others 2002). The results showed a band of high risk along the length of the Pacific coast including WA, OR and CA, which included currently infested areas, but most of the high-risk area was in the East, centered in the Southern Appalachian Mountains.

Federal and state forest management agencies in seven eastern states encompassing most of the high risk area joined in pilot tests of early detection survey methods in 2003. Objectives were to field test survey methodology, develop diagnostic capacity, and determine *P. ramorum* status. The risk map guided sampling intensity. *Rhododendron* spp. and *Kalmia latifolia* were examined for foliar symptoms and *Quercus* spp. bark for bleeding cankers on 4-100 meter transects per survey location. Diagnosis for the presence of *P. ramorum* was by nested PCR (Hayden and others 2004) conducted on symptomatic tissue at laboratories located in six of the seven cooperating states. Foliar samples consisted of a 10-leaf composite while bark samples came from single cankers. Quality assurance of diagnostic results was

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accomplished on replicate samples from half of all transects at a separate laboratory. Over 1,100 bark and foliage samples were collected from the forested perimeter of 54 woody ornamental nurseries and from 118 general forest areas during the growing season of 2003 (*Table 1*). *P. ramorum* was not detected in any sample.

Survey methods were slightly modified and implemented in a greatly expanded survey in 2004 in response to the discovery that *P. ramorum*-infected ornamental nursery stock had been shipped throughout the United States during the previous year. The target host list was expanded to eight foliar and three bark host genera. Top survey priority was assigned to the perimeters of trace forward woody ornamental nurseries. A total of 36 states encompassing all of the projected high and moderate risk area conducted surveys in 2004. The cumulative total of locations surveyed is now 1,119, and 5,633 symptomatic tissue samples have been tested. *P. ramorum* was detected by these methods during 2004 only in two samples from *Q. agrifolia* growing in Golden Gate Park, San Francisco, CA. This area is in the heart of the CA quarantine area surrounded by the infested counties of Marin, Contra Costa, Alameda and San Mateo. These results strongly suggest that *P. ramorum* is not native to eastern North American oak forests. Further, they demonstrate that the pathogen is not yet widely established in forest landscapes outside of currently regulated areas on the Pacific coast, even in close proximity to woody ornamental nurseries located in high risk areas receiving infected stock.

Table 1—*Number of locations surveyed, samples collected, and diagnostic results for Phytophthora ramorum detection surveys in U.S. forests in 2003 and 2004.*

Year of survey	Location type	Number of locations	Number of samples	*P. ramorum* positive
2003	Nursery perimeter	54	279	0
	Forest	118	837	0
	Subtotal	172	1,116	0
2004	Nursery perimeter	681	3,207	0
	Forest	266	1,310	2[1]
	Subtotal	947	4,517	2
Grand total		**1,119**	**5,633**	**2**

[1]*P. ramorum* detected in San Francisco County, CA

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Phytophthora ramorum Experience and Approach in the Netherlands[1]

M.H.C.G. Steeghs[2] and J. de Gruyter[2]

Abstract

Phytophthora ramorum was found for the first time in the Netherlands in 1993. In 2001 a risk analysis was done. The results initiated a program to investigate the spread in the Netherlands and to develop measures to prevent further spread. The measures outside the nurseries gave rise to intensive discussions with the managers and owners of these areas. In 2002 the European Union (E.U.) Commission Decision aimed at preventing introduction and spread of the pathogen and gaining insight in the distribution came into force. During the first season of inspections 4 percent of the nurseries growing host plants had *P. ramorum* infections on rhododendron or viburnum. In the second season of inspections the number of infections at the nurseries dropped to 1.5 percent and in the current season, 2004-2005, the incidence of *P. ramorum* has dropped even lower. The measures appear to be effective. Outside the nurseries 2 percent of the inspected rhododendron sites had infections. During the follow-up survey at the infested sites two infestations in *Quercus rubra* were found. The owners had to remove the infestation or prevent people from entering the area. Eradication from the Netherlands territory is not considered feasible. In addition to these government measures there is also a national campaign to encourage owners/managers of green spaces to remove infested plants and as a result lower the inoculum pressure.

Key words: infestation, Netherlands, nurseries, plant quarantine policy, public and private green spaces

Nurseries

At the nurseries Naktuinbouw, Netherlands Inspection Service for Horticulture, inspects the host plants under responsibility of the Plant Protection Service. In case of a suspicion the follow-up activities are handed over to the Plant Protection Service. The Plant Protection Service conducts the diagnoses and imposes the treatment measures. For the diagnosis the Taqman ITS-PCR is used combined with isolation of the pathogen. To determine the type of *P. ramorum* the isolates are tested with PCR-RFLP based on Cytochrome oxidase subunit 1 gene. Since the beginning of the inspections for *P. ramorum* the number of infestations has

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declined sharply from 4 percent in 2002-2003 to 1.5 percent in 2003-2004 to 0.5 percent in the first part of the season in 2004-2005. In addition to the effectiveness of the measures, awareness by the growers and a shift in the assortment contributed to the decline. Infestations were found in rhododendron, viburnum and in one case in *Taxus* x *media*. During the first season the majority of the infected lots were *Viburnum* x *bodnantense* Dawn.

Public and private green spaces

During the preliminary survey outside the nurseries in 2001 infestations were found only in rhododendron. Based on this experience the extensive survey of 2002-2003 was directed towards rhododendron. Sites with rhododendron and at least one other "potential" host plant were selected for inspection. Infestations were found at 30 of the 1400 inspected sites with rhododendron. Because of the known susceptibility of *Vaccinum myrtillus* an additional 100 sites were inspected. No additional infestations were found. The infestations in rhododendron were mostly found in old plants growing in the shade. During the follow-up inspections two infections were found in *Quercus rubra*, and none in the other "potential" host plants. In this survey more than 900 samples were tested of 18 different "potential" host plants.

Treatment measures included complete removal of the plants or cutting back the plants to below 50 cm. At 50 percent of the sites where infested rhododendron had been cut back, re-growth was partly found infected. Relying on the symptoms, newly developing shoots get directly infected from the stumps. Another observation was that the delimitation for the measures at the larger infestations was in general too narrow. The measures have been adjusted for these deficiencies. The period that the infestation remains under observation is extended to at least one year. The widespread occurrence of the disease combined with the difficulties in eradication led to the conclusion that eradication of *P. ramorum* from the territory of the Netherlands is not feasible.

Policy

The aim of the program is to protect the indigenous plants by trading healthy plant material, eradication at the nurseries and containment outside. At the nursery level the program is successful. For the public and private green spaces the conclusion is that especially the larger infected sites with a high inoculum pressure pose a risk for our indigenous plants.

The containment measures are based on the strong recommendation to remove the infestation and if the infestation is not removed the owner has to make sure that the area is not accessible. Until now all owners, with one exception, have chosen to remove the infestation. Taking into account the spread outside the nurseries it is not possible for the government to locate all infestations. Therefore a national campaign has been initiated to lower the inoculum pressure. The aim is that the owners/managers of the public or private green spaces take responsibility

in removing infestations. Part of this campaign is intensive communication and the development of management programs. The management programs are directed at removing the infestation as well as improving the growing practices. Practices include the use of non-host species, planting of host plants under conditions where the pathogen doesn't thrive, maintenance of plantations with rhododendron, and removing shade trees. It is also recognized that there can be variation to the recommended management practices. The primary expenses are the costs of removal and destruction of infested plant material. The costs can be reduced by more than 60 percent by allowing the plant material to be chipped and left outside. More feasible, but adequate measures, make it easier for the owner to follow the recommendations.

The sustainability of this approach still needs to be proven, but with more and more fungi being identified as a threat to the public and private green spaces, the efforts by managers and owners of the public and private green spaces will be required in addition to the efforts by the government.

Pre-epidemic Mortality Rates for Common *Phytophthora ramorum* Host Tree Species in California[1]

T.M. Barrett[2]

Abstract

Understanding the impacts of *Phytophthora ramorum* on forests will require knowledge of pre-disease distribution, abundance, and rates of change for affected species. This study estimated pre-epidemic mortality rates for nine common host tree species: bigleaf maple (*Acer macrophyllum*), California bay laurel (*Umbellularia californica*), California black oak (*Quercus kelloggi*i), canyon live oak (*Quercus chrysolepi*s), coast live oak (*Quercus agrifolia*), Douglas-fir (*Pseudotsuga menziesii*), madrone (*Arbutus menziesii*), coastal redwood (*Sequoia sempervirens*), and tanoak (*Lithocarpus densiflorus*) using inventory data from 1981 to 1984 and 1991 to 1994 statewide inventories of private (and some public) forest land. Mortality rates that were developed represent the average annual mortality for trees that were at least 12.5 cm in diameter at 1.4 m above the ground (dbh) at the time of the first (1981-1984) inventory. Natural mortality for all trees was estimated at 0.5 percent of trees per year, with rates for individual host species ranging from 0.1 to 0.6 percent. Overall, growth exceeded natural mortality and harvest for most host species between 1981 to 1984 and 1991 to 1994. Tanoak, the most numerous tree species in the quarantined counties, increased in volume and number of trees by more than 15 percent over the decade.

Key words: forest change, forest health, forest monitoring, oak decline, sudden oak death

Introduction

As of January of 2005, 14 coastal counties in California (Alameda, Contra Costa, Humboldt, Lake, Marin, Mendocino, Monterey, Napa, San Mateo, Santa Clara, Santa Cruz, San Francisco, Solano and Sonoma) were quarantined for *Phytophthora ramorum*. There are more than two million hectares of forestland in this quarantined

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area, with more than two-thirds of the forest in private ownership.[3] Among other factors, the extent of the affected area, the prevalence of host species for *P. ramorum* within the forestland, and the associated mortality of some common host species result in the possibility of substantial effects on forest ecosystems.

Monitoring the impacts of the disease on forests requires knowledge of pre-disease distribution and abundance of affected species. Most tree species have populations that are not in equilibrium from decade to decade, with natural and human-caused impacts, such as wildfire, climate change, forest management, and development, resulting in long-term change even for very large geographical regions. Therefore, in addition to knowledge of pre-disease distribution and abundance, pre-disease rates of change (mortality, recruitment, and growth) are also important for understanding disease effects.

Unfortunately, mortality is one of the most difficult of commonly measured forest attributes to estimate precisely for large regions. Reasons include its relative infrequency, spatially and temporally correlated occurrence, and the necessity of having inventory systems in place before the time period of interest. In this paper, mortality rates for host tree species in California are developed using available statewide inventory data for the time period 1981-1984 to 1991-1994. The estimated rates are accompanied by additional information on net change in quarantined counties to provide context for their interpretation.

Methods

Mortality was calculated using data from a 1981-1984 statewide inventory of forestland in California that was remeasured in 1991-1994. The inventory was conducted by the USDA Forest Service Forest Inventory and Analysis program and excluded forestland on national forests and lands reserved from timber harvesting (Waddell 1996). There were 4,824 sampling units (plot locations) distributed throughout the state; 1010 of these were located in timberland and were used for estimating tree mortality on timberland. I also used a supplementary woodland inventory with 1218 sampling units; of these, 180 plots were located on accessible woodland and were used for estimating mortality of woodland trees. At the initial visit, live trees that were 17.5 cm in diameter at 1.4 m above ground (dbh) were sampled with a basal area prism on five points located around a central subplot; smaller trees were sampled with five fixed-area plots. The field crew tagged trees greater or equal to 12.5 cm dbh and recorded the azimuth and distance to trees to aid

[3] California inventory 2001-2003 (unpublished data on file), Forest Inventory and Analysis, Pacific Northwest Research Station, Portland, Oregon.

in identification at the next inventory. At the 1991-1994 inventory, trees that had died in the intervening years were recorded as either (1) harvested; (2) culturally killed (including girdling or spraying with herbicide); or (3) naturally dead (USDA Forest Service 1994). Trees that died and were subsequently harvested probably would have been recorded as harvested.

Annual mortality was based on the number of growing seasons between measurement dates for the plots. Annual mortality was calculated as a simple arithmetical average over the remeasurement interval, rather than an annually compounded value. The relatively short remeasurement interval resulted in only small differences between these methods. I used combined ratio estimators from Cochran (1977) to calculate mortality and standard error. Point-in-time estimates were made using double sampling for stratification (Cochran 1977). The mortality rates are an estimate of the total number of trees that died in the population divided by the total number of initial live trees in the population. The probability of inclusion for mortality trees was based on the 1981-1984 inventory, adjusted for plots on land that transferred to national forest ownership or reserved status.

The estimated forest land in the population was 4.8 million hectares. Because of the difficulty of reestablishing plots when land is disturbed, mortality from permanent conversion of forest land to other uses, such as residential development or roads, is not included in rates below. The estimated rate of conversion of forest to nonforest between 1981-1984 and 1991-1994 was less than 1 percent per decade; even if all trees on these plots were killed, it would be a minor component of mortality statewide. Plots where the owner denied access to the property add some additional uncertainty to estimates.

Host species for which mortality rates were estimated included bigleaf maple (*Acer macrophyllum*), California bay laurel (*Umbellularia californica*), California black oak (*Quercus kelloggii*), canyon live oak (*Quercus chrysolepis*), coast live oak (*Quercus agrifolia*), Douglas-fir (*Pseudotsuga menziesii*), madrone (*Arbutus menziesii*), coastal redwood (*Sequoia sempervirens*) and tanoak (*Lithocarpus densiflorus*). Other host species were either not considered trees (e.g., Shreve's oak) or were uncommon enough in California to preclude meaningful estimates of mortality (e.g., California buckeye).

Results

When all tree species in California were combined, the estimated rate of natural mortality was 0.5 percent per year (*table 1*). When culturally killed and harvested

trees are included, overall mortality doubled to 1.0 percent per year. Standard errors were high, particularly for woodland species such as coast live oak. Coastal redwood had a very low rate of natural mortality of 0.1 percent per year. Mortality from harvesting and cultural treatments was higher for commercial species (Douglas-fir and redwood) and tanoak, a frequent associate.

Table 1—*Average annual mortality in California from 1981-84 to 1991-94 by species for trees >= 12.5 cm dbh*[1]

	Average annual mortality[2]		**Average annual natural mortality**	
Species	**Rate**	**SE**[3]	**Rate**	**SE**
	----------*Percent of initial live trees* ---------			
California black oak	0.76	0.18	0.58	0.15
Coast live oak	0.59	0.33	0.49	0.29
Tanoak	1.39	0.29	0.36	0.08
Bigleaf maple	0.56	0.28	0.47	0.27
California-laurel	0.69	0.34	0.45	0.29
Canyon live oak	0.40	0.19	0.27	0.18
Douglas-fir	1.20	0.17	0.30	0.06
Pacific madrone	0.84	0.22	0.56	0.17
Redwood	1.53	0.37	0.10	0.03
All California tree species	1.05	0.11	0.49	0.07

[1] Excludes national forests, reserved forests, and nonforest.
[2] Includes trees that were harvested, culturally killed, or died of natural causes.
[3] SE is standard error.

These rates can serve as a baseline for future monitoring of the effects of the disease. However, whether the population of a particular tree species is at equilibrium depends on how natural and human-caused mortality is balanced with regeneration and growth. Because the age and size distribution of trees can also change, population trends may differ depending on whether these are assessed by number of trees, biomass, foliage, or other attributes.

In this case, I chose to look at (1) numbers of trees and (2) volume as indicators for change in the populations of host tree species. Using volume as an indicator is useful because it allows comparison to published estimates of change on California timberlands between the late 1960s and 1981-1984; in addition, it is typically very closely correlated with above-ground tree biomass. In the fourteen California counties under quarantine in January of 2005, the *P. ramorum* host species bigleaf maple, California black oak, California bay laurel, canyon live oak, Douglas-fir, and tanoak all showed volume increases between 1981-1984 and 1991-1994 (*table 2*).

Table 2—*Initial volume in 1981-84 and change in volume from 1981-84 to 1991-94 by P. ramorum host species for 14 California counties under quarantine in January, 2005*[1,2]

		Annual net change in volume		
Species	**1981-84 Volume**	**Change**	**SE**[3]	**Change over decade**
	----------*Thousand cubic meters*----------			---- *Percent* -----
Bigleaf maple	2,327	+ 64	15	+ 28
California black oak	15,565	+ 200	48	+ 13
California laurel	12,614	+ 326	89	+ 26
Canyon live oak	10,182	+ 182	62	+ 18
Coast live oak	29,632	+ 322	185	+ 11
Douglas-fir	82,503	+ 1,520	338	+ 18
Pacific madrone	30,015	+ 370	84	+ 12
Redwood	124,674	+ 481	654	+ 4
Tanoak	38,554	+ 952	191	+ 25
All 9 species	346,065	+4,418	957	+ 13

[1] Does not include national forests or lands reserved from timber harvesting
[2] Volume calculated for trees at least 12.5 cm dbh, stump to 10 cm top, discounted for cull.
[3] SE is standard error

However, looking at the same populations in terms of number of trees shows somewhat different results (*table 3*). Canyon live oak, tanoak and Douglas-fir still show large percentage increases in the quarantined region. The difference in the increase for Douglas-fir volume (an 18 percent increase) compared to the increase in number of trees (a 28 percent increase) is characteristic of a shift in the population from larger to smaller trees. California black oak and coast live oak had higher estimates of change in volume (*table 2*) than change in numbers of trees (*table 3*). This is the result of a shift in the size distribution from smaller to larger trees, perhaps caused by low recruitment of trees crossing the 12.5 cm dbh threshold.

Table 3—*Initial trees in 1981-84 and change in trees from 1981-84 to 1991-94 of P. ramorum host species for 14 California counties under quarantine in Jan. 2005*[1, 2]

		Change in tree numbers		
Species	**1981-84 Trees**	**Net change**	**SE**[3]	**Change over decade**
	1000 trees	---------- *1000 trees / decade* ------		*Percent*
Bigleaf maple	6,175	+ 1,666	704	+ 27
California black oak	34,311	- 105	794	- 0
California laurel	48,051	+ 5,714	3,420	+ 12
Canyon live oak	25,017	+ 4,253	2,652	+ 17
Coast live oak	84,014	+ 757	4,293	+ 1
Douglas-fir	76,472	+ 21,109	4,103	+ 28
Pacific madrone	70,796	- 938	1,816	- 1
Redwood	114,322	+ 5,527	4,386	+ 5
Tanoak	159,927	+ 28,553	5,740	+ 18
All 9 species	637,272	+ 69,023	12,116	+ 11

[1] Does not include national forests or lands reserved from timber harvesting
[2] All trees with dbh ≥ 12.5 cm
[3] SE is standard error

Discussion and Conclusion

One question of interest is whether these rates of change were unique to the time period of 1981-1984 to 1991-1994. For the north coast region of California, hardwood growing stock volume on timberland was reported to increase by an average annual rate of 2.7 percent from 1964-1967 to 1981-1984 (Lloyd and others 1986). For the central coast region, hardwood growing stock volume was estimated to increase by an average annual rate of 1.1 percent between 1968-1971 and 1981-1984 (Colclasure and others 1986). For the state, Bolsinger (1988) reported net annual volume growth on timberland of 3.5 percent for tanoak, 3.1 percent for Pacific madrone, 3.5 percent for California bay laurel, and 2.4 percent for California black

oak.[4] The 1981-1984 to 1991-1994 average annual volume increases reported here for forest land in the quarantined counties (*table 2*) are 1.7 percent for tanoak, 1.1 percent for Pacific madrone, 2.2 percent for California laurel, and 1.6 percent for California black oak.

If the 1960s to 1980s values were combined with the 1980s to 1990s values, then cumulative volume increases for these four species would range from 40 to 60 percent over 20 years. The estimates cannot actually be combined because the geographic regions in the published reports do not coincide with the quarantined counties discussed in this paper. However, the increase of volume reported here for hardwood timberland species (bigleaf maple, California black oak, California bay laurel, Pacific madrone, and tanoak) appears to be consistent with a longer term trend in coastal California counties between 1964-1971 and 1991-1994.

Ultimately, populations of host species will be determined by levels of disease resistance (Garbelotto and others 2001) along with the pre-disease distribution and abundance of host tree species. The results shown here suggest that in the 10 years prior to the first observation of the disease, several host tree species were undergoing significant shifts in total numbers and volume, along with changes in individual tree size distribution within the population. Effects of the disease are most likely to be compared to conditions prior to its discovery, or as ongoing measurements as the disease progresses. However, it also may be useful to keep in mind that the populations of species affected by *P. ramorum* were not in an equilibrium state in the decades prior to the discovery of the disease.

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[4] Values in Lloyd, Moen, and Bolsinger (1986), Colclasure, Moen, and Bolsinger (1986) and Bolsinger (1988) are for unreserved timberland outside of national forests. Bolsinger (1988) values cited here do not include harvest, which was 6 percent of net annual growth.

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Sudden Oak Death Disease Progression in Oaks and Tanoaks[1]

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Key words: California black oak, coast live oak, *Phytophthora ramorum*, survival analysis, tanoak

Abstract

In March 2000, we established twenty disease progression plots in Marin County to monitor the progress of sudden oak death symptoms in coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and tanoak (*Lithocarpus densiflorus*) (McPherson and others 2005). Plots were located to encompass a variety of habitat types and species combinations, ten in China Camp State Park and ten in Marin Municipal Water District. We monitored every coast live oak (731), California black oak (52), and tanoak (181) with a stem diameter greater than 2.5 cm, four times a year. Trees were evaluated by symptom and not sampled for *Phytophthora ramorum* to avoid affecting disease progression. Bleeding coast live oaks were found to follow a consistent and predictable sequence: bleeding, then beetle colonization, followed by emergence of *Hypoxylon thouarsianum* reproductive structures, and then death. Results are reported for the period 2000 through 2003. For all three species, the combined number of bleeding and dead trees increased by 2003, with the greatest increases seen in tanoaks. The percentage of living coast live oaks that were bleeding remained relatively constant, at around 25 percent. The response of California black oaks was very similar to coast live oaks. The proportion of bleeding tanoaks increased from 39 percent to 72 percent. Through 2003, every plot with symptomatic coast live oaks in 2000 showed increased numbers of bleeding and dead trees. Approximately 50 percent of bleeding coast live oaks were under active attack by ambrosia and bark beetles (Coleoptera: Scolytidae) or showed

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past beetle activity during each March sampling period, from 2000 to 2003. Beetles had colonized every bleeding coast live oak that died during this study while each was still alive. Beetles colonized tanoaks much less consistently than coast live oaks during the study period. Structural failure on the main stem (bole) occurred in bleeding coast live oaks with a much greater frequency than in asymptomatic trees of this species, or in either bleeding California black oaks or tanoaks.

We used two approaches, Weibull regression and Cox Proportional Hazards models (Lee and Wang 2003), to describe the expected survival of coast live oaks and tanoaks, based on the disease progression symptoms described above (status in 2000). Weibull regression is a parametric model that requires assumptions about the distribution of the data and can be used to project survival trends beyond the time frame of the input data. The Cox Proportional Hazards model is non-parametric, thus requiring fewer assumptions about the data, but cannot be used to predict future outcomes. The models provided very similar estimates for survival of trees in the following initial disease categories; asymptomatic, bleeding only and bleeding with beetle colonization. Survival times were derived from the Weibull distribution (*fig. 1*). The median survival time for coast live oaks declined considerably as a function of disease state: asymptomatic, 29.5 yr; bleeding only, 7 yr; and bleeding with beetles, 2.6 yr. The presence of *H. thouarsianum* fruiting bodies in addition to beetle activity did not change survival. The median survival times for tanoaks also declined in this order: asymptomatic, 12.6 yr; bleeding only, 8.7 yr; and bleeding with beetles, 2.9 yr. The Weibull model was used in a health analysis to estimate the median asymptomatic time for tanoaks and coast live oaks, as a function of stem diameter (dbh). For both species, the predicted asymptomatic times declined as diameter increased, with the greater decline seen in tanoaks (*table 1*).

The impacts of sudden oak death on its principal fagaceous host trees continued to increase from 2000 through 2003. This effect was greatest in tanoaks. Scolytid beetles were consistently associated with approximately half the bleeding coast live oaks at any date. The tunneling activity of these beetles may contribute to structural failure in living trees. Stem diameter was positively associated with bleeding symptoms in both coast live oaks and tanoaks. Among all bleeding coast live oaks, beetles were positively associated with larger diameter trees (*fig. 2*). The Weibull and Cox Proportional Hazards models predict that bleeding tree survival will decline following beetle attacks and found that the presence of beetles was a better predictor of mortality than *H. thouarsianum* fruiting. These models also suggest that time from initial symptoms to death is similar for coast live oaks and tanoaks. The accuracy of the model-derived estimates is constrained by the limited period of observation and uncertainty about the timing of the first appearance of each disease stage. However, this initial effort to establish a time sequence for sudden oak death should facilitate

comparisons of disease progression in different host species and species combinations if *P. ramorum* becomes established in different geographic areas.

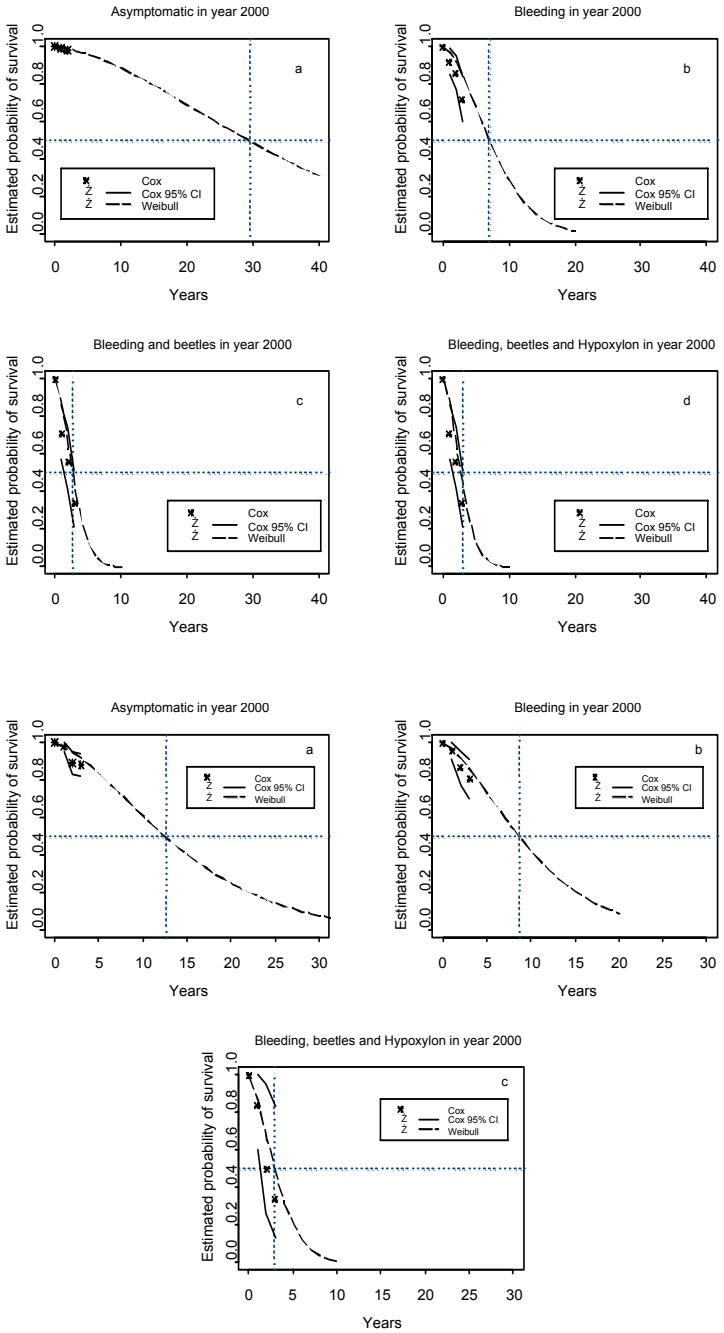

Figure 1—Weibull and Cox Proportional Hazards survival models for same-symptom cohorts of coast live oak (the four upper graphs) and tanoak (the three lower graphs); vertical dashed lines denote the predicted median survival times. All regressions were statistically significant ($p < .001$).

Table 1—*Weibull health analysis of coast live oaks and tanoaks, showing the median asymptomatic time in years (± standard error) as a function of stem diameter (dbh) in 2000.*

	Diameter (dbh) in cm					
Species	**10**	**20**	**40**	**60**	**70**	**80**
Coast live oak	18.5 (4.8)	15.9 (3.5)	11.4 (2.0)	8.1 (1.6)	-	5.8 (1.5)
Tanoak	3.8 (0.4)	3.2 (0.3)	2.3 (0.4)	1.6 (1.8)	1.4 (0.5)	-

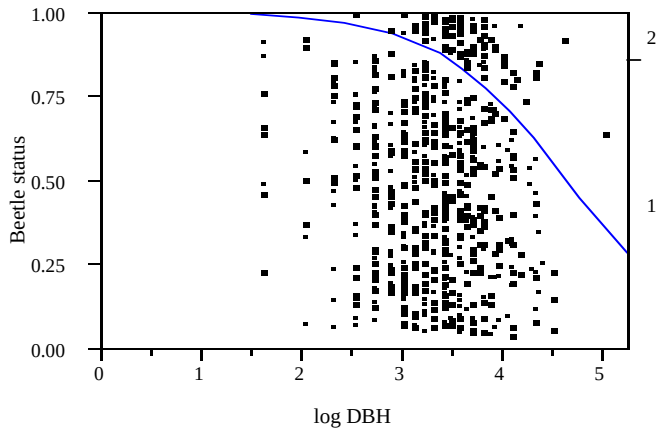

Figure 2—Beetles colonized bleeding coast live oaks as a function of stem diameter. Beetle status; 1 = no colonization, 2 = beetle colonization; analyzed by logistic regression ($p < 0.001$).

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Disease Risk Factors and Disease Progress in Coast Live Oak and Tanoak Affected by *Phytophthora ramorum* Canker (Sudden Oak Death)[1]

Tedmund J. Swiecki[2] and Elizabeth Bernhardt[2]

Abstract

This paper reports on five years of observations in a case-control study examining the role of tree and site factors on the development of *Phytophthora ramorum* stem canker (sudden oak death) in coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). In September of each year from 2000 through 2004, we collected data on *P. ramorum* symptoms, tree condition, midday stem water potential (SWP), and various other factors in 150 circular plots (8 m radius). Each plot was centered around a case (symptomatic) or control (asymptomatic) plot center tree. Plots were located at 12 locations in the California counties of Marin, Sonoma and Napa in areas where *P. ramorum* canker was prevalent in 2000. Between September 2000 and September 2004, the percentage of symptomatic coast live oak trees in the plots increased slightly, from 23 percent to 24 percent. Over the same period, the percentage of symptomatic tanoaks increased from 31 percent to 43 percent. Between 2000 and 2004, mortality due to *P. ramorum* increased from 4 percent to 9 percent in coast live oak and from 12 percent to 23 percent in tanoak. About 58 percent of coast live oak and 47 percent of tanoak study trees with disease symptoms in 2000 progressed to a more advanced disease severity class by 2004. In both species, some symptomatic trees developed callus tissue along at least part of the canker margin where canker expansion was apparently inhibited. In some infected trees, cankers have not changed in size for several years and appear inactive. Diffuse canopy dieback commonly develops in coast live oaks with advanced *P. ramorum* canker symptoms that survive for at least several years. Most coast live oaks and tanoaks with *P. ramorum* canker symptoms have maintained relatively high stem water potential (SWP) levels and do not show progressive increases in water stress as disease develops. For coast live oak, trees with low water stress (high SWP) are more likely to develop *P. ramorum* canker, but subsequent disease progress in symptomatic trees is not related to SWP. Only two of numerous bark characteristics were associated with *P. ramorum*

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canker in coast live oak. The presence of unweathered, brown bark in bark furrows was the only bark surface characteristic that was positively correlated with disease. This characteristic may be associated with faster rates of bole radial growth, and is consistent with other analyses indicating that faster-growing coast live oaks may have a greater risk of developing *P. ramorum* canker than slow-growing trees. Bark thickness was positively correlated with both the risk of developing *P. ramorum* canker and the likelihood of disease progress among infected trees. Because bark thickness also increases with stem diameter, it is possible that the lack of cankers on small coast live oak stems and branches could be related to their relatively thin bark.

Key words: bark morphology, bark thickness, *Phytophthora ramorum,* resistance, stem water potential, sudden oak death, water stress

Introduction

Phytophthora ramorum, the causal agent of sudden oak death, causes bleeding bark cankers on the main stems of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and several other oak species in California (Garbelotto and others 2001, Rizzo and others 2002). The bark cankers can expand over time and eventually girdle susceptible trees. The sapwood-decaying fungus *Hypoxylon thouarsianum*, ambrosia beetles (*Monarthrum* spp.), and oak bark beetles (*Pseudopityophthorus* spp.) are commonly associated with *P. ramorum*-infected trees in later stages of decline (Garbelotto and others 2001).

We initiated a long-term study to follow disease progress and evaluate disease risk factors in the summer of 2000, shortly after *P. ramorum* (then unnamed) was identified as the cause of sudden oak death. Most of the study trees are coast live oak, but we collected parallel data on tanoak at two locations for comparative purposes. We used a case-control study design to test whether various tree factors and plot/stand factors were related to the development of *Phytophthora* bole cankers in coast live oak in areas where the disease was common. Models based on results from the first three years of this project (Swiecki and Bernhardt 2001, 2002a,b) were the first to document that California bay laurel (*Umbellularia californica*) cover and density near coast live oak is significantly correlated with disease risk. Other predictors of disease risk in coast live oak included canopy dominance (canopy sky exposure), plot canopy cover, stem water potential (SWP, a measure of water stress), tree decline associated with other agents, stem diameter, and multiple main stems. Based on the effects of predictors related to tree growth rate, we inferred from these models that trees with faster growth rates (associated with larger diameter, higher

SWP, greater sky exposure, lack of decline from other agents) had an elevated risk of *P. ramorum* canker.

By collecting data on disease status over time in these plots, we have been able to determine how the disease status of these trees has changed over time, improving our overall concepts of disease risk and disease progress in this population. In addition, between 2000 and 2004 we have collected data on additional variables that were not considered in the original study design. In particular, bark morphology characteristics, including bark thickness, have been included in analyses reported in this paper.

Repeated observations on the trees in these study plots over the past five years has also provided detailed information on long-term symptom development and disease progress in this cohort. These observations include ratings of canker expansion, the abundance of *H. thouarsianum* stromata and beetle boring, changes in canopy health, branch and main stem failures and September water stress levels, as indicated by SWP. From these observations, we have been able to document patterns of disease progress that were not apparent when sudden oak death was originally described in California (Garbelotto and others 2001, Rizzo and others 2002).

Methods

Plot selection

The methods used to select study locations and plots have been previously described (Swiecki and Bernhardt 2002a). During September 2000, we established plots and collected data at 12 study locations (*table 1*). Locations were chosen in areas where *P. ramorum* was prevalent at that time.

At each study location, we collected data in circular 8 m radius (0.02 ha) plots, each of which was centered around a case or control tree. Case trees had only early symptoms of *P. ramorum* canker (bleeding cankers), with the exception of nine coast live oak cases that had minor amounts of beetle boring or a few small *H. thouarsianum* stromata, typically on a single scaffold or on a localized portion of the bole. Control trees were lacking any *P. ramorum* canker symptoms. In 2000, we established 128 coast live oak plots (53 cases, 75 controls) and 22 tanoak plots (10 cases, 12 controls).

Table 1— *Locations of plots and host species studied*

Location number	Location	County	Number of plots	Tree species
1	Marin Municipal Water District (MMWD) watershed - Azalea Hill area	Marin	12	coast live oak
2	MMWD-Pumpkin Ridge south	Marin	16	coast live oak
3	MMWD-Pumpkin Ridge north	Marin	11	coast live oak
4	MMWD-Phoenix Lake area	Marin	11	coast live oak
5	China Camp SP - Miwok Meadows area	Marin	16	coast live oak
6	China Camp SP - SE Buckeye Point area	Marin	12	coast live oak
7	Woodacre (Private land)	Marin	12	coast live oak
8	Lucas Valley (Private land)	Marin	12	coast live oak
9	Muir Woods NM / Mt. Tamalpias SP	Marin	10	tanoak
10	Wall Road (Private land)	Napa	13	coast live oak
11	Marin County Open Space land, Novato	Marin	13	coast live oak
12	Jack London SP	Sonoma	12	tanoak

Disease ratings

Trees in plots were evaluated each year in September of 2000 through 2004. Tree species were rated as trees if they had at least one stem 3 cm in diameter at 137 cm height (DBH).

For all canker host trees (coast live oak, tanoak, and California black oak) in the plots, we noted whether *P. ramorum* canker symptoms were present on the main stems and the stage of disease development. We did not assess tanoaks for the presence of *P. ramorum* twig cankers. Trees were classified as having early symptoms if only bleeding cankers were present. We defined late disease symptoms as including cankers and *H. thouarsianum* stromata and/or beetle boring. Trees killed as a result of *P. ramorum* cankers were classified as the dead *P. ramorum* symptom class. Initially symptomatic trees that showed no bleeding or other evidence of disease progress for at least two consecutive years were classified as having inactive infections if cankers were still visible and were rated as asymptomatic if external canker symptoms had disappeared. Severe decline (poor condition likely to result in tree death within 10 years) and death due to agents other than *P. ramorum* (primarily canker rot fungi) were also evaluated for each tree.

Disease ratings were based primarily on visual assessments. Chipping of bark to expose or sample cankers was minimized to avoid affecting future observations on the study trees. For some symptomatic trees, tissue pieces were placed into PARP semi-selective media (Erwin and Ribeiro 1996) to isolate the pathogen. Identifications of cultures were made by specialists at the lab of David Rizzo (University of California, Davis). Isolations were primarily restricted to atypical cankers and new cankers that appeared after 2000. In a few instances, isolations attempted in successive years have yielded negative results one year and positive results the next year. Because isolations can result in false negative results, especially in trees with older cankers sampled late in the season, trees with very typical *P. ramorum* cankers were rated as symptomatic even if isolation results were negative. For a few trees with ambiguous disease symptoms, tree disease symptoms in later years of the study were used to retrospectively reassign disease status for data analysis. The majority of these reassignments were trees rated as symptomatic in 2000 that were reclassified as asymptomatic due to a lack of symptoms in all subsequent years.

We collected additional detailed information on the extent of *P. ramorum* canker symptoms in plot center trees and plot trees used for additional water potential measurements (extra SWP trees, see below). Trunk girdling by *P. ramorum* cankers, *H. thouarsianum,* and beetles were scored by estimating the percent bole circumference girdled (0 to 6 scale described below) as if all affected areas were projected onto the same cross section. In 2003, we also recorded the apparent height above the soil of the lower margin of the lowest *P. ramorum* canker and the upper margin of the highest *P. ramorum* canker for coast live oak trees only. Bleeding, externally visible bark symptoms, and in some trees, limited chipping of outer bark, were used to delimit the extent of cankers. We also used the number of bleeding areas to estimate the number of *P. ramorum* cankers present each year.

We also assessed overall (chronic) canopy thinning (none, slight, definite), the presence of epicormic branches (none, few, numerous), and recent canopy dieback (0 to 6 scale, below). Decay impact ratings (none, low, moderate, high) were made by assessing the probability that current apparent decay levels would negatively impact tree health or survival. The assessment of decay impact was based on the type(s) of decay present, location of decay within the tree, and the estimated extent of decay as rated by a trained observer. Additional details of the tree health rating methods are presented in Swiecki and Bernhardt (2001).

Stem water potential determinations

In September of each year (2000 to 2004), we collected midday stem water potential (SWP) readings on the center tree in each plot during the peak midday period (about 1300 to 1530 PDT). SWP methods followed those outlined by Shackel (2000) and have been previously reported (Swiecki and Bernhardt 2002a). In addition, starting in 2001, we took SWP readings on plot trees other than the plot center tree (extra SWP trees) in 45 of the 150 plots for comparative purposes. One extra SWP tree was used in 44 plots; the remaining plot had two.

Additional tree and plot variables

Plot center trees and the 47 extra SWP trees were also rated for origin class (seed or coppice), stem count, DBH, and amount of crown exposure to overhead sunlight (0 to 6 scale, below). Plot variables recorded included plot slope and aspect, total basal area, tree counts by species, plot canopy cover, woody understory cover, cover of selected tree species and poison oak (*Toxicodendron diversilobum*). We also recorded the disease status of all other coast live oak, California black oak, and tanoak trees in the plot with respect to *P. ramorum* and other pathogens, and counts of regeneration of these three species. Coast live oak, black oak and tanoak trees other than the plot center tree are collectively referred to as plot trees in this paper.

In 2003, we collected data on physical characteristics of the bark of coast live oak plot center and extra SWP trees at all locations except 9 and 12, which did not include coast live oaks. We used a variety of descriptors to rate bark morphology of the lower bole. Bark characteristics rated included the abundance (none, trace, low to moderate, moderate to dense) and location of epiphytic lichens and mosses, various surface bark morphologies (striate, checkered, smooth, furrowed, irregular), the presence of shallow, medium, or deep bark fissures, the presence of deep bark cracks, and the presence of non-weathered, brown bark in the center of furrows or fissures. We rated the relative abundance of this last characteristic using the 0 to 6 scale in the 2004 evaluations.

We used the following arcsine-transformed percentage scale for most ocular estimates of percentages: 0 = not seen, 1= less than 2.5 percent, 2 = 2.5 percent to 19 percent, 3 = 20 percent to 49 percent, 4 = 50 percent to 79 percent, 5 = 80 percent to 97.4 percent, 6 = more than 97.5 percent.

Bark thickness

Bark thickness was measured using a needle-type bark probe, which functioned in the same way as the one described by Gill and others (1982). The bark probe measures the distance between the outer bark surface and the wood using a blunt-ended 1.9 mm diameter steel probe that is pushed by hand pressure through the bark layers. The probe tip does not penetrate the wood, so the depth of penetration, measured to the nearest millimeter, indicates total bark thickness at the point of insertion. All bark depth measurements were made in "average" bark depth areas, avoiding atypically high or low spots.

To determine the optimal height to measure bark thickness, we conducted a small pilot study using 19 trees located beyond the edges of the study plots. For these trees, we measured bark thickness at each of the cardinal compass directions at 0.5, 1, 1.5, and 2 m above the ground surface. The four readings at each height were averaged to give a mean bark thickness reading for each tree at each height.

Based on the results from the pilot study (discussed in Results below), we selected 1 m as the height for measuring bark thickness for other trees in the study. To minimize possible effects associated with bark probing on study trees, bark thickness data in 2003 was measured only on dead trees in the plots and extra SWP trees. The status of the bark at each point of measurement (live, dead but moist, dead and dry) was recorded. For dead trees and dead areas of living trees, bark was chipped open so that its thickness could be measured while viewing the bark in a radial or cross section. This prevented errors associated with gaps between wood and dead bark or penetration of the probe into decayed wood. On live trees, the bark area to be probed was sprayed with 70 percent isopropanol prior to insertion of the bark probe, which was cleaned with 70 percent isopropanol before each probe. After determining that bark probing of live trees did not initiate disease or cause significant damage, we expanded bark probe measurements to include all remaining plot center trees in fall 2004.

To adjust thickness measurements to account for shrinkage due to drying, we conducted a separate study of radial bark shrinkage upon drying. Bark thickness measurements were made using the bark probe on 35 fresh sections of coast live oak. Stems were then allowed to air dry and bark thickness was remeasured. For 22 samples, we also obtained measurements for partially dried bark (i.e., dead but moist status). The average percent shrinkage in thickness for all samples was 22.5 percent for fresh to dry bark and 12 percent for fresh to dead but moist bark. These shrinkage values were used to adjust individual bark thickness measurements of

dead/dry or dead/moist bark samples to the estimated fresh bark thickness prior to averaging and analysis.

Statistical analyses

We used JMP® statistical software (SAS Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $P \leq 0.05$. The likelihood ratio chi square statistic was used to test the significance of difference of proportions in 2×2 contingency tables. Effects of year and tree variables on SWP in 2000 to 2004 were tested using repeated measures analysis of variance. We used linear regression to test for associations between continuous outcomes (e.g., SWP) and continuous or categorical predictor variables. The nonparametric Spearman test was used to test for correlations between pairs of categorical variables (e.g., those using the 0 to 6 rating scale). We also used analysis of variance (F-tests) or t-tests to test whether mean levels of continuous variables differed between groups such as cases and controls.

We developed logistic regression models to examine the effects of factors on the binary disease outcome (plot center tree is diseased, i.e., a case) or a binary disease progress outcome (disease progress observed). We screened possible predictor variables using univariate logistic regressions, examined correlations between predictor variables, and tested whether models were strongly influenced by outlying observations by comparing models with and without extreme outliers. We also used recursive partitioning (also known as regression trees, CART™, etc.) to develop preliminary models, investigate interactions between predictors, and determine optimum thresholds for creating binary variables. This procedure splits data in a dichotomous fashion according to a relationship between the predictor and outcome values, creating a tree of partitions. Each partition is chosen to maximize the difference in the responses between the two branches of the split.

We developed multivariate models using a stepwise procedure. Factors were generally considered for entry into the multivariate models if odds ratios from univariate models were significant at $P \leq 0.10$. The significance level of each factor reported in the final models should be interpreted as if it were the last factor added to the model. We also calculated Akaike's information criterion (AIC) to compare the fit of alternative models. For models constructed for a given data set, smaller AIC values indicate better model fit.

Results

Symptom development and disease progress 2000 to 2004

Overall disease incidence and mortality

Among 122 live tanoaks that were asymptomatic in 2000, 21.3 percent showed disease symptoms by September 2004. These presumably represent both new infections and trees with latent infections that developed visible symptoms during the observation interval. Among 473 live coast live oaks that were asymptomatic in 2000, 23 (4.9 percent) showed disease symptoms by 2004. This increase in disease incidence is significantly less than that seen in tanoak (likelihood ratio $p<0.0001$). More than 50 percent of the newly symptomatic coast live oaks first exhibited symptoms in 2003. In contrast, the largest yearly increases in the number of newly symptomatic tanoaks were observed in the 2001 and 2004 ratings.

Although newly symptomatic trees have been observed among both tanoak and coast live oak between 2000 and 2004, only tanoak shows a steady net increase in disease over this period (*fig.1*). Including recently-killed trees present at the start of the study in the base tree population, the overall *P. ramorum* canker incidence in tanoak has increased from 31 percent in 2000 to 43 percent in 2004 (*fig.1*). For coast live oak, overall *P. ramorum* canker incidence increased only from 23 percent in 2000 to 24 percent in 2004 (*fig.1*). In coast live oak, 16 of the 124 live coast live oak trees that had *P. ramorum* canker symptoms for at least the first two years of the study have been reclassified as asymptomatic due to a lack of apparent cankers in later ratings. Losses in symptomatic coast live oaks largely offset the gains due to new symptom development, leading to almost no net change of *P. ramorum* canker incidence in coast live oak over the period. In contrast, none of the tanoaks with *P. ramorum* symptoms in 2000 had become asymptomatic by 2004.

As seen in *fig. 1*, mortality due to *P. ramorum* has occurred at significantly higher rates in tanoak than in coast live oak (likelihood ratio $p<0.0001$). In addition, most mortality occurred among trees that were already symptomatic in 2000. Among live trees that had *P. ramorum* canker symptoms in 2000, 35 percent of tanoaks (n=34) and 26 percent of coast live oaks (n=124) had died by 2004. Among trees that were asymptomatic in 2000, 6 percent of tanoaks but only 0.4 percent of coast live oaks developed *P. ramorum* canker symptoms and died by 2004.

Futhermore, symptomatic tanoaks typically died more rapidly than coast live oaks. Among tanoaks with *P. ramorum* canker symptoms that were live in 2000 and had died by 2004 (n=21), 57 percent died within one year and 81 percent died within two years after symptoms were initially observed. In contrast, for coast live oaks (n=34), 26 percent died within one year and 62 percent died within two years after *P.*

ramorum symptoms were initially observed. These totals include trees that developed symptoms after 2000 as well as trees that were symptomatic in 2000, so some trees may have been symptomatic longer than the periods noted.

P. ramorum canker has been the main cause of mortality in tanoak study trees (*fig. 1*). Because most tanoak trees in the two tanoak study locations are relatively young and vigorous, only one tanoak has died due to agents other than *P. ramorum.* This was a 4 cm DBH root sprout originating from a tree which died and failed before the study started. The sprout died as the result of root disease associated with the failed stem. In contrast, canker rots and other wood decay fungi are relatively common in the coast live oak study locations, and these agents are associated with decline and death of numerous coast live oaks in the study (*fig. 1*).

In trees with multiple stems, each stem generally functions independently with respect to *P. ramorum* canker. Death of main stems generally occurs independently and is related to canker severity on each stem. We recorded symptom data for individual stems in plot center and extra SWP trees for coast live oaks and tanoaks. Of the 70 multistemmed trees in this subset, 14 of 56 coast live oaks and 10 of 14 tanoaks had symptoms of *P. ramorum* canker in at least one main stem (*fig. 2,* left). However, in a substantial percentage of these symptomatic trees (7 coast live oaks and 7 tanoaks), main stems with and without *P. ramorum* symptoms were present in the same tree. By 2004, there were more multistemmed trees with a mixture of both live stems and stems killed by *P. ramorum* than multistemmed trees in which all stems had been killed by *P. ramorum* (*fig. 2,* right).

Many trees killed by *P. ramorum* cankers produce basal sprouts that can persist for at least several years after the death of the top. In addition, some trees with *P. ramorum* cankers that have experienced bole failures while still alive have subsequently produced vigorous epicormic sprouts on the remaining portion of the bole. In 2004, 81 percent of tanoaks (33/39) and 43 percent of coast live oaks (22/51) with tops killed by *P. ramorum* cankers over the study still had live basal sprouts or epicormic sprouts arising after bole failure. The data on sprouting and disease in multistemmed trees support our general field observations that, in most trees with *P. ramorum* canker, the decline of the top is directly related to the presence of stem cankers and is not associated with decline due to pre-existing root disease.

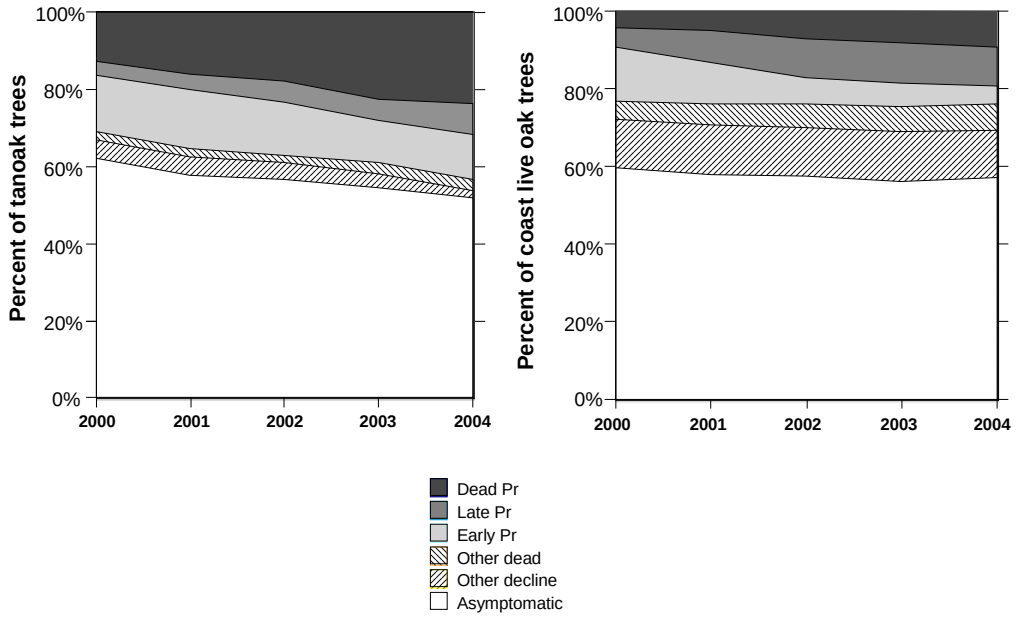

Figure 1— Changes in health of all tanoak (n=187) and coast live oak (n=655) study trees from September 2000 to September 2004. **Dead Pr** = tree dead as a result of *P. ramorum;* **Late Pr** = live trees with *P. ramorum* cankers plus beetle boring and /or *H. thouarsianum* fruiting bodies; **Early Pr** = live trees with *P. ramorum* cankers only; **Other dead** = tree dead due to agents other than *P. ramorum;* **Other decline**=tree in severe decline due to agents other than *P. ramorum;* **Asymptomatic**= no evident symptoms of *P. ramorum* infection or decline due to other agents.

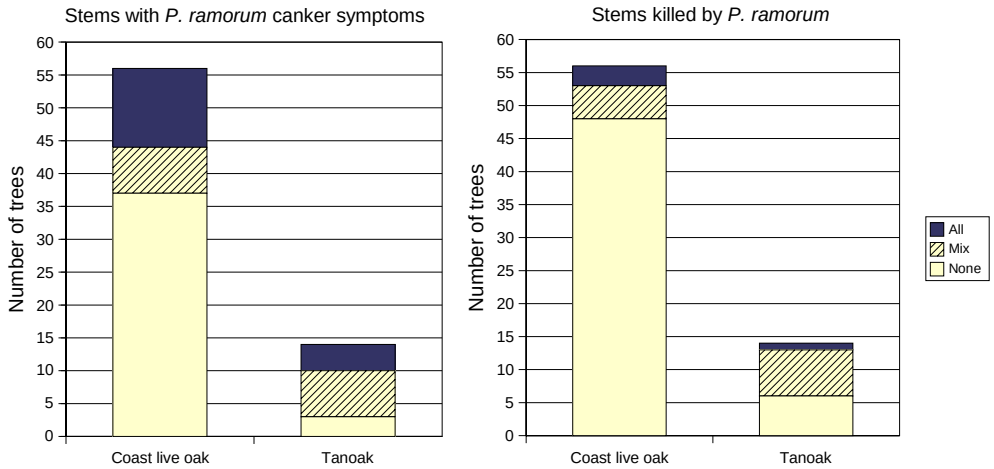

Figure 2 — Occurrence of *P. ramorum* canker symptoms (left) or *P. ramorum*-related mortality (right) among individual main stems of multistemmed coast live oak (n=56) and tanoak (n=14) in September 2004. **All** = all main stems symptomatic or dead; **Mix** = mixture of asymptomatic and symptomatic (left) or live and dead (right) stems; **None** = no stems symptomatic or dead.

Symptom development

In coast live oak and tanoak trees that were killed by *P. ramorum* canker, early symptoms typically consisted of bleeding bark cankers, but cankers did not necessarily bleed in all years. Recent bleeding (within the previous 6 to 12 months) was seen in 26 percent of the live tanoaks with *P. ramorum* symptoms each year from 2002 through 2004. In comparison, recent bleeding was seen in 39 percent of the live, symptomatic coast live oaks in 2002 and 2004, and in 54 percent of the live, symptomatic coast live oaks in 2003.

Over time, most *P. ramorum* cankers expanded in both radial and longitudinal directions. The number of apparently separate cankers tended to decrease over time, either because adjacent cankers merged or because the true extent of cankers became more obvious over time. We rated the percent of the bole circumference girdled by *P. ramorum* cankers in the plot center and extra SWP trees at each annual evaluation. For both tanoak (n=17) and coast live oak (n=52) trees with *P. ramorum* canker symptoms, the mean 2004 *P. ramorum* girdling rank of trees with only early disease symptoms was significantly less than the girdling rank of trees that had progressed to late disease symptoms or died (*table 2*). All of the trees that had died were at least 50 percent girdled by *P. ramorum* cankers.

Table 2— *Percent of total main stem circumference affected by* P. ramorum *cankers in tanoaks (n=17) and coast live oaks (n=52) by disease stage in 2004*

	Mean (sd) *P. ramorum* canker girdling rating[1]		
Species	**Early symptoms**	**Late symptoms**	**Trees killed by *P. ramorum***
Tanoak	2.60 (0.55) a[2]	4.44 (0.73) b	5.00 (0) b
coast live oak	2.36 (1.36) a	3.97 (1.17) b	4.67 (1.07) b

[1] Percent of main stem circumference girdled by *P. ramorum* cankers estimated using 0 to 6 scale (see methods).

[2] Means followed by different letters are significantly different ($p<0.05$) according to Tukey-Kramer HSD test.

We collected data on the apparent height of the upper and lower margins of *P. ramorum* cankers in coast live oak plot center and extra SWP trees to help determine an appropriate height range for studying the relationships between bark characteristics and disease. The lower margins of all measured cankers (n=60) occurred within 1.3 m of the soil grade, with most (80 percent) occurring within the lower 0.4 m of the bole. The mean height for all lower canker margins was 27 cm (s.d. 37). The height of the upper canker margin of the highest cankers was quite variable, with an overall mean of 1.11 m (s.d. 56) above the soil grade and a range from 0.1 to 2.2 m above soil level. Only about 25 percent of the upper canker margins were higher than 1.5 m.

Late symptoms of *P. ramorum*-related decline include ambrosia beetle boring and/or stromata of *H. thouarsianum* that develop on larger cankers and may extend well beyond the cankers around and up the stem. All of the coast live oak study trees with *P. ramorum* symptoms that died between 2000 and 2004 had beetle boring and/or stromata of *H. thouarsianum* in the year prior to mortality and almost all trees had both secondary agents visible in the year that death was recorded (*table 3*). However, small diameter tanoaks did not consistently exhibit beetle boring and/or *H. thouarsianum* stromata (late symptoms) before being killed by *P. ramorum* cankers (*table 3*). The trees that had no evident beetle boring or *H. thouarsianum* stromata in the year that they died ranged from 4.5 to 17 cm DBH. Hence, although many smaller tanoaks were apparently killed by *P. ramorum* cankers alone, secondary agents were consistently present in coast live oaks with *P. ramorum* cankers that died.

Table 3— *Number of tanoaks and coast live oaks with P. ramorum cankers that also had beetle boring or H. thouarsianum stromata in the year that tree mortality was recorded*

Species	**beetle boring**	***H. thouarsianum*** stromata	**no beetles or *H. thouarsianum***	**Total trees**[1]
tanoak	7	4	7	15
coast live oak	28	29	0	29

[1] Data set includes plot center trees and extra SWP trees that were live in 2000 and died by 2004 and other plot trees that were live in 2001 and died by 2004.

Many *P. ramorum*-infected coast live oaks experienced major failures before the top had died. Between 2000 and 2004, we observed 56 failures (bole failures or branch failures ≥20 cm diameter) among 153 coast live oaks with *P. ramorum* symptoms. This 36 percent failure rate in *P. ramorum*-infected trees is significantly greater (likelihood ratio $p<0.0001$) than the 4 percent failure rate seen over this period among 484 coast live oaks without *P. ramorum* symptoms.

Limited symptom development

Although symptom development commonly proceeds as described above, some trees have shown a lack of symptom progress beyond the early or late symptom stage over the course of the study. Overall, 47 percent of the tanoaks and 58 percent of the coast live oaks with early or late *P. ramorum* canker symptoms in 2000 advanced to a more advanced symptom class (late or dead, respectively) by 2004.

Among the smaller set of trees for which we have ratings of the percent of the main stem girdled by *P. ramorum* cankers, three of 17 (18 percent) symptomatic tanoaks

and 16 of 60 (27 percent) coast live oaks showed no apparent disease progress between 2000 and 2004 based on either canker girdling rating or symptom class. Cankers in most of these trees exhibited little or no bleeding and appeared to be inactive. These counts include all trees that were rated as symptomatic at least three of the five years, and excludes 16 coast live oaks that had been rated as having *P. ramorum* canker symptoms for at least two years and were reclassified as asymptomatic by 2004. These trees initially had small cankers that had subsequently been inactive for several years.

Over the past several years, we have also noted the production of apparently healthy callus at the edge of *P. ramorum* cankers in coast live oak and tanoak trees (*fig.3*), especially in trees that have shown little or no symptom progress from year to year. Callus may either partially or completely encircle cankers. Callus development was noted in the detailed disease ratings for three of 17 live tanoaks and 22 of 52 live coast live oaks with *P. ramorum* cankers in 2004.

Figure 3— Callus development around inactive *P. ramorum* cankers in coast live oak (left) and tanoak (right). Arrows indicate callus edge.

Many trees with late *P. ramorum* canker symptoms have developed elevated levels of diffuse canopy dieback during the study (*fig. 4*). This gradual dieback and canopy thinning occurs in trees with extensive cankers that survive for at least several years. It is distinct from the rapid dieback of the entire canopy ("sudden death") seen in trees that die within one to two years from the appearance of stem cankers. Among *P. ramorum*-infected trees that were still alive in 2004, canopy dieback rating was positively correlated with the *P. ramorum* stem girdling rating (Spearman R=0.555,

p=0.034, n=14 for tanoak; Spearman R= 0.367, p=0.020, n=40 for coast live oak). Diffuse canopy dieback also increases over time in trees that are in severe decline due to other mortality agents, such as canker rot fungi (*fig. 4*).

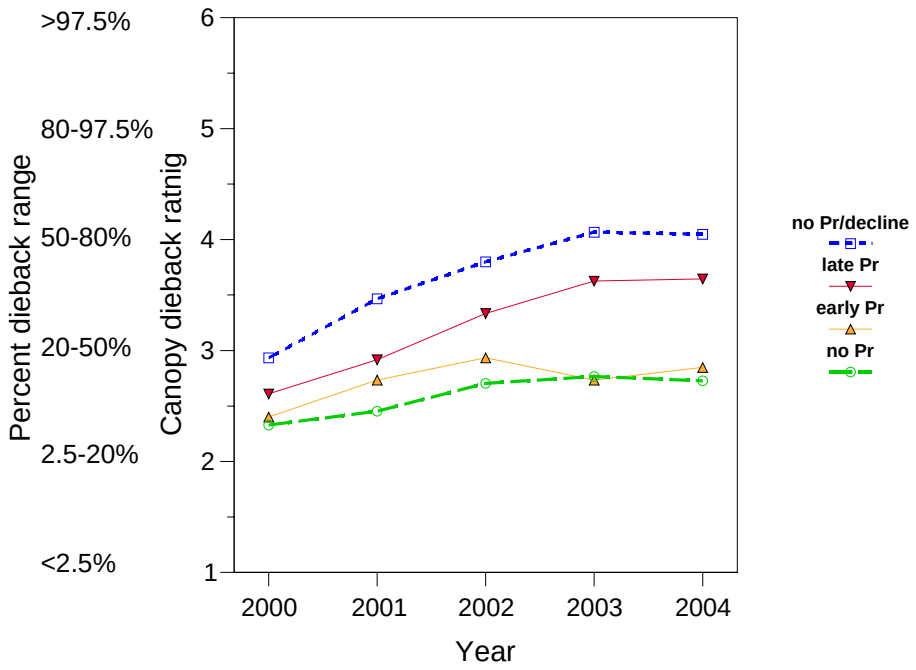

Figure 4— Canopy dieback ratings from September 2000 to September 2004 in coast live oak that were still living in 2004; **no Pr/decline**=trees in severe decline due to agents other than *P. ramorum*, **late Pr**=live trees with *P. ramorum* cankers plus beetle boring and /or *H. thouarsianum* fruiting bodies; **early Pr**=trees with *P. ramorum* cankers only, **no Pr**=non-declining trees with no evident symptoms of *P. ramorum* infection.

Stem water potentials of coast live oaks and tanoaks

In a repeated measures analysis of variance of SWP, the effects of year and species were highly significant (p<0.0001), but the interaction between these factors was not (p=0.071). Tanoaks consistently had higher average SWP readings than coast live oaks (*fig. 5*). Overall mean SWP fluctuated from year to year, apparently in response to the level of precipitation in the previous year (*fig. 5*). For coast live oak, the study location average SWP was positively, but not strongly, correlated with the rainfall for the location and year (adjusted R^2=0.080, p=0.026, n=10). Our rainfall data were from nearby weather stations rather than from the actual study sites, which may have weakened the apparent correlation.

Stem water potentials (SWP) measured in September of 2000, 2001, 2002, 2003 and 2004 were highly correlated from year to year for individual coast live oak and tanoak trees. A correlation matrix analysis showed that SWP readings from individual trees in different years were significantly correlated (p<0.0001) in all

combinations, with adjusted R^2 values ranging from 0.71 to 0.85. Although mean SWP varied from year to year, we found that the SWP of most trees shifted up or down by an amount that approximated the overall mean year to year difference. After removing the overall year to year differences, the SWP of individual trees were quite consistent from year to year. This suggests that relative SWP levels measured over the study period were likely to be similar to those that existed prior to the start of the study, i.e., during the period when most trees were initially infected.

To determine whether the SWP of the plot center tree (case or control) was representative of the water status of the plot as a whole, we compared SWP readings for 2001 through 2004 from plot center trees with the extra SWP trees present in 45 of the 150 plots. Readings between pairs of trees from the same plot (n=45) were significantly correlated in all four years (adjusted R^2 varied between 0.519 and 0.784 in the different years). This suggests that much of the variation in SWP is related to the available soil moisture level within the plot, which is influenced by local factors including soil type and depth, slope, aspect, and vegetative cover. Hence, SWP of any tree in a plot (center tree or a different tree) provides an indication of tree water stress levels within the plot as a whole.

Because year to year variations in SWP tend to obscure directional changes in SWP over time, we used repeated measures analysis of variance to test whether SWP was influenced by disease progress. For this analysis, we compared SWP of coast live oaks that were asymptomatic in 2000 through 2004 (n=107) to trees that had progressed from early to late *P. ramorum* symptoms between 2000 and 2004 (n=22). In this analysis, only the effect of time was significant ($p<0.0001$); neither disease status (asymptomatic vs. early to late) nor the interaction between disease status and time were significant. The same overall results are obtained if all live trees with *P. ramorum* canker in 2004 (n=36) are compared with asymptomatic trees. These results indicate that year to year changes in SWP were not affected by disease status, i.e., SWP in trees with *P. ramorum* did not change directionally over time.

Although the year-adjusted SWP of most trees with *P. ramorum* canker did not change over time, a few trees did show SWP changes. In several coast live oaks with late *P. ramorum* symptoms that were in the process of turning brown due to stem girdling, SWP readings were generally at least 1 MPa lower than previous readings, a difference well in excess of the overall annual change. In contrast, SWP readings on root sprouts of trees killed by *P. ramorum* were typically similar to or higher than readings made on the same trees before death of the top. This indicates that at least some portion of the root system continued to function after the death of the canopy in these trees.

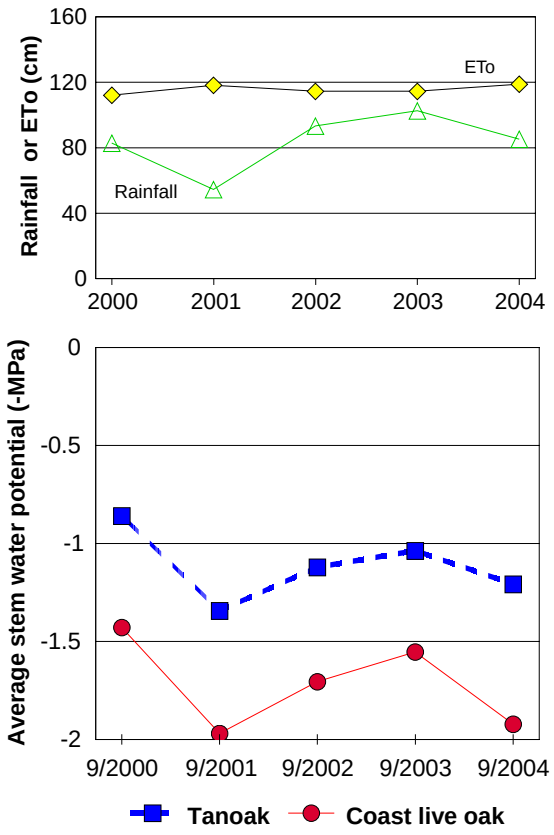

Figure 5— Average stem water potential for coast live oaks and tanoaks (bottom graph) across all locations and years compared with potential evapotranspirtation (ETo) and average seasonal rainfall (top graph); ETo is for September of previous year through August of listed year from the California Irrigation Management Information System (CIMIS) station in Santa Rosa; rainfall is the mean for all study locations calculated from the nearest weather station data for each location.

Bark characteristics and disease

Bark thickness

Initial bark thickness measurements were made at multiple heights (0.5, 1, 1.5, and 2 m) and the four cardinal directions on 19 healthy coast live oaks located beyond plot edges. We used analysis of variance to test whether bark thickness varied with cardinal direction or height above ground. This analysis showed no effect of cardinal direction but a significant linear decrease in bark thickness over the range of 0.5 to 2 m above grade ($p<0.0001$). For this sample, average bark thickness at 2 m (1.92 cm) was 70 percent of the mean bark thickness at 0.5 m (2.75 cm).

Based on the bark thickness data from these initial measurements and data on the vertical distribution of stem cankers (above), we selected 1 m as the standard sampling height for bark measurements in a sample of plot trees. Bark at this height was near its maximum thickness and measurements could be performed more readily

than at the lower (0.5 m height). Furthermore, most *P. ramorum* cankers on coast live oak study trees were found near or below this height.

Overall, the mean adjusted bark thickness of trees with *P. ramorum* canker symptoms in 2004 was significantly greater than that of the asymptomatic trees (t-test $p<0.0001$, *fig. 6*). Furthermore, mean bark thickness was greatest among trees that had already died and lowest among trees that still had only early symptoms of disease in 2004. Trees with late disease symptoms in 2004 had intermediate bark thickness levels (*fig. 6*). Given that most of these trees were symptomatic in 2000, this suggests that symptoms progressed more rapidly in trees with thicker bark. Bark thickness was also positively correlated with other indicators of disease severity, including the number of bleeding cankers in 2001 (adjusted $R^2=0.101$, $p=0.008$) and the rating of the percentage of the bole girdled by *P. ramorum* cankers in 2001 (adjusted $R^2=0.098$, $p=0.009$).

Among all (symptomatic and asymptomatic) coast live oaks, bark thickness increased in a nonlinear fashion with increasing stem diameter (*fig. 7*), with an upper plateau near 5.5 cm. As seen in *fig. 7*, bark thickness can vary by 2 cm or more for trees with a given stem DBH. Bark thickness increased as the tree's sky exposed canopy rating increased (adjusted $R^2=0.17$, $p<0.0001$ for quadratic regression line), suggesting that bark thickness is generally greater in more dominant trees. In addition, among coast live oaks without *P. ramorum* canker symptoms, trees that were severely declining due to other agents or factors had significantly thinner bark (mean 2.2 cm, n=23) than non-declining trees (mean 2.7 cm, n=113; t-test $p<0.0001$). Overall, these data suggest that bark thickness was generally greater in more vigorous, faster-growing coast live oaks. These dominant, fast-growing coast live oaks also had a higher risk of developing *P. ramorum* canker symptoms than suppressed, slow-growing oaks in the same stands.

Bark surface characteristics

Most bark characteristics, including the abundance and location of epiphytic lichens and mosses, various bark morphologies (striate, checkered, smooth, furrowed, irregular), and the presence of bark fissures of various depths occurred at nearly equal frequencies in symptomatic and asymptomatic trees and were thus not predictors of disease status. However, one bark characteristic was a significant predictor of the case (disease) outcome in both single variable and multivariate models. This was the presence of non-weathered, brown bark in the center of furrows or fissures that resulted from recent bark expansion (*fig. 8*).

To further explore this factor, we estimated the proportion of bark fissures on the lower bole that contained unweathered brown bark using the 0 to 6 scale. Ratings were made on plot center trees in 2004. This quantitative brown fissures variable was a significant predictor of the case outcome in both single and multivariate models, as noted below. However, brown fissures were not significantly correlated with variables related to disease progress, including the amount of *P. ramorum* girdling and progress in disease symptoms between 2000 and 2004. This suggests that this bark characteristic may be related to risk of infection, but not to later canker expansion.

Average brown fissure ratings were significantly lower among severely declining trees without *P. ramorum* symptoms (mean 0.30, n=20) than among relatively healthy (non-declining) asymptomatic trees (mean 2.2, n=96; t-test $p<0.0001$). Brown fissure ratings also declined as decay impact ratings increased. Furthermore, brown fissure rating was positively correlated with sky exposed canopy rating (adjusted $R^2=0.31$, $p<0.0001$, n=123), and negatively correlated with plot canopy cover (adjusted $R^2=0.12$, $p<0.0001$, n=123). These correlations suggest that brown fissures are more common in trees that are likely to have at least moderate growth rates. However, brown fissure ratings were not correlated with either stem diameter or bark thickness. Hence, although brown fissures and bark thickness may both be related to tree vigor or growth rate, they appear to reflect different ways that bark morphology can be affected by growing conditions.

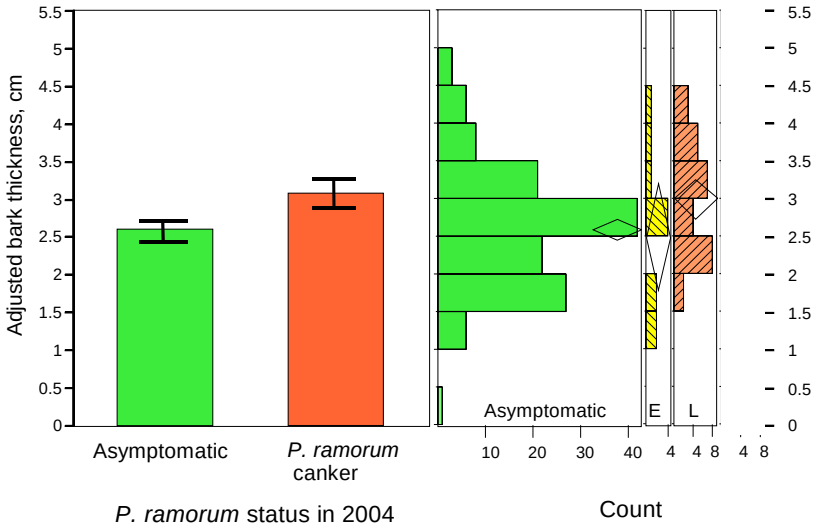

Figure 6—Relationship between coast live oak bark thickness and *P. ramorum* disease status in 2004; bark thickness readings in dead bark samples were adjusted to account for shrinkage due to drying; error bars (left graph) and outer points of means diamonds (histograms, right) denote 95 percent confidence intervals of the means; *P. ramorum* symptoms in histograms are E=early (bleeding cankers only), L=late (cankers + beetles and/or *H. thouarsianum*), D=dead; n=203

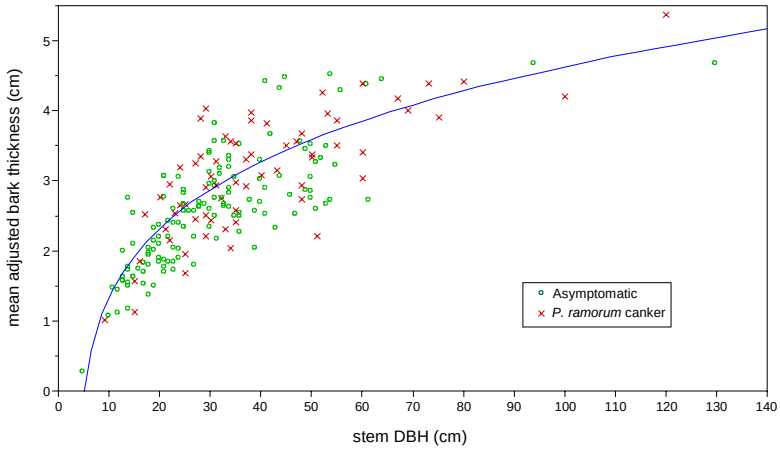

Figure 7— Relationship between bark thickness at 1 m height and stem diameter (at 1.37 m height, DBH) for coast live oaks with or without symptoms of *P. ramorum* canker; fitted curve is $e^{(\text{adjusted bark thickness})} = -1.52 + 0.471(\text{DBH}) + 0.00573(\text{DBH})^2$, adjusted R^2=0.62, p<0.0001, n=202

Figure 8 - Images of coast live oak bark on the left have unweathered brown bark within bark fissures, presumably resulting from recent bark expansion. Unweathered brown areas are lacking in bark fissures shown on the right.

Disease risk models for coast live oak

We used recursive partition models and logistic regression to model the risk of *P. ramorum* infection in plot center trees. The outcome variable for these analyses included all plot center trees that had expressed likely *P. ramorum* canker symptoms through September 2004, including some initially symptomatic trees in which disease had apparently become inactive. Including these non-progressing trees maximized our ability to detect factors that may be related to infection but not later disease progress. For these models, tree and plot variables that could be considered outcomes related to disease were not tested as explanatory variables. For instance, diffuse canopy dieback was not included as a predictor because our data (above) suggest that it is a consequence of *P. ramorum* canker.

We constructed recursive partition disease risk models using both plot center trees only (n=128) and a larger data set that included extra SWP trees (n=168). The two initial splits were the same for both data sets, but subsequent splits varied between the data sets. In both models, the best initial split of the data was based on the brown fissure rating (0 and 1 vs. ≥2). About 90 percent of the trees with brown fissure ratings of 1 or less were controls. Within the group with brown fissure ratings of 2 or more, the next split for both models was based on 2002 SWP. About 80 percent of the trees with 2002 SWP <-2 MPa (relatively high water stress) were controls. Other splits in the models were based on further division by 2002 SWP (disease risk higher with high SWP/low water stress), the number of overstory and understory California bay in the plot (disease risk higher with more bay), sky exposed canopy (disease risk higher with greater canopy exposure), and bark thickness (disease risk higher with thicker bark).

We also developed logistic regression models for the case outcome using only the plot center tree data set. Two optimized models with similar fit based on AIC are shown in *table 4*. The two models differ only in that the SWP variable is replaced by a binary bark thickness variable (bark thickness greater than 3.3 cm). The optimum split for the binary bark thickness variable was determined from recursive partition models. Bark thickness was not significantly correlated with SWP overall. However, SWP was somewhat higher (t-test p=0.08) in trees with bark thickness greater than 3.3 cm than in trees with thinner bark. SWP readings from all years could be substituted for each other in the model, with 2002 SWP being the most significant. We used 2001 SWP in the model because it was the earliest year that SWP was measured in both plot center and extra SWP trees.

Table 4— *Logistic regression models for* P. ramorum *canker disease risk in coast live oak*

	Model 1[1]		Model 2[1]	
Predictor variables	**P level[2]**	**Odds ratio (CI)[3]**	**P level[2]**	**Odds ratio (CI)[3]**
Brown bark fissures rating (0-6 scale)	<0.0001	53.28 (10.01-363.68)	<0.0001	64.27 (10.85-531.30)
California bay cover in plot (0-6 scale)	0.0285	8.21 (1.25-61.11)	0.0009	21.06 (3.35-162.43)
More than 2 stems	0.0029	8.27 (1.98-46.72)	0.0028	9.11 (2.06-53.46)
2001 SWP (MPa)	0.0372	19.28 (1.88-396.59)		
Bark thickness>3.3 cm			0.0128	3.87 (1.33-12.33)

[1] The Akaike Information Criterion (AIC) for models 1 and 2 are 123.56 and 127.76 respectively. Overall significance levels of for both models were p<0.0001
[2] Likelihood ratio test significance level
[3] Odds ratios and 95 percent confidence intervals; odds ratios greater than 1 indicate that a factor is positively associated with the case (disease) outcome

Predictors in the model included only one plot variable, California bay cover, which is related to local inoculum density. Disease risk increases as bay cover in the plot increases, presumably because the amount of inoculum present in the plot also increases with bay cover. Other predictors are tree variables that may be related to host susceptibility. SWP can be considered both a tree and plot factor because SWP levels within a plot are correlated as noted above. Wetter conditions within plots (indicated by higher SWP levels) may favor host susceptibility, inoculum production, and the duration of suitable infection periods.

To validate the model, we used the model to predict the disease outcome in the extra SWP trees, which were not used to develop the models. For this smaller data set (8 cases, 32 controls), model one correctly predicted 72 percent of the outcomes and model two correctly predicted 69 percent of the outcomes. The case outcome was strongly underpredicted. However, the bay cover variable is based on cover in the 8 m plot around the plot center tree. Actual bay cover in an 8 m radius plot around the extra SWP trees could differ somewhat from this value, possibly contributing to poorer prediction of the case outcome.

Disease progress models for coast live oak

We also developed models for coast live oak to determine whether any of the factors we rated were related to disease progress or resistance in trees that were initially infected (*table 5*). This data set includes 58 coast live oaks (plot center and extra

SWP trees) which were alive and had *P. ramorum* canker symptoms in 2000. Of these, 42 showed disease progress, as shown by canker expansion and/or progression to a later disease stage (e.g., early to late or dead).

Indicators of initial disease severity, including canker count in 2001 and *P. ramorum* canker girdling rating in 2000 or 2001 are highly significant predictors of disease progress (likelihood ratio $p<0.0001$; AIC 37.4, 46.5, 44.7, respectively). Canker count was a somewhat better predictor of disease progress than canker girdling rating. Trees with greater numbers of cankers and/or a higher girdling rating in 2000 or 2001 were more likely to show disease progress than trees that initially had few and/or small cankers. The mean canker rating in 2000 was significantly higher (t-test $p=0.031$) for those that showed disease progress than for those that did not (mean ratings 3.0 and 2.1, respectively). However, because almost all of these trees were symptomatic at the start of the study, larger and more cankers in 2000 and 2001 can also be interpreted to be the result of disease progress up to that point. Thus, although canker severity at a given point in time may be a good indicator of future disease progress, these variables tell us little about factors that might predispose trees to greater disease progress.

The best multivariate model that excludes the initial disease severity variables is shown in *table 5.* When initial disease severity variables are excluded, bark thickness is the best single predictor of the disease progress outcome (likelihood ratio $p<0.0075$, AIC 64.5). As shown in *fig. 6*, this factor is positively correlated with disease risk and the disease stage in 2004, which is a component of the disease progress outcome.

The other factors in the model are more difficult to interpret. Disease was more likely to progress in trees located in plots with no or low levels of madrone cover. Recursive partition models showed that disease progress occurred in 88 percent of the trees in plots without any madrone cover but only occurred in 52 percent of the trees in plots with madrone cover. Furthermore, the likelihood of disease progress decreased as madrone cover increased. However, plots with the highest levels of madrone cover (greater than 20 percent) were only found at three of the 10 coast live oak study locations (five, six, and seven), so this variable may be partially confounded with other location-specific factors. Within the study areas, madrone tends to occur on soils that appear to be more shallow and are often more rocky than soils that are dominated by other tree species. This may indicate that coast live oaks growing in more unfavorable sites are less likely to show disease progress. However, SWP levels did not vary with madrone cover in these plots, and SWP was not a significant predictor of disease progress. The third factor in the model, poison oak

cover at least 2.5 percent, is not significant in a single variable model. In the multivariate model, the higher level of poison oak cover is positively correlated with disease progress.

Table 5— *Logistic regression model for* P. ramorum *disease progress in coast live oaks*[1]

Predictor variables	P level[2]	Odds ratio (CI)[3]
Bark thickness	0.0457	17.97 (1.054-450.6)
Madrone cover	0.0236	0.051 (0.00304-0.671)
Poison oak cover >2.5 percent	0.0488	7.878 (1.010-184.9)

[1]Overall significance level for the models is p<0.0021, AIC=61

[2] Likelihood ratio test significance level

[3] Odds ratios and 95 percent confidence intervals; odds ratios greater than 1 indicate that a factor is positively associated with the case (disease progress) outcome

Discussion

Over the course of the study, levels of disease and mortality due to *P. ramorum* have been consistently higher in tanoak than in coast live oak. Compared with tanoak, coast live oak had lower initial levels of disease observed in 2000, fewer trees that developed new symptoms between 2000 and 2004, and more trees that became asymptomatic after showing some early disease symptoms. These results support the overall consensus that tanoak is more susceptible to *P. ramorum* canker than coast live oak. In addition, our data suggest that conditions favorable for new bole infections occur more regularly in the tanoak study sites than in the coast live oak sites. Although apparent bole infection rates in tanoak have increased fairly steadily throughout the study period, most of the apparently new coast live oak bole cankers developed between the 2002 and 2003 ratings. This interval had the first relatively wet spring of the study period. Davidson and others (2005) found that *P. ramorum* inoculum levels in rainwater sampled in a coast live oak woodland in Sonoma County were greatly elevated in spring 2003 relative to the two previous years.

After five years of observation, we have been able to document several different symptom progression patterns in trees with *P. ramorum* canker. The “sudden oak death” pattern presented in the original descriptions of the disease (Garbelotto and others 2001, Rizzo and others 2002) involves fairly rapid canker expansion followed by a rapid dieback of the entire canopy of affected stems. Presumably many of the trees that were dead at the time of the initial evaluation in 2000 had followed this

pattern. Since that time, up to about 81 percent of tanoaks and about 62 percent of coast live oaks that have died have followed this rapid decline pattern. However, especially in coast live oak, cankers caused by *P. ramorum* are frequently smaller and less aggressive. Trees with less aggressive cankers follow different disease patterns, including apparent symptom remission in some trees.

Most tanoaks (65 percent) and coast live oaks (74 percent) with *P. ramorum* canker symptoms in 2000 were still alive in 2004. In coast live oak, trees with extensive late-stage cankers commonly develop diffuse canopy dieback (*fig. 3*, Swiecki and Bernhardt 2002c, 2004) that can eventually lead to canopy thinning. Some slow-declining tanoak and coast live oaks have developed healthy callus tissue around the margins of old *P. ramorum* cankers that do not appear to be expanding. Callus development is evidence of a host resistance response, but it is not clear whether callus development actually limits canker expansion or whether it is simply represents the normal host wound closure response acting in areas where pathogen activity has ceased for other reasons.

In addition, cankers in some coast live oaks and tanoaks have not expanded or oozed in several successive years and appear to be inactive. In the most extreme case of arrested disease progress, *P. ramorum* canker symptoms appear to have gone into remission in at least 16 coast live oaks. Because *P. ramorum* canker was not confirmed by culturing all symptomatic trees at the start of the study, it is possible that some of these trees represent trees that were not actually infected and had bleeding due to other causes. However, disease has failed to progress in some trees in which *P. ramorum* has been confirmed. Furthermore, the strong correlation between disease progress and initial disease severity suggests that disease progress is commonly limited in trees that have only a few small cankers. In addition, *P. ramorum* is typically difficult to recover from cankers in the latter part of the dry season of the year, and from older, relatively inactive cankers (D. Rizzo, personal communication). This suggests that the activity of *P. ramorum* varies over time within infected hosts. If *P. ramorum* cankers in coast live oak are limited at an early stage of development due to a combination of host resistance and low initial levels of infection, affected trees may be able to recover more or less completely.

Given that *P. ramorum*-infected trees have consistently had higher average SWP levels (i.e., lower water stress) than asymptomatic trees, we conclude that water stress is not a significant predisposing factor for the development of *P. ramorum* canker in coast live oak. To the contrary, trees with chronically high levels of water stress in September have a lower risk of developing *P. ramorum* canker. Because *P. ramorum* canker occurs more commonly in trees with both relatively low levels of

water stress and high canopy exposure (Swiecki and Bernhardt 2001, 2002a,b,c) with high potential transpiration rates, it is also unlikely that these are trees that had any significant impairment of root function prior to infection.

Furthermore, in coast live oaks with *P. ramorum* canker, the decline of the tree's canopy is driven primarily by the girdling of the bole rather than loss of root function. In *P. ramorum*-affected trees that show gradual canopy decline, canopy dieback reduces leaf area and hence transpiration demand. Consequently, symptomatic trees show no decrease in SWP and sometimes develop higher SWP levels over time despite symptom progress. If root decay or dysfunction was the primary cause of top dieback, lower SWP readings should have been common in symptomatic trees, rather than being limited to the few trees that are in the final stages of collapse due to extensive sapwood decay.

Many *P. ramorum*-killed coast live oaks and tanoaks have also produced vigorous basal sprouts or epicormic sprouts on the lower portions of failed stems that have survived for several years. This is evidence that at least some portion of the root system survives in these trees. In addition, independent disease progress among different stems observed in multistemmed trees (*fig. 2*) and canker girdling ratings (*table 2*) provide further evidence that decline of *P. ramorum*-infected trees is directly related to the number and extent of stem cankers, and is not primarily related to root disease. Although root pathogens, e.g., *Armillaria mellea,* eventually affect some trees with late *P. ramorum* canker symptoms, we have seen very little evidence of root disease among any of the trees in this study. Hence, several independent lines of evidence indicate that decline and death of trees with *P. ramorum* canker symptoms are not associated with pre-existing root disease or overall tree decline related to root dysfunction, but are a direct consequence of girdling by *P. ramorum* cankers.

Despite its relatively large host range, *P. ramorum* causes bark cankers on the main stems of only a few species under field conditions. This suggests that tree species that develop main stem cankers have relatively unique bark characteristics that permit *P. ramorum* to infect and colonize living bark tissues on mature main stems. For coast live oak, bark thickness and unweathered brown tissue within bark fissures were the only bark characteristics we rated that were positively correlated with *P. ramorum* disease risk. The latter characteristic appears to represent relatively rapidly expanding regions of the outer bark. Slow growing trees, including highly suppressed understory trees and declining trees, have very few or no fissures showing this characteristic, and are also at low risk for developing *P. ramorum* canker (Swiecki and Bernhardt 2001, 2002a,b,c).

Bark expansion zones may represent sites that are more easily breached by *P. ramorum* zoospores. It is also possible that the outer periderm in these areas may be thin enough that substances might diffuse from these areas when the bark surface is wet. If *P. ramorum* zoospores were chemotactically attracted to these zones, greater aggregation of zoospores cysts might occur in these areas, leading to a greater likelihood of successful infection. Bark fissures may also remain wet longer than other areas of the bark, increasing the chance of successful infection. These areas of the bark deserve further attention in studies of the infection process in coast live oak. The correspondence between brown fissures and high-moisture bark channels imaged by MRI (Florance, these proceedings) should also be investigated.

Bark thickness is the first variable we have measured in coast live oak that appears to be correlated with both disease risk (*table 4*) and disease progress (*table 5, fig. 6*). Coast live oak bark is relatively thick and consists primarily of living tissue, with only a thin (a few mm) outer layer of dead periderm (rhytiderm) in most trees. Trees with thicker bark appear to be more likely to become infected by *P. ramorum* and, once infected, disease appears to progress faster in trees with thicker bark. These findings are consistent with field observations that *P. ramorum* cankers do not naturally occur in either small diameter coast live oaks or in the smaller-diameter upper stems of coast live oak.

Several lines of evidence from this study indicate that *P. ramorum* canker is most likely to affect relatively vigorous or fast-growing coast live oaks, and is not primarily a disease of stressed trees. As we have previously reported, coast live oaks with *P. ramorum* canker tend to have more dominant canopy positions (higher levels of sky-exposed canopy) and larger stem diameter, and are less likely to be declining due to other agents or factors including canker rot and overtopping (Swiecki and Bernhardt 2001, 2002a,b,c). In addition, *P. ramorum*-infected trees have consistently had higher average SWP levels (i.e., lower water stress) than asymptomatic trees. Increased bark thickness and the presence of unweathered bark in bark fissures are additional variables that correlated both with faster tree growth and greater risk of *P. ramorum* canker. Although we examined a sample of increment cores to investigate recent growth rates, several technical issues made this approach impractical (Swiecki and Bernhardt 2003).

Because many of the factors that are related to faster growth are correlated with each other, it is difficult to determine which, if any, actually play important roles in affecting disease risk. For example, dominant and /or larger trees may intercept more inoculum, bark expansion zones may constitute favorable infection courts, and thicker bark may provide a better substrate for *P. ramorum* growth. However,

additional investigations are necessary to determine whether these and/or other factors correlated with tree growth rate actually contribute to disease risk.

Although we have identified a number of variables that can serve as predictors of disease risk in this study (*table 4*, Swiecki and Bernhardt 2001, 2002a,b,c), we have found very few good predictors of disease progress in infected trees. This is in part due to the fact that the data set for disease progress analyses is much smaller than the disease risk data set. Overall, initial disease levels observed in 2000 or 2001 were the best predictors of later disease progress, although these predictors are not completely distinct from the outcome. However, the significance of the factors in the model presented in *table 5*, shows that some tree and plot variables may help to predict the risk of disease progress in infected trees. Long-term data from a wider data set, such as all plot trees in this study, could be used to develop more robust disease progress models for coast live oak. However, we do not currently have detailed data, including bark thickness, needed to test disease progress models for the entire plot tree population.

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Surveying and Monitoring Sudden Oak Death in Southwest Oregon Forests[1,2]

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Key words: monitoring, mortality detection surveys, Oregon, *Phytophthora ramorum*, sudden oak death

Abstract

Phytophthora ramorum, the causal agent of sudden oak death, was first discovered in Oregon in July 2001 by aerial survey (Goheen and others 2002). Alerted to the situation in California and experienced in aerial tree mortality surveys, cooperators from the USDA Forest Service and the Oregon Department of Forestry planned a pilot survey for *P. ramorum* to become familiar with what was perceived to be the high risk type: forest stands with tanoak components within approximately 16 km of the Oregon coastline and along highly traveled road corridors leading to and from California. One hundred thirty seven thousand hectares were surveyed in total, but almost immediately upon starting the fixed-wing based portion of the survey, patches of recently killed tanoaks were spotted and mapped.

Helicopter surveys were then used to describe the sites, eliminate miscalls and obtain an accurate location for use in on-ground navigation. Ground-checking efforts followed, consisting of locating mapped trees, isolating from symptomatic plant tissue in the field directly onto plates of CARP media, transporting samples to Oregon State University and Oregon Department of Agriculture laboratories, and identifying samples with traditional culturing or DNA extraction methods. Trees and shrubs in the vicinity of dead tanoaks were also examined for symptoms. An additional 224,200 ha were surveyed and ground-checked in October 2001. By the end of 2001, over 100 infected trees on nine infested sites were detected within a 23 km^2 area in the extreme southwest corner of the state near the city of Brookings, Curry County. Individual sites ranged in size from 0.2 to 4.5 ha and altogether totaled approximately 16 ha. Tanoak (*Lithocarpus densiflorus)* was the dominant affected species. Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*) were found to be infected to a much lesser degree and when infected were located in close proximity to or directly underneath infected tanoaks. An eradication effort was begun in fall 2001; affected host vegetation and healthy host plants within a 15 to 30 m buffer zone were cut, piled and burned.

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1 Presented at IUFRO Working Party 7.02.09 Mtg., *Phytophthora* in Forests and Natural Ecosystems, Sept. 2004, Freising, Germany
2 A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California
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Each year since 2001, continued systematic aerial surveys to detect tanoaks killed by *P. ramorum* have been conducted in early summer and mid autumn. Surveyed area has ranged from 90,000 to 150,000 ha using a risk-based approach to approximately 750,000 ha when covering the entire tanoak host type. Sites with dead tanoaks mapped from the air have increased from 41 in 2001, to 178, 292, and 307 in 2002, 2003, and 2004, respectively. In most years 100 percent of all mapped dead tanoaks have been visited and evaluated; difficulty accessing trees due to steep, rugged terrain has made visiting a few of the mapped trees impossible in some years. The number of *P. ramorum*-infected tanoaks confirmed each year has decreased since the initial detection of the pathogen from 85 to 48 to 36 in 2002, 2003, and 2004, respectively. A total of approximately 24 ha within a 30 km^2 area are considered infested as of September 2004.

P. ramorum is not the only agent associated with tanoak mortality in southwestern Oregon forests (Hansen and others 2003). Other mortality agents identified include *P. nemorosa*, *Armillaria mellea*, *A. gallica*, herbicides and mechanical damage. *P. nemorosa* causes bleeding symptoms quite similar to *P. ramorum*, making field diagnosis challenging. It is most often associated with widely scattered, single tree mortality. *Armillaria* species are found on trees with obvious *Phytophthora*-caused cankers as well as on trees with no evidence of *Phytophthora* infection.

In the course of ground-checking aerial surveys and monitoring eradication treatments, new hosts for *P. ramorum* were found and described. These include poison oak (*Toxicodendron diversiloba*) with visible bleeding and necrotic phloem symptoms typical of *P. ramorum*'s oak and tanoak hosts, and salmonberry (*Rubus spectabilis*) and cascara (*Rhamnus purshiana*), both foliar hosts with watersoaked leafspots (Davidson and others 2003).

Detecting *P. ramorum* from the air is nearly impossible when oak mortality is not visible or when *P. ramorum*-caused symptoms are restricted to leafspots and twig blights in understory species. Ground-based surveys for *P. ramorum* have been done in many areas within the known host type. These include roadside transect surveys designed to detect *P. ramorum* on leafspot and twig dieback hosts, surveys designed for early detection of *P. ramorum* within the host type but outside of known disease areas, transect surveys of host vegetation targeted along popular trails and in high-use campgrounds near the Winchuck and Chetco Rivers, and extensive watershed-level monitoring inside and outside the regulated area by baiting streams with tanoak and rhododendron leaves. In Oregon forests, new, positive *P. ramorum* finds have almost always resulted from either aerial detection surveys or eradication treatment perimeter plots and reconnaissance; *P. ramorum* has not been detected outside of known infested areas in either roadside or targeted ground surveys. It has been recovered from most streams draining infested sites, was recovered once from a stream within the affected area but not associated with a known infested site, and has never been recovered from monitored streams outside of the generally infested area.

Searching for *P. ramorum* in forest settings is challenging. Success associated with detecting *P. ramorum* in Oregon's tanoak forests is due to many factors. Funding for early detection is readily available. Experienced aerial surveyors work with the latest mapping technology. Cooperating field crews from several agencies and laboratories are committed to timely ground checking and sample processing. Landowners have given access to their properties for ground checking, treatment and

monitoring. In Oregon, *P. ramorum* primarily affects tanoaks, therefore, recently killed tanoaks can be used to indicate the most likely areas of disease. Despite new occurrences of *P. ramorum*, distribution of the pathogen remains limited to a relatively small area in Curry County. Surveys as described in this paper will be continued in both the known affected area and across the ranges of the host species.

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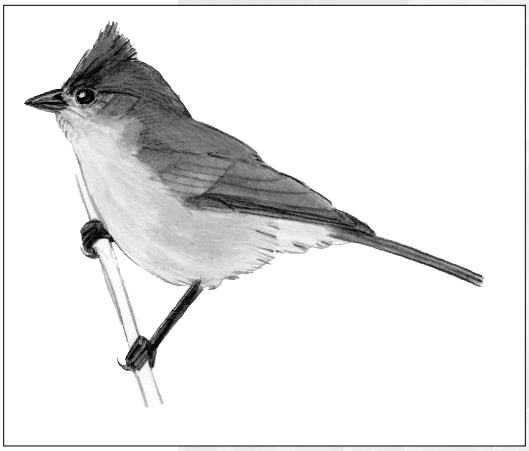
Management II



The Response of Saprotrophic Beetles to Coast Live Oaks Infected With *Phytophthora ramorum*[1]

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

Key words: sudden oak death, bark and ambrosia beetles, attraction

Abstract

Saprotrophic ambrosia and bark beetles (Coleoptera: Scolytidae) tunnel into the bark overlying cankers caused by *Phytophthora ramorum* in coast live oaks, *Quercus agrifolia*. These insects are characteristically reported to colonize freshly dead or moribund trees (Furniss and Carolin, 1977). However, the initial attacks by these beetles on *P. ramorum*-infected coast live oaks are limited to the bleeding cankers. In a disease progression study (McPherson and others, 2005), we found that beetles were present in about 50 percent of all bleeding trees in each of four years and every bleeding tree that died following beetle colonization had been attacked while the foliage was healthy. We have several lines of evidence from field studies suggesting that beetles play significant roles in sudden oak death (SOD). Beetles preferentially colonize bleeding cankers, catastrophic bole failure of bleeding trees is strongly associated with beetle tunnels, ambrosia beetles, particularly *Monarthrum* spp., require sound, undecayed wood, and the development of *Hypoxylon thouarsianum* fruiting bodies consistently follows beetle colonization.

The study described here was designed to quantify the attraction of these beetles to *P. ramorum*-infected trees. In July 2002, mature *Q. agrifolia* were 1) inoculated with *P. ramorum* (80), and 2) wounded but not inoculated (40). Half of each group was sprayed with insecticides to prevent beetle colonization. Wire traps coated with Tanglefoot were placed on the insecticide-treated trees to catch insects and monitored throughout 2003. Beetles were identified to species and to sex where possible. Infected trees attracted significantly more beetles than mock-inoculated trees. Of the seven beetle species most commonly trapped, six

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were in the family Scolytidae. Strongly skewed sex ratios were found for two of the four most abundant species. The greatest response to inoculated trees occurred within the first year following inoculation. Beetles had colonized more than half the bleeding trees not treated with insecticide through July 2004, killing 13 percent of this group within two years of inoculation.

Because only 72 percent of inoculated trees developed bleeding cankers, we analyzed beetle responses to bleeding vs. non-bleeding trees. Repeated measure analyses of various insect counts showed significant treatment (bleeding vs. non-bleeding) and treatment by insect species interaction effects. Bleeding trees attracted significantly more beetles than non-bleeding trees for the five collection periods in 2003. Beetle abundance was greater for the July-October period than during other months. We trapped five ambrosia beetle species (*Monarthrum scutellare*, *M. dentiger*, *Xyleborinus saxeseni*, *Xyleborus californicus*, *Gnathotrichus pilosus*), one bark beetle species (*Pseudopityophthorus pubipennis*) and one false powderpost beetle species (*Scobicia declivis*). For each species, significantly more beetles were trapped on bleeding than on non-bleeding trees (*Fig. 1*). Abundances of *X. californicus* were insufficient for analysis. Sex ratios were strongly male biased in the *Monarthrum* spp. and female biased in *X. saxeseni*. Through July 2004, four (14 percent) of the bleeding trees that were not sprayed with insecticides had died, in contrast to one sprayed tree.

The study provides direct evidence for the association of saprotrophic beetles with the death of trees infected by *P. ramorum*. The principal host materials of *P. pubipennis* are dead branches on live trees, and severely weakened, or recently killed trees. In Europe, false powderpost beetles are known to attack well-seasoned, dead timber. The roles that these beetle species play in SOD are not clear. However, their consistent association with discrete cankers on live native trees infected with an introduced pathogen suggests that these insects affect the etiology of the disease.

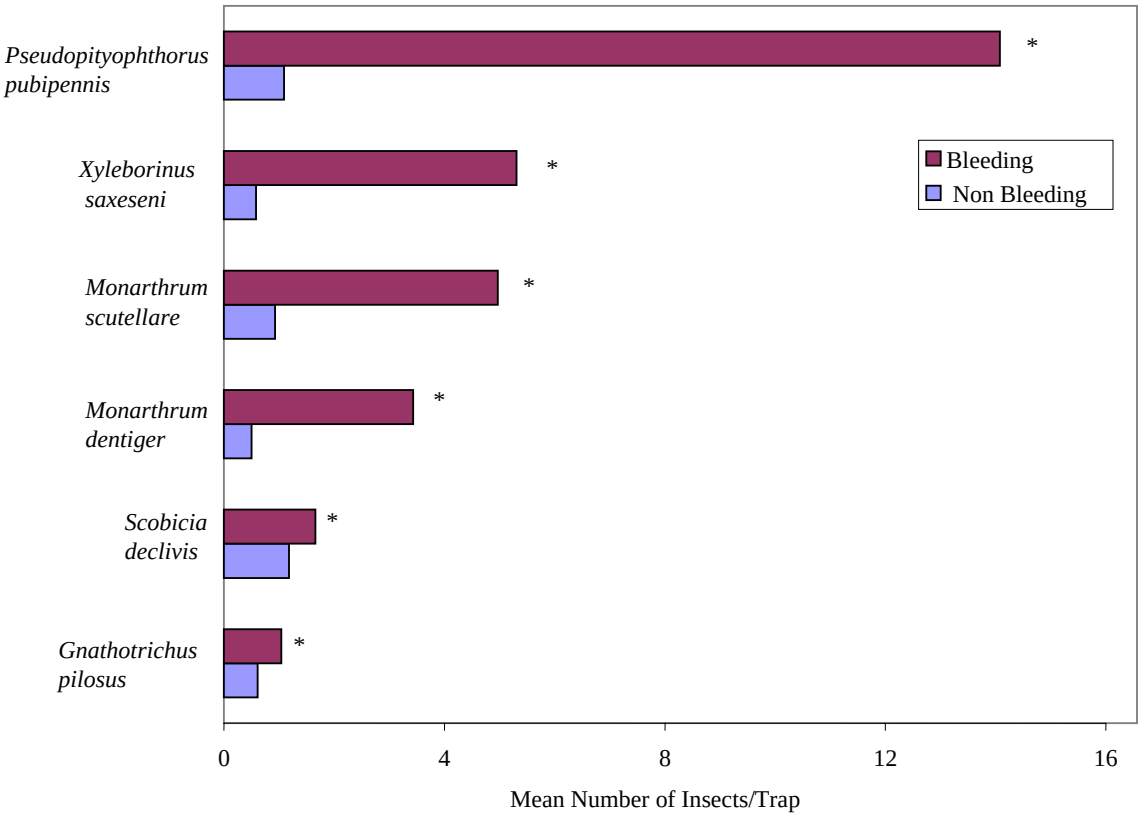

Figure 1— Species composition of bark, ambrosia & false powderpost beetles trapped at bleeding and non-bleeding trees; asterisks denote statistically significant differences ($p < .05$).

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Chemical Ecology of Sudden Oak Death/ Ambrosia Beetle Interactions[1]

Frances S. Ockels[2], Pierluigi Bonello[2,3], Brice McPherson[3] , and David L. Wood[3]

Key words: ambrosia beetles, coast live oak, *Phytophthora ramorum*, volatile

Abstract

Coast live oaks, *Quercus agrifolia*, infected with *Phytophthora ramorum* in California produce a characteristic sequence of symptoms and signs. Ambrosia beetles consistently tunnel into the bark of bleeding cankers in naturally infected trees. In field monitoring conducted since 2000, every bleeding coast live oak that subsequently died had been colonized by beetles while bleeding cankers were the only symptom of *P. ramorum* infection. Although the function of these beetles in decline and death of infected trees has not been confirmed, they likely disrupt nutrient and water conduction and accelerate structural failure (McPherson and others 2005). In mechanically inoculated trees, beetles colonize the cankered areas within nine months of inoculation. This behavior is not consistent with the reported feeding ecology of the saprotrophic insects (Chamberlain, 1958; Furniss and Carolin, 1977; Wood, 1982; McPherson and others 2005). In an inoculation study, we used traps to monitor the response of beetles to both infected and wounded but uninfected trees. Traps placed on inoculated trees caught significantly more scolytid beetles than traps on trees that were only wounded. Six species of saprotrophic beetles are now known to be attracted to these infected trees. This directed response to native oaks infected by a putative introduced pathogen points to the production of volatile chemical attractants (kairomones). Volatile samples were collected in two ways. Firstly, samples of volatiles emitted from the bark of infected and control oaks were collected in the field by strapping the mouths of 1-L plastic bottles directly onto the bleeding produced by cankers on inoculated trees, and over the site of the "inoculation holes" on the uninfected, but wounded trees. The point where each bottle rested against the bark was sealed to minimize mixing with external air. Alternatively, the "blood," the dark colored exudate released by cankers, was placed in a vial and sealed. In both cases, samples were collected 24 h later by exposing solid phase micro-extraction (SPME) fibers to the atmosphere inside the bottles/vials for 30 min. The samples were analyzed by gas

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chromatography-mass spectrometry (GC-MS) using the method of Martin and others (2003). Identification of eluting compounds was based on matching with natural product spectra contained in the Wiley Registry of Mass Spectral Data and the NIST Library. Preliminary results from GC-MS analysis identified, with a high confidence level, 4-ethylphenol, 4-ethlyguaiacol, and 4-propylguaiacol among the peaks. These compounds are volatile phenolics. Phenolics are known to be involved in host defense responses to microbial pathogens. Coast live oaks may respond to *P. ramorum* infection and canker development by producing volatile phenolics that in turn are attractive to ambrosia beetles. Future work to test this hypothesis includes: collecting additional volatiles for GC-MS analysis, initiating quantitative studies, and conducting field experiments to test behavioral effects of the identified compounds.

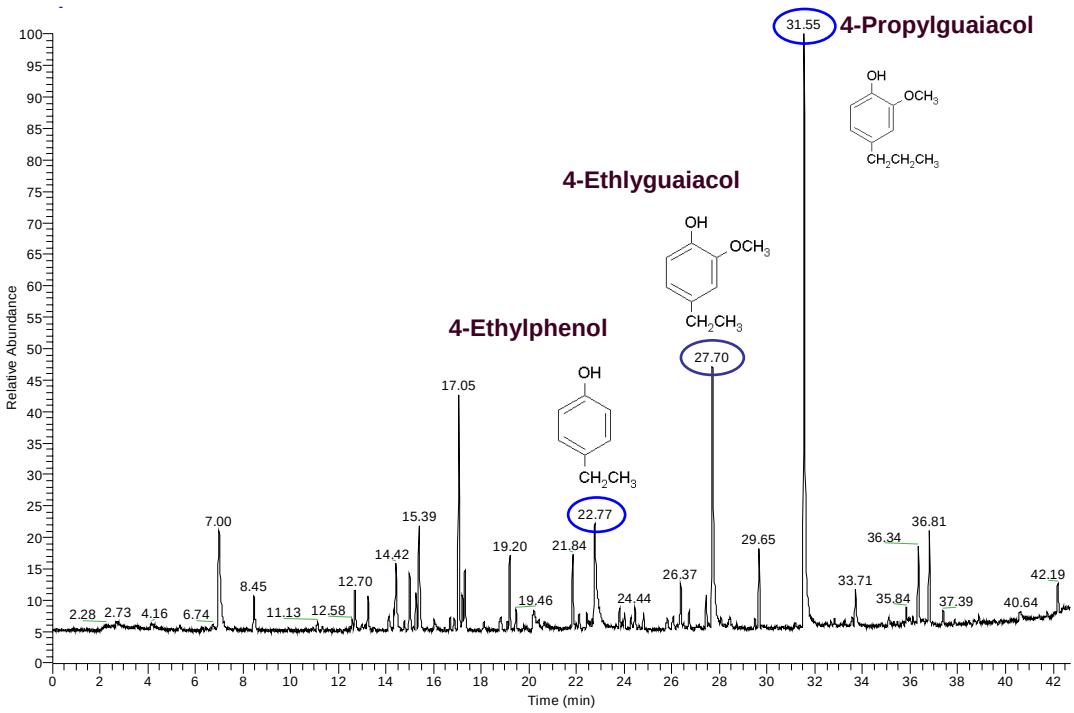

Figure 1— Gas chromatogram of the "blood" displaying subset of compounds identified by GC-MS analysis.

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Relationships Between *Phytophthora ramorum* Canker (Sudden Oak Death) and Failure Potential in Coast Live Oak [1]

Tedmund J. Swiecki[2], Elizabeth Bernhardt[2], Christiana Drake[3], and Laurence R. Costello[4]

Abstract

In autumn 2002, we conducted a retrospective study on coast live oak (*Quercus agrifolia*) failures in Marin County, California, woodlands affected by *Phytophthora ramorum* canker (sudden oak death). The objectives of this case-control study were to quantify levels of bole, large branch, and root failure in these woodlands and determine how various tree and stand factors are related to failure potential. Non-failed trees were used as a control population to identify factors that might contribute to tree failure. Rates of bole and large branch (more than 20 cm diameter) failures were significantly higher between about July 2001 and December 2002 than in the period from 1992 through July 2001. Bole failures were the most common type of failure. Based on the estimated date of failure, for the years 1992 through 1996, boles failures occurred in 0.5 percent of the trees each year. The incidence of bole failures increased to 5 percent per year between mid-2001 and the end of 2002. Among recent failures (2001-2002), 39 percent of the bole failures and 30 percent of the scaffold (primary branches arising from the main stem) failures occurred in live stems. Most root and root crown failures also occurred in live trees. Among trees with recent failures (2001-2002), 83 percent showed symptoms of *P. ramorum* canker. Branch, scaffold, and bole failures were strongly associated with advanced symptoms of *P. ramorum* canker, which include evidence of wood degradation by *Hypoxylon thouarsianum* and/or various wood boring beetles.

Early *P. ramorum* canker symptoms, consisting of only bleeding cankers without secondary invasion, were not associated with an increased likelihood of failure. Wood decay was the most consistent and important factor influencing failure potential. Decay was present and rated as a contributing factor in almost all failures. Fruiting bodies of *H. thouarsianum* and

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other wood decay fungi, decay columns, and canker rot symptoms were significantly more common among failures than among non-failed controls. Several variables indicative of wood decay were highly significant predictors of failure in both recursive partition and multivariate logistic regression models. Beetle boring was also significantly more common among failures than among non-failed trees. Other factors associated with increased failure potential include overtopping of the tree by other trees, local alteration of the stand canopy due to dead or failed trees, multiple trunks, multiple branches arising from the same point, and asymmetric canopy shape. Failures in both live and dead trees were largely influenced by the same factors.

Key words: tree failure, wood decay fungi, *Hypoxylon thouarsianum,* ambrosia beetles

Introduction

Phytophthora ramorum, the causal agent of sudden oak death, causes bleeding bark cankers on the main stems of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and several other oak species in California (Garbelotto and others 2001). The bark cankers can expand over time and eventually girdle susceptible trees. The sapwood-decaying fungus *Hypoxylon thouarsianum*, ambrosia beetles (*Monarthrum* spp.), and oak bark beetles (*Pseudopityophthorus* spp.) are commonly associated with *P. ramorum*-infected trees in later stages of decline (Garbelotto and others 2001). These agents also attack declining trees or branches that are not infected with *P. ramorum*.

Prior to this study, there were anecdotal reports that trees infected with *P. ramorum* were more likely to fail than noninfected trees. Because *P. ramorum* cankers primarily affect the bark, cambium, and occasionally some of the outer sapwood (Rizzo and others 2002), it appeared unlikely that *P. ramorum* alone would increase the failure potential of infected trees. Preliminary data released by the University of California, Richmond, Forest Products Laboratory (Shelly 2002) indicated that *P. ramorum* does not directly cause significant losses in wood density of tanoak.

However, wood decay fungi that are associated with *P. ramorum* cankers, such as *H. thouarsianum,* do significantly reduce wood density and strength. Increased levels of wood decay associated with *P. ramorum* cankers could therefore increase the likelihood of failure in trees with sudden oak death. In addition, wood boring beetles, especially ambrosia beetles, are often associated with decay in trees with *P. ramorum*

canker. Previous studies had not addressed whether beetle boring contributes meaningfully to failure potential.

To investigate the relationship between sudden oak death and tree failure, we conducted a retrospective study on tree failure in coast live oak woodlands affected by *P. ramorum.* Our objectives were to quantify levels of failure in these stands and determine how various tree and stand factors were related to failure potential. Factors under study included *P. ramorum* canker symptoms, colonization by *H. thouarsianum* and other decay fungi, beetle boring, tree defects, and stand characteristics.

Methods

Study site selection

To observe adequate numbers of both failed trees and trees with *P. ramorum* symptoms, we selected six coast live oak stands in Marin County, California, in which both conditions were common. The stands used in this study included permanent sudden oak death research plots we had established in September 2000 as part of a related study (Swiecki and Bernhardt 2001, 2002a,b, 2005). We had visited these stands annually beginning in September 2000 and already had observations on overall disease levels and the timing of some recent failures. The study areas all had relatively dense mixed hardwood forests dominated by coast live oak and California bay (*Umbellularia californica*), with lesser amounts of California black oak and madrone (*Arbutus menziesii*). The study area elevations ranged from 30 to 180 m.

Survey design

At each location we surveyed a wide area around the pre-existing plots from the related (Swiecki and Bernhardt 2001, 2002a,b, 2005) study. Field data were collected over a period of about six weeks, beginning in late October 2002. Final observations were made at all locations in December 2002. Boundaries of the surveyed areas were determined from aerial imagery of the sites and readings from a GPS receiver (Garmin® GPS76 used with an external, boom-mounted antenna). Surveyed areas at the six locations ranged in size from 0.95 to 2.08 ha , based on polygons of the study sites created with ArcView® GIS software.

Branch failures were counted only if they were at least 20 cm diameter at the point of failure. Bole, root crown, and root failures were counted if they affected main stems at least 15 cm diameter at 137 cm height (DBH). Within each of the study areas, we catalogued all failures of coast live oak and California black oak that exceeded these

minimum size thresholds and appeared to have occurred within the past 10 years. We recorded GPS coordinates of trees with rated failures. Seven of the failures (2.3 percent) were secondary failures, i.e., failures that occurred because the trees were hit by material from an adjacent tree failure. These failures were excluded from the study results.

We used a case-control study design to investigate relationships between various tree and plot factors and failure potential. Cases were 106 coast live oak trees with recent failures (occurring within about 1.5 years of the survey date) that exceeded the minimum size thresholds noted above. Cases were selected randomly from observed recent failures, and were spatially stratified to ensure that cases were distributed throughout the study areas to the degree possible. The number of cases rated per location ranged from 11 to 25.

Controls were 170 non-failed coast live oaks within the sampled stands where the failures were located. The number of controls rated per location ranged from 22 to 37. We sampled both near controls, i.e., the nonfailed tree closest to a given failure, and far controls, i.e., non-failed trees located away from failures. Overall, 103 controls were located within 10 m of a failed tree and 71 were more than 10 m from a failure. Far controls were included to ensure that variation in site factors (e.g., slope, aspect) was not constrained, since such factors tend to be similar among trees that are near each other. Both types of controls were sampled without bias and were not matched to cases with respect to tree form or other characteristics. This allowed us to investigate a wide variety of tree and site factors as potential explanatory variables in the statistical models.

After cataloging all failures, we made a complete count of all coast live oak and California black oak trees with DBH of 10 cm or more within each study area.

Variables assessed

For all failed trees within the study areas, we recorded various characteristics of the failures, including: site of failure (bole, branch, etc.); diameter at the point of failure, major factors contributing to the failure (decay, cavity, beetle boring, structural defects, wounds). We estimated the failure date using a variety of indicators including the amount of oxidation and weathering of the broken wood surface, the condition of foliage, if any, degradation and loss of fine twigs, the amount and type of debris that had accumulated on exposed surfaces, and knowledge of recent failure dates based on previous visits to the sites. Failure dates were assigned to one of the following time classes: (1) July 2002 to December 2002; (2) January to June 2002; (3) July to December 2001; (4) before July 2001 but within the previous 5 years; (5)

between 5 and 10 years ago. The first three time intervals were subsequently combined to form the recent failure age class (i.e., within 1.5 years of the survey).

The data listed above were collected for failures in 141 coast live oaks and 13 California black oaks that were not used as cases. In addition, for a small number of failed trees (24 coast live oaks and five California black oaks), factors contributing to failure were not rated. These were older (failed more than 1.5 years before the survey) and/or relatively small-diameter failures that were not observed until the complete count of trees within the study locations was conducted in December 2002. These 29 trees are included in overall failure percentages but are excluded from some analyses.

For recent failures (failed within 1.5 years of the survey) that were assigned as cases, we assessed the above variables and a number of additional variables related to the failure. These included whether the failure was associated with previous failures; thickness of failed wood; failure direction; height of failure above the ground; depth of beetle boring; and number of induced (secondary) failures. We also determined the status of the failed part at the time of failure (live or dead). Status was generally obvious for very recent failures. For somewhat older failures, we inferred the status at the time of failure from factors such as the degree of leaf retention and orientation of dead leaves, evidence of post-failure wilting of leaves and stems, moisture level in the wood, the pattern of breakage on impact, and sunburning of bark on large stems, which commonly occurs after failures of live stems.

For all cases and controls, we also collected a wide variety of data on tree form, condition, and site characteristics. These included ground slope and aspect; canopy exposure to overhead light and to wind from the side; presence of recent changes in stand density around the subject tree; number and diameters of stems over 3 cm DBH; presence and severity of symptoms related to *P. ramorum* canker, including the extent of beetle boring damage and *H. thouarsianum* sporulation; the presence, type, extent, and location of decay and cavities; type(s) fungal fruiting bodies present; overall tree condition, and the presence of a variety of structural defects that may contribute to failure. Tree defects rated included those used in the California Tree Failure Report Program (CTFRP) rating form (Edberg and others 1993). A complete description of all variables assessed in failed trees and controls is reported in Swiecki and Bernhardt (2003).

Evaluations of *P. ramorum* canker on case and control trees were based on visual symptoms, including external bleeding and discolored bark tissue visible when the outer bark surface is sliced away (Rizzo and others 2002). For 51 trees failed and control trees in this study that were also located within the pre-existing plots from our

other study, disease evaluations had been made in September 2000 and 2001 as well as in the year of the survey. *P. ramorum* has been isolated from symptomatic trees at all of the study locations by ourselves and/or other researchers. However, because isolation efficiency would likely have been low due to both the time of year that trees were evaluated and the fact that many of the failed trees were already dead, we did not attempt to isolate *P. ramorum* from symptomatic trees in this study.

Statistical analyses

We used JMP® statistical software (SAS® Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $p \leq 0.05$. Only one failure per tree was used for all statistical model building. For the few case trees in which more than one failure was scored, one failure was randomly selected to be included in the recursive partition and logistic regression analyses.

We used the likelihood ratio chi square statistic to test for independence of variables in 2×2 or larger contingency tables. We used the recursive partitioning platform in JMP® to develop models relating various predictor variables to the case (failure) outcome. The platform recursively partitions data in a dichotomous fashion according to a relationship between the predictor and outcome values, creating a tree of partitions. Each partition is chosen to maximize the difference in the responses between the two branches of the split. Splitting was done interactively and was stopped when an endpoint had fewer than five trees in it or consisted of all failures or controls. After splitting, models were pruned upward to minimize the misclassification rate. We also calculated and compared k-fold crossvalidated G^2 statistics (k=5) for candidate models to assess relative improvement in fit when building models. Unless they were associated with a large change in the crossvalidated G^2, we also pruned splits in which both sides of the split had a majority of the same outcome (cases or controls).

For logistic regression models of the case (failed) versus control (not failed) outcomes, the likelihood ratio chi square was used to test the significance of each effect in the model. We also calculated Akaike's information criterion (AIC) to compare the fit of alternative models. For models constructed for a given data set, smaller AIC values indicate better model fit.

Results

Overall failure rates

Within the six study areas, we recorded data on 1540 coast live oak and California black oak trees (*table 1*). We catalogued 308 failures that were within the age range (past 10 years) and size classes (at least 20 cm diameter for branch failures, at least 15 cm diameter for bole or root failures) that were used as cutoffs for the study. The catalogued failures occurred in 297 trees, some of which had multiple large failures. An additional 105 failures slightly below the size thresholds were observed in the study areas. With one exception (a bole failure less than 15 cm diameter), these were branches that were between 10 and 20 cm in diameter. Trees with these smaller failures are not included in the analyses of failures discussed below.

Table 1— *Coast live oak and California black oak failures by type and study site characteristics at the six study locations.*

Location	Area[1] (ha)	Total trees surveyed[2]	Percent of trees with failures	Percent bole failures[3]	Percent branch failures[3]	Percent root and root crown failures[3]
2	1.984	489	8	70.5	25.0	4.5
3	1.494	286	20	62.5	28.6	8.9
5	2.077	204	23	37.2	51.0	11.8
6	1.471	186	31	67.3	22.4	10.3
8	1.668	163	33	35.8	49.1	15.1
11	0.949	212	20	41.3	56.5	2.2
Totals	9.644	1540	19	52.6	38.3	9.1

[1] Areas are based on flat GIS polygons and underestimate actual surface area due to significant ground slopes at many sites.
[2] Includes only coast live oak and California black oak trees within surveyed areas.
[3] Percent of all failures occurring within the previous 10 years that were above the following size thresholds: branch failures greater than 20 cm diameter, bole, root, and root crown failures of stems greater than 15 cm DBH.

The percentage of failed trees differed significantly between the six locations (likelihood ratio test $p<0.0001$) and ranged from 8 percent to 33 percent. The relative prevalence of bole, branch, and root failures also varied significantly by location overall (likelihood ratio test $p=0.0001$). The majority of the failures within the target size and age ranges were bole failures (*table 1*).

California black oak was a minor component of the woodlands in the study, comprising only 5 percent of the oak trees included in the sample. Of all trees with failures, 6.8 percent were California black oak. Overall failure rates for coast live oak (19.1 percent) and California black oak (23.7 percent) were not significantly different.

Failure dates

Several types of failures were much more common in the 1.5 years before our survey than in the preceding decade (*fig. 1*). Bole failures showed the greatest rate of increase overall, but the rate of branch failures also changed significantly over time (likelihood ratio test $p<0.0001$). Root and root crown failure rates did not differ significantly over the three time intervals. None of the study areas had burned in the 10 years prior to the survey and were not subject to other factors that would remove evidence of older failures. Recent failures (within 1.5 years of survey) were common at all study sites, constituting between 45 percent and 74 percent of the observed failures at the different sites.

About one third of the failures that occurred in 2002 apparently occurred in the first half of the year, although the precise timing of these failures could not be determined. However, of 112 recent failures observed, 27 still had green leaves and moist wood, indicating that these failures had occurred within a month or less of the survey. At least six trees failed between successive visits to a site during autumn 2002. We also heard six nearby tree failures between September and December 2002, all of which occurred during calm weather. Our observations indicate that recent failures in the study areas had occurred throughout the year and many occurred in the absence of severe weather during summer and fall 2002.

Condition of failed treesP. ramorum symptoms

In general, the presence of characteristic *P. ramorum* disease symptoms in failed trees could be rated with confidence only for trees that had failed in the previous 1.5 years, so the analysis in this section is restricted to that subset of trees (*fig. 2*). We also scored trees with less definitive *P. ramorum* symptoms as questionable when noting their most likely disease status. Trees with questionable *P. ramorum* disease symptoms are excluded from *fig. 2*, although the overall trends do not change if the questionable trees are included with their most likely disease categories. Trees with *P. ramorum* symptoms failed at a significantly higher rate (likelihood ratio test $p<0.0001$) than trees lacking *P. ramorum* symptoms. Trees with symptoms of *P.*

ramorum canker constituted 83 percent of the trees with failures but only about 31 percent of the non-failed control trees.

The overwhelming majority of recent failures occurred in trees that had *P. ramorum* symptoms and were either dead or had late symptoms of disease, i.e., beetle boring and/or *H. thouarsianum* fruiting bodies present (*fig. 2*). None of the recorded recent failures occurred in trees displaying only early *P. ramorum* canker symptoms, i.e., bleeding cankers only (*fig. 2*). This result indicates that increased risk of failure is associated with degradation of wood by decay fungi and/or beetles following the appearance of *P. ramorum* canker symptoms, not with *P. ramorum* cankers alone.

Most of the recently failed trees that lacked *P. ramorum* canker symptoms were either dead or in decline due to other disease agents (*fig. 2*), most commonly canker rots or other wood decay fungi. Trees killed by these decay fungi usually experienced multiple failures before they died. Among trees without *P. ramorum* symptoms, the proportion of dead and declining trees was significantly greater among cases than among controls (likelihood ratio test $p<0.0001$). Of the 29 recently failed trees without *P. ramorum* canker symptoms, 41 percent were in decline and 34 percent were dead due to other agents. In comparison, among the 113 non-failed controls without *P. ramorum* symptoms, 11 percent were in decline and none were dead. Only a small percentage of non-declining live trees exhibited recent failures above the size thresholds (*fig. 2*).

Bole failures in trees with *P. ramorum* symptoms occurred across the full range of size classes represented in the population (median diameter at failure 37 cm; mean 38.7 cm, standard deviation [sd] 16.7), with the majority of bole failures occurring in trees between 20 and 60 cm diameter at the point of failure. In contrast, most bole failures in trees lacking *P. ramorum* symptoms occurred in stems less than 25 cm (median diameter at failure 23.5 cm; mean 28.4 cm, sd 14.7). These small diameter trees with bole failures were typically suppressed understory trees that were colonized by wood decay fungi.

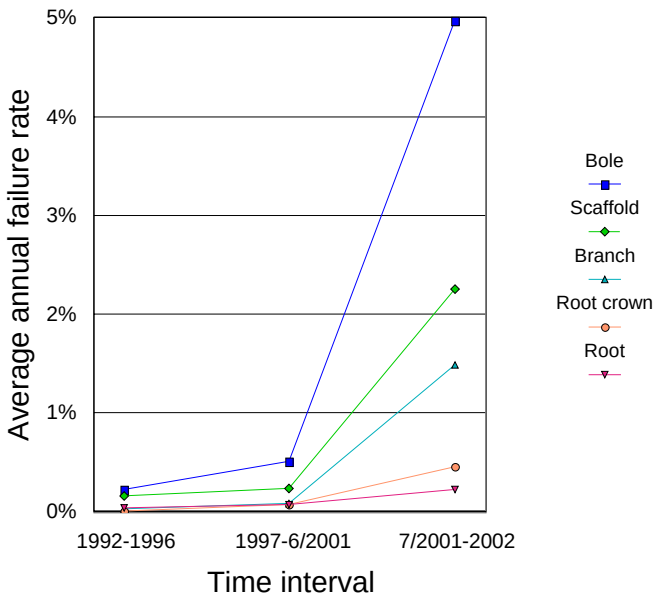

Figure 1—Estimated annual failure rates by failure type for the standing tree population of coast live oak and California black oak within the study areas over three time intervals. The base tree population size was adjusted downward for each interval by subtracting trees with bole, root crown, and root failures that occurred in the previous interval(s) (n=1540, 1521, and 1476, respectively, for the three intervals).

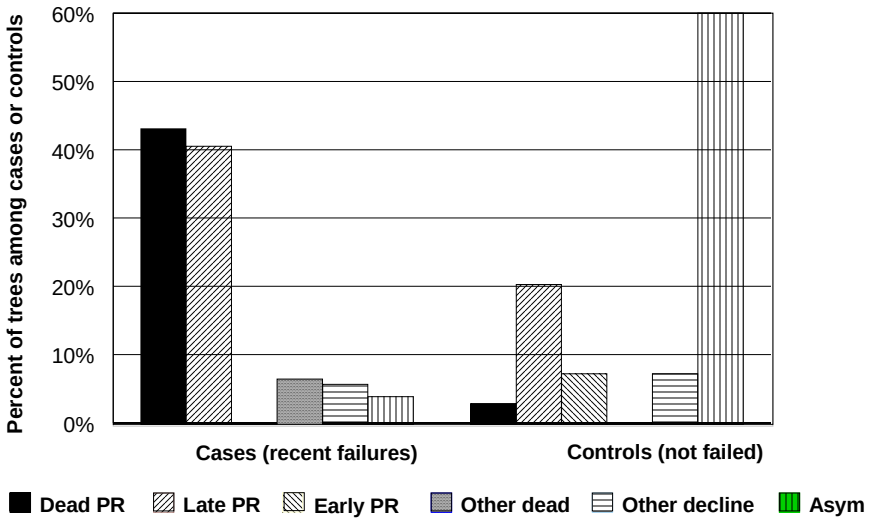

Figure 2— Disease status for coast live oaks with recent (within previous 1.5 years) failures and nonfailed control coast live oaks. Failures and controls with uncertain *P. ramorum* symptoms are excluded; missing columns indicate 0 percent. **Dead PR**= dead trees with extensive *P. ramorum* symptoms; **Late PR**= live trees with *P. ramorum* canker symptoms plus beetle boring and /or *H. thouarsianum* fruiting bodies; **Early PR**= live trees with *P. ramorum* canker symptoms but no secondary organisms; **Other dead**= dead trees lacking *P. ramorum* symptoms, mortality due to other agents; **Other decline**= live trees lacking *P. ramorum* symptoms, in severe decline due to other agents; **Asym**= no evident symptoms of either *P. ramorum* canker or decline due to other agents (n=155 failures and 162 controls).

Most trees with failures were either dead at the time of failure or the failed part was dead at the time of failure. This includes dead main stems of multi-stemmed trees that were classified as having late *P. ramorum* symptoms because they still had one or more live stems. Within the study areas, 75 percent (131/174) of the dead trees had failures above the threshold size. However, a substantial number of live stems also failed. More than 28 percent of all failures occurred in live stems among trees for which the status (living or dead) at the time of failure could be determined (primarily failures occurring within the past 1.5 years). Live stem failures constituted 39 percent of the bole failures and 30 percent of the scaffold (primary branches arising from the main stem) failures. Root and root crown failures also occurred predominantly in live trees.

Factors associated with tree failure

For most failures, we noted the major factors that appeared to have contributed to failure (*fig. 3*). These data do not include older failures for which contributing factors could not be reliably identified. Factors were scored as contributing to failure only if present at levels that were high enough to feasibly affect failure potential. Wood decay was evident in the broken wood surface of almost every failure and was judged to be a major factor contributing to failure in over 96 percent of the observed failures (*fig. 3*). Cavities, beetle boring, and structural problems also contributed to failure in a sizeable number of trees, but in all of these trees, decay was also scored as a contributing factor. For many trees, multiple factors were scored as contributing to failure. Wounds contributed to failure in only a single tree.

In addition to these general ratings, we also made detailed observations on decay, cavities, beetle boring, structural defects, and other factors that might be related to failure potential in case and control trees. We used these and other factors in the statistical models described below to determine which factors were related to failure risk.

Wood decay and cavities

For case trees, we estimated the percent of the stem cross-sectional area at the point of failure that was affected by decay or cavities. Most failed stems showed high levels of decay (*fig. 4*). Cavities were present in only 27 percent of the failures, and only 9 percent had cavities affecting more than half of the cross sectional area (*fig. 4*).

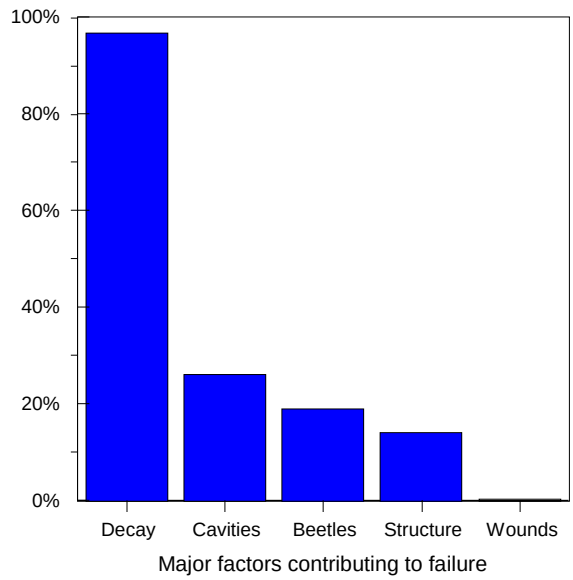

Figure 3— Frequency of major factors contributing to failures (n = 259 failed coast live oak and 13 failed California black oak).

Estimates of decay in non-failed control trees were based on external symptoms only. Although the amount of decay present in controls was probably underestimated, 58 percent of the controls had no obvious decay and only 1 percent of the controls had more than 50 percent cross-sectional decay. These observations suggest that severe wood decay was much less common in non-failed trees than in failed trees.

We probed exposed cavities in non-failed trees to gauge their extent, so cavity ratings of controls are more directly comparable to cavity ratings in cases, at least for open cavities. The distribution of cavity ratings (recoded to three levels for contingency table analysis) did not differ significantly between cases and controls (likelihood ratio test p=0.129). Most observed cavities in both cases and controls were small, and such small cavities did not appear to greatly increase the likelihood of failure in coast live oak.

Cavities and related defects, such as wounds from previous failures and decayed branch stubs, were not consistently present at the point of failure, but 64 percent of the recorded failures were near previous branch or stem failures. The distance between the defect and the point of failure averaged 0.42 m (range 0 to 2 m). When such defects occurred at or very near to the point of failure, they commonly represented a point of structural weakness that contributed to the failure. In other situations, especially where the defects were further from the point of failure, the defects were areas where decay organisms had gained entry into the tree or indicators of decay columns that contributed to both recent and earlier failures on the same stem.

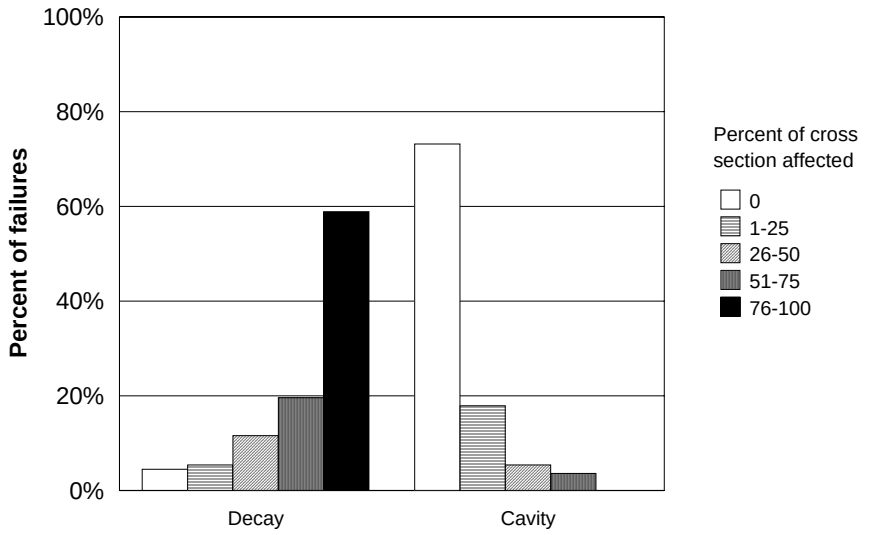

Figure 4—Percent of cross-sectional area at the point of failure affected by decay and cavities among failures scored in coast live oak case trees.

Trees affected by *P ramorum* canker commonly showed fruiting of the wood decay fungus *H. thouarsianum*. *H. thouarsianum* fruiting bodies (stromata) were present on 91 percent of the cases that had *P. ramorum* canker symptoms, but on only 39 percent of cases without *P ramorum* symptoms. *H. thouarsianum* stromata were present on 82 percent of all case trees but only 21 percent of non-failed control trees (likelihood ratio test $p<0.0001$).

For both cases and controls, we attempted to quantify *H. thouarsianum* infections based on the distribution and density of stromata to determine whether such variables could be used to estimate failure potential. Among all trees, the average rating for the percent of stem circumference with *H. thouarsianum* stromata was significantly higher in cases than controls (t-test $p<0.0001$). To more rigorously test whether distribution of stromata was a predictor of failure potential, we repeated the analysis after excluding trees without *H. thouarsianum* from the analysis. For the 128 trees with *H. thouarsianum* stromata, the percentage of stem circumference with stromata was a significant predictor of failure (likelihood ratio test $p=0.001$). Although the likelihood of failure increased as the percent of the circumference with *H. thouarsianum* stromata increased, the density of *H. thouarsianum* stromata on the stem was not a significantly predictor of failure.

Other decay fungi were also significantly associated with failure risk. The incidence of canker rot symptoms was higher among cases (64 percent) than controls (31 percent) (likelihood ratio test $p<0.0001$). Fruiting bodies of the canker rot fungus *Inonotus andersonii* and *Phellinus* spp., most commonly *P. gilvus,* were also significantly more common among cases than controls. Fruiting bodies of *I.*

andersonii were found only on failed trees. *P. gilvus* often seemed to fill a niche similar to that of *H. thouarsianum* as an opportunistic but somewhat aggressive colonizer of stem areas affected by *P. ramorum* canker.

Beetle boring

Boring by beetles, particularly ambrosia beetles, was significantly more common in cases than controls (likelihood ratio test $p<0.0001$). Among cases, the incidence of beetle boring was 86 percent. In comparison, beetle boring was noted on the lower 2 m of the main stem(s) in 30 percent of controls. Ambrosia beetles were associated with 79 percent of the failures in case trees, although they were frequently not present at levels that were likely to contribute to failure potential (*fig. 3*).

Ambrosia beetle boring was also closely associated with advanced *P. ramorum* canker symptoms. Obvious *P. ramorum* canker symptoms were present in 88.5 percent of the 122 trees (cases +controls) that had evidence of ambrosia beetle boring. Only four trees with ambrosia beetles were rated as free of *P. ramorum* symptoms; the remaining ten were of uncertain disease status.

In failed trees, ambrosia beetle boring was almost exclusively limited to portions of the wood that showed incipient to advanced decay. All of the cases that had beetle boring present also had wood decay present. In contrast, of the failures that had wood decay, 17.8 percent had no ambrosia beetles and 11.6 percent had no evident beetle boring of any type.

We measured the maximum depth to which beetle tunnels extended into the wood on 92 failed stems. The maximum observed depth was 15 cm from the cambium. Thus, beetle boring can potentially reach the center of stems with a below-bark diameter of 30 cm or less. However, the mean depth of boring was 7.3 cm and 90 percent of all ambrosia beetle tunnels extended 10 cm or less into the wood. Depth of boring was not significantly correlated with stem diameter, so as the diameter of the failed wood increased, the proportion of the cross section mined by beetles decreased. Consequently, beetle boring was less likely to directly contribute substantially to loss of wood strength and failure potential in large diameter stems than in small diameter stems.

Structural defects

We scored all cases and controls for the presence of the structural defects listed on the CTFRP tree failure assessment form (Edberg and others 1993) and several other

common defects. For failed trees, we also noted whether any of the observed defects were likely to have contributed to the failure.

Overall, many defects were equally common in cases and controls, but several defects were noted more frequently in trees with failures than in non-failed trees (*table 2*). These included decay columns, cavities, one sidedness, and cracks or splits. Although tree structure was scored as a main causative factor in only 20 percent of all failures (*fig. 3)*, structural defects were commonly scored as playing a contributing role in failures (*table 2*). For instance, as noted earlier, many failures occurred in close proximity to defects such as cavities and decayed branch stubs. In such situations, these defects may have contributed to failure potential even though they were not the primary cause of failure. In general, structural defects did not substantially increase failure potential in trees that had little or no decay. However, in trees with substantial amounts of decay, failures were more apt to occur in stems that were also compromised by structural defects.

Only one defect, excessive tree lean, was scored as being present at a significantly higher incidence in controls than in cases. However, we believe that this may be an artifact associated with rating failed trees. Particularly for bole and root crown failures, it can be difficult to estimate the pre-failure amount of lean after failure has occurred.

We coded multiple trunks and co-dominant stems as a single defect type to maintain consistency with the CTFRP reporting form. As shown in *table 2*, the incidence of this combined defect category did not differ significantly between cases and controls. However, stem count data show that 44 percent of trees in this category have a single bole but co-dominant leaders. If trees with multiple stems were considered separately, the percentage of multi-stemmed trees was significantly greater among cases (41 percent) than controls (28 percent) (likelihood ratio test $p=0.035$). In addition, stem count was recorded for all trees within the study areas during the tree count. Using coast live oak data from the entire study area ($n=1431$, excluding secondary failures), bole and root crown failures were significantly more likely to occur in multi-stemmed trees than in single stemmed trees (likelihood ratio test $p<0.0001$ and $p=0.0002$, respectively). The incidence of branch and root failures did not differ between single stem and multi-stemmed trees. In the study areas, most coast live oaks with multiple stems originated from coppice sprouting. Many of these sprout-origin trees have unbalanced canopies and defects at the base of the tree, such as cavities and decay, which may make them more prone to bole and root crown failure.

Table 2— *Frequencies of common structural defects in coast live oak cases (failed trees) and controls (nonfailed trees)*

Defect	Frequency in controls (percent)	Frequency in cases (percent)	Significance[1]	Contributed to failure[2] (percent of cases)
Canopy one sided	58	78	0.0003	94
Multiple trunks/ codominant stems	58	62	ns	35
Hollow branch stubs	53	58	ns	48
Excessive lean	52	27	<0.0001	83
Crook or sweep in bole	50	43	ns	80
Decay column	39	84	<0.0001	84
Embedded bark in crotch	25	15	ns	63
Multiple branches arising at one point	24	30	ns	63
Heavy lateral limb	19	16	ns	82
Cavities	16	38	<0.0001	73
Canopy top heavy	6	8	ns	100
Cracks or splits	2	13	0.0004	86

[1] Significance level of likelihood ratio test comparing frequency of the defect in the controls with that in the cases
[2] For cases only, all defects present were also evaluated as to whether may have contributed to the observed failure.

Multivariate models of factors associated with failure

We developed two types of multivariate models, recursive partition and logistic regression models, to identify factors associated with failure. Both types of models can be used to identify variables that predict an outcome, e.g., the case (failure) outcome in our case-control study. The two types of models are constructed in different ways and can therefore produce somewhat different sets of significant factors. Recursive partition models (other names for the overall technique include decision trees, CART™, etc.) are developed in a sequential, dichotomous fashion. At each step in the analysis, the procedure selects the explanatory factor that maximizes the difference in the responses between the two branches of the split. Subsequent splitting of the partitions is made in the same fashion. In recursive partition models, the algorithm accounts for the possibility that a predictor variable may affect the outcome differently in the presence or absence of another factor, or that the effects of

one predictor variable depend on the levels of another. In logistic regression models, such relationships need to be modeled using interactions.

Given the wide variety of variables measured and the fact that some of these variables are highly correlated with each other, the data can be fitted to a number of alternative models. Our objective in constructing models was to explore the relationships between explanatory variables and determine which variables or sets of related variables could be used to identify trees that are most likely to fail.

Recursive partition models

We constructed the recursive partition model for the case outcome (all failure types) starting with 43 possible predictor variables. Candidate predictor variables included defect types, indicators of disease status, variables describing the distribution and intensity of beetle boring and *H. thouarsianum* sporulation, and variables related to the site and the tree's position within the stand. The recursive partition model for all failure types is summarized in *fig. 5*. The presence of *H. thouarsianum* fruiting bodies provided the best first split of the data. Trees with *H. thouarsianum* were predominantly (82 percent) cases, whereas the group lacking *H. thouarsianum* were mostly (80 percent) controls. The second split for both halves of the partition tree was based on the presence of *Phellinus* fruiting bodies, most commonly *P. gilvus*. Cases were more likely than controls to have *Phellinus* fruiting whether or not they also had *H. thouarsianum* sporulation. Further splits were based on sky exposed canopy (higher percentage of cases where exposure was less than 50 percent), the presence of *I. andersonii* fruiting bodies (only present in failed trees), the distribution of *H. thouarsianum* stromata around the stem (cases more likely to have stromata around at least 50 percent of the stem circumference) and the presence of multiple branches arising from a single point, often as co-dominant stems (this defect was more common in cases).

We also constructed recursive partition models using bole and root crown failures as the outcome variable (not shown). This model used the same initial split (*H. thouarsianum* presence) as the model for all failures, and also included splits based on *Phellinus* presence and sky exposure. However, other variables included in this model differed from those in the model for all failures. Among trees with *H. thouarsianum* and lacking *Phellinus,* increased likelihood of failure was associated with one-sided (i.e., unbalanced) tree canopies, dead trees, and multi-stemmed trees.

Logistic regression models

Some of the explanatory variables that we evaluated are highly correlated with each other. In constructing logistic regression models, highly correlated explanatory

variables can often be substituted for each other with relatively little change in overall model fit. Furthermore, highly correlated variables, when included in the same model, will often fail to be significant, even when they are known prognostic factors. The effects of the variables cannot be separated; only combined effects can be estimated. Variables related to the presence and abundance of beetle boring, *H. thouarsianum* sporulation, and the late or dead *P. ramorum* canker status were highly correlated. Variables describing these three factors usually cannot be included in the same model, but they can be substituted for each other in the model without greatly affecting model fit.

The best fitting models do not necessarily constitute the best models from the standpoint of predicting failure. Some variables are more readily detected or more precisely rated in failed trees than in intact trees, or vice versa. For instance, although both internal decay and ambrosia beetle boring are strongly associated with failures, they are much more evident on failed wood surfaces than in intact trees. While these factors can be fitted into multivariate models, they may not be as useful for predicting failure in intact trees. We avoided using variables in the reported models that were likely to be biased due to differences in ratings of the factors in intact and failed trees.

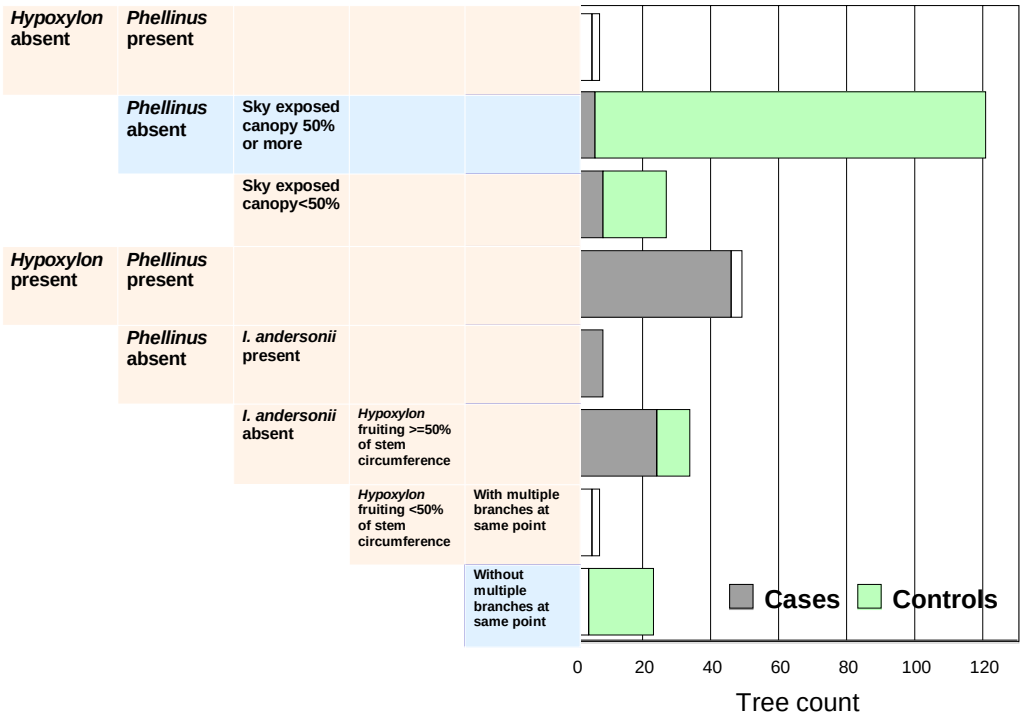

Figure 5— Summary of results of recursive partitioning model of all coast live oaks with failures (cases, n=106) and nonfailed controls (n=170).

Table 3 shows parameters for two models with nearly identical fit (based on AIC values) using combined data for all failure types. Model one includes the overall *P. ramorum* symptom status as a variable. Compared with asymptomatic trees or trees with only bleeding cankers (early *P. ramorum* symptoms), trees with more advanced disease symptoms were much more likely to fail. In model two, the presence of beetle boring and greater distribution of *H. thouarsianum* sporulation around the stem are both positively associated with failure. One or both of these agents were present in all trees with late *P. ramorum* symptoms. The other six variables that these two models have in common have nearly identical significance levels and odds ratios in the two models. Failure was more likely to occur in dead trees, trees that had *Phellinus* sporulation or canker rot symptoms, and trees in tree neighborhoods altered by adjacent or nearby failures or mortality. Failure potential also increased with the number of stems, but decreased with increasing levels of canopy exposure to the sky (i.e., increased dominance). No other predictors were consistently fitted into the best overall models for all failures.

Using the logistic regression models, a high probability of failure was predicted only if a number of factors are simultaneously present at levels that favor failure. This is consistent with field observations. We observed that even dead trees, which have an intuitively high failure potential, generally did not have large-diameter failures unless they exhibited other symptoms associated with decay and degradation (e.g., beetle boring, *H. thouarsianum* or *Phellinus* sporulation) or poor structural characteristics (e.g., multiple stems).

Overall failure patterns in trees with P. ramorum *canker symptoms*

Coast live oaks with severe *P. ramorum* canker symptoms typically followed one of several general patterns of failure characterized by different types of wood decay. These patterns were evident in both this study and in our related long-term disease progress study (Swiecki and Bernhardt 2004). The first failure pattern was common in trees with diameters up to about 30-40 cm. Many of these trees were healthy and sound prior to the onset of *P. ramorum* canker symptoms. These trees commonly experienced bole failures characterized by moderate to heavy colonization of the failed stem cross section by ambrosia beetles and extensive to nearly complete wood decay that originated in the outer sapwood and progressed inward. Fruiting of *H. thouarsianum* and/or *P. gilvus* was commonly present, suggesting that these were the among the primary sapwood decay fungi. Trees commonly failed after the top was

completely dead, although some trees with green tops showed this failure pattern. Bole failure was commonly the first failure above the threshold size that occurred in these trees. This pattern of failure was not been observed in trees without *P. ramorum* canker symptoms, largely because no other mortality factors present in these areas kill otherwise vigorous trees in this size class.

A second failure pattern was seen in larger diameter trees that (1) had extensive *P. ramorum* canker symptoms, (2) were dead at the time of failure, and (3) were apparently healthy and largely unaffected by wood decay fungi prior to developing *P. ramorum* canker symptoms. Although the boles of these trees were also attacked by ambrosia beetles and *H. thouarsianum,* the depth of sapwood decay was insufficient to cause bole failure over the short term. As a result, these trees have remained standing for several to many years after death. The first above-threshold failures occurring in these trees were branch failures that resulted from wood decay and tunneling by wood-boring beetles. Boring by buprestid and cerambycid beetle larvae was commonly more prominent than ambrosia beetle boring in failed branches from these standing dead trees. After several years, progressively larger branches have failed as various wood decay fungi have degraded the wood. Some of these trees have eventually failed at the bole or root crown, especially if the tree canopy was unbalanced. Trees without *P. ramorum* canker symptoms in the study areas generally did not show this overall mortality/failure pattern.

A third failure pattern was seen in trees that had substantial amounts of pre-existing wood decay due to established infections by canker rot or other decay fungi. These failures often occurred in living stems and were characterized by extensive heartwood decay columns that were sometimes associated with cavities. In some trees, sapwood decay and beetle boring associated with *P. ramorum* canker symptoms had approached or merged with these heartwood decay columns, causing a critical loss in structural integrity that resulted in failure. Fruiting bodies of *I. andersonii* and, less frequently, *P. robustus* were sometimes associated with this type of decay and failure. However, fungal fruiting bodies were lacking on many failed trees with extensive heartwood decay. This failure pattern was involved in most of the large-diameter (greater than 50 cm diameter) bole, root crown, and branch failures, but also occurred in smaller diameter stems. Unlike the two previous patterns, failure due to extensive heartwood decay commonly occurred in trees without *P. ramorum* canker symptoms, although it appears that *P. ramorum* canker symptoms increased the likelihood of failure in trees which already had substantial amounts of heartwood decay.

Table 3— *Parameters and significance levels for multivariate logistic regression models for the case outcome (any failure over threshold size) in coast live oak (n=106 cases, 170 controls).*

	Model 1[1]		**Model 2**[1]	
Predictor variables	**P level**[2]	**Odds ratio (CI)**[3]	**P level**[2]	**Odds ratio (CI)**[3]
P. ramorum symptoms late or dead (vs. early or none)[c]	<0.0001	13.5 (5.28-39.1)	--	--
Beetle boring present	--	--	0.0030	5.99 (1.84-20.48)
Rating of percent stem circumference with *Hypoxylon* sporulation	--	--	0.0441	4.64 (1.04-23.2)
Number of stems from ground	0.0029	50.5 (3.64-787)	0.0038	42.5 (3.21-656)
Phellinus present	<0.0001	15.5 (5.17-56-2)	<0.0001	16.0 (5.21-59.5)
Canker rot present	<0.0001	5.35 (2.36-13.03)	0.0001	4.85 (2.12-11.8)
Tree dead	<0.0001	17.1 (5.39-65.8)	0.0001	13.5 (3.58-58.5)
Sky exposed canopy rating	<0.0001	0.0181 (0.00235-0.115)	<0.0001	0.018 (0.00232-0.117)
Altered neighborhood	0.0139	4.19 (1.32-14.9)	0.0032	5.80 (1.76-21.9)

[1] The Akaike Information Criterion (AIC) for models 1 and 2 were 171.3 and 174.3, respectively. Overall significance levels of for both models were p<0.0001.
[2] Likelihood ratio test significance level
[3] Odds ratios and 95 percent confidence intervals; odds ratios greater than 1 indicate that a factor is positively associated with the case (failure) outcome

Discussion

In the *P. ramorum*-affected coast live oak woodlands we studied, failure rates have increased markedly over the last five years (*fig. 1*). In particular, rates of bole failure and large branch failure were greatly increased in the 1.5 years prior to our survey (*fig. 1*). Most of the recent failures have occurred in trees with extensive *P. ramorum* canker symptoms that were already dead or had late disease symptoms, characterized by the presence of *H. thouarsianum* and/or wood-boring beetles. Failure risk in trees with *P. ramorum* canker symptoms was substantially increased only if the trees had been attacked by secondary wood degrading organisms.

Failure rates for the 1.5 year interval preceding the study survey were probably near the maximum that is likely to be observed in these stands in connection with the current disease outbreak. Observations from permanent plots have shown that few additional coast live oaks in these areas have developed *P. ramorum* symptoms

between 2000 and 2004 (Swiecki and Bernhardt, these proceedings). Because more than 80 percent of the recent failures in this study occurred in trees with *P. ramorum* symptoms that were already dead and/or were colonized by secondary organisms (*fig. 2*), once most of these diseased trees have failed, the annual failure rate should decline substantially. Within our permanent plots, nearly 80 percent of trees with *P. ramorum* symptoms that were dead or had late symptoms in 2000 had failed by 2004, with the greatest number of failures occurring in 2002 and 2003 (Swiecki and Bernhardt 2004, unpublished). Hence, unless or until a large number of the remaining asymptomatic trees in these stands develop severe *P. ramorum* canker symptoms, the annual failure rate should decrease. If results in these plots are typical, we would expect that an outbreak of *P. ramorum* canker in a coast live oak stand is likely to be followed in several years later by a corresponding spike in the tree failure rate among killed and late-stage diseased trees.

Because *P. ramorum* can kill some trees relatively quickly, particularly trees that are otherwise some of the more vigorous trees in the stand (Swiecki and Bernhardt 2002a,b, 2004, these proceedings), we have found patterns of tree failure that are unique to trees with *P. ramorum* canker symptoms. These include main stem failures due to decay that originates in the sapwood and large branch failures occurring on large-diameter standing dead trees that were killed by *P. ramorum* canker. Hence, coast live oak stands affected by *P. ramorum* have both higher failure rates and different types of failures than are typical in stands that are free of *P. ramorum*.

Several lines of evidence implicate wood decay as the primary factor influencing failure potential in the stands we studied. Decay was present and rated as a contributing factor in almost all failures. Fruiting bodies of various wood decay organisms, decay columns, and canker rot symptoms were significantly more common among cases than controls. Also, variables related to decay were highly significant in both recursive partition and multivariate logistic regression models. The reduction in wood strength due to decay is a fundamental cause of almost all of the failures we observed. Similarly, in a study of oaks with stem failures conducted in the aftermath of Hurricane Hugo, 96 percent of failed (not windthrown) trees had internal decay (Smiley and Fraedrich 1992). Decay has also been implicated in failures among natural conifer stands (Coates 1997, Ruel 2000, Dunster 1996), but not among planted Monterey pine in a park setting (Edberg and others 1994).

We collected samples of decayed wood from many of the recently failed trees in this study to identify decay fungi present using DNA probes (M. Garbelotto, unpublished). Partial results compiled to date confirm that most decay samples were colonized by one to several wood decay fungi. In many of the samples, wood decay

fungi identified by DNA detection methods were not observed fruiting on the affected stems. Further analyses of these samples will provide greater insight into the interactions of various decay fungi in coast live oaks affected by *P. ramorum*.

Fruiting bodies of *H. thouarsianum,* a sapwood-decaying fungus, were present on 82 percent all recent failures and 91 percent of the recent failures with *P. ramorum* canker symptoms. *H. thouarsianum* appears to be responsible for much of the sapwood decay seen in failures of trees with *P. ramorum* canker symptoms. Although the biology of *H. thouarsianum* has not been studied, most *Hypoxylon* species and similar fungi in the Xylariaceae colonize living hosts either as endophytes or through latent infections of woody tissue, and function as opportunistic pathogens of stressed trees (Ju and Rogers 1996). *Biscogniauxia atropunctata* and *B. mediterranea,* both of which were previously classified as species of *Hypoxylon*, initiate latent infections in healthy oaks and subsequently colonists decay the inner bark and sapwood when trees that have been stressed by drought (Sinclair and others 1987).

Wood degradation by wood boring beetles may also contribute to failure risk, but because beetle damage was so highly correlated with the presence of decay, we were not able to completely distinguish between the effects of decay and beetle boring. The lack of heavy beetle boring activity at the point of failure in many failed trees suggests that beetle boring is not the primary factor influencing failure potential. Nonetheless, the presence of extensive beetle activity is an indicator of increased failure potential in trees with *P. ramorum*, whether the effect is due to beetle tunneling itself or beetles are serving as an indicator of associated decay. This is especially the case in smaller–diameter trees that follow the first pattern of failure described above.

In a recent study on log sections from two failed coast live oak trees with *P. ramorum* cankers, sapwood decay appeared to proceed faster in logs that were heavily colonized by ambrosia beetles than in logs treated with permethrin to inhibit beetle colonization (Svihra and Kelly 2004). Logs used in that study were initially sound, about 40 cm in diameter or less, and were observed for 411 days. If beetle boring accelerates sapwood decay caused by *H. thouarsianum* and/or other fungi, beetles may play a role in determining how soon failure occurs in *P. ramorum*-infected trees that follow the first failure pattern described above. However, branch failures in large diameter trees (pattern 2 above) and failures in trees with existing heartwood decay (pattern 3) are less likely to be strongly influenced by amounts of beetle boring in the bole. It is far from certain that reducing scolytid beetle attack with insecticides will attenuate failure potential in trees that follow these two latter failure patterns.

Relatively few structural defects were strongly associated with tree failures in this study. Also, structure-related defects associated with failure in single-variable models (*table 2*) were not the same as those in multivariate models (*fig. 5, table 3*). Structural defects such as multiple stems or branches at one point, one-sided canopy distribution, and cavities either create a point of structural weakness or lead to uneven distribution of stress in the bole or branches. In coast live oak, it appears that sound wood is typically strong enough to prevent failure in the presence of these defects. Due to growth patterns in these forests, many live oak canopies are highly asymmetric and imbalanced, but failure rates are low among live, non-decayed trees. However, when wood strength is lost due to decay, these same defects may precipitate failure. Hence, in coast live oak, variables related to decay are generally much stronger predictors of failure potential than are structural defects related to canopy imbalance.

Two factors related to local stand structure were significant in various failure models. The first, sky-exposed canopy, measures the degree of overtopping or tree dominance within the canopy. In all models, lower levels of sky exposed canopy (i.e., greater amounts of overtopping) were associated with a higher risk of failure. Some of this effect is related to the presence of high levels of wood decay in severely suppressed understory trees, which generally did not exhibit *P. ramorum* canker symptoms. In addition, cooler, moister conditions in the understory may increase the activity of wood decay fungi in these trees.

The second variable related to stand structure in the models is the altered neighborhood variable. This factor indicates that failures in these stands tend to occur near other dead and/or failed trees. We have previously shown (Swiecki and Bernhardt 2001, 2002a,b) that *P. ramorum*-infected trees in these stands are clustered on a very local scale (i.e., within 8 m radius plots). Hence, spatial clustering of failures seen in this study is probably due in large part to the underlying spatial clustering of *P. ramorum* canker. However, many tree canopies also overlap and interlock in many of these stands, so loss of adjacent trees may result in loss of direct support and increased wind exposure, thereby increasing failure potential.

Results from the multivariate recursive partitioning and logistic regression models indicate that multiple factors contribute significantly to the chance that a given tree will fail. Given that several patterns of failure are found in these stands, it is logical that no single variable serves as a satisfactory predictor of failure. A high failure potential typically exists when multiple factors are at levels that favor failure. The logistic models also provide an idea of the relative magnitude of the effects of each factor. For example, late *P ramorum* canker symptoms (i.e., beetles and/or *H.*

thouarsianum present), tree death, and the presence of *Phellinus* sporulation have a bigger impact on failure potential than the presence of beetle boring, canker rot symptoms, or altered neighborhoods (*table 3*). Factors modeled as continuous variables are more difficult to rank because the range of observable values must be considered when interpreting the odds ratios. Among factors modeled as continuous variables, sky exposed canopy ratings have the greatest overall impact on calculated failure probabilities, followed by the number of stems and *H. thouarsianum* girdling.

The retrospective design used in our study allowed us to evaluate a large number of failures over a short time period and develop models that can be used to predict failure risk. We have used this data to develop guidelines for assessing failure potential in coast live oak stands affected by *P. ramorum* canker (Swiecki and Bernhardt 2003). Although we were able to evaluate many characteristics in failed trees, some factors were clearly assessed with less accuracy on fallen trees than standing ones, e.g., lean and some canopy distribution characteristics. In addition, most trees were evaluated many months after failure had occurred. Levels of some factors, such as the presence of fruiting bodies, may have been different at the time of observation than at the time of failure. If this is so, even if a factor (e.g., decay caused by *Phellinus*) is related to failure, the rated variable (e.g., *Phellinus* sporulation) may be less useful as a predictor than is implied by the models.

These limitations can be overcome by conducting a prospective evaluation of trees that may fail. Because annual failure rates are typically low, to observe sufficient numbers of failures and the factors associated with them, prospective studies generally require very large sample sizes and observations over an extended time period. In our related study (Swiecki and Bernhardt 2001, 2002a,b, 2004, these proceedings), we are prospectively evaluating tree failure in *P. ramorum*-affected stands. This prospective data will be used to refine models developed in our retrospective study.

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The Current Situation With *Phytophthora ramorum* in England and Wales[1]

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Abstract

Since the first finding of *Phytophthora ramorum* in England in April 2002, an intensive campaign, supported by the European Community (EC) and national legislation, has been conducted to locate and eradicate all interceptions and outbreaks of *P. ramorum*. A summary of the findings made during these surveys is presented, along with an initial analysis of progress towards eradication.

Key words: Phytophthora ramorum, sudden oak death, hosts, symptoms of infection, surveillance, eradication

Introduction

Surveillance for *Phytophthora ramorum* in England and Wales commenced in July 2001 and the first finding of the pathogen was confirmed on a nursery in England in April 2002. In response to this finding, emergency national legislation was adopted in May 2002, followed by EC legislation in November 2002. These official controls currently include a ban on the import of susceptible host material from affected parts of the USA and plant passporting controls on the movement of *Rhododendron*, *Viburnum* and *Camellia* within the European Union (EU). Member States in the EU are also required to conduct official surveys and to take action at least to prevent the spread of *P. ramorum*.

In the United Kingdom (UK), intensive surveillance has been conducted by the Plant Health and Seeds Inspectorate (PHSI) and by the Forestry Commission and Forest Research. Similar surveillance has also been conducted in Scotland and Northern Ireland. Within England and Wales, PHSI aim to make at least one visit each year to all commercial premises that trade in susceptible material, with wholesale nurseries receiving at least four visits a year. Targeted surveys of parks, gardens and 'wild' locations have also been undertaken, some in partnership with other agencies such as English Nature, and the Forestry Commission have made two random surveys of woodlands in 2004. In addition, a targeted programme of port inspections has been made to monitor intra-community trade.

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Action is taken to eradicate all findings of *P. ramorum* in the whole of the UK. At nurseries and garden centres, all infected plants, and susceptible hosts within a 2m radius are destroyed, and susceptible plants grown within a 10m radius are held for at least three months and monitored for signs of infection. Whilst under official hold these plants must not be sprayed with anti-*Phytophthor*a fungicides, in order to prevent symptom suppression. In addition, hygiene measures are required and a programme of follow up visits is implemented. Eradication action is also required against findings at managed and unmanaged sites, such as parks, gardens and wild areas.

Hosts and Symptoms

P. ramorum has been found on a range of ornamental plants and trees at nursery and retail premises, and on managed (e.g. parks and gardens) and unmanaged land. Non-tree, plant species found to be infected in England and Wales include: *Camellia* spp., *Hamamelis* spp., *Kalmia latifolia*, *Laurus nobilis*, *Leucothoe fontanesiana*, *Pieris* spp., *Rhododendron* spp., *Syringa vulgaris*, *Taxus baccata* and *Viburnum* spp. Characteristic symptoms of infection are leaf blight, and dieback of infected shoots. Dieback has only occasionally been observed in *Camellia, Kalmia and Syringa*, however, and has not yet been noted on *Laurus nobilis* or *Leucothoe spp*. In addition, *Griselinia littoralis, Magnolia spp.,* and *Parrotia persica* are recently discovered hosts, for which Koch's postulates have yet to be completed. All three new genera exhibit leaf blight, with *Griselinia littoralis* and *Magnolia x soulangeana* showing evidence of dieback also.

Mature specimens of the following trees have been found to be infected: *Aesculus hippocastanum, Castanea sativa, Drimys winterii, Fagus sylvatica, Fraxinus excelsior, Nothofagus sp., Quercus cerris, Quercus falcata, Quercus ilex, Taxus baccata* and *Umbellularia californica*. Leaf blight has also been a characteristic symptom of *P. ramorum* in trees, with examples recorded on *Castanea sativa, Drimys winterii, Fraxinus excelsior, Quercus ilex, Taxus baccata* and *Umbellularia californica*. *Castanea sativa*, *Quercus ilex* and *Taxus baccata* have also exhibited some young shoot dieback. Lethal bleeding cankers have been observed on *Aesculus hippocastanum, Fagus sylvatica, Nothofagus obliqua, Quercus cerris* and *Quercus falcata*.

Outbreak Statistics and Trends

A total number of 462 outbreaks has been recorded up to the end of December 2004 at 398 sites. Of these, 376 outbreaks (at 324 sites) have been found at nurseries and garden centres, and 86 outbreaks (at 74 sites) have been on managed/unmanaged land. Eradication has been more successful at commercial premises, where 82 percent of outbreaks have been eradicated, than in managed/unmanaged land, where to date only 27 percent of outbreaks have been eradicated.

Several encouraging trends have been observed when comparing inspection figures between 2003 and 2004 For example, despite a 66 percent increase in number of inspections, the number of new outbreaks found decreased by 30 percent, and the number of findings on passported material fell by 89 percent. Furthermore, the percentage of sites at which *P. ramorum* was confirmed also dropped between 2003 and 2004. For nurseries and garden centres, the percentage of sites affected was 5.1 percent in 2003 and 3.0 percent in 2004, and for managed/unmanaged land, the percentage of sites affected was 8.0 percent in 2003 and 3.9 percent in 2004.

In summary, import and plant passporting controls appear to have reduced substantially the amount of *P. ramorum*-infected material moving in commercial trade. Good progress has also been made at eradicating *P. ramorum* from nurseries and garden centres, although repeat findings of *P. ramorum* on commercial premises continue to cause concern. In contrast to commercial premises, eradication of *P. ramorum* from parks, gardens and wild areas appears to be a more protracted process.

Epidemiology of *Phytophthora ramorum* Infecting Rhododendrons Under Simulated Nursery Conditions[1]

S.A. Tjosvold[2], D.L. Chambers[2], S. Koike[3], and E. Fichtner[4]

Abstract

The current understanding of diseases caused by *Phytophthora ramorum* and their dynamics in nursery crops is almost entirely derived from casual field observations. The objectives of the study are to help understand basic biological factors such as, inoculum viability, dispersal, and infectivity that influence disease occurrence and severity in a rhododendron nursery. The experiments were conducted under conditions that simulate a commercial containerized rhododendron nursery regime in the central coast of California. This extended abstract presents information on the findings of the first year of a two-year study.

Key words: Phytophthora ramorum, epidemiology, inoculum, irrigation, soil

Stream Water Inoculum

We need to know whether naturally-infested stream water could infect nursery hosts if the water is used for irrigation. Stream water in Lompico creek (Felton, California) has been monitored regularly since December 2003 and viable inoculum has consistently been detected (with pear baiting) during rainy winter and early spring conditions (December 2003 to April 2004), and only later in dry conditions when infected California bay (*Umbellularia californica*) leaves were blown into the stream by strong winds (June, 2004). Rhododendron stock (*Rhododendron* 'Cunninghams White') was irrigated, beginning February 24, 2004 until the present, with water pumped from the stream. When viable inoculum was detected in the stream, inoculum was also detected in irrigation water at several points along the irrigation-water distribution system, including at the irrigation sprinkler heads. Infectivity of stream water was evaluated under drip and sprinkler irrigation techniques. To date, no infection has been detected in rhododendron stock.

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[1] A version of this paper was presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18-21, 2005, Monterey, California.

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Soil Inoculum

We need to know the conditions that soil-borne inoculum could be important in infecting rhododendron stock. The surface 1 cm of container soil in rhododendron stock was mixed with 100 infected rhododendron leaf disks (5 mm diameter). Each inoculated container was surrounded by non-infested container stock at nursery-block spacing. Observations were made to determine if soil-borne propagules could be dispersed and cause disease. Evaluation occurred in winter (with rainfall) and later with drip or sprinkler irrigation. Infection only occurred on leaves touching soil that contained infected leaf disks. In an associated inoculum viability experiment, 100 infected leaf disks were placed in sachets (to facilitate removal) and buried to 1 cm depth in the container soil planted with rhododendron. The monitored soil/plants were placed in the field or in a greenhouse. The sachets were periodically removed and leaf disk viability was assessed by plating with selective media. In the field, viability of recovered disks declined from 100 percent (week 0) to 0 percent in 38 weeks and has produced zoospores (with soil flooding) for up to 12 weeks. In the greenhouse, leaf disks began to disintegrate at 28 weeks but those that could be recovered nearly all remained viable at each evaluation (up to 38 weeks). See *Figure 1*.

Aerial Inoculum

The dynamics of aerial dispersal from infected plants to adjacent and nearby plants needs to be understood. Experiments were designed to evaluate “long” distance dispersal (up to 4 meters) and “short” distance dispersal, within a pot-to-pot spacing in a nursery block. From December 2003 to the conclusion of this experiment in June 2004 (at the end of the rainy season), new infections were only detected on plants in the “short” distance experiment, immediately next to and up to about 30 cm away from a centrally-located artificially-infected plant, and on other parts of an artificially infected plant. In the “long” distance experiment, no infections were detected, and no inoculum was detected in rain traps located 1 to 4 meters away from the infected plant during rain events. The presence and relative concentration of the propagules were detected on inoculated leaves following rain events by washing leaves with deionized water in the early morning and collecting the wash. Detection occurred in the early spring (February, 2004) but not during or following March, 2004, when extraordinarily warm and dry conditions existed. At this writing (January, 2005) a new dispersal experiment has been established and inoculum has been collected in rain traps up to 0.5 meters away from inoculated plants during a series of four strong storms in a two-week period.

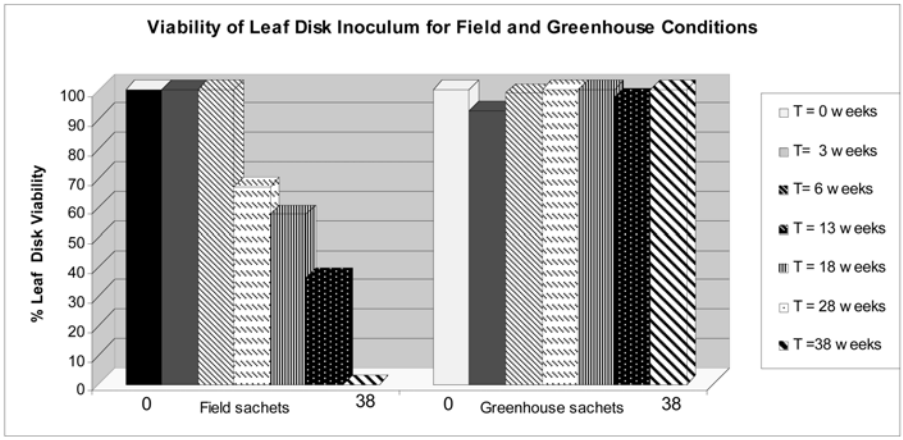

Figure 1. Viability of leaf disk inoculum based on recovery on selective media. Inoculum mixed in top 1 cm of soil in containerized rhododendron stock growing in field and greenhouse conditions.

Converting Biology Into Regulations: U.S. *Phytophthora ramorum* Quarantine as a Case Study[1]

Susan J. Frankel[2] and Steven W. Oak[3]

Abstract

Regulation of *Phytophthora ramorum*, cause of sudden oak death and other diseases, has resulted in endless challenges for regulators, and the forest and nursery industries in the United States. This paper outlines the process used to design U.S. *P. ramorum* quarantines and explores some of the biological paradoxes presented by having to develop regulations for a recently discovered pathogen. Disregard for Koch's postulates, limitations of PCR and cultural diagnostic techniques, the perils of basing rules purely on published literature, and the precautionary principle are discussed. The current status of U.S. and California regulations are compared to demonstrate the difficulties of limiting pathogen spread using generally accepted regulatory processes. The economic and social impacts of the U.S. *P. ramorum* quarantine are also presented along with suggested steps to improve quarantine procedures.

Key words: Phytophthora ramorum, sudden oak death, quarantine policy

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Who Pays for Sudden Oak Death? An Econometric Investigation of the Impact of an Emerging Pathogen on California Nurseries[1]

J.K. Gilless[2], J. Tack[2], and A. Peterson Zwane[2]

Key words: Phytophthora ramorum, sudden oak death, nurseries

Abstract

While there is a great deal of scientific uncertainty about the nature and control of *Phytophthora ramorum*, there is growing concern that its economic costs will be significant. Indeed the repercussions at the nursery level of a positive test for the presence of *P. ramorum* may include severely impacted current and future cash flows. Given this possibility, nurseries that are susceptible to *P. ramorum* infestation may reassess their production strategy. Firms may choose to increase expenditures to prevent *P. ramorum* infestation, or they may choose to reduce production of *P. ramorum* host plants, thus limiting exposure to infestation risk. Optimal production strategies may reduce the expected cost of *P. ramorum* by affecting either the probability of infestation or the magnitude of the loss incurred in the event that infestation occurs.

In this paper, we use a unique data set to identify the characteristics of firms that are impacted by *P. ramorum* (e.g., their location, size, and target market). We also estimate econometrically the partial equilibrium relationship between realized costs of *P. ramorum* control and nurseries' crop mix. We motivate the empirical work with a simple model of firm behavior in the presence of regulatory inspection and uncertainty. The model is related to previous work on efficient resource management to control pests short of eradication and the pollution abatement decision when regulatory compliance may be imperfect.

The data employed in this research was collected via survey during a three-month period in 2004. This confidential and timely data contain qualitative and quantitative information about many nursery characteristics not reported by the California Agricultural Census surveys. The sample of firms represented covers a wide geographic area and includes both large and small firms with varying production mixes. All California nurseries that (i) were considered a

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candidate for producing at least one type of *P. ramorum* host plant and (ii) operate their growing facility on at least five acres of land were contacted and asked to participate in the survey. In order to account for the non-random sampling nature of our data, we use Heckman two-step estimation to correct for sample selection bias.

In the empirical section of the paper we develop two separate regression equations: we predict changes in (either fixed or marginal) cost as a function of firm characteristics, and we predict *P. ramorum* /Non- *P. ramorum* host product mix as a function of changes in costs and firm characteristics. The explanatory variables in both regressions include grower characteristics such as size, location, and sales destinations. Changes in the cost of production arise largely as a result of changes in water treatment and irrigation practices, fungicide programs, labor practices, and inventory management.

We discuss the bias resulting from the endogeneity of the production mix and cost and present possible bias-correcting techniques including instrumental variables. Our results allow us to identify firm characteristics that may predict the relative impact of *P. ramorum* on profitability, and describe nurseries' behavioral responses to a possible extension outward of the zones of infestation boundaries. These findings can assist regulators in identifying which firms and regions may benefit most from technical assistance.

Understanding the Disposal and Utilization Options for *Phytophthora ramorum* Infested Wood[1]

John Shelly[2], Ramnik Singh[2], Christine Langford,[2] and Tad Mason[3]

Key words: Phytophthora ramorum, sudden oak death, wood properties, firewood, biomass, salvage lumber, disease monitoring

Abstract

Removing trees inflicted with the sudden oak death (SOD) disease is often necessary because of hazard issues or homeowner/landowner desires. An alternative to disposal of this material is to find acceptable uses for this diseased material. A series of studies is being conducted to help understand the risk of spreading the *Phytophthora ramorum* infested wood through disposal and utilization activities. Two collection yards were set up in California for wood suspected of being infected with *P. ramorum*, one in Marin County and one in Santa Cruz County. More than 1,155 tons of woody, *P. ramorum* host material has been processed through the collection yards since May 2003, most from the removal of hazard trees. This material was converted into fuel for biomass power plants, firewood, and lumber. In addition to operating the collection yards, this project included periodic monitoring for *P. ramorum* spores during various stages of the processing and transportation by sampling the delivered host material and monitoring the host vegetation growing in and around the collection yards.

The periodic sampling of woody materials transported to and processed in the collection yards yielded a small number of positive cultures of *P. ramorum* isolated from a variety of the unprocessed and processed materials. Of the 418 samples collected, the following tested positive: two of 84 chip samples, two of eight California bay laurel leaf samples, eight of 145 samples of freshly split firewood, one of 85 samples of firewood air dried for about 6 months, nine of 93 grinder and saw dust samples, and one of three rainwater runoff samples. Furthermore, the sampling results of the host vegetation growing in and around the collection

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yards confirmed the presence of *P. ramorum* at each site before operations began but continued sampling has not revealed any significant infestation increase.

This early data suggests that alternatives to disposal exist for *P. ramorum*-infested wood. Furthermore, although *P. ramorum* can be isolated from various stages of processing at the collection yards, no evidence has been gathered to support the hypothesis that the collection, sorting, and processing activities in the collection yards increases levels of *P. ramorum* infestation in and around the sites.

Introduction

This project was initiated in response to concerns raised by the California Oak Mortality Task Force (COMTF) that disposing or using *P. ramorum* infested woody materials would spread this pathogen that is potentially lethal for many hardwood trees across the country. The potential to spread plant pathogens by moving infected plants and plant materials from one location to another is well documented in the literature (Erwin and Ribeiro 1996; Garbelotto 2003; Davidson and others 2002). The list of host plants for *P. ramorum* continues to grow with the possibility that many of the important tree species of California's forests could become hosts (Garbelotto and others 2003). Unlike most other *Phytophthora* species, *P. ramorum* cankers are found highly associated with the bark of host trees (Storer and others 2001). Also, two beetles of the Scolytidae family, the western oak bark beetle (*Pseudopityophthorous pubipennis*) and the oak ambrosia beetle (*Monarthrum* sp.) are also known to be highly associated with this disease in infected trees (Storer and others 2001). However, the role these beetles play in the spread of the pathogen is not well understood since the high incidence of attack appears to coincide with the latter stages of the disease (Furniss and Carolin 1977). Although it is considered unlikely that the beetles play a significant role in pathogen spread they could be a valuable tool for measuring *P. ramorum* activity in a region.

Based on an initial list of host plants and the realization that suitable hosts could be found throughout the country, public agencies were quick to regulate the transport of infested material and implement quarantine zones. The California Department of Food and Agriculture established intrastate regulations in 2001 (Anon. 2005) followed by the enactment of federal interstate regulations by the U.S. Department of Agriculture Animal and Plant Health Inspection Service (Anon. 2002). Both sets of regulations were enacted before studies of *P. ramorum* infested wood could be completed. Recognizing that the likely pathways for the spread of *P. ramorum* are by water, soil, infested plant materials, and perhaps by air, the regulations encourage that infested material be quarantined and left in place if possible. However, it is not

always possible to leave infested material at the site of the infestation. Many *P. ramorum* infested trees are in urban areas, public areas, or adjacent to roads or utility lines. Such trees are often considered hazard trees and they must be removed. The removal of large numbers of these trees results in high, unbudgeted expenditures and often a disposal problem. As a woody material, this biomass could generate some value as consumer products but it was unclear if utilization activities can contribute to the spread of the pathogen. Under current regulations, if host plants and plant materials including wood and unprocessed wood products are transported outside of a regulated county they become subject to intensive inspection and permitting. Based on the premise that *P. ramorum* in trees thrives only on leaves or directly beneath the bark, wood and wood products can be exempt from the regulations as long as they are free of bark. However, the actual risk of spreading the pathogen by moving wood and wood products, with or without bark, was not well understood.

The goals of the project reported in this paper were to study the risks of moving material infested with *P. ramorum* from the site of its initial infection to a central location or processing facility and to identify potential uses for the infested material. The approach used to gauge the risk was to track the infestation levels of *P. ramorum* and bark beetles in and around collection yards during the life of this project. Two collection yards for *P. ramorum* infested wood were set up and actively managed under this project. The Marin County collection yard, located at Marin Resource and Recovery in San Rafael, California, opened in May 2003 and operated for 20 months at the time this paper was written. The Santa Cruz County yard, located at the Santa Cruz County transfer station in Ben Lomond, California, was opened in December 2003 and operated for 13 months. Both yards are scheduled to close by July 2005.

Objectives

The overall objectives of the project included:

- Design a comprehensive disposal and utilization plan for Marin and Santa Cruz Counties that incorporates the views of public agencies and private enterprise.
- Develop protocols for the removal and transportation of *P. ramorum* infested woody material and for monitoring the potential pathogen spread
- Evaluate the relationship between transporting and processing *P. ramorum* infested wood and the risk of spreading the pathogen

- Evaluate the basic wood properties of *P. ramorum* infested wood and the value-added utilization potential and implement the distribution of *P. ramorum* infested wood to appropriate markets.

Methods

To create a disposal and utilization plan that addressed the objectives of the project, a series of meetings was conducted with representatives of interested organizations and stakeholders. These included state and county agencies responsible for the removal and disposal of woody biomass as well as tree service companies, arborists, land managers, and commercial users of woody biomass. An outcome of these meetings was the establishment of two collection yards to serve as a central location for the drop-off of host trees suspected of having SOD.

Consultations with state regulators and other researchers studying SOD led to a set of protocols used in this project to define the rules of participation in the project and the methods used to monitor the activities. Only host material suspected of being infected with *P. ramorum* and identified by a person having received training in *P. ramorum* symptoms was accepted at the collection yards. This material had to be removed and transported to the collection yard by a tree service company or other qualified expert following the transportation guidelines distributed to all potential participants and also posted on the project internet web site (http://groups.ucanr.org/sodbusters). Once at the yard, the *P. ramorum* infested material temporarily stored at the site was physically separated from other woody biomass operations. This material was stored until enough volume was collected to justify moving it to a utilization market. During this sequence of events the material, the site, surrounding environment, and processing activities were monitored for the presence of *P. ramorum* and bark beetle activity according to the protocols described in an earlier progress report (Shelly and others 2004).

In summary, the following monitoring was conducted according to the schedule outlined in *table 1*.

- Determined base line levels of infestation in the area surrounding the collection yard by inspecting all of the following host plants found in circular plots (6 m radius) spaced 20 meters apart along transects emanating from the collection yard.
    - Coast live oak (*Quercus agrifolia*) – inspected for SOD symptoms

    - California bay laurel (*Umbellularia californica*) – collected symptomatic leaves from trees taller than 2 meters that were used for laboratory examination (see culture techniques below)
- Monitored beetle levels by capturing insects in ethanol-baited, "Lindgren" beetle traps and periodically counting the catch of scolytids.
- Periodically sampled collection yard materials
    - Delivered *P. ramorum* infested woody biomass (chips, branches, leaves, and logs)
    - *P. ramorum* susceptible rhododendron plants (*Rhododendron var. Colonel Coen*), potted and placed in the collection yard (sentinel rhododendrons)
    - Airborne dust generated during unloading of *P. ramorum* infested material from trucks (only at the Marin County yard and the Soledad biomass power plant)
    - Rain water runoff from chip piles (only at the Marin County yard)
- Periodically sampled materials during processing activities
    - Airborne dust generated during wood grinding operations
    - Small wood particles that fall from the grinders (grinder fines)
    - Firewood with bark as it was processed
    - Sawdust from sawmill as logs were processed into lumber
- Periodically sampled processed firewood as it dried

Table 1 – *Schedule of sampling at each collection yard (M = Marin county collection yard, SC = Santa Cruz County collection yard, SM = sawmill site in Davenport, S = Soledad biomass power plant).*

Item Sampled	Every 2 weeks	Every Month	Every 2 months	Every 6 months	As needed
Host plants in sample plots				M,SC, SM	
Sentinel rhododendrons			M, SC	SM, S	
Beetles		M, SC	SM		
Rain water runoff from collection yard					M
Delivered chip material and CA bay laurel leaves					M, SC
Delivered sawlogs					SC
Airborne dust generated during delivery	M				S
Grinder fines and sawdust					M, SC, SM
Lumber					SM
Freshly split Firewood					M, SC, SM
Stored firewood				M, SM	

Culture techniques

All chip, wood particle, dust, water and solid wood samples were subjected to a pear baiting technique to determine the presence of *P. ramorum*. Hard, green d'Anjou pears without wounds or bruises were partially submerged for 3 days at 20 °C in deionized water containing the material being tested. The pears were then removed from the water and air dried at 20 °C for 2-5 days. Any characteristic *Phytophthora* lesions that appear on the pears were isolated and placed on an agar growth medium prepared with ampicillin, rifampicin, and pimaricin (PARP) using sterile techniques. Organisms that grew on the PARP plate were examined for *P. ramorum* characteristics to determine the presence of the pathogen (Blomquist and Kubisiak 2003). Leaves collected from host plants were not pear baited, the *Phytophthora* lesions on the leaves were directly plated on to PARP.

The Plant Pest Diagnostics Center, California Department of Food and Agriculture (CDFA) in Sacramento performed DNA analysis using polymerase chain reaction (PCR) techniques to confirm the presence of *P. ramorum* on all plates that grew organisms with *P. ramorum* characteristics.

Utilization analysis

Data gathered on the properties of the infested wood collected in the study area included tree size and form, gross wood characteristics, wood moisture content at the time of the diseased wood delivery, and wood density. Moisture content was measured on solid wood specimens taken from the processed firewood and logs selected for lumber processing. The same specimens were also used to measure the density. These properties were used to determine the level of degradation in the wood and to identify the best use for the material. Products considered included biomass powerplant fuel, firewood, and lumber.

Results

A total of 1,155 tons of potentially *P. ramorum* infested wood was delivered and processed at the two collection yards (*table 2*). In the 20-month period from May 2003 to December 2004, the Marin County collection yard received 856 tons from 14 private tree service companies and three public works departments. The Santa Cruz County collection yard received 299 tons in the 12-month period from December 2003 to December 2004, all as hazard tree removals under contact of the County of Santa Cruz during the 6-month period from December 2003 to June 2004. At the Marin County yard the deliveries were tracked by species. Of the 929 trees removed and delivered to the yard, 761 (82 percent) were tanoak (*Lithocarpus densiflorus*), 160 (17 percent) were coast live oak (*Quercus agrifolia*), five (0.5 percent) were California black oak (*Quercus kelloggi*), and three (0.3 percent) were California bay laurel (*Umbellularia californica*). Although the deliveries could not be tracked by species at the Santa Cruz County yard, the species breakdown is assumed to be similar based on the inventory of hazard trees by the county in which tanoak made up about 75 percent of the material and coast live oak 21 percent.

Table 2 – *Breakdown of the SOD-diseased material delivered to the Marin County collection yard and the Santa Cruz County collection yard.*

Tree Species	Marin (19 months)		Santa Cruz (12 months)	
	# of Trees	Tons	# of Trees	Tons
Lithocarpus densiflorus (tanoak)	761	na	na	na
			na	na
Quercus agrifolia (coast live oak)	160	na	na	na
Quercus kelloggi (California black oak)	5	na	na	na
Umbellularia californica (California bay laurel)	3	na	na	na
Total	929	856		299
Average deliveries/active month		45		46

Of the total 1,155 green tons of material collected, about 84 percent were processed into biomass powerplant fuel, 14 percent converted into firewood, and 2 percent processed into lumber. The commercial biomass power plants that received the biomass fuel from this project were in Rocklin, Woodland, and Soledad, California. All of the biomass fuel chips processed at the Santa Cruz County yard were sent to the Soledad facility, a total of 283.4 green tons. The Marin County yard processed 649.5 tons of biomass fuel chips that were distributed to all three of the powerplants listed above.

Monitoring for changes in P. ramorum *infestation*

Host vegetation

A baseline of *P. ramorum* infestation was established before each collection yard began operations by sampling the host vegetation in and around each collection yard. The same trees were reexamined every 6 months throughout the duration of the project to determine changes in the level of the disease. The baseline survey of *P. ramorum* infestation at the Marin County site in the spring of 2003 showed that 69 percent of the bay laurel trees in all plots were infected with *P. ramorum*. This overall infestation level decreased to 61 percent in the spring of 2004 after 1 year of SOD-diseased wood collection activities. This was due to a few trees that exhibited *P. ramorum* symptoms initially but were no longer symptomatic in subsequent sampling. This may not actually indicate a real drop in infestation but it could be a reflection of the natural variation of the symptoms. Although the rate of infestation appeared to be greater closest to the collection yard (*figure 1*), this is believed to not be influenced by the collection yard activities. The collection yard is located at the base of a steep slope and all of the plots closest to the collection yard are at the base of the slope. The higher percentages of infected trees at 450 feet from the collection yard were likely a reflection of higher natural *P. ramorum* infestations expected at the bottom of a steep slope. The observed lower infestation rates in the fall were a reflection of the difficulty in sampling infected bay laurel leaves in the fall when most of the infected leaves have fallen from the tree.

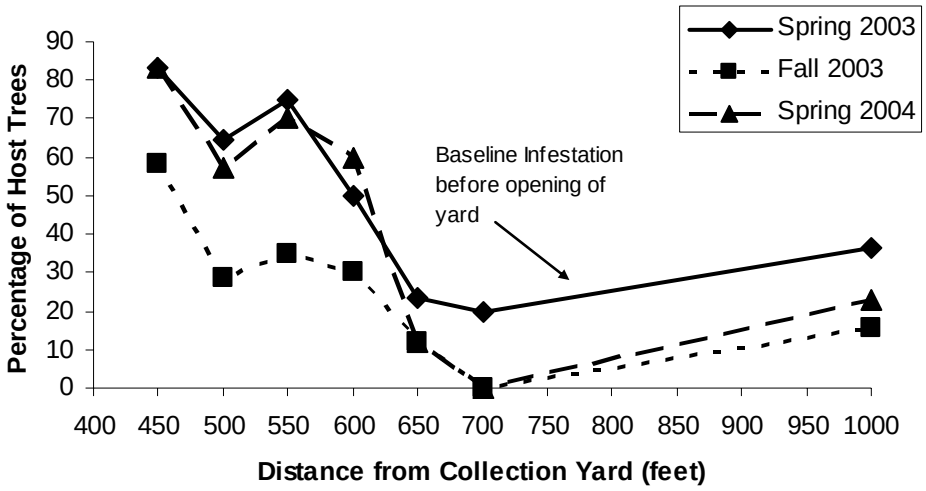

Figure 1 – Percentage of host trees in each sample plot at the Marin County collection yard that are infected with *P. ramorum*.

Similarly, no changes were observed with the coast live oak trees in the sample plots that could be explained by the collection yard activities. The overall level of symptomatic coast live oak trees in all transects remained constant, but one additional coast live oak developed symptoms in a control plot far from the collection yard activities and one of the symptomatic trees in a plot close to the collection yard became symptom free.

None of the sentinel rhododendron plants placed in the collection yard developed positive *P. ramorum* cultures. Similar results were found at the Santa Cruz County collection yard where the slight changes observed in the infestation were seasonal and also located in plots far from the collection yard activities.

Beetle activity

Bark beetle activity in and around the two collection yards is summarized in *figure 2*. The beetles were most active in both yards in the spring/early summer months with a secondary period of activity in the fall. This is consistent with the expected life cycle of bark beetles. During late May to early June 2004 the beetle count in the trap inside the Marin County yard reached a peak of 2,352 beetles. This dramatic increase in bark beetle activity coincided with numerous deliveries of *P. ramorum* infested hazard trees. A similar peak also occurred at the same time in the Santa Cruz County yard; this peak was also associated with numerous deliveries of *P. ramorum* infested hazard trees. The beetle counts in the control D and E baited traps, which were

located the furthest from the collection yard, were generally quite a bit less than the counts inside the collection yards with the exception of the August 2003 peak for control two at the Marin County site. This control two count was in the plot farthest from the collection yard in an area with many dead and dying coast live oak trees and is believed to be a reflection of normal beetle activity and not related to the collection yard activities. With the exception of the traps adjacent to the collection yards that may be influenced by the collection yard activities, the other traps showed a good record of the background beetle activity in the area.

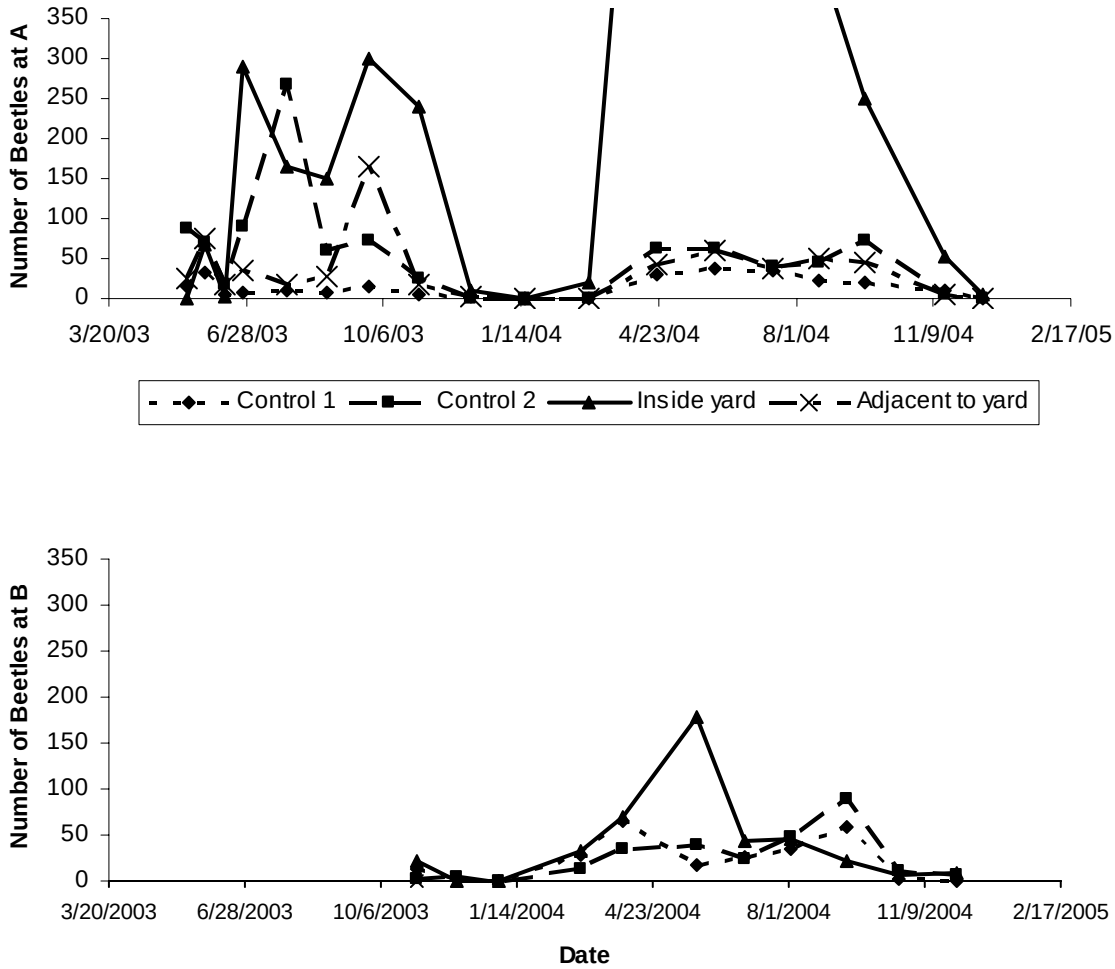

Figure 2 – Scolytid activity at the collection yards (A is Marin County, B is Santa Cruz County).

Monitoring of delivered materials

The results of the monitoring activities for *P. ramorum* in the host material delivered to and processed at both collection yards are presented in *table 3*. To date, *P. ramorum* has been cultured from the following specimens:

- two of eight bay leaf samples recovered from the chips bin in the collection yard
- two of 84 samples of chips from the collection bins
- eight of 145 pieces of freshly split firewood
- one of 85 pieces of firewood dried for about 6 months
- nine of 93 specimens of grinder and saw dust collected next to the wood grinders and sawmill
- one of three samples of rainwater runoff from chip piles

None of the samples from air-borne dust in the collection yards, near the processing equipment, or the sentinel rhododendron plants have tested positive for *P. ramorum*.

Table 3–*P. ramorum monitoring of P. ramorum infested material delivered or processed at both collection yards.*

Sample	Marin C. Yard		Santa Cruz C. Yard		Sawmill		Powerplant	
	# tested	*P. ramorum* positive	# tested	*P. ramorum* positive	# tested	*P. ramorum* positive	# tested	*P. ramorum* positive
Chips	63	1 (1%)	21	1 (1%)				
Grinder fines	12	0 (0%)						
Fresh firewood	100	8 (8%)			45	0 (0%)		
Air-dried firewood	85	1 (1%)						
Air-borne dust	125	0 (0%)			6	0 (0%)	12	0 (0%)
Grinder/saw dust	41	8 (20%)	11	1 (9%)	41	0 (0%)		
Sentinel rhododendrons	14	0 (0%)	4	0 (0%)	2	0 (0%)		0 (0%)
Bay laurel leaves	8	2 (25%)						
Rainwater	3	1 (33%)						

Wood properties and lumber quality

Twenty-four logs (15.5 tons total weight) of *P. ramorum* infested logs were selected for sawlog potential at the collection yard and delivered to the sawmill in Davenport, CA. The species mix of the sawlog test was 21 tanoak, two coast live oak, and one California black oak. The yield and physical properties of the logs and lumber produced is summarized below in *table 4*. The average moisture content of the heartwood and softwood lumber was 84 percent and 74 percent respectively. The specific gravity (oven dry mass/green volume) of the tanoak processed was 0.57.

The logs were processed into 8-foot long lumber measuring 5/4-inch thick and various widths of 3-inch, 4-inch, and 6-1/4". All of the solid wood residue from the sawmilling operation was processed into firewood and the sawdust was collected and stored on site for future analysis and potential utilization. The total log volume of 412.84 ft^3 (4,954 board feet (bf)) produced 188.89 ft^3 (2393 bf) of green, 5/4-inch thick lumber or a lumber yield of 47.8 percent. The remaining 52.2 percent of the log volume not converted into lumber was either sawdust or a solid wood residue that was converted to firewood.

Table 4 – *Green lumber yield and wood properties of test sawlogs by species and log quality.*

Species	Log Quality	Number of logs	Avg. MC (%)	Avg. SG	Avg. Diameter (in)	Green Lumber Vol. per log (bf)	Yield (%)
Tanoak	Good	9	92.1	0.55	19.5	103.1	47.0
Tanoak	Moderate	8	81.2	0.60	17.7	87.9	50.5
Tanoak	Low	3	76.0	0.60	21.9	113.4	45.2
Coast live oak	Good	3	75.3	0.70	20.8	102.8	43.7
CA black oak	Good	1	90.6	0.56	17.9	98.0	56.5
Overall Average		24	84.8	0.57	19.3	99.7	47.8

The logs were evaluated for quality on the basis of log form (straightness and taper), presence and level of decay, level of beetle infestation, and the presence of heart stain (known to be highly correlated with drying defects). The lumber yield varied from 45.2 percent to 50.5 percent but this variation was not correlated with log quality, the moderate category resulted in the highest yield. Similarly, no trend was identified between lumber yield and log diameter (*fig. 3*), moisture content, or specific gravity. Plots of yield with these factors resulted in plots similar to *figure* 3.

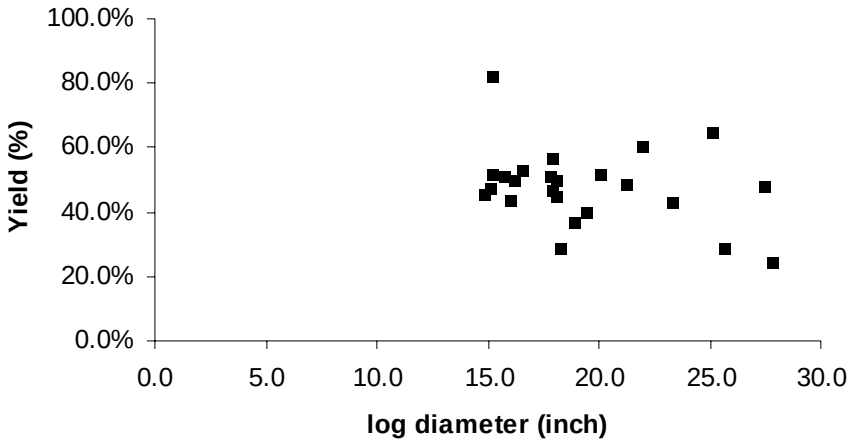

Figure 3 – Green lumber yield by log diameter.

Discussion

The collection yards have been successful in collecting *P. ramorum* infested wood. The Marin County collection yard was the most active, with the support of at least a dozen tree service companies and the county of Marin. The Santa Cruz collection yard was very active during the Santa Cruz County hazard tree removal project but it proved to be a challenge to encourage the local tree service companies to participate in the collection program. These yards have collected about 1200 green tons of woody biomass from sudden oak death host trees, mostly tanoak (~ 80 percent) and coast live oak (~ 15 percent). The potential for this material having viable *P. ramorum* spores is higher than in the general population of host material because the yards only accepted material that exhibited the combination of symptoms associated with the disease. However, since the presence of *P. ramorum* was not positively identified in each tree the yards undoubtedly accepted some dead and dying trees that were *P. ramorum* free.

The high correlation between the deliveries of host woody biomass to the yards and the bark beetle count inside the collection yard suggests that even though the beetles may not be a vector for *P. ramorum*, they are highly associated with the *P. ramorum* infested wood. Because most of the wood delivered was from hazard trees that were dead for more than 4 months, it is suggested that the beetles were not attracted to the wood once in was in the yard but rather they came into the yard in the dead wood. Also, since the beetle counts in the traps away from the yard did not show a corresponding rise, the beetles did not appear to spread far from the collection yard. Although the relationship between massive beetle attack and tree death is not fully understood, this evidence of large numbers of beetles associated with the wood

collected suggests that the beetles play an important role in the disease complex and further study is needed.

The data gathered from the collection yard activities and the periodic sampling for *P. ramorum* in and around the collection yard will be used in future studies to analyze the risk of spreading *P. ramorum* by transporting and processing biomass and wood products. Although *P. ramorum* was found in 23 of the 418 specimens collected (~ five percent), all of the positive cultures, except for firewood, were collected in unprocessed material at the collection yard or at the primary processing equipment (grinders and saws). Although no positive cultures have been obtained at the end uses of biomass power plant fuel, firewood air-dried longer than 6 months, or in processed lumber, the eight positive cultures collected from the fresh split firewood and the one positive from the partially dried firewood emphasize the need to further study the survivability of *P. ramorum* in processed firewood. It is encouraging that none of the firewood specimens dried longer than 6 months have tested positive. Also of interest is the observation that about 2/3rds of the positive *P. ramorum* cultures were collected during the winter and early spring months, the rainy months. This data suggests that *P. ramorum* is most active during the wet weather season of the year and that the risk of spreading *P. ramorum* is higher during these months.

The final evaluation of the potential lumber quality of *P. ramorum* infested logs cannot be reported until the analysis of the kiln-dried lumber is complete. However some preliminary observations are possible. The average specific gravity of 0.57 and the average green moisture content (MC) of 85 percent are slightly lower than the previously reported values of 0.60 specific gravity and 90 percent MC, but is certainly within the range of expected normal variation (Shelly and Jackovics 2001). The 48 percent yield of green lumber reported in this study is markedly lower than the 60 percent green lumber conversion previously reported by Shelly and Jackovics (2001). The extra deterioration of wood quality from the decay and insect infestations in the logs surely accounts for this lower yield. The final conclusions cannot be made until the kiln dried lumber is evaluated for quality but the yield is expected to decrease another five to ten percent as a result of drying defects created during kiln drying. This suggests that although lumber can be produced from *P. ramorum* infested trees, the expected low yield of high quality lumber may limit the economic potential.

Summary

The data reported on in this study indicate that viable *P. ramorum* spores can be collected from dead and dying sudden oak death host trees that are delivered to

collection yards. However, no evidence has been gathered to support the hypothesis that the collection, sorting, and processing activities in the collection yards influences *P. ramorum* infestation levels at the site.

Biomass powerplant fuel and lumber are considered good uses for *P. ramorum* infested wood if care is taken in isolating the grinder dust and sawdust generated during processing. Firewood may be a good use but more information is needed to understand the survivability of *P. ramorum* in firewood. The relative ranking of these options as a best use is dependent on the expected value of the product and any extra costs associated with handling the material to limit the risk of pathogen spread. This economic potential will be evaluated in future work.

Acknowledgements

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Extended Abstract on the Potential for *Phytophthora ramorum* to Infest Finished Compost[1]

Steven Swain[2] and Matteo Garbelotto[2]

Abstract

The survival rate of *Phytophthora ramorum* was assessed when introduced at high rates into composts of varying provenance and curing time, produced by both "turned windrow" and "forced air static pile" techniques. Survival in some compost media was high and statistically indistinguishable from positive controls (P<0.01), while other sources were statistically indistinguishable from negative controls (P<0.01). The difference between positive and negative controls was large and highly significant (P<0.01). We found no significant differences in survival rates between compost processing techniques or curing time. No compost material was so suppressive of *P. ramorum* that it could not be re-infested after the composting process was finished. This suggests that measures should be taken to insure that finished compost is not contaminated by infested greenwaste.

Key words: pathogen, eradication, green-waste, survival, quarantine

Introduction

Phytophthora ramorum is a (presumably) introduced plant pathogen that is currently under both state and federal quarantines. Because many cities within the infested region have curbside greenwaste collection, it becomes of public import to understand how this pathogen's survival and dispersal may be affected by the composting process (Garbelotto, 2003). Studies to determine *P. ramorum's* ability to survive the composting process are currently underway. However, even if this pathogen is killed by the composting process, it may be possible for it to survive in finished compost if it is introduced after the thermophilic "pathogen reduction" phase, and thereby may be inadvertently transported to new locations. This abstract

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outlines studies conducted to ascertain the degree to which *P. ramorum* can colonize finished compost. By "finished" we are here referring to compost which has completed its thermophilic phase, and has now cured for at least one month.

Materials and Methods

This experiment consisted of compost samples of varying ages and produced by varying techniques, which were exposed to *P. ramorum* in a water medium. After exposure, the samples were allowed to dry, and then were pear baited as outlined in Erwin and Ribeiro (1996), or in some cases was directly plated onto PARP media (Erwin and Ribeiro, 1996). Formal statistical analysis was done on a pair-wise basis using the non-parametric Fisher's Exact test.

The techniques used to produce the compost that the samples were taken from included turned windrow and forced air static pile composting. Two different compost ages were used in this assessment as well: (1) compost cured for more than one month; and (2) compost cured for less than one week. The inoculum sources that the compost samples were exposed to included *Umbellularia californica* leaves infected with two different strains of *P. ramorum*, or V-8 agar plugs containing those same two strains. The V-8 agar plugs were separated from the compost by a perforated physical barrier in water, while the *U. californica* leaves were in direct contact with the compost. Filter paper was substituted for compost for our negative controls. The entire experiment was replicated twice, and several portions three times.

Results

All composts tested evinced *P. ramorum* recovery rates that were statistically distinguishable from zero. Survival in some compost media was high and statistically indistinguishable from positive controls ($P<0.01$), while other sources were statistically indistinguishable from negative controls ($P<0.01$). The difference between positive and negative controls was large and highly significant ($P<0.01$). We found no significant differences in survival rates between compost processing techniques or curing time.

Discussion

The recovery rates of *P. ramorum* from most of our compost samples were statistically indistinguishable from filter paper, which suggests that these composts

are not any better substrates for this pathogen's survival than any other material. However, recovery rates obtained from one compost pile was both statistically indistinguishable from the positive controls and significantly higher than with filter paper or either of the other composts used. This suggests that some composts may actually facilitate the long term survival of *P. ramorum* better than other materials.

At present, we cannot explain the source variation found between our high and low recovery samples, as they came from different composting facilities, so a number of confounding factors may be involved. These factors may include the base materials going into the compost (Hoitink and Boehm, 1999), the moisture, carbon availability, and pathogen diversity of the pile (Soares and others 1995) and the frequency and efficiency of turning operations (Churchill and others 1995). The most apparent difference between the samples coming from these two windrow composting facilities is that the facility producing the low recovery samples has specially designed windrow turning equipment (Scarab compost turner), while the turned windrow facility producing the high recovery samples uses front end loaders to turn their materials.

Conclusion

No compost material was so suppressive of *P. ramorum* that it could not be re-infested after the composting process was finished. There is a significant amount of variation among composts as to how well *P. ramorum* can survive, and there is some evidence to support the idea that *P. ramorum* may be able to live as a saprophyte using certain finished composts as substrate. This suggests that measures should be taken to insure that finished compost is not contaminated by infected greenwaste.

Acknowledgements

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Ecological Evidence of Intensive Cultivation of Oaks by California Indians: Implications for the Treatment of Sudden Oak Death[1]

Lee Klinger[2]

Key words: sudden oak death, California Indians, agriculture, oaks

Abstract

The native oaks of California are remarkable for being among the oldest and largest oak trees in the United States besides their great age and size, these trees possess various idiosyncrasies in their arrangements and shapes that appear to defy basic principles of ecology and population biology. When taken together, these and other associated patterns present an anomalous situation that cannot be explained using our current scientific understanding of old-growth forests. In this paper I describe several abnormal features of California oaks recorded during ecological surveys in the Coast Range and the Sierra Nevada foothills in 1997 and 2003. These features are all plainly visible, expressed as gross variations in the character of the oaks and the surrounding soils. In the search for a plausible and parsimonious explanation of these anomalies, I am drawn by previous experiences to consider the wisdom and practices of the native people.

In the oak savannas of California the various species of native oaks occur as individual large trees or in clusters of a few, with broad canopies and thick trunks that indicate the trees are many centuries old. Most have trunks that branch oddly into multiple large boles. Numerous trunks have unusually bent and trailing forms. Open grasslands adjacent to the oaks are in certain places distinctly terraced, especially on steeper slopes and in draws. Young trees are notably few or absent in oak savannas. The predominance of old oaks and a lack of regeneration means that the population of oaks in these savannas is not sustainable, nor has it been sustainable for several hundred years. In recent years elevated levels of dieback have occurred among the older oaks. This mortality is sometimes (though not always) associated with the sudden oak death (SOD) pathogen (*Phytophthora ramorum*).

Within the oak savannas that exhibit some regeneration, there is seen a cohort-related dimorphism in the growth forms of the old (bent and branched) vs. young (straight and unbranched) oaks. Seen also is a similar dimorphism occurring within individual old oaks

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which exhibit two developmental phases, an earlier bent-and-branched phase and a more recent straight-and-unbranched phase. Along with these peculiar dimorphisms we find other phenomena, like oddly-placed fire scars, scatterings of seashell and bone fragments under the oaks, and, on the trunks of certain oaks, lime-rich crusts which cover the bark.

In the search for an explanation, it is first apparent that natural forces cannot readily account for these unusual features. It appears more reasonable that the shell fragments and the strange forms of oaks (and other trees) are the result of plant husbandry practices by the local Indians who formerly occupied these areas. The trailing, bent, and many-boled forms of the old oaks are quite likely the result of coppicing, pruning, and training by native people mainly for the purposes of maximizing the size of the tree canopy and, thus, the production of acorns for food. The low, lateral-tending branches also would have greatly facilitated the gathering of the acorns. Oak trees established since white settlement were not traditionally tended and so have taken on normal, more upright and unbranched growth forms. It is well known that local people regularly burned the land to improve its fertility, and archeological evidence suggests that large quantities of seashells, bones, ashes, and mineral-rich rocks were stockpiled in huge middens in order to make mineral fertilizers.

Thus, it is apparent to this author that the California Indians were not simple hunter-gatherers, but instead were sophisticated farmers who practiced a sustainable kind of agriculture that involved the careful cultivation of oaks and other food-bearing trees in vast orchards. Today this ancient wisdom casts light on a promising treatment for SOD simply by ameliorating acidity and improving mineral nutrition which makes trees healthy and able to resist attacks by insects and pathogens. The many ancient oaks that still thrive in our landscape stand as testimonials to the long-term efficacy of this treatment in keeping the SOD pathogen and other insects and pathogens under control.

Eradication of *Phytophthora ramorum* in Oregon Forests—Status After 3 Years[1]

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

Keywords: sudden oak death, Oregon, eradication, *Phytophthora ramorum*

Abstract

Sudden oak death (SOD) was first discovered in Oregon forests in July 2001 near the city of Brookings. Since September 2001 we have been attempting to eradicate the pathogen by cutting and burning all infected host plants and adjacent apparently uninfected plants. Eradication currently is in progress on approximately 42 sites, totaling 72 acres. The majority of sites are on private land. All eradication funding has come from the USDA Forest Service.

The number of infected trees discovered each year has decreased since we first discovered the pathogen in Oregon (*table 1*). Most new infected trees tend to occur very near eradication sites, and usually in a northward direction as result of spread by wind and rain. In 2004 we found only 30 new infected trees (nine new sites and fice extensions to existing sites), all near existing infested sites. This was a major improvement in the rate of new discoveries compared to previous years, and suggests that the eradication effort is slowing spread of the pathogen.

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

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Despite several new occurrences of *Phythopthora ramorum* in 2003 and early 2004, distribution of the pathogen in Oregon forests remains limited to a very small area near Brookings. Repeated aerial surveys and ground-checks have failed to detect the pathogen in forests beyond this area. The forested area in Oregon under regulation by the Oregon Department of Agriculture and USDA- APHIS is 11 mi^2. Intensive and extensive monitoring and eradication activities in Oregon forests likely will continue for several years.

Table 1—*Occurrence of P. ramorum and Sudden Oak Death patches near Brookings Oregon; aerial and ground surveys combined, December 30, 2004.*

Year	Number of tanoaks infected with *P. ramorum*	Number of new disease patches	New area undergoing eradication each year (acres)
2001	100+	9	40
2002	85	12	8
2003	48	12	12
2004	30	9	12
Total	263+	42	72

Trace Forward, Perimeter, and National Nursery Surveys for *Phytophthora ramorum* in Texas[1]

D.N. Appel[2], T. Kurdyla[2], R.F. Billings[3], K.S. Camilli[4], and A. Purdy[3]

Abstract

Four nursery surveys for *Phytophthora ramorum* have been, or are currently being, conducted in Texas. These consist of the original Pilot Survey during 2002-2003, the Trace Forward Survey during spring 2004, the National Survey during summer and fall 2004, and the U.S. Department of Agriculture Forest Service Perimeter Survey during summer and fall 2004. Four different agencies, including Texas A&M University, the Texas Department of Agriculture (TDA), the Texas Forest Service, and U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS PPQ) have been involved with one or more of the surveys. We discuss the Trace Forward and the Perimeter Surveys, each of which has focused on the 112 nurseries that received plant materials from Monrovia Nursery in California. The Perimeter surveys have been completed for 42 of the nurseries. Both surveys were conducted according to the systematic approaches devised and published by USDA APHIS and the USDA Forest Service. Samples were collected, returned to the laboratory, and processed for isolation of *Phytophthora* spp. on a selective medium (PARP). ELISA was performed for a subsample of the Trace Forward Survey and replicate samples in the Perimeter Survey were sent to Mississippi State University for processing by PCR. A total of 1620 samples were collected from the Trace Forward nurseries, consisting of six genera and 15 species of plants. There were 50 positive PARP isolations, of which 33 were *P. ramorum*. These specimens were taken from five nurseries. Another five nurseries have been confirmed for *P. ramorum* through samples submitted by USDA APHIS through the Texas A&M University, Texas Plant Diagnostic Laboratory. ELISA only had a 55 percent success rate in correctly diagnosing PARP positive samples for *P. ramorum*. Alternatively, the ELISA test had only a 3 percent false positive rate. One hundred thirteen samples have been collected in the Perimeter Survey. There are 32 species, of which 17 were oaks (*Quercus* spp.). No *Phytophthora* spp. have been isolated from these specimens, nor have any of the replicate samples tested positive for *P. ramorum* with PCR, indicating that there is little likelihood that the pathogen has escaped from the Trace Forward nurseries. Nursery operators, landscape maintenance personnel, natural resource managers and homeowners have benefited from these surveys by a better understanding of the threat posed by *P. ramorum* in Texas.

Key words: Phytophthora ramorum, sudden oak death, national survey

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Small Mammal and Herpetofaunal Abundance and Diversity Along a Gradient of Sudden Oak Death Infection[1]

Kyle Apigian[2], Letty Brown[2], Jenny Loda[3], Sarah Toas[4], and Barbara Allen-Diaz[2]

Abstract

We studied the effects of sudden oak death (SOD) on coast live oak woodlands in the San Francisco Bay Area. Small mammals and herpetofauna were sampled in the spring and fall of 2002 and 2003 along a gradient of *Phytophthora ramorum* infection. Coverboards along a grid were sampled in the spring to determine herpetofaunal numbers and diversity while Sherman live traps were used to measure small mammal populations. Descriptive analyses indicate that the California slender salamander (*Batrachoseps attenuatus*) was the most common herpetofauna present on all SOD sites, but did not show a consistent pattern with *P. ramorum* infection. Wood rats (*Neotoma fuscipes*) were found only on our two most heavily affected sites. Deer mice (*Peromyscus maniculatus*) were most common on heavily affected sites, while Piñon mice (*Peromyscus truei*) and brush mice (*Peromyscus boylii*) were more common on less affected sites. Observed patterns of abundance may be related to some aspects of habitat change related to SOD, such as increased downed, woody debris, crown cover reduction, and changes in overstory tree species. However, there may be many other covariates that impact the abundance of these groups (for example, geography, climate, competitors, and predators) that are unrelated to SOD. Our study design may have not been powerful enough to detect effects of SOD; natural variations in population size make larger sample sizes necessary to detect potentially small effects of habitat change. Additionally, it is important to monitor these populations for longer than two seasons to potentially detect differences.

Key words: small mammals, herpetofauna, sudden oak death, *Phytophthora ramorum*

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Pacific Yew as a Host for *Phytophthora ramorum* in California Forests[1]

J.C. Bienapfl[2], J.W. Zanzot[2], and D.M. Rizzo[2]

Abstract

English yew (*Taxus baccata*) has been reported as a host of *Phytophthora ramorum* in nurseries in Great Britain. In 2004, branch dieback was observed on Pacific yew (*T. brevifolia*) in a Mendocino County, California, forest and *P. ramorum* was recovered. Symptoms on Pacific yew, similar to those reported for English yew, included discolored needles and shoot dieback. The Pacific yews were found in the understory of a Douglas-fir/tanoak forest associated with *P. ramorum* infected bay (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*). Other *P. ramorum* hosts (e.g., *Vaccinium ovatum, Lonicera hispidula*) were present and yielded cultures of the pathogen. A survey of the area resulted in the detection of three *P. ramorum* infected yew shrubs. Disease progression resulted in branch dieback, but death of individuals was not observed.

Pacific yew is the only plant species known as a natural host for both *P. ramorum* and *P. lateralis. Phytophthora lateralis* is the closest known relative to *P. ramorum.* It causes Port-Orford cedar root disease and has been reported as a root pathogen of Pacific yew. Pacific yew twigs and seedlings were inoculated with both *Phytophthora* species to investigate symptom expression, potential interactions between these two pathogens, and to demonstrate Koch's postulates.

Key words: Phytophthora ramorum, sudden oak death, Pacific yew, *Taxus brevifolia*

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First Report of *Pyracantha koidzumii* as a Host for *Phytophthora ramorum*[1]

Stephan C. Briere[2], Sarah Llewellyn[2], and Gary Kristjansson[2]

Abstract

Phytophthora ramorum was confirmed for the first time in the new host plant *Pyracantha koidzumii* cv. "Victory" at the Centre for Plant Quarantine Pests at the Canadian Food Inspection Agency. *Pyracantha koidzumii*, known commonly as scarlet firethorn, is a perennial shrub in the family Rosaceae. It was found during an emergency action to screen all plants shipped into Canada from the Monrovia Nursery in Azusa, California, during the summer of 2004. Foliage was sampled at a nursery in British Columbia, Canada, and sent to our laboratory for official testing for *P. ramorum*. Tissue was plated onto PARP isolation medium and incubated for one week. Suspect mycelia were then plated onto five percent V-8 medium for morphological determination. Morphological identification was confirmed by PCR. The two original plants were shipped directly to the laboratory for confirmatory isolation. *P. ramorum* was then recovered and re-confirmed from one of the plants. Koch's postulates were conducted and confirmed that *P. koidzumii* was a susceptible host for *P. ramorum*.

Key words: sudden oak death, *Phytophthora ramorum, Pyracantha koidzumii*

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Predicting the Potential for Establishment of *Phytophthora ramorum* in the Oak Forests of the North Central States, USA[1]

S.D. Cohen[2] and R.C. Venette[3]

Abstract

Phytophthora ramorum the causal agent of Sudden Oak Death, is known to cause significant mortality in susceptible host species in forest ecosystems of northern California and southwestern Oregon. Additionally, the potential exists to move the fungal pathogen to new locations in the United States via nursery stock. For example, in March 2004, plants confirmed positive for *P. ramorum* in southern California were distributed to 39 other states (APHIS update, August 27, 2004). The pathogen was not detected in the North Central states (Illinois, Indiana, Michigan, Minnesota, and Wisconsin) that received shipments. Future shipments could move the pathogen into the North Central states, but for establishment, *P. ramorum* must encounter susceptible hosts and suitable climatic conditions. Because susceptible hosts grow in this region, we focused on predicting the area of establishment of *P. ramorum* in the North Central states.

In nature, *P. ramorum* produces sporangia, zoospores, chlamydospores and possibly oospores for dispersal and survival. Climatic factors affect dispersal and survival of the pathogen. If climate factors regulate establishment of the pathogen, it should be possible to predict the potential establishment of the pathogen in the North Central states of the United States. The purpose of this study is to compare two approaches to climate modeling that predict the likelihood of establishment of *P. ramorum* in the North Central states.

Key words: Disease distribution, pathogen survival, climate mapping, climate modeling, CLIMEX

Each climate model requires a different set of data (for example, climate matching requires the known distribution of the pathogen and eco-climatic index relies on the physiological responses of the pathogen to climate variables). Prior to climate modeling, a baseline climate data set containing 1,589

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meteorological stations with United States climate normals (monthly average maximum temperature, minimum temperature, and precipitation from 1970-2000) was imported into the climate analysis software, Climex, ver. 2.0 (Sutherst and Maywald 1985). Climate similarity analysis was conducted by comparing climatic conditions at five locations in California (Fairfield, Richardson Grove State Park, Richmond, Monterey, and Upper San Leandro) to 979 locations in the North Central Region using the Match Climates function within Climex. Sites in California delimited known occurrences of *P. ramorum* in the field. Composite similarity (for temperature and moisture combined) was expressed in an index scaled between 0 and 100 percent. Results from Climex were exported as the percentage maximum to ArcView, ver. 3.2a and a contour data layer created by interpolation.

The second climate analysis, based on population growth and survival parameters, generates an eco-climatic index. In this analysis, temperature parameters from Werres and others (2001) and soil moisture parameters from Brasier and Scott (1994) describe conditions needed for population growth of the pathogen are entered into the Climex software. Climex generates an Eco-climatic Index (EI; 0 - 100 scale) that describes the general potential for population growth at a given location given climatic constraints. Suitable plant hosts are assumed to be present. Index values were exported to ArcView to create a contour data layer by interpolation. Predicted results from the Eco-climatic Index were validated by overlying the location of *P. ramorum* finds in the field (through 12 Dec 2003) on the map of climate suitability. We then examined the frequency of finds among suitability classes. These locations were not used to develop the model; thus, they provide independent validation. The observed frequency of finds in each suitability class was tested against the expectation (assuming a random distribution) that finds should occur in direct proportion to the area occupied by each suitability class. Unsuitable and marginally suitable areas were combined into a single class for analysis.

In general, climatic similarity of the North Central states to *P. ramorum* sites in California varied between 40 and 60 percent, with more southern states generally being more similar. It is unclear whether risk of establishment decreases in direct proportion to reduced similarity. Prediction of establishment using this approach requires a larger dataset drawn from diverse geographic locations. In this case, locations confirmed positive for *P. ramorum* are clustered in a small area and are not adequate to predict potential establishment areas on a larger geographic scale.

Of the two methods, climate suitability based on an Eco-climatic Index proved to be a more effective method for predicting the establishment of *P. ramorum* in the North Central states**.** Areas highly favorable (EI=26 to 52) for establishment of *P. ramorum* in California were found along the coast of Washington, Oregon and northern California. Favorable sites (EI=11 to 25) were further inland from the coastline of Washington, Oregon and California. Potential areas of establishment were limited by the amount of rainfall and soil moisture. Within the North Central states, highly favorable areas for establishment (EI=26 to 52) did not occur. Favorable areas for establishment for *P. ramorum* were found in the lower portions of Illinois, Indiana, Missouri, and Ohio. Marginal areas (EI=1 to 10) for establishment occurred throughout the North Central states and represent areas that may support persistent survival of the pathogen. The model predicted the distribution of *P. ramorum* well. The majority of *P. ramorum* finds in the field occurred in areas predicted to be climatically favorable or very favorable for establishment of the pathogen. No finds of *P. ramorum* occurred in areas that were

predicted to be unsuitable. The frequency of finds among suitability classes differed significantly from expectation assuming a random distribution (df=2, $\chi 2$=643, P<0.001).

In conclusion, Eco-climatic analysis based on physiological responses of *P. ramorum* to climate proved to be a better predictor of potential establishment in the North Central states. *P. ramorum* is most likely to encounter a climate suitable for establishment in Missouri, Illinois, Indiana, and Ohio. Surveys of susceptible hosts should be concentrated in the southern portions of higher risk states.

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Mechanisms Underlying Differences in Inoculum Production by *Phytophthora ramorum* in Mixed-Evergreen Versus Tanoak-Redwood Forest in California[1]

Jennifer M. Davidson[2], Elizabeth J. Fichtner[3], Heather A. Patterson[3], Kristin R. Falk[3], and David M. Rizzo[3]

Abstract

Timing of inoculum production by *Phytophthora ramorum* occurs later in mixed-evergreen forest, as opposed to tanoak-redwood forest in California. This lag may be due, in part, to a greater decrease in the inoculum reservoir in California bay laurel (*Umbellularia californica*) leaves, the main source of inoculum, during the hot, dry summer months in mixed-evergreen forest. In our experiments, we focused on the mechanisms underlying this phenomenon. Using detached leaf inoculations, we compared the susceptibility of the California bay laurel populations and the aggressiveness of the corresponding pathogen populations between the two forest types. Although no difference in percent leaf area affected was observed between California bay laurel populations, lesion size was significantly smaller on leaves from mixed-evergreen forest regardless of origin of isolates, suggesting the potential for a smaller source of primary inoculum in this ecosystem. We also estimated chlamydospore production on, or inside, inoculated and naturally infected leaves from both forest types using clearing techniques. Surprisingly, chlamydospore production was zero in over 98 percent of inoculated leaves, and there was no conclusive evidence of chlamydospore presence in naturally infected bay leaves from either site. Summer measurements suggested that California bay laurel leaves from mixed-evergreen forest have significantly lower moisture content and water potential than bay laurel leaves from tanoak-redwood forest. Further studies are planned to address the influence of tree water potential on survival of *P. ramorum* in California bay laurel leaves.

Key words: cross inoculation, dormancy, sudden oak death, summer survival, transmission

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Detection and Distribution of *Phytophthora ramorum* in Soils[1]

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Abstract

Failure to detect *Phytophthora ramorum* in soils throughout the year does not denote absence of viable propagules, rather the inability to detect soilborne chlamydospores. The objectives of this study were: 1) to compare pear and rhododendron bait materials for detection of sporangia and chlamydospores, and 2) to assess the seasonal distribution of *P. ramorum* within redwood-tanoak forest soils. Soil was infested with sporangia or chlamydospores, baited with either pears or rhododendron leaves, then symptomatic bait tissues were transferred to selective medium. Chlamydospores were not detected by either bait material; however, rhododendron leaves were more sensitive than pears for detection of sporangia. Soil and leaf litter samples were collected monthly, from December 2003 to January 2005, under host trees, including *Sequoia sempervirens*, *Lithocarpus densiflorus*, and *Umbellularia californica*. *P. ramorum* was recovered from soils from December 2003 through June 2004, with the highest frequency of recovery occurring under *U. californica* and minimal recovery under *L. densiflorus*. Overall, recovery of soilborne inoculum was low, but underestimated given the limitation of baiting techniques for detection of chlamydospores. Future studies will address quantitative detection of pathogen propagules in soil and investigate the role of chlamydospores in pathogen survival.

Keywords: Phytophthora ramorum, sudden oak death, soil, baiting, detection

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Progress Toward the Development of a Model to Quantify the Efficacy of Detection Strategies for *Phytophthora ramorum*[1]

Yashika Forrester[2], Betsy Randall-Schadel[3], Allan Hogue[4], David Oryang[4], and Robert McDowell[4]

Abstract

The emergence of sudden oak death and the continuing appearance of new incidences have prompted a coordinated federal, state and local effort to manage the risk of spreading the causal agent, *Phytophthora ramorum*, through nursery stock within the USA. The wide host range, the commonality of symptoms with other pathogens, and environmental influences complicate detection of this pathogen. A mathematical model is under development to evaluate the effectiveness of different strategies to detect *P. ramorum*. The model uses event sequence diagram analysis to compare selected strategies. An event sequence diagram sequentially illustrates individual events that can occur in a system along with their subsequent probabilities. Aggregated expert judgment data are used to supplement empirical data. The advantages and disadvantages of the novel use of this model are highlighted.

Key words: Phytophthora ramorum, sudden oak death

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A Case Study to Evaluate Ground-Based, Wildland Survey Methods for *Phytophthora ramorum* in Coast Live Oak Stands in California[1]

Susan J. Frankel[2], Sylvia Mori[3], Janice Alexander[4], Don Owen[5], Jennifer Davidson[3], and David M. Rizzo[6]

Abstract

In a case study, we evaluated observers' accuracy in detecting *Phytophthora ramorum* and their ability to estimate percentage of infested area in coast live oak (*Quercus agrifolia*) stands in California. The study compared visual detection of symptoms on California bay laurel (*Umbellularia californica*) and oaks with results from three permanent 1-hectare plots. Each plot had 100 percent survey of all stems and varied in infection level (Sonoma County - 84 percent infection; Napa County - 68 percent infection; and Marin County - four percent infection). In 50 randomly selected plots at each site, an expert and novice observer noted the presence or absence of *P. ramorum*. These observations were used to test the following null hypotheses: (1) expert and novice observers have the same detection ability; and (2) expert and novice observers can accurately detect symptoms. The overall analysis shows the experts' assessments of infection level were closer to true than the novices' assessments. The observers tended to over-estimate the amount of infection by larger amounts when less disease was present. The novice assessment was 23 percent higher for the plot with four percent infection. The expert assessments were within the 95 percent confidence interval of the estimated proportion, indicating that a trained observer can accurately detect symptoms of *P. ramorum* in coast live oak stands in California.

Key words: Phytophthora ramorum, survey methods

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Introduction and Objectives

This study evaluates the precision and accuracy of wildland surveyors at estimating infection by *Phytophthora ramorum*. We re-measured three permanent 1-hectare plots in coast live oak forests where infection levels of *P. ramorum* had previously been determined by Jennifer Davidson (Fairfield Osborn Preserve, Skyline Park, and Pacheco Open Space). In addition, we compared accuracy for detecting *P. ramorum* symptoms between two surveyor teams, one expert and one novice.

Methods

Sampling design

The plot attribute to be estimated was proportion of *P. ramorum* hosts with disease symptoms. We decided that a desirable level of observer accuracy would be within +/- 10 percent of the true proportion and we chose a sampling design to achieve this level of accuracy with a precision of 90 or 95 percent confidence. We split the 1-hectare plots into 100 subplots of 10 x 10 meters for ease of sampling. Prior to sampling, we determined the minimum sample size required for a 10 percent margin of error (|True proportion - estimated proportion|= 0.1) with a 95 percent confidence. We evaluated the minimum sample size needed using Lenth's sample size calculation by comparing field survey results to the model. (For more calculations use: http://www.stat.uiowa.edu/~rlenth/Power/). This calculation was done assuming the greatest variance possible, a proportion of 0.5 - the worst-case scenario. In our example, assuming the plot had 50 percent infection level we would survey 50 0.01-hectare subplots for presence or absence of *P. ramorum* (*fig. 1*).

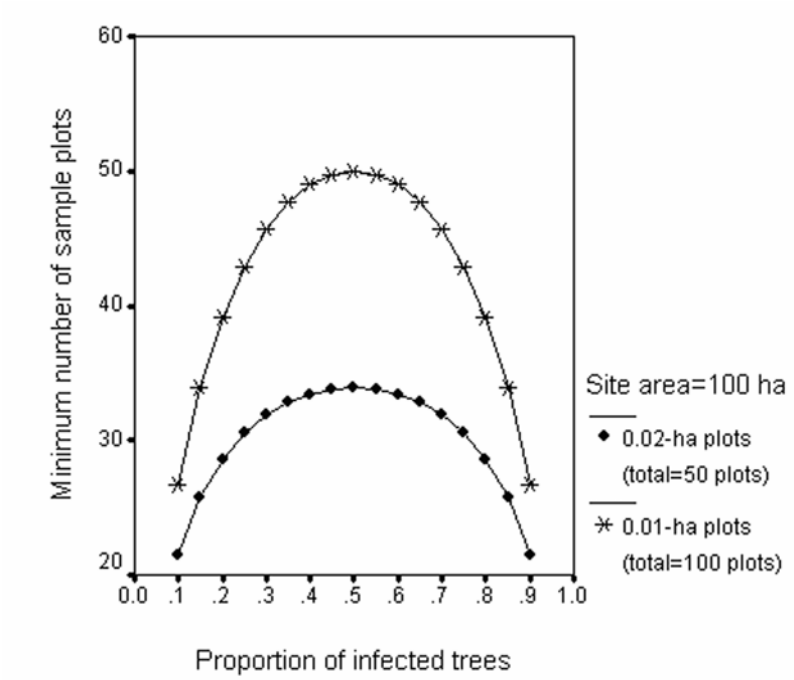

Figure 1 —Minimum number of sample subplots within a one hectare block.

Data collection and lab analysis

In October 2003, we assembled two survey teams and visited the three oak woodland sites. We defined the novice team as having only an introductory training in symptom recognition, while the expert team had many years experience in field forest pathology. Neither team knew the true proportion of infection in any of the three plots prior to sampling. For each site, 50 0.01-hectare subplots were chosen at random within the 1-hectare plot. Each team visited the 50 subplots and noted presence or absence of the pathogen based on visible symptoms. When no host plants were present in a subplot, another subplot was chosen, at random, as a replacement. For each subplot, each team also collected samples to be analyzed for confirmation of *P. ramorum* symptoms. Lab samples were collected, labeled, and shipped the same day following the standard California Department of Food and Agriculture (CDFA) protocol using PDR slips, and sent to Cheryl Blomquist, CDFA in Sacramento.

Data analysis

Permanent plot data from Davidson provided the true proportion values (mean infection level) for comparison with survey team results. These data represented 100 percent samples, and therefore, were considered accurate of the true infection levels. Results were analyzed by presence or absence of *P. ramorum* in the 50 randomly selected subplots at each site. The following results compare the expert and novice team results against the true results for each plot and the amount of agreement

between the two teams. The data were analyzed with SAS (v.9.1.3) GENMOD procedure.

Results and Discussion

Fairfield Osborn Preserve had the highest infection level (84.1 percent) followed by Skyline (68.2 percent), with the Pacheco site showing almost no infection (four percent). The expert team's assessment of infection level was closer to true than the novice team's assessment, though both teams tended to estimate higher levels of infection when the true infection level was very low. However, due to the lack of replications, it is not possible to make statistically valid inferences about the interaction level of experience and level of *P. ramorum* infection. We can only conclude that the true proportions were within the 95 percent confidence interval of the expected mean for FOP and Skyline, but not for Pacheco *(table 1 and fig. 2*), and that the expert team was significantly more accurate than the Novice team (p=0.083).

Table 1 – *Estimated proportions compared to the true proportions using initial true information and corrected true information (new P. ramorum infection.*

		True % of *P.r.*	Estimated% of *P.r.*	Lower 95% bound	Upper 95% bound	True % of *P.r.* within 95% CI?
FOP	Expert	84.1	86.4	76.3	92.6	Yes
FOP	Novice	84.1	91.4	83.5	95.7	Yes
Pach	Expert	4.0	9.5	4.9	17.7	No
Pach	Novice	4.0	15.0	8.4	25.4	No
Sky	Expert	68.2	68.4	55.8	78.8	Yes
Sky	Novice	68.2	78.4	66.9	86.7	Yes

Laboratory results confirmed that trained observers can use visual symptoms in Coastal Evergreen Forests (mix of coast live oak, California bay laurel, and so forth) to estimate the level of *P. ramorum* infestation in a stand. A lesser-trained observer may not be as accurate as a professional, but estimates of disease were still within acceptable levels, with the caveat that proportions will likely be over-estimated by any observer. We cannot say what additional amount of training would be necessary to move a novice to the expert level, but the novice level of accuracy may be acceptable depending on the survey objectives.

Because surveys may be more likely in areas of low disease occurrence - as with early detection efforts - actual disease proportion may be much lower than any estimated proportion, even with well-trained surveyors. Also, we found that the amount of time and difficulty of the survey increases greatly in areas where the

pathogen is not present or present at low levels (less than approximately 25 percent). Due to leaf drop on bay and new symptom expression, the time lag between establishment of the true disease proportion on the permanent plots and our survey may have introduced some error. In fact, there were three cases at the Skyline site where the lab analysis confirmed *P. ramorum* in areas not noted by the initial permanent plot data.

This is a case study without replication, not a generic recipe for survey methodology. Each survey needs to be designed to meet specific objectives, terrain, and vegetation. For example, transects may be more efficient than plots when actually surveying a site for *P. ramorum*. Also, results of our study, as in any other, are dependant on the size of the sampling area. A smaller subplot size could have greatly changed our estimates of infection at any of the three sites we visited.

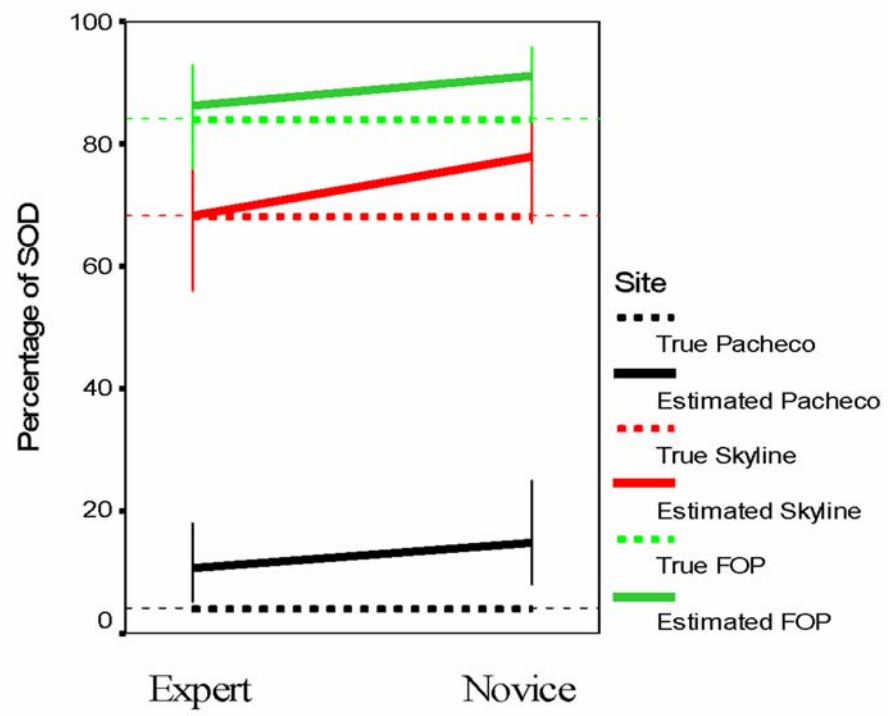

Figure 2 —Estimated levels of infection and 95 percent confidence interval for expert and novice compared to the true levels at the different sites.

Summary Conclusions

- Sample size based on 50 percent disease incidence was adequate for accurately determining the infection rate at three sites with varying infection levels.
- Laboratory results confirmed that visual symptoms can be used in Coastal

Evergreen Forests to estimate the level of *P. ramorum* infestation in a stand.

- Experts are better than novices, though both surveyors overestimated when infection level was low.

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Addressing *Phytophthora ramorum* in California: Programs of the USDA Forest Service and California Department of Forestry and Fire Protection[1]

Susan J. Frankel[2] and Mark Stanley[3]

Abstract

Since 2000, the U.S. Department of Agriculture Forest Service and California Department of Forestry and Fire Protection have been collaborating on a comprehensive program to address sudden oak death, caused by *Phytophthora ramorum*, in California, investing over $12 million of state and federal funds. The program's primary objectives are pathogen containment and maintenance of public safety, which are accomplished through monitoring, management, research, education and outreach. Monitoring efforts have included surveys of Southern California and the Sierra Nevada, as well as watershed, vegetation, and aerial surveys of high-risk terrain in and surrounding California's 14 infested, coastal counties. Education and outreach programs, utilizing www.suddenoakdeath.org, and collaborative efforts via the California Oak Mortality Task Force (COMTF), have trained thousands of professionals in pathogen detection, treatment, and management. Quarantine implementation, suppression efforts, hazard tree reduction, and debris disposal programs for *P. ramorum* are also on-going; a program overview is provided.

Key words: sudden oak death, *Phytophthora ramorum*, management, monitoring

Introduction

Since 2000, the U.S. Department of Agriculture Forest Service (USDA-FS) and California Department of Forestry and Fire Protection (CDF) have been collaborating on a comprehensive program to address sudden oak death, caused by the pathogen *Phytophthora ramorum*, in California, investing over $12 million of state and federal funds. The primary objectives of the program are pathogen containment and maintenance of public safety, which are accomplished through monitoring, research, management, education and outreach. The program is carried out via grants to cooperators at over 15 universities or agencies. The California Oak Mortality Task Force (COMTF) provides program coordination and collaboration, as well as education and outreach activities.

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Monitoring

Early detection and some delimitation monitoring is carried out via aerial survey, watershed, and ground-based vegetation survey. In 2004 the following projects were implemented:

1. Aerial Survey (Jeff Mai, USDA Forest Service, and Walter Mark, Cal Poly San Luis Obispo)
2. Watershed Monitoring (David Rizzo, University of California, Davis)
3. Ground-Based Targeted Risk Survey (Ross Meentemeyer, Sonoma State University)
4. California National *P. ramorum* Survey (Don Owen, CDF, Redding)
5. North Coast Recreation Area Monitoring (Yana Valachovic and Chris Lee, University of California, Cooperative Extension, Humboldt and Del Norte Counties)
6. Reported finds follow-up (David Rizzo and John Bienapfl, University of California, Davis)

A risk model to help in planning and survey design is maintained by Meentemeyer, Sonoma State University. An interactive, GIS database for all wildland detections is managed by Kelly and Tuxin, University of California, Berkeley. Support for diagnostic services is provided to Rizzo, University of California, Davis and Garbelotto, University of California, Berkeley. Sierra Nevada and Southern California surveys have not detected *P. ramorum* in those ecosystems. Project results are posted in the monitoring section, at www.suddenoakdeath.org.

Management

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

1. Northern California (Del Norte, Humboldt and Mendocino Counties) - *P. ramorum* wildland infections are few and isolated from other infestations. Our strategy is an aggressive slow-the-spread program. A suppression project was carried out in Southern Humboldt County to remove symptomatic plants in an old-growth redwood forest near Redway in February 2004. Cooperators include the Humboldt County Department of Agriculture and University of California Cooperative Extension, Humboldt and Del Norte Counties.

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

Throughout the infested area, sanitation and quarantine compliance are emphasized**.** In infested areas, all fire crews in California clean their equipment prior to departure. USDA-FS, CDF, and parks personnel are trained annually in quarantine compliance and sanitation for all operations. Plant debris collection sites (http://groups.ucanr.org/ SODBusters/) are operating in Santa Cruz and Marin Counties and California-funded hazard tree removal along highways and other high-use areas.

Education and Outreach

The California Oak Mortality Task Force (COMTF) is an umbrella organization that brings together all groups working on sudden oak death (*P. ramorum)* in California. Education and outreach are carried out via www.suddenoakdeath.org, a monthly newsletter, informational meetings, training sessions, talks, and a public affairs program. In 2004, 11 training sessions on nursery and forestry aspects of *P. ramorum* reached well over 1,000 professionals. The education/outreach program is implemented by Janice Alexander, Outreach Specialist, University of California Cooperative Extension, Marin County; Katie Palmieri, Public Information Officer, University of California, Center for Forestry, Berkeley; and Chris Lee, Sudden Oak Death Specialist, University of California Cooperative Extension, Humboldt and Del Norte Counties.

Anatomical Examination of *Phytophthora ramorum* Infection in *Camellia*[1]

Elizabeth Geltz[2], Jessica McHugh[2], Lisa Baird[2], Sibdas Ghosh[3], Peter Thut[3], and Mietek Kolipinski[4]

Abstract

In spring 2004 *Phytophthora ramorum* infection of *Camellia* plants was reported at multiple nursery sites in California. Our research was initiated to examine the mode of infection of *Camellia* plants with *P. ramorum* at the whole plant and cellular level. *Camellia* leaves were infected with *P. ramorum* via zoospores or plug inoculation. Leaf samples were harvested 3 hours and 1, 2 and 4 days after infection and fixed immediately for light and electron microscopy. Examination with light and scanning electron microscopy indicated that possible anatomical pathways for infection of *Camellia* leaves include stomates and large sub-epidermal oil glands located on abaxial surfaces of leaves. Preliminary scanning electron microscopy in our laboratory indicates that stomates are the most likely site of initial infection.

Key words: Phytophthora ramorum, electron microscopy, camellia leaf

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Applications of Fungicides to Protect Four Hosts From Foliar Infection by *Phytophthora ramorum* in Curry County, Oregon[1]

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Key words: Phytophthora ramorum, fungicide treatment, Stature®, Agri-Fos®

Introduction

Oregon's *Phytophthora ramorum* eradication effort in tanoak/conifer forests in Curry County has greatly reduced levels of infection, but has not completely eliminated *P. ramorum* from some affected sites. Two fungicides, Stature® (dimethomorph and mancozeb) and Agri-Fos® (mono and dipotassium salts of phosporous acid), were tested for their ability to protect Pacific rhododendron (*Rhododendron macrophyllum)*, evergreen huckleberry (*Vaccinium ovatum*), Oregon myrtlewood (*Umbellularia californica*), and tanoak (*Lithocarpus densiflorus*) from foliar infection.

Field and Laboratory

At two different field locations, a total of 12 uninfected plants of each host species were treated; three uninfected plants of each species were selected as controls. Fungicides were applied at 1X and 2X their recommended rates to six plants of each host. Applications were made to all plants in February; one half of the selected plants in each species were treated again in April. In May, the new foliage of plants that had received a single application of fungicide was treated. Leaves were collected from each treated plant at set weekly intervals after treatment, removed to the laboratory, and inoculated with *P. ramorum*.

In the laboratory, all leaves from each plant were wounded a single time with a sterile push pin. Half of the leaves were then inoculated with a mycelial plug of an A1/European genotype isolate (03-74-N11A) of *P. ramorum* and the other half with a mycelial plug of an A2/North American isolate (4143) of *P. ramorum*.

The inoculated leaves were placed in a sealed moisture chamber maintained at room temperature for one week. At that time, the inoculated leaves were photographed and analyzed visually for disease incidence and for leaf necrosis with the program Assess.

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[1] An abstract of a poster presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California.

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Reisolation of *P. ramorum* on standard PARP media or DNA extraction and PCR analysis was performed for all samples.

Results

Overall, treatment effects using foliar application of Stature® and Agri-Fos® to Pacific rhododendron, evergreen huckleberry, Oregon myrtlewood, and tanoak as applied in this field study were limited. We were consistently able to reisolate *P. ramorum* from symptomatic and asymptomatic leaf tissues regardless of fungicide treatment.

We observed significantly reduced lesion area for both A1 and A2 isolates with application of Stature® to newly-emerged tanoak leaves at one week after fungicide application. We observed significantly reduced lesion area for mature evergreen huckleberry leaves treated with Agri-Fos® and inoculated with the A2 isolate of *P. ramorum*.

Regardless of treatment, inoculation with the A1 isolate of *P. ramorum* consistently produced significantly larger lesions than the A2 isolate on Pacific rhododendron, evergreen huckleberry, and Oregon myrtlewood, but not on tanoak leaves.

Leaf necrosis was significantly greater on newly emerged Pacific rhododendron leaves than on mature leaves inoculated with either the A1 or the A2 isolates of *P. ramorum.* Lesion size was greater on new evergreen huckleberry leaves than mature leaves when inoculated with A2. On tanoak and the other hosts (although not quite so obvious), as the new foliage matured it seemed to become less susceptible to infection.

Acknowledgements

Funding for this project was provided by the USDA Forest Service Pesticide Impact Assessment Program (FS-PIAP). Special thanks are extended to South Coast Lumber Company for providing field sites and other assistance.

Amplified Fragment Length Polymorphism Assessment of Population Diversity in California Bay Laurel (*Umbellularia californica*)[1]

Sarah Gordon[2], Richard Whitkus[2], Ross Meentemeyer[3], and Brian Anacker[3]

Abstract

Umbellularia californica is a major foliar host for *Phytophthora ramorum*. The population genetics of a host influences the spread of pathogens through the host population, however no information is available on the population genetics of *U. californica.* We investigated the population genetics of *U. californica* using Amplified Fragment Length Polymorphism (AFLP) in a study of the factors underlying the spread of *P. ramorum* through the Sonoma Valley. Genomic DNA was extracted from leaves from 97 populations of *U. californica*, representing 1293 adults and 2119 juveniles. Forty-six AFLP primer pairs have been surveyed, resulting in six pairs providing a strong signal. We surveyed two geographically proximate populations to find a combination of markers that provides representative genetic diversity. Comparing individual primers, noticeable variation was seen in the number of scorable loci (45 to 82), percent polymorphism (60 to 75 percent), total diversity (HT: 0.096 to 0.131), and between population diversity (GST: 0.071 to 0.105). One combination of three primers (186 loci) gave very similar diversity values when compared to all six primers (340 loci). This study produced a solid basis for conducting a population genetics study of an estimated 6000 *U. californica* trees in the study area and generating useful data for modeling the spread of *P. ramorum.*

Key words: AFLP markers, population genetics, *Umbellularia californica*

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Persistence of *Phytophthora ramorum* After Eradication Efforts in Oregon Tanoak Forests[1]

E.M. Hansen[2] and W. Sutton[2]

Abstract

In Oregon, *Phytophthora ramorum* is confined to a small area of forest land in the southwestern corner of the state, where it is subject to an ongoing eradication effort. Infected trees and adjacent host plants are destroyed. Intensive monitoring of the sites for the presence of the pathogen follows treatment. Here we report part of that monitoring effort, focused on rainwater and streams flowing out of treated areas, and persistence of *P. ramorum* in soil and on plants growing around the stumps of infected tanoak trees. Rainwater and streams were baited with leaves of tanoak and rhododendron, soils were baited with pear fruits and tanoak and rhododendron leaves, and susceptible Douglas-fir and redwood seedlings were planted around stumps. In addition, other sprouting host plants were examined for infection. *P. ramorum* is still recovered from streams 30 months after site treatment, although no new plant infections have been associated with these streams. The pathogen was initially recovered from tanoak sprouts around 88 percent of the 43 sampled stumps, from soil around 14 percent of the stumps, and from other sprout plants around seven percent of the stumps. Douglas-fir and redwood seedlings were not infected. Recovery rates after 30 months are currently being assessed.

Key words: Phytophthora ramorum, sudden oak death, eradication

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Effect of Chemicals on Hyphal Growth, Sporangia Production and Zoospore Germination of *Phytophthora ramorum*[1]

Tamar Y. Harnik[2] and Matteo Garbelotto[2]

Abstract

Phytophthora ramorum, causal agent of the sudden oak death, has 23 regulated hosts. Some of these, such as coast live oak, tanoak and California bay laurel, play a key role in the ecology of California forests. There is one commercially available, preventative treatment for two hosts, coast live oak and tanoak. New treatments that employ easier and cheaper application methods and protect both foliar and trunk/twig hosts are needed. Other products in the market registered to control other *Phytophthora* pathogens may be effective for controlling *P. ramorum*.

The ability of three chemicals to inhibit different stages in the life cycle of *P. ramorum* (hyphal growth, sporangia production and zoospore germination) was tested *in vitro*. The chemicals Subdue® (metalaxyl), Agrifos400® (phosphite) and Champ® (copper hydroxide) were tested at different concentrations against 12 North American *P. ramorum* isolates. Additionally, we conducted *in planta* experiments on controlling the pathogen using foliar spray on California bay laurel. Isolates varied in response to the treatment, but no isolates were tolerant to any of the chemicals. All the chemicals were effective in inhibiting all life cycle stages tested. *In planta*, copper hydroxide was very effective in controlling infection on California bay laurel leaves up to six weeks after treatment.

Key words: phosphite, metalaxyl, copper hydroxide, fungicide, California bay laurel

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Quantitative Resistance to *Phytophthora ramorum* in Tanoak[1]

Katherine Hayden[2] and Matteo Garbelotto[2]

Abstract

Tanoak (*Lithocarpus densiflorus*) is among the hosts most heavily impacted by sudden oak death; up to 70 percent of tanoaks are infected, with correspondingly high mortalities, on individual sites. However, patches of healthy tanoak are often observed immediately adjacent to patches with heavy mortality, suggesting that there may be variability for resistance in the system. We report on a preliminary study of resistance to infection by *Phytophthora ramorum* within a single tanoak population. Sixty saplings grown from acorns collected on the Six Rivers National Forest were inoculated, using both standard underbark inoculations and a wounding leaf inoculation. There was significant variability in lesion size among individuals resulting from both techniques. Stem lesion area was positively correlated with leaf lesion area. Further, there was a significant block effect, whereby trees in south-facing rows of the lathehouse had smaller lesions than those in north-facing rows. This effect was less pronounced for the leaf assay. We conclude that there is variability in resistance to *P. ramorum* within tanoak populations, and that leaf inoculations provide a convenient, and easily replicated, assay for resistance.

Key words: Lithocarpus densiflorus, resistance, inoculation studies

[1] An abstract of a poster presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California.

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Distribution and Pathogenicity of *Phytophthora nemorosa* and *P. pseudosyringae* in Coastal Forests of California[1]

Camille E. Jensen[2], Allison C. Wickland[2], and David M. Rizzo[2]

Abstract

Phytophthora nemorosa and *P. pseudosyringae* are two recently described species that have been recovered during studies of *P. ramorum*, the causal agent of sudden oak death. The distribution of *P. nemorosa* and *P. pseudosyringae* was compiled from a number of surveys of coastal California and Oregon. *P. nemorosa* was isolated from *Umbellularia californica, Sequoia sempervirens* and *Lithocarpus densiflorus* in redwood and mixed-evergreen forests from Monterey County, California, to Coos County, Oregon. *P. pseudosyringae* was isolated from *U. californica* and *Quercus agrifolia* in mixed-evergreen forests and coast live oak woodlands from San Luis Obispo County to Humboldt County, California. Inoculation experiments were conducted to satisfy Koch's postulates and verify pathogenicity of *P. nemorosa* and *P. pseudosyringae* on these tree species. Based on re-isolation of the pathogen and lesion lengths significantly different than controls ($P<0.05$), *S. sempervirens, Q. agrifolia, L. densiflorus,* and *U. californica* were confirmed as hosts of *P. nemorosa* and *Q. agrifolia* and *U. californica* as hosts of *P. pseudosyringae*. The ecology of *P. nemorosa* and *P. pseudosyringae* is very similar to *P. ramorum,* however, these species do not cause landscape level tree mortality.

Key words: Phytophthora nemorosa, Phytophthora pseudosyringae, Umbellularia californica

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Inoculation Trials With *Phytophthora ramorum* on Moorland Species[1]

Stefan Wagner[2], Katrin Kaminski[2], and Sabine Werres[2]

Abstract

Moorland plants are important for landscape and garden culture in many European countries. Studies on the host range of *Phytophthora ramorum*, in other words first infection trials with the moorland genera *Calluna* and *Erica*, should give a first impression on the susceptibility of these plants. Two cultivars each of *Calluna vulgaris* and *Erica gracilis* were infected with four different *P. ramorum* isolates. Wounded and non-wounded twigs were inoculated with zoospores or mycelium. First results indicate that *P. ramorum* can infect *C. vulgaris* and *E. gracilis*. *E. gracilis* seemed to be more susceptible than *C. vulgaris*. Both inoculation with zoospores and with mycelium resulted in infection. The experiments with unwounded plant material indicate that *P. ramorum* can infect the healthy (twig) tissue. There was a difference in the aggressiveness of the tested isolates; isolates from the United States exhibited the highest aggressiveness. There were also several interactions between inoculation method, inoculum, cultivar and isolate. These must be verified in further experiments.

Key words: Phytophthora ramorum, Calluna vulgaris, Erica gracilis, infection.

Introduction

In many European countries the Moorland genera *Calluna* and *Erica,* which are produced in specialized nurseries, are important for landscape and garden culture. Until now, only a single plant of *Calluna vulgaris*, infected with *Phytophthora ramorum*, has been detected in a Polish nursery. Thus, the aim of our studies was to test whether *P. ramorum* can infect cultivars of *C. vulgaris* and *E. gracilis* and therefore pose a potential hazard for these European plants. Furthermore, the best inoculum and inoculation method should be evaluated for further tests. The present study is part of the research project RAPRA ("Risk analysis for *Phytophthora ramorum*", a recently recognized pathogen threat to Europe and cause of the sudden oak death in the United States), which is funded by the European Union and is important for the estimation of the potential host range of *P. ramorum* in Europe.

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Material and Methods

Detached twigs of *C. vulgaris* ('Amethyst', 'Long White') and *E. gracilis* ('Weißes Schloß', 'Glasers Rote') were tested. Three European isolates (1376, from *Viburnum tinus* "Eve Price", UK; 1577 from *Rhododendron catawbiense*, Germany; 1578 isolated from *Rhododendron grandiflora*, U.K.) and one American isolate (from *Vaccinium ovatum*) of *P. ramorum* were tested. Isolates were cultivated on carrot piece agar (CPA five percent) at 20° C with 16 hour white light for 14 days. Induction of zoospore release was achieved by flooding the colonies with five ml of sterile distilled water, then incubated at 4 °C for one hour, followed by 60 minutes at room temperature. Twigs and sterile distilled water were used for the negative control. Mycelium plugs, eight mm in diameter, were taken from the edge of an actively growing colony. Sterile CPA was used for the negative control. Wounded and unwounded twigs were dipped with the apical end into zoospore suspension for 5 seconds. Wounded twigs were pressed into a mycelium plug and placed on a wet sterile swab. Twigs were incubated for 6 days in a wet chamber at 20° C with 16 hours light. Seven twigs were used for each trial.

Verification of the infection rate was calculated by the length of discoloration in mm followed by PCR amplification using specific ITS-Primer for *P. ramorum* (http://www.bba.de/inst /g/pramorumneu/pramorum start.pdf). Kruskal-Wallis ANOVA was used on ranked means with $P \leq 0,05$.

Results

Both *C. vulgaris* and *E. gracilis* could be infected with *P. ramorum. 9*6 percent of the *Calluna* twigs and 99 percent of the *Erica* twigs showed discoloration. The length of discoloration was significantly longer on the *Erica* twigs (*fig.1*). The susceptibility of the two *C. vulgaris* cultivars differed significantly, in contrast to the two *Erica* cultivars (*fig. 2*). The U.S. isolate 1403 caused longer discoloration than the three European isolates (*fig. 3*). Both zoospores and mycelium caused discoloration on the twigs but the damage was significantly higher with zoospores. In comparison to non-wounded plants, wounding did not increase the length of necrosis after inoculation with zoospores (*fig. 4*).

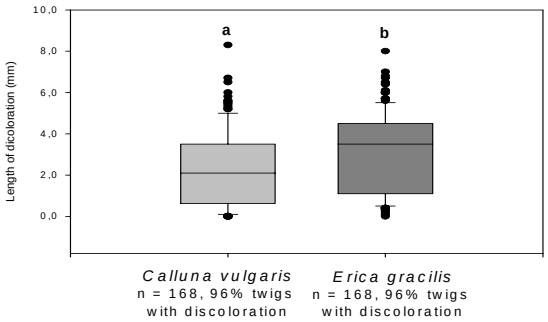

Figure 1: Disease rate by *C. vulgaris* and *E. gracilis*.

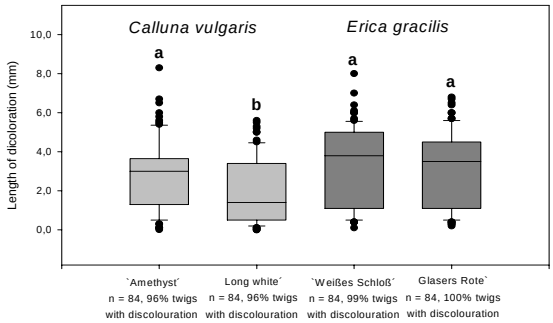

Figure 2: Susceptibility of different *C. vulgaris* and *E. gracilis* cultivars.

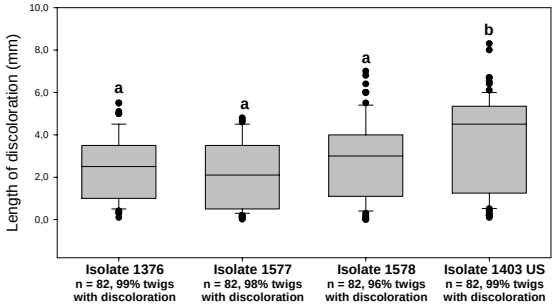

Figure 3: Disease rate in dependence of the *P. ramorum* isolate.

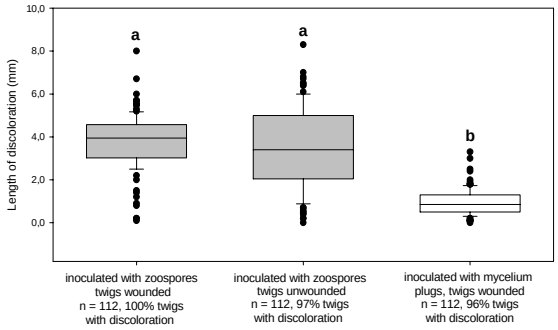

Figure 4: Influence of the inoculum and inoculation method.

Infectivity and Survival of *Phytophthora ramorum* in Recirculation Water[1]

Heinrich Beltz[2], Thomas Brand[2], Katrin Kaminski[2], Dankwart Seipp[2], Stefan Wagner[3], and Sabine Werres[3]

Abstract

In a 2-year project the spread and survival of *Phytophthora ramorum* will be studied in a simulation system for water recirculation.

Key words: Phytophthora ramorum, containerized nurseries, recycling water, spread

Introduction

Phytophthora ramorum has been detected in diseased plants and contaminated soil, but also in water samples. Until now, there was little known in Central Europe about the survival of this pathogen in water, and the possible correlation between inoculum density in water and infection rate. An understanding of the epidemiology in water is of great importance for container nurseries because they use recirculated water from the container stands for irrigation.

Survival and infection rate during different seasons will be studied in a 2-year project (funded by the U.S. Department of Agriculture Forest Service) that started in 2004. The results should provide a better understanding of the possible spread of *P. ramorum* in water.

Material and Methods

Simulation System - The open-air, simulation system includes nine separate container areas (stands) with separate water recycling systems. Each container stand is approximately 15 m_ in length. Similar to commercial container nurseries, potted plants in each stand were arranged on a waterproof sheet. From each stand the surplus water from irrigation and natural rain was collected in a water reservoir with a capacity of about 1,000 liters. Each container stand had its own reservoir. From these reservoirs the water was taken for overhead irrigation with nozzle pipes. The temperature of the water in the reservoirs and the ambient data were recorded continuously.

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[1]An abstract of a poster presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California

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Water - The reservoirs were filled with ground and pond water. Water quality was identical to that used by the growers. Because it is an open-air, simulation system, the recycled water mixed with natural rainwater during the season.

Plant Material - Rooted cuttings of *Rhododendron* hybrid 'Cunningham's White' were taken. The plants originated from the Bad Zwischenahn Research Centre and from a local nursery; 328 of these plants were put onto each container area in May 2004.

Inoculum and Inoculation - The isolate *P. ramorum* BBA 9/95 (German origin) was cultivated on five percent Carrot Juice Agar (CJA) according to (Werres and others 2001) at 20 °C in the dark for 14 days. For inoculation the actively growing cultures were cut into small pieces and mixed thoroughly with water and sediment in each reservoir. Two different inoculum densities were chosen: for the "Low inoculum density" the cultures of 12.5 Petri dishes (9-cm diameter), for the "High inoculum density" the cultures of 25 Petri dishes were prepared for each reservoir. For the control treatment, sterile CJA of 12.5 Petri dishes was used. Three replicates for each inoculum density treatment, as well as for the negative control, were used. First inoculation of the water was in 2003 (pretrial). Second inoculation was in June 2004.

Calculation of Infection and Survival Rate - The infection rate has been evaluated weekly by visually assessing disease symptoms. The "pattern" of disease spread within a single container area was also examined. As soon as disease symptoms occurred, the infected plants were taken to the laboratory for direct isolation.

Survival of *P. ramorum* in the water reservoirs during the season was studied with the *Rhododendron* leaf test (Themann and others 2002a,b) in April (prior to inoculation), in July, and in October. Per reservoir, 10 *Rhododendron* leaves were placed directly on the water surface of each reservoir. *P. ramorum* infection of the baits was determined with specific PCR-primer (http://www.bba.de/inst /g/pramorumneu/pramorum start.pdf) and by direct isolation to test the fitness of the propagules.

Preliminary results

In 2004 disease symptoms characteristic for *Phytophthora* occurred. Reisolation and identification of the species of *Phytophthora* has not yet been finished. At present reliable results are not yet available. Disease symptoms could be observed on the stem tips as well as on the stem base and/or on leaves. Disease symptoms occurred mainly from June to the beginning of September. From July to mid-August there seemed to be no correlation between the outbreak of symptoms and the position of the plants on the container stands. In one replication there was an increase of diseased plants near the end of August starting around a single plant.

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Ectomycorrhizal Diversity Associated With Tanoaks (*Lithocarpus densiflorus*)[1]

Nicholas Kordesch[2], Sarah Bergemann[2], and Matteo Garbelotto[2]

Abstract

Ectomycorrhizal fungi are important symbionts of many temperate plant species. *Phytophthora ramorum* has caused widespread mortality of tanoaks along California's coastal regions. Because tanoaks form ectomycorrhizal associations, local extinction may impact ectomycorrhizal symbionts. Our goal in this study is to identify the predominant ectomycorrhizal fungi from tanoak and to determine the relative abundance of taxa. To determine the community composition of ectomycorrhizal fungi, 15 plots were established in tanoak stands near the town of Whitethorn, California in Humboldt County, California. Root tips were sorted from soil core sections of equal volume, bulked and amplified using general primers that target the ITS region and a portion of the 28s rDNA. To differentiate taxa, melt- temperature profiles were preformed for 80 PCR products cloned from each plot. Samples with unique melt-temperature profiles were sequenced to differentiate taxa. From 382 ITS sequences, 83 ectomycorrhizal taxa were detected. Taxa with the broadest spatial distribution included *Cenococcum geophilum, Lactarius, Phialaphora, Russula,* and *Tomentella.* This is the first comprehensive study of the ectomycorrhizal composition of tanoak. Information collected from this study can be used as baseline information for future studies to investigate the impacts of *P. ramorum* on the ectomycorrhizal fungi of tanoak.

Key words: community, ectomycorrhiza, species richness

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Epidemiological Aspects of *Phytophthora ramorum* in Redwood Forests of the Coast Range of California: A 3-Year Look[1]

Patricia E. Maloney[2], Shannon C. Lynch[2], and David M. Rizzo[2]

Abstract

Understanding the epidemiology and disease dynamics of *Phytophthora ramorum* in redwood forest communities will allow researchers and land managers to predict the effects on forest structure, as well as other key ecosystem processes. As part of a long-term study on the epidemiology and ecology of *P. ramorum* in redwood forests, 120 plots were established and censused yearly since 2002. The main hosts and tree species are coast redwood (*Sequoia sempervirens*), tanoak (*Lithocarpus densiflorus*), and bay laurel (*Umbellularia californica*). Baseline disease incidence across the 120 plots was 17 percent in the initial sampling in 2002. Overall disease incidence on the plots increased in 2003 and 2004 to 24 and 29 percent, respectively. This was an increase by 42 percent in 2003 and only 19 percent in 2004. We have also found that mortality rates of tanoak have been elevated in *P. ramorum*-infested locations as compared to uninfested locations. The number of new, individual host trees becoming infected has declined, but new infections are still occurring. Explanations for increases, decreases or leveling off may be a function of pathogen and host biology, as well as interannual variation in climate affecting *P. ramorum* sporulation and dispersal. Mortality rates and rates of disease increase are discussed further.

Key words: *Lithocarpus*, tanoak, disease ecology, disease progression

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Sensitivity and Specificity of Inspection and Testing Procedures for *Phytophthora ramorum* on Nursery Stock[1]

Robert McDowell[2], Betsy Randall-Schadel[3], and Shawn Robertson[3]

Abstract

Targeted sampling is used to detect defective or hazardous items in populations, but is routinely criticized as a non-probability sampling method with drawbacks, including the inability to make statistically valid inferences about the sampled population. Recent advances in stratified discovery sampling theory have remedied this defect. We apply this new technique to evaluate sampling and testing protocols to detect *Phytophthora ramorum*-infected nursery stock. Stratified discovery sampling relies on identifying sub-populations (with easily identifiable characteristics) that are enriched with the defective items. By incorporating test sensitivity and the relative frequencies of the indicator characteristic in defective and non-defective subpopulations, we can infer the true prevalence in the entire population. The target category is symptomatic plants. Assuming a screening (ELISA) test sensitivity of 0.95 and conditional probabilities of plants showing symptoms of 0.5 for infected and 0.05 for uninfected plants, the resulting target population has a disease prevalence 10 times higher than the entire population. Thus, a sample size of 40 in a targeted population is equivalent to a sample size of 400 in the general population.

Key words: Phytophthora ramorum, stratified discovery sampling

[1] An abstract of a poster presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California.

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Survival of *Phytophthora ramorum* in Tanoak and Rhododendron Leaves[1]

I. McLaughlin[2], W. Sutton[2], and E. Hansen[2]

Abstract

Wild rhododendron and tanoak leaves inoculated with *Phytophthora ramorum* were placed in the field. Leaves were shaded or unshaded, and placed above ground or buried. Leaves were collected at intervals across two or three months. More than 90 percent of leaves were PCR positive. In the fall trial, 89 percent of buried leaves and less than 50 percent of surface leaves were culture positive for *P. ramorum*. In the summer trial, however, about 50 percent of buried leaves were culture positive and less than five percent of surface leaves were culture positive. Sporulation occurred on 32 percent of inoculated leaves that were culture positive.

Key words: soil, sporangia, chlamydospores

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Multihyphal Structures Formed by *Phytophthora ramorum* on Inoculated Leaves of Mediterranean Shrubs[1]

Eduardo Moralejo[2] and Enrique Descals[2]

Abstract

Multihyphal reproductive structures produced by the plant pathogen *Phytophthora ramorum*, similar to sporodochia of the mitosporic fungi, have been repeatedly observed one to two weeks after zoospore inoculation of detached leaves and fruits of an assortment of Mediterranean sclerophyll shrubs. The morphology of these structures and their morphogenesis and position on the host tissue were examined by dissecting, compound and scanning electron microscopy. Initials of the fruit bodies developed subcuticularly or subepidermally as small hyphal aggregates by repeated branching, budding, swelling and interweaving, eventually coalescing into prosenchymatous stromata. These always emerged through the adaxial side of the leaf by rupture of the overlying host tissue. Occasionally, short sporangiophores bearing clusters of 20 to 100 sporangia formed above and were associated with the stroma. Packed clusters of chlamydospores were sometimes formed instead. Full descriptions of the already proposed term 'sporangioma' and the newly proposed 'chlamydosorus' are given. Their biological significance is discussed.

Key words: Phytophthora ramorum, sporulation, sporangioma, chlamydosorus

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Monitoring *Phytophthora ramorum* Distribution in Streams Within Coastal California Watersheds[1]

Shannon K. Murphy[2], Chris Lee[3], John Bienapfl[2], Yana Valachovic[3], and David M. Rizzo[2]

Abstract

Thirty-six locations were established in winter-spring 2004 to monitor for the presence of *Phytophthora ramorum* in perennial watercourses throughout coastal northern California. The areas of focus include Alameda, Contra Costa, Del Norte, Humboldt, and Mendocino counties. These counties have limited *P. ramorum* detection, but are high-risk areas for *P. ramorum* infestation. Two sites in Sonoma County were included as a baseline for successful recovery of *P. ramorum*. *Rhododendron* leaves were placed in mesh bags and secured in watercourses for 7- to 21-day intervals year-round to bait for *Phytophthora* species. Recovered symptomatic leaves were plated on *Phytophthora*-selective media. *P. ramorum* was recovered at all sites with *a priori* knowledge of *P. ramorum* forest infestation. We recovered *P. ramorum* at three sites downstream of known forest infestations. One site is along the South Fork Eel River, approximately eight km downstream of a known infestation near Redway. Additionally, *P. ramorum* was recovered at two sites without a prior known forest infestation in Briones East Bay Regional Park. Stream monitoring provides a useful method of early detection for infestations of *P. ramorum*. Future work will include the additional monitoring at the southern extent of the known range of *P. ramorum*, including Monterey and San Luis Obispo counties. In addition, we will address research questions related to quantification, spread, and survival of this pathogen in watercourses.

Key words: Phytophthora ramorum, water monitoring, *Phytophthora* baiting,

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Monitoring of *Phytophthora ramorum* in the North Central United States[1]

Frances S. Ockels[2], Pierluigi Bonello[2], and Manfred Mielke[3]

Abstract

Participating in the 2004 USDA Forest Service Sudden Oak Death (*Phytophthora ramorum*) monitoring program, our lab surveyed nursery perimeters and forest sites and processed samples from Ohio, Indiana, Illinois, Missouri, Iowa, and Wisconsin. As part of these protocols, samples were stored in an ice chest on sealed coolant and shipped to laboratory for detection of *P. ramorum* within 72 hours of collection, using PCR-based procedures. Results showed that 89 samples from Ohio, 100 samples from Indiana, 75 samples from Iowa, 75 samples from Missouri, and 219 samples from Wisconsin were free of the pathogen. During surveys, the process of storing the samples on coolant and shipping every two days became cumbersome in terms of expense and convenience. Thus, we explored the feasibility of desiccating the samples as a way of preserving tissues at ambient temperature. Symptomatic leaves were placed in a plastic bag in the presence of a sachet containing Drierite. Fungal DNA extracted from Drierite-desiccated samples was successfully amplified by PCR using universal ITS primers. However, further work is needed to show that *P. ramorum*-infected leaves can be desiccated and still allow for PCR-amplification of *P. ramorum* DNA. Currently, our lab is not permitted to work with *P. ramorum* infected material.

Key words: Phytophthora ramorum, monitoring, desiccate

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Genotyping *Phytophthora ramorum* Isolates From Nurseries in the United States Using PCR-RFLP and Microsatellite Analyses[1]

Rodrigo Olarte[2], Matteo Garbelotto[3], and Kelly Ivors[4]

Abstract

The risk of introducing the important plant pathogen *Phytophthora ramorum* outside of its present natural range is of extreme concern; however, the detection of *P. ramorum* in U.S. nurseries is increasing. The USDA has confirmed the presence of *P. ramorum* in nurseries in 21 states that received plants from an infested, southern California nursery and others. This nursery produces approximately 15 million landscape plants annually, of over 2200 varieties. Although it remains unknown whether these new introductions will lead to an outbreak of sudden oak death (SOD) in the affected states, many potential susceptible hosts of *P. ramorum* are widely distributed in forest ecosystems, and many of the affected states have climatic conditions conducive for SOD. As part of an effort to genotype new nursery infestations, we characterized 19 of these isolates from 13 states with cox I-RFLP banding patterns (Kroon and others 2004) and microsatellite analyses (Ivors and others, unpublished data). All isolates screened produced the typical 'U.S.' RFLP pattern and showed no variation among 14 microsatellite loci. These results indicate that all isolates analyzed consist of a single clone genotype, identical to isolates established in the wild in California and Oregon. This discovery is in contrast with findings from other nursery infestations in Oregon and Washington, where both the European and U.S. genotypes have been identified in infested blocks of plants.

Key words: *Phytophthora ramorum*, microsatellite

Reference:

Kroon, L.P.N.M.; Verstappen, E.C.P.; Kox, L.F.F.; and Bonants, P.J.M. 2004. **A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*.** Phytopathology 94, 613-620.

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Surveying for *Phytophthora ramorum* in Urban and Wild Forests Throughout Western Oregon[1]

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Abstract

In 2004, Oregon received information indicating *Phytophthora ramorum*-infected nursery stock may have been shipped to 52 nurseries throughout western Oregon. The pathogen was detected in nine of the nurseries in seven counties (Clackamas, Josephine, Lane, Lincoln, Marion, Multnomah, and Washington). Because the pathogen may have been present at these nurseries for approximately 12 months, the urban and wild forests nearby were surveyed. As the *P. ramorum*-susceptible plants were not contiguous in these areas, the USDA Forest Service-Sudden Oak Death (*P. ramorum*) National Detection Survey for Forests protocol was used to provide a systematic survey strategy. Four transects were established around each nursery and around a tenth nursery (Columbia County) where *P. ramorum* was found. All susceptible plants within each transect were visually surveyed and samples collected for laboratory testing. Five subsamples per plant were tested with DAS ELISA to identify *Phytophthora*-infected plants. DNA was extracted from ELISA-positive samples by spin column chromatography and then tested with nested PCR and with multiplex PCR. A total of 782 samples were collected from 40 transects. One sample collected in Marion County tested ELISA-positive. DNA testing showed the *Phytophthora* infecting the plant was not *P. ramorum.* Based on these survey results, *P. ramorum* did not spread from the infected stock within the nurseries into the natural environment in these eight counties in western Oregon.

Key words: Phytophthora ramorum, forest survey, nursery perimeter

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Detached-Leaf Assays With *Phytophthora ramorum*: Are They Valid?[1]

Jennifer L. Parke[2], Melody L. Roth[2], and Caroline J. Choquette[2]

Abstract

Detached leaves have been used by several researchers to investigate host susceptibility to *Phytophthora ramorum*, evaluate fungicide efficacy, and investigate other aspects of *P. ramorum* biology. This is because large-scale, whole-plant testing is restricted to quarantine facilities that are not available to most researchers. Detached leaves offer several advantages: greater reproducibility due to similar size and age of leaves, increased replication, more consistent delivery of inoculum, uniform incubation conditions, localization of the inoculation site to specific parts of the leaf, more accurate quantification of disease, conservation of space, and ease of handling. Disadvantages of the detached leaf assay are that some host defense responses may be compromised, movement and behavior of certain systemic fungicides may be atypical, and detached leaves may not adequately reflect the variability of whole plants in terms of leaf age and leaf orientation. We describe some of the factors that influence the outcome of detached leaf assays and consider conditions under which this assay can be a useful and reliable indicator of whole plant response.

Key words: inoculation methods, pathogenicity tests, host susceptibility

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Low Genotypic Diversity of *Phytophthora ramorum* in North America Revealed by Microsatellite Markers[1]

S. Prospero[2], E.M. Hansen[2], and L.M. Winton[3]

Key words: Phytophthora ramorum, microsatellites

Abstract

The newly discovered pathogen *Phytophthora ramorum* causes an emerging forest disease in California and Oregon. *P. ramorum* affects a broad range of hosts, including tanoak (*Lithocarpus densiflorus*) and several species of oak (*Quercus* spp.), on which it causes stem cankers, leaf blight, and/or twig dieback. Knowledge of the population diversity of *P. ramorum* may provide important information on the origin of the pathogen (native vs. introduced), its reproductive mode (recombinant vs. clonal), and its evolutionary history. In this study we investigated the population structure of *P. ramorum* in North America by using microsatellite markers.

A total of 350 isolates of *P. ramorum* were used for our study. Most of these isolates (189) were recovered from 2001 to 2004 from different hosts in forests of Oregon and California (10 isolates). Seven isolates were obtained from soil samples and 22 were obtained from stream baits. The remaining 132 isolates were recovered from infested nurseries in Oregon and Washington. DNA was extracted from pure cultures using standard protocols.

All isolates of *P. ramorum* were screened at 10 microsatellite loci. The di-nucleotide and tri-nucleotide loci (CA_{15}, CGA_8, TCG_9, TTG_6, TCG_7, and CAC_8) were isolated from an enriched genomic library as described by Prospero and others (2004). The four tetra-nucleotide loci ($AAAC_6$, $TATC_{32}$, $GTGA_8$, and $TCCG_{11}$) were identified by screening the genome of *P. ramorum* (available online at: http://genome.jgi-psf.org) for the most common tetra-nucleotide repeats according to literature. For each locus, specific primers were designed using the program Primer3. In each primer pair, the forward primer was labeled with a fluorescent dye (either 6-FAM or HEX) for detection on a capillary sequencer. The products obtained by PCR amplification of the loci were sized on an ABI 3100 sequencer. Results were analyzed using GeneScan and Genotyper software.

Our results show that the field isolates (in other words, from forests, soil and streams) of *P. ramorum* belong to seven different North American genotypes, whereas eight nursery isolates belong to a European genotype. All ten microsatellite loci differentiate North American and European genotypes but only two tetra-nucleotide loci ($TATC_{32}$ and $TCCG_{11}$) show variation within North American isolates. About 95% of the field isolates from Oregon belong to the same genotype. Rare genotypes

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were found both in the field and in nurseries. The ten isolates from California belong to a genotype that is very rare in Oregon. The index of association value for all *P. ramorum* isolates is significantly higher ($P < 0.001$) than the value calculated from 1000 artificially recombined datasets.

Our study shows a low genotypic diversity of *P. ramorum* in North America, confirming the results of AFLP analysis (Ivors and others 2004). This strongly clonal population structure may suggest a recent introduction of the pathogen. The observed index of association value indicates that, despite the presence of both mating types, the North American population of *P. ramorum* is not recombining. We are now screening more isolates to determine: (1) if the North American population of *P. ramorum* is geographically subdivided (for example, Oregon vs. California), and (2) the origin of the rare nursery genotypes.

References

Ivors, K.L.; Hayden, K.J.; Bonants, P.J.M.; Rizzo, D.M.; and Garbelotto, M. 2004. **AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*.** Mycological Research 108: 378-392.

Prospero, S.; Black, J.A.; and Winton, L.M. 2004. **Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death.** Molecular Ecology Notes 4: 672-674.

Nursery Cultural Practices and Physiological State of Nursery Stock on Susceptibility to *Phytophthora* Species, including *P. ramorum*[1]

Steven J. Scheuerell[2], Robert G. Linderman[3], Niklaus J. Grunwald[3], and E. Anne Davis[3]

Abstract

The physiological state of nursery stock can be altered by nursery cultural practices, particularly fertilization. Fertilization practices influence plant growth rate and tissue nutrient concentrations throughout the growing season, which in turn influences the susceptibility of plants to infection by pathogens. Experiments using rhododendron and lilac are underway to relate infection by *Phytophthora* spp., including *P. ramorum*, to increasing concentrations of soil-applied and foliar nitrogen, iron, and silica. Other experiments are planned to assess how leaf age and tissue nutrient concentration affect the susceptibility to infection (by *P. ramorum*) by performing detached leaf assays on material collected from commercial nurseries over the growing season. In addition, compost-amended potting medium and compost teas will be tested for the biological control of root and foliar infection caused by *Phytophthora* spp., including *P. ramorum.*

Key words: Phytophthora ramorum, fertilization

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Environmental Limits to Infection by *Phytophthora ramorum* in Tanoak and California Bay Laurel[1]

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Abstract

Very little is known about conditions that might limit infection of *Phytophthora ramorum*. This study examines two factors hypothesized to limit both infection rates and the extent of disease caused by *P. ramorum* in intermediately resistant tanoak (*Lithocarpus densiflorus*) and California bay laurel (*Umbellularia californica*). Six trees of each species were sampled in the fall of 2004 at Samuel P. Taylor State Park, in Marin County. To find the maximum and minimum temperatures that result in zoospore infection of leaves we inoculated leaves of each species for one day at five temperatures. After this incoulation period, we incubated the leaves at ideal growth conditions (19° C) for ten days. To determine the maximum and minimum inoculum zoospore concentrations for leaf infection we inoculated leaves of each species at five concentrations, and incubated them at 19° C. To determine how lesion size varies within these conditions, we measured the lesion area on each infected leaf. Data were analyzed both in terms of infection rates (plus or minus infection) and infection extent (lesion size). This study will aid others who are developing risk assessment for native oak forests, for whom knowledge about conditions in the environment that limit infection by *P. ramorum* is crucial.

Key words: Phytophthora ramorum, temperature limitations

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Evaluating the Survival of *Phytophthora ramorum* in Firewood[1]

John Shelly[2], Ramnik Singh[2], Christine Langford[2], and Tad Mason[3]

Abstract

Many of the *Phytophthora ramorum* host species are a favored source for the commercial firewood industry. This study addresses the viability of *P. ramorum* in processed firewood and analyzes the unknown risk of spreading *P. ramorum* by transporting and storing firewood products produced from *P. ramorum*-diseased trees. Preliminary data from an earlier study suggested that *P. ramorum* spores could survive on firewood for at least 6 months. Positive *P. ramorum* cultures were isolated on eight of 49 freshly cut specimens and one of 30 specimens that had aged (air-dried) for 6 months. Following these initial results, a larger study was initiated. Approximately 7 tons of split firewood was selected from tanoak trees exhibiting the symptoms of sudden oak death, and stored at a commercial firewood operation in Marin County with ambient temperature, humidity and rainfall data being monitored and recorded. A similar amount of firewood, selected from the same diseased trees, is stored at the Richmond Field Station, University of California, on an outside concrete pad with simulated rain exposure reflecting periods of exceptionally high rainfall. Firewood specimens, as well as water collected from the firewood pile runoff, are periodically tested for presence of viable *P. ramorum* propagules.

Key words: Phytophthora ramorum, firewood, disease spread, sudden oak death, tanoak, Marin County

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Development of Detection Methods for *Phytophthora ramorum* in Georgia[1]

Pauline Spaine[2], William Otrosina[2], and Stephen Fraedrich[2]

Abstract

Since 1995, large numbers of tanoaks and oaks have been dying in the coastal counties of California. The death of these trees has been attributed to *Phytophthora ramorum*, the pathogen responsible for the syndrome commonly called Sudden Oak Death. In April 2004, *P. ramorum* infected nursery stock was shipped from California to many eastern states, including Georgia. At present, 16 nurseries in Georgia have confirmed the presence of infections of *P. ramorum* on nursery stock. In addition, some plants were sold to the general public before the nursery stock could be quarantined. *P. ramorum* has a wide host range that includes eastern oaks and ericaceous plants species. Many genera of east coast plants could support *P. ramorum* sporulation, particularly the ecologically and economically important eastern red oaks. This study will evaluate various methods of detection and baiting for *Phytophthora* propagules in water and on vegetation from different ecotypes throughout Georgia. Temperature and humidity will be monitored throughout the year to characterize the range of these parameters in Georgia forests, where potential *P. ramorum*-susceptible hosts occur. This information will help determine if southeastern forests contain microhabitats suitable for *P. ramorum* development. Our preliminary surveys of bleeding cankers on oaks indicated a broad assortment of organisms living in these cankers. The organism most often found associated with these cankers has been the fungal species *Mortierella* and potential antagonists, such as *Trichoderma*. Results from baiting techniques for waterways, streams and rivers will be compared to native water mold populations found associated with southern oaks and other indigenous plant species.

Key words: *Phytophthora ramorum*, baiting, waterways, microclimate, eastern oak species

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Sudden Oak Death Bike Tire Scrubber[1]

Peter Thut[2], Rocky Chavez[2], Desaree Williams[2], Sibdas Ghosh[2], Lisa Baird[3], Mietek Kolipinski[4], and Bruce Badzik[5]

Abstract

One source of inoculum for potential dispersal of *Phytophthora ramorum* is infested sediment on bicycle tires of visitors entering open-space land. This is a serious concern as recreational users may travel from an infested to a non-infested area in a short period of time. A prototype scrubber has been built to address this issue for mountain bike users of public parks and recreation areas. The device reduces accumulated soil and mud, decreasing the potential inoculum load on tires of mountain bikes before they leave an infested area. This scrubber is intended for areas without electricity, pressurized water, or personnel, and must be affordable, simple to operate, and easy to maintain at trailheads. The current design is essentially a trough of tough bristles through which the rider walks their bike. The motion of the bike provides all the power. In preliminary tests 75 percent of the adhered sediment was removed from the tire tread. Comparison with a competing design is discussed and recommendations for installation at trailheads are made. This scrubber is suggested as part of an overall system of reducing invasive species transport by humans.

Key words: Phytophthora ramorum, invasive species, mountain bikes

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Phytophthora ramorum Research and Control in North Coastal California[1]

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Key words: Phytophthora ramorum, survey, stream baiting, treatment, North Coast

Abstract

A number of factors distinguish the north coast counties of California (Mendocino, Humboldt, and Del Norte Counties) from other counties currently infested by, or vulnerable to, *Phytophthora ramorum.* The North Coast is still largely uninfested, with limited infections in southern Humboldt and central Mendocino Counties, but at high risk for introduction and spread of the pathogen, primarily because of climate and vegetation. Accordingly, cooperators from the University of California Cooperative Extension, the University of California, Davis, and the California Department of Forestry and Fire Prevention have launched an integrated, adaptive monitoring and treatment program for the North Coast that involves implementation of a variety of research and treatment approaches.

Over the past year, research efforts have focused on a sampling of over 75 heavily visited landmark areas, trails, campgrounds in state and county parks, and recreation areas known to have year-round use from central Mendocino County to the Oregon border. The first 100 m of each selected trail were surveyed out to 10 m from each side of the trail; symptomatic vegetation was sampled and surveyors noted sampled tree/shrub locations and selected environmental variables. For parking lots and campgrounds, all vegetation within the campground or parking lot area, as well as within a 10-m strip around the perimeter, was surveyed. *P. ramorum* was recovered from only two locations (three percent) near the known infestation in Redway, one along a trail and one beside a parking lot.

An additional research project involves continuous sampling of 31 strategically located streams from south of the Anderson Valley in Mendocino County to the Smith River near Oregon. In Mendocino County, the pathogen was recovered from one stream with no forest infestation immediately adjacent but approximately three km upstream; in Humboldt County, the pathogen was recovered from two streams that were near to the previously known Redway infestation (one with infestation adjacent to the stream, the other downstream approximately eight km). Stream monitoring plans for 2005 include the addition of eight to ten new stream sites near Redway and Garberville, three to four new stream sites on tributaries to the Klamath River, one to two new sites on tributaries to the Mattole River in southwestern Humboldt County, and one new site on the South Fork Winchuk River near Oregon.

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[1] An abstract of a poster presented at the Sudden Oak Death Second Science Symposium: The State of Our Knowledge, January 18 to 21, 2005, Monterey, California.

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Treatment of the infested area in Humboldt County near Redway has involved tree removal and site sanitation in an effort to reduce inoculum load and slow the spread of the pathogen. The pathogen was recovered post-treatment in the South Fork Eel River downstream from Redway, demonstrating the persistence of the infestation owing to several factors, such as incomplete cooperation from affected landowners, potential as-yet-unknown infected trees in the area, and possible pathogen presence in site soil. Future treatment options for the Redway sites, and an additional infested area near Garberville, will focus on a comparative study of other methods, including those that do not require whole-tree removal, such as pruning lower branches and chemical treatment. Ten-to-twenty-meter treatment buffers will also be installed to aid suppression efforts, where landowner permission can be obtained.

In addition to these research and treatment efforts, intensive education and outreach efforts, targeting a variety of interested parties and stakeholders throughout the North Coast area, have been completed and are in on-going development.

Characterization of European and American *Phytophthora ramorum* Isolates Due to Their Morphology and Mating Behavior *in vitro* With Other Heterothallic *Phytophthora* Species[1]

Sabine Werres[2] and Katrin Kaminski[2]

Abstract

The colony pattern, vegetative growth rate, sporangium and chlamydospore size and the mating behavior of 94 *P. ramorum* isolates (67 from Europe and 27 from North America) were examined. There were significant differences between *P. ramorum* isolates of mating type A1 and A2. The A1 isolates showed a homogenous colony pattern, grew faster and had bigger chlamydospores than the A2 isolates. Furthermore, none of them produced oogonia with *P. cambivora*. The A2 isolates were much more heterogenic and most of them accepted *P. cambivora* as a mating partner. The morphological character of the single A2 isolate from Europe (BBA 26/02) was similar to the A1 isolates; the mating behavior was similar to that of the A2 isolates. Differences within the studied *P. ramorum* population seemed to be more influenced by the mating type than by the source (for example, nursery, forest) of the isolate.

Key words: Phytophthora ramorum, mating in vitro, morphology

Introduction

The heterothallic *Phytophthora ramorum* is a severe pathogen on hardy ornamentals and trees in Europe and North America. Since its first detection and description (Werres and others 2001), many isolates have been collected and identified. Initial studies indicated that there was genetic and phenotypic variation between the European and North American *P. ramorum* populations (Brasier and Kirk 2004, Hansen and others 2003, Ivors and others 2004). To date, however, there have not been many detailed morphological studies with a wide range of isolates from both continents and none with regard to differences between the two mating groups. In our studies *P. ramorum* isolates were characterized, by morphology and their mating behaviour *in vitro*, with other heterothallic *Phytophthora* species.

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Material and Methods

Ninety-four *P. ramorum* isolates (67 from Europe and 27 from North America) were examined. Isolates from Europe were from *Rhododendron* (48), from *Viburnum* sp. (18) and from *Quercus rubra* (1). The North American isolates came mainly from *Quercus* spp. (5), *Lithocarpus densiflorus* (3), *Umbellularia californica* (3), *Arbutus menziesii* (2) and *Sequoia sempervirens* (1), but also from *Rhododendron* spp. (four from Canada, four from the USA), from *Viburnum* sp. (1) and from *Vaccinium* sp. (2). One isolate originated from a soil sample. Colony pattern, vegetative growth rate, sporangium and chlamydospore size and mating behavior were measured and calculated according to Werres and others (2001). Statistical analyses were made using a *t*-test and Mann-Whitney Rank Sum Test ($p<=0.05$).

Results

The A1 isolates showed no distinct colony pattern, but the A2 isolates showed more compact colony morphology, a flattening colony edge, or grew with sectors. Vegetative growth rate at optimum temperature was significantly higher for A1 than for A2 isolates. A single European A2 isolate (BBA 26/02) grew as fast as the A1 isolates (3.4 mm d-1). There was no significant difference between sporangia size and L:B ratio of A1 and A2 isolates. Chlamydospore size of A1 isolates was significantly higher than that of A2 isolates. Only one A2 isolate from Europe produced chlamydospores as big as A1 isolates (mean 56.4 µm).

All isolates could be paired successfully with the tester strains, except three isolates from the USA. All European isolates paired with heterothallic *Phytophthora* species of mating type A2, except the Belgian isolate BBA 26/02. Seven North American isolates (including the four Canadian isolates from nurseries) could be determined as mating type A1, 17 as mating type A2. The most successful mating partner was *P. cryptogea* (for 79.2 percent of all isolates), followed by *P. cinnamomi* (50 percent), *P. drechsleri* (17.3 percent) and *P. cambivora* (13.6 percent). There was a clear separation between isolates of mating type A1 and A2: none of the A1 isolates could be paired with *P. cambivora* while 83.3 percent of the 18 A2 isolates accepted this mating partner, as well as the single European A2 isolate (BBA 26/02). On the other hand 68.5 percent of the 73 A1 isolates accepted *P. cinnamomi*, while only 27.8 percent of the A2 isolates produced gametangia with this species.

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Surveying for *Phytophthora ramorum* in Ornamental Nurseries, Home Landscapes, and Forests in Georgia, USA[1]

Jean L. Williams-Woodward[2]

Abstract

Six ornamental production nurseries in Georgia were surveyed for *Phytophthora ramorum* in 2003. No *P. ramorum* was detected in 946 plant samples (508 nursery and 438 nursery perimeter/forest). Other *Phytophthora* species, mostly *P. cinnamomi* and *P. parasitica*, were recovered from *Rhododendron* and *Pieris* leaves from 50 nursery and 2 perimeter samples. Trace forward surveys in 2004 identified 14 retail nurseries that received *P. ramorum*-infected camellia plants from Monrovia Nurseries, Azusa, California. Adjacent plants, as well as the forested nursery perimeters were surveyed, and *P. ramorum* was not detected. From January 2003 to March 2004, Georgia received *ca*. 28,000 plants from Monrovia Nurseries in Azusa. A collaborative effort to recover purchased suspect *P. ramorum*-infected plants was initiated by the Georgia Department of Agriculture, Georgia Forestry Commission, and The University of Georgia Cooperative Extension Service. Three *Camellia* samples were confirmed positive out of 221 home landscape samples. All known infected plants were removed, and *P. ramorum* was not detected in a survey of landscape plants, soils, or forested environ away from the infected plant. *P. ramorum* was introduced into Georgia. However, there is no evidence that *P. ramorum* has spread or become established within retail nurseries.

Key words: sudden oak death, landscape, nursery, survey, establishment

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Plotless Evaluation of *Phytophthora ramorum* Incidence in Oaks and Tanoaks in Two Different Forest Types in California[1]

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Abstract

Sudden oak death (SOD) has a patchy distribution in the forests of central California. Both this fact and the requirement that individual trees must be inspected present considerable challenges to efforts to estimate the extent of this disease and resulting mortality at landscape scales. We sought to develop unbiased estimates of these disease parameters in two forests that differed in species composition and apparent impacts of the disease. In 2001, we initiated monitoring studies in the 600-ha China Camp State Park (CCSP) in Marin County and the 1070-ha Soquel Demonstration State Forest (SDSF), in Santa Cruz County. CCSP, which has shown evidence of severe disease since the late 1990s, features coastal oak woodlands, montane hardwood, and montane-hardwood-conifer forest types. SDSF, reported to be much less affected, is primarily redwood-tanoak woodland. The host species of interest in CCSP were coast live oak (*Quercus agrifolia*) and California black oak (*Q. kelloggii*), and in SDSF, tanoak (*Lithocarpus densiflorus*), coast live oak, and Shreve oak (*Q. parvula* var. *Shrevei*). We used a point-centered quarter, transect-based approach (Engemann and others 1994) to estimate *P. ramorum* infection and mortality in these species. For this plotless method, we arrayed parallel transects, 500 m apart, in a north-south alignment, to cover each site. Sampling nodes were placed every 100 m on each transect and GPS coordinates were recorded.

Key words: Phytophthora ramorum, coast live oak, tanoak, Shreve oak, point-centered quarter population density estimation

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At each node, the nearest oak or tanoak in each cardinal direction (up to four per node) was permanently labeled, azimuth and distance from the node center were recorded and the tree was evaluated for SOD symptoms. Evaluation was based on symptoms alone (bleeding, scolytid beetle tunneling, and the presence of *Hypoxylon thouarsianum* reproductive structures) to avoid manipulating trees and possibly changing disease status through wounding. Each stem greater than 2.5 cm diameter at breast height (dbh) was treated as an individual tree. Up to four trees were evaluated in each of 89 (CCSP) and 139 (SDSF) sites. Trees in SDSF were re-evaluated in 2003, and in 2004 in CCSP. An additional four nearest neighbor trees were added to the study in 2004 for CCSP. Where bleeding was observed in 2004, samples were collected, plated onto PARP media at the site, cultured in the lab, and checked for the presence of *Phytophthora* spp.

In CCSP, the proportion of symptomatic coast live oaks declined by 2004, but mortality among those bleeding in 2001 doubled (Table 1). Numbers of both bleeding and dead black oaks increased. In SDSF, the proportions of coast live oaks and tanoaks that were bleeding and dead increased by 2003, but Shreve oaks showed little response to the presence of *P. ramorum* in the forest (Table 2). Scolytid beetles had colonized bleeding coast live oaks in CCSP in both 2001 (66 percent) and 2004 (59 percent). Among bleeding California black oaks in CCSP, these percentages were 24 percent in 2001 and 28 percent in 2004. *Phytophthora* (not yet identified to species) was cultured from 39 percent of bleeding coast live oak cankers (n = 44) and 50 percent of bleeding California black oak cankers (n = six).

Table 1— *China Camp State Park symptomatic and dead trees, 2001 and 2004.*

		Percent Bleeding		Percent Dead	
Species	n	2001	2004	2001	2004
Coast Live Oak	364	23	13	10	21
Black Oak	52	23	28	14	25

Table 2— *Soquel Demonstration State Forest symptomatic and dead trees, 2001 and 2003.*

		Percent Bleeding		Percent Dead	
Species	n	2001	2003	2001	2003
Tanoak	393	13	26	2	7
Shreve Oak	95	2	4	1	4
Coast Live Oak	29	10	20	3	14

Plotless evaluation of SOD was intended to provide both initial estimates of disease incidence and resulting mortality and information on rates of change. The decline from 2001 to 2004 in the proportions of coast live oaks that were bleeding, combined with the increase in mortality, may indicate that many of the trees that were symptomatic in 2001 were the most susceptible,

many of which have since died. Relatively few trees that were bleeding in the 2004 monitoring were asymptomatic in 2001. This may be evidence for the existence of resistance to *P. ramorum* in the population. California black oaks showed slight increases in bleeding proportions. The trends for both species are consistent with results from disease progression plots over the same period in CCSP (McPherson and others 2005). The strength of the association of scolytid beetles with bleeding coast live oaks has also been observed in the same Marin County disease progression plots. The lower proportions of symptomatic and dead trees for all species in SDSF are consistent with the hypothesis that the introduction of *P. ramorum* into this isolated drainage in Santa Cruz County was more recent than in Marin County, where the disease was first noticed and where its impact is considerably greater. The lower response of Shreve oak to the presence of *P. ramorum* inoculum in SDSF might indicate that this species is less susceptible to *P. ramorum* than the other fagaceous hosts identified in California. However, very little is known about the ecology of Shreve oak, a species that was only recently recognized to be a major component of the mixed evergreen forests of the Santa Cruz Mountains.

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Thwarting *Phytophthora ramorum*: An Interactive Poster on Breaking the Disease Cycle[1]

Betsy Randall-Schadel[2] and Scott Redlin[3]

Abstract

A proposed disease cycle was presented in an interactive format. The lack of a single mitigation measure to effectively eliminate *Phytophthora ramorum* from nursery stock suggested the value of a diagram highlighting points in the disease cycle, where multiple mitigations may be applied. For each point, potential mitigation measures are listed. The diagram was compiled from scientific sources, including published papers and proceedings of science panels. The proposed *P. ramorum* disease cycle is being presented as an interactive poster to prompt and capture discussion.

Key words: *Phytophthora ramorum*, nursery stock, mitigation measures

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Phytophthora ramorum on *Calluna vulgaris*, *Photinia fraseri*, and *Pieris japonica* in Poland[1]

Leszek B. Orlikowski[2], Aleksandra Trzewik[2], and Grazyna Szkuta[3]

Abstract

After recovery of *Phytophthora ramorum* from rhododendron and cowberry (*Vaccinium vitis-idaea)* in 2000 to 2002, disease symptoms were observed on three other ornamentals from July to August 2003. Symptoms usually occurred after at least three drizzly days at 18 to 23º C. On heather (*Calluna vulgaris*), symptoms were observed only on cv. Peter Sparkes. Shoot apices turned brown to dark brown for two to five cm. Older invaded shoots formed shepherd's crooks. On Photinia (*Photinia fraseri*), symptoms were observed only on leaves. Petioles were dark brown and necrosis spread on blades. On some leaves, brown or dark brown spots were observed at the apex or middle of leaf blades. Spots usually enlarged and leaves fell quickly. On leaf spots transferred to moist chambers, one to two mm brown-black drops appeared within two to three days at 20 to 22º C. *Pieris* shoot symptoms were similar to *Rhododendron*. Morphological and molecular methods indicated *P. ramorum* was the A1 mating type. The pathogen grew best at 20 to 25º C (range 2 to 28º C). After pairing three test isolates with A2 mating type of *P. cryptogea*, generative structures formed. Three isolates from heather, *Photinia*, *Pieris* and three cultures from *Rhododendron* caused similar lesions. Most rhododendron and heather cultivars inoculated with *P. ramorum* isolates developed necrotic symptoms.

Key words: Phytophthora ramorum, Calluna vulgaris, Photinia fraseri, new hosts

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Comparative Evaluation of Real-Time PCR (TaqMan®) With Isolation for Diagnosis of *Phytophthora ramorum*[1]

Kelvin Hughes[2,3], Ruth Griffin, Jenny Tomlinson, Neil Boonham, Victoria Barton, Patricia Giltrap, Ellie Hobden, Lynn Walker, Gilli Humphries, Ann Barnes, Paul Beales, Alan Inman, and Charles Lane[3]

Key words: Phytophthora ramorum, ramorum canker, dieback and leaf blight, sudden oak death, TaqMan®

Abstract

Since 2001, the Central Science Laboratory (CSL) in York, has been involved with diagnosis of *Phytophthora ramorum* from various ornamentals and tree hosts including rhododendron, viburnum, pieris, camellia, lilac, Virginian witch-hazel, American southern red oak (*Quercus falcata*), Holm oak (*Q. ilex*) and yew. This has been performed using both traditional means, such as isolation, baiting and morphological assessment, as well as serological lateral flow devices and PCR. This poster describes comparative testing on 320 plant samples for *P. ramorum* using isolation and a real-time PCR (TaqMan®) assay developed at CSL and under evaluation by U.S. Department of Agriculture-APHIS. Each sample was sent to CSL by Department for the Environment, Food and Rural Affairs, (DEFRA) Plant Health and Seeds Inspectorate between October 2003 and February 2004. These were surface- decontaminated with water then split into two equal parts. One half was plated out onto semi-selective agar (P_5ARP[H]) and examined microscopically after six days incubation (20° C, 12h light /12h dark) for the presence of *P. ramorum*. From the second half, DNA was extracted using a Nucleospin DNA extraction kit, and this was tested with the TaqMan® assay taking two days. This contained reagents for specific amplification of *P. ramorum* DNA and a universal plant gene (cytochrome oxidase (COX)) as an internal reaction control. In this trial of 320 samples, over 98.7 percent of the samples, gave the same result for both isolation and TaqMan® PCR with 25 samples (7.8 percent) testing positive for *P. ramorum*.

However, for four of the 320 samples, the test results did not agree; two samples were negative by isolation, but positive by TaqMan® for *P. ramorum,* possibly indicating that *P.*

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ramorum was dead and could not be cultured. Two samples were positive by isolation for *P. ramorum,* positive by TaqMan® for the COX assay, but negative by TaqMan® for *P. ramorum*. We feel that this indicates that *P. ramorum* DNA was not extracted from these samples or its presence was below a detectable level for diagnosis by TaqMan® PCR. In conclusion, this trial demonstrated that isolation and TaqMan® PCR are equally reliable and robust diagnostic assays for diagnosis of *P. ramorum* for plant material in the U.K.

Acknowledgements

Funding for this work was provided by DEFRA Plant Heath Division and all material was processed under DEFRA licence for handling non-indigenous plant pathogens.

Development of Molecular Diagnostics for *Phytophthora kernoviae* a New *Phytophthora* Threatening U.K. Trees, Woodlands, and Ornamental Plants[1]

Kelvin Hughes[2, 3], Ruth Griffin[3], Neil Boonham[3], and Alan Inman[3]

Key words: Phytophthora kernoviae, Phytophthora taxon C, bleeding canker

Abstract

In autumn 2003, a new *Phytophthora* species, informally designated as *Phytophthora kernoviae* and previously known as *Phytophthora* taxon C, was isolated by the Central Science Laboratory (CSL) from rhododendron bushes from a woodland area in southwest England. Concurrent with this, Forest Research (FR) isolated a similar *Phytophthora* from a large bleeding canker present on a mature beech tree (*Fagus sylvatica*) and from an adjacent rhododendron at a second southwest site. *P. kernoviae* has since been isolated from soil and also found on other plant species: *Liriodendron tulipifera, Pieris formosa, Magnolia stellata, Michelia* sp., *Gevuina avellana, Fagus sylvatica* and *Quercus ilex.* Morphological assessment by CSL and FR (Brasier and others 2004, 2005) revealed these isolates to be the same species having papillate, caducous to mouse-shaped sporangia (average size 39.6 x 25.6 µm, range 30 to 50 x 20 to 32 µm), being homothallic with amphigynous atheridia approximately 11 x 10 µm and oogonia with an average diameter of 25 to 27 µm. Sequence information from the internal transcribed spacer regions (ITS one and two) of the nuclear ribosomal gene repeat were also aligned with data provided from Dr. David Cook of the Scottish Crop Research Institute. These sequences were identical to each other and distinct from other sequences on the Genbank sequence database, with the closest match to *P. boehmeriae*. Further alignment of this sequence data with that from 45 other *Phytophthora* isolates, representing 10 *Phytophthora* species, including *P. ramorum* and *P. boehmeriae,* has allowed us to identify areas for PCR primer design. Based on these areas, and areas in the cytochrome oxidase gene (subunits I and II), TaqMan® primers and probes have been designed and are currently being evaluated on pure cultures and plant samples from the U.K. Work is also under way to use *P. kernoviae* primers for on-site use and to profile *P. kernoviae* isolates with molecular methods including AFLP.

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Isozyme Genotyping of *Phytophthora ramorum* Reveals Fixed Heterozygosity at Three Loci Suggesting a Hybrid Origin[1]

Willem A. Man in't Veld[2]

Abstract

Five enzyme stains, comprising seven loci, were used to characterize 37 strains of *Phytophthora ramorum* originating from Europe and America. All strains were monomorphic at all putative loci, except *Ldh-2*, which was polymorphic. At the dimeric *Gpi* and *Ldh-2* loci, three banded patterns, and sometimes five banded patterns (*Ldh-2*), were present indicating heterozygosity. The presence of four alleles at *Ldh-3* indicates polyploidy. I concluded that the American and European strains are conspecific. The heterozygosity at *Gpi, Ldh-2* and *Ldh-3* suggests that *P. ramorum* has been involved in hybridization events in its evolutionary past, because fixed heterozygosity is generally considered to be the hallmark of hybridization. This hybridization event may have triggered the problematic mating behavior as observed in laboratory experiments. When in the presumed common center of origin, outcrossing would have taken place regularly; gene flow between the two mating types should have resulted in homozygosity at the three heterozygous loci forementioned, at least in some strains. This, however, was not observed. In addition, sequence analysis of the Cox I gene and AFLP data showed consistent differences between the two mating types. This apparent lack of gene flow suggests that the two mating types have been reproductively isolated in their recent evolutionary past, and that they may represent two species *in statu nascendi*.

Key words: dimeric enzymes, fixed heterozygosity

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The Effects of Sudden Oak Death on Wildlife-Can Anything Be Learned From the American Chestnut Blight?[1]

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Abstract

American chestnut (*Castanea dentata*) was largely removed from eastern North American forests by chestnut blight, a disease caused by the introduced fungus *Cryphonectria parasitica*. In 1900, American chestnut comprised nearly 25 percent of eastern deciduous forests in the United States. From 1910 until 1950, chestnut blight spread over 81 million ha of forestland, profoundly affecting the ecology and economy of eastern forests, especially in the Appalachian area. In 1995, Sudden Oak Death (SOD) was identified near Mill Valley, California. Caused by the fungus *Phytophthora ramorum*, the disease has since spread to 14 coastal California counties, killing thousands of tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) trees. SOD threatens coastal woodlands throughout California and Oregon, as well as some eastern U.S. and European hardwood forests. Present-day issues in the conservation and management of California oak woodlands are disturbingly reminiscent of the widespread concern for the future of chestnut in the early 1900s. Insight into chestnut blight's effects on wildlife, however, comes from only a few newspaper reports and study of the few remaining mature trees. The profound changes in forest composition and structure unquestionably reduced the eastern forest's carrying capacity for many species of wildlife. Hopefully we can anticipate and shape the future, so that the high diversity and abundances of wildlife in California coastal oak woodlands will persist.

Key words: American chestnut blight, *Cryphonectria parasitica*, sudden oak death, *Phytophthora ramorum*, wildlife

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Elimination of *Phytophthora ramorum* in the Tunnel Composting Process[1]

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Key words: Phytophthora ramorum, Rhododendron, tunnel composting, sanitation

Introduction

Phytophthora ramorum is the causal agent of sudden oak death, which has a devastating effect on a wide range of host plants (Werres and others 2001, Rizzo and others 2002). In contrast to infected trees, which seem to be a dead-end host, *Rhododendron* and *Umbellularia californica* seem to play a key role in the spread of this pathogen, probably by acting as main inoculum source (Swiecki and Bernardt 2002, Garbelotto and others 2003). Partly due to its aggressiveness, the pathogen is placed on the E.U.-Alert list. Phytosanitary regulations on such pathogens require destruction of infected plant material. Several composting or heat treatment techniques have been tested recently to determine the effect on the survival of *P. ramorum* (Garbelotto 2003, Harnik and others 2004). In the present research, the use of tunnel composting for the elimination of *P. ramorum* in infected plant material was investigated *in situ*.

Tunnel composting is a closed system composting method, and therefore, suitable for composting plant material infected with quarantine organisms, such as *P. ramorum*. The essence of this method is that the plant debris is composted under strict temperature and moisture conditions, using hot circulating air, which is blown through a perforated floor and the plant material. The actual composting process can be divided into two phases: pre-composting and after-composting. The pre-composting phase consists of a pasteurisation step, in which the debris is heated up to a minimum of 60° C (140º F) for at least 10 hours, followed by a drying step, which takes approximately four days. The actual composting takes place during the after-composting phase in which the material is kept at 48° C (118º F). This part of the

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process takes at least 12 days. The composting is followed by a filtration step. Particles larger than 15 mm are removed from the end product and incinerated or composted again.

Methods

The effect of the above mentioned pasteurisation step on the survival of *P. ramorum* was investigated. For this experiment, plant material was collected from wildtype *Rhododendron catawbiense* showing *P. ramorum*-like symptoms. The material was divided in 20 shoot samples, as well as 20 samples of leaves. Within a sample, each individual piece of material was cut in half to obtain two sets of 'identical' subsamples. To prove the viability of the pathogen, one set of subsamples was tested in the presence of *P. ramorum* using a leaf-baiting test, followed by a Taqman ITS-PCR assay for the detection of the pathogen.

The remaining subsamples were placed in small nylon nets, which were hidden in large coarse woven plastic nets among approximately 15 litres of *Rhododendron* plant debris. The nets containing the plant debris and the test material were placed in a filled tunnel-composting chamber. After 10 hours at 60° C, the recovered samples were tested in the described baiting test, again followed by Taqman ITS-PCR.

Results and Discussion

The results of the bait and the Taqman PCR, which was conducted on the first, non-composted set of subsamples, showed that *P. ramorum* was present in 37 samples (18 leaf and 19 shoot samples). Three samples did not show presence of viable *P. ramorum*. Their corresponding 'identical' subsamples in the second set were not composed, but acted as a negative control in the baiting test on the composted material.

After the pasteurisation, 27 samples (13 leaf and 14 shoot samples) were recovered, the other ten samples were lost in the process. None of the recovered samples showed presence of viable *P. ramorum*. Our conclusion is that *P. ramorum* is eliminated in the pasteurisation step of the tunnel-composting process.

Garbelotto (2003) found that *P. ramorum* infected material was sanitized after two weeks exposure to 55° C. Recently, Harnik and others (2004) demonstrated that *P. ramorum* survives at least one week at 55° C in infected leafs of *U. californica*. In our present study, we concluded that a five degree (Centigrade) higher temperature

shortens the exposure time needed. This is supported in a recent experiment by Harnik and others (2004) using infected California bay laurel leaves. They showed that a short period at 60° C, in combination with 22 hours in a vacuum, is sufficient to eliminate the pathogen. The main advantage of the tunnel composting pasteurisation step is that the eradication of *P. ramorum* is completed in only 10 hours, which makes tunnel composting a highly effective option for the elimination of *P. ramorum* in infected plant material.

Table 1—*Results of the baiting test on leaf (a) and shoot (b) material, before and after pasteurisation at 60°C for 10 hours.(+)* P. ramorum *present, (-)* P. ramorum *absent, (nt) not tested/not recovered from the composting installation.*
a) Leaf materia

	Sample Number																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**Tot**
Before composting	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	18/20
Post-treatment	-	-	-	-	-	-	-	-	-	-	-	-	nt	-	nt	nt	nt	nt	nt	nt	0/13
b) Shoot material																					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**Tot**
Before composting	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19/20
After 10h at 60°C	-	-	nt	-	-	-	-	-	-	-	-	-	-	-	-	nt	nt	nt	nt	nt	0/14

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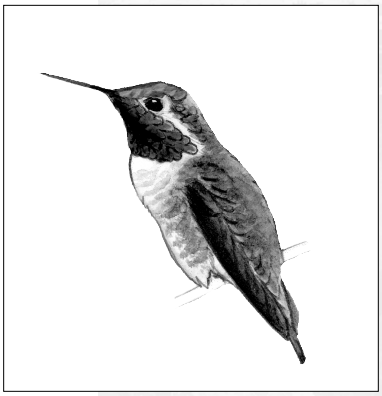
Index



Index

A

Afzal-Rafii, Zara 123
Alexander, Janice 37, 503
Allen-Diaz, Barbara 179, 191, 494
Anacker, Brian 515
Apigian, Kyle 191, 494
Appel, D.N. 493
Appiah, Alex 167
Aveskamp, Maikel M. 279, 560

B

Badzik, Bruce 542
Baird, Lisa 512, 542
Balci, S. 57
Balci, Y. 57
Barker, Ian 143
Barnes, Ann 554
Barrett, T.M. 371
Barton, Victoria 554
Bauer, L. 534
Beales, Paul 554
Beals, Kathryn 119
Beltz, Heinrich 287, 523
Bennett, Lynne 455
Bergemann, Sarah 61, 193, 526
Berger, Philip 47
Bernnardt, Elizabeth 383, 427
Bienapfl, John C. 495, 531, 543
Billings, R.F. 493
Bilodeau, G.J. 139
Bonants, Peter 129, 135, 145
Bonello, Pierluigi 423, 532
Boonham, Neil 143, 554, 556
Boore, Jeffrey 157
Boschee, M. 534
Brand, Thomas 287, 523
Brasier, Clive 31, 101, 105, 109
Briere, Stephan C. 496
Brown, Anna 31, 105
Brown, Letty B. 179, 494
Buja, Kelly 361

C

Camilli, K.S. 493
Chambers, Dave L. 263, 269, 459
Chastagner, G.A. 77, 239
Chavez, Rocky 542
Choquette, Caroline J. 291, 535
Cohen, S.D. 497
Colunga, Manuel 329
Costello, Laurence R. 427
Cushman, J. Hall 161

D

Davidson, Jennifer M. 500, 503
Davis, E. Anne 83, 265, 299, 538
de Cock, A.W.A.M. 139
De Dobbelaere, Isabelle 79, 241
de Gruyter, J. 279, 367
de Vries, Ineke 135, 145
Denman, Sandra 31, 105, 109

Descals, Enrique 183, 530
Dodd, Richard S. 119, 123, 125
Drake, Christiana 427
Duchaine, C. 139

E

Eales, Stephen 167
Eggers, J. 57
Erbilgin, Nadir 419

F

Falk, Kristin R. 500
Fichtner, Elizabeth J. 165, 459, 500, 501
Fischer, Keyt 193
Fischer, Lisa 345
Florance, Edwin R. 91
Forrester, Yashika 502
Fowler, Glenn 329
Fraedrich, Stephen 541
Frangioso, Kerri 193
Frankel, Susan J. 463, 503, 509

G

Garbelotto, Matteo 61, 63, 87, 125, 129, 131, 143, 145, 149, 157, 263, 483, 517, 518, 526, 533, 539
Geltz, Elizabeth 512
Ghosh, Sibdas 512, 542
Gilless, J.K. 465
Giltrap, Partricia 554
Goheen, Ellen Michaels 27, 113, 413, 489, 513
Gordon, Sarah 515
Gottschalk, K.W. 57
Griffin, Ruth 554, 556
Grigoriev, Igor 157
Grunwald, Niklaus J. 83, 157, 538
Guo, Q. 333

H

Hadj-Chikh, Leila 193
Hamelin, R.C. 139
Hansen, Everett M. 27, 63, 77, 141, 239, 259, 413, 489, 516, 529, 536
Harnik, Tamar Y. 87, 125, 131, 517
Hayden, Katherine 518, 539
Hesse, C. 141
Heungens, Kurt 79, 241
Hobden, Ellie 554
Hogue, Allan 502
Hong, Chuanxue 147
Hüberli, Daniel 87, 125, 129, 131
Hughes, Kelvin 143, 554, 556
Humphries, Gilli 167, 554
Hunter, Stephen 51

I

Inman, Alan 554, 556
Ivors, Kelly 129, 135, 143, 145, 157, 533

J

Jennings, Philip 167, 273
Jensen, Camille E. 519
Jirka, Amy 345
Jones, Jonathan M. 45
Juzwik, J. 57

K

Kanaskie, Alan 27, 259, 413, 489, 513, 534
Kaminski, Katrin 287, 520, 523, 545
Karchesy, Joseph J. 67
Kelly, N. Maggi 333, 379, 549
Kelsey, Rick G. 67
Kirk, Susan 101, 105, 109
Klinger, Lee 181, 487
Koenig, Walter D. 195
Koike, S. 269, 459
Kolipinski, Mietek 512, 542
Kong, Ping 147
Kordesch, Nicholas 61, 526
Kristjansson, Gary 139, 496
Kubisiak, Thomas L. 113, 147
Kurdyla, T. 493

L

Lamour, Kurt 157
Lane, Charles 455, 554
Langford, Christine 467, 540
Lee, Chris 531, 543
Lévesque, C.A. 139
Lewis, Carrie D. 291, 295
Liddell, Debbie 167, 273
Linderman, Robert G. 83, 265, 299, 538
Linzer, Rachel 149
Liu, D. 333
Llewellyn, Sarah 496
Lockley, David 167
Loda, Jenny 494
Long, R. 57
Lotz, Elizabeth 361
Lüttmann, Ralf 287
Lynch, Shannon C. 165, 501, 527

M

MacDonald, W.L. 57
Maes, Martine 79, 241
Magarey, Roger 329
Mai, Jeffrey A. 345
Maloney, Patricia E. 527
Man in't Veld, Willem A. 558
Manter, Daniel K. 67
Mark, Walter 345, 361
Marshall, Jack 543
Martin, Frank N. 153
Mason, Tad 467, 540
Mayer, Wasima 123
McDonough, Sam 167, 273
McDowell, Robert 502, 528
McHugh, Jessica 512
McLaughlin, I. 529
McPherson, Brice A. 275, 379, 419, 423, 549
McWilliams, Michael 413, 489
Meentemeyer, Ross 161, 361, 515
Meshriy, Matthew 87, 131
Metz, Timothy 61
Mielke, Manfred 532
Miles, Lori 87, 131
Monahan, William B. 195
Moralejo, Eduardo 183, 530
Mori, Sylvia R. 379, 503
Moritz, Max A. 169
Murphy, Shannon K. 69, 531, 543

N

O

O'Dell, Brenda 125
O'Hara, Kevin L. 185, 187
Oak, Steven W. 365, 463
Ockels, Frances S. 423, 532
Odion, Dennis C. 169
Olarte, Rodrigo 533
Orlikowski, Leszek B. 553
Oryang, David 502
Osterbauer, Nancy 27, 413, 489, 513, 534
Otrosina, William 541
Owen, Don 503

P

Parke, Jennifer L. 291, 295, 513, 535
Parry, Nicola 455
Patterson, Heather A. 500
Peterson, Ebba 193
Posner, Martin 287
Propero, S. 536
Purdy, A. 493
Putnam, Nik 157

Q

R

Randall-Schadel, Betsy 285, 502, 528, 552
Reaves, Jimmy L. 11
Redlin, Scott 285, 552
Reeser, P. 141
Reuther, Keefe 87
Richardson, Patricia A. 147
Riley, K.L. 77, 239
Rizzo, David M. 21, 63, 69, 73, 149, 165, 275, 361, 495, 500, 501, 503, 519, 527, 531, 543
Robertson, Shawn 528
Rokhsar, Daniel 157
Rose, Joan 101, 105, 109
Roth, Melody L. 291, 513, 535

S

Scheuerell, Steven J. 538
Schinsing, Lilly 539
Schmidt, Doug 263
Seipp, Dankwart 523
Shaari, D. 333
Shea, Patrick J. 7
Shelly, John 467, 540
Singh, Ramnik 467, 540
Slawson, David 455
Smith, W.D. 365
Spaine, Pauline 541
Spencer, Mark 185
Standiford, Richard B. 275, 379, 419, 549
Stanley, Mark R. 17, 509
Steeghs, M.H.C.G 367
Stonehouse, Jackie 167, 273
Storer, Andrew J. 275, 379, 419, 549
Suslow, Karen 41
Suttle, S. 534
Sutton, Wendy 27, 77, 141, 239, 259, 413, 489, 516, 529
Svihra, Pavel 275, 379, 419
Swain, Steven 483
Swiecki, Tedmund J. 383, 427
Szkuta, Grazyna 553

T

Tack, J. 465
Tempel, Douglas J. 211, 233, 559
Thut, Peter 512, 542
Tietje, William D. 211, 233, 305, 559
Tjosvold, Steve A. 263, 269, 275, 459
Tkacz, B.M. 365
Toas, Sarah 494
Tomlinson, Jenny 143, 554
Tooley, Paul W. 153
Tripathy, Sucheta 157
Trippe, Aaron 513, 534
Trzewik, Aleksandra 553
Turner, Judith 167, 273
Tyler, Brett 157

U

Ufer, Thorsten 287

V

Valachovic, Yana 531, 543
van Baal, P.J.M. 279
Venette, R.C. 497
Verstappen, Els 135

W

Wagner, Art 129
Wagner, Stefan 287, 520, 523
Walker, Lynn 554
Waring, Kristen M. 187
Webber, Joan 31, 105, 109
Werres, Sabine 287, 520, 523, 545
Wessels, Hans-Peter 287
Whitkus, Richard 515
Wickland, Allison C. 73, 519
Wiejacha, Kataryzna 135
Williams, Desaree 542
Williams-Woodward, Jean 548
Wingelaar, Johanneke 560
Winslow, Donald E. 211, 305, 559
Winton, L.M. 141, 536
Wood, David L. 275, 379, 419, 423, 549
Woolsey, Lynn 3

X

Y

Z

Zanzot, J.W. 495
Zhao, Wenxia 113
Zingaro, Ralph 181
Zwane, A. Peterson 465